ASSESSMENT OF BACTERIOLOGICAL QUALITY OF *RASTRINEOBOLA ARGENTEA* ALONG ITS VALUE CHAIN IN LAKE VICTORIA, MWANZA, TANZANIA

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ABSTRACT

A cross-sectional study design was used to assess bacteriological contamination in *Rastrineobola argentea* along its value chain in Lake Victoria, Mwanza. *Rastrineobola argentea* were collected from different points along the chain. Fresh, dried on raised racks and on ground samples of *R. argentea* were purposively collected from landing sites, drying areas and retail markets. Bacterial counts were performed and *Escherichia coli* and *Salmonella* spp were identified using bacteriological standard procedures. Antibiotic susceptibility testing was performed on Muller Hinton Agar. The findings showed a significant difference ($p<0.05$) in bacterial counts between ground and raised racks dried *R. argentea*. Results for *E. coli* quantification showed that fresh *R. argentea* had significant difference ($p<0.05$) in mean counts to those dried on raised racks and ground at processing and market levels. Overall prevalence of *Salmonella* spp along the chain was 15.8% (n=120). The prevalence of *Salmonella typhimurium* and unidentified *Salmonella* spp in fresh *R. argentea* were 20% and 5% (n=40) respectively. Prevalence of *S. typhimurium* in *R. argentea* dried on ground at processing and markets were 20% and 15% (n=20) respectively. *Salmonella* spp were not detected in *R. argentea* dried on racks. The overall prevalence of *E. coli* was 43.3% (n=120). Prevalence of 62.5% (n=40) was found in fresh *R. argentea* and 70% (n=20) in those dried on ground at market while none of *R. argentea* dried on racks were contaminated. *Salmonella* spp and *E. coli* were resistant to ampicillin, tetracycline and co-trimoxazole, however were sensitive to norfloxacin and ciprofloxacin. *Escherichia coli* showed sensitivity to chloramphenicol too. Therefore, sundried *R. argentea* using raised racks is recommended as it is more hygienic than drying on ground to provide quality products for consumptions. Antibiotics susceptibility revealed that *R. argentea* contains antibiotic resistant *Salmonella* spp and *E. coli* of public health implications.

DECLARATION

I, Zebedayo Baniga do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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Prof. R. H. Mdegela	Date
(Supervisor)	

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DEDICATION

This work is dedicated to my beloved parents Mr and Mrs Songelaeli Baniga Nkopi, my beloved family, brothers: Raphael Songelaeli Baniga and John Songelaeli Baniga with their families for their valuable assistance in completion of this work.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
a_w	Water activity
cfu/g	Colony forming unit per gram
DNA	Deoxyribonucleic acids
FAO	Food and Agriculture Organisation
GMP	Good Manufacturing Practices
HACCP	Hazards Analysis Critical Control Points
ISO	International Standards Organisation
Lab	Laboratory
Ltd	Limited
MOFEA	Ministry of Finance and Economic Affairs
MOLFD	Ministry of Livestock and Fisheries Development
MPN	Most Probable Number
mpn/g	Most probable number per gram
NFQCL	National Fish Quality Control Laboratory
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
Pvt	Private
rpm	revolutions per minute
s	seconds
SD	Standard Deviation
SE	Standard Error
spp	Species
SPSS	Statistical Package for Social Science

TASP II	Trade and Agriculture Support Programme Phase II
UK	United Kingdom
USD	United States Dollar
WHO	World Health Organisation
µg	microgram
µl	microlittre
µM	micromole
Σ	Summation
χ^2	Chi-Square

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Rastrineobola argentea (silver cyprinid/sardines) are among the most important commercial fish species found in Lake Victoria (LVFO, 2012). They are commonly named *dagaa* in Tanzania, which is the collective name for all species of sardines. In Lake Tanganyika, there are two different species which are *Limnothrissa miodon* and *Stolothrissa tanganicae*, while in Lake Nyasa are known as *Engraulicypris sardella* (Kirema-Mukasa, 2012). Additionally, sardines from marine water include *Sardinella neglecta*, *Carcharinus falciformis* and *Anchoviella commersonii* (MOLFD, 2014). The catch composition shows that *R. argentea* constitute 72.3% of the total landing fish species from Lake Victoria followed by *Lates niloticus* (20.1%), *Haplochromines* (5.4%), *Oreochromis niloticus* (1.7%), *Protopterus* (0.2%) and other species (0.1%) (MOLFD, 2014).

According to National Economics Survey in 2009, about 30% of consumed animal protein in Tanzania was from fish at 8.0 kg per capita (MOFEA, 2009). *Rastrineobola argentea* are veritable source of proteins for both human as well as animals (Sifuna *et al.,* 2008). The market of *R. argentea* is currently expanding especially at regional level. This is attributable to the high demand from consumers in eastern and southern Africa as well as the accessibility and affordability of sardines over other sources of animal protein (Kirema-Mukasa, 2012). The situation can also be explained by the increased awareness among consumers on nutritional value of *R. argentea* and limited availability of food in neighbouring countries particularly in Southern Sudan and Democratic Republic of Congo (DRC) (Kabahenda *et al.,* 2011).

The regional markets of *R. argentea* include countries like Democratic Republic of Congo (DRC), Zambia, Malawi, Sudan, Mozambique, Zimbabwe, South Africa and other countries around Lake Victoria such as Kenya, Uganda, Rwanda and Burundi (LVFO, 2012). The main marketing challenges in all these countries are the poor quality and uncertain safety of marketed *R. argentea* (Kabahenda and Hüsken, 2009). Therefore, there is a need to improve the quality and safety of *R. argentea* in order to increase local, regional and international markets, as well as safeguard consumers from potential health risks.

Contamination of *R. argentea* with *Salmonella* spp, *Escherichia coli* (*E. coli*) and *Vibrio cholerae* (*V. cholerae*) are among the public health challenge of concern (Onyuka *et al.,* 2011). These microorganisms are causative agents of diseases like salmonellosis and gastroenteritis in human (Chagas *et al.,* 2013). *Rastrineobola argentea* can be contaminated by these bacteria during drying, from handlers, bird's faeces and contact surfaces. Another source of bacterial contamination may be the fishing environment and contaminated water used for washing before drying (Rao *et al.,* 2014).

The lake may become contaminated with wastewater directed into the lake from industries, hospitals, pharmaceuticals industries, houses and agriculture (Bolarinwa *et al.,* 2011). Furthermore, wastewater may contain some antibiotics residues at low doses which may account for the emergence of antibiotic resistant bacteria in fish. Humans could become infected with these antibiotic resistant bacteria via consumption of contaminated fish or water (Collard *et al.,* 2007). Antibiotic resistance is an emerging issue of global concern with serious threats to public health (Levy, 2001; Okeke *et al.,* 2005). Previous studies demonstrated antibiotic resistance in water, fish and/or *R. argentea* from different water bodies. These include studies by Byamukama *et al.* (2005) in Uganda and Onyuka *et al.* (2011) in Kenya who reported contamination of *R. argentea*

with resistant bacteria to different antibacterial agents. Mishra *et al.* (2010); Bolarinwa *et al.* (2011) and Sudheesh *et al.* (2013) reported contamination of microbial hazards in fish from different water bodies which had multidrug resistant pathogens.

1.2 Problem Statement and Justification

1.2.1 Problem Statement

The processed *R. argentea* from Lake Victoria, Mwanza Tanzania has low quality and uncertain safety for human and animal consumption (Bille and Shemkai, 2006). There are many contributing factors that are responsible for poor quality. The major factors include enteric bacteria such as *Salmonella* spp*, E. coli* and *V. cholerae* contamination of fishing environment, unhygienic post-harvest handling and processing methods (Onyuka *et al.,* 2011). Additionally, the poor processing and handling practices during transportation, storage and the selling environment at the market places contribute to the poor quality of *R. argentea*. Finally, poor packaging can also play a role to the low quality of *R. argentea* produced (Kabahenda and Hüsken, 2009).

The poor quality and questionable safety of *R. argentea* has many implications for fishermen and communities that consume the fish. These include the spread of food borne diseases, economic constraints for the fishermen leading to reduced production due to limited market availability and increased post-harvest losses due to market rejection. Ultimately, the poor quality and safety greatly limit the profits of the fishermen as well as local and regional market access of what could be a valuable source of protein for human consumption.

1.2.2 Justification of the Study

The Lake Victoria is shared by three countries notably Tanzania (49%), Uganda (45%) and Kenya (6%) (Mhongole, 2009; MOLFD, 2013). Despite having studies on

bacteriological contamination in *R. argentea* from Uganda and Kenya, limited information exists in Tanzania. Moreover, *R. argentea* is a valuable source of protein for human and animal consumption provided that it is hygienically fished, processed, stored and distributed. These conditions would ensure high quality and safety of sardines (Kabahenda *et al.,* 2011; Sudheesh *et al.,* 2013). However, these conditions are not currently being met leading to poor quality of *R. argentea* produced.

1.3 Objectives

1.3.1 Main Objective

The main objective of this study was to assess the bacteriological quality of *R. argentea* along its value chain.

1.3.2 Specific Objectives

The specific objectives of the study were;

i. To assess the microbial loads in *R. argentea* along the value chain
ii. To determine the magnitude of contamination in *R. argentea* with *Salmonella* spp and *E. coli* along the value chain
iii. To establish antibiotic resistance patterns to isolated *Salmonella* spp and *E. coli* from *R. argentea* along the value chain

1.3.3 The Research Questions

The research questions of the study were;

i. What are the extents of microbial loads in *R. argentea* along the value chain?
ii. What is the magnitude of contamination of *R. argentea* with *Salmonella* spp and *E. coli* along the value chain?
iii. What are the antibiotic resistance patterns of isolated *Salmonella* spp and *E. coli* from *R. argentea* along the value chain?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview

Food quality and safety is threatened by bacterial contamination which poses public health risks (Jeyasanta *et al.,* 2012). This is a concern in the production of *R. argentea* for human and animal consumption. Unhygienic post-harvest handling and processing methods undermine the quality and safety of *R. argentea* for consumption (Akande and Diei-Ouadi, 2010). This is due to presence of physical and biological hazards like sand, grasses and bird's faeces along the value chain (Sifuna *et al.,* 2008; Onyango *et al.,* 2015). Therefore, a major challenge that Tanzania is facing in regional and international markets of *R. argentea* produced from Lake Victoria is the poor quality and questionable safety. Currently, most of *R. argentea* harvested from Lake Victoria do not meet the criteria set by fish and fishery product standards for quality assurance (Tanzania Standard 402, 1988). As a result, this reduces and limits the exports of *R. argentea* to regional and international markets.

Regional export data indicates an initial increase in exportation of *R. argentea* from 2005 to 2010 which was followed by a decline from 2011 to 2014 (Table 1). This was associated with economic factors and quality reasons among traders in the regional markets. The main reason for the decline in exportation was a decrease in demand in Kenya due to poor quality where *R. argentea* were widely used in animal feeds. This was caused by adulteration of the products by sand and snails (Ibengwe, 2010). Kenya began importing *R. argentea* from Uganda because the processed sardines were of higher quality for animal consumption compared to Tanzania (Mondoka, E.M. personal communication, 2015). The decline in Kenyan demand resulted in decreased exportations

of *R. argentea* from Tanzania and a drop in both production and income generation earned from the regional markets (Table 1).

Table 1: The local and export data and values in dollars of *Rastrineobola argentea* from Lake Victoria, Tanzania 2005 to 2014

Year	Regional Markets (Metric Tons)	Value in USD Regional markets	Local Markets (Metric Tons)
2005	1 277 887.24	935 419.40	No records
2006	1 371 385.10	683 628.60	No records
2007	4 694 315.60	3 962 348.60	37 116 693
2008	7 633 884.00	4 840 560.90	40 381 442
2009	7 650 662.50	2 664 819.10	36 173 108
2010	8 200 490.40	36 011 572.40	44 200 329
2011	6 229 818.37	4 279 462.30	44 187 594
2012	7 691 329.00	5 797 334.85	40 113 307
2013	6 261 246.00	5 310 924.89	38 252 955
2014	6 089 940.00	12 183 128.80	41 925 052
Average	5 710 095.821	7 666 919.984	40 293 810

Source: Tanzania Fisheries Development Division 2015

For the local markets (in country), the production of *R. argentea* was consistent showing that over 90% of the harvest were marketed within the country (LVFO, 2012). In this regard, there is a need of expanding markets especially in the region through improving the quality and ensuring the safety of processed *R. argentea*. The improvement of *R. argentea* will be enhanced by strengthening the production along the value chain through monitoring and implementation of Good Manufacturing Practices (GMP) and Hazards Analysis Critical Control Points (HACCP) in order to produce good quality and safe products. The good quality and safety of *R. argentea* will have local impacts by increasing the income of fishermen around Lake Victoria as well as contribution to economic growth for the country as a whole.

