

**ZOONOTIC BACTERIA AND THEIR EXPOSURE FACTORS IN AQUACULTURE IN
CHIRUNDU AND SIAVONGA DISTRICTS OF ZAMBIA**

BY

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE
IN ONE HEALTH ANALYTICAL EPIDEMIOLOGY**

**THE UNIVERSITY OF ZAMBIA
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DECLARATION

I, Bertha Chitambo, do hereby declare to the Senate of the University of Zambia that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor concurrently submitted for higher degree award in any other institution.

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CERTIFICATE OF APPROVAL

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ABSTRACT

This study aimed to identify bacterial pathogens with zoonotic potential found in fish in Chirundu and Siavonga districts of Zambia. Accordingly, a total of 63 fish and 59 water samples from three fish farms (ponds and cages) were aseptically collected and bacteria isolated from the skin, gills, liver, spleen and intestines of fish and from the water samples. Isolates were identified through standard bacteriological methods through culturing, morphological characteristics, Gram staining characteristics and a series of biochemical tests. The following well-known fish pathogens were identified with attendant prevalence of, *Aeromonas* 13.2%, *Lactococcus/Streptococcus* 4.2% and *Staphylococcus* 18.1%. Other bacteria with varying significance as fish pathogens were also identified including *Acinetobacter* 2.1%, *Aequorivita* 1.4%, *Aerococcus* 1.4%, *Bacillus* 2.1%, *Bordetella* 2.1%, *Carnobacterium* 10.4%, *Citrobacter* 3.5%, *Clostridium* 2.1%, *Corynebacterium* 1.4%, *Dermatophilus* 1.4%, *Enterococcus* 2.1%, *Escherichia coli* 0.7%, *Flavobacterium* 4.2%, *Klebsiella* 6.9%, *Lactococcus* 2.1%, *Listeria* 0.7%, *Micrococcus* 6.9%, *Planococcus* 1.4%, *Proteus* 1.4%, *Pseudomonas* 6.3%, *Rhodococcus* 1.4%, *Shewanella* 1.4%, *Streptococcus* 0.7% and *Vagococcus* 0.7%. *Aeromonas*, *Bacillus*, *Clostridium*, *Escherichia coli*, *Klebsiella*, *Lactococcus*, *Listeria*, *Staphylococcus* and *Streptococcus* were of zoonotic importance. The three farms had various production types from which the general exposure factors were identified following visual determination. All key determinants in the exposure pathway were categorized under demographical, biological, environmental and/or management factors. These were then processed individually before being combined into a final framework to inform the impact pathway using the spider web analysis to aggregate exposure factors. The current study provides baseline information for future reference and implementation of public health guidelines regarding fish disease and the health of consumers in the country.

DEDICATION

To the late Beatrice Nthanga Chitambo, you shall forever be missed and loved mum.

ACKNOWLEDGEMENTS

First and foremost, praises and glory be to the Almighty God for carrying me through my research work, I am nothing without Him.

I would like to express my deep and sincere gratitude to the ACEIDHA (Africa Centre of Excellence for Infectious Diseases of Humans and Animals) project for having accorded me this rare opportunity. Thank you to the entire administration and technical staff for facilitating this wonderful and unique two years of study. I would like to appreciate my supervisors Professor Bernard M. Hang'ombe and Professor Musso Munyeme for providing invaluable guidance throughout the research and challenging me enough to the point of producing excellence. I would also like to thank the technical and advisory staff at the University of Zambia for all the assistance, special appreciation goes to Mr. Evans Mulenga for faithfully and diligently helping me with the heavy workload in the field and laboratory.

I am extremely grateful to my family for their love, prayers, caring and sacrificing for educating and preparing me for the future. I also express my thanks to my sisters and brothers for their support and prayers and my wonderful partner Dr. James Chanda, you have been so supportive. I am extending my thanks to my friends and research colleagues. Finally, my thanks go to Palabana fisheries, Kariba Harvest and Yalelo fish farms for providing access for me to sample from their farms and also to the people who supported me to complete the research work directly and indirectly.

TABLE OF CONTENTS

DECLARATION	i
CERTIFICATE OF APPROVAL.....	ii
ABSTRACT.....	iii
DEDICATION.....	iv
ACKNOWLEDGEMENTS.....	v
LIST OF TABLES.....	ix
COPYRIGHT.....	xi
LIST OF ABBREVIATIONS AND SYMBOLS.....	xii
CHAPTER ONE.....	1
INTRODUCTION.....	1
1.1. Background Information.....	1
1.2. Problem statement.....	3
1.3. Justification.....	4
1.4. Research Question.....	5
1.5. Objectives of the study.....	6
1.5.1. Overall objectives.....	6
1.5.2. Specific objectives.....	6
CHAPTER TWO.....	7
LITERATURE REVIEW.....	7
2.1. Trends in Fisheries and Aquaculture.....	7
2.2. Aquaculture production systems in Zambia.....	9
2.3. Aquatic Health management in Aquaculture.....	14

2.3.1. Aquatic Health Problems associated with aquaculture production systems	14
2.4. Significant zoonotic Bacteria Pathogens observed in aquaculture establishments	18
2.5. Principal Pathways of Zoonotic pathogens in aquaculture	23
2.5.1. Hatchery	23
2.5.2. Nursery.....	24
2.5.4. Harvesting, Marketing and Consumer	32
2.6. Prevention and control	35
CHAPTER THREE	37
MATERIALS AND METHODS.....	37
3.1. Study Area.....	37
3.2. Study Design	38
3.3. Sample Size	38
3.4. Collection of samples	38
3.5. Isolation and identification of isolated Bacteria.....	39
3.5.1. Isolation of bacteria	39
3.5.2. Identification of the isolated bacteria	39
3.5.3.Exposure Pathway Assessment	41
3.5.4.Constructing Impact Pathways	41
3.6. Data Analysis	41
3.6.1. Ethics Consideration.....	42
CHAPTER FOUR.....	43
RESULTS	43
4.1. Descriptive Results	43
4.2. Sample proportion by sample type under secondary sampling units.....	44

4.3. Sample proportion by Strata	45
4.4. Sample proportion by Production systems	46
4.5. Bacterial Isolation and Identification.....	47
4.6. Bacterial Isolates stratified according to the different Farms(origin).....	49
4.7. Identification of Exposure Factors.....	52
CHAPTER FIVE	55
DISCUSSION	55
CHAPTER SIX.....	64
CONCLUSION AND RECOMMENDATIONS	64
6.1.Conclusion.....	64
6.2.Recommendations.....	64
REFERENCES	65

LIST OF TABLES

Table 2.1: Typology of aquaculture systems in Africa.....	10
Table 3.1: Interpretation of Triple Sugar Iron Agar reaction slants	40
Table 4.1: Sampling proportions according to Production Type	43
Table 4.2: Sampling proportions according to Sample Type	45
Table 4.3: Sampling proportions according to Strata	46
Table 4.4: Sampling proportions according to Production System	46
Table 4.5: Cross tabulation of Strata versus Production Systems	47
Table 4.6: Proportions of Bacterial Isolates at genus level based on morphological characteristics, Gram-staining and biochemical test screening	48
Table 4.7: Cross tabulation of Identified Bacterial isolates at genus level by strata	49
Table 4.8: General Exposure Factors identified from the visual determination	51

LIST OF FIGURES

Figure 2.1: Fish output (t) by contribution from aquaculture and fisheries	12
Figure 2.2: Aquaculture landscape in Zambia	13
Figure 2.3: Conceptual diagram of equilibrium among hosts, pathogens and the environment in aquatic systems	17
Figure 2.4: Hatchery design at one of the fish farms in Chirundu district.....	17
Figure 2.5: The technique of application of hormone mixed feed to the early fry in the nursery hapas	25
Figure 2.6: Buckets being used for harvesting eggs and fry in an open pond.....	26
Figure 2.7: Egg cleaning in the hatchery to remove deade ones using a scoop.....	27
Figure 2.8: Hapa arrangement used for rearing either fry or fingerling/juvenile fish.....	29
Figure 2.9: Floating cages on lake Kariba.....	30
Figure 2.10: Schematic representation of distribution channels of tilapia products.....	33
Figure 3.1: Study areas – commercial fish farms in Chirundu (study site 1) and in Siavonga (Study sites 2 & 3).	37
Figure 4.1: Increased bacterial load in terms of production type, with out-growers having a bacterial load of around 20 indicating an exponential increase in bacteria isolated from older fish as compared to fingerlings.....	51
Figure 4.2: Exposure factors overlapping with each other.....	53
Figure 4.3: Flow chart showing exposure pathway evaluation of environmental, biological, demographical and management factors inclusive of an impact pathway.....	54

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percent
°C	Degrees Celsius
ACEIDHA	Africa Centre of Excellence for Infectious Diseases of Humans and Animals
ACF	Agricultural Consultative Forum
ARB	Antibiotic Resistant Bacteria
CI	Confidence Interval
DOF	Department of Fisheries
EHEC	Enterohemorrhagic <i>E. coli</i>
EIEC	Enteroinvasive <i>E. coli</i>
EPEC	Enteropathogenic <i>E. coli</i>
ETEC	Enterotoxigenic <i>E. coli</i>
FAO	Food and Agricultural Organization
FDA	Food Drug Administration
FSRP	Food Security Research Project
G	Grams

Hrs	Hours
IAPRI	Indaba Agricultural Policy Research Institute
N	Number
OIE	World Organization for Animal Health
PSU	Primary Sampling Units
SADC	Southern African Development Community
SIM	Sulphur Indole Motility
SSU	Secondary Sampling Units
TSI	Triple Sugar Iron
UNZABREC	University of Zambia Biomedical Research Ethics Committee
WHO	World Health Organization
χ^2	Chi-square
ZDA	Zambia Development Agency

CHAPTER ONE

INTRODUCTION

1.1. Background Information

Fish continues to be an ever-important nutritional component worldwide as a source of energy, protein and a range of essential nutrients and vitamins. In addition, fish provided about 3 billion people with almost 20% of their average per capita intake of animal protein and 4.3 billion people with about 15% of such protein by fish globally (FAO, 2012). Consumption of fish is a part of many people's diet that in part is due to variations in culture, religious beliefs, food habits and socioeconomic status. People with different cultural and religious backgrounds often bring their values and practices that affect their food choices. For example, the traditional Chinese diet has more than 80% of its calories from grains, legumes and vegetables, and 20% is from animal protein, fruits and fats. The calcium is obtained from either tofu or consuming small fish with bones because most Asians are lactose intolerant (Goh et al., 2018). Food selection is also affected by religious beliefs. For instance, many Hindus are vegetarians though fish is an exception for some as the only meat they can consume (Collins et al., 2009). Protein from fish is a crucial nutritional component for some densely populated countries where total protein intake levels may be low (HLPE, 2014). In 2017, about 17% of all animal protein and 7% of all protein consumed globally were accounted for by fish (FAO, 2020a). This continuous supply of fish has led to the rearing of aquatic animals. Aquaculture continues to grow faster than other major food production sectors due to an increase in the global population. The demand for fish food products

is also increasing globally. The leveling off of production from capture fisheries and most fishing areas reaching their maximum potential shows that sustaining fish supplies from capture fisheries will, therefore, not be able to meet the growing global demand for aquatic food. Aquaculture provides an opportunity to bridge the supply and demand gap of fish food in most parts of the world (Subasinghe et al., 2009). It is developing, expanding, and intensifying in almost all regions of the world. China was the largest producer of farmed fish in 2016. Other largest producers included India, Indonesia, Viet Nam, Bangladesh, Chile, Egypt and Norway (FAO, 2020a).

The increase in aquaculture production and consumption has seen an increased possibility of contracting zoonotic infections either from handling or ingesting fish and fishery products (Haenen et al., 2013). The principal pathogens acquired from fish are *Aeromonas hydrophila*, *Edwardsiella tarda*, *Mycobacterium marinum*, *Streptococcus iniae*, *Vibrio vulnificus* and *V. damsela* (Lehane and Rawlin, 2000). The principal zoonotic pathogens are *Mycobacterium species*, *Streptococcus iniae*, *Clostridium botulinum* and *Vibrio vulnificus* (Gauthier, 2015). These pathogens are all indigenous to the aquatic environment, have also been associated with disease outbreaks in food fish. They are usually associated with a puncture or open wounds in humans during processing with fishermen and fish handlers representing the historical human population highly exposed to fish zoonotic pathogens (Boylan, 2011a). Outbreaks are often related to management factors, such as the quality and quantity of nutrients in the water and high stocking density, which can increase bacterial loads on the external surface of the fish. As a result, diseased or contaminated fish are more likely to transmit the infection to humans (Haenen

et al., 2013). The significant increase of aquaculture activities in Zambia may predispose consumers and processors to possible bacterial zoonotic diseases..

1.2. Problem statement

Zambia has in the recent past seen a steady rise in aquaculture practices and production in the fisheries sector continued its upward trend in the last decade by an increase of 7.6% in 2019 (IAPRI, 2020) though still in its infant stage of development compared to livestock agriculture. However, fish production is vital to Zambia's economy as a source of food and employment. Zambia is among the largest aquaculture producers in sub-Saharan Africa. Being the largest aquaculture producer in the Southern African Development Community (SADC), Zambia has seen various actors coming into play (Kaminski et al., 2019). These include small-scale, smallholder and commercial farmers that aid in further aquaculture development. With such growth comes about fish diseases which raise public health concerns and this leads to economic losses. The cage or pond systems that have recently been established have led to situations where the incidence of bacterial diseases is rising as fish is concentrated in one place or kept at high densities (Keeley, 2013). In such a scenario, the need arises to look at bacteria zoonotic organisms associated with aquaculture. The investigation should encompass likely pathways to determine prevention, control measures, and enhancement of knowledge on this topic due to insufficient research work done in Zambia as more and more people are venturing into aquaculture with little to no knowledge. In addition, no information exists on bacteria zoonotic infections in aquaculture in Zambia.

