

**MICROBIAL CONTAMINATION OF NILE TILAPIA (*Oreochromis niloticus*)
AND AFRICAN CATFISH (*Clarias gariepinus*) FINGERLINGS REARED IN
HATCHERY TANKS AT SAGANA, JAMBO, AND MWEA FISH FARMS**

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SCIENCE (MICROBIOLOGY) IN THE SCHOOL OF PURE AND APPLIED
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DECLARATION

This thesis is my original work and has not been presented for award of a degree in any other university or for any other award.

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DEDICATION

This work is dedicated to my late grandfather Isaac Ndinwa and my late grandmother Damaris Wangeci who worked honestly and tirelessly to empower their families and kin with education and knowledge as a basis of life.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BW	Body Weight
CFU	Colony Forming Unit
dH ₂ O	Distilled Water
DO	Dissolved Oxygen
FAO	Food and Agriculture Organization
FFEPP	Fish Farming Enterprise Productivity Programme
NARDTC	National Aquaculture Research Development and Training Center
pH	Potential Hydrogen
RAS	Recirculation Aquaculture Systems
SARNISSA	Sustainable Aquaculture Research Networks in Sub-Saharan Africa.
Spp	Species
SPSS	Statistical Package for Social Sciences
SS	Salmonella-Shigella Agar
TCBS	Thiosulfate Citrate Bile Salt Sucrose
TDS	Total Dissolved Solids
TL	Total Length
TVC	Total Viable Count
WHO	World Health Organization

ABSTRACT

In Kenya, aquaculture continues to expand as a source of food production and livelihood in all the counties. Mortality of Nile Tilapia and African Catfish fingerlings constitute the biggest loss in extensive and intensive fish farms. The main objective of this study was to determine contamination by bacteria and fungi in Nile Tilapia and African Catfish fingerlings. The study was carried out from March 2014 to July 2014 in fish farms with different water sources, hatchery systems and feed sources. These were; Jambo fish farm in Kiambu County which utilizes borehole water and has a recirculation aquaculture system also known as (RAS). Sagana and Mwea fish farms in Kirinyaga County. Four weeks old and weighing 5-7 g, Nile Tilapia (*Oleochromis niloticus*) and African Catfish (*Clarias gariepinus*) 60 each in number were stocked as follows; in every hatchery tank 10 fingerlings of each species regardless of sex. Data obtained from samples was analyzed using descriptive statistics. Fish fingerlings samples and fish feeds were collected from three fish farms and taken to the Laboratory. The bacterial load of the samples was determined using the pour plate method. Identification and characterization of various isolates were based on gram-staining technique, biochemical tests and subculture on selective media. Fungi were isolated on SDA agar, Macroscopy of hyphae and mycelium, microscopic identification of subcultured fungal isolates using LCBS stain and Mycology charts. The mean of bacteria isolated in the two fish species from three farms per cfu/mL was as follows: African Catfish had $9.00 \pm 3.85a$, $27.75 \pm 2.85a$ and $21.67 \pm 4.82a$ for the *Bacillus* spp, *Escherichia* spp and *Salmonella* spp, respectively while Nile Tilapia had $8.58 \pm 3.68a$, $25.25 \pm 3.54a$ and $22.83 \pm 4.95a$, for the *Bacillus* spp, *Escherichia* spp and *Salmonella* spp, respectively. *Penicillium* spp 33.1 ± 2.5 cfu/g and *Rhizopus* spp 27.6 ± 2.8 cfu/g occurred less frequently. There was a significant variation ($p = 0.015$) in the means of bacteria contaminants isolated in Sagana fish farm from Nile Tilapia and African Catfish species. *Escherichia* spp in the skin did not vary significantly ($P = 0.0684$) between the fish species, however African catfish skin had the highest 27.7 ± 2.8 *Escherichia* spp contamination. There was also significant ($p < 0.0001$) variation in *Bacillus* spp contamination in fingerlings skins from different farms. The occurrence of *Klebsiella* species 22.0 ± 4.7 cfu/ml, *Salmonella* species 22.80 ± 4.9 cfu/ml, *Streptococcus* spp 25.0 ± 5.4 cfu/ml while *Vibrio* spp had a mean of 20.0 ± 3.6 cfu/ml. There was no significant ($P = 0.4857$) interaction between fish species and farm in determining the *Klebsiella* spp in fish skin. Formulated feeds were more contaminated (25.58) than commercial feeds (1.15) (Table 4.6). Formulated feeds had higher contaminations attributable to high moisture, poor storage facilities, handling and preparation methods. For physiochemical parameters, Salinity varied from 155.47 ± 6.49 to 94.70 ± 0.33 mg/L in Sagana and Mwea respectively with Mwea recording the least levels of salinity. Water temperature in the morning ranged from $20.55 \pm 0.49^\circ\text{C}$ to $25.30 \pm 0.26^\circ\text{C}$. In the evening time, there was also a significant variation in the levels of temperature with Sagana recording the highest at 27.10 ± 0.63 , followed by Jambo 25.00 ± 0.41 and Mwea had the lowest temperature of 20.15 ± 0.43 . Variations of salinity, temperature oxygen levels are among the parameters which determine the contamination for the two fish species among the three farms. It is recommended that all farms supplying fingerlings apply frequent assessment of water parameters, fish feeds and water analysis. Fish farmers should be educated on best practices in aquaculture in order to prevent bacterial and fungal contamination.

CHAPTER ONE

INTRODUCTION

1.1 Background information

As a food-producing industry, aquaculture is growing fast and has made enormous contribution to economic development and global food security. However, the output in Africa has stagnated over a decade (FAO report, 2012). Kenyan aquaculture industry has not been growing fast for decades now until recently, when the government-funded Economic Stimulus Program increased fish farming nationwide. This noble program has facilitated poverty alleviation, spurred economic growth and strengthened livelihoods among Kenyan fish farmers. Indeed, national aquaculture production grew from 1,000 MT/y in 2000 (equivalent to 1% of national fish production) to 12,000 MT/y, representing 7% of the national harvest, in 2010. The production is projected to hit 20,000 MT/y, representing 10% of total production and valued at USD 22.5 million by the year 2020. The dominant aquaculture systems in Kenya include earthen and lined ponds, dams, and tanks distributed across the country (FAO, 2017).

Intensification of aquaculture practices like use of contaminated water from rivers and formulated feeds leads to the increase of a number of pathogens (FAO report, 2010). Fast and unrestrained advancement of infectious agents in aquatic creatures and unselective usage of antibiotics to avert them have led to occurrence of multidrug resistant microorganisms in aquaculture (Adebayo-Tayo *et al.*, 2012). These factors are currently very important for researchers and fish farmers. Contamination due to

these agents is documented to be one of the major limitations in the aquaculture production since the initiation of the sector. Apart from obstructing the development, they too hinder the sustainability of the global fish sector (Bondad-Reantaso *et al.*, 2005).

Various types of bacterial, fungal, viral and parasitic contamination affect cultured fish. Examples comprise motile aeromonads, vibriosis, columnaris, saprolegianiosis, edwardsilloosis and furunculosis (Budiati *et al.*, 2013). Most of these gram negative and gram positive bacteria are widely spread in aquaculture and are susceptible in a large number of mostly freshwater species. Disease management strategies up to date have been based mainly on drug therapy. Microorganisms that are drugs resistant have emerged lately. However, it has accelerated difficulties associated with excesses of drugs in cultured fisheries, and receptiveness towards ecological contamination. Problems originating from constant use of chemotherapeutics have triggered attention on different approaches to management of disease (Adebayo - Tayo *et al.*, 2012).

Kenya is endowed with several aquatic resources with aquaculture potential. The country has varied climatic and topographical areas, extending to parts of the Indian Ocean coastline, large rivers, Lake Victoria, swamps and several wetlands, which support many wild fish species (Okechi, 2004). These water resources vary from brackish and marine to cold and warm fresh waters that are frequently used in ponds for fish production and sustainability. Warm water fish farming in ponds was started in Kenya in 1920's, originally by rearing species of Nile Tilapia, Carp and the African Catfish (Nyonje *et al.*, 2011). The rearing of fish in rural Kenya was promoted in

1960's by the government through campaign dubbed; "eat more fish". As a result, farming of Nile Tilapia stretched out rapidly, with the establishment of many small ponds, especially in Western and Central counties of Kenya (Ngugi *et al.*, 2007). In 1970's there was a decline in the number of productive ponds, majorly due to inadequate extension services, poor quality fingerlings, and inadequate training for extension officers. Fish farming in Kenya had characteristics of low-level farm management, little production and few ponds (Ngugi and Manyala, 2006). Shitote *et al.* (2013) reported that there are no major improvements made to change the situation.

Renewal of fish farming in Kenya was catapulted by research programs start-ups for determining the practices that were best in fish pond culture, renovation of several government fish rearing facilities and training program that was intensive for fisheries extension workers (Mwangi, 2008). Research conducted recently and on-fish farm trials, indicated that fish farmers have been trained on application of suitable methods and good management practices which will result in better yields and a high income (Orina and Maina, 2014).

Diseases have been a major constraint in the development of aquaculture sector. Many factors cause the spread of disease including, poor quality of water, lack of quality feeds, poor knowledge of disease prevention and treatment by the farmers, poor pond and tank management, high stocking densities, and lack of proper advice on fish farm management (Onyia and Sogbesan, 2011). Aquaculture is growing into new dimensions and is quickly intensifying and varying in Africa countries. The increase has brought about hatchery tank contaminations that are now a main

restriction to the culture of fish species. This in turn slows economic and social development (Orina and Maina, 2014). As a food producing industry, it has experienced the fastest growth with a yearly growth rate of 9.9% since 1972, likened to only 1.4 % for culture fisheries and 3.8 % for terrestrial farmed fish over the same period (Musa *et al.*, 2012).

Global aquaculture development and demand has grown immensely in the last five decades from a million tonnes in 1950 to more than 60 million tons by 2010 (FAO report, 2012). A cost of US \$ 70.3 billion was reaped from this production. Of this, 70 % was produced from China while 22 % was produced from the rest of the Pacific and Asia (FAO, 2015). However, as production levels rise with movement of fish intensifying, health issues for the consumers are ignited. Kenya, currently with more than 400 aquaculture farms, has a potential for growth of fisheries sector (Mwangi, 2008). Climatic diversity, natural resources and features endow the country thus make it favorable for the rearing of a wide diversity of aquaculture species. However, though it has not been computed, only a small portion of these resources are utilized (Munguti *et al.*, 2014).

Hatcheries in Kenya are mainly concentrated on aquaculture of the Nile Tilapia and African Catfish which are the popular farmed fish species (Ngugi and Manyala, 2004). Kenya and other developing countries fish production is practiced mainly in semi-intensive ponds which allow low-cost fingerlings to be produced because of cheap feed supplements and fertilizers that are used in hatcheries. About 90 % of aquaculture production in Kenya is led by Nile Tilapia species followed closely by

African Catfish. Among the most notable constraint of cultured fish are infectious diseases which impede the realization of aquaculture and its full potential (Munguti *et al.*, 2014).

The most severe contamination in Nile Tilapia production are bacterial contaminations which cause 80 % of deaths in both the cultured as well as the wild fish and is the main cause of economic losses (Klesius *et al.*, 2006). Aeromonads, Flexibacteria and Vibrios are some of microorganisms that contaminate both freshwater and salty fish present on the skin and gills of fish. Fish present with symptoms of scale loss, ulcerated tail, fin rot and hemorrhage. *Aeromonas* and *Pseudomonas* species are ubiquitous facultative bacteria, whose potential pathogenicity is in producing toxins in fish tissues (Shoemaker *et al.*, 2007). The two bacteria species belong to the flora of water ecosystem and therefore are present in water for domestic and animal use. In the fish environment and body most pathogenic bacteria reside on/in apparently normal fish (Shoemaker *et al.*, 2006).

Ten percent of all cultured aquatic animals are estimated to die because of infectious disease. Ecological circumstances like poor water quality, fluctuations in temperature, poor nutrition, overcrowding, poor handling and transportation which are common in intensive fish allow stressful conditions to the fish and they make the fish more vulnerable to a wide variety of pathogens (Njoku *et al.*, 2015). Stress in fish results in poor feeding, deformity and cannibalism, reduced growth and survival rate. All these predispose the fish to contamination and disease leading to a reduction in fish health status and eventual mortality (Budiati *et al.*, 2013).

Many researchers in aquaculture (Ibrahim and Elnaggar 2010; Omondi *et al.*, 2001, and Rasowo *et al.*, 2007), recommend assessment of water quality parameters in fish farms since they are likely to affect growth, disease and general performance of cultured fish in ponds and hatchery tanks. However, water quality parameters alone cannot explain the observed variations in growth in fish. There is need therefore, to employ a more inclusive approach that would not only provide the link between the routine water quality assessment procedures, but also sources of water for hatcheries. Feeding the fingerling with commercial or formulated feeds and numbers of fingerlings in each system and the size of the hatcheries are also prime factors (Raburu, 2003). This research sought to investigate physicochemical parameters and their effect on bacterial and fungal contaminations levels in the three fish farms of Kenya. Since literature on research on fish diseases in Kenya is scarce, this research design offers answers to improving the quality of fingerlings sold to fish farmers and thus its subsequent contributions to fish health. This would provide an alternative potential for poverty alleviation, improve livelihood especially for the population of a region which registers a development rate of less than 2% per annum (SARNISSA Report, 2010).

1.2 Problem statement and justification

1.2.1 Problem statement

Kenyan fish farms are characterized by microbial contamination, where some fish ponds harbour bacteria and fungi that cause serious health conditions to human beings (Karimi, 2015). Insufficient availability and quality of fingerlings for stocking are key constraints for the development of Kenyan aquaculture. In addition to the escalating

demand for African Catfish (*C. gariepinus*) fingerlings for stocking fish ponds, this species is also used as bait fish in capture fisheries, leading to increased demand every year (Ngugi *et al.*, 2007). Lack of adequate laboratory facilities for the analysis of the health status of fish products is a great hindrance to adequately convince the international markets of the product status in the country (FAO, 2009). Hatcheries and ponds are adjoined to rivers and are filled with water taken directly from the river. This leads to contamination of farm-raised fish especially young fingerlings. Formulated feeds preparation, poor water quality and farm practices are part of the contamination passageway (Emikpe, 2011).

The inadequate number of trained personnel in most government fish and research institutes has delayed effective advancement of aquaculture in Kenya. There are few training programs for current and prospective fish farmers thereby affecting acquisition of knowledge (Munguti *et al.*, 2014).

Even where research has been carried out, new findings in aquaculture may not be acquired by many farmers in time due to the poor linkage between researchers and farmers (Mwangi, 2008). For both theory and policy, the study is result oriented as it would add to existing literature on current situation of diseases in fish farms in Kenya. In terms of policy the study will inform stakeholders in the sector about the causes and status of contaminants and limitations especially to small scale fish farmers. This will set the guidelines and lasting solutions to control the constraints in the aquaculture sector (Ngugi and Manyala, 2004).

1.2.2 Justification of the study

In Kenya, there are many constraints hindering development of aquaculture, related to policy and socio-economic issues. Policy on the certification of fingerlings that are supplied to small scale fish farmers is non-existent. Currently, the quality of fingerlings from the country is not certified in some farms that sell fingerlings. Fish farmers who buy fingerlings from certified fish farms sources become frustrated because of low fish yields and high mortality (Nyandat and Owiti, 2013). Large scale commercial feeds are scarce and expensive to common fish farmers in Kenya and thus farmers prepare formulated feeds on farm which leads to contamination due to poor preparation, processing and storage practices. The use of locally available materials as fish feeds formulations have been experimented, however many investors fear to take up fish feed production venture because the ingredients for formulating feeds are expensive (FAO, 2013).

1.3 Study hypotheses

- i. The fingerlings of Nile Tilapia and African catfish are not significantly contaminated with bacteria and fungi in Mwea, Sagana and Jambo fish farms
- ii. The commercial and formulated feeds are significantly contaminated with bacteria and fungi in Mwea, Sagana and Jambo fish farms.
- iii. There is no significant variation in the physico-chemical parameters of water used in Mwea, Sagana and Jambo fish farms.

1.4 Objectives of the study

1.4.1 General objective

To investigate the contamination of Nile Tilapia and African Catfish fingerlings in Sagana, Mwea and Jambo fish farms by bacterial and fungal agents.

1.4.2 Specific objectives

- i. To determine the contamination of Nile Tilapia and African catfish fingerlings with bacteria and fungi in Mwea, Sagana and Jambo fish farms
- ii. To determine variation in the physicochemical parameters of water used in Mwea, Sagana and Jambo fish farms.
- iii. To determine the feed type and its effect on bacterial and fungal contamination levels in Nile Tilapia and African Catfish fingerlings.

1.5 Significance of the study

This study sought to address the problem of fish contamination in aquaculture farms. Data from this study has many practical implications. The results will be used to educate the farmers on the variability of physico-chemical parameters and their effects on fish. The owners of fish farms will be enlightened on the best source of water and fish feeds to use to rear their fingerlings. Currently there are limited studies on fish diseases in most fish farms in Kenya. Therefore, this research work will be a contribution to existing data and stakeholders of fish farming sector. The public wishing to start fish farming as an income generating venture will be enlightened especially on how to ensure that they carry out proper management of their fish

fingerlings, hatchery tanks and fish feeds to avoid contamination that could affect both their fish and humans.