Economically, fisheries sector contributes 1.4% to the national Gross Domestic Production (GDP) per annum (MOLFD, 2014). Approximately 6.67 million USD per year is earned by the country from export of *R. argentea* to regional markets (Table 1). The lake fisheries also contribute to employment where over 4 million people are involved in fisheries activities (MOLFD, 2010; MOLFD, 2014). The majority of people involved in fisheries activities are fish traders (MOLFD, 2010). Among the people involved in fisheries activities 5.1% (202 654) are fishermen and fish farmers. Out of 202 654 people 9.5% (19 223) are fish farmers and 90.5% (183 431) are fishermen (MOLFD, 2014).

2.2 Fishing and Handling of *Rastrineobola argentea* Post Fishing

Usually *R. argentea* fishing takes place during the night when moon is not visible (Sharpe *et al.,* 2012). The fishing is normally done using a purse seine net with approximately 6 mm mesh size and open peddled canoes or motorised boats. However, ice isn't used to preserve the catch (Nath *et al.,* 2013). The rationale behind fishing at night when the moon is not visible is the predator-prey interaction between *R. argentea* and some aquatic insects. These insects are physiologically attracted to light emitted by paraffin lanterns. The attraction of insects to the light results in attraction of the *R. argentea* to their food. (Legros and Masette, 2010). This is the reason why during fishing of *R. argentea,* fishermen use the light from paraffin lanterns attached to the pole of boats (Sharpe *et al.,* 2012).

As reported by LVFO (2012), fishing of *R. argentea* is associated with lunar variation which can lead to variation in the duration between fishing and offloading at landing sites. This duration can be a short period of time or a long period of time. Ice isn't employed as a preservation method and the practice of making large piles of sardines

can lead to high bacteria counts and sometimes spoilage due to bacteria and lipid oxidation (Adams and Moss, 2008; Mhongole, 2009).

There are sources of bacterial contamination of *R. argentea* from Lake Victoria. The primary source is the contaminated water in which *R. argentea* are fished and the secondary sources of contamination are the poor post-harvest handling of fish (LVFO, 2012). Other sources of contamination include the unhygienic conditions of the fishing boats and the absence of ice for preservation immediately after fishing to halt bacterial multiplication and avoid post-harvest spoilage of *R. argentea* (Onyango *et al.,* 2015). Additionally, most contamination occurs during drying rather than in the market since sun drying of *R. argentea* is mostly done on the ground at landing sites along the beaches (Ibengwe, 2010). These also serve as potential sources of bacterial contamination during drying. This is attributed to the presence of birds (Fig. 1) and insects in the drying areas, as well as lack of latrines in some landing sites. However, the raised rack drying process of *R. argentea* tends to produce good quality and safe products for human consumption due to implementation of GMP resulting in less bacterial contamination (LVFO, 2012).

Figure 1: Great Egret (*Casmerodium albus*) and Crowned cranes birds found around the drying area of *Rastrineobola argentea* at one of the landing site

The consequence of drying *R. argentea* on the ground leads to products that are of poor quality and questionable in safety for human and animal consumption. Additionally, the drying method leads to high post-harvest losses especially during the rainy season (Akande and Diei-Ouadi, 2010). These post-harvest losses reduce the contribution of fisheries sector into the National economy. Hence, all these increase poverty levels of fishermen and the community involved in fisheries activities.

2.3 The Growth of Bacteria in Dried *Rastrineobola argentea*

The growth of bacteria in food substances depends on factors that support their growth. One of the essential factors includes availability of water activity. Water activity (a_w) is a term describing the availability of unbound water to food molecules which can support the growth of microorganism and is presented as a percentage (Bell and Kyriakides, 2009). The water activity is not the same as moisture content but they do relate to each other, where an increase in moisture content leads to an increase in water activity. Water activity differs between groups of organisms. For instance *Salmonella* spp and *E. coli* grow better at minimum of a_w of 0.94, other bacteria at 0.90 and moulds can survive at lowest a_w compared to bacteria (ICMSF, 1996; Relekar *et al.,* 2014). Although most bacteria cannot grow on dried *R. argentea* (food), those that survived the drying process can grow when there is increase of a_w (Bell and Kyriakides, 2009). Bacteria can remain alive on dried food for prolonged periods of time to weeks in presence of proteins derived from body fluids, but quickly resume to their activity upon rehydration (Beuchat *et al.,* 2011).

Fresh fish have a_w of 0.99 which supports the growth of bacteria while dried foods have a_w of 0.80 (Bell and Kyriakides, 2009). The bacteria which were present in fresh *R. argentea* can survive at drying process and grow again upon the increase of water activity

in products. This is due to improper storage conditions which may lead to increase of moisture contents on *R. argentea*. At the market, the exposure of *R. argentea* to moisture contents at storage and selling environment will result to raised a_w hence growth of bacteria (Petterson and Ranjitha, 2009). Therefore, bacterial stability in dried products during processing and storage depends on their moisture content (Samad *et al.,* 2009).

2.4 Impact of *Salmonella* species and *Escherichia coli* in Public Health

Pathogenic bacteria in fish and fishery products are classified into three groups based on the source of the pathogens (Lyhs, 2009). These include indigenous bacteria which belong to the natural microflora of the fish. They include *Clostridium botulinum*, non-pathogenic *Vibrio* spp and *Aeromonas hydrophila*. Non-indigenous enteric bacteria which are also present in fish due to faecal contamination. Some examples are *Salmonella* spp, *Shigella* spp and pathogenic and non-pathogenic *E. coli*. The third class includes bacteria which result from cross contamination during food processing or storage. These include *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium perfringens* (Lyhs, 2009).

2.4.1 *Salmonella* species

Salmonella spp are among the group of bacteria in the family *Enterobacteriaceae* commonly known as enteric bacteria which are Gram-negative and rod shaped that live in gastrointestinal tracts of warm-blooded animals (Romich, 2008). The genus *Salmonella* comprises about 2 579 serovars (Grimont and Weill, 2007). The serovars which are most frequently isolated in food borne illnesses in humans are *Salmonella enteritidis* and *Salmonella typhimurium* (Soumet *et al.,* 1999; Herikstad *et al.,* 2002; Raufu *et al.,* 2014). Globally, *S. enteritidis* is the most prevalent species (Amini *et al.,* 2010; Hassanein *et al.,* 2011) and is followed by *S. typhimurium*. Another species, *S.*

weltevreden, is confined to Asia (Acha and Szyfres, 2003). These *Salmonella* spp are responsible for salmonellosis which is a worldwide health problem in humans and animals. The disease is normally transmitted through eating contaminated food with faecal materials (David *et al.,* 2008). *Salmonella* infections are the second leading cause of bacterial food-borne illness in the United States. Approximately 95% of cases of human salmonellosis are associated with consumption of contaminated foods such as meat, poultry, eggs, milk, seafood and fresh produce (Acha and Szyfres, 2003; Raufu *et al.,* 2014). *Salmonella* spp are also responsible for typhoid in human in which an estimated 12-33 million cases of typhoid fever occurs globally each year and mortality rate of 10% to 30% reported in Asia and Africa respectively each year (Romich, 2008). Specific *Salmonella* spp which are responsible for typhoid in humans are *Salmonella typhi* and *Salmonella paratyphi* A and B (Acha and Szyfres, 2003; Romich, 2008).

Similarly, sources of *Salmonella* spp in fish and aquatic environment are attributed to human activities (Gürakan *et al.,* 2008). Water bodies carry animals, plants and human wastes from point and non-point sources and channel to the lake via rivers. This creates a favourable environment for bacterial growth (Olgunoğlu, 2012).

2.4.2 *Escherichia coli*

Escherichia coli are coliform bacteria, Gram negative and normal intestinal flora which ferment lactose (Romich, 2008). The presence of *E. coli* in water and other food matrices indicate their contamination with faecal materials of animals and/or human origin. Therefore, *E. coli* are used as indicators of polluted areas with faecal materials originating from warm blooded animals (Tallon *et al.,* 2005). Different categories of *E. coli* are present and include both pathogenic and non-pathogenic strains. The pathogenic *E. coli* strains includes *Enterotoxigenic* (ETEC), *Enteropathogenic* (EPEC),

Enterohaemorrhagic (EHEC) and *Enteroinvasive* (EIEC) which cause serious human diseases like urinary tract infections (UTI) (Alao and Chukwujioke, 2013). Their transmission is through the consumption of water and food that is contaminated with faecal materials (Doyle, 1990; Romich, 2008). Other strains of *E. coli* are verocytogenic like the *E. coli* 0157:H7 and diarrheagenic *E. coli* (DEC) which are major cause of diarrhoea in children and are of public health importance in developing countries (Moyo *et al.,* 2007).

2.4.3 Antibiotic Resistance Patterns

The emergence of antibiotic resistant bacteria is a serious global problem which has been classified by World Health Organisation (WHO) as an important aspect in public health (Collard *et al.,* 2007). Also World Organisation for Animal Health (OIE) and the Food and Agriculture Organisation (FAO) recognise the spread of multiple antimicrobial resistant pathogenic bacteria as a growing threat to human and animal health globally (OIE Manual, 2012). The spread of antimicrobial resistance in non -typhoid *Salmonella* spp isolates in humans is attributed to the use of antimicrobial agents in food animal production (Angulo *et al.,* 2000; Threlfall *et al.,* 2000). The antibiotic resistant bacteria can be transmitted to human through water and food when they are contaminated with these bacteria and cause major threat to public health (Okeke *et al.,* 2005).

Other studies have reported the presence of antibiotic resistant bacteria like *Salmonella* spp and *E. coli* in water, *R. argentea* and in other fish. Sifuna *et al.* (2008) reported the contamination of sundried *R. argentea* with *Salmonella* spp and *E. coli* from Lake Victoria, in Kenya and noticed that these bacteria were resistant to two or more antimicrobial agents. Similarly, Onyuka *et al.,* (2011) reported *R. argentea* contamination with *S. typhimurium* (49.6%), *E. coli* (46.6%) and *V. cholerae* 01 (2.8%). All isolates were resistant to more than two antibiotic agents.

2.4.4 Processing Methods of *Rastrineobola argentea*

There are different methods used for processing *R. argentea*, including salting, frying and sun drying. Sun drying is done on the ground and on raised racks mostly along the beaches (Kabahenda and Hüsken, 2009). Raised racks are special constructions built using sticks for fish drying purposes (Fig. 2). They are approximately 10 to 15 m long, 1.5 m wide and rise to 1 m high at the area of processing or drying (Oduor-Odote, 2010). The top of the racks are covered from one end to another by either nylon or used fishing nets. The raised racks improve efficiency of drying by facilitating the air circulation between and around sardines and provide good quality and safe *R. argentea* (Oduor-Odote, 2010).

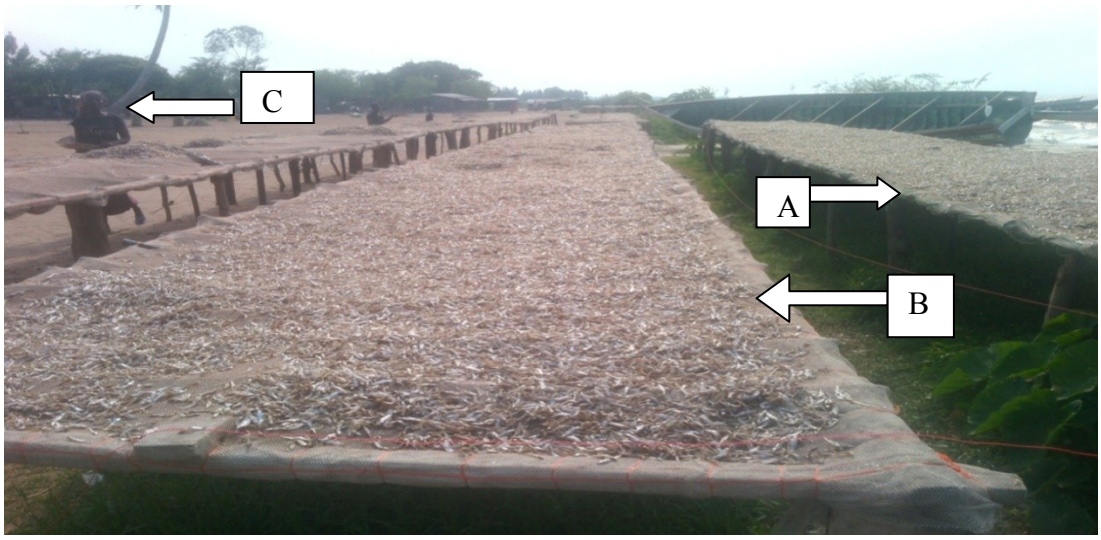

Figure 2: Sun-drying of *Rastrineobola argentea* on raised racks found at Igombe landing site

Legend: A: Raised racks B: *Rastrineobola argentea* on raised racks C: Processor taking care of *R. argentea* being dried as to prevent birds from access to the products.