1.3. Justification

Zambia is among the largest aquaculture producers in sub-Saharan Africa focusing on species from the *Cichlidae* family. Attempts to raise indigenous species of fish in dams and earthen ponds brought about the start of fish farming in the 1950s in Zambia (FAO, 2012). The country's estimate of fish production from aquaculture was 32, 888 tons in 2017 (Alexander and Mwango, 2018). With the expansion of the industry, many international players have established themselves on the market (Bwalya et al., 2020). Land-based systems and floating cages are the two levels at which fish farming is practiced. Land-based system dominates among small-scale farmers in the central areas of the country, around Lusaka, Copperbelt and Northern Province (ACF/FSRP, 2009; Musuka and Musonda, 2012; Musuka and Musonda, 2013; Mwango et al., 2016). Cage farming by commercial farmers on Lake Kariba started in 2001 on the Zambian side (Bwalya et al., 2020). It is growing in other areas like the Luapula province.

With the benefits realized from the growth in aquaculture, the main question still remains “where and in what circumstances is aquaculture an option in livelihood improvement, economic development fostering and an improvement of food security as well as curbing or reduction of the public health risks that come with it in terms of zoonotic pathogens and zoonotic diseases (Beveridge et al., 2010). An effective answer to this question is vital to identify significant pathogens along the aquaculture production pathway with zoonotic potential. As with any other industry which involves the intensification of biological production, it will inevitably result in problems, particularly in diseases of infectious origin and aquaculture is no exception (Håstein et al., 2005). Ecological changes affect pathogen epidemiology and evolution and may trigger the emergence of novel diseases. Aquaculture radically alters the ecology of fish and their

pathogens. It is through the alteration of the pathogen's epidemiology and introduces a specific form of pathogen mortality. Selection of virulence and disease dynamics are affected due to the above changes. Fish farming does lead to the evolution of more virulent pathogens (Pulkkinen et al., 2010).

Although increased disease severity driven by intensive farming practices is of great concern in food production, the role of evolutionary change in disease is not well understood in these environments (Sundberg et al., 2016). Therefore, more research is appropriate to investigate the prevalent zoonotic bacterial isolates present at various production pathways..

This study focused on fish farms in Chirundu district of Lusaka Province and Siavonga district of Southern Province where a substantial number of farms are. A study in this area helps understand various production systems practiced in Zambia and their public health involvement, biosecurity measures to be put in place and gaps therein. This is important because, after this study, knowledge, and understanding of the most prevalent zoonotic bacterial pathogens detected at various points of the production pathways will be established. It will serve as a basis for tackling gaps in biosecurity measures, hence, better prevention and control measures. It will give aquaculturists, policymakers, and relevant authorities a reference point in establishing preventive and control measures.

1.4. Research Question

1. What are the zoonotic bacterial pathogens associated with aquaculture in Zambia?

2. What are their likely pathways in the production chain?

1.5. Objectives of the study

1.5.1. Overall objectives

To identify zoonotic bacterial pathogens associated with aquaculture in Zambia and determine the likely pathways into the production chain.

1.5.2. Specific objectives

1. To determine the presence of bacteria zoonotic pathogens in fish grown in an aquaculture environment in Zambia.
2. To conduct a visual onsite determination of exposure factors based on-farm practices by aquaculture in Zambia.

CHAPTER TWO

LITERATURE REVIEW

2.1. Trends in Fisheries and Aquaculture

Global aquaculture has grown dramatically and has seen an enhancement in people's diets globally due to the significant growth in fish consumption because of diversification. A substantial change in the activities involved in the production, response to changes in the availability of fish stocks (for fisheries) and changes in the environmental state of the marine system driven by climatic and other changes is what diversification in Fisheries and aquaculture is. New genetic strains of fish have emerged in aquaculture due to diversification strategies which include management practices more suitable to changed conditions. These include recirculating aquaculture systems, integrated multitrophic aquaculture and offshore aquaculture. These have provided additional opportunities for using new species in aquaculture because of high market demand (Piracha, 2015). This market demand includes export opportunities, funding opportunities, competition, climate change, environmental and social factors. Currently, Asia dominates the aquaculture industry. In 2011, freshwater aquaculture production was 44.3 million tons (29%) of world fisheries production. Asia accounted for 95% of this production-China was the leading country (FAO, 2020b). Several factors have contributed to rapid growth in this region due to pre-existing aquaculture practices, population and economic growth, relaxation in the regulatory framework and expanding export opportunities (Bostock et al., 2010). These projections show that the prudent development of aquaculture is essential for the provision of

fish as a crucial nutritional component worldwide. Africa has seen enormous growth in aquaculture because of various factors. These include favorable economic climate, the growing strength of domestic markets due to rising demand for fish from a rapidly emergent middle class and the increasing price of wild-caught fish (Piracha, 2015; Tschirley et al., 2015).

Aquaculture addresses several issues, the most important one being poverty and food insecurity through several routes and various scales. For example, these provide smallholder farmers with means to diversify production, providing nutrient-rich food for their families and surplus for sale. The micro to large-scale aquaculture enterprises provides fish for sale, farm income creation and employment opportunities throughout the value chain. The value chain consists of the hatchery, fish feed manufacture, nursing, raising fish, logistics, marketing and consumers: all these stages require people to do the job, hence employment creation and respond to high market demands (Beveridge et al., 2010). To keep up with the demand, aquaculture production of food fish will need to increase to 102 million tonnes (>90% increase) by 2025 (FAO, 2016). The 2020 latest FAO report on the State of World Fisheries and Aquaculture reveals that a total global capture fisheries production reached the highest level ever recorded at 96.4 million tonnes, an increase of 5.4% from the average of the three previous years (FAO, 2020b).

In 2017, fish accounted for about 17% of total animal protein and 7% of all proteins consumed globally (FAO, 2020b). It is enhanced by people's diets worldwide due to the significant growth in fish consumption because of diversification, non-religious attributes and high nutrient value.

2.2. Aquaculture production systems in Zambia

Zambia is a country richly endowed with natural resources best suited to the production of aquaculture (Nsonga and Simbotwe, 2015). Rivers, lakes and swamps provide the basis for aquaculture development and constitute fifteen (15) million hectares of water in Zambia (Genschick et al., 2017). This endowment provides the foundations to support significant economic growth and development in aquaculture (Musumali et al., 2009; Mushili and Musuka, 2015). These natural resources are suited to the development of aquaculture (ACF/FSRP, 2009). Hence, aquaculture development programs have the positive potential to create new jobs, improve food security among poor households, remove variability in terms of household income flow and increase farm-level efficiency and sustainability (Kaliba et al., 2007).

Aquaculture production systems have a typology used that in Africa is differentiated by their extensive, semi-intensive and intensive system as seen in Table 2.1 (adapted from Hecht, 2007). These systems are either extensive or intensive based on the technologies, species and level of capital investment, labor and management practice they employ. These three systems exist in Zambia through its government. They use a narrow typology as small-scale or large-scale production systems (Genschick et al., 2017). The period 2005-2019 saw a positive upward trend in the overall aquaculture production yield and capture fisheries in Zambia, shown in figure 2.1.

Table 2.1: Typology of aquaculture systems in Africa (adapted from Hecht, 2007)

Categories & characteristics	Extensive	Semi-intensive	Intensive
Culture systems	Earthen ponds	Earthen ponds and cages	Cages, raceways, tanks and earthen/concrete ponds
Species	Polyculture (tilapia spp. Catfish and/or wetland species)	Polyculture and/or monoculture (Tilapia and African catfish or some monoculture (tilapia)	Mainly monoculture of tilapia, carp or catfish
Management input	Low to medium	Medium to high	High
Labor needs	Family labor to low requirement for external labor	Medium	Low to high (more capital intensive)
Capital costs	Low to medium	Medium	Medium to high
Operational costs	Zero to low	Medium	High
Business orientation	Low to medium	Medium to high	High
Integration with other farm incentives	Medium to high	Low to high	Low
Feeding	Zero to medium, mostly through fertilization	Scheduled to unscheduled using mainly farm-made feeds	Scheduled intensive feeding using commercial feed or farm-made feed
Fertilization	Zero to medium	Medium to high	Zero to high
Integration in value chain	Zero to medium, mostly isolated and little access to inputs and markets	Medium to high, access to inputs but little access to markets	High, key players in value chain
Socioeconomic characteristics	Mostly rural poor and subsistence-based	Rural to peri-urban, low to middle income households	Rural to urban, middle to high income, business owners
Level of communication	Household activity	Household to farm operation	Full commercial farm operation and business

In Zambia, commercial fish farming is at two levels; land-based (uses ponds and tanks with or without re-circulatory systems in Lusaka, Copperbelt and Northern Provinces), and cage aquaculture is on Lake Kariba (Musuka and Musonda, 2012). The aquaculture system a farmer

adopts depends mainly on the geographical location where land with a gentle slope is desirable (avoid flood-prone areas), water availability and ideal temperatures (Kapetsky, 1994; Brummett et al., 2008b). Clay-type soils have good water holding properties since the soil type should not allow more seepage than 10-20mm per day (Stander, 2000). Other factors to be considered are resources for feeding and improvement of water quality through aeration and partial water exchange depending on the intensity of the establishment. The individual financial situation of the farmer also determines the level of intensification employed that impacts the type of system chosen (Slonski, 2005). Aquaculture production has seen a remarkable increase in the past few years in Zambia due to the large-scale commercial aquaculture sector growth (Genschick et al., 2017). Commercial fish farming as a principal economic activity is based on money-making (profit) by actively participating in the market through the sale of all kinds of fish products (dried fish, smoked fish, fish oil, fish meal) and other related farm-produce (corn, poultry, groundnuts, beef, soybean, milk). Commercial fish farming is market-oriented that may include processing for export (ACF/FSRP, 2009).

In 2019, production in the fisheries sector increased from 118,799 metric tonnes (MT) in 2018 to 127, 822 MT in 2019 accounting for a 7.6% increment as shown in figure 2.1 (IAPRI, 2020). This growth was seen in both capture fisheries and aquaculture production with figure 2.2 showing the aquaculture landscape in Zambia as of 2014 (Genschick et al., 2017).

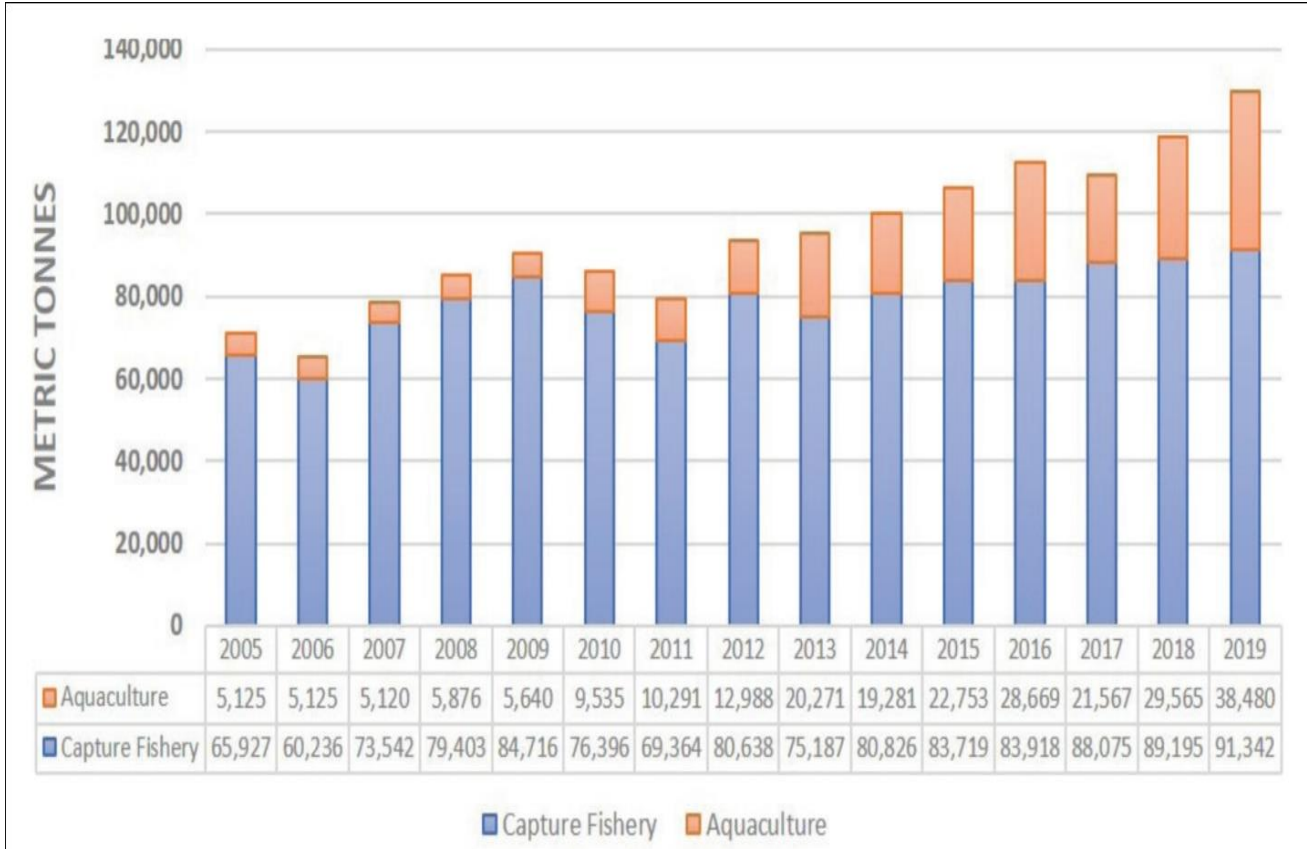


Figure 2.1: Fish output (tonnes) by contribution from aquaculture and capture fisheries (2005-2019) (DOF, 2020)