The data collected will be used to identify common bacterial and fungal contaminants in hatchery tanks in study fish farms. In addition, data obtained on the physico-chemical parameters will serve as an eye opener to the fish farmers on how best to manage their water quality.

CHAPTER TWO

LITERATURE REVIEW

2.1 Aquaculture growth in Kenya

Aquaculture growth pattern in Kenya is similar to that of many African countries. Commercial aquaculture in Kenya involves the production of *Oreochromis niloticus*, *Clarias gariepinus* (African catfish), and *Oncorhynchus mykiss* (rainbow trout) (Mwangi, 2008). Nile Tilapia and African catfish are mainly produced as mono- or poly-culture under semi-intensive production systems while the rainbow trout is produced in intensive production systems using raceways and tanks (Mbugua, 2008). While all the species are produced for the food fish market, there has been increasing demand for baitfish for the capture of Nile perch in Lake Victoria (Mwangi, 2007).

In this regard, the ministry in charge of fisheries development introduced fish farming enterprise productivity program (FFEPP) in 2009 geared mainly towards increasing and strengthening fish farming in the country (Obiero *et al.*, 2014). An initiative that has so far seen the Kenyan government injecting over Kshs.6 billion to establish 1000 fish ponds in each of the forty-seven counties (Musa *et al.*, 2012). This was followed by stocking of the ponds with fingerlings most of which have been harvested, marketed and ponds restocked. The stimulus program has enabled the fisheries industry grow from 722 ha to 20,000 Ha since inception with production growing from 4,220 tons in 2009 to over 19,000 tons in 2011 (Nyonje *et al.*, 2011). This has resulted in Kenya being recognized as the fastest aquaculture growing country in Africa. The FFEPP has led to job creation, poverty alleviation, and food security and

fisheries industry growth in a span of 3 years. Some of the areas that have attracted farmers' interest as well as tremendous growth are hatchery management for fingerlings production and feed supply to hatcheries and grow-out farms (Mwangi, 2008). However, this has not gone on without challenges. Challenges is quality of fingerlings by producers, high mortality rates of fingerlings, lack of quality fish feeds, poor water quality and contamination in hatcheries (Nyonje *et al.*, 2011).

2.2 Aquaculture policy in Kenya

Due to financial challenges, an operational field based system that provides an active aquaculture development program lacks in Kenya. The number of field work personnel who are trained and understand diseases of fish is grossly inadequate (Charo *et al.*, 2004). Many of the available extension officers do not possess understanding and equipment necessary to transfer knowledge and skills to fish farmers effectively (Orina *et al.*, 2014). An extension program implemented in the 1990's influenced aquaculture sector negatively as it focused on output. In 2005 a functional ministry of Fisheries was set up that revived the sector. The move was expected to provide full-time extension workers who would receive training to help support the growing industry (Mbugua *et al.*, 2008).

2.3 Current status of fingerlings sector in Kenya

Various authors such as (Munguti *et al.*, 2014, Shitote *et al.*, 2013 and Obiero *et al.*, 2014) have reported inadequate and poor quality fingerlings as the key limitation in aquaculture development in Kenya. In addition, the demand for Nile Tilapia and African Catfish fingerlings for rearing has intensified. These two species are most

preferred leading to increased demand every year. In 2007, the department of fisheries development projected that the yearly demand in Lake Victoria for fingerlings was ten million m/y for aquaculture (Musa *et al.*, 2012).

According to the Ministry of Fisheries Development 2010 report fish produced from farming in that year was 12,153 MT valued at KShs 2.6 million. Nile tilapia contributed 75 percent (or 9,115 MT) of the total fish produced while African catfish contributed 18 percent (or 2,188 MT) (Charo *et al.*, 2010). Quality fish status can be achieved by obtaining same-sex fingerlings using sex reversal techniques and hybridization. However, technical knowledge and advanced facilities are required for such initiatives and are therefore unpopular among fish farmers (Munguti *et al.*, 2014).

2.4 Constraints in the Kenyan aquaculture sector

Inadequate knowledge of aquaculture ventures and slow economic performance of several fish farming enterprises cost of fish feeds and diseases impede Kenyan aquaculture sector (Nyonje *et al.*, 2011). More prominently, the insufficient supply of quality formulated and commercial feeds and certified fish fingerlings have been limiting factors to the progress of aquaculture (Omondi *et al.*, 2001). Most farmers have lost hope in this sector as they incur massive losses due to stocking of their ponds with fingerlings of poor quality and contaminated formulated feeds (Ngugi and Manyala, 2004). Aquaculture growth is affected by lack of an all-inclusive aquaculture policy, poor management systems in farms, lack of strong field-based research. Furthermore, the private sector is never keen to venture into this sector. The

Economic stimulus program brought an explosive interest in fish farming, new challenges attributed to environmental degradation and pollution, biosecurity, and the spread of diseases (Musa *et al.*, 2012).

2.5 Management of water quality in aquaculture

Suitable water quality is very important for the production of fingerlings that are healthy. When management of water quality parameters is abandoned, fingerling production is bound to be low and of a compromised quality. Declining dissolved oxygen (DO) levels is a frequent water quality parameter in many fish farms and impacts negatively on fish production (Nzungu, 2011). When dissolved oxygen level fall to below 4 parts per million (ppm) for long, fingerling growth can be severely slowed, a lot of efforts should be put to keep DO levels above 4 ppm. Low DO may not directly kill fingerlings however stress from such conditions lowers diseases resistance of the fish (CLIMAWAT, 2011). It is advisable that this parameter is checked in the morning and evening in all weather in aquaculture systems that are well stocked and getting large sources of fish feed. Low oxygen problems can be projected when daily DO records are kept for each pond and aeration improvement equipment put into place to avoid any deviations (Mwaura, 2005).

2.6 Current status of fish feed sector

Most fish farmers in Kenya approximated at 90% formulate own feeds from agricultural raw materials, such as wheat bran, maize bran, soya bean, copra cake, rice bran, groundnut bran, wheat and fish meal. There is no quality check for these feeds which then become sources of contaminations for both bacterial and fungal

contaminations (Munguti *et al.*, 2011). Most quality feeds are very expensive, not easily available and exceeds the reach of the majority of farmers. Further research ought to be carried out on feed formulations. Studies on nutrition feeds have focused on application of cheap and obtainable feed elements due to the high cost of fishmeal (Ngugi *et al.*, 2007, Nyonje *et al.*, 2011).

2.6.1 Fingerlings survival in hatcheries

African Catfish and Nile Tilapia culture thrive well under diverse Kenya climatic patterns, existence during cultivation of eggs and from hatching to mature size. (Yousif, 2002) evaluated on how the success of hatching African catfish eggs in hatcheries is effected by chemicals treatment like potassium permanganate, formaldehyde, sodium chloride and hydrogen peroxide. Based on ease of availability and cost, the study recommended a 1,000 ppm sodium chloride treatment of African Catfish and Nile Tilapia eggs for routine use by small scale fish farmers to heighten egg hatchability. Research on increased survival from hatching to fingerlings stages is scarce. Fingerlings stage survival in earthen and concrete ponds ranges from 0.6 to 21 fry/m². A survival rate of 30 percent or from 1 to 7 grams fingerling weight is regarded best (Ezeri *et al.*, 2009).

2.7 Species suitable for culture in Kenya

Culture systems for fish farming in Kenya include intensive and extensive culture of Nile Tilapia and African Catfish. It is practiced by start-ups farmers and experienced fish farmers in static ponds, and in raceways. The species used at any fish farm are mainly endemic to the region and more or less appropriate to the agro climatic zone.

Furthermore a warm water fish like Nile Tilapia is mainly reared in a freshwater environment while African Catfish are reared in the same agro climatic region as Nile Tilapia (Liti *et al.*, 2005). The challenge with African catfish production is high mortality of fingerlings, especially during the first 14 days after the eggs hatch (Ngugi *et al.*, 2004).

2.7.1 Nile Tilapia (*Oreochromis niloticus*)

This is the third most commonly farmed fish after carp and salmon. It had global production of 1.50 million metric tonnes (mmt) by the year 2005 and is anticipated to increase to 4.0 mmt by the year 2016. However, a limiting factor in Nile Tilapia fingerlings culture production is outbreak of disease due to contaminants (Ebtsam *et al.*, 2002). It is documented that frequent drugs and probiotics used to prevent bacteria, fungal, viral, and parasitic diseases in many Nile Tilapia farms and hatcheries have led to resistance (FAO Report, 2010). Tilapia is a cichlid fish which is endemic to Central and Eastern Africa. They grow fast and have increased acceptance to a variety of environmental ecosystem. They are also able to reproduce in captivity and have short generation times. Nile Tilapia feeds on low trophic levels of zooplanktons and accepts artificial feeds immediately after yolk-sac absorption (Charo *et al.*, 2010). Nile Tilapia culture however, is heavily constrained by the limited availability of good quality fingerlings. Although it breeds easily, large-scale production of fingerlings is hampered by poor spawning that occurs among the females within the population, even in the same age group. Nile Tilapia reaches maturity in earthen and concrete ponds at age of 5-6 months (Xu *et al.*, 2007).

2.7.2 Natural distribution and habitat of Nile Tilapia

Tilapia's origin is central and North Africa and the Middle East. The species is a tropical freshwater and estuarine species that ingests a wide variety of natural foods. Nile Tilapia prefers water that is shallow, still waters on the lakes and wide rivers with sufficient green vegetation (Ngugi *et al.*, 2007). Nile Tilapia is famous for its ability to endure hypoxic and even anoxic settings for a short time. As a result, the species is best suited to hypereutectic situations in static aquacultures as compared to other species. Moreover, it is notable fish present in temperate and tropical waters are limited by incipient levels of oxygen which can probably occur in form of hypoxia (Mwangi *et al.*, 2007).

2.7.3 Environmental tolerance ranges

Nile Tilapia reportedly fares well in any water environment except for torrential rivers. Some of the key limiting issues to its distribution are DO, salinity, pH and temperature (Musa *et al.*, 2012). In Tilapia, temperature endurance limits reportedly lie between 11 and 42°C. Furthermore, fluctuations in the levels of dissolved oxygen are not a major limiting factor for Tilapia, as they can tolerate levels as low as 3-4 mg/l (Pailwal *et al.*, 2009).

2.7.4 African Catfish (*Clarias gariepinus*)

The African Catfish is a dominant freshwater fish which can grow to between 8-59 kgs and between 0.5 and 2 m long (Al-harbi *et al.*, 2012). It is a heavy boned, flat headed fish with premaxilla and lower jaw pointed teeth arranged in several rows and four pairs of long sensory organs known as barbells around its mouth giving it a

similar appearance to a cat, hence the name Catfish and is endemic to Central and Eastern Africa (Ngugi *et al.*, 2004). They grow fast and tolerate numerous environmental factors. Furthermore they are able to breed in confinement and have short generation times. African Catfish feed on low trophic levels of zooplanktons and accept synthetic feeds instantly after yolk-sac absorption (Okechi, 2004). Naturally air-breathing African Catfish has an extended bony body shape that is also characterized by long dorsal and anal fins and a smooth body with no scales. It has variation in colour from dark green at the dorsal fin and is often mottled with shades of slightly silver tint and grey while the underside is a pale cream to white (Charo *et al.*, 2000).

2.7.5 Natural distribution and habitat

The diversity and natural habitat of African Catfish covers most of the South America and African continent. This fish species is distributed in many countries of Africa as it adapts easily to environmental and rearing conditions (Al-Harbi and Uddin, 2010). The tolerance status of African Catfish to many different habitats makes it the best for aquaculture activities, but mostly it is taken to be a fresh water species. It thrives well in slow moving rivers, floodplains, dams and muddy ponds. It can bear ecosystem with low dissolved oxygen and high in turbidity and is the only fish type that is found in muddy zones of drying rivers (Ajayi, 2012).

2.7.6 Environmental tolerance ranges

The African Catfish can survive in extremely harsh conditions in the reared environment as it is able to withstand adverse changes in the physicochemical

parameters and even survive for longer time without water since they make use of a specialized supra gills organ in their gill epithelium. These branches above the gill arc tissue are a large paired chamber which is specifically adapted for air breathing and allows it to survive on muddy area even when weather patterns dictate otherwise (Boyd, 2007). Salinity level of 0 to 10 %, water temperatures between 8 and 37°C and wide pH ranges are well tolerated by the fish. High rates of growth of between 25 and 33°C are exhibited by the African Catfish with an optimum growth recorded at 30°C. The survival of fish in diverse environmental ranges makes it able to tolerate these extreme conditions and allow it to adapt even in moist areas of sand (FAO Report, 2010).

2.8 Nutritional needs of fingerlings

Fingerlings demand for nutritional supplement is very vital for their fast growth and health. Feeds manufacturers must make sure that the feeds have all the required nutritional sources (Munguti *et al.*, 2011). Floating and sinking fish feeds are very popular in most aquaculture farms. Unless the fingerlings receive adequate and the right feeds they will become weak and more vulnerable to disease agents and this affects their subsequent rearing status. Vitamin C deficiency may cause skeletal deformities and may be particularly widespread (FAO Report, 2014). Feeding African Catfish poorly for a prolonged period causes them to lose about 9 percent of their weight. There should be total increase of up to 18 % of their weight during the cold seasons if the fish are fed properly, 1 percent of their total body bulk either on substitute days or on days when surface water temperatures are 54 degrees F or above

in mid-afternoon. Healthy fingerlings can be achieved by applying balanced feed rations, storage and preparation practices (Munguti *et al.*, 2012).

2.9 Stress and its effect on fish diseases

While Nile Tilapia and African Catfish are very tolerant to most of the environmental factors, stressful conditions can affect them adversely and make them more susceptible to diseases. Stress can result from nutritional differences especially due to vitamin and protein imbalances, environmental quality culture conditions, physicochemical and biological interference for example polluted water sources, crowding of fish, contaminated feeds, transportation and organic enrichment. Nile Tilapia can suffer from many different infections; such as bacterial, parasitic and viral diseases as well as fungal infections (Mwajuma *et al.*, 2010).

Bacterial pathogens due to small gram negative rods belonging to the group Enterobacteriaceae, Pseudomonaceae and Vibrionaceae cause tissue septicemia and ulcerative disease conditions in fingerlings (Dutta *et al.*, 2010). Most fish irrespective of the species are susceptible to Streptococci bacteria which is world-wide in distribution. In intensive and extensive aquaculture fish farms; the morbidity and mortality are documented to reach 100 % and 70 % respectively (Klesius *et al.*, 2009).

2.9.1 Nile Tilapia diseases

Diseases of Nile Tilapia are a major constraint in aquaculture and are caused by bacteria, fungi or viruses. Adverse water quality parameter changes and other stressors do not cause adverse effects on this species of fish as they are more resistant

than most other commercial aquaculture species (Shoemaker *et al.*, 2006). Disease process is affected most by stress, contaminated feeds, physiochemical parameters in the water and stocking densities. The introduction of a pathogen by any means into a hatchery tank, can be accelerated by some of these factors. Eradication of an infectious agent generally involves reducing the stocking densities, use of uncontaminated feeds and water and introduction of various antibacterial and antifungal agents (Klesius *et al.*, 2008). Diseases can be avoided by proper management of the rearing unit. The introduction of contaminated fingerlings, contaminated feeds and poor water quality are the commonest sources of disease in hatchery tanks (Shoemaker *et al.*, 2007).

2.9.2 Bacteria contamination

The major bacterial contaminations among tropical fish are a great concern because they constitute the biggest economic loss in farms. They are caused by both gram negative and gram-positive bacteria like *Aeromonas*, *Edwardsiella*, *Pseudomonas*, *Flavobacterium*, *Mycobacterium* and *Streptococcus*. Haemorrhagic septicaemias are caused by *Aeromonas* species and are considered as the most common bacteria in fish culture systems. Edwardsiellosis is associated with *Edwardsiella tarda* and Columnaris disease is caused by *Flavobacterium columnaris*. Among the virulent bacteria, *Streptococcus iniae* is connected with substantial mortalities of cultured African Catfish and Nile Tilapia. This bacterium has been reported in most farms worldwide (Emikpe *et al.*, 2011).

Most bacteria act as opportunistic pathogens and are therefore ever present in water. The severity and onset of most bacterial contaminations is mainly generated by stressors such as high and low temperature in water, low dissolved oxygen, high ammonia concentration and heavy dissolved solid waste (Ajayi, 2012). As a result of transformation of small-scale fish farms into large scale commercial production undertakings, bacterial impurities may upsurge, which proportionally rises with the incidence and concentration of environmental deterioration (Xu *et al.*, 2007).

2.9.3 Fungal contamination

The most vital contamination of African Catfish and Nile Tilapia under culture conditions are fungal contaminations particularly in the early life of fingerlings and numerous internal and external tissues (Hussein *et al.*, 2001). Fungal contaminations have great impact in the sector worldwide and are caused mainly by contaminated formulated feeds and overstocking of fingerlings in hatchery tanks (Haroon *et al.*, 2014). Fungal contamination also affects fish that have been affected by temperature shift, mechanical injury, or lesions from other illnesses. The two core fungal contaminations that affect Nile Tilapia and African Catfish cultured in hatcheries in the world are *saprolegniasis* and gill rot (Siddique *et al.*, 2009).