Traditional methods used for processing of *R. argentea* include the predominant method of sun drying on the ground (Fig. 3) and, less commonly on raised racks. Other methods are smoking, frying and rarely freezing of fresh *R. argentea.* Nevertheless, the bacteriological quality of *R. argentea* sundried on raised racks and on ground is not yet

established. Therefore, this study will provide information on the quality and safety aspects of *R. argentea* dried on raised racks against those dried on ground.

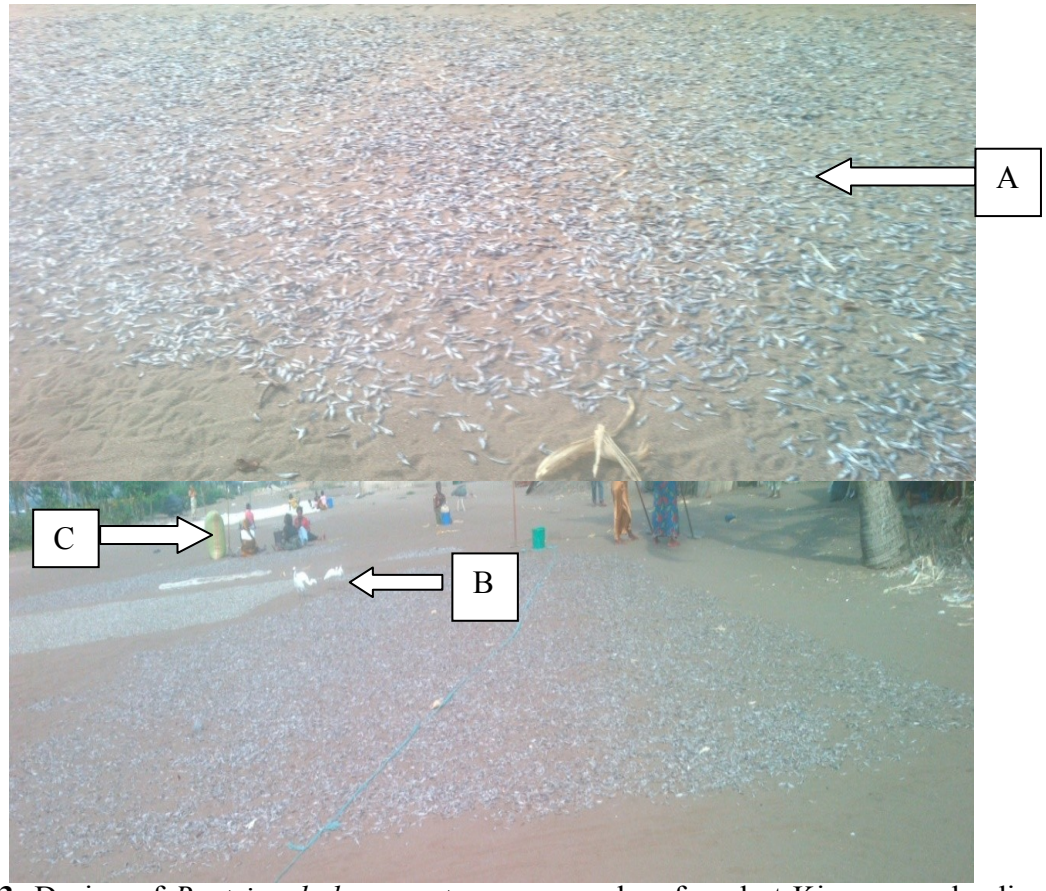

Figure 3: Drying of *Rastrineobola argentea* on ground as found at Kigangama landing site

Legend: A: *Rastrineobola argentea* drying on ground B: Birds having access to *R. argentea*

C: Packaging of sundried *R. argentea*

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area and Sampling Sites

The study was carried out in Mwanza basin along Lake Victoria-Tanzania. The selected sampling points were Bwiru beach/landing site, Igombe landing site, Kijiweni beach and Kigangama landing site. These sites were selected because they are located in Mwanza basin and have relatively high availability of *R. argentea* fished from Lake Victoria, Mwanza. Also, the areas were easily accessible. Other sampling sites were Kirumba market, Mwanza city market, Mkuyuni market and Nyakato market due to the large population of consumers of *R. argentea* in these markets. The samples were collected and transported to National Fish Quality Control Laboratory (NFQCL) for bacteriological analyses.

3.2 Study Design

This was a cross-sectional study design which was conducted on *R. argentea* collected from the study areas and submitted to the laboratory for bacteriological analysis. The sampling and sample analysis were done during October 2014 to April 2015.

3.3 Sample Size

The sample size was estimated based on the prevalence value reported from the previous studies conducted on *R. argentea*. The formula used was;

$$n = \frac{(Z_{\alpha})^2 \times P(1-P)}{d^2}$$ (Daniel, 1999).

Where, P is the prevalence value = 8.11% as reported by Sifuna *et al.* (2008); Z_{α} is the statistical level of confidence of 95% = 1.96; n is estimated number of samples; d^2 is the precision equals to 5% and 1 is a constant.

After the values given were substituted in equation; total samples calculated were equals to 114, where each sample was equivalent to 0.5 kg. During sampling and transportation, some samples could be damaged; therefore the estimated number of sample for this study was added with 5% of the calculated sample size (5.7 approximate 6) to cover the ones which could be damaged. Therefore the total number of samples for this study was 120.

Therefore, a total of 120 samples of *R. argentea* from different points along the value chain in Lake Victoria were collected and analysed, each sample was equivalent to 0.5 kg. Forty samples of fresh *R. argentea* were collected from the boats, 40 samples on processing/ sun drying where 20 dried on raised racks and 20 dried on ground and 40 samples from the market level where 20 were dried on raised racks and 20 on ground. All samples were aseptically collected and transported to the laboratory for analysis as described in subsection 3.6.

3.4 *Rastrineobola argentea* Value Chain along Lake Victoria

A value chain is a sequence of target oriented combinations of production factors that create a marketable product or service from its conception to the final consumption (Herr, 2007). The value chain for *R. argentea* starts from fishing and offloading the fish at landing site, processing (drying, frying, salting and freezing) marketing and to the consumers (Fig. 4). In every stage within the value chain there are essential stakeholders, where at fishing and offloading fishermen are key actors. Processing stage involves *R. argentea* processors that include driers, at the market sellers and finally to the animal and human consumers. In this study, the samples were collected from the identified sampling points (Fig. 4) along the value chain to assess the bacterial contamination in *R. argentea*.

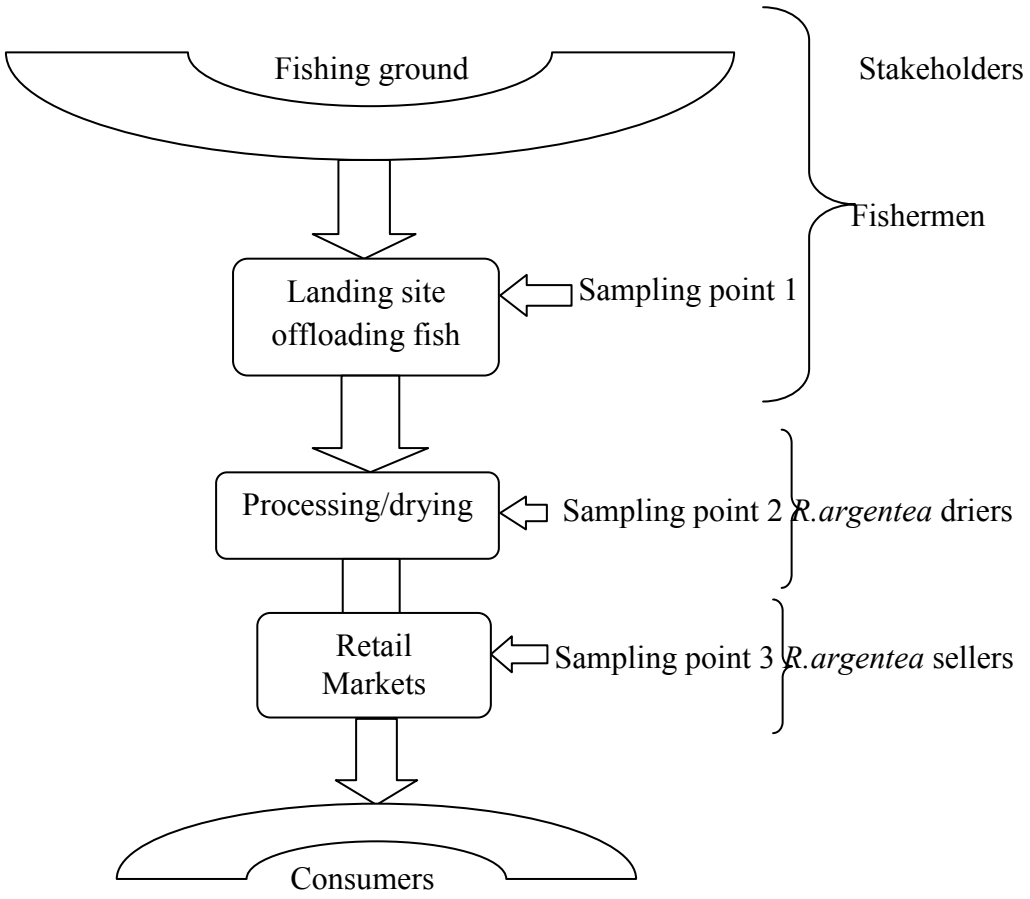

Figure 4: Sketch diagram to show the *Rastrineobola argentea* value chain

3.5 Sampling and Sample Storage

A total of 60 *R. argentea* dealers were involved in this study in which samples were collected from, 20 fishermen and 40 driers and sellers. The samples were collected purposively from these dealers in different points along the value chain from landing sites to retail markets. The sample handling techniques and transportation to NFQCL for analysis were done according to the nature of the samples. Fresh *R. argentea* were collected from the boats at landing sites using sterile plastic bags and preserved in cool box (previously disinfected using 70% spirit) packed with ice cubes. Two samples of fresh *R. argentea* were collected from selected fishermen during offloading at landing sites. The selection of fishermen was done randomly. Other samples were collected immediately after being sundried on raised racks and on ground where, two samples from each *R. argentea* drier were collected using sterile plastic bags and were carried into dry

and disinfected container. The samples from the markets involved *R. argentea* dried on raised racks and on the ground where, two samples were collected from each *R. argentea* seller at the market level. The fresh samples were stored by refrigeration and the dry samples were stored at room temperature until analysis. The samples were analysed on the same day of sampling.

3.6 Sample Preparation and Microbiological Analysis

3.6.1 Sample preparation

During sample preparation, 25 g of samples were aseptically weighed and mixed with prepared sterile 225 ml of Buffered Peptone Water (BPW) (Oxoid® Ltd., CM0509, Basingstoke, Hampshire, England) then transferred into sterile stomacher bags and homogenised using stomacher for 60 seconds. Then, after being homogenised analysis were done as per the in-house protocols documented for each parameter in test methods used for routine sample analysis at NFQCL.