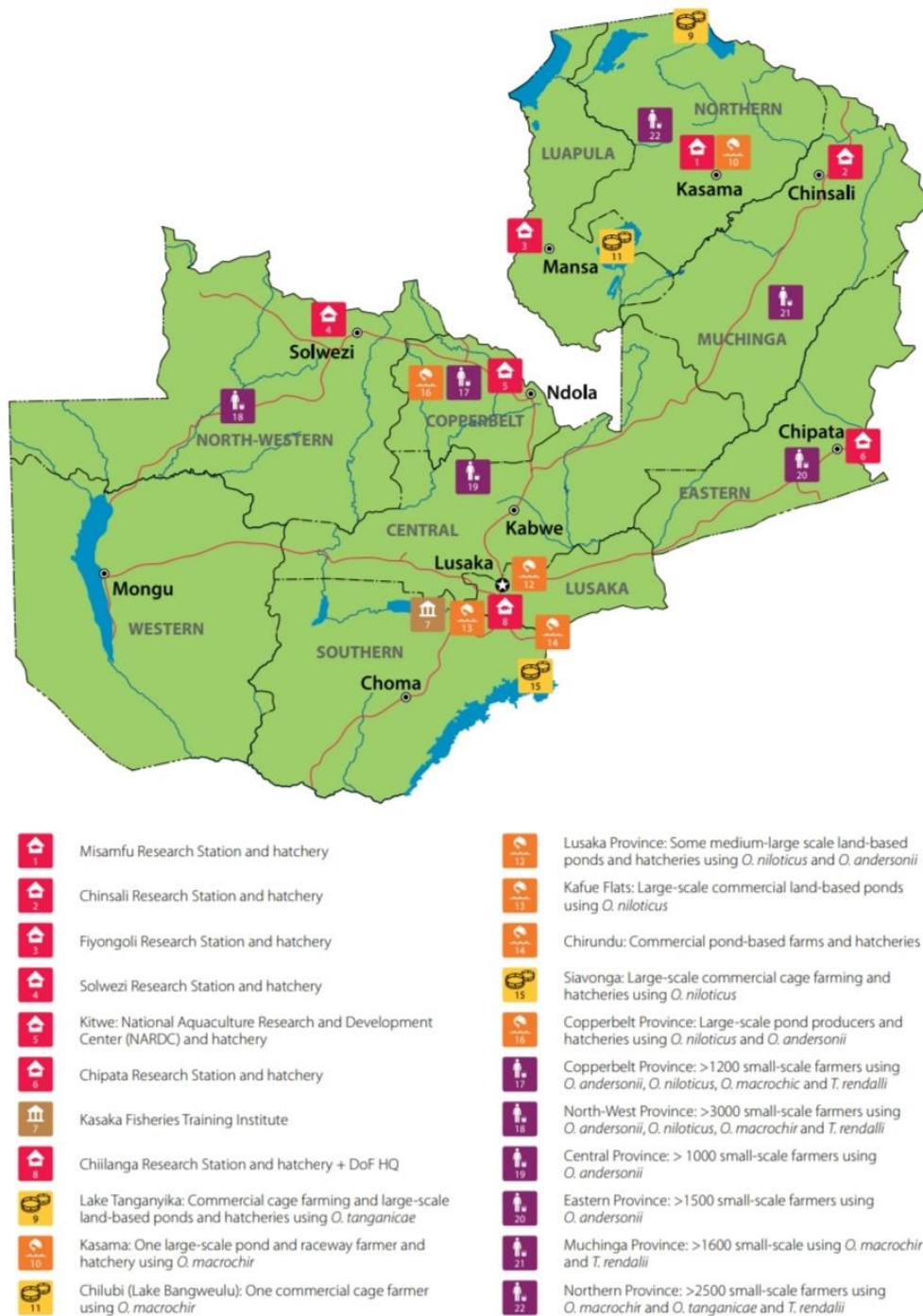


Figure 2.2: Aquaculture landscape in Zambia (Genschick et al., 2017)

2.3. Aquatic Health Management in Aquaculture

It is imperative to master the art of disease prevention rather than disease treatment to successfully maintain fish health. Fish health management includes good water quality management, nutrition and good sanitation practices. With this said, no substitute exists for proper animal husbandry in fish health and management (Francis-Floyd, 1997). The backbone for good husbandry is to keep good records that should be readily available for immediate reference in case of fish disease outbreaks. These records should contain information such as dates fish were stocked, size of fish at stocking, source of fish, feeding rate, growth rate, daily mortality and water quality (Parker, 2012). For early detection of problems when they occur, daily observation at regular intervals is vital as it will give an overview of the behavior of fish and feeding activity. Good husbandry in fish health goes hand-in-hand with proper biosecurity practices. Biosecurity in aquaculture includes measures that minimize the risk of introducing infectious pathogens or diseases and the risk that these infections will leave the premises and spread to other sites (Yanong and Erlacher-reid, 2012).

2.3.1. Aquatic Health Problems associated with aquaculture production systems

Fish production systems vary from extensive to intensive depending on the stocking density of culture organisms, level of input and degree of management (Funge-Smith and Phillips, 2001). An extensive system is where there is the utilization of a huge area of natural water bodies to achieve marginal fish production. This involves the use of lakes, rivers, reservoirs and streams to manage fish production and crops at a renewable sustainable level. The intensive culture system involves the maximum production of fish within a given mass of water body obtained by the use of artificial and sometimes natural fish food (Oddsson, 2020). Examples include indoor

hatcheries, cages, ponds and enclosure systems to produce fish at maximum attainable levels (Ikeogu et al., 2010). Integrated fish farming defined as a combination of livestock production (mainly chickens and pigs) and fish farming is also practiced (Petersen et al., 2002). Differences in the environment created in culture systems make diseases to prevail, examples include *Citrobacter freundii* infections (from pig manure), *Listeria monocytogenes* (from sheep/goat waste), *Cryptosporidium* (from cattle), *Salmonella hartford* and *Plesiomonas shigelloides* infections (associated with well-water contaminated by poultry manure) (Guan and Holley, 2003).

An impairment of the normal state of a living animal or plant body or one of its parts is referred to as a disease (Merriam-Webster, 2017). Various factors do contribute to disease causation and can be divided into intrinsic and extrinsic factors, particularly physiochemical changes and water contaminants (Ikeogu et al., 2010). Metabolism, feeding and other activities within the aquatic environment make up intrinsic factors that essentially become environmental stressors (factors that cause stress to fish in their natural habitat). The disease is usually a result of stress caused by environmental change and the creation of a suitable environment for the growth and development of bacterial, viral, fungal and parasitic disease-causing organisms. Stress weakens the immune system of fish and disease susceptibility is increased (Ikeogu et al., 2010).

Extrinsic factors originate outside the aquatic environment. They include aquatic contaminants which enter the aquatic environment through water pollution (Ikeogu et al., 2010). Disease predisposition in fish is usually the result of an interaction between the host (fish) susceptibility, disease-causing agent (pathogen) and environmental factors (stressors) (Sarker et al., 2013). This

is dependent on the interaction and cyclical balance among the pathogens, hosts and their environment as shown in figure 2.3 below.

Host variables of importance that determine the degree of resistance and susceptibility to the disease include actions of the immune system generally and specifically the influence of genetic characteristics (species, age, size, development), nutrition and reproductive needs (Hedrick, 1998). These are all encompassed in the immune system whose function is to defend the host against diseases that impact optimal biological, chemical and physical health (Huicab-pech et al., 2016). Lastly, the host, pathogen and environmental balanced relationships are maintained by the host-pathogen system and the primary regulator of this interaction being the immune system as shown in figure 2.3.

The causative microorganisms of bacterial diseases in fish are essentially naturally occurring bacterial flora of fish that originate from the environment in which the fish swim (Roberts, 2012). Different kinds of pathogens do exist. The ones described above are opportunist pathogens that invade the tissues of a fish host rendering it susceptible to infection by stress factors or other disease processes. Facultative pathogens survive in the absence of a host. They are associated with structures such as capsules, flagella, hair, endospores and cytoplasmic inclusions (Huicab-pech et al., 2016). Pathogens directly related to host infection and contained within structures such as cell walls, plasma membranes, cytoplasm, ribosomes and nuclear inclusions are called obligatory pathogens.

In fish, few bacterial species appear to be obligatory pathogens since they do not multiply to any significant extent outside the host, yet, they may survive in the aquatic environment for a variable time. Disease caused by these primary pathogens is usually stress-mediated. Clinically healthy-appearing fish yet latently infected generally do not succumb to infection provided favorable environmental conditions prevail. This kind of fish may remain long-term carriers of the pathogen infecting other fish when subjected to stress (Roberts, 2012).

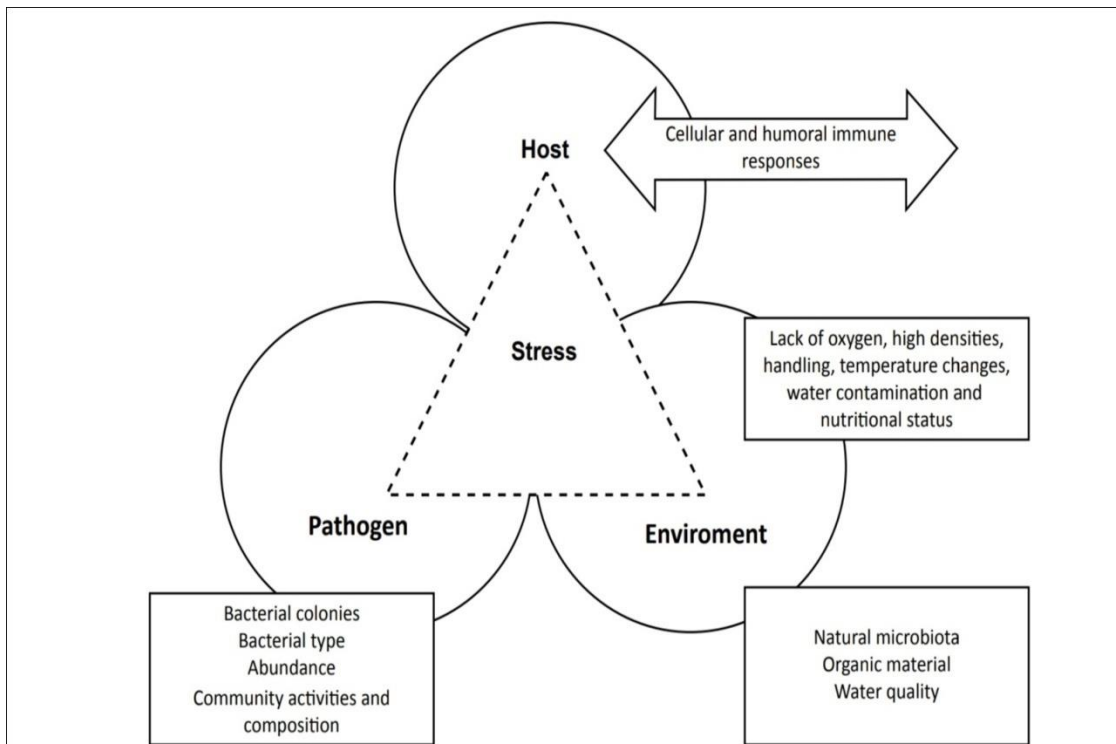


Figure 2.3: Conceptual diagram of equilibrium among hosts, pathogens and the environment in aquatic systems (Huicab-pech et al., 2016).

The pathogenicity and infection of an organism are associated with stress and environmental dynamics such as natural microbiota, organic material and water quality (Figure 2.3). Temperature, pH, salinity, oxygen, ammonium, nitrates and nitrites are vital to water quality (Huicab-pech et al., 2016). Stress is a result of significant deviations from the normal. Several

factors in aquaculture induce stress in fish. These are determined by the period of adaptation to changes in their aquatic environment, lack of oxygen, high stocking densities, handling and changes in the temperature. Pollutants in the water associated with symptoms point to the lack of balance among the host, pathogen and aquatic environment (Huicab-pech et al., 2016). Environmental stress factors predisposing fish to the clinical disease include water with high organic load (this favors multiplication of bacteria), rapidly changing temperatures, overcrowding, trauma and transportation. These factors are seen mainly in intensive fish culture systems (Roberts, 2012).

2.4. Significant zoonotic Bacterial Pathogens observed in aquaculture establishments

Various zoonotic bacteria have been implicated in causing disease from fish to man. These include *Lactococcus garvieae*, *Escherichia coli*, *Aeromonas hydrophila*, *Vibrio cholerae*, *Listeria monocytogenes*, *Edwardsiella tarda*, *Streptococcus iniae*, *Vibrio vulnificus*, *Mycobacterium spp* (*M. marinum*, *M. fortuitum* and *M. chelonae*), *Photobacterium damsela subsp. damsela*, *Vibrio alginolyticus*, *Erysipelothrix rhusiopathiae*, *Salmonella spp.*, *Clostridium spp* (*C. botulinum* and *C. perfringens*), *Campylobacter jejuni* and *Plesiomonas shigelloides* (Asante et al., 2019). Some of these bacteria are only pathogenic for humans such as *Erysipelothrix rhusiopathiae* that has no apparent effect in fish and is found on fish skin and mucus. It is obtained during contact when handling animal tissues and usually finds its way in humans through an existing wound or injury by being bitten and cut by fins during harvesting or egg/fry collection (Lowry and Smith, 2007). Among these bacteria, *V. cholerae* and *E. coli* cause disease in humans and are associated with no symptoms in fish because fish are reservoirs of these bacteria (Halpern and Izhaki, 2017). The other bacteria like *Lactococcus garvieae*,

Aeromonas hydrophila, *Listeria monocytogenes*, *Edwardsiella tarda*, *Streptococcus iniae*, *Vibrio vulnificus*, *Mycobacterium* spp., *Photobacterium damsela* subsp. *damsela*, *Vibrio alginolyticus*, *Salmonella* spp., *Clostridium* spp (*C. botulinum* and *C. perfringens*), *Campylobacter jejuni* and *Plesiomonas shigelloides* are pathogenic for both humans and fish and lead to disease development in both (Asante et al., 2019).

Various microorganisms with zoonotic significance have been isolated from fish and are mainly bacteria. Many of these pathogens are an indigenous part of the aquatic environment and fish can get them from their environment (Rahman et al., 2020). *Erysipelothrix rhusiopathiae* is one of the zoonotic bacteria which affects both humans and non-human animals through direct contact with cutaneous wounds on fish (Boylan, 2011a). *E. rhusiopathiae* although being a fish-borne pathogen that causes systemic skin diseases in marine mammals, there is no reported disease in fish caused by it (Rahman et al., 2020). *E. rhusiopathiae* causes disease in humans called erysipeloid and in animals, the disease is called erysipelas (Gauthier, 2015). People working on fish farms, fish processing plants and fishery industries are directly vulnerable to transmission of this pathogen during handling and processing of live and dead fish, hence the disease is sometimes called fish-handler's disease (Reboli and Farrar, 1989). Purple or red skin discolorations gives this disease another name called 'fish rose' because of the designated skin symptoms (Reboli and Farrar, 1989; Wang et al., 2010). Localized infection such as painful self-limiting cellulitis and edema is common with this pathogen and systemic infection is rare, though it can still cause serious endocarditis (Gorby and Peacock, 1988; Reboli and Farrar, 1989; Rahman et al., 2020). This bacterium has the ability to survive in frozen conditions; therefore, fish handlers must take extra precaution during handling of both fresh and frozen fish (wearing

protective sleeves and/or gloves) during change of nets in ponds and cages (Principe et al., 2016).