Members of Saprolegniaceae in Oomycetes class commonly cause fungal contaminations. Global research further asserts that *Saprolegnia*, *Chylo*, *Aphanomyces* and *Branchiomyces* are the most pathogenic species (Iqbal *et al.*, 2013). Seldom, members of class Zygomycetes and *Aspergillus* in the class hypomycetes are related to fish contaminations. In Africa, a key challenge in most hatcheries more so in newly

stocked fish systems is caused by genus *Saprolegnia* (Isyagi *et al.*, 2006). The contaminations are normal in stressed or immuno-compromised fingerlings produced by compromised immunity, damage and poor treatment, or they are subordinate to other contaminants (Adebayo –Tayo *et al.*, 2012).

2.9.4 Saprolegnia species

It is caused by a water fungus of the genus *Saprolegnia*. Some of the species are commensals whereas other causes diseases due to mechanical injury, stress or pre existing infection. Saprolegniales exhibit cotton like growth of fungus adherent to the gills or skin of fish. Most of the species are saprophytic naturally present in the environment and are considered opportunistic pathogens (Siddique *et al.*, 2009). They are secondary invaders requiring previous injury or external tissues from mechanical abrasion or other primary pathogens. It is a fungus that resides on the branchiole vessels affecting respiratory tissues of fish (Hussein *et al.*, 2001).

2.9.5 Factors influencing fungal pathogenesis

The fungal agents infect injured skin or open cuts on fish, and are secondary contaminations. The fungi grow to protect the injured sites and then spread to the adjacent tissue around it (Igbal *et al.*, 2012). Prominent fungi abolish the surrounding tissue due to digestive proteins being produced and as a result fungal contamination keeps on to extent, engrossing nutrients from dead and living tissues. If the fungi cover the gills of the fish, it may cause respiratory complications or even death if levels are severe (Siddique *et al.*, 2009).

2.9.6 Monitoring of water quality

This refers to the assessment of the physical, chemical or biological parameters that affect fish production. It should be done in the morning and in the evening and any variation noted. The most limiting factor in fingerlings production is water quality (Ibrahim *et al.*, 2010). It is hard for any farm to predict, manage and understand the quality of water in its systems. The quality of water affects directly the growth rates, efficiency of feeds, feeds conversion and growth. Temperature affects the development of fungal contaminations in fish. When temperatures are low outbreak due to disease occur, but high temperatures are also implicated to cause outbreaks (Mwaura, 2005). Water quality and its frequent checking (temperature, dissolved oxygen content, pH and salinity) are crucial in detecting issues in a fishery. The use of a multi meter that can measure most of the parameters is recommended (Adebayo *et al.*, 2012).

2.10 Fish hatchery in aquaculture

The fish hatchery is where the young fish are held and produced in large quantities year after year and distributed to fish growers. It has become an important part of modern fish culture all over the world. The preconditions of a fish hatchery are: qualitatively and quantitatively enough water, sufficient land for the ponds, hatchery tanks, buildings, electricity, transportation facilities and manpower (FAO report, 2010).

2.10.1 Fish hatchery requirement

Exhaustive fish rearing rarely succeeds without an effective hatchery management. It encompasses a long variability in activities in breeding stretching from the assemblage, choice and management of the accessories. The design of the hatchery and operation mode contributes significantly to prevention of diseases in fingerlings (Adekoya *et al.*, 2006, Akankah *et al.*, 2011). Proper site selection, size and type of hatchery and building materials play a vital role in any effective fingerlings hatchery. The most important necessity of fingerlings hatchery includes, clean yet sufficient quality water and enough land for the site of hatchery tanks (Charo *et al.*, 2010).

2.10.2 Re-circulatory systems in Hatchery

Recirculation systems in aquaculture are designed with an inlet and outlet pipes for easy supply of water in to the hatchery. They are concrete, plastic or glass especially for holding fingerlings up to mature age. The advantage of recirculation system is that it occupies less land and many numbers of fish can be reared. (Adediran, 2002) In many African countries, there is poor quality of water and this affects some recirculatory systems as most infectious agents are trapped in the systems causing emergence of contaminations. Recirculating aquaculture systems though expensive are becoming popular aquaculture method mainly for small scale private farms. Because water recirculates as it is recycled, redistributing fish rearing systems apply a less water necessary as compared to the traditional fish production tank or pond systems (Akankali *et al.*, 2011).

The development of an efficient water treatment system is the most important consideration in recirculating systems. Waste brought about by uneaten feeds in aquaculture systems and silt must be cleared frequently to control the proliferation of hatchery contaminations (Abowei *et al.*, 2011).

2.10.3 Hatchery tank design

An affluent water recycle system should consist of tanks, inlet pipes, storage area, and other apparatus. A hatchery must be managed and operated by knowledgeable and dedicated staff. The final aim is to come up with a structure and to regulate progressions that can be simulated by others, with spaces for adjustments to fit other site-specific features. For continuous clearing action the design must have a flow through rearing unit and the baffle gap should be between 3-8 cm for every unit. The distances are spaced approximately equal to the pond hatchery width (Adediran, 2002). While hatchery design can be applied as the preferred model to design the system aquaculture is a fast emerging science and the latest scientific advancement should be considered into the end design. Climate variability, reliable water availability of and sources of power, the know-how to construct and manage the hatchery and water quality are just a few site specific of considerations that could affect the choice to continue in hatchery design (Al-hassan *et al.*, 2012).

2.10.4 Quality parameters of water

Physiochemical parameters such as pH, dissolved oxygen and temperature are some of the most important in aquaculture. The condition of water quality measures is influenced by the pH of water, like the harmfulness of nitrogen and ammonia.

Therefore, pH is a vital factor to be examined and controlled regularly. The pH of a rearing unit should be between 7.0 and 8.0 (Raburu, 2003). The capacity of water to neutralize acidity is termed as alkalinity. Bicarbonate and carbonate ions are the biochemical entities involved in water alkalinity. Recirculating systems workers therefore need to frequently check on alkalinity levels in borehole water sources so that hydrated lime is added to sustain a neutral pH value (SARNISSA, Report, 2010).

Environmental system of aquaculture is a one that comprises numerous variables of water parameters. Temperature, total dissolved solids and dissolved oxygen and salinity are critical parameters (Subasinghe *et al.*, 2009). Nevertheless, dissolved oxygen is very vital and crucial parameter, calling for continuous and frequent checking in aquaculture production systems. This is owing to the datum that its reduction is an indicator of disease presence in hatchery system (Nzungu, 2011).

2.10.5 Dissolved oxygen

Fingerlings require high levels of dissolved oxygen concentration as they have high metabolic rates. This should not go below 4-5ppm at any given time. The provision of adequate aeration system for optimal maintenance level of oxygen must be maintained at levels appropriate for fish rearing (Kahareri, 2003). For an effective growth the installation of an effective bio filter system that has complete accessories is paramount. Dissolved oxygen concentration in the system must be maintained in adequate proportion. Carbon dioxide must be continuously removed as oxygen is installed (Apha, 2005).

2.10.6 Aeration systems

Aeration systems in aquaculture must be efficient to allow for air changes especially to lower dissolved oxygen levels in recirculating units in hatchery. For efficient transport of water systems, one has to consider the effectiveness of bio filter installed in the aerators. Ordinary aeration strategies include propeller aspirators, paddle wheels, and vertical-lift pumps (Boyd *et al.*, 2007). The large sizes of some fish tanks cause turbulence in the fish tank. Oxygenation in the tanks is achieved by means of air diffusers which help lower the pressure at the bottom of the tank. The diffusers blow smaller bubbles in the tanks and rises up in the tank. Oxygen moves from the bubbles into the tanks. As a result, shallow tanks are preferred as they enable more oxygen diffusion. Membrane diffusers are the most recommended for this recirculation systems (Oparaku *et al.*, 2013).

2.10.7 Fish stocking density

Fish rearing tanks that evaluate recirculation in hatcheries have a system that access stocking densities in a given area of a tank. Fish can be stocked in high densities if they are fed small bits only to maintain the basic needs regardless of the species (Ezeri *et al.*, 2009). The organic matter status of feeds triggers oxidation and therefore oxygen is used up. Any leftover feed and fecal matter from fingerlings has direct impact on health status. Farms should only stock the right numbers of fingerlings per unit (Nguyen *et al.*, 2008).

2.10.8 Hatchery operation

In Kenya, African Catfish and Nile Tilapia rearing is very intensive and ranges from small to big fish farms. There are currently many public and a few private owned hatcheries which supply fingerlings all over the country (Adekoya *et al.*, 2006). Fingerlings supplied from government fish farms to stock their ponds make 90 percent of the farmers in the aquaculture sector. This situation results in dire undersupply to ponds, as the count of fingerlings is inadequate. The fact that most farms lack trained personnel to manage their hatchery operations also affects the quality of fingerlings (Munguti *et al.*, 2014).

Because of the low production of hatcheries in Kenya, there is an acute fingerlings shortage. The boost of the sector by European Union in 2015 at the tune of 4.5 billion has caused anxiety in small scale fish farmers as the demand increases (FAO report, 2012). There are no policies to certify Nile Tilapia and African Catfish that are produced by fish farmers and this affects the quality of the fingerlings. Grow-out facilities modern technology is used to set up. Methods like selective breeding have been useful to recover the development rate of fingerlings through cooperative research program and the training of hatchery staff on operation and maintenance. This would improve the quality of the fingerlings, protect genetic diversity and reduce inbreeding (Ngugi *et al.*, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted in two counties of central Kenya (Kiambu and Kirinyaga). The three fish farms included in this study are situated in different climatic zones and have different sources of water, feeding types, hatchery systems and managerial styles of rearing fingerlings. All the farms rear Nile Tilapia and African Catfish fingerlings for sell out purposes to farmers in Kenya (Figure 3.1). All the fish farms have a re-circulated tank system but of different design (Figure 3.1, 3.2 & 3.3 a & b) respectively.

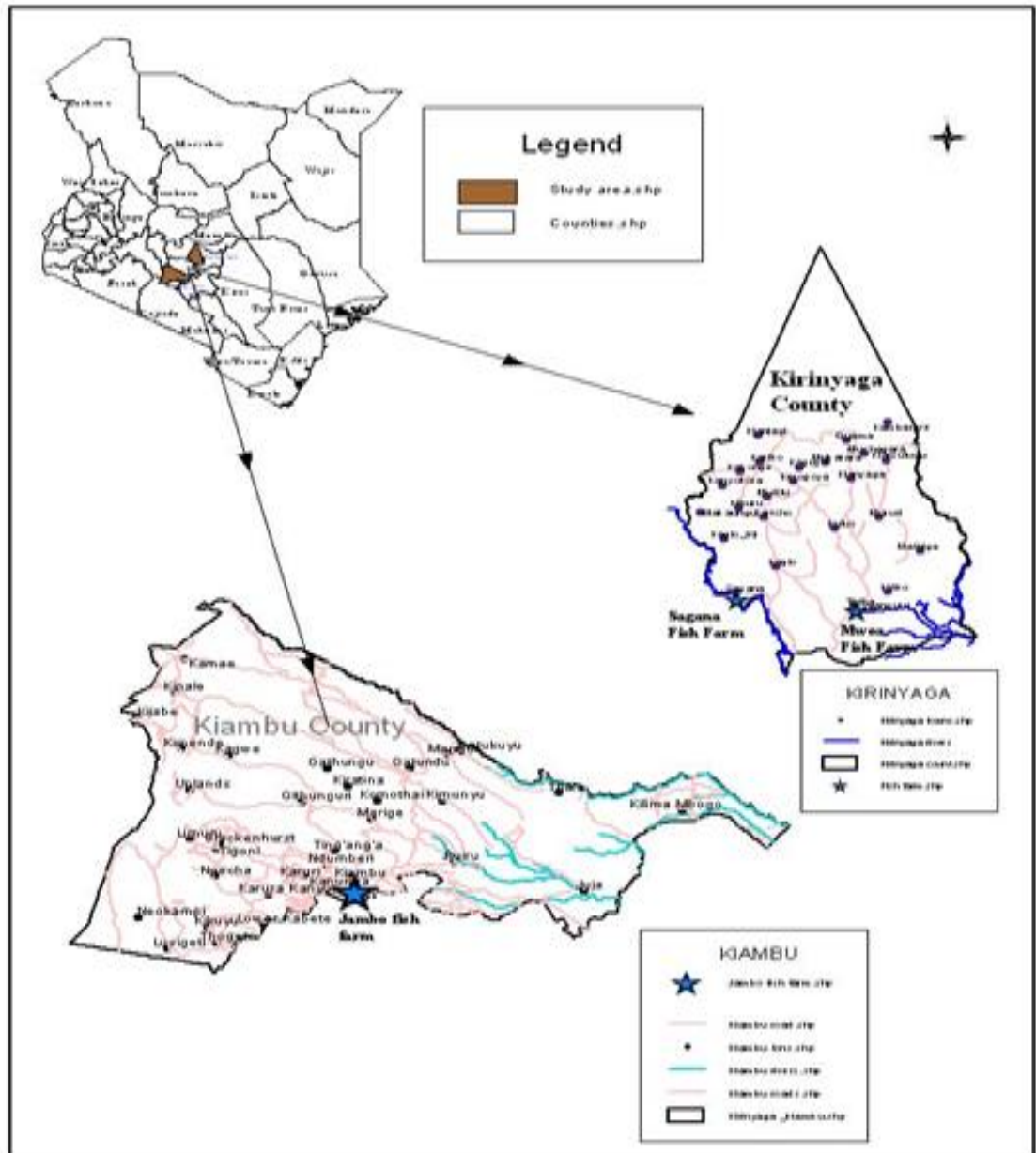


Figure 3.1: Map of Kiambu and Kirinyaga Counties from Google maps; Star points indicate the study farms

3.2.1 Jambo fish farm

The Jambo fish farm is a Dutch owned fish farm located on the Northern By-pass Road ($0^{\circ} 32'S$, $37^{\circ} 11'E$), roughly 10 km off Nairobi city on the Northeast at an

altitude of 1,100m. The Ministry of Fisheries Kenya recognizes the farm to breed quality African Catfish and Nile Tilapia fingerlings. Dutch owners Fleuren and Nooijen have over twenty-five years of experience in production of African catfish fingerlings using state of the art Recirculation aquaculture systems (RAS).

The farm has invested in a solar powered water heating system and on latest design of recirculation aquaculture system (RAS), which include a degassing system and specialized commercial feeds feeding system. The farm utilizes a borehole source of water which is constantly pumped into the hatchery after filtration. This maintains physicochemical parameters of water at standard accepted levels (Plate 3.1).

Aquaculture sector in Kenya has been redefined by Jambo fish farm by rearing the fish in tanks which can hold 1500 mature African Catfish and Nile Tilapia. To enhance growth these tanks are placed inside a greenhouse whose temperature is controlled. Per one harvest the farm has a capacity of producing 1.5 tonnes of mature fish. They have more than ten permanent members of staff to take care of the fish on the farm.



A)

B)

Plate 3.1: A & B) Photos of a recirculated aquaculture system(RAS) with an inbuilt bio filter, an aerator and a degassing unit at Jambo fish Farm

3.2.2 Sagana fish farm

The fish farm popularly known as The National Aquaculture Research Development Center (NARDTC) Sagana Fish Farm, is located in Sagana town, Kirinyaga County ($0^{\circ} 39'S$, $37^{\circ} 12'E$), roughly 105 km off Nairobi on the Northeast with an altitude of 1,230 m. The source of water is river Ragati which is also used by peasant farmers around Karatina and Sagana area. Sagana fish farm has been central to aquaculture development in Kenya since 1963. Enhancement and restoration started then, under the support of the Belgian government through a project funding. The building and a facelift of existing ponds began in 1996. Sagana now has earthen and concrete ponds, which were built for aquaculture research, production of fingerling, spawning and rearing activities.

Sagana fish farm is still one of the leading suppliers of African Catfish and Nile Tilapia fingerlings at affordable rates to farmers in the vast Mount Kenya region and beyond. The farm also produces its own formulated fish feed. The provision of quality fingerlings has been necessitated by research activities at the farm. It has concrete and glass aquaria hatcheries (Plate 3.2). The farm is operated by the fisheries department of the government of Kenya, which assigns officers to the station for this purpose. The farm has a good water quality and fish disease laboratory at the station through donor support.



a)



b)

Plate 3.2: a) Image of glass aquaria (b) Image of concrete hatchery at Sagana fish farm

3.2.3 Mwea Fish Farm

Mwea Fish farm is a private owned fish farm located near Karoti Girls High school next to Ngurubani town ($0^{\circ} 49'S$, $37^{\circ} 19'E$), Kirinyaga County, approximately 180 km Southeast of Nairobi at an altitude of 1,110 m above mean sea level on the Embu/ Nairobi highway and is accessible throughout the year through a maintained rough road. Aquaculture activities are practiced in 19 ponds and the most reared species are Tilapia and Catfish. In addition, there are 8 plastic hatchery fingerlings holding tanks. The fish ponds are constructed on loam soils and measure 300 sq meters. The tributary of Sagana River, Nyamindi is the primary water source which also serves the rice and French beans farms in the vast Mwea division. The farm has a feed preparation room, a hatchery and a large feed store. It has eight hexagonal holding tanks and nineteen earthen ponds where fish farming is practiced (Plate 3.3). Consultancy services are carried out to equip fish farmers with the basic fish farming management skills as well as training and research activities.



a)

b)

Plate 3.3: (a & b) Plastic tanks with inlet and outlet pipes at Mwea fish farm

3.3 Research Design

The study used experimental research design. Data derived from the research was coded and entered in MS excel. Laboratory work was carried out at Sagana aquaculture laboratory and Karatina University Biological Sciences Laboratory. Sixty fingerlings each of Nile Tilapia and African Catfish (5.6 gm in average and 7.0 cm in length) in each of the three farms were sampled at different occasions for a period of three Months. Physico-chemical parameters were determined for one month.