3.6.2 Microbiological analysis

3.6.2.1 Total Plate Counts

The Total Plate Counts (TPC) were performed as per ISO 4833 (2003) for the enumeration of microorganisms-colony count technique at 30^{o}C. The mixture in subsection 3.6.1 was the first dilution and further serial dilutions were made up to 8th dilutions. Then 1 ml from the selected and prepared dilutions was drawn and inoculated into sterile petri dish. Then Plate Count Agar (PCA) (Oxoid® Ltd., CM0325, Basingstoke, Hampshire, England) autoclaved at 121˚C for 15 min and maintained in water bath at 45°C to 47^{o}C during the time of analysis was pour plated and was mixed well. Thereafter, PCA plates were left to solidify at room temperature for 10 to 15 min and incubated at temperature of 30°C for 72 hours. After incubation time, colonies were

counted using colony counter and computed as per ISO 7218 and reported in colony forming unit per gram (cfu/g). Appendix 1 shows the raw data of microbial counts obtained. The formula used for computing results for microbial loads was:

$$N = \frac{\Sigma C}{V\,(1.1 \times d)} \qquad \text{(ISO 7218, 2007)}$$

Where: N is the total number of colony forming unity;

ΣC is the summation of bacteria colonies in respective dilutions

V is the volume inoculated in the petri dishes

d is dilution factor

1.1 is a constant

3.6.2.2 *Salmonella* species

The detection of *Salmonella* was done as per ISO 6579 (2002). The stomacher bags contained 225 ml of BPW with 25 g of samples were incubated at 37°C for 24 hours for pre-enrichment. Then 0.1 ml of culture was transferred into test tubes containing 10 ml of Rappaport Vassiliadis Broth (RVs) (Oxoid® Ltd., CM0866, Basingstoke, Hampshire, England) sterilised at 115°C for 15 min and 10 ml Müller Kauffman Tetrathionate-Novobiocin broth (MKTTn) (Oxoid® Ltd., CM1048, Basingstoke, Hampshire, England) for enrichment then incubated at 42°C and 37°C respectively for 24 hours. From the two enriched test tubes, a loopful culture was streaked into petri dishes containing boiled Xylose Lysine Deoxycholate (XLD) (Oxoid® Ltd., CM0469, Basingstoke, Hampshire, England) and Bismuth Sulphate Agar (BSA) (Oxoid® Ltd., CM0201, Basingstoke, Hampshire, England) and incubated at 37°C for 24 hours. The suspected colonies of *Salmonella* spp in XLD were pink with or without black centres and in BSA produced the characteristic brown to black colour with metallic sheen. The suspected colonies were streaked onto Nutrient Agar (NA) (Oxoid® Ltd., CM0001, Basingstoke, Hampshire, England) sterilised at 121°C for 15 min as purification and incubated at 37°C for 24

hours. The colonies were confirmed biochemically using Triple Sugar Iron agar (TSI) (Oxoid® Ltd., CM0277, Basingstoke, Hampshire, England) (sterilised at 121˚C for 15 min) slant test tubes and serologically using slide agglutination with *Salmonella* O and H group of antiserum (Rapid Lab Ltd, UK).

About 3 to 4 of purified colonies of *Salmonella* were suspended in 5 ml of diluents and incubated at 37°C for 3 hours. One drop of antiserum was placed on one end of microscopic glass slide and another end was placed with 0.3 ml of sterile diluents (0.1% BPW) as control. Then bacteria suspensions of *Salmonella* from the initially prepared solution were placed onto the drops of antiserum and diluents placed earlier on the slide and then mixed thoroughly. The slide was shaken gently for at least one minute before reading and interpreting of the results. Agglutination observed on slide was a positive result for *Salmonella* spp. The positive control used was *Salmonella typhimurium* ATCC No 13311 and NCTC 74 (Public Health England, Mutton, UK) available from routine quality control of NFQCL whereas a drop of diluents was treated as a negative control.

Molecular identification of *Salmonella* species using Polymerase Chain Reaction (PCR) based assay

This study intended to identify *S. typhimurium* and *S. enteritidis* as bacterial contaminant in *R. argentea*. Diagnostic polymerase chain reaction was used for confirmation of *Salmonella* colonies previously identified by biochemical tests.

Deoxyribonucleic Acids (DNA) Extraction

Deoxyribonucleic acids (DNAs) were extracted as per the protocol according to Dashti *et al.* (2009). Briefly, *Salmonella* colonies were mixed with 200 µl of sterile distilled water in eppendorf tubes at room temperature and then the mixture were heated in water bath at

80°C for 15 min. Thereafter, the eppendorf tubes with their contents were centrifuged at 5 000 rpm (equivalent to 7 277 xg) for 20 min and the supernatant containing the DNA templates were transferred into another new eppendorf tube and stored at -20°C, whereas pellets were discarded. During transportation, eppendorf tubes containing DNA were packed into cool box containing ice cubes and transported to Sokoine University of Agriculture Molecular Biology Laboratory for the identification of *Salmonella* spp, *S. typhimurium* and *S. enteritidis* by PCR.

Identification of *Salmonella typhimurium* and *Salmonella enteritidis*

Two primer sets targeting the Sef167-Sef478 gene of *S. enteritidis* and the Fli15-Tym gene of *S. typhimurium* were used, together with primer set for identification of the ST11–ST15 gene specific for the genus *Salmonella* (Table 2) in a single reaction (Osman *et al.,* 2011; Moussa *et al.,* 2012).

Table 2: List of primers used in Polymerase Chain Reaction based assay for the identification of *Salmonella* species

Target sequence	Primers	Primer sequence	Products size base pair (bp)	Reference
Random sequence	ST11 F	GCCAACCATTGCTAAATTGGCGCA	429	
	ST15 R	GGTAGAAATTCCCAGCGGGTACTGG		Soumet *et al*.,1999
*Fli*C gene	Fli15 F	CGGTGTTGCCCAGGTTGGTAAT	559	
	Tym R	ACTCTTGCTGGCGGTGCGACTT		Moussa *et al.,* 2012
*Sef*A gene	sef167 F	AGGTTCAGGCAGCGGTTACT	312	
	sef478 R	GGGACATTTAGCGTTTCTTG		Moussa *et al.,* 2012

Legend: F is forward and R is Reverse

Deoxyribonucleic acids (DNA) amplification

Salmonella spp identification was carried out using conventional PCR amplification with TECHNE TC-4000 PCR machine (Bibby Scientific Ltd). The reaction was performed as multiplex in a final reaction volume of 25 µl containing 0.8 µM concentration of each primer, 2 x Dream Taq Green PCR Master Mix (Inqaba-Biotec, Nairobi, Kenya) and 5 µl

of template DNA. The master mix solution used for the detection contained the reagents as shown in Table 3.

Table 3: The master mix solutions with their volumes used in Polymerase Chain Reaction

Primers/PCR Reagents	Volume (µl)
F-ST11	0.2
R-ST15	0.2
F-Sef167	0.2
R-Sef478	0.2
F-Fli15	0.2
R-Tym	0.2
Dream Taq Green PRC Master Mix	12.5
Double Distilled Water	6.3
DNA Template	5.0
The final volume	25

Legend: F means Forward and R is Reverse; PCR Polymerase Chain Reaction; DNA means Deoxyribonucleic acids

Polymerase Chain Reaction cycling conditions were adjusted as follows: Initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 56°C for 1.5 min and extension at 72°C for 30s, with a final extension at 72°C for 10 min (Osman *et al.,* 2011; Moussa *et al.,* 2012).

Gel electrophoresis

By using micropipette, 10 µl of PCR products were loaded into electrophoresis of 1.2% agarose gel containing 0.1 µl ml^{-1} GelRed Nucleic Acid Stain dived in 1x TBE (Tris Borate EDTA) buffer. The separation on gel electrophoresis was conducted under 70 voltages for 1 hour along with 50 bp-2000 bp ladder (Bionexus, Inc) as molecular weight DNA marker. The gel was visualised under UV-trans illuminator and the photos for the results were taken for records. According to the primers used the expected bp size for *Salmonella* spp is 429 bp, *S. typhimurium* is 559 bp and *S. enteritidis* is 312 bp.

3.6.2.3 Enumeration of *Escherichia coli*

Enumerations of *E. coli* were performed as per ISO 7251: 2005 (MPN). From the mixture in the subsection 3.6.1, multiple tubes technique, a set of three test tubes with double strength of sterile Lauryl Tryptose Broth (LTB) (Oxoid® Ltd., CM045, Basingstoke, Hampshire, England) (sterilised at 121˚C for 15 min) for each sample were inoculated with 10 ml of inoculums. Further, a set of three test tubes per dilution contained sterile LTB single strength with Durham tubes inside were inoculated with 1 ml of inoculums for each and incubated at 37°C for 48 hours. Turbidity and gas formations on Durham tubes after incubation time showed the suspected positive and then were transferred by using a loop into the test tubes that contained 10 ml of sterilised EC Broth (Oxoid® Ltd., CM 0853, Basingstoke, Hampshire, England) at 121˚C for 15 min, and then incubated in a shaking water bath at 44.5°C for 24 hours. Positive test tubes with gas formation in Durham tubes were transferred using loop into prepared test tubes that contained 5 ml of sterile Tryptone broth (Oxoid® Ltd., CM0087, Basingstoke, Hampshire, England) then incubated at 44.5°C for 24 hours to 48 hours in shaking water bath. After the incubation, Indole test was performed on each test tubes using 0.3 to 0.5 ml of Kovac's indole reagent (HiMedia Laboratories Pvt. Ltd Mumbai R008, India). A red ring formation on test tubes was regarded as positive of *E. coli*. Positive control used was *Escherichia coli* ATCC 8739 (Oxoid Limited Remel Inc, UK). The results were reported in mpn/g. The raw data were recorded for the enumeration of *E. coli* as shown (Appendix 2). The PCR based assay was not used for the identification of the type of *E. coli* contaminated in *R. argentea*. However, the objective of *E. coli* analysis was to assess the possible contamination of *R. argentea* with faecal materials since are the good indicator bacteria for faecal contamination.

3.7 Antibiotic Sensitivity Test

The isolated *Salmonella* spp and *E. coli* were subjected to different antibiotics for sensitivity test as per standard disc diffusion method (Sensitivity Disk) on Muller-Hinton Agar (HiMedia Laboratories Pvt. Ltd Mumbai, India). The discs used for the study contained the following antibiotics with their concentration in microgram (µg); ampicillin (Ax) 10 µg, tetracycline (TE) 30 µg, co-trimoxazole (COT) 25 µg, streptomycin (S) 10 µg, kanamycin (K) 30 µg, gentamicin (GEN) 10 µg, ciprofloxacin (CIP) 30 µg, norfloxacin (Nx) 10 µg erythromycin (E) 15 µg and chloramphenicol (C) 30 µg (HiMedia Laboratories Pvt. Ltd Mumbai, India). These antibiotics were selected because are frequently used for the treatment of diseases caused by enteric bacteria in humans.

Additionally, the isolated bacteria were purified on NA and incubated at 37°C for 24 hours. Then colonies of *Salmonella* spp were inoculated into 5 ml of sterile nutrient broth and for *E. coli*, colonies were inoculated into 5 ml of sterile LTB media and incubated at 37°C for 18 to 24 hours to resuscitation the bacteria cells. The resuscitated bacterial cells were adjusted to a turbidity equivalent to a 0.5 McFarland standard. Sterile cotton wool swabs were dipped into recovered isolates in nutrient broth and LTB for *Salmonella* spp and *E. coli* respectively were swabbed onto Muller-Hinton Ager plates to produce a confluent lawn of bacterial growth. After the inoculum on the plates was dried, the antibiotic discs were aseptically placed using sterile forceps on the plates of Muller-Hinton Agar and incubated at 37°C for 24 hours. The results were recorded based on the inhibition zone size (Bauer *et al.,* 1966) and were interpreted as described in Clinical and Laboratory Standards Institute (2009). *Salmonella* spp and *E. coli* were classified as sensitive, intermediate or resistant according to the specifications stated in the disc manufacturers (HiMedia Laboratories Pvt. Ltd Mumbai, India).

3.8 Data Analysis

All data were entered and stored in Microsoft Office Excel 2007. The data were analysed using Statistical Package for Social Science (SPSS) software. The microbial counts obtained in cfu/g were converted into $\log_{10}$ forms and then analysed using descriptive statistics to obtain means and standard deviation. Then means were compared using ANOVA. Further Post Hoc analysis for comparison of means was done using Turkey Kramer. The proportions of *R. argentea* contaminated with *Salmonella* spp and *E. coli* were analysed using descriptive statistics. The proportions were compared using χ^2 test. For all analysis, the significant difference were judged at $P<0.05$. Moreover, descriptive statistics were also used to analyse the frequency distribution of antibiotics resistance in *Salmonella* spp and *E. coli* isolated in *R. argentea* along the value chain.

CHAPTER FOUR

4.0 RESULTS

A total of 120 samples were collected from four landing sites, four drying areas and four markets along the value chain in Lake Victoria, Mwanza Tanzania. Overall bacterial loads are summarised in Table 4. The highest counts were observed in fresh *R. argentea* and lowest in the ones dried on raised racks. The contamination with *Salmonella* spp and *E. coli* were significantly ($p<0.05$) higher in fresh *R. argentea* than in dried ones Table 7 and 8.