Lactococcus garvieae affects a wide range of wild fish species (both marine and freshwater fish), giant prawns from freshwater and wild marine mammals. This bacterium causes a condition called lactococcosis in fish which is severe hyperacute hemorrhagic septicemia and is of high mortality rates. It is a rare pathogen with a low virulence in human infection though it can cause endocarditis, bacteremia, peritonitis and/or bacterioascites, discopondylitis, hip prosthetic infection, acalculous cholecystitis and meningitis (Kim et al., 2013; Gibello et al., 2016; Malek et al., 2019). Transmission pattern of this bacterium from fish to humans is via direct contact with contaminated raw fish during handling and processing as well as through the consumption of contaminated raw or undercooked fish and seafood (Gibello et al., 2016).

Vibrio species are also among the zoonotic pathogens isolated from fish that also affect human beings. Several *Vibrio* species, at least 12 are of zoonotic potential (Haenen et al., 2013). Among these 12, four of them (*V. cholerae*, *V. parahemolyticus*, *V. damsela* and *V. vulnificus*) are usually involved in human infections (Austin, 2010; Zhang et al., 2016). *Vibrio* infections are contracted through consumption of contaminated raw or undercooked fish and seafood and serious symptoms are seen which include diarrhea, vomiting and dehydration (Zereen et al., 2019). Accidental ingestion of contaminated fish products (contaminated with fecal matter) and direct transmission through broken, wounded skin are part of transmission routes in humans. Cross-contamination has recently been reported with zoonotic pathogens like *Listeria monocytogenes* in ready-to-eat fish products (Chen et al., 2016).

Mycobacterium species also fall under bacteria with zoonotic potential from fish to humans. They are opportunistic pathogens that reside in aquaria environment and water supplies (Yanong et al., 2010). In this group, three of the *Mycobacterium* species of importance are *M. marinum*, *M. chelonae* and *M. fortuitum* which are the main concerns in aquaculture with *M. marinum* as a well-known zoonotic pathogen (Rahman et al., 2020). *Mycobacterium marinum* is a ubiquitous waterborne bacterium with an optimal growth temperature of around 30°C (Clark and Shepard, 1963). It is prevalent all over the world in marine water, brackish water and freshwater (Beecham et al., 1991; Hashish et al., 2018). *M. marinum* poses a zoonotic concern due to the production of granulomatous lesions in human skin and deep tissues. In addition, it also causes cutaneous infections manifested by ulcers, skin nodules and nodular lymphangitis when there is exposure to abrasions and lacerations with fish spines occurring at a fish pond, in the fish processing industry or through contaminated pond/cage water (Jernigan and Farr, 2000; Aubry et al., 2002; Wongworawat et al., 2003; Hashish et al., 2018). The infections caused by *Mycobacterium* species are called mycobacterioses and fish acquire these infections through consumption of contaminated food and polluted water (Gauthier et al., 2003; Nenoff and Uhlemann, 2006). In viviparous fish, transovarian transmission was also recorded (Hashish et al., 2018).

In humans, *M. marinum* enters the skin through minor traumata arising from resting the elbows on the border of the fish pond/cages, also through handling fish or following trauma attributed to infected foreign bodies like wood splints (Beecham et al., 1991) as well as skin injuries during processing and preparation of seafood/fish (Clark et al., 1990; Hashish et al., 2018). Direct

injuries caused by fins of fish or bites during pond/cage cleaning or water changing activities are part of ways through which this bacterium infects humans (Bhatty et al., 2000; Jernigan and Farr, 2000). The incubation period of *M. marinum* infections in humans ranges between 3 weeks to 9 months (Jernigan and Farr, 2000).

Infections with *M. marinum* in humans may be hypothetically classified into four clinical categories in order to guide therapeutic options (Bhatty et al., 2000). Type 1 is commonly observed in immunocompetent patients and is known as single or limited (1-3) lesions marked by superficial cutaneous infection which appear in form of crusted or ulcerated nodules or verrucous plaques. These take weeks to months to develop after contact with infected fish (Aubry et al., 2002). Type 2 infections with *M. marinum* consist of numerous lesions (more than 3) which include inflammatory nodules or sporotrichoid spreading pattern or with abscess and granulomas in immune suppressed patients. Infection starts with distal inoculation and then progresses to nodular lymphangitis (Hashish et al., 2018). Type 3 has involvement of deep infections associated with or without skin infection signs like arthritis, osteomyelitis, tenosynovitis and bursitis. Type 4 infection is disseminated infection involving the lungs and other systemic manifestations. Bacteremia though rare may occur in severely immunocompromised patients (Bhatty et al., 2000). It has also been noted that *M. marinum* infection also results in intranasal lesions (Asakura et al., 2016).

In summary *M. marinum* is a naturally multi-drug resistant organism with treatment being solely dependent on the investigators and their personal experiences (Edelstein, 1994).

2.5. Principal Pathways of Zoonotic pathogens in aquaculture

Aquaculture though being the fastest growing food processing industry has brought about the threat of disease as a primary constraint and risk to sustainable growth of the aquaculture sector as well as a public health risk with associated zoonoses (Bondad-Reantaso et al., 2001). Infections that are transmitted from animals to humans either directly or by a carrier are referred to as zoonoses or zoonotic diseases (Farzadnia and Naeemipour, 2020).

Various pathways are involved in the movement or spread of these disease causing pathogens in both fish and humans. A pathway can be described as a path, route, trail or a course (Neely et al., 1970). In this case, a pathway will be defined as an entry point of fish pathogens into the human society or community as fish is a carrier of these pathogens into the human population. The following are the points where there is a likelihood that human beings will come into contact with these pathogens:

2.5.1. Hatchery

Fish fry and fingerlings are very essential to fish farmers. They are the basic input which enables repetition of the production cycle and regular supply of high quality fish seed is necessary to support any viable aquaculture industry (Genschick et al., 2017). Africa's biggest barrier to aquaculture development was the access to secure supplies of high quality feed and seed (Dey et al., 2006; FAO, 2006b; Brummett et al., 2008; Beveridge et al., 2010). In Zambia the growth of commercial aquaculture has experienced major changes in the fish seed supply sector where large-scale aquaculture producers are developing their own hatcheries that produce mixed-sex and male sex-reversed fingerlings for grow-out purposes (Genschick et al., 2017).

Hatcheries are usually designed with a particular species in mind, nonetheless, there are elements common to most hatcheries which include good water quality maintenance, and therefore having adequate water storage is cardinal as shown in figure 2.4. Biosecurity is also very important and phytosanitary systems such as foot baths are to be maintained at the entrance of hatcheries and protective wear (gumboots, overalls and gloves) has to be worn by workers in the hatcheries (Sids, 2020).



Figure 2.4: Hatchery design at one of the fish farms in Chirundu district.

2.5.2. Nursery

The purpose of this phase is for growing or nursing fry to fingerlings. This phase usually determines the variation in size of fish the farm will have upon reaching market size. It is during this nursery stage that fries are fed with hormone mixed feed to reverse females to males because

of their faster growth rate when compared to females (Megbowon and Mojekwu, 2014). The treatment administration of male steroid (17 alpha-methyl testosterone) to recently hatched fry to induce development of the undifferentiated gonad tissue of generic female to testicular tissue thus functioning reproductively as males is called sex reversal in tilapia species (Forgako, 2016). This is achieved by feeding recently hatched fry with feed that has been mixed with the hormone for the duration of 21 days in hapas as shown in figure 2.5.



Figure 2.5: The technique of application of hormone mixed feed to the early fry in the nursery hapa.

A number of activities do take place in the hatchery and nursery which include hatching of harvested eggs and rearing of fish through the early stages of life (Clement et al., 2018). During the harvesting period, the eggs and fry are moved from the brood stock ponds/cages to the hatchery using harvesting buckets in most cases (figure 2.6) and if these are contaminated with bacteria pathogens, these pathogens are carried into the hatchery. Opportunistic bacteria in addition to other causative agents of disease in fish fall under the common causes of fish diseases

in hatcheries and early rearing systems (nurseries) (Faruk and Anka, 2017). Common bacteria pathogens in the hatchery and nursery include *Pseudomonas spp.*, *Aeromonas spp.*, *Francisella spp.*, and *Streptococcus spp* (Mohamed and Subasinghe, 2017). *Pseudomonas*, *Aeromonads* and *Vibrios* have been reported to cause morbidity and mortalities of fish seed in hatcheries and nurseries with *Flavobacterium ulcerative* disease characterized by the presence of hemorrhagic surface ulcers (Faruk and Anka, 2017).



Figure 2.6: Buckets being used for harvesting of eggs and fry in an open pond

Diseases in hatcheries and nurseries can spread via various items which are moved around during day-to-day operations and these include things like buckets, nets, siphon hoses, clothing, footwear and other materials. These can accidentally come into contact with sick fish or pathogens and spread diseases (Mohamed and Subasinghe, 2017). Humans can get these diseases through direct contact with fish (live or dead) or contaminated water when cleaning eggs by

removing dead ones using a scoop in the trays in the hatchery as shown in figure 2.7. Bacteria penetration can be facilitated by skin wounds or damage to the skin during handling of both live or dead fish as well as accidental ingestion of contaminated water with these pathogens (Lowry and Smith, 2007; Gauthier, 2015).



Figure 2.7: Egg cleaning in the hatchery to remove dead ones using a scoop

2.5.3. Grow -out

The grow-out process is where the fingerlings or juveniles are grown to market size. Various methods of fingerling/juvenile production commonly practiced in Zambia are cage, pond and hapa methods. A hapa is a cage-like structure that is either rectangular or square net impoundment placed in a pond for holding fish for various reasons (fry rearing of fingerling/juvenile rearing or for stocking brooders for easy harvesting of fry and eggs). They are made up of fine mesh netting material with mesh size that cannot allow fry or fish to escape (Towers, 2015) as shown in figure 2.8. These methods consist of collection of fry from spawning

units and stocked into ponds or cages or hapas which can either be fertilized or not for rearing to fingerling/juvenile stage until they reach market size (Towers, 2015).

Open ponds are the simplest and most common method of tilapia production which serve for both breeding and rearing of fish. Brooders are stocked in these ponds in the ratio of 1:3 or 1:4 (1 male to either 3 or 4 females). Harvesting is done every 15 to 21 days though the frequency depends on average water temperatures of above 25 °C.



Figure 2.8: Hapa arrangement used for rearing either fry or fingerling/juvenile fish.

Cage culture has seen a rapid increase in use and has become the most recent innovation in fish production in most countries (Hasimuna et al., 2019). Cage culture consists of rearing of fish

within fixed or floating net enclosures separated by frameworks made of bamboo, wood or metal and placed in sheltered or open shallow portions of lakes, rivers, bays and estuaries (Masser and Woods, 2008) as shown in figure 2.9. It is one of the most common intensive forms of aquaculture practiced.



Figure 2.9: Floating cages on Lake Kariba

A number of activities are practiced in aquaculture in order to have high yields of fish with less input in terms of promoting growth and aiding in the growth of plants for food for the fish. In order to achieve such growth promoters in terms of feed additives and use of livestock manure has been seen to be on the rise.

In ponds and hapas the use of livestock manure has been practiced in fish farming with little attention to the environmental effects that this can cause. This manure causes pond/hapa water to become fertile resulting in more food organisms, hence high fish production (Jie-yi et al., 1988). Livestock manure (from poultry, cattle, pigs or sheep/goats) unfortunately can take in zoonotic pathogens from animals to fish which can have adverse effects on the consumers. This manure is consumed directly by fish or supports the growth of phytoplankton leading to high yields with low input (Little, 2017). This facilitates the spread of many pathogens (Tacon, 1990). Among these are some bacterial pathogens of fish that can cause disease in humans like *Aeromonas spp.*, *Vibrio vulnificus*, *Streptococcus iniae* and *Mycobacterioses* (Ghittino et al., 2003; Rathore et al., 2005). *Enterobacteriaceae* may reach aquaculture production systems through livestock manure and cause infection in humans via handling or consumption of carrier fish (Jie-yi et al., 1988).

Bacterial infections being one of the major constraints in aquaculture development has led to antimicrobials taking the upper hand in the treatment and prevention of some of these bacterial infections (Salah et al., 2009). The challenge though is how to use these antimicrobials wisely to minimize the risk of resistance (FAO/OIE/WHO, 2006). There is exposure of these antimicrobials to the pond/hapa environment either through human and animal waste or via integrated fish farming and this has caused a surge in the number of food-borne infections caused by antibiotic resistant bacteria (ARB) (Teuber, 1999).

A lot of benefits of feeding antibiotics at low or subtherapeutic concentrations have been realized in food animals which include improvement in feed conversion efficiency and reduction in

subclinical disease (Walton, 1983). The challenge, however, of using antibiotics as feed additives is that there is development of ARB in the environment (Smith et al., 2003). In most developing countries, the use of antimicrobial drugs for treating humans and animals is unregulated and these are purchased from market stalls, pharmacies or general stores without prescriptions (Ayukekbong et al., 2017).

Waters with high organic load (which can result from excess feed accumulating at the bottom of the cage/pond or hapa) tend to favor multiplication of bacteria coupled with rapidly changing water temperatures; overcrowding and trauma are some of the commonly encountered environmental stress factors that predispose fish to clinical disease. This is commonly seen in intensive fish culture systems like cages (Roberts, 2012; Huicab-Pech et al., 2016).

The small surface area to volume ratio in caged fish as compared to ponds or raceways results in competition and pronounced disparity in food acquisition among individual fish leading to more contact between the fish (Beveridge, 2004). High incidences of injuries leading to open wounds are commonly observed in such cases due to competitive and defensive feeding behavior like high speed, spreading of fins and jaw protrusion at biting (Helfman et al., 2009). Diseases do result due to horizontal transmission from fish to fish through cannibalism and skin injuries as well as from the aquatic environment to fish and one such common disease is Streptococcosis (MSD Animal Health, 2006).