Table 3.1: Experimental layout of the research tanks in Sagana, Mwea and Jambo

Tank Dimension	Hatchery tank system	Nile Tilapia stocked	African Catfish stocked	Feeding regime
Jambo fish farm				
1m x 1m x 1m	Fingerlings tank 1	10	0	Commercial feeds
1m x 1m x 1m	Fingerlings tank 2	0	10	Commercial feeds
1m x 1m x 1m	Fingerlings tank 3	10	0	Commercial feeds
1m x 1m x 1m	Fingerlings tank 4	0	10	Commercial feeds
Sagana Fish Farm				
4m x 2.5mx 1m	Concrete tank	0	10	Commercial feeds
4m x 2.5mx 1m	Concrete tank	10	0	Formulated feeds
30 cm x 60 cm x 30 cm	Aquaria 1 tank	0	10	Formulated feeds
30 cm x 60 cm x 30 cm	Aquaria 2 tank	10	0	Commercial feeds
Mwea Fish Farm				
1.5 m x 1.5m	Plastic tank 1	10	0	Formulated feeds
1.5m x1.5m	Plastic tank 2	0	10	Commercial feeds
1m x 1m	Plastic tank 3	10	0	Formulated feeds
1m x 1m	Plastic tank 4	0	10	Commercial feeds
		(n)= 60	(n)= 60	

3.3.1 Stocking density in each hatchery

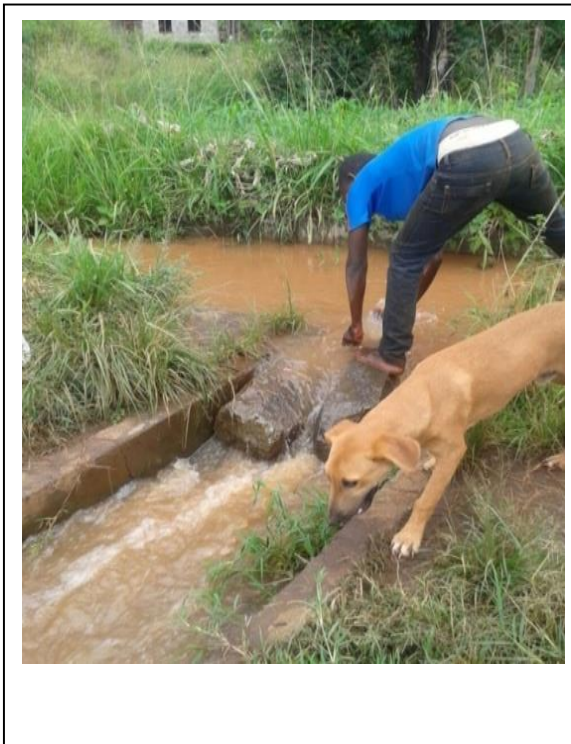
This experiment was conducted in each of the three farms in four tanks per farm in hatcheries as shown in (Table 3.1). Fingerlings of African Catfish and Nile Tilapia were stocked at 10 fingerlings per tank. Each farm had four tanks with each tank holding 10 Nile tilapia and 10 African catfish fingerlings respectively. All the tanks in each farm had 20 African Catfish and 20 Nile Tilapia fingerlings (n equals $60 \times 2=120$). The fingerlings were of $6.5\text{g} \pm 1$ in weight and $5.5 \pm 1\text{cm}$ in length. Floating pelleted feeds of commercial and formulated feeds were given twice daily at 08:30 am and 04:30 pm, 6 days per week in different tanks. All fingerlings were subjected to normal feeding rates without any deviation.

3.4 Laboratory analyses

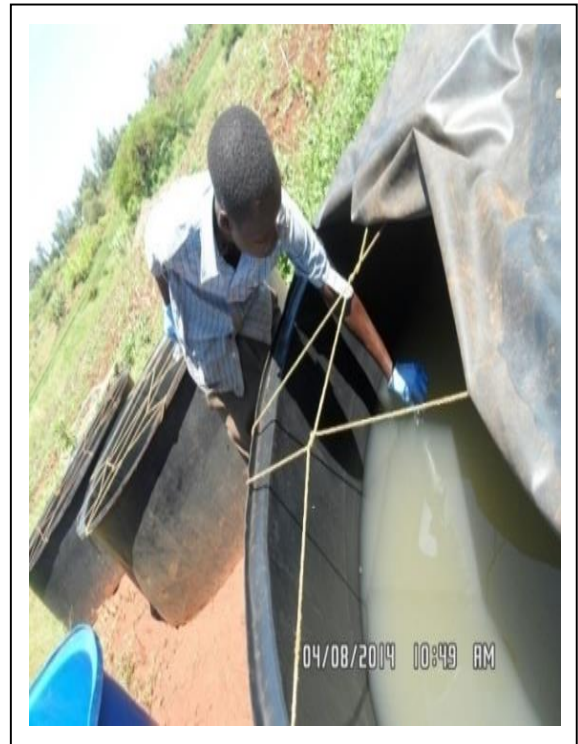
3.4.1 Determination of water quality parameters

At each fish farm, water samples were collected directly into pre-sterilized universal bottles of 10 ml. At the hatchery tanks, the universal bottles were opened aseptically, then held at their bases and submerged to a depth of about 20 cm with the mouth facing upwards. Samples were taken by filling the bottles to the top to exclude air. A total of 12 samples were collected which was consistent with method described by (Samie *et al.* 2011). The tanks were stirred before the samples were collected. The water samples were then placed in ice cool box at a temperature of 4°C after which they were transported to Karatina University, Biological Science Laboratory for bacterial and fungal analysis. All the samples were analyzed within 24 hours.

Samples of water were obtained from sampling sites using sterilized 250 ml universal bottles 15 to 20 cm underneath surface of water from three positions in each hatchery system (Plate 3.4 a & b). On reaching the laboratory, bacteriological and fungal examination was done on the samples of water from each farm, separately. A 10^{-4} serial dilution was made using 0.85 % (w/v) sterile NaCl. Using an automatic pipette, 0.1 ml of the serial dilution was drawn and then inoculated by spread plate technique onto Nutrient agar media plates in duplicate.



a)



b)

Plate 3.4: (a & b)

Inlet tunnel water from river Ragati at Sagana fish farm.

Water sampling activity in hexagonal tanks at Mwea fish farm.

3.4.2 Physico-chemical assessment

The pH, water temperature, Dissolved Oxygen (DO) and Redox potential (ORP) was measured in the field at each monitoring site using a Multimeter in accordance with standard methods (APHA, 2005). All instruments were calibrated prior to use. All physicochemical parameters were analyzed within 24 hours of sample collection. Sterile universal bottles were pre-cleaned, dried and stored in a dust free environment as described by Cheesbrough (2006). Two samples of water were collected from rivers and borehole for each sampling farm and labeled appropriately. Borehole water samples collected from taps were allowed to run for ten minutes before sample collection for physicochemical parameters. For DO determinations, the mouth of the tap was heated for five minutes with a spirit lighter to destroy microorganisms, and the tap water allowed to run for 5 minutes prior to sample collection. All water samples were stored in an insulated cooler containing ice maintained at 4°C and delivered to the laboratory. The results of analysis were expressed as milligrams per liter except temperature measured as degree centigrade (°C) CLIMAWAT (2011).

3.4.3 Collection and processing of fingerlings samples

Live hatchery reared African Catfish and Nile Tilapia fingerlings were collected from concrete, plastic and glass tanks. Sampling was done between 10.00 am at periodic interval of two weeks for duration of three months. Five samples each from each species of fingerlings were randomly collected from four tanks from each of the three farms using hand nets and placed aseptically into polythene containers. Fish samples were transported in clean polythene bags directly to the Laboratory of Biological Science Department, Karatina University within 2 hours of sampling. The fish

samples were then sacrificed by denying them water washed and rinsed in sterile water (Plate 3.4 and 3.5).

3.4.4 Bacteria isolation from fingerlings

One gram (1 g) of each fingerlings gills and skin sample was serially diluted up to 10^{-5} of the fifth test tube, 1 ml of an appropriate dilution was inoculated on Nutrient agar plates and the plates were incubated for 24 h at 30°C. After 24 h, sterile wire loop was used to pick the isolate from the plate and was streaked on a freshly prepared sterile nutrient agar plates, then it was incubated for 24 h at 30°C in order to get pure cultures. The plates were then incubated in various selective media like Macconkay agar, TCBC agar, Blood agar and XLD agar at 37°C for 24hours and colonies formed were enumerated. Representative microbial colonies were isolated, characterized presumptively by gram staining before biochemical tests were carried out. The biochemical tests for identification of bacterial isolates includes the following: Coagulase test the catalase test, citrate utilization tests, urease test and carbohydrate fermentation test.

3.4.5 Isolation of bacteria from fish feeds

Ten grams (10 g) of labeled feed sample was homogenized with 90 ml sterile distilled water for 3 min using a blender. They were then cultured after serial dilution of up to 10^5 . Bacterial colonies upon growth were picked up in pure form and sub cultured on selective and differential media. Identification of all isolates was carried out by cultural, morphological characteristics, motility and biochemical tests (Plates 3.6 and 3.7). Aggregate aerobic heterotrophic bacterial tallies of commercial feeds and

formulated feeds was assessed by growth in all the plates incubated at 25°C, and colonies were determined using a digital colony counter at 24 and 48 h after inoculation. Plates having between 30 and 300 colonies were used to compute population of bacteria, stated as colony-forming units per unit sample. To identify purified isolates to genus level basic tests, namely Gram's stain reaction was done followed by subculture on selective and differential media and biochemical tests as per the schedule described in the District laboratory manual for tropical countries (Cheesbrough, 2006).



a)



b)

Plate 3.5:(a) Workers pelleting formulated fish feeds without gloves at Mwea fish farm

(b) Commercial fish feeds sample from Jambo fish farm

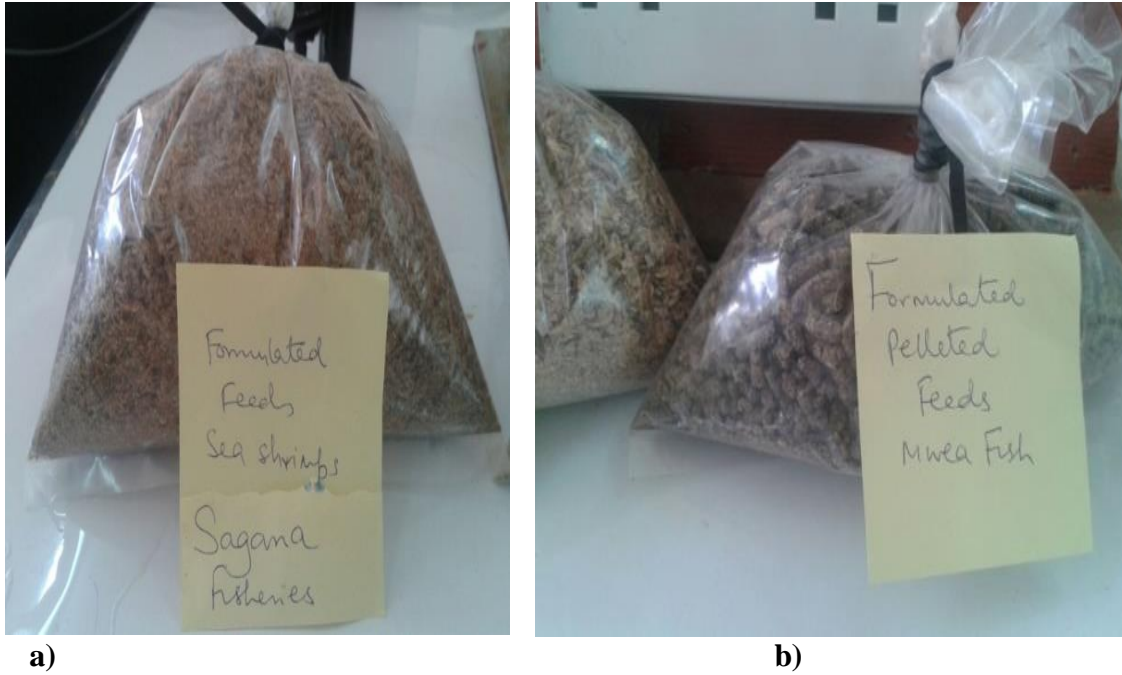


Plate 3.6: (a and b) Formulated Fish feeds sample for laboratory analysis from Sagana fish farm



Plate 3.7 : (a) Poor feeds storage practices at Mwea fish farm
(b) Standard aerated fish feed store at Jambo fish farm

3.4.6 Determination of total viable count (TVC) within a bacterial suspension

To determine the total viable count, plates were incubated at 37 °C for 24-48 hours. The number of colonies in a particular dilution was multiplied by the dilution factor to determine TVC which was expressed as mean Logarithm colony forming units (CFU \pm SD/ml). TVC was measured at three time intervals. Single colony was further sub cultured until pure culture was obtained.

For convenience the results are given as CFU/ml for liquids and CFU/g for solids. The number of bacteria (CFU) was calculated per milliliter or gram of sample by dividing the number of colonies by the dilution factor. The number of colonies per ml reported reflected precision of the method and did not include more than two significant figures. Those counts within 30-300 colony forming units (cfu) were reported as total viable count (TVC).

The CFU/ml was calculated using the formula: Cfu/ml = (no. of colonies x dilution factor) / volume of culture plate. Following a suitable growth period, number of colonies that had grown on each plate was enumerated. For best results, only plates harboring colony counts between ranges of 25-250 colonies as this provides an accurate representation of the bacterial titer.

3.4.7 Identification of Bacteria

3.4.7.1 Colony characteristics

Colony characteristics such as: shape, size, surface texture, edge, elevation, color and opacity developed after 24 hours of incubation at 37°C were recorded.

3.4.7.2 Gram staining

The gram staining technique was used to differentiate the gram positive from gram negative isolates based on the gram staining technique described by Christian Gram in 1884 (BD Diagnostic's, 2009). The principle of the test is based on the cell wall properties of the two bacterial classes. A smear of the isolate was made and fixed on a grease free slide and passed over a flame. Firstly, crystal violet was poured on the smear which was rinsed off after one minute; lugols iodine (which is the mordant) was then poured on the smear and rinsed off after one minute. Few drops of ethanol-alcohol were then used to decolorize the smear which was rinsed off after 5 seconds. Lastly, fuchsin (which is the counter stain) was poured on the smear and was rinsed off after one minute. The slide was air dried afterwards, immersion oil was then dropped on the slide (a drop) which was placed under the microscope and viewed using a magnification of x100.

3.4.8 Biochemical tests

Biochemical tests were carried out as described by Cheesbrough (2006) to determine the identity of bacteria isolates with reference to Bergey's Manual of Determinative Bacteriology.

3.4.8.1 Sugar fermentation test

This test shows the ability of microorganisms to ferment certain sugars. The sugar fermentation test was performed by inoculating a loopful of culture of the organisms into each tube containing five basic sugars (e.g. dextrose, sucrose, lactose, maltose and mannitol) separately and incubated for 24 hours at 37°C. Acid production is

indicated by the color change from reddish to yellow in the medium and the gas production is noted by the appearance of gas bubbles in the inverted Durham's tube.

3.4.8.2 Catalase test

This test was used to differentiate bacteria which produce the enzyme catalase. To perform this test, a small colony of good growth pure culture of test organism was smeared on a slide. One drop of catalase reagent (3% H₂O₂) was then added onto the smear. The slide was observed for bubble formation. Formation of bubble within few seconds was the indication of positive test while the absence of bubble formation indicated negative result (Cheesbrough, 2006).

3.4.8.3 Indole test

Two milliliter of peptone water was inoculated in 5 ml of bacterial culture and incubated at 37 °C for 48 hours. Kovac's reagent (0.5 ml) was added, mixed well and examined after 1 minute. A red color in the reagent layer indicated Indole formation. In negative case, there is no development of red color (Cheesbrough, 2006).

3.4.8.4 Methyl Red test

The test was conducted by inoculating a colony of the test organism in 0.5 ml sterile glucose phosphate broth. After overnight incubation at 37°C, a drop of methyl red solution was added. A red coloration was positive and indicated an acid pH resulting from the fermentation of glucose. A yellow coloration indicated negative result (Cheesbrough, 2006).