Table 4: Bacterial counts and range in *Rastrineobola argentea* analysed in different sampling points

Stage of production	Status/ Method of drying	Number of samples (n)	Mean ± SD (log cfu/g)	Range (log cfu/g)
Fresh	Fresh	40	6.67 ± 0.71	5.58-7.90
At processing level	Dried on raised racks	20	4.19 ± 0.58	3.15-4.86
	Dried on ground	20	6.36 ± 0.73	4.80-7.93
At market level	Dried on raised racks	20	4.96 ± 0.57	3.89-5.88
	Dried on ground	20	7.07 ± 0.60	5.74-7.91

Legend: n is the number of sample in each stage along the chain
SD is a Standard Deviation
Cfu/g is the colony forming unity per gram

4.1 Bacteria Counts on Fresh *Rastrineobola argentea* from the Boat at Landing Site

Bacterial loads in fresh *R. argentea* ranged from 6.0 to 7.2 $\log_{10}$ cfu/g (Fig.5). Kigangama landing site recorded the highest bacteria mean counts followed by Kijiweni and Bwiru landing sites, while Igombe landing site recorded the lowest counts. The difference in mean counts was significantly difference between groups ($p<0.05$). Comparison of means demonstrated the difference in mean count between Igombe and

Kijiweni together with Kigangama landing sites. Mean counts for Bwiru was significantly different to Kigangama landing sites (Fig. 5).

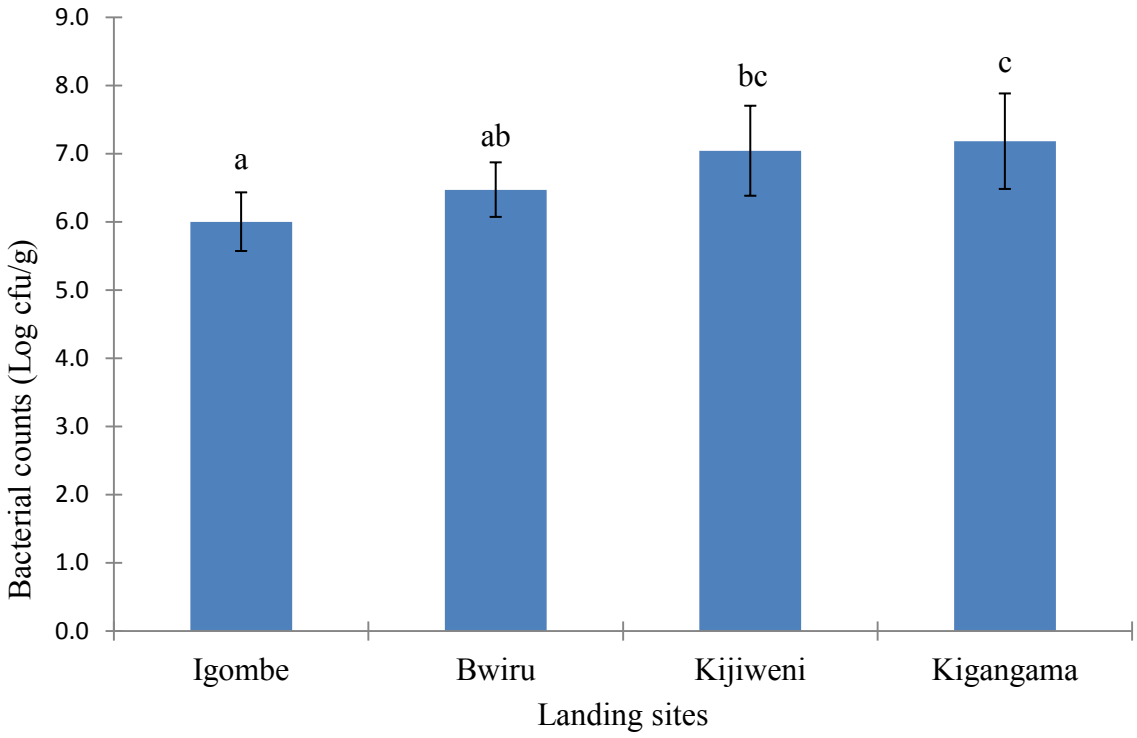

Figure 5: The bacteria counts on fresh *Rastrineobola argentea* from four landing sites

Legend: Letter 'a', 'b' and 'c' show the comparison of mean counts between the landing sites, where those that shared the same letter indicate no significant difference ($p>0.05$) and the ones indicate different letter shows the significant difference ($p<0.05$) n=40. Cfu/g is colony forming unit per gram

4.2 Bacteria Counts on Sun Drying of *Rastrineobola argentea* at Landing Site

The mean bacterial counts in *R. argentea* dried on raised racks at processing level ranged from 4.0 to 4.3 $\log_{10}$ cfu/g (Fig. 6). Kigangama and Bwiru landing sites had the highest bacteria mean counts among the *R. argentea* dried on raised racks, followed by Igombe landing site, while Kijiweni landing site had the lowest bacteria mean counts (Fig. 6). There was decrease in bacterial mean counts of *R. argentea* in all landing sites between fresh and those dried on raised racks. No significant difference ($p>0.05$) in bacteria mean

counts was observed between the four landing sites. Additionally, comparison of means showed no significant difference ($p>0.05$) observed in bacterial counts in landing sites.

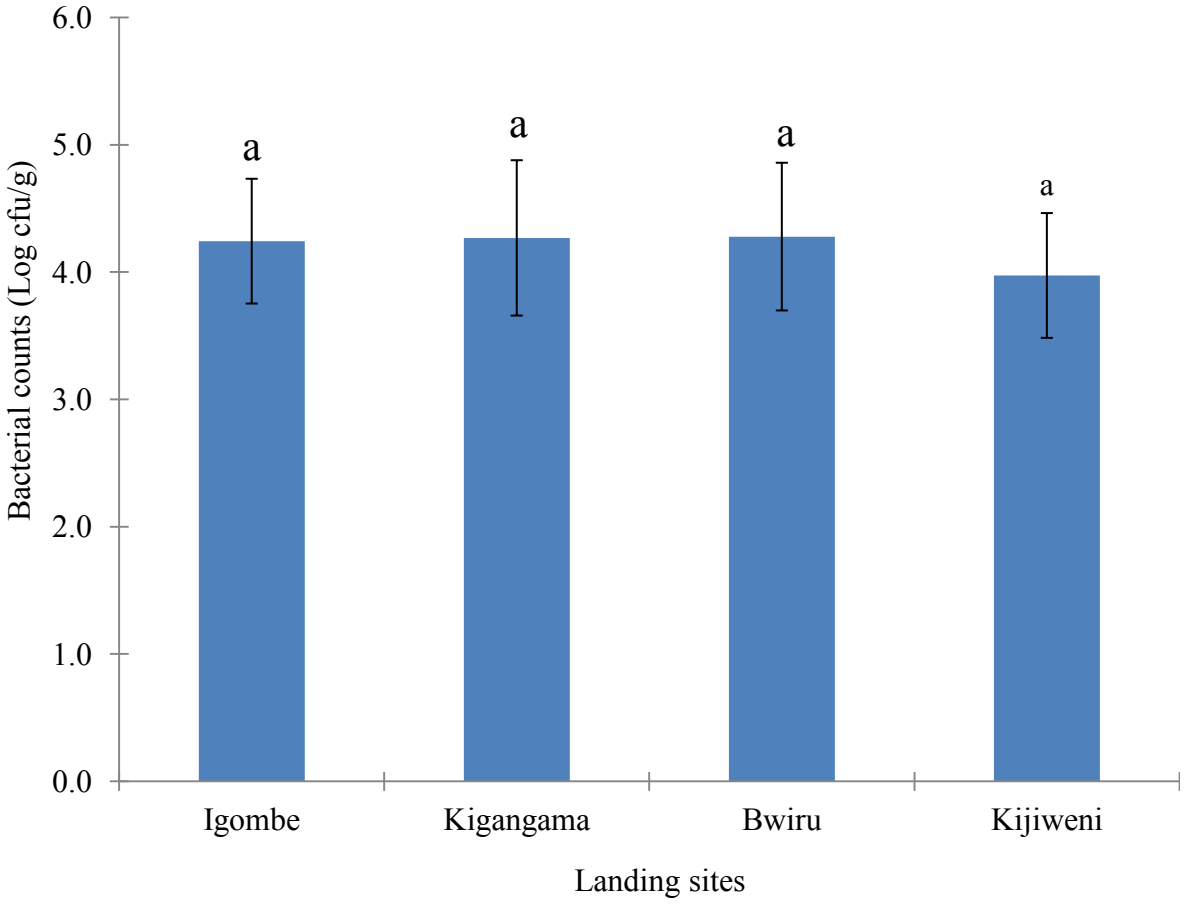

Figure 6: Bacterial mean counts of *Rastrineobola argentea* dried on raised racks at processing level

Legend: The same letter 'a' means no significant difference between the landing sites. Cfu/g is the colony forming unit per gram. (n=20).

Result of mean bacterial counts of *R. argentea* dried on ground showed that Igombe landing site recorded the highest bacteria mean counts of 6.7 $\log_{10}$ cfu/g followed by Kigangama landing site while Bwiru landing site had the lowest counts of 5.8 $\log_{10}$ cfu/g (Fig 7). No significant difference ($p>0.05$) was observed in mean counts between landing sites. The comparison of mean showed that Igombe landing site had significant difference ($p<0.05$) from Bwiru landing site. However, no significant difference ($p>0.05$) was observed between Igombe and Kigangama together with Kijiweni landing sites. As

well, no significant difference in mean counts was observed for Bwiru, Kigangama and Kijiweni landing sites.

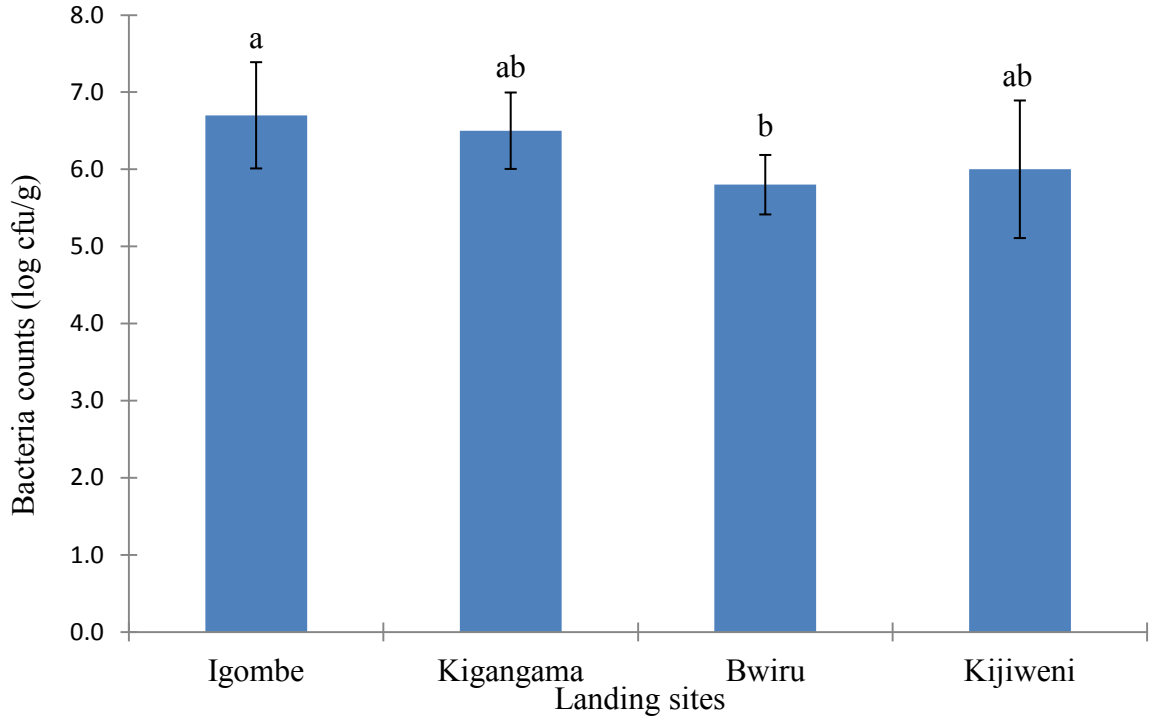

Figure 7: Bacterial mean counts in *Rastrineobola argentea* dried on ground at processing level

Legend: Letters 'a' and 'b' show the comparison of means between the landing sites, where those with the same or shared the letter indicate no significant difference and the one indicate different letter shows the significant difference.