Pathogens like *Aeromonas* and *pseudomonas spp* are common fish pathogens. The *Aeromonas spp* is a group of aquatic bacteria which are ubiquitous in freshwater environment. *Salmonella*

spp are commonly associated with integrated fish farming due to common use of livestock manure as fertilizer in the ponds/hapas or cages (FAO, 2020c). The other bacteria like *Enterococci* are commensals in humans and animals and cause gastrointestinal tract disturbances (Treitman et al., 2005).

2.5.4. Harvesting, Marketing and Consumer

Aquaculture just like any other business venture depends on the marketability of the products and the wise and efficient use of the available resources (Bardach et al., 1974). Tilapia industry for example can be categorized into three subsystems of aquaculture: procurement (includes buying of production inputs like seed, feed and labor), transformation (this involves the production process where seed stock is reared to marketable sizes) and marketing (Ruddle and Grandstaff, 1978). Marketing as a subsystem in aquaculture encompasses domestic and foreign exchange as well as various marketing intermediaries and consumers (Matlala et al., 2013).

There are many activities involved in the marketing system of fish and these range from harvesting, processing, selling and consuming which can be referred to as marketing channel, distribution channel or supply chain (Matlala et al., 2013). Production cost, operating costs, competition among producers, and degree of processing, production scale and consumers' demand are some of factors that mainly affect the marketing system in the production systems (Pillay, 1994).

In tilapia products production or aquaculture products production, the marketing channel can be divided into two (figure 2.10). The first marketing channel involves distribution of products from

the producer to domestic markets then to the consumer whereas the second channel involves farmers selling the entire production of live-fish to middlemen and then the fish is distributed according to the market outlet demands.

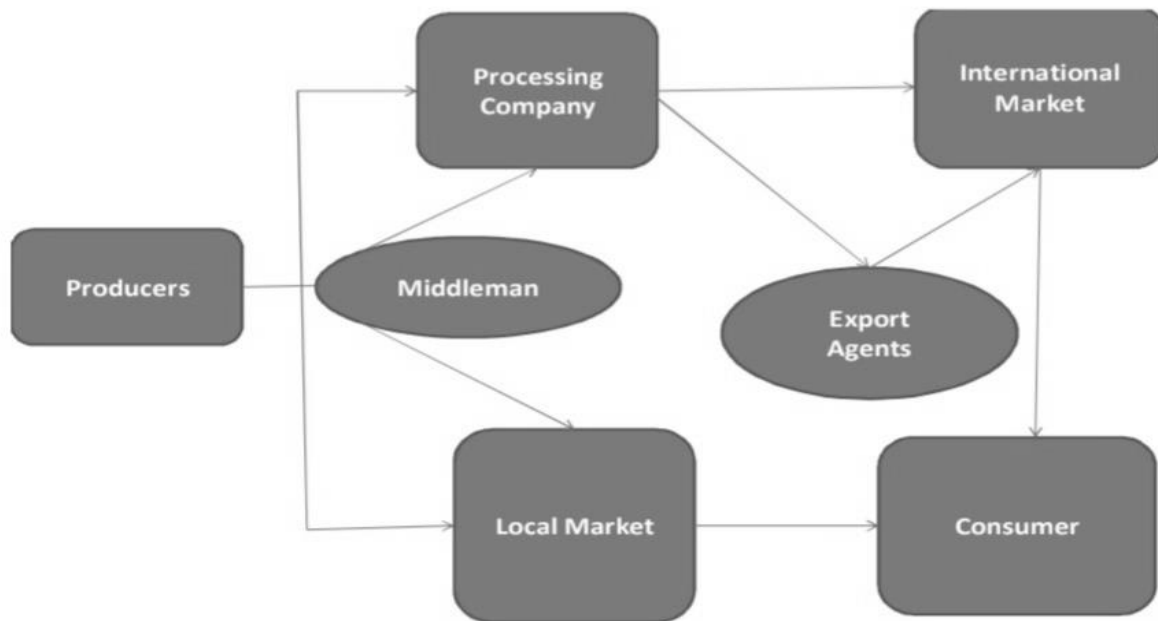


Figure 2.10: Schematic representation of distribution channels of tilapia products(Matlala et al., 2013).

Processed products are also made available to the consumers in form of frozen whole fish, frozen fillet, fresh fish fillet, salted and/or smoked products, canned and roasted fish fillet (Matlala et al., 2013).

Zoonotic bacteria can be transmitted via various routes to humans (Smolinski and Hamburg, 2003; Pal, 2005). According to (Raissy and Branch, 2020) the following are the various routes

with the most common transmission route being transmission from fish species via oral route through the consumption of untreated food (64%), transmission through skin contact and cutaneous ulcers (23%) and transmission through water contaminated with bacteria (19%). Contaminations during handling, processing and preparation are additional ways in which fish can be contaminated. Various contributing factors can also be implicated such as storage and transmission at inappropriate temperatures, contamination by infected fish handlers, or cross-contamination with fish or aquatic environment during harvest (Iwamoto et al., 2010).

Dietary choices especially consumption of raw and undercooked seafood/fish have led to an increase of fish zoonotic diseases via oral route and people more susceptible to infection are those with underlying disorders, particularly liver disease (Howden et al., 2009). Bacteria such as *Aeromonas hydrophila*, *Edwardsiella tarda*, *Streptococcus iniae*, *Mycobacterium spp.* (*M. marinum*), *Erysipelothrix rhusiopathiae*, *Vibrio vulnificus* and *Vibrio damsela* are commonly transmitted via skin contact and cutaneous ulcers (Lehane and Rawlin, 2000). People working in fish abattoirs and fish-processing plants (plants that process fish meal) are at higher risk because of close contact between them and fish/tissues of fish when slaughtering and processing (Acha and Szyfres, 2005; Haagsma et al., 2012; Pal et al., 2013).

Transmission through water contaminated with bacteria (*Salmonella*, *Shigella*, pathogenic *Escherichia coli*, *Yersinia*, *Brucella* and *Edwardsiella*) is common for farms located in polluted areas where excreta is used as fertilizer and faecal effluents from human sewage, animal farms or wild animals is also used for such purposes and people at high risk are the fish farm workers.

With the increased production and consumption of aquatic animals by humans, comes the risk of developing zoonotic diseases and this is on the rise worldwide (Haenen et al., 2013). Fishermen and fish-processing workers who are frequently exposed to fish, their products and their environment are at higher risk.

Common infections acquired through consumption of raw or undercooked fish, contaminated fish or through exposure to an open wound or cutaneous ulcer to an aquatic environment or fish are *Vibrio* infections and the most common species associated with such infections are *Vibrio parahaemolyticus* (associated with sporadic infections and gastroenteritis outbreaks) and *Vibrio vulnificus* which is particularly virulent among patients with liver disease and iron storage disorders (Novotny et al., 2004; Iwamoto et al., 2010).

Zambia is amongst other African countries that have experienced significant growth in aquaculture albeit from low baseline due to increasing intensification and commercialization in the value chain (Kaminski et al., 2018). Value chains are defined as the full range of processes required to bring a product from its conception to its end use (Kaminski et al., 2019).

2.6. Prevention and control

Prevention and control of aquatic zoonotic diseases is not a single approach action but rather a combination of different strategies (Assefa and Abunna, 2018). These strategies involve good husbandry practices such as good water quality management, good sanitation and good nutrition as well as maintaining optimum environmental conditions (optimal temperatures and pH). The focus of effective fish health management is on reducing stressful conditions, prevention of

pathogen introduction, proper use of drugs and vaccines for their intended purpose. It is highly observed that fish reared under favorable conditions is well-nourished, less prone to most disease-causing pathogens and there is less need to resort to chemotherapy since less stress and favorable environmental conditions lead to self-cure (Meyer, 1991).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Area

The study area for this research was fish farms in Chirundu district of Lusaka province and Siavonga district of Southern Province (figure 3.1).

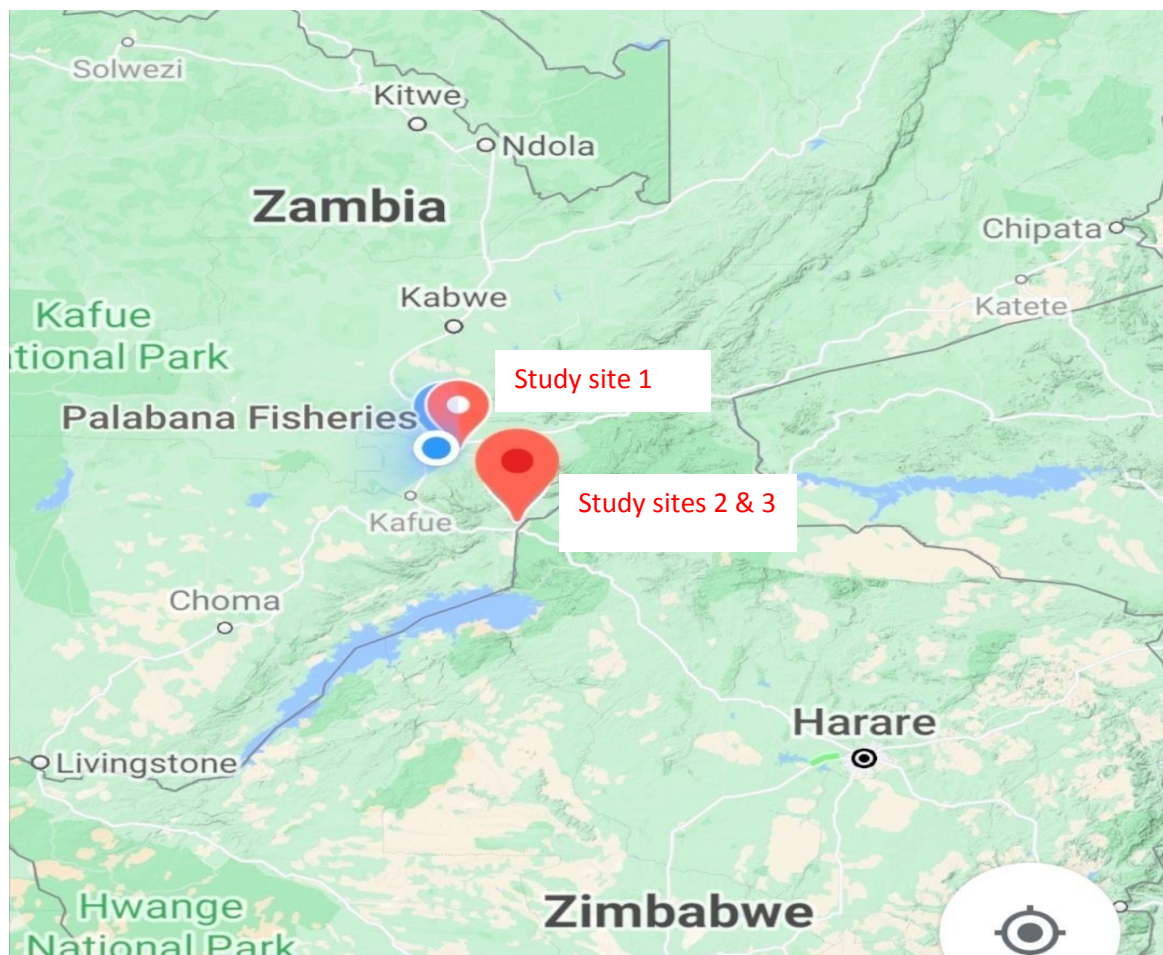


Figure 3.1: Study areas – commercial fish farms in Chirundu (Study site 1) and Siavonga (Study sites 2 & 3). Source: Google maps.

These districts were selected as they have many fish farms. Farms in these districts include all strata of farms such as large scale, medium scale, and small-scale farmers (Kaminski et al., 2019). The farmers in the different categories mentioned have different levels of aquaculture management skills, with the commercial farmers being the most experienced as they can afford experienced and skilled labor. The small-scale farmers have little management practice skills (Mainza and Musuka, 2016). All the three farms involved in this study are commercial fish farms.

3.2. Study Design

This study used a purposive sampling design, where the selection parameter included the farms with fish grow-out ponds or cages and to produce a sample size that was logically representative of the population of interest.

3.3. Sample Size

Sampling according to (OIE, 2016) guidelines. The recommended minimum number of adult fish (>150g) sampled for outbreak investigation is a sample size of 5 (Midlyng et al., 2000). Following this, from each farm, five cages/ponds were selected. The minimum number of fish collected per cage/pond was five fish, hence the summary below;-

Sampling frame of 5 cages/ponds; 5 fish from each cage/pond

Total Sample Size = 25 fish/farm

3.4. Collection of samples

A total of 77 fish were collected from 3 purposively selected farms from the following districts; Chirundu (1 farm) and Siavonga (2 farms). Two farms practiced a pond production system (1 farm from Chirundu district and one from Siavonga district), and one farm from Siavonga

district practiced a cage production system. The forty-five (45) water samples collected included the source of water for these farms, the Kafue River (water source for farm A) and the Kariba dam (water source for farms B and C) in Chirundu and Siavonga districts respectively. The collected fish and water were transported in cooler boxes to the Microbiology Laboratory at the School of Veterinary Medicine, Department of Paraclinical Studies, University of Zambia.

3.5. Isolation and identification of isolated Bacteria

3.5.1. Isolation of bacteria

Live fish were humanly killed by a sharp blow on the head and put in sealed packs with ice. Bacteriological swabs were taken from any gross lesions observed on the fish' skin then fish surfaces were disinfected by spraying with 70% ethanol. The fish were aseptically dissected to obtain tissue samples of the gills, liver, spleen and intestines. The water samples and swabs from fish tissues were inoculated individually on MacConkey agar, Nutrient agar and Blood agar (HiMedia Laboratories, India). The plates were incubated at 37°C for 24 hrs to 48 hrs, and pure cultures were obtained by subculturing.