3.4.8.5 Voges-Proskauer Test

The Voges-Proskauer test is used to differentiate between members of the Enterobacteriaceae family based on their pattern of glucose metabolism. Two milliliter of sterile glucose phosphate peptone water was inoculated with the 5 ml of test organisms and incubated at 37°C for 48 hours. A very small amount of creatine was added and mixed. Three milliliter of sodium hydroxide was added and mixed well. The bottle cap was removed and left for an hour at room temperature. It was observed closely for the slow development of a pink color for positive cases. In the case of negative cases, there was no development of pink color (Cheesbrough, 2006).

3.5 Direct plating on Selective media

To enumerate the species of bacteria contamination of medical importance various selective media were used. Briefly, 1ml aliquot of Nutrient agar isolates were mixed with selective media and poured onto solid agar plates and allowed to settle for five minutes then on to agar surfaces, streaking was done.

The following culture media were used for this analysis: Thiosulphate citrate bile salts sucrose (TCBS) agar was used to screen for *Vibrio* species; Xylose lysine deoxycholate (XLD) agar to screen for *Shigella* species; Salmonella shigella (SS) agar was used for *Salmonella* species while Dihydroxycholate hydrogen sulphate lactose (DHL) agar was used to screen for *Escherichia* species. The entire agar plates with the processed samples from the three fish farms were thereafter inverted and incubated at 37°C for 24 hours' period. There after results were read and interpreted based on colony on their respective agar plates.

3.5.1 Sample processing for fungal isolation

All fish samples collected for each fish species were surface sterilized with 70% ethanol and rinsed with two changes of sterile distilled water. A 10 g tissue portion of each fish species was cut from the gills and skin with a sterile forceps, grinded aseptically in mortar and pestle and mixed in 90 mL of sterile peptone water. From this mixture, 20 microliters was plated in duplicates on Sabourand Dextrose Agar (SDA) sterile plates supplemented with streptomycin to inhibit bacterial growth. The inoculated plates from each sample were incubated at room temperature (25°C) for four days. The cultures were examined for growth at regular intervals.

Morphological examination included growth rate, general topography, surface and reverse pigmentation if any. Microscopic identification of positive fungal cultures was carried out using the method described by (Murray *et al.* 2005). Each different appearing culture was transferred with a sterile needle to a slide, teased apart and stained with Lactophenol cotton blue and examined microscopically. A drop of the stain was placed at the centre of a clean slide and a fragment of the fungus colony 2-3 mm from the colony edge using an inoculating needle and placed on the stain and teased gently. A cover slip was placed gently so that the conidia were not dislodged from the conidiophores. This was then examined using a microscope at low and higher power for the presence of characteristic mycelia and fruiting structures. A mycology morphology chart was used to compare and confirm the morphologies (Lecker, 1999). All observed colonies were sub cultured to obtain pure cultures which were subsequently isolated and identified using morphological characteristics, spore

formation, the production of fruiting bodies and biochemical reactions. Colony identification was made easier by the use of mycology atlases.

3.6 Data analysis

Data collected was entered in excel spread sheets and stored in a computer. Before analysis, the data was transformed using the formula $\text{LN}(x-1)$ where x represented the observed value and LN represented the natural log. The data transformed data was then analysed using two-way analysis of variance. Feed type, fish type and farm were considered as independent factors in each case. Where means were different, post hoc test at 0.05% was performed using Tukey's HSD test. The two way ANOVA analysis and post hoc tests were done using SAS software version 9. Only P. value for the transformed data were reported

CHAPTER FOUR

RESULTS

4.1 Physico-chemical parameters in the three fish farms

The physico-chemical parameters values of the water samples obtained from the borehole and rivers tested in the morning time are shown in table 4.1. A variation was significant at ($p < 0.05$) evident in the measures between the water sources. Salinity varied from 94.70 ± 0.33 to 155.47 ± 6.49 mg/L in both Mwea fish farm and Sagana fish farm respectively, with Mwea recording the least levels of Salinity. The differences in the mean levels of dissolved oxygen among the three fish farms were significant ($p = 0.001$).

Dissolved oxygen levels were significantly different ($p=0.001$) across the fish farms. Jambo fish farm recorded the highest levels of dissolved oxygen of 2.00 ± 0.16 while Sagana and Mwea fish farms had no variation in the levels of dissolved oxygen of 0.05 ± 0.00 and 0.05 ± 0.01 respectively. There was no significant ($p= 0.163$) interaction observed in the levels of total dissolved solvent among the Sagana and Mwea fish farms, though Jambo fish farm recorded the highest level of 65.50 ± 1.32 . Significant variations ($p = 0.01$) were observed in the levels of pH between the Jambo and Mwea and Sagana fish farms. Jambo fish farm had a pH of 7.80 ± 0.03 , Mwea 8.03 ± 0.05 and Sagana 7.32 ± 0.18 .

Water temperature in the morning ranged from $20.55 \pm 0.49^\circ\text{C}$ to $25.30 \pm 0.26^\circ\text{C}$. No significant variation was observed in the levels of oxidation-reduction potential (ORP)

among the three fish farms as indicated by a calculated p value of 0.987. The ORP ranged from -80.50 ± 1.04 to -78.50 ± 12.04 .

Table 4.1: Comparison of physico-chemical parameters in Sagana, Jambo and Mwea fish farms in the morning

Farm	Salinity (mg/L)	DO (mg/L)	TDS (mg/L)	pH	Temp °C	ORP (mV)
Sagana	155.47 ± 6.4	5.0 ± 0.00^a	$58.83 \pm 3.$	7.32 ± 0.1	20.55 ± 0.4	78.50 ± 12.04
	9^c		20	8^a	9^a	
Jambo	131.15 ± 2.6	2.00 ± 0.1	$65.50 \pm 1.$	7.80 ± 0.0	20.50 ± 0.6	80.50 ± 1.04
	6^b	6^b	32	3^b	5^a	
Mwea	94.70 ± 0.33	5.0 ± 0.01^a	$58.25 \pm 1.$	8.03 ± 0.0	25.30 ± 0.2	80.25 ± 7.20
	a		38	5^b	6^b	
p-						
value	<0.001	<0.001	0.163	0.010	<0.001	0.987

Mean values followed by the same letter within the same column are not significantly different from one another at $P \leq 0.05$.

In the morning, a significant disparity ($p < 0.001$) in the levels of salinity in the three fish farms was observed with Sagana recording the highest levels of 155.47 ± 6.49 mg/L and Mwea 94.70 ± 0.33 mg/L (Table 4.1). Dissolved oxygen also showed a significant variation ($P < 0.001$) with Sagana fish farm recording the highest levels of 5.00 ± 0.16 mg/L while Jambo the least of 2.00 ± 0.16 mg/L. Additionally, there was a significant variation ($P < 0.001$) in the levels of pH, temperature and oxidative reduction potential among the three fish farms (Table 4.1).

Table 4.2: Physico-chemical parameters in Sagana, Mwea and Jambo Fish farms in the evening

Farm	Salinity (mg/L)	DO (mg/L)	TDS (mg/L)	pH	Temp °C	ORP (mg/L)
Sagana	138.17±1.0 1 ^c	6.0±0.01 ^a	60.83±3.1 8 ^a	8.18±0.1 7 ^a	27.10±0.6 3 ^c	-09.67±2.93 ^a
Jambo	131.15±2.6 6 ^b	3.50±0.23 c	81.25±1.0 3 ^b	9.33±0.2 5 ^b	25.00±0.4 1 ^b	-85.75±1.80 ^b
Mwea	87.75±0.42 ^a	3.3±0.25 ^b	84.75±1.1 1 ^b	9.75±0.0 9 ^b	20.95±0.4 3 ^a	-92.00±9.01 ^b
P value	<0.001	<0.001	<0.001	<0.001	<0.001	0.012

Mean values followed by the same letter within the same column are not significantly different from one another at $P \leq 0.05$.

In the evening, there was a significant variation ($P < 0.001$) in the levels of salinity in the three fish farms with Sagana recording the highest levels of 138.17±1.01mg/L and Mwea 87.75±0.42mg/L. Dissolved oxygen also showed significant variation ($P < 0.001$) with Mwea fish farm recording the least levels of 3.3±0.23mg/L⁻¹ followed by Jambo 3.50±0.23 while Sagana the highest at 6.0±0.01 mg/L (Table 4.2). In addition there was a significant variation ($P < 0.001$) in the levels of pH and temperature. Total dissolved solids and oxidative reduction potential among the three fish farms were not significant different (Table 4.2).

Table 4.3: Mean Interaction of Physico-chemical parameters at morning and evening between the three fish farms

Parameter	Sagana		Jambo		Mwea	
	Morning mean±SE	Evening mean±SE	Morning mean±SE	Evening mean±SE	Morning mean±SE	Evening mean±SE
SALINITY(mg/L)	155.47±6.49	138.17±1.01	131.15±2.66	131.15±2.66	94.70±0.33	87.75±0.42
DO(mg/L)	5.0±0.01	6.0±0.01	2.00±0.16	3.50±0.23	5.0±0.01	3.3±0.25
TDS(mg/L)	58.83±3.20	60.83±3.18	65.50±1.32	81.25±1.03	58.25±1.38	84.75±1.11
PH	7.32±0.18	8.18±0.17	7.80±0.03	9.33±0.25	8.03±0.05	9.75±0.09
TEMP(°C)	20.55±0.49	27.10±0.63	20.50±0.65	25.00±0.41	25.30±0.26	20.95±0.43
ORP(mV)	-78.50±12.04	-109.67±2.93	-80.50±1.04	-85.75±1.80	-80.25±7.20	-92.00±9.01

Values given are Mean ±SE. DO, Dissolved oxygen; TDS, total dissolved solids; ORP, oxidation reduction potential. DO - Dissolved oxygen, TDS - Total Dissolved Solids, pH - Acidity or Alkalinity of a solution, TEMP - Temperature and ORP - Oxidation reduction potential. Values (Means ±SE) P≤0.05

According to two way ANOVA dissolved oxygen (DO) concentration was significantly different in all the farms during the morning and evening time (Table 4.3). The highest value of DO was observed in Sagana farm while the lowest in Jambo regardless of the time. The water Temperature was significantly (P< 0.0001) different in all the sites especially at evening time as compared to morning time. The highest temperature was recorded at Sagana farm at evening time while the lowest value recorded was in Jambo farm at morning time.

There was significant (P<0.0001) difference in the Ph across all the farms regardless of the timings. High Ph was observed in the evening time in all farms with Mwea

recording the highest while Sagana farm had the lowest (Table 4.4). Total dissolved solids was significantly different ($P < 0.0001$). TDS of was highest at evening across all farm and varied between 58.25mg/L and 65.50 in the morning and 60.83 and 84.75mg/L in the evening.

Table 4.4: Correlation matrix showing relationship in physico-chemical parameters in the three fish farms

		Salinity	DO	TDS	pH	TEMP	ORP
Salinity	Pearson Correlation	1					
	Sig. (2-tailed)						
DO	Pearson Correlation	.266	1				
	Sig. (2-tailed)	.611					
TDS	Pearson Correlation	-.409	-.318	1			
	Sig. (2-tailed)	.420	.539				
PH	Pearson Correlation	-.590	-.104	.933**	1		
	Sig. (2-tailed)	.217	.845	.007			
TEMP	Pearson Correlation	-.038	.754	-.183	.109	1	
	Sig. (2-tailed)	.944	.084	.729	.837		
ORP	Pearson Correlation	.304	-.104	-.884*	-.935**	-.202	1
	Sig. (2-tailed)	.558	.845	.019	.006	.702	
	N	6	6	6	6	6	6

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

The relationship between the parameters in the water samples was established using Pearson correlation analysis. The result showed that increase in water salinity resulted

in an increase in dissolved oxygen (DO) ($r = 0.266$) and an increase in ORP ($r = 0.304$). When there was a decrease in Salinity, there was an increase in Total dissolved solvents ($r = -0.409$), increase in pH ($r = -0.590$), increase in water temperature ($r = -0.038$). (Table 4.4).

Table 4.5: Relationship between the physico-chemical parameters on fish bacteria contamination

		Bacteriaload	Salinity	DO	TDS	pH	Temp	ORP
Bacteriaload	Pearson Correlation	1						
	Sig. (2-tailed)							
Salinity	Pearson Correlation	-.013	1					
	Sig. (2-tailed)	.992						
DO	Pearson Correlation	.995	-.114	1				
	Sig. (2-tailed)	.065	.927					
TDS	Pearson Correlation	-.985	.186	-.997*	1			
	Sig. (2-tailed)	.111	.881	.046				
PH	Pearson Correlation	-.298	-.951	-.199	.128	1		
	Sig. (2-tailed)	.807	.201	.872	.918			
Temp	Pearson Correlation	.417	-.914	.508	-.569	.743	1	
	Sig. (2-tailed)	.726	.266	.661	.615	.467		
ORP	Pearson Correlation	-.675	-.729	-.596	.537	.906	.389	1
	Sig. (2-tailed)	.528	.480	.593	.639	.279	.746	
	N	3	3	3	3	3	3	3

*. Correlation is significant at the 0.05 level (2-tailed).

Contamination of fingerlings by bacteria was influenced positively by an increase in DO ($r = 0.995$) and Temp. ($r = 0.417$) but negatively influenced by Salinity ($r = -0.013$), TDS ($r = -0.985$), pH ($r = -0.298$) and ORP ($r = -0.675$). However, the influences of the parameters were not significant ($P > 0.05$), (Table 4.5).

Table 4.6: Comparison of means of bacteria isolated in two species of fish from the three fish farm per CFU/mL

Treatment	<i>Bacillus</i> spp	<i>Bac trans</i>	<i>Escherichia</i> spp	<i>Eco trans</i>	<i>Klebsiella</i> spp	<i>Kleb trans</i>	<i>Salmonella</i> spp	<i>Saltrans</i>	<i>Staphylococcus</i> spp	<i>Staph trans</i>	<i>Streptococcus</i> spp	<i>Strep trans</i>	<i>Vibrio</i> spp	<i>Vibtrans</i>
Feed type														
African catfish	9.00±3.85a	1.11±0.47a	27.75±2.85a	3.29±0.12a	22.08±4.76a	2.35±0.50a	21.67±4.82a	2.33±0.50a	19.58±5.70a	2.17±0.48a	25.08±5.42a	2.43±0.52 a	18.50±4.06a	2.23±0.48a
Nile tilapia	8.58±3.68a	1.09±0.47a	25.25±3.54a	3.14±0.17a	21.00±4.60a	2.32±0.50a	22.83±4.95a	2.37±0.50a	16.83±5.40b	2.01±0.47b	20.83±4.59b	2.31±0.49b	20.25±4.40a	2.29±0.49a
Farm														
Jambo	0.001	0.001	12.88±.41b	2.59±0.10b	0.001	0.001	0.001	0.001	11.38±1.12b	2.49±0.19b	0.001	0.001	0.001	0.001
Mwea	26.38±0.80a	3.31±0.03a	32.75±2.00669a	3.50±0.10a	29.88±0.91b	29.88±0.92b	35.38±1.02a	3.59±0.03a	43.25±1.11a	3.79±0.025a	35.13±2.51a	3.57±0.08a	28.25±1.42a	3.37±0.05a
Sagana	0.001	0.001	33.88±1.89a	3.54±0.05a	34.75±1.13a	34.75±1.13a	31.38±2.12a	3.46±0.07a	0.001	0.001	33.75±1.19a	3.54±0.04a	29.88±1.45a	3.42±0.05a
P values														
Fish species	0.4590	0.4545	0.2260	0.0684	0.2813	0.2228	0.3996	0.3379	0.0030	0.0017	0.0138	0.0210	0.1642	0.1543
Farm	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Fish species*farm	0.5740	0.5677	0.2652	0.1078	0.4857	0.4139	0.0222	0.0238	0.0682	0.0035	0.1491	0.1883	0.0680	0.0542

Values (Means ±SE) followed by dissimilar letters along the columns are significantly different at P≤0.05, Two way ANOVA, Tukey's HSD-test)

Based on two way analysis of variance, there was no significant ($p= 0.4590$) variation of *Bacillus* spp contamination in different species of fingerlings. African Cat fish skin had more (9.00) *Bacillus* spp contamination. *Bacillus* spp in fish skin varied significantly (0.0001) from farm to farm. Mwea fingerlings skins had the highest (26.38) *Bacillus* spp contamination. There was significant ($p = 0.5677$) interaction between fish spp and farm in determining *Bacillus* spp in the fingerlings skins. *Escherichia* spp in the skin did not vary significantly ($P= 0.0684$) between the fish species, however African cat fish skin had the highest (27.75) *Escherichia* spp contamination. There was also significant ($p<0.0001$) variation in *Bacillus* spp contamination in fingerlings skins from different farms. Sagana farm had the highest (33.88) *Escherichia* spp in fingerlings skin. There was no significant interaction ($P = 0.48139$) between between fish type and farm in influencing *Escherichia* spp in fingerlings skin. *Klebsiella* spp contamination did not vary significantly ($p = 0.2813$) in the skin between the fish species. African cat fish had the highest contamination (22.08). There was significant ($p<0.0001$) difference in *Klebsiella* contamination in fish skin between the various farms. Sagana had the highest contamination by (34.75) *Klebsiella* spp while Jambo had least (0.001). There was no significant ($P= 0.4857$) interaction between fish species and farm in determining the *Klebsiella* spp in fish skin.