Cfu/g: colony forming unit per gram; (n=20)

Additionally, the means of bacterial counts for fresh, dried on raised racks and on ground *R. argentea* were compared within each landing site. The result showed that at Igombe landing site, fresh *R. argentea* had no significant difference ($p>0.05$) to those dried on ground. However, there was significant difference ($p<0.05$) between *R. argentea* dried on raised racks and fresh together with those dried on ground at the same landing site (Fig. 8). At Kigangama landing site, fresh *R. argentea* had no significant difference ($p>0.05$) in mean counts to the ones dried on ground. This is contrary to *R. argentea* dried on raised racks, which had significant difference ($p<0.05$) in bacterial mean counts to fresh

and those dried on ground. For Bwiru landing site, the result showed fresh *R. argentea* had no significant difference ($p>0.05$) to those dried on ground. However, there was significant difference ($p<0.05$) in bacterial mean counts between *R. argentea* dried on raised racks and fresh together with those dried on ground at the same landing site. At Kijiweni landing site revealed significant difference ($p<0.05$) in mean counts between each products, that is fresh, dried on ground and on raised racks (Fig. 8).

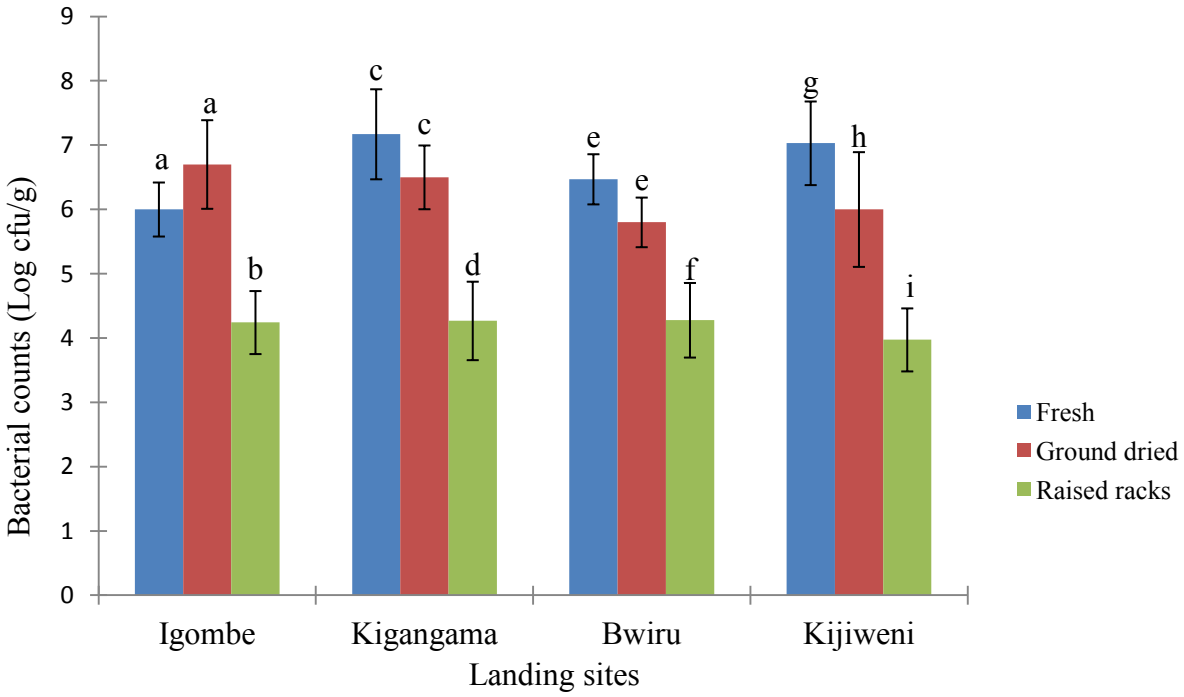

Figure 8: Comparison of bacteria mean counts of fresh, dried on raised racks and on ground *Rastrineobola argentea* within each landing site

Legend: Letters show the comparison of mean counts within the landing site, where those with the same letter indicate no significant difference ($p>0.05$) and the ones indicate different letter shows the significant difference ($p<0.05$) to others.

Cfu/g is the colony forming unity per gram

Result of bacterial counts in *R. argentea* dried on raised racks were compared to those dried on ground at processing level and showed that, *R. argentea* dried on raised racks had the lowest bacteria mean counts of 4.2 $\log_{10}$ cfu/g compared to those dried on ground

which had the highest bacteria mean counts of 6.4 $\log_{10}$ cfu/g. The difference was significant ($p<0.05$) in bacterial counts between the two drying methods.

4.3 Bacteria Counts in Dried *Rastrineobola argentea* at Market Level

At market level, for *R. argentea* dried on raised racks, the results of bacteria mean counts ranged from 4.7 to 5.2 $\log_{10}$ cfu/g (Fig. 9). Mkuyuni market recorded the highest bacteria mean counts followed by Kirumba and Mwanza city markets, while Nyakato market recorded the lowest bacteria mean counts. The mean counts were comparable between four markets ($p>0.05$). Additionally, the comparison of means observed no significant difference between the markets ($p>0.05$).

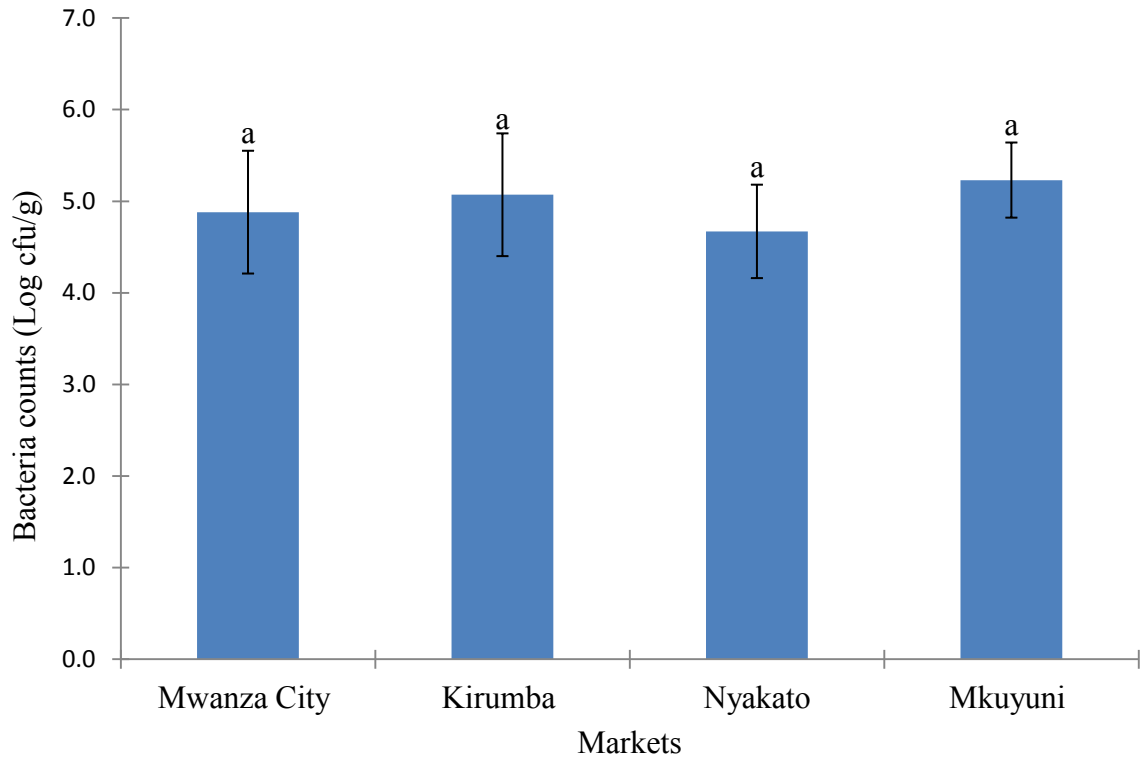

Figure 9: Bacterial mean counts of *Rastrineobola argentea* dried on racks at market level

Legend: The same letter 'a' means no significant difference ($p>0.05$) between the markets

Cfu/g is the colony forming unity per gram; (n=20)

The result presented in Fig. 10 show the comparison of bacteria mean counts of *R. argentea* which were dried on ground and sold in four different markets. The results ranged from 6.6 to 7.5 $\log_{10}$ cfu/g. Mwanza city market registered the highest bacteria mean counts, while Nyakato market had the lowest bacteria mean counts. The mean counts were comparable between the markets ($p>0.05$). However, the comparison of means observed significant difference ($p<0.05$) in counts between Mwanza city and Nyakato markets while noticed no significant difference ($p>0.05$) between Nyakato and Mkuyuni together with Kirumba markets.

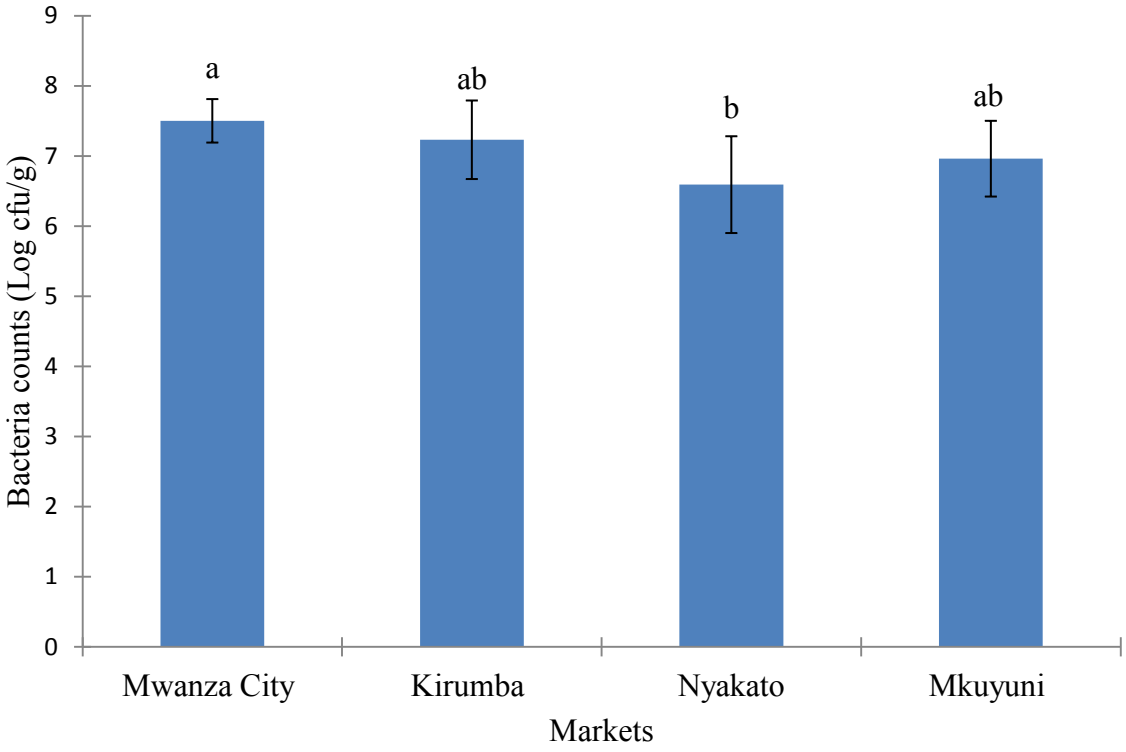

Figure 10: Bacterial mean counts of *Rastrineobola argentea* dried on ground at market level

Legend: Letters 'a' and 'b' show the comparison of mean counts between the market, where those with the same/shared letters indicate no significant difference ($p>0.05$) and the one indicate different letter shows the significant difference ($p<0.05$). Cfu/g means colony forming unity per gram; (n=20)

Furthermore, the bacteria mean counts in *R. argentea* dried on raised racks and on ground for each market were compared. The comparison shows that within each market there was significant difference ($p<0.05$) in bacteria mean counts between *R. argentea* dried on raised racks and on ground (Fig. 11).