3.5.2. Identification of the isolated bacteria

The isolates were identified following the previously described methods (MacFaddin, 2000; Starliper, 2011). In brief, colony morphology, shape, color, pigmentation, hemolytic activity, size, edges and elevation were determined and isolates were grouped accordingly. The representative isolates from each group were subjected to Gram-staining, motility testing and some biochemical tests including Simmon's Citrate, SIM (Sulphur, Indole, Motility) test, Triple Sugar Iron test (TSI), Urease and carbohydrate 'sugar' utilization tests (Esculin, Galactose, Raffinose, Salicin, Maltose monohydrate, Xylose, Mannitol, Trehalose, Insulin, Sorbitol, Lactose monohydrate and Glucose). Further identification of isolates was according to Buller (2004).

Table 3.1: Interpretation of Triple Sugar Iron Agar reaction slants

Appearance	Reactions
Acid butt: yellow, alkaline; slant: red	Glucose fermented
Acid throughout medium: butt and slant yellow	Glucose and sucrose and /or lactose fermented
Gas bubbles in butt and medium frequently split	Gas production
Butt shows blackening	Hydrogen sulphide produced
Unchanged or alkaline butt and slant: medium red throughout	None of the three sugars fermented

Source: (Provost, 1985)

3.5.3. Exposure Pathway Assessment

The three farms had various production types. Farm A consisted of 18 production types (river, hatchery, hatchery outlet, fixation pond, sex reversal pond, broodstock, empty pond, draining pond, male pond, out-grower pond, stabilization pond, *Andersonii* pond, rejects pond, direct breeding pond, *Macrochir* pond, *Niloticus* pond, mixed pond and fingerling pond). Farm B had two production types (juvenile cage and out-grower cage) and farm C encompassed three production types (fingerling pond, out-grower pond and the lake). The general exposure factors identified from the visual determination were demographic factors, biological factors, environmental factors and management factors. In this study, demographic factors involved characteristics defining the fish population and management factors involved practices associated with the entire technical operations of the farm and monitoring of daily activities adopted by aquaculture farmers (Samah and Kamaruddin, 2015). Biological factors related to the description of necessary routes for exposure of fish and humans to the bacterial pathogens while environmental factors dealing with the ecology were either abiotic or biotic that influence the living organisms in this case fish (Arthur et al., 2009).

3.5.4. Constructing Impact Pathways

A conventional sequence of steps in the logical framework formulation was used (Couillard et al., 2009). The steps followed several logical sequences (stepwise approach). Firstly, the problem was identified, after which deterministic factors were graded and analyzed according to a specific key factor. Through this study, we were able to stratify these into four overarching key factors (pillars): - Demography, biology, environment and management. These were processed

individually before being combined into a final framework to inform the impact pathway using the spider web analysis to aggregate exposure factors.

3.6. Data Analysis

All observations were recorded in Microsoft Excel 2007 and later transferred to StataSE 12 (www.stata.com) for analysis. Descriptive statistical analysis of quantitative bacterial counts and measurements of location were used to describe the outcome. The results were presented in percentages/proportions, and the difference in the distribution of predictor variables was considered significant if the p-value was less than 0.0001 at a 95% confidence interval. Pearson's chi-square was used to test the significance.

3.6.1 Ethics Consideration

Ethical approval was granted by the University of Zambia Biomedical Research Ethics Committee (UNZABREC) (C0000474). Fish farmers consented (written/oral) before farm visitations and sample collection was done. Fish safety and welfare was considered such that appropriate collection methods were used and aseptic techniques were observed when collecting water and sampling fish.

CHAPTER FOUR

RESULTS

4.1. Descriptive Results

Three (3) farms, which made up the primary sampling units (PSU), were recruited under this study. In terms of production systems, farm A was an exclusive fingerling production farm, and the other two (Farms B and C) were grow-out fish farms. A total of 44 (15 fish & 29 water) samples were collected from farm A, 34 (19 fish & 15 water) samples from farm B and a total of 44 (29 fish & 15 water) samples were from farm C giving a subtotal of 122 (63 fish & 59 water) samples from all three farms. All three farms are commercial fish farming farms.

Table 4.1: Sampling proportions according to Production Type

Production Type	Number of bacterial isolates (n=170)	% Proportion of isolates`	(95% CI)
<i>Andersonii</i> pond	3	1.8	*
Broodstock	4	2.4	(0.1 – 4.7)
Direct breeding pond	1	0.6	*
Draining pond	4	1.8	(0.1 – 4.7)
Empty pond	1	0.6	*
Fingerling pond	21	12.4	(7.4 – 17.3)
Fixation pond	1	0.6	*
Hatchery	1	0.6	*
Hatchery outlet	1`	0.6	*
Juvenile cage	11	6.5	(2.7 – 10.2)
Lake	5	2.9	(0.4 – 5.5)
<i>Macrochir</i> pond	1	0.6	*
Male pond	1	0.6	*
Mixture pond	1	0.6	*
<i>Niloticus</i> pond	12	7.1	(3.2 – 10.9)
Out-grower cage	84	49.4	(41.8 – 57)
Out-grower pond	1	0.6	*
Rejects pond	4	2.4	(0.1 -4.7)
River	2	1.2	*
Sex reversal pond	3	1.8	*
Stabilization pond	8	4.7	(1.5 – 7.9)

*The figures were too low to create a confidence interval.

The secondary sampling units (SSU) consisted of fish, vegetation and water for bacterial isolation from the two (2) production systems (Cage and Pond Production Systems). Two different types of samples were collected from each farm. Farm A comprised of 29 water samples and 15 fish samples. 15 water samples were each collected from farm B and C with Farm B having 19 fish samples and farm C 29 fish samples. Water samples were collected to test for bacteria since it makes up the habitat for the fish. Table 4.1 outlines the results for the sampling proportions according to the production type.

Of the production types in table 4.1 above, the ones with the highest number of isolates were the broodstock, draining pond, rejects pond, lake, stabilization pond, juvenile cage, *Niloticus* pond, fingerling pond and out-grower pond in increasing order.

Out of the 21 production types, farm A had 18 different production types within its pond production system namely; river, hatchery, hatchery outlet, fixation pond, sex reversal pond, broodstock, empty pond, draining pond, male pond, out-grower pond, stabilization pond, *Andersonii* pond, rejects pond, direct breeding pond, *Macrochir* pond, *Niloticus* pond, mixed pond and fingerling pond whilst Farm B had only two production types within its cage production system namely; juvenile cage and out grower cage and farm C had three (3) production types these were fingerling pond, out-grower pond and lake.

4.2. Sample proportion by sample type under secondary sampling units

Sampling was across different production types (Table 4.1), with two types of samples being collected (Table 4.2). A total of 59 water samples (29 from farm A and 15 samples were each

collected from farms B and C) and 63 fish samples (15 from farm A, 19 from farm B and 29 from farm C) were collected from all the 3 farms. Table 4.2 indicates the proportions that were sampled from fish which made up 54.7% (at 95% CI: 47.1-62.3%), with the rest being samples from water. In total, 122 samples were collected.

Table 4.2: Sampling proportions according to Sample Type

Sample type	Number of bacterial isolates (<i>n</i>=170)	% Proportion of isolates	(95% CI)
Water	77	45.3	(37.7 – 52.9)
Fish	93	54.7	(47.1 – 62.3)

4.3. Sample proportion by Strata

The secondary sampling units came from three study strata. The first strata was farm A, followed by farm B and the last was farm C. Farm A had 44 samples in total (29 water samples and 15 fish samples), farm B had 34 samples (15 water samples and 19 fish samples) and 44 samples were from farm C (15 water samples and 29 fish samples). From the secondary sampling units, farm A had more bacterial isolates from water and in farm B more isolates came from fish samples with more bacterial isolates coming from water samples in farm C.

Table 4.3: Sampling proportions according to Strata

Strata	Number of bacterial Isolates (n=170)	% Proportion	(95% CI)
		of isolates	
Farm A	60	35.3	(28 – 42.6)
Farm B	61	35.9	(28.6 – 43.2)
Farm C	49	29.9	(21.9 – 35.7)

4.4. Sample proportion by Production systems

There were two production systems involved in the study according to the production systems that the farms under this study practiced. Farms A and C used the pond production system while farm B uses the cage production system. A total of 88 samples were from the pond production system and 34 were from the cage production system. The sampling proportions by production systems are indicated in table 4.4. Majority of the samples, ($n = 109$), representing 64.1% (at 95% CI: 56.8-71.4%) being from the Pond system (Table 4.4).

Table 4.4: Sampling proportions according to Production System

Production System	Number of bacterial isolates (n=170)	% Proportion	(95% CI)
Cage System	61	35.9	(28.6 – 43.2)
Pond System	109	64.1	(56.8 – 71.4)

When the production systems were stratified against the farms, it became clear that farm A and farm C practiced the pond system, whilst farm B practiced the cage system (Table 4.5).

Table 4.5: Cross-tabulation of Strata versus Production Systems

Strata [Farm Systems]	Production Systems	
	Cage	Pond
Farm A	0	60
Farm B	61	0
Farm C	0	49

In terms of production systems against strata, there was a significant difference between the pond and cage production system given that the $\chi^2 = 170$, ($p < 0.0001$).

4.5. Bacterial Isolation and Identification

Out of the 63 fish and 59 water samples collected from all the 3 farms, 5 fish from farm B were diseased. These diseased fish presented with symptoms such as exophthalmos, skin ulcers, whitish cloudy eyes, abnormal swimming (swimming backwards and in circles) and ulceration around the mouth. All other fish collected were apparently healthy. A total of twenty-seven (27) bacterial genera were identified. Some bacteria were not properly identified and were presented as ‘unidentified bacteria’. These were from farm B and farm C (Table 4.6).

Table 4.6: Proportions of Bacterial Isolates at genus level based on morphological characteristics, Gram-staining and biochemical test screening

Identified Bacterial Isolates (Genus)	Number of bacterial isolates (n=153)	Percent proportion (%)
<i>Acinetobacter</i>	3	2
<i>Aequorivita</i>	2	1.3
<i>Aerococcus</i>	2	1.3
<i>Aeromonas</i>	19	12.4
<i>Bacillus</i>	3	2
<i>Bordetella</i>	3	2
<i>Carnobacterium</i>	15	9.8
<i>Citrobacter</i>	5	3.3
<i>Clostridium</i>	3	2
<i>Corynebacterium</i>	2	1.3
<i>Dermatophilus</i>	2	1.3
<i>Enterococcus</i>	3	2
<i>Escherichia coli</i>	1	0.7
<i>Flavobacterium</i>	6	3.9
<i>Klebsiella</i>	10	6.5
<i>Lactococcus</i>	3	2
<i>Lactococcus/Streptococcus</i>	6	3.9
<i>Listeria</i>	1	0.7
<i>Micrococcus</i>	10	6.5
<i>Planococcus</i>	2	1.3
<i>Proteus</i>	2	1.3
<i>Pseudomonas</i>	9	5.9
<i>Rhodococcus</i>	2	1.3
<i>Shewanella</i>	2	1.3
<i>Staphylococcus</i>	26	17
<i>Streptococcus</i>	1	0.7
<i>Vagococcus</i>	1	0.7
Unidentified bacteria	9	5.9

The unidentified bacteria represent 5.9% of the bacterial isolates grown from fish and water. Ten of the bacteria species failed to grow after sub-culturing and these were not accounted for together with the yeast in the tabulations.

4.6. Bacterial Isolates stratified according to the different Farms (origin)

After accounting for yeasts, and the unidentified bacteria, a total of 160 bacterial isolates grouped according to genus were identified (Table 4.7).

Table 4.7: Cross-tabulation of Identified Bacterial isolates at genus level by strata

Identified Bacterial Isolates (Genus)	Strata			Total
	Farm-A	Farm-B	Farm-C	
<i>Acinetobacter</i>	2	1	0	3
<i>Aequorivita</i>	2	0	0	2
<i>Aerococcus</i>	0	0	2	2
<i>Aeromonas</i>	18	1	0	19
<i>Bacillus</i>	1	1	1	3
<i>Bordetella</i>	0	3	0	3
<i>Carnobacterium</i>	3	7	5	15
<i>Citrobacter</i>	4	0	1	5
<i>Clostridium</i>	2	0	1	3
<i>Corynebacterium</i>	0	1	1	2
<i>Dermatophilus</i>	0	1	1	2
<i>Enterococcus</i>	1	2	0	3
<i>Escherichia coli</i>	1	0	0	1
<i>Flavobacterium</i>	3	2	1	6
<i>Klebsiella</i>	10	0	0	10
<i>Lactococcus</i>	0	3	0	3
<i>Lactococcus/Streptococcus</i>	0	1	5	6
<i>Listeria</i>	1	0	0	1
<i>Micrococcus</i>	0	7	3	10
<i>Planococcus</i>	0	1	1	2
<i>Proteus</i>	2	0	0	2
<i>Pseudomonas</i>	0	8	1	9
<i>Rhodococcus</i>	1	1	0	2
<i>Shewanella</i>	0	1	1	2
<i>Staphylococcus</i>	9	4	13	26
<i>Streptococcus</i>	0	1	0	1
<i>Vagococcus</i>	0	0	1	1
Unidentified Bacterial	0	5	4	9
Total	60	51	42	153

Further, these were stratified according to the area of production or strata (Table 4.7). Farm A had the highest number of bacterial isolates identified at 60 in total followed by farm B at 54, whilst farm C had 46. In farm A all bacteria isolated were successfully identified whilst some

bacteria were not identified in farms B and C and yeast was identified from these two farms. Of the identified bacteria genera, *Aeromonas spp* was the most isolated bacteria in farm A whereas farm B and C, *Pseudomonas* and *Staphylococcus spp* were the most isolated bacteria respectively.

Across all the three farms, after removal of yeast and unidentified bacteria, *Staphylococcus spp.* accounted for 18.1% across all the three strata and was the most commonly isolated bacterial species, followed by *Aeromonas spp* (13.2%), then followed by *Carnobacterium* (10.4%). These were isolated in both water and fish samples.

Bacteria isolates were further stratified according to production systems and sample types. The pond production system accounted for more bacterial isolates constituting 106 isolates compared to the cage production system which had 54 isolates. Out of the two sample types, fish samples accounted for 87 isolates whilst water samples accounted for 73 isolates.

When the bacterial load was determined against the age of the fish, starting with sampling from the fingerlings through to the out-growers, to the broodstock, it was found that the bacterial load increased with the increasing age of the fish (Figure 4.1).