There was no significant ($P= 0.3379$) variation in *Salmonella* spp contamination in the skin between the different fish species, however Nile Tilapia skin had the highest contamination by (22.83) *Salmonella* spp. The farms differed significantly ($p=0.0001$) in *Salmonella* spp contamination in skin of fish from the farms. Mwea farm had the

highest (35.38) *Salmonella* spp in fingerlings skin while Jambo farm had the least contamination (0.001). There was significant ($p=0.0238$) interaction between farm and fish type in determining *Salmonella* spp contamination in fish skin. *Staphylococcus* spp varied significantly ($p=0.0017$) between the skin from different fish types. African cat fish skins had the highest (19.580) *Staphylococcus* species contamination. There was also significant ($p<0.0001$) difference in *Staphylococcus* spp contamination in fish skin between the various farms. Mwea farm recorded the highest (43.25) *Staphylococcus* spp in fish skins while Sagana farm recorded the least (0.001) contamination. There was significant (0.0035) interaction between fish type and farm in determining *Staphylococcus* spp in the three farms.

Streptococcus spp contamination in fish skin varied significantly (0.0210) between the fish species. African cat fish had the highest contamination (25.08) *Streptococcus* spp in their skins. There was also significant ($p<0.0001$) difference in *Streptococcus* contamination on fish skins between the different farms. Mwea farm had the highest (35.13) *Streptococcus* spp while Jambo had the lowest (0.001). There was no significant ($P= 0.1883$) interaction between fish type and farm in determining the streptococcus spp in fish skin. There was no significant (0.1543) difference in *vibrio* spp contamination in the skins of different fish type. Nile tilapia skins had the highest contamination of (20.25) *vibrio* spp. There was significant ($p < 0.0001$) variation in *vibrio* spp contamination in the fingerlings skin between the various farms. Mwea farms had the highest (28.25) *vibrio* spp contamination in fish skin while Jambo farm had the lowest (0.001). There was also significant (0.0542) interaction between fish spp and farm in determining *vibrio* spp contamination in fish skin (Table 4.6).

Table 4.7: Comparison of means of bacteria isolated in formulated and commercial feeds in the three fish farm per CFU/mL

Treatment	E. coli	Eco trans	Salm spp	Salm trans	Staph spp	Staph trans
Farm						
Jambo	4.13±1.74c	1.08±0.42b	0.001	0.001	2.875±1.20b	0.93±0.36b
Mwea	18.75±4.96b	2.69±0.31a	17.625± 5.75369a	2.28±0.51b	20.75±6.10a	2.69±0.36a
Sagana	24.38±6.04a	2.98±0.29a	23.375± 4.87317a	2.97±0.29a	24.38±6.10a	2.97±0.29a
Feed type						
Commercial	7.67±0.81b	2.10±0.10b	4.92±2.03b	1.15±0.34b	6.42±0.74b	1.95±0.10b
Formulated	23.83±5.29a	2.39±0.51a	22.42±4.90a	2.36±0.50a	25.58±5.57a	2.44±0.52 a
P values						
Farm	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Feed type	<.0001	0.0156	<.0001	<.0001	<.0001	<.0001
Farm*feed type	<.0001	<.0001	<.0001	0.0004	<.0001	<.0001

Values (Means ±SE) followed by dissimilar letters along the columns are significantly different at $P \leq 0.05$, Two way ANOVA, Tukey's HSD-test)

Significant differences ($p < 0.0001$) of *Escherichia* spp in feeds was observed in feeds from different farms, feeds from Sagana farm had the highest *Escherichia* spp (24.38) contamination while Jambo farm recorded the least (4.13) spp in feeds. Formulated feeds had significantly ($p < 0.0156$) higher *Escherichia* spp (23.83) while commercial feeds had (7.67) *Escherichia* spp. There was also significant ($p < 0.0001$) interaction between farm and feed type in determining *Escherichia* spp contamination in feeds. There was also significant ($p < 0.0001$) difference in *Salmonella* spp between the farm feeds. Sagana farm feeds had the highest *Salmonella* spp (23.375). Jambo farm feeds had the least *Salmonella* spp (0.001). There was also significant ($p < 0.0001$) variation

in *Salmonella* spp contamination between the feed types. Formulated feeds had higher *Salmonella* spp (22.42) while commercial feeds had lower salmonella spp (4.92). There was significant ($p=0.0004$) interaction between feed type and farm in determining feed type and farm in determining the *Salmonella* spp contamination.

Staphylococcus spp contamination in feeds varied significantly ($p<0.0001$) between the farms. Sagana farm feeds had the highest (24.38 *Staphylococcus* spp) while Jambo farm feeds had the lowest (2.875) *Staphylococcus* spp. Formulated feeds had significantly ($p<0.0001$) higher *Staphylococcus* spp (25.58) while commercial feeds had (6.42). There was significant interaction ($p<0.0001$) between farm and feed type in determining *Staphylococcus* spp contamination in the feeds (Table 4.7).

Table 4.8: Comparison of means of Fungi isolated in two species of fish in the three fish farm per CFU/g

Treatm ent	<i>Rhizop us spp</i>	<i>Rhiztr ans</i>	<i>Penicil lium spp</i>	<i>Penist rans</i>	<i>Asperg ilus spp</i>	<i>Asper trans</i>	<i>Mucou r</i>	<i>Mucot rans</i>
Fish type								
African cat fish	17.75± 3.97a	2.20±0 .47a	22.33± 3.79a	2.94±0 .22a	21.58± 3.84a	2.89± 0.23a	21.58± 4.82a	2.33±0 .50a
Nile tilapia	18.58± 4.69a	2.19±0 .48a	23.50± 3.40a	3.08±0 .16a	26.00± 3.75a	3.15± 0.18b	19.67± 4.52a	2.26±0 .49a
Farm								
Jambo	0.001	0.001	9.38±1. 25b	2.26±0 .18b	8.13±1. 46b	2.14±0 .14 b	0.001	0.001
Mwea	26.88± 3.22a	3.27±0 .14a	26.25± 3.25a	3.25±0 .13a	29.63± 2.63a	3.39±0 .10 a	28.25± 2.68a	3.34±0 .11a
Sagana	27.63± 2.82a	3.32±0 .11a	33.13± 2.51a	3.51±0 .08a	33.63± 2.67a	3.53±0 .08a	33.63± 1.76a	3.54±0 .05a
P values								
Fish type	0.7824	0.7824	0.7024	0.3923	0.1186	0.0344	0.4023	0.4057
Farm	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Fish species *farm	0.5475	0.4683	0.6427	0.5302	0.9848	0.3703	0.7929	0.8354

Values (Means ±SE) followed by dissimilar letters along the columns are significantly different at $P \leq 0.05$, Two way ANOVA, Tukey's HSD-test)

According to two way analysis of variance there was no significant ($p = 0.7824$) difference in *Rhizopus* spp contamination on the different fish species skins. However there was higher number (18.58) of *Rhizopus* spp on the skins of Nile tilapia spp. There was significant ($p < 0.0001$) variation in *Rhizopus* spp contamination in skin between the three fish farms. Highest *Rhizopus* spp (27.63) was recorded in fish from Sagana farm, while the lowest (0.001) was recorded on skins of fish from Jambo fish

farm (Table 4.6). There was no significant ($p = 0.5475$) interaction between fish type and farm in determining the *Rhizopus* spp contamination on the fish skin.

According to the results, there was no significant ($p = 0.3923$) variation in *Penicillium* spp on the skins of the two fish species. Highest number (23.50) of *Penicillium* spp contamination was recorded in Nile tilapia fish skins. There was significant ($p < 0.0001$) difference in *Penicillium* spp in fish skin between various fish farms. Lowest (9.38) *Penicillium* spp in skin was observed in Jambo farm while highest (33.13) *Penicillium* spp in skin was recorded in fish from Sagana fish farm. There was no significant interaction ($p = 0.5302$) between fish and farm in determining the *penicillium* spp in fish skin. *Aspergillus* spp varied significantly (0.0344) between the two species of fish skins. Nile tilapia had higher (26.00) *Aspergillus* spp contamination on the skin than African catfish. There was significant ($p < 0.0001$) variation in contamination *Aspergillus* spp on the skin of fish from various farms. Fish from Jambo farm had lower (8.13) *Aspergillus* spp contamination while fish from Sagana (33.63) had the highest *Aspergillus* spp in the skin. There was also no significant (0.3703) interaction between fish species and farm in determining *Aspergillus* spp contamination on fish skin.

Mucour spp contamination did not vary significantly ($p = 0.4057$) in the skins of the two types of fish. Nile tilapia still had higher (2.26) *mucour* spp contamination on its skin. The various farms varied significantly ($p < 0.0001$) in *mucour* spp on skins of fish from these farms. Fish from Sagana had the highest (3.54) *mucour* spp contamination on their skin while those from Jambo had the lowest (0.000). There

was no significant ($p=0.8354$) between farm and fish type in determining the *Mucor* spp in fish skin (Table 4.8).

Table 4.9: Comparison of means of bacteria isolated in formulated and commercial feeds in the three fish farm per CFU/g

Treatment	<i>Rhizopus</i> spp	<i>Rhiztr</i> ans	<i>Penicillium</i> spp	<i>Penist</i> rans	<i>Aspergillus</i> spp	<i>Asper</i> trans	<i>Mucou</i> r	<i>Mucot</i> rans
Feed type								
Commercial	7.25±2.02b	1.58±0.36b	6.42±1.39b	1.77±0.23b	7.25±1.71b	1.79±0.28b	6.00±1.93a	1.42±0.33a
Formulated	15.42±3.69a	2.10±0.45a	19.75±4.58a	2.26±0.49a	19.92±4.51a	2.27±0.49a	11.17±3.70a	1.72±0.42a
Farm								
Jambo	0.001	0.001	1.63±0.89b	0.61±0.31b	0.88±0.58b	0.37±0.25b	0.50±0.33b	0.28±0.18b
Mwea	18.00±3.63a	2.82±0.19a	21.88±5.04a	2.92±0.26a	20.38±4.77a	2.81±0.29a	18.38±3.40a	2.86±0.17a
Sagana	16.00±2.81a	2.70±0.21a	15.75±4.00a	2.52±0.33a	19.5±3.77a	2.89±0.20a	6.88±2.96a	1.57±0.38a
P values								
Feed type	0.0016	0.0018	<.0001	0.0287	<.0001	0.0211	0.0689	0.3061
Farm	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001
Feed type*farm	0.0277	0.0507	<.0001	<.0001	0.0004	0.0009	0.1101	0.1381

Values (Means ±SE) followed by dissimilar letters along the columns are significantly different at $P \leq 0.05$, Two way ANOVA, Tukey's HSD-test)

Based on two way analysis of variance there was significant ($p = 0.0018$) in fish feeds between the commercial and formulated fish feeds. Formulated fish feeds had higher contamination (15.42) species. There was significant variation ($p < 0.0001$) in *Rhizopus* spp in feeds from various farms. Mwea fish farm feeds had the highest contamination (18.00) by *Rhizopus* spp while Jambo fish farm feeds had the lowest contamination (0.001). There was significant interaction ($p = 0.050$) between feed type and farm in determining rhizopus spp contamination in feeds. There was significant variation ($p < 0.0287$) in *Penicilium* spp in different feeds (Table 4.9). Formulated feeds had higher contamination (19.75) of *penicilium* spp. There was significant variation ($p < 0.0001$) in *penicillium* spp contamination in feeds from different farms. Mwea farm feeds had the highest *penicillium* spp (21.88) while feeds from Jambo farm had the lowest penicillium contamination (1.63). There was significant interaction ($p < 0.0001$) between farm and feed type in determining *penicilium* spp in feeds.

Aspergillus species contamination varied significantly ($p = 0.0211$) in feeds. Formulated feeds had the highest contamination (19.92) number of *Aspergillus* species. *Aspergillus* species in feeds also varied significantly ($p < 0.0001$) from farm to farm. Mwea farm had the highest (20.38) number of *Aspergillus* spp while Jambo farm had the lowest contamination (0.88). There was significant ($p = 0.0009$) interaction between farm and feed type in determining *Aspergillus* spp in feeds. There was no significant ($p = 0.3061$) variation in *Mucour* spp in feeds, however formulated feeds had high (11.17) number of *Mucour* species contamination.

There significant difference in *Mucour* ($p < 0.0001$) species in feeds from different farms. Mwea farm feeds had the highest (18.38) *Mucour* spp, while Jambo farm feeds had the lowest (0.50) *Mucour* spp contamination. There was no significant ($P = 0.1381$) interaction between farm feeds and farm in determining *mucour* species in feeds.

4.5 Cultural characteristics and morphology of bacteria isolates

Samples from fish gills, skin, water and fish feeds were cultured on nutrient agar. The isolates of gram negative colonies formed cream to white colonies with a few samples having yellow colonies when cultured on plates (Plate 4.1). Morphological features observed on most nutrient agar plates revealed that *Escherichia* species, *Staphylococcus*, *Aeromonas*, *Bacillus* species formed cream and round colonies.

Densities of total aerobic bacteria were above the normal count of 30 colony forming units per gram of colonies. Water samples from Mwea and Sagana fish farms had different species of family Enterobacteriaceae like *Salmonella*, *Shigella* and *Klebsiella* (Plate 4.2).

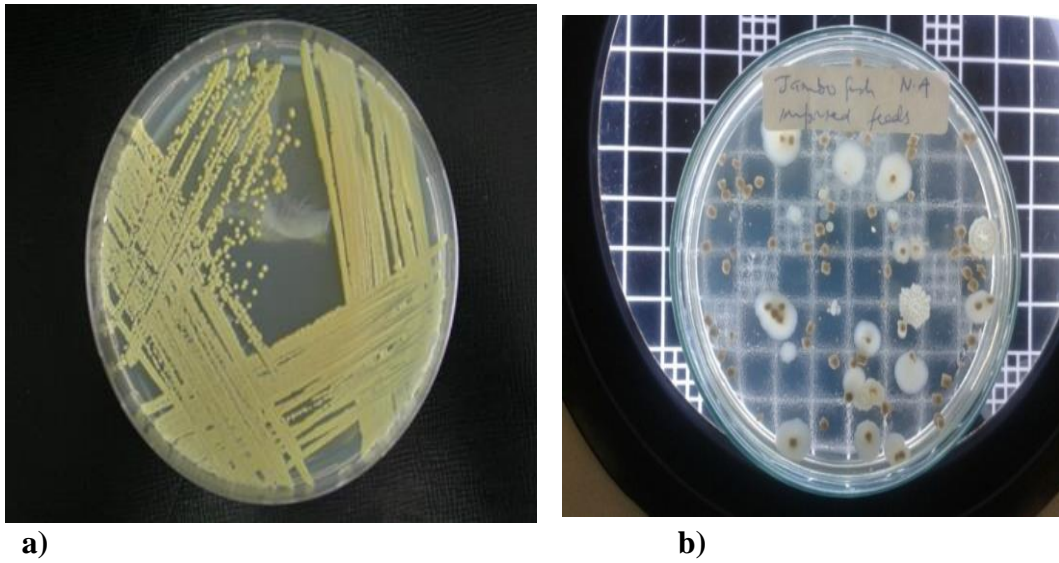


Plate 4.1: (a) Minimal growth of *Staphylococcus* species on Nutrient agar, commercial feed sample, Jambo fish farm

(b) Typical *Micrococcus* species colonies isolated from hexagonal tanks, Mwea fish farm

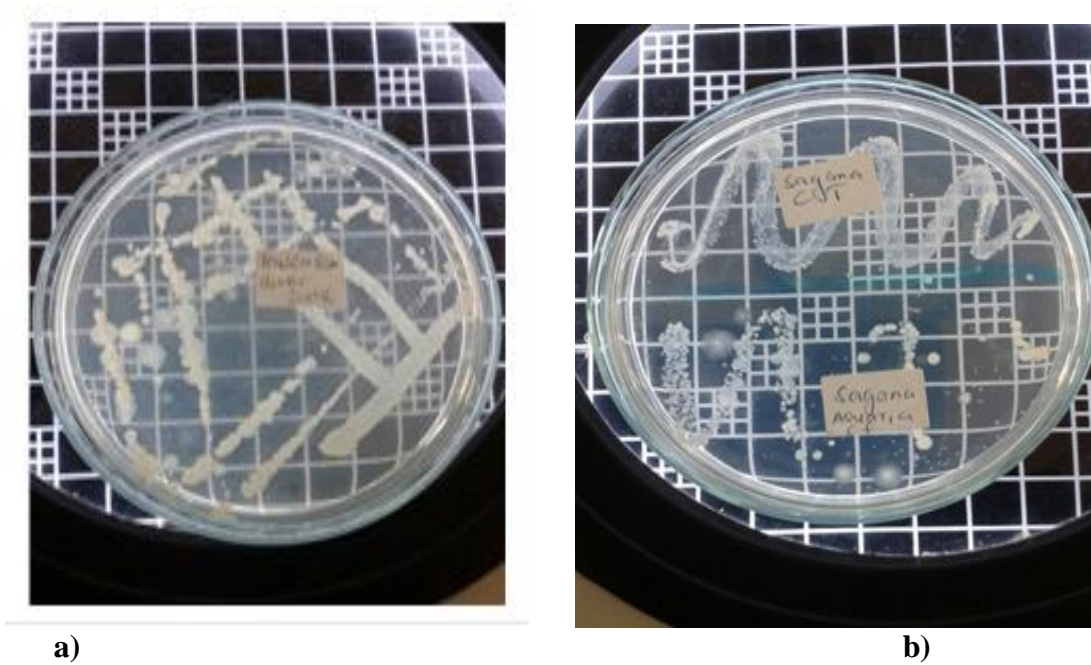


Plate 4.2: (a) Cream *Salmonella* species colonies isolates on Nutrient agar, from river water source, Mwea fish farm