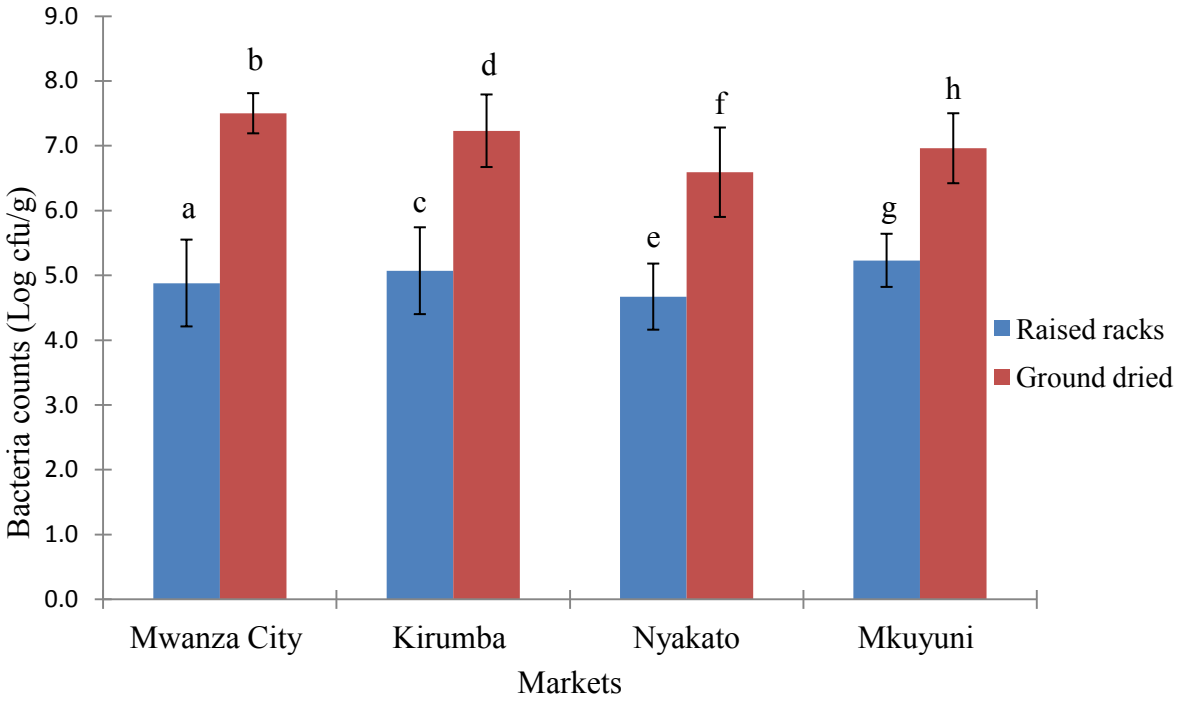

Figure 11: Bacteria counts in *Rastrineobola argentea* which were dried on raised racks and on ground from four different markets

Legend: Letters in Fig. 11 showed the comparison of mean counts within the market, where those indicating different letter are significant difference ($p<0.05$).
Cfu/g means colony forming unity per gram

At the market, the results of bacteria counts in *R. argentea* which were dried on raised racks and on ground methods were compared. The *R. argentea* dried on raised racks had the lowest bacteria mean counts of 5.0 $\log_{10}$ cfu/g, while those dried on ground had the highest bacteria mean counts of 7.1 $\log_{10}$ cfu/g. In fact, these results showed significant difference ($p<0.05$) in bacteria counts between the two drying methods.

4.4 Enumeration of *Escherichia coli* in *Rastrineobola argentea* as an Indicator of Faecal Pollution

Results of enumeration of *E. coli* in *R. argentea* along the value chain showed that the mean counts in fresh *R. argentea* were ranged from 1.9 to 3.1 $\log_{10}$ mpn/g. Kigangama landing site recorded the highest counts followed by Kijiweni and Igombe landing site, while Bwiru landing site had the lowest counts (Table 5). The results showed comparable mean counts between landing sites ($p>0.05$). The comparison of means observed significant difference ($p<0.05$) between Bwiru and Kigangama landing sites, while not significant difference ($p>0.05$) to Kijiweni and Igombe landing sites (Table 5).

Table 5: Enumerated *Escherichia coli* in fresh and dried *Rastrineobola argentea* at landing and processing sited of *Rastrineobola argentea* (log mpn/g)

Landing sites	Fresh (Mean ± SE)	Number of samples (n)	Dried on ground (Mean ± SE)	Number of samples (n)	Dried on raised racks (Mean ± SE)	Number of samples (n)
Kigangama	3.1 ± 0.62a	10	2.7 ± 0.59d	5	0.3 ± 00e	5
Kijiweni	2.4 ± 0.60ab	10	1.8 ± 0.63cd	5	0.3 ± 00e	5
Bwiru	1.9 ± 0.56b	10	0.5 ± 0.17c	5	0.3 ± 00e	5
Igombe	2.2 ± 0.53ab	10	1.9 ± 0.66cd	5	0.3 ± 00e	5

Legend: SE is a Standard Error; n is the number of samples in each landing site.

The letters in Table 5 show the comparison of mean counts between and within the landing site. Where, the same or shared letter indicates no significant difference ($p>0.05$) between landing sites, while different letter show significant difference ($p<0.05$) between and within the landing site for fresh, dried on ground and raised racks *R. argentea*.

Mpn/g is the most probable number per gram

At processing/drying level, *R. argentea* dried on raised racks had 0.3 $\log_{10}$ mpn/g mean count which implies that, all landing sites, that is Kigangama, Bwiru, Igombe and

Kijiweni landing sites were not contaminated by *E. coli*. However, for those dried on ground at processing level the results for *E. coli* mean count ranged from 0.5 to 2.7 $\log_{10}$ mpn/g with Kigangama landing site recording the highest mean counts followed by Igombe and Kijiweni landing sites. This was contrary to Bwiru landing site which show the lowest mean counts (Table 5). The results as well showed comparable counts ($p>0.05$) between drying areas. For the *R. argentea* dried on ground the comparison of means showed Bwiru landing site was significant difference ($p<0.05$) to Kigangama landing sites while was not significant difference ($p>0.05$) to Kijiweni and Igombe landing sites (Table 5). However, means comparison showed a significant difference ($p<0.05$) between *R. argentea* dried on raised racks and those dried on ground within each landing site.

Turning attention at market level, for *R. argentea* which were dried on raised racks, the result of *E. coli* mean counts ranged from 0.3 to 0.7 $\log_{10}$ mpn/g. Mwanza city market registered the highest *E. coli* mean counts followed by Nyakato and Kirumba markets while Mkuyuni had no *E. coli* counts. This counts were also comparable between markets ($p>0.05$). The comparison of means observed comparable counts between the markets ($p>0.05$). Finally, *R. argentea* dried on ground which were in the market had *E. coli* mean counts that ranged from 1.3 to 2.5 $\log_{10}$ mpn/g. Nyakato market recorded the highest counts of *E. coli* followed by Mkuyuni and Kirumba markets, while Mwanza city market had the lowest counts (Table 6). The mean counts had no significant difference between the markets ($p>0.05$). Means comparison also observed no significant difference ($p>0.05$) between and within the markets. Also, the comparison of means observed that *R. argentea* dried on ground had significant difference ($p<0.05$) to those dried on raised racks within each market. Overall, the mean bacteria counts of fresh *R. argentea* was significant difference ($p<0.05$) to the ones dried on raised racks and on ground at processing and the market level.

Table 6: Enumerated *Escherichia coli* in *Rastrineobola argentea* at market level (log mpn/g)

Markets	Dried on ground (Mean ± SE)	Number of samples (n)	Dried on raised racks (Mean ± SE)	Number of samples (n)
Mwanza city	1.3 ± 0.53a	5	0.7 ± 0.30b	5
Mkuyuni	2.1 ± 0.52a	5	0.3 ± 0. 00b	5
Nyakato	2.5 ± 0.60a	5	0.6 ± 0.20b	5
Kirumba	1.9 ± 0.40a	5	0.5 ± 0.23b	5

Legend: The letters in Table 6 show the comparison of mean counts between the markets. The same letter indicates no significant difference ($p>0.05$) between the markets, different letters show a significant difference ($p<0.05$) between *R. argentea* dried on racks and on ground within the market; Mpn/g means most probable number per gram; SE is Standard Error and 'n' the number of samples in each market.

4.5 The Prevalence of *Escherichia coli* in *Rastrineobola argentea* along its Value Chain

The overall prevalence of *E. coli* contamination in *R. argentea* along the value chain was 43.3% (n=120). The result indicate the prevalence of *E. coli* in fresh *R. argentea* was 62.5% (n=40) and those dried on ground at market was 70% (n=20). Additionally, the prevalence of *E. coli* contamination in *R. argentea* dried on raised racks at market level was 15% (n=20). However, there was no contamination with *E. coli* observed in *R. argentea* dried on raised racks at processing level (Table 7).

Table 7: The prevalence of *Escherichia coli* on *Rastrineobola argentea* along its value chain

Type of samples	Number of samples (n)	Proportions (%)
Fresh	40	62.5
Drying on raised racks	20	0
Drying on ground	20	50
Markets dried on racks	20	15
Markets dried on ground	20	70
Total samples	120	43.3

Legend: n is the number of samples in stages of processing

The results showed significant difference ($p<0.05$) in *E. coli* contamination between fresh *R. argentea* and those dried on ground at processing level, while there was no significant difference ($p>0.05$) between fresh *R. argentea* and those at the markets which were dried on ground. A significant difference ($p<0.05$) in prevalence of *E. coli* was observed between fresh *R. argentea* and those dried on raised racks at processing. Significant difference ($p<0.05$) was realised in contamination between fresh *R. argentea* and those dried on raised racks at the market. Additionally, there was significant difference ($p<0.05$) in *E. coli* prevalence between *R. argentea* dried on raised racks and those dried on ground at processing level. Similarly, significant difference ($p<0.05$) was observed between *R. argentea* dried on raised racks and those dried on ground at market level. There was also the significant difference ($p<0.05$) in prevalence of *E. coli* between *R. argentea* dried on raised racks at processing and at the market, while no significant difference ($p>0.05$) observed between *R. argentea* dried on ground at processing and at the market.

4.6 Prevalence of *Salmonella* species in *Rastrineobola argentea* along its Value Chain

Table 8 show the prevalence of *Salmonella* spp contamination in *R. argentea* along the value chain. Overall, the prevalence of *Salmonella* spp contamination in *R. argentea* was

15.8% (n=120). The prevalence of *Salmonella* spp in fresh *R. argentea* was (20%, n=40) and those dried on ground at processing/drying was 30% (n=20). The prevalence of *Salmonella* spp contamination in *R. argentea* at the market for the ones dried on the ground was (15%, n=20). However, none of *R. argentea* dried on raised racks at processing and the market level were contaminated with *Salmonella* spp. The identified *Salmonella* spp which were contaminated in *R. argentea* are shown in (Table 8).

Table 8: Prevalence of *Salmonella* species in *Rastrineobola argentea* along its value chain

Type of samples	Number of samples (n)	Unidentified *Salmonella* spp (%)	*S. typhimurium* (%)	*S. enteritidis* (%)
Fresh	40	5	20	0
Dried on raised racks	20	0	0	0
Dried on ground	20	10	20	0
Markets dried on racks	20	0	0	0
Markets dried on ground	20	0	15	0
Total	120	3.33	12.5	0

Legend: n is the number of samples in each stage along the chain

% is the prevalence of *Salmonella* spp contamination in each stage along the chain

There was significant difference ($p<0.05$) in *Salmonella* contamination between fresh *R. argentea* and those dried on raised racks at processing and market level, while no significant difference ($p>0.05$) was observed between fresh *R. argentea* and those dried on ground at processing and at the market. In addition, there was significant difference ($p<0.05$) in prevalence of *Salmonella* spp contamination between *R. argentea* dried on raised racks and those dried on ground at processing. Also, there was significant difference ($p<0.05$) between *R. argentea* dried on raised racks and those dried on ground at market level.

4.7 Confirmation of *Salmonella* species by Polymerase Chain Reaction

The analysis of *Salmonella* spp in this study was confirmed using biochemical, serological and molecular technique by PCR using specific primers (Table 2). The results showed that a total of thirty two samples out of 120 were biochemically confirmed positive as *Salmonella* spp, out of these 21 were serologically confirmed as positives and 19 out them were confirmed as positive by using PCR technique. The PCR was used also to identify the contamination of *R. argentea* with *S. typhimurium* and *S. enteritidis*. *Salmonella typhimurium* were detected at around 559 bp and *Salmonella* spp at around 429 bp as shown in Fig 12. However, no *S. enteritidis* was detected.