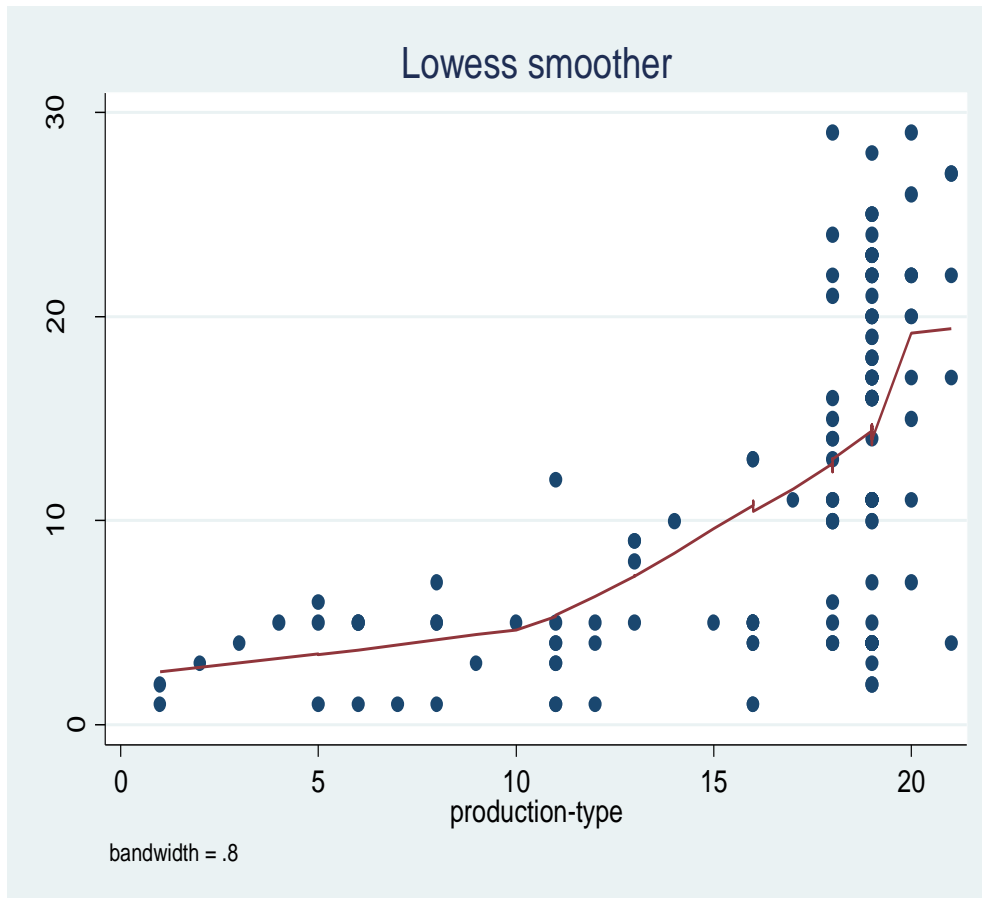


Figure 4.1: Shows an increase in bacterial load in terms of production type, with out-growers having a bacterial load of around 20 indicating an exponential increase in bacteria isolated from older fish as compared to fingerlings.

4.7. Exposure factors

The identified exposure factors from visual determination on the three farms under the study are shown in Table 4.8. The cage production system comprised the out-grower cage and the juvenile cage as its production types whilst the other types of production fell under the pond system.

Table 4.8: General Exposure Factors identified from the visual determination

Demographic factors	Biological factors	Environmental factors	Management factors
Hatchery	Hatchery	River	Hatchery
Sex reversal pond	Breeding stock	Fixation pond	Sex reversal pond
Breeding stock	Male pond	Sex reversal pond	Out-grower pond
Male pond	Out-grower pond	Empty pond	Fingerling pond
Out-grower pond	Andersonii pond	Draining pond	Rejects pod
Andersonii pond	Niloticus pond	Stabilization pond	Juvenile cage
Rejects pond	Machrochir pond	Out-grower pond	
Machrochir pond	Mixed pond fingerling pond	Out-grower cage	
Niloticus pond	Out-grower cage	Lake	
Fingerling pond	Juvenile cage	Hatchery outlet	
Out-grower cage			
Juvenile cage			

The significant factors fell under the pond system and it was more significant given the $\chi^2 = 82$, ($p < 0.0001$).

4.8. Identification of Exposure Factors

Four main aggregates were identified from the data collected: Demographical, Biological, Environmental and Management factors. When the analysis was undertaken controlling for production systems, demographical factors covered a wider area, followed by biological factors (Figure 4.2). Environmental factors were closely associated with biological factors whilst management factors overlapped across all factors albeit as an outlier (Figure 4.2).

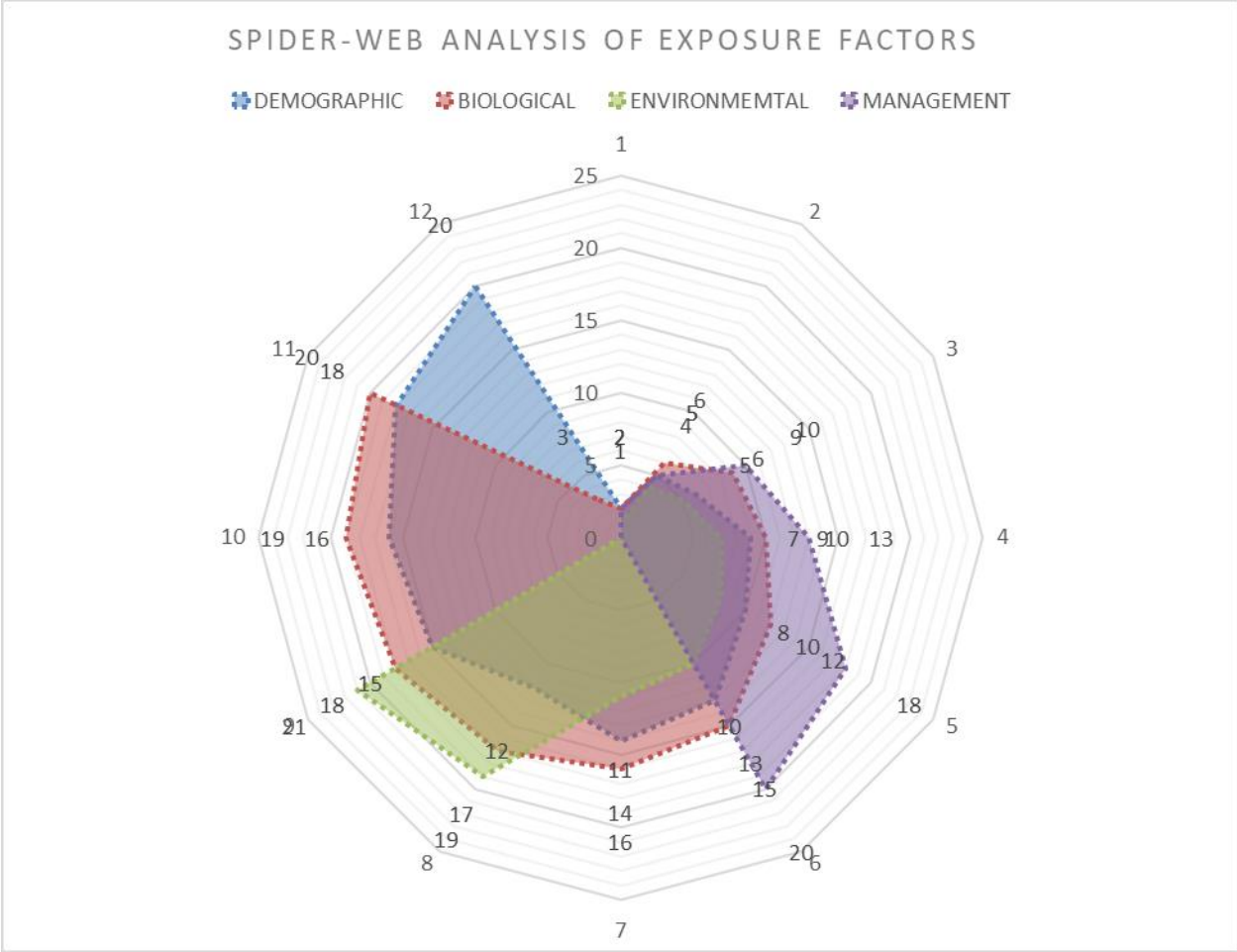


Figure 4.2: Shows how exposure factors overlap with each other. NOTE: demographical factors cover a wider area followed by biological, then environmental and last management factors.*: 1= river, 2= hatchery, 3= hatchery outlet, 4= fixation pond, 5= sex reversal pond, 6= broodstock, 7=empty pond, 8= draining pond, 9= male pond, 10= out grower pond, 11= stabilization pond, 12= *Andersonii* pond, 13= rejects pond, 14= direct breeding stock, 15= *Macrochir* pond, 16=*Niloticus* pond, 17= mixture pond, 18= fingerling pond, 19= out-grower cage, 20= juvenile cage, 21= lake

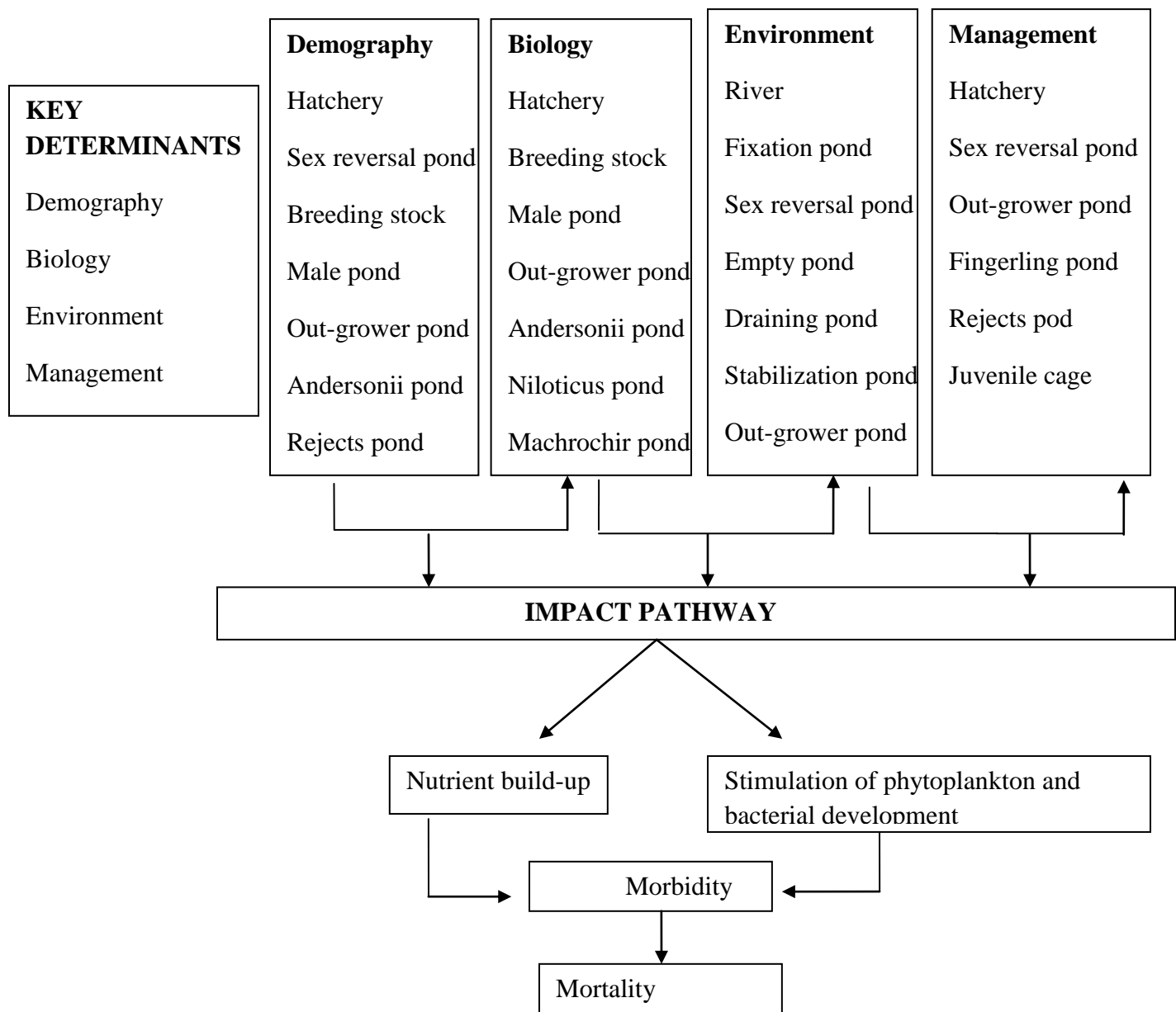


Figure 4.3: flow chart showing exposure pathway evaluation of environmental, biological, demographical and management factors inclusive of an impact pathway.

CHAPTER FIVE

DISCUSSION

Knowledge about prevalent etiological agents and those with zoonotic potential is necessary for the implementation of proper public health guidelines, management and control of disease in a susceptible population.

In this study, we identified the different genera infecting *Oreochromis* species especially *Oreochromis niloticus* and amongst these, those with zoonotic potential were also determined. Based on bacterial culture, morphology, Gram-staining characteristics and a series of biochemical evaluations and classification, a total of 27 different genera of bacteria were isolated. Culturing was done at 37 °C for the identification of these bacteria since zoonotic bacteria was of great importance in this study. This would mean that if zoonotic bacteria grow at this temperature then they would grow in humans, survive and cause disease. This included bacteria known to cause serious disease in fish and those of varying significance as fish pathogens. 15 bacteria genera in eels were reported (Joh et al.,2013) with 3 genera (*Aeromonas*, *Citrobacter* and *Pseudomonas spp*) common between the two studies. However, Newaj-Fryzul and others isolated 13 genera from Tilapia in Trinidad (Newaj-Fyzul et al., 2008), with 5 genera (*Pseudomonas*, *Staphylococcus*, *Enterobacter*, *Bacillus* and *Aeromonas spp.*) common between the two studies.