(b) White, circular *Escherichia* species colonies on Nutrient agar plate at Sagana fish farm

Table 4.10: Identity of the bacteria isolates on selective culture media

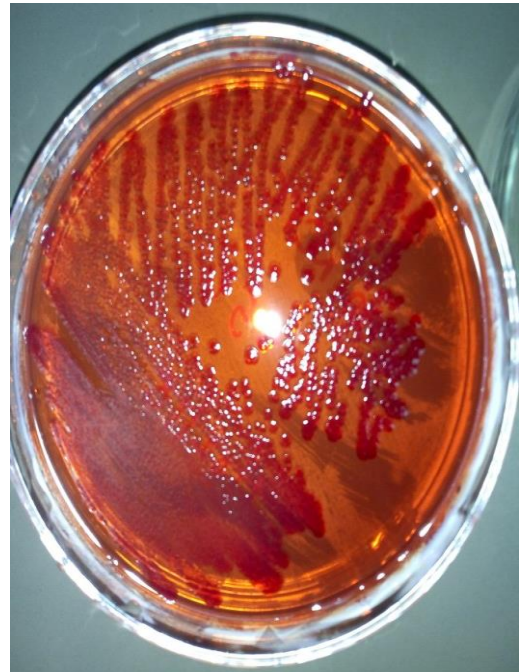
Organism	Colony Characteristics	Culture media
<i>Vibrio</i> species	Large yellow flat colonies	TCBS
<i>Escherichia</i> species	Medium pink red colonies	MCCA
<i>Salmonella</i> species	Medium pale colonies with black centers	SS
<i>Klebsiella</i> species	Pink colonies	MCCA
<i>Shigella</i> species	Medium pink red colonies	XLD
<i>Bacillus</i> species	Colonies with wide zone of beta haemolysis	BA
<i>Pseudomonas</i> species	Large colonies, undulate & beta haemolysis	BA

TCBS=Thiosulphate citrate bile sucrose; DHL=Dihydroxycholate hydrogen sulphate lactose;
 SS= Salmonella shigella; XLD= Xylose lysine deoxycholates; MCCA= Mac Conkay agar;
 BA=Blood agar

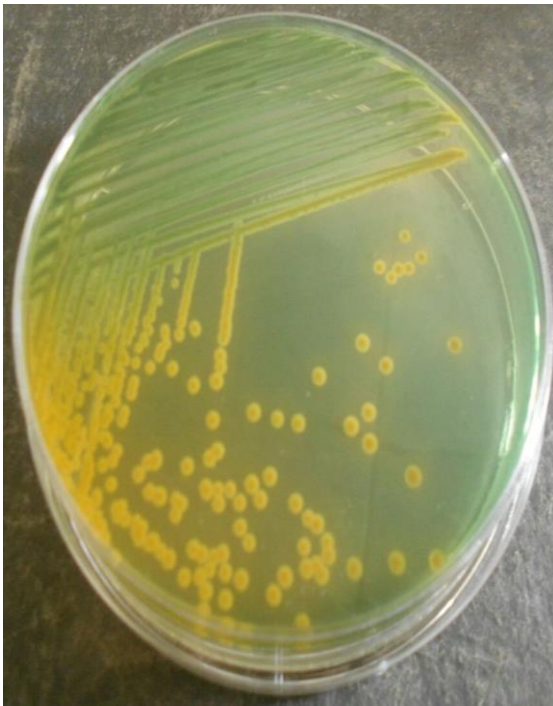
The table above shows the general morphological characteristics of isolates on their respective culture media.



a)



b)



c)



d)

- Plate 4.3:**
- a) Colony morphology by *Klebsiella* spp on MacConkay media
 - b) Lactose fermentation by *Escherichia* spp on MacConkay media.
 - c) Large yellow flat colonies *vibrio* spp on TCBS selective media
 - d) Red to yellow *Pseudomonas* spp colonies change due to the degradation of xylose, lactose and sucrose on XLD agar.

Table 4.11: Biochemical tests of bacteria isolated from African Catfish and Nile Tilapia fingerlings

Cultural characteristics and biochemical reactions of isolated bacteria from various samples of <i>Clarias gariepinus</i> and <i>Oleochromis niloticus</i>																
Sample Code		Gram staining	Shape	Motility	Lactose	Glucose	sucrose	Mannitol	MR	V P	Cit	Ox	Maltose	Indole	Catalase	Probable organism
Mwea isolate 1	Rhizoid, flat or effuse, tentate, Transparent, milky	negative	Rods	-	AG	AG	AG	AG	+	-	-	-	A-	negative	positive	<i>E. Coli</i>
Mwea isolate 2	irregular, Low convex, entire, Transparent, Milky	positive	cocci in chains	-	AG	AG	AG	No Gas & acid prod					No Gas & Acid prod	negative	negative	<i>Streptococcus Spp</i>
Mwea isolate 3	Rhizoid, low convex, tentate, Transparent, Creamy	negative	Tetrads	-	AG	AG	AG	No Gas & acid prod				+	No Gas & Acid prod	negative	positive	<i>Micrococcus Spp</i>
Sagana isolate 1	Rhizoid, flat or effuse, tentate, Transparent, milky	negative	Rods	-	AG	AG	AG	AG	+	+	-	-	A-	negative	positive	<i>E. Coli</i>
Sagana isolate 2	Circular, low convex, tentate, opaque, creamy.	positive	cocci in Cluster	-	A-	A-	A-	A-					A-	Neg	Pos	<i>Staphylococcus Spp</i>
Sagana isolate 3	Rhizoid, low convex, tentate, Transparent, Creamy	negative	tetrads	-	AG	AG	AG	No Gas & acid prod	-	-			No Gas & Acid prod	negative	negative	<i>Micrococcus Spp</i>
Sagana Isolate 4	filamentous, Raised edge, undulate, Transparent, Milky	negative	Rods	+	No Gas & Acid prod	A-	No Gas & Acid Prod	No Gas & acid prod				+/-	NO Gas & Acid prod	Neg	Pos	<i>Pseudomonas Spp</i>
Sagana isolate 5	Irregular, Raised edge, entire, Tentate	positive	cocci in chains	-	AG	AG	AG	No Gas & acid prod					No Gas & Acid prod	negative	negative	<i>Streptococcus Spp</i>
Jambo isolate 1	Irregular, Flat or Effuse, Fimbriate, Opaque, Milky	negative	Rods	-	AG	AG	AG	AG	+	+	+	-	-G	-	positive	<i>Klebsiella Spp</i>
Jambo isolate 2	Circular, low convex, tentate, opaque, creamy.	positive	cocci in Cluster	-	A-	A-	A-	A-					A-	-	+	<i>Staphylococcus Spp</i>

KEY: AG - Acid and Gas Production; A-: Acid production; G- Gas Production; -- No Gas Production; = No Gas and Acid Production

4.6 Cultural characteristics and biochemical reactions of isolated bacteria from various samples of African Catfish and Nile Tilapia fingerlings

The study established that bacteria isolates differed in regard to biochemical characteristics. Gram negative bacteria isolates from Mwea and Sagana fish farms which microscopically appeared as short rods were subjected to biochemical reactions and were confirmed to be Indole positive and Catalase positive. The isolates produced acid and gas with mannitol. The same isolates did not react with lactose even after multiple repetitive tests (Plate 4.3a).

Other gram negative bacteria had short rods, coagulase positive, catalase negative and produced acid and gas in mannitol. They produced acid only in lactose, glucose and fructose. After reacting bacteria isolates with hydrogen peroxide solution some produced acid in fructose, galactose and glucose but were catalase and coagulase positive. More isolates which were confirmed to be gram positive were coagulase and catalase negative. They produced acid and gas in glucose, galactose, fructose and lactose (Table 4.10).



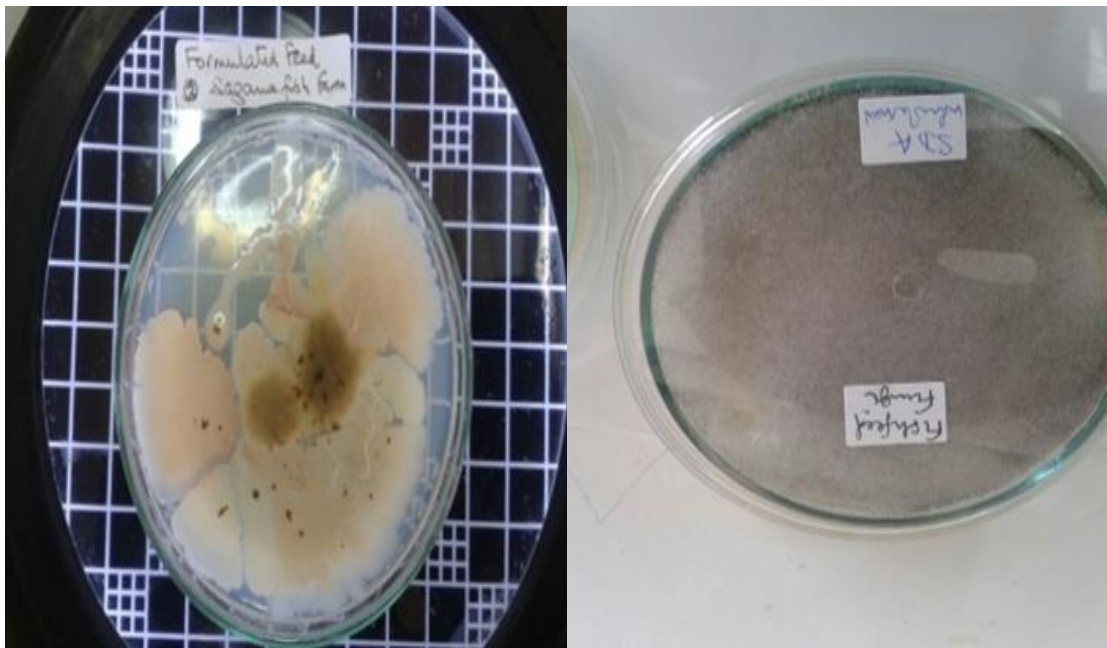
Plate 4.4 a) Biochemical characterization of *Shigella* spp, *Escherichia* spp *Salmonella* and *Vibrio* spp isolates from Sagana fish farm using selective media.



Plate 4.5: a) Biochemical characterization of *Shigella* spp, *Escherichia* spp *Salmonella* and *Vibrio* spp isolates from Mwea fish farm using selective media.

4.7 Colony morphology of fungal agents on Sabourand Dextrose Agar

Isolates of *Mucor* species were differentiated from those of *Aspergillus* species according to the colour pigmentation of the growing mycelia (Plate 4.5 a) and b) after 4 days of incubation at 25°C. The reverse colour of the SDA plates ranged from weak orange colour to a bright cadmium orange colour.



a)

b)

Plate 4.6: (a) Typical *Mucor* species growing on SDA agar from formulated feeds, Mwea

(b) Typical *Aspergillus* species with characteristic swarming of the plate

4.8 Cultural characteristics and morphology of fungal isolates

The isolates of *Aspergillus* species formed green to black colonies with a few samples having yellow colonies when cultured on SDA (Plate 4.7). Morphological features observed on most SDA plates revealed that *Aspergillus* species formed white mycelia with spreading dark green, green or yellow colonies. The conidia were olive green

with some being overlaid by olive yellow colonies. Some fungus produced brown sclerotia in some samples. Microscopic characteristics of *Penicillium* species showed smooth globose conidia. The conidiophores appeared wide and roughened and were uncolored. Some hyphae were septate with hyaline appearance (Plate 4.6b).



Plate 4.7: *Rhizopus* species Rhizoids are present; sporangiophores nodal in origin (x400)

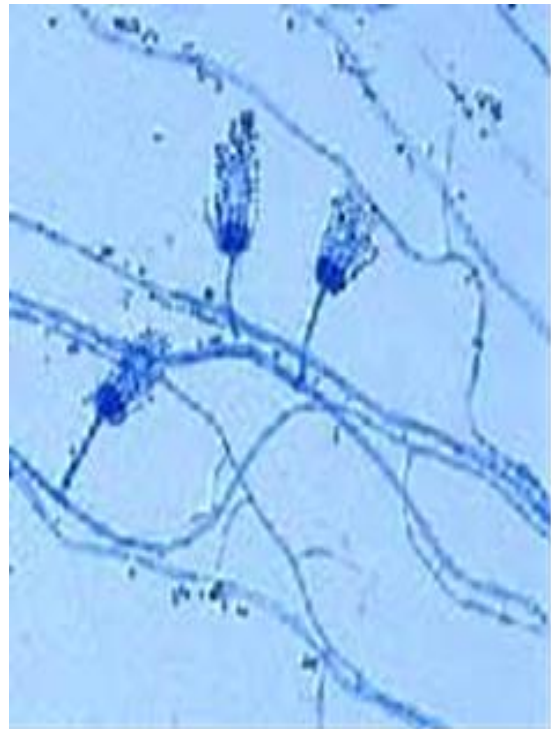


Plate 4.8: *Penicillium* species Conidiophores characteristically forming a brush-shaped structure (x400)

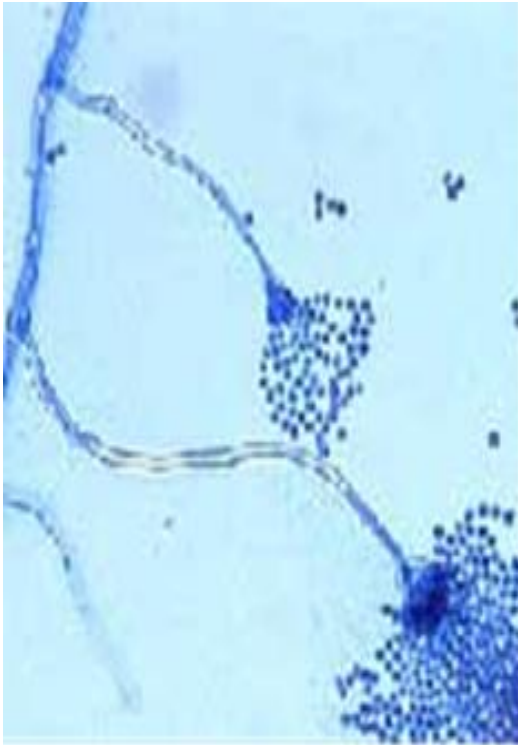


Plate 4.9: *Aspergillus* species showing characteristic of Mycelium septate and hyaline with unbranched conidiophores

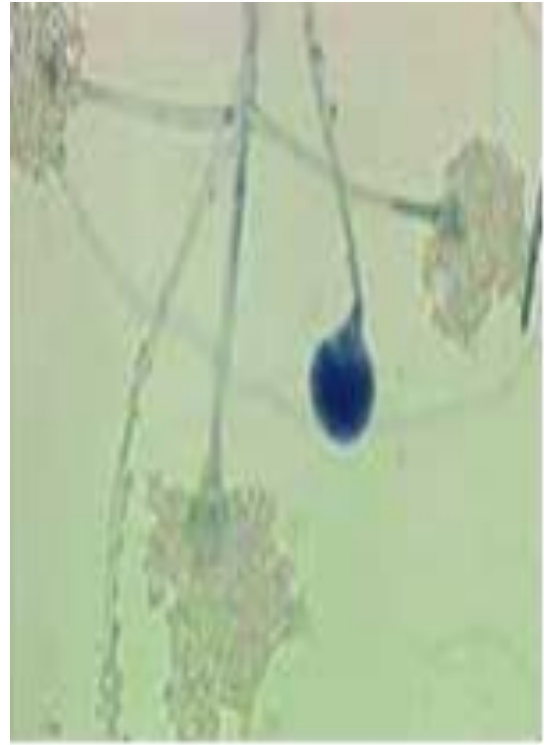


Plate 4.10: Spores of *Mucor* species (Mg= X400) with Unbranched sporangiophores. No rhizoids

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Physico-chemical parameters

The was variation observed in dissolved oxygen in Mwea and Sagana fish farms in the present study, this could be attributed to contamination of river water sources and holding of water in the hatchery tanks for a long time without changes, small sizes of hatcheries especially in Sagana fish farm was a contributing factor. (Shoemaker *et al.*, 2000) documented that significant correlation exists between number of fish and contamination levels. The results are similar to previous studies by (Adedeji *et al.*, 2011) who found that variations of various water parameters do affect the abundance of bacterial and fungal contamination levels.

Salinity levels were varied among the farms based on water source. Sagana fish farm recorded the highest levels of salinity while Jambo and Mwea farms had no variation in the levels of salinity although it was within the acceptable limit (CLIMAWAT, 2011). The rise in salinity in Sagana could be attributed to increase in deposition of organic materials into the river water from farms along the river Ragati. Similar results have also been reported in Nigeria (Adebayo *et al.*, 2012). Drastic changes of salinity may also alter the phytoplankton fauna and their population densities and lead to instability of the ecosystem. Lowering the salinity by more than 5ppt, at each time of water exchange, is not recommended (Boyd, 2007).The Total dissolved solvent among the three fish farms varied significantly though Mwea fish farm recorded the

highest levels especially at evening time (Table 4.3). This could be due to uneaten feeds, dead and decaying organic matter.

Fishes are Poikilothermic animals and their metabolic rate is regulated by the temperature of the Surrounding water. Temperature has a pronounced effect on chemical and biological processes. According to (Carvalho *et al.*, 2004), all life processes of living organisms are physicochemical in nature and are therefore affected by temperature.

Temperature and pH plays a vital role in determining the effectiveness of digestive enzymes in the fish as a whole (Makinde *et al.*, 2015). A study by (El-sayed, 2002), showed temperature influences fish growth, specifically on the sensitive fingerling stage. According to present study, the physico-chemical parameter range, pH and temperature were considered suitable for aquaculture according to (El-sayed, 2002). Temperature values during the sampling time (morning) differed significantly across the three fish farms. The highest recorded temperature and the lowest were within the recommended limits of 20°C to 30°C (Kahareri, 2003). The World Health Organization recommends 27°C as the permissible limit for fish growth and productivity. (Laibu, 2015) reported similar findings in research done in Meru County. Most studies on salinity if the water source is from bore hole usually have more consistent parameters compared to river water, and is less likely to be contaminated by pathogens and other pollutants (Boyd, 2005).

In addition, the value of pH concentration varied temperature and oxidative reduction potential among the three fish farms. (Utete *et al.*, 2013) found that significant variation in the water parameters was due to shift in weather patterns and the fact that fingerlings had utilized most of the oxygen. Regarding the seasonal variation, the highest prevalence irrespective of the agent was in the evening. This result is triggered by elevated temperature of water which is important in division of bacteria and fungal isolates which consequently make the fingerlings susceptible to contamination in the hatchery. This is confirmed by the results of (Shoemaker *et al.*, 2000), (Adedeji *et al.*, 2011) and (Klesius *et al.*, 2008), (Uddin and Al-harbi, 2012) reported that temperature of water correlated with the load of bacteria in fish. In the present study, temperature of water ranged from 26 to 27.5°C. They revealed that mesophiles and psychrotrops bacteria grew in the ranges of 15 to 47°C and -5 to 35°C, respectively.

pH is a general measure of the acidity or alkalinity of a water sample and is indicated on a scale of 0-14. The mean values recorded in the morning at Sagana, Jambo and Mwea fish farm were slightly lower than the (WHO, 2004) This may be attributed to anthropogenic activities such as sewage disposal and use of fertilizers in agricultural lands of the study area. The pH values for boreholes in Jambo fish farm are similar to those reported by (Abowei, 2011) for Nigeria study, which focused on Salinity, DO, Ph and water Temperature in Nkoro river, Niger delta. The European Union (EU) protection pH limit for aquatic life and aquaculture is in the range 6.6-9.0 (Bond, 2005). The pH obtained for river and bore-hole water in all the three farms was in this range. However the water from the borehole sources can also easily corrode the water

piping due to the acidic nature of the water therefore increasing the Total dissolved solids.

Study on dissolved oxygen shows significant differences between the three farms that agree with (Boyd, 2007) who reported that as culture period in hatcheries progresses there is usually low dissolved oxygen in Fingerlings tanks. This could be due to the rise of fingerlings biomass against a standard hatchery surface area (Shoemaker *et al.*, 2008). Water quality parameters, Temperature and ORP are correlated and had an effect on the bacterial and fungal contamination levels. It has been observed that differences in susceptibility among African Catfish and Nile Tilapia fingerlings mainly attributed to the African Catfish feeding behavior (Adedeji *et al.*, 2011). When the ORP value is high, there is lots of oxygen present in the water. This means that bacteria that decompose dead tissue and contaminants can work more efficiently. In general, the higher the ORP value, the healthier the borehole or river water is.

An analysis of dissolved oxygen measures the amount of gaseous oxygen dissolved in an aqueous solution. The maintenance of adequate dissolved oxygen concentrations is critical for the survival of aquatic biota. Ideally, increased temperature causes an increase in the metabolic activity of the fish while reducing the DO content in the system (Nzungu, 2011). WHO recommends a temperature range of between 25 °C and 32 °C for good performance of fish (WHO, 2006). High water temperature enhances the growth of thermo-tolerant microorganism. DO levels in river and borehole water depend on the physical, chemical, and biological activities of water body (Yousif, 2002) and (Utete *et al.*, 2013). DO range obtained in borehole water

from Jambo fish farm and well water was above (3.0 mg/L) (Fig. 2c), indicating anaerobic and healthy state of the source. Low levels of DO in both surface and groundwater could probably result from presence of materials of high organic content leading to oxygen depletion (Raburu *et al.*, 2009). Lack of oxygen indicates a higher rate of deoxygenation due to biological decomposition of organic matter. This finding implies a high degree of organic pollution in rivers Nyamindi and Ragati water.

Total Dissolved Solids (TDS) is a measure of both anions and cations concentration in a water body. The major anions and cations of TDS include bicarbonates, sulphates, hydrogen, silicate, chloride, calcium, magnesium, manganese, sodium, potassium, nitrates, and phosphates (Laibu, 2015). TDS values recorded in this study in the three fish farms were low in comparison to the maximum acceptable limit of 500 mg/l required for aquaculture (WHO, 2003) as well as for drinking and other domestic purposes. Total dissolved solids indicate the salinity behavior of water. In the study area, TDS values varied in Sagana and in Jambo (Table 4.3), in the morning time. In the evening Mwea fish farm had the highest levels in the evening. All the samples analyzed were found within the standard permissible limit. The borehole samples had the highest TDS values than the river water source. Boreholes and river sources from all the farms contained desirable level of TDS (55.0 mg/L to 85.0 mg/L). This could be due to low tidal influence, soil weathering, leaching and percolation of dissolved ions from waste materials, and industrial discharges. The study results were in the range shown by other investigators like (Al-harbi and Siddiqui, 2000), (Ibrahim *et al.*, 2010) APHA, 2005 and (Boyd, 2007) that was between the recommended values by WHO and EMA.

5.1.2 Bacteria Contamination

The findings implied that there exists a significant difference in the bacteria mean in Nile Tilapia in Sagana fish farm. This results agree with (Ajayi, 2012) who isolated gram negative and gram positive bacteria having used Nutrient agar as a media of isolation. There was a significant variation in the mean of bacterial spp. present in the African Catfish as indicated by *Escherichia* spp, recording the highest mean while *Vibrio* spp. had the lowest mean as shown in (Table 4.4). This high bacteria population may be due to the discharge of waste material into water bodies upon which the fingerlings fed and rain water finding its way to the rivers and ending to the hatchery tanks (Klesius *et al.*, 2003). The presence of the *Vibrio* spp could be as a result of fish excreta (Adedeji *et al.*, 2011), and the presence of the organic matter from the feed could have encouraged their presence. The presence of *Vibrio* spp noticeable in the tank fed on formulated feed could be due to the fact that organic matter in the feed deposited favored the proliferation of *Vibrio* spp. River water sources to Sagana and Mwea fish farms had wastewater effluents, combined sewer overflows, run-off from urban land, animal waste, and municipal waste sludge disposed into these two farms. Jambo fish farm had minimal bacterial and fungal contamination rates since it utilized water from borehole source.

In Mwea fish farm, there was an overall variation in the means of bacteria species though not significant among *Bacillus*, *Klebsiella* spp. and *Vibrio* spp. There was also no variation in *Escherichia* spp., *Salmonella* spp. and *Streptococcus* spp. The results are not in contradiction with previous research by (Shoemaker *et al.*, 2000), (Adedeji *et al.*, 2011) and (Budiati *et al.*, 2013); that the above agents are important human

bacterial contaminations found in aquaculture water sources. The results also agree with previous studies that *Salmonella* spp, *Streptococcus* spp. and *Vibrio* spp. have been isolated from African Catfish and Nile Tilapia fish samples (Ajayi *et al.*,2009; Radu *et al.*, 2003). Most bacteria species identified, were present in *Clarias gariepinus* (Table 4.4), which include both pathogenic and normal flora. These bacteria species found in the gills and skin of *C. gariepinus* and *Oleochromis niloticus*. These studies were similar to the ones isolated in cultured *C. gariepinus* by Emikpe *et al.* (2011) and (Al-harbi and Uddin 2010).

The results strongly suggest that Jambo fish farm had low levels of bacteria and fungal contaminants while Sagana and Mwea with predominantly higher levels of the agents than expected. Some normal floral of humans such as *Staphylococcus spp* and *Escherichia spp* were found predominant in the formulated feeds from Mwea and Sagana fish farm. This could be based on the practices in the two fish farms such as poor handling of feeds during could have led to introduction of bacteria contaminants. Run-off from roads was found to carry animal wastes into river water course and ponds therefore contributing to contamination (Emikpe *et al.*, 2011). Kirinyaga county, river Nyamindi and river Ragati had run off from farms and road draining to the river, a factor that could contribute to river contamination.

The tank water could be a source of contamination of the African catfish cultured in them. This is in agreement with (Adedeji *et al.*, 2011) and (Budiati *et al.*, 2015) reported that the most contaminated water source for African catfish ponds was borehole while river water was the least contaminated water source. Contamination of

ponds could result from indiscriminate deposition of human and animal excreta. During rainy seasons, environmental wastes including fecal matter are washed from polluted lands into water bodies such as fish ponds. This could be the reason why enteric organisms were isolated from the African Catfish and Nile Tilapia water source.

The results indicated that there was variation in the levels of bacterial and fungal contaminants isolated from Nile Tilapia and African Catfish fingerlings tissue sites respectively. This study does not agree with (Adedeji *et al.*, 2011) who found that there was no significant difference between all gills and skin samples of their total colony forming unit regardless of the species. These results are in congruence with previous study by (Igbal, 2010) that contaminated rivers supply water to hatcheries for aquaculture and this acts as main source of contaminants because of deposit of indiscriminate animal and human excreta and other environmental wastes to the natural water especially in the rainy season (Abowei, 2011). *Vibrio* species positive colonies were associated with large flat yellow colonies which ferment sucrose on TCBS agar. *Escherichia* species colonies were associated with pink/red round shaped colonies on DHL agar. *Shigella* species were linked with pale colonies on SS agar and pink- red colonies which do not ferment lactose on XLD agar while *Salmonella* species were associated with pale colonies with black centers which do not ferment lactose on SS agar plates. *Klebsiella* species colonies were mucoid and yellow which fermented lactose. *Streptococcus* species were associated with haemolysis which was exhibited by most isolates. *Bacillus* species on blood agar exhibited beta hemolytic colonies which had irregular perimeters with swarming from the site of initial

inoculation. (Musefiu *et al.* 2011) reported similar bacterial species in their study of the bacterial flora of *Clarias gariepinus* and *Oreochromis niloticus* from Ibadan South west Nigeria.

5.1.3 Fungal contamination

Investigations from this study revealed that *Aspergillus* spp, *Mucor* spp, *Penicillium* spp., and *Rhizopus* spp were found to be contaminants associated with African catfish and Nile tilapia fingerlings reared in three fish farms. *Mucor* spp. and *Aspergillus* spp were the dominant fungi recorded in association with the fingerlings contamination in this study (Tables 4.8). *Penicillium* spp occurred less frequently compared to other contaminants.

The isolation of fungal contaminants; *Aspergillus* spp, *Mucor* spp, *Penicillium* and *Rhizopus* species in both Sagana and Mwea fish farms and not Jambo fish farm indicates that water quality and its source have a significant effect on presence of contaminants. Furthermore, the use of formulated feeds in the two farms unlike Jambo fish farm which uses commercial feeds had an effect on contaminants isolated from feeds. Mwea fish farm storage of the formulated feed was wanting and mould formation was seen from the bags containing the feeds. (Mohammed *et al.*, 2012) isolated *Aspergillus flavus* species in both Nile Tilapia and African Catfish that proves contamination of aquacultures fishes with Mycotoxin producing fungi is possible. The fungi contaminants isolated from the formulated feeds especially from Mwea and Sagana fish farms have the potential to generate Mycotoxins. These findings are

similar to those reported in Pakistan by (Haroon *et al.*, 2014) and (Fayioye *et al.*, 2008)..

Ecological differences play an important role in species diversity of fungi that develop on both fish and eggs (Hussein *et al.*, 2001) and (Iqbal and Khan, 2001). Interaction of physiochemical factors generally has influence on the diversity of water molds (Paliwal and Sati, 2009). *Aspergillus* species contamination varied significantly in feeds. Formulated feeds had the highest contamination of *Aspergillus* species. *Aspergillus* species in feeds also varied significantly from farm to farm. Mwea farm had the highest number of *Aspergillus* spp while Jambo farm had the lowest contamination. There was significant interaction between farm and feed type in determining *Aspergillus* spp in feeds.

Four different fungal genera, *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor* species were isolated and identified from the formulated feed samples collected during the study. The significant increase of fungal loads from feeds samples could be attributed to high moisture levels; hence the production of moulds by the fungi may have been deposited on feed pellets. Formulated feeds had higher contaminations mainly because of the moisture, poor storage facilities handling and preparation methods. This agrees with (Siddique *et al.*, 2009) findings, that the above factors as the cause of fungal contamination in fish feeds. The study reported that fish fed on contaminated feed developed complications and die. This led to the conclusion that bad storage of feed constituents, high moisture and feeds raw materials can lead to contamination with aflatoxins due to mould formation (WHO, 2007), (Iqbal and Mughal, 2012),

(Haroon *et al.*, 2014). The source of fungal contaminations can also be as a result of consumption of fungal contaminated feed present in the hatchery tanks. Moreover, the decomposition of these feed also add to increase in fish contamination. There might be certain other conditions in the pond which favour the possibility of fungal contamination; particularly, dirty tanks, uneaten feeds and unchanged water. Fungal contaminants pose wide range of contamination in fish farming mainly due to mismanagement of tanks. Fungi isolated from this present study are in consonance with findings by others authors however, (Junaid *et al.*, 2010), (Iqbal *et al.*,2012) reported that *Penicillium* spp., *Aspergillus* spp., and *Rhizopus* spp. are normal mycoflora present in most fish farms. However they may cause diseases under favourable predisposing environment.

5.2 Conclusions

- i. The fingerlings from Mwea and Sagana fish farms were found to be significantly contaminated with both gram negative and gram positive bacteria regardless of the species of fish.
- ii. The pathogenic bacteria: *Salmonella*, *Vibrio*, *Escherichia* and *Pseudomonas* species were isolated indicating high contamination and a high risk of fingerlings to infection.
- iii. All the Physio-chemical parameters varied from one site to another and the Temperature, Dissolved oxygen and Salinity were found to be within the recommended values by WHO for aquaculture.
- iv. In regard to study farms, Jambo farm hatchery that utilized borehole water had

lower contamination as compared to Mwea and Sagana fish farms which had higher contamination due to use of contaminated water from river source. Therefore borehole water can be recommended for aquaculture.

- v. The contamination by pathogenic bacteria, fungi and variability of physico-chemical parameters were influenced majorly by the source of water and feeding type used.
- vi. Formulated feeds which were used in Mwea and Sagana fish farms were a source of both bacteria and fungal contamination.

5.3 Recommendations

Based on the findings from this study:

- i. In order to evaluate contamination of fish fingerlings, formulated fish feed and water sources must be monitored regularly for any deviations.
- ii. Aquaculture water regardless of source should be routinely examined from different points for example, source, reservoirs, storage tanks, hatchery tanks for bacteria and fungal contamination.
- iii. The fish farmers should be trained on best practices on how to prepare, store and use formulated fish feeds.
- iv. Workers working in fish farms must wear personal protective equipment, fishing gears and must be frequently decontaminate hatchery tanks and feed stores.
- v. Molecular techniques like Pyrosequencing should be used to characterize bacteria from various samples to identify specific contaminants.

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**APPENDIX I: GUIDELINES FOR PHYSICO-CHEMICAL PARAMETERS
OF WATER IN AQUACULTURE**

Physico-chemical parameter	WHO (2006)	EMA (1997)
Temp °C	27 °C	35 °C
pH	6.5-9.0	6.0 - 9.0
Conductivity (µs/cm)	1000	1000
Turbidity (NTU)	1000	1000
Dissolved oxygen (mg/l)	5.0	5.0
Nitrates (mg/l)	50.0	50.0
Ammonia (mg/l)	0.5	0.5
Sulphates (mg/l)	100	-