Among the bacteria identified, some of these bacteria are common pathogens known for causing heavy mortalities in fish worldwide. These include *Aeromonas spp.*, (*A. sobria* and *A.*

hydrophila) which have been isolated from several cases involving high virulent strains (Nielsen et al., 2001; Hossain et al., 2014; Zeng, 2020). *Aeromonas spp* was one of the most common isolated bacteria in this study at 13.2% of all isolates from the samples from the three farms sampled. Indeed, similar findings have been reported in Uganda (Wamala et al., 2018) and other parts of the world (Joh et al., 2013). Some researchers have also reported similar findings and this can be due to a fact that *Aeromonas spp.*, are ubiquitous in nature in the aquatic environment, the similarity of production systems and fish species involved in the different studies (Wamala et al., 2018).

The prevalence of the other pathogens from this study was;- *Acinetobacter* 2.1%, *Aequorivita* 1.4%, *Aerococcus* 1.4%, *Bacillus* 2.1%, *Bordetella* 2.1%, *Carnobacterium* 10.4%, *Citrobacter* 3.5%, *Clostridium* 2.1%, *Corynebacterium* 1.4%, *Dermatophilus* 1.4%, *Enterococcus* 2.1%, *Escherichia coli* 0.7%, *Flavobacterium* 4.2%, *Klebsiella* 6.9%, *Lactococcus* 2.1%, *Listeria* 0.7%, *Micrococcus* 6.9%, *Planococcus* 1.4%, *Proteus* 1.4%, *Pseudomonas* 6.3%, *Rhodococcus* 1.4%, *Shewanella* 1.4%, *Streptococcus* 0.7% and *Vagococcus* 0.7% (Buller, 2004). Therefore, based on this study, the pathogens most commonly present in these fish farms include *Klebsiella*, *Pseudomonas*, *Carnobacterium*, *Staphylococcus* and *Aeromonas*. The bacteria species isolated from the five sick fish included *Pseudomonas*, *Aeromonas*, *Staphylococcus* and *Lactococcus*. *Micrococcus*, *Flavobacterium*, *Aequorivita*, *Bacillus*, *E. coli*, *Proteus*, *Listeria*, *Clostridium*, *Acinetobacter*, *Rhodococcus*, *Lactococcus*, *Planococcus*, *Corynebacterium*, *Lactococcus/Streptococcus*, *Shewanella*, *Bordetella*, *Streptococcus*, *Aerococcus*, *Vagococcus*, *Dermatophilus* and *Citrobacter* species were generally low. This shows that they were not major contributors to the clinical disease encountered in this study in the observed sick fish.

Other studies have documented known pathogens including *Aeromonas spp*, *Staphylococcus spp*, *Lactococcus spp* and *Streptococcus spp* to be frequently isolated from diseased fish similar to what was observed in this study in the diseased fish. Among the disease symptoms observed were skin ulceration, abnormal swimming (swimming in circles), blindness (whitish appearance of the eyes) and exophthalmos. Other authors have reported similar findings with *Aeromonas spp* (Sreedharan et al., 2012), *Lactococcus garvieae* (Patrick and Woo, 2011; Roberts, 2012; Helmy and Atallah, 2015), *Streptococcus iniae* (Baiano, 2009; Pretto-Giordano and Scarpassa, 2015) and *Streptococcus agalactiae* (Jafar et al., 2008; Iregui et al., 2014). Theoretically, Streptococcosis affects all sizes of fish, though; bigger fish are more susceptible to the disease (Wendover et al., 2018).

Pseudomonas (Amutha and Kokila, 2014), *Flavobacterium* (Huicab-Pech et al., 2016) and *Streptococcus spp* (Anshary et al., 2014; Iregui et al., 2014; Pretto-Giordano and Scarpassa, 2015) have been documented as well in warm water regions similar to climatic conditions in Zambia. Bacteria from the family *Streptococcus* are among the opportunistic bacteria in nature that reside in the natural aquatic environment. Poor husbandry and excessive stocking densities have been associated with disease causation by this family of bacteria (Roberts, 2012).

The majority of the other bacteria isolated have been associated with a few cases of disease in fish in other countries with varying pathogenicity. Such cases include *Pseudomonas spp* (*P. aeruginosa*) (Thomas et al., 2014), *Citrobacter spp* (Svetlana et al., 2003), and *Klebsiella spp* (Hu et al., 2021). It was quite difficult to associate these bacteria with disease in this study since

they were isolated from apparently healthy fish. No information was readily available to us about the pathogenicity of *Planococcus spp*, *Shewanella spp*, *Dermatophilus spp*, and *Micrococcus spp* in fish. Even in this study, they were only isolated from apparently healthy fish. The pathogenic nature of many fish pathogenic bacteria though ubiquitous in the aquatic environment is dependent on stress in the fish host to cause disease (Walters and Plumb, 1980; Barton et al., 2002; Lio-Po and Lim, 2014). Intensification of fish farming which is a practice characterized by high stocking density, increased human interference and low water quality coupled with the current trend of environmental deterioration which is on the rise may make these rare bacteria more important fish pathogens in the near future (Wamala et al., 2018).

Various bacterial species have been involved in disease outbreaks in fish with the most widely implicated bacteria being *Streptococcus/Lactococcus* (Belém-Costa and Cyrino, 2006; Musa et al., 2009; Amal and Zamri-Saad, 2011; Abdelsalam et al., 2013; Ahmed., 2013; Haenen et al., 2013; Amutha and Kokila, 2014; Pretto-Giordano and Scarpassa, 2015) and *Aeromonas* (Austin, 2007; Noga, 2010; Roberts, 2012; Huicab-Pech et al., 2016) due to their opportunistic nature. These bacteria can survive in a dormant state in the environment and when there is environmental and/or host destabilization, they then invade host tissues. *Aeromonas* was one of the most isolated bacteria at 13.1% from all 3 farms.

Bacillus, *Carnobacterium* and *Rhodococcus spp* were among the bacteria isolated in fish and have been shown to have probiotic properties as lactic acid-producing bacteria (Takyi et al., 2012). *Citrobacter* is known to be a commensal in the aquatic environment and surrounding soils (Roberts, 2012; Takyi et al., 2012). *Klebsiella* and *Staphylococcus* are also opportunistic in

nature just like *Streptococcus* and may manifest mainly in heavily stressed fish as shown by the study with *Staphylococcus* being the most isolated bacteria at 18.1% across all farms (during sampling, the farms were in the harvesting period and fish might have been stressed due to excessive handling). These bacteria can cause public health risks, same with *Enterococci* species which are associated with human fecal pollution in water (Marcel et al., 2013). The *Flavobacteriaceae* family included the *Aequorivita* and *Flavobacteria* genera. *Flavobacteria* are mostly associated with living and dead phytoplankton within the natural aquatic environment (Bowman and Nichols, 2002). The *Corynebacteria* genera have bacterial species which have been associated with widespread disease conditions affecting the kidney in many fish species other than *Oreochromis niloticus* species (Buller, 2004; Patrick and Woo, 2011). Other recent bacterial species like the *Acinetobacter* species have been labeled as emerging fish pathogens in other fish species (Kościńska et al., 2014).

Ponds had a significantly higher prevalence of bacteria as compared to cages with a chi-square value of 170 ($p < 0.0001$). This can be associated with high stocking density of fish, reduced water quality, increased human activity and animal interaction, water re-circulation, stock movement and high organic matter especially associated with earthen ponds. Infection and transmission of bacteria in aquaculture are facilitated by the high density of fish. Surrounding soils can lead to direct contamination of pond waters by bacteria, hence, contributing to the observed high prevalence and diversity of bacteria in earthen ponds unlike in cages.

Some bacteria were not properly identified. These were characterized by inconsistent biochemical reactions at conventional biochemical tests and unacceptable profiles when run

against bacteria profiles as presented by Buller (2004). Two of the most prominent reasons that could have led to the failure of identifying these bacteria are; firstly, there could have been gaps in the diagnostic ability of the techniques used and secondly, these bacteria could represent new genera/species of bacteria infecting fish.

Other than causing disease in fish, some of these bacteria have been able to infect humans and cause disease. In this study, nine (9) of the 27 genera were identified as bacteria with zoonotic potential meaning they were capable of causing disease not only in fish but in humans too. These bacteria are *Bacillus spp*, *Clostridium spp*, *E.coli spp*, *Klebsiella spp*, *Lactococcus spp*, *Listeria spp*, *Staphylococcus spp* and *Streptococcus*.

In aquaculture, bacteria pathogens pose a great threat to public health. *Bacillus spp* is one such bacterium (FDA, 2020). *Bacillus* species are more prevalent in the environment because of their spore-forming ability just like *Clostridium* species. These bacteria can contaminate food. *Bacillus* species can be found in various foods and fish is no exception. Humans get infected by this pathogen from fish through consumption of raw fish contaminated with the pathogen in foods like sushi (Özdemir, 2019). Two forms of intoxication do occur in humans with *Bacillus spp.*, diarrhea-causing form which starts from 6 to 15 hrs after consumption and the other form causes vomiting and nausea (McDowell et al., 2020). Everyone is susceptible to both forms.

Clostridium species are also spore-forming organisms that are ubiquitous in nature and the spores can contaminate fish and its products. *Clostridium botulinum* and *Clostridium perfringens* are the ones of great concern to public health. *C. botulinum* poses a high health risk to consumers

resulting in botulinum disease which has a high incidence and high fatality rates (Cortés-Sánchez, 2021). Various symptoms are seen with this bacterium including lethargy, double vision, abdominal swelling, paralysis and finally death. With *C. perfringens*, the commonly observed symptoms are abdominal cramps and diarrhea (Sabry et al., 2016). Everyone is susceptible to both pathogens though *C. perfringens* is more common in children and old people (FDA, 2020).

Escherichia coli (*E. coli*) is reported as one of the most common cause of food poisoning in many countries in the world (Del Rio-Rodriguez et al., 1997) and most of them are non-pathogenic though certain strains of the bacterium are responsible for four types of illness: gastroenteritis or infantile diarrhea caused by enteropathogenic *E. coli* (EPEC) (Cardozo, 2013), traveler's diarrhea caused by enterotoxigenic *E. coli* (ETEC), bacillary dysentery caused by enteroinvasive *E. coli* (EIEC) and hemorrhagic colitis caused by enterohemorrhagic *E. coli* (EHEC). Varying symptoms are observed for the four different types of illness with the common ones being abdominal pain, diarrhea, vomiting, fever, electrolyte imbalance, dehydration and general body discomfort. EPEC is most common in infants though all types tend to severely affect the very young and elderly (FDA, 2020).

Listeriosis is a result of being infected with *Listeria monocytogenes* and the most susceptible groups are pregnant women, newborns and the immunocompromised. This bacterium produces mild-flu like symptoms in all individuals, however, in the susceptible groups, it can result in more severe symptoms like septicemia, meningitis, encephalitis, spontaneous abortion and stillbirth (FDA, 2020).

Humans get infected with these bacteria organisms through oral route through consumption of raw or undercooked fish, though, others like *Lactococcus*, *Streptococcus* and *Staphylococcus spp* can find their way into the human body via abraded, wounded skin or through injuries causing by fish fins or fish spikes during handling and processing procedures in certain food preparation

In conclusion, the consideration of food safety with the nutritional organoleptic and commercial characteristics constitutes the total quality of food and is very important to public health (De la Fuente and Corona, 2010). The importance and adoption of food safety cannot be stressed further with the relationship that exists between safety production technologies, food handling and consumer health.

In aquaculture, the need for the hazard analysis critical control points (HACCP) is a great tool to safeguard the public health aspect. HACCP is a preventive system of food safety controls. It is a system designed to identify the significant hazards (factors) associated with the products or operations and establish procedures to monitor that these hazards are controlled (Gall et al., 1995). The challenge, however in aquaculture is that HACCP is being implemented at a more reduced pace even though it has to be applied throughout the food chain from primary production to final consumption (Alberto, 2015).

In this study, four types of factors that could give rise to a hazard in food safety were identified as demographical, biological, environmental and management factors. These factors can occur at various stages of the food chain (farm to table) which involves the contamination of food by different causal agents which can be of biological origin (parasites, viruses, bacteria, fungi, or

prions) or chemical (heavy metals, natural toxins or organic compounds), risking the health of consumers (De la Fuente and Corona, 2010; Huertas-Caro et al., 2019). Some of the causes of the loss of food safety are inadequate or absent hygiene conditions and practices, high degree of handling and use of contaminated water or raw materials (De la Fuente and Corona, 2010; Rodríguez-Cavallini et al., 2010; Huertas-Caro et al., 2019).

This study showed various bacteria in the aquaculture set up from the three farms sampled. These included zoonotic and food spoilage bacteria. Knowledge about these bacteria helps formulate policy and regulations regarding the national aquaculture trade development strategy for public health and consumer safety.

As with many research activities, limitations were present. In our case, time and resources (financial) were among the limiting factors. A longitudinal study was going to be suitable instead of the cross-sectional study done. It would account for environmental changes (temperature, pH) and seasons that affect the microbiota in the water where fish live and the microbiota of fish. More time and financial resources are needed. Sampling and analysis biases were the two forms of biases encountered in this study. To lower sampling bias, three different farms were involved in the sampling process. Sampling was spread across the different production systems (cage and pond systems) and it was non-probabilistic, hence our analysis did not involve the use of the more statistical tools, thereby reducing the levels of biases in this study.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

In this study, 27 bacterial genera were isolated from the fish and water collected from the 3 fish farms. Out of the 27 bacterial genera, nine were identified as zoonotic with zoonotic potential and these included *Aeromonas*, *Bacillus*, *Clostridium*, *E. coli*, *Klebsiella*, *Lactococcus*, *Listeria*, *Staphylococcus* and *Streptococcus*. The exposure factors were categorized under demographical, biological, environmental and/or management factors. These were then processed individually before being combined into a final framework to inform the impact pathway using the spider web analysis..

6.2. RECOMMENDATIONS

The following are the recommendations based on the study findings:

1. There is a wide array of opportunistic bacterial genera isolated from the fish farms involved in the study. Management of these establishments must invest in acquiring knowledge on these potential pathogens and put up appropriate precautionary measures to maintain an optimal environment of less stress for the fish.
2. A study involving human settlements and animal husbandry practices as a contributing source of the isolated bacteria found in this study should be considered in the future.
3. In the future, a longitudinal study is more suitable to include an account for changes in the environment (seasonality).

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