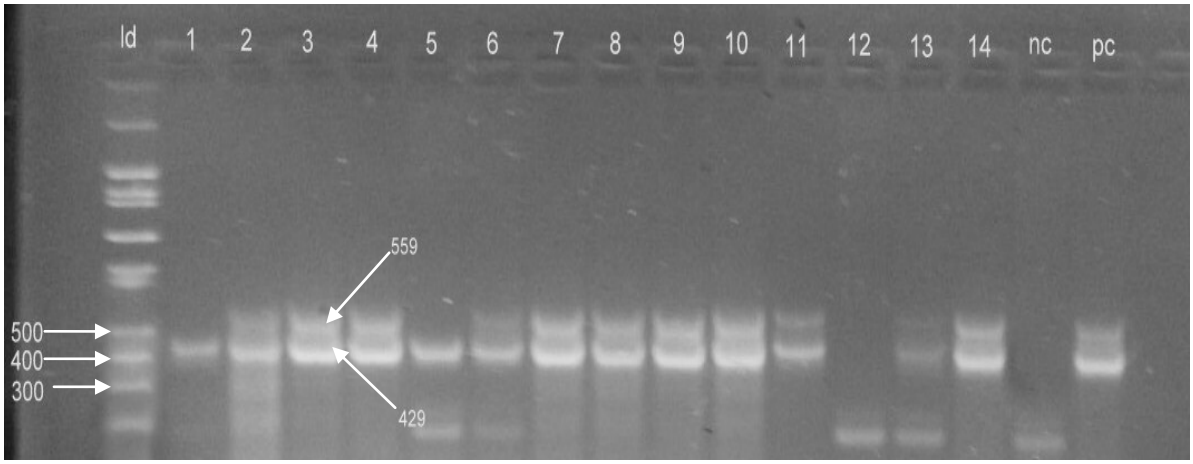

Figure 12: *Salmonella* species in gel electrophoresis

Legend: ld is a ladder DNA marker, lanes 1, 5 and 13 show the positives for unidentified *Salmonella* spp. Lanes 2, 3, 4, 6, 7, 8, 9, 10, 11 and 14 show the positives results for *S. typhimurium*. Lanes 15 and 16 are negative (NC) and positive (PC) controls respectively. Positive control used was *S. typhimurium* ATCC 13311 and NCTC 74.

4.8 Antibiotic Susceptibility of *Salmonella* species and *Escherichia coli* isolated from *Rastrineobola argentea*

The antibiotic susceptibility results show that, *Salmonella* spp were resistant to ampicillin, tetraclycline and co-trimoxazole also showed intermediate resistant to some antibiotics. However, all *Salmonella* spp were sensitive to ciprofloxacin and norfloxacin (Table 9).

Table 9: Antibiotic resistant patterns for *Salmonella* species (N = 19)

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
Ampicillin	0 (0)	2 (10.5)	17 (89.5)
Tetracycline	0 (0)	2 (10.5)	17 (89.5)
Co-trimoxazole	0 (0)	0 (0)	19 (100)
Streptomycin	0 (0)	14 (73.7)	5 (26.3)
Kanamycin	0 (0)	15 (78.9)	4 (21.1)
Gentamicin	0 (0)	14 (73.7)	5 (26.3)
Erythromycin	0 (0)	16 (84.2)	3 (15.8)
Chloramphenicol	0 (0)	17 (89.5)	2 (10.5)
Norfloxacin	19 (100)	0 (0)	0 (0)
Ciprofloxacin	19 (100)	0 (0)	0 (0)

Legend: N is the number of *Salmonella* spp confirmed positives

% is the percentage of susceptibility, intermediate and resistance of *Salmonella* spp to specific antibiotic.

Antibiotics susceptibility results show that, *E. coli* were resistant to ampicillin, tetracycline and co-trimoxazole and show intermediate resistant to some antibiotics. However, all *E. coli* isolates were sensitive to chloramphenicol, norfloxacin and ciprofloxacin (Table 10).

Table 10: Antibiotics resistant patterns for *Escherichia coli* (N = 52)

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
Ampicillin	0 (0)	8 (16)	44 (84)
Tetracycline	0 (0)	10 (20)	42 (80)
Co-trimoxazole	0 (0)	6 (12)	46 (88)
Streptomycin	0 (0)	32 (62)	20 (38)
Kanamycin	0 (0)	34((66)	18 (34)
Gentamicin	0 (0)	30 (58)	22 (42)
Erythromycin	0 (0)	41 (79)	11 (21)
Chloramphenicol	52 (100)	0 (0)	0 (0)
Norfloxacin	52 (100)	0 (0)	0 (0)
Ciprofloxacin	52 (100)	0 (0)	0 (0)

Legend: N is the number of *E. coli* confirmed positives in the study.

% is the percentage of susceptibility, intermediate and resistance of *E. coli* to specific antibiotic.

CHAPTER FIVE

5.0 DISCUSSION

This study was carried out to assess the bacteriological quality of *R. argentea* along the value chain in Lake Victoria Mwanza, Tanzania. Specifically, the study aimed to assess the microbial loads, the magnitudes of contamination of *R. argentea* with *Salmonella* spp and *E. coli* along the value chain. The isolated *Salmonella* spp and *E. coli* were subjected to antibacterial susceptibility testing. Overall results showed high microbial loads, high contamination with *Salmonella* spp and *E. coli* on fresh and *R. argentea* dried on ground compared with those dried on raised racks. The isolates showed resistance to more than two antibiotics.

The results of mean bacterial counts in fresh *R. argentea* was 6.7 $\log_{10}$ cfu/g, which was beyond the limit of 5 $\log_{10}$ cfu/g set by Tanzania Standards (TZS 402, 1988) and East African Standards (EAS 826, 2014) for fish and fishery products specifications. The reasons for high bacteria counts in fresh *R. argentea* was probably due to the fact that fishermen do not use ice for preservation of sardines immediately after fishing to halt bacterial growth. Additionally, the high bacteria counts in fresh *R. argentea* was due to the human activities associated with the landing sites like agriculture activities, disposing untreated households and industrial wastes which contaminate water in the Lake Victoria via water runoff. The results are in agreement with those reported by Adams and Moss, (2008) and Mishra *et al.* (2010) that freshly caught fish had high bacteria counts. Also, improper cleaning of boats used to carry *R. argentea* could have contributed to high bacteria counts through cross contamination of the fish with bacteria in boats. Overall between four sampling sites of fresh *R. argentea*, Kigangama and Kijiweni landing sites had high bacteria counts compared to Bwiru and Igombe landing sites. These differences in mean counts were attributable to human activities carried out around the landing sites

leading to contamination of the lake with wastes generated. The landing sites with high bacterial mean counts were associated with high human activities and produce wastes which channelled into the lake as a result cross contaminate the *R. argentea* at landing site. This was contrary to landing sites which had low bacterial mean counts.

At the processing level, the drying method contributed greatly to the bacteria counts of the sundried *R. argentea*. The *R. argentea* dried on raised racks had the mean bacterial counts of 4.2 $\log_{10}$ cfu/g, which were within acceptable limit based on standards TZS 402, (1988) and EAS 826, (2014) for sundried *R. argentea* specifications. The reason was due to an improved processing method that limits cross contamination of *R. argentea* with bacteria from the drying environment and birds were prohibited from accessing the sardines being dried. The prevention of birds from accessing the *R. argentea* being dried on raised racks was not done using special protection, but the processors were attentive to bird access to their products. Their reason explained was not because birds can contaminate *R. argentea* with bacteria, but mainly with physical hazards like sand, feathers and bird's faeces which reduce the value in the markets. Since the *R. argentea* dried on raised racks are mainly for export in regional markets, processors pay more attention during drying in order to ensure good products for their customers. This could explain why processors who dry using raised racks do wash *R. argentea* before drying to remove debris contaminated during fishing and offloading at landing site. However, the *R. argentea* dried on ground had the mean bacteria counts of 6.4 $\log_{10}$ cfu/g, which was beyond the acceptable limit set by TZS 402 (1988) and EAS 826 (2014) standards for fish and fishery products specifications. This was attributed to the fact that the *R. argentea* dried on the ground were subjected to cross contamination with bacteria from drying environment since they were dried on dirty areas. The drying areas were also prone to birds, which were allowed to roam around drying areas and had access to the *R.*

argentea being dried. The birds might contribute to high bacteria counts to the sun dried *R. argentea*. This result is consistent to the one reported by Onyango *et al.* (2015) of the presence of high bacteria counts beyond the acceptable limit in sundried *R. argentea*. Between drying areas where samples were collected, the *R. argentea* dried on ground at Igombe landing site had significantly high bacterial mean counts compared to Bwiru landing site though was not significant different to Kigangama and Kijiweni landing sites. The difference could be due to an exposure of the drying areas to cross contamination of *R. argentea* with the wastes from animals and human since the particular landing sites are used for both fish and *R. argentea* landings from the lake, as a result the landing site become populated with people involved in fishing activities around the landing site.

At the market level, the *R. argentea* that were dried on raised racks had mean bacteria counts of 5.0 $\log_{10}$ cfu/g that met the acceptable criteria set by standards for fish and fishery products specifications. However, there was significant difference ($p<0.05$) in bacteria counts in *R. argentea* immediately following the raised racks drying process and those that were dried by that method, being sold in the market. The *R. argentea* dried on ground sold in the markets had mean bacteria counts of 7.1 $\log_{10}$ cfu/g which was beyond acceptable limit set by standards. The result showed a significant difference ($p<0.05$) in mean counts between immediately after ground dried *R. argentea* and the ones dried by the same method sold in the markets. The increase in bacteria mean counts in *R. argentea* immediately following racks and ground drying methods and the ones sold in the markets being dried with both methods might be due to the packaging, transportation and storage conditions that allowed the increase in moisture contents resulting in increased water activity in *R. argentea* which favour the growth of bacteria. The result is in agreement with that reported by Sudheesh *et al.* (2013) of the presence of high bacteria counts in

dried fish found in the retail markets. The high bacteria counts indicate the poor condition of the market like the presence of waste disposal near the market that may increase insect proliferation on the products leading to high bacteria counts in *R. argentea*. Among four markets, Kirumba, Mwanza city and Mkuyuni markets had high bacteria mean counts compared to Nyakato market. The reason for high counts could be due to accumulation of waste generated from the market where, the rate of wastes generation in the market become high compared to the rate of wastes removal. This situation provides ample time for more insects proliferations on the products from the wastes leading to cross contaminate the *R. argentea* being sold in the market.

The overall along the value chain, the results for bacteria counts showed that, fresh *R. argentea* had high counts compared to the counts at processing level. This implies that, sun drying process has an impact of reducing the bacteria being contaminated in products to acceptable limit for the ones dried on raised racks in this study. However, for *R. argentea* which were dried on ground though sun drying process reduce the bacteria counts compared to fresh, but the products did not comply with quality standards as so reported by Onyango *et al.* (2015) that *R. argentea* dried by traditional sun drying method on ground had microbial counts which were beyond the acceptable limit set by standard. At the market level, the results showed that, for the *R. argentea* dried with both methods and sold in the markets, there was an increase in bacterial mean counts compared to the ones at processing level. The reason behind could be poor handling practices of *R. argentea* after being dried. These include handling at packaging, truck used for transportation, storage and the selling environment at the markets which favour the growth of bacteria in the products. Generally, the sun drying of *R. argentea* on ground should be avoided because it poses a public health risk for human and animals that are key consumers of sardines.

The analysis of *Salmonella* spp was confirmed biochemically, serologically and by molecular technique using PCR. The results showed that out of 32 biochemically and 21 serologically confirmed *Salmonella* spp, only 19 were confirmed and identified positive by using PCR. This implies that PCR is the best confirmation method for *Salmonella* spp over biochemical followed by serological methods. In addition, PCR gave the identification of the type of *Salmonella* spp contamination in *R. argentea,* which are of public health importance while biochemical method did not.

Enteric bacteria are one of the major causes of gastroenteritis in humans (Ezung and Abraham, 2014). The results reported show *R. argentea* were contaminated with *E. coli, S. typhimurium* and unidentified *Salmonella* spp. According to standards of fish and fishery products specification, *Salmonella* spp and *E. coli* are not supposed to be present in fish and fishery products intended for animals and humans consumption. Fresh *R. argentea* were the most contaminated with *S. typhimurium*, unidentified *Salmonella* spp and *E. coli* followed by the ones sundried on the ground. Though *E. coli* are the normal flora to fresh *R. argentea* gastrointestinal tracts sometimes can contain pathogenic ones. Therefore, their presence may cause health problems to humans when consumed since *R. argentea* are consumed without being eviscerated. The presence of *Salmonella* spp and *E. coli* in *R. argentea* was possibly due to cross contamination with contaminated water at landing sites and in the environment in which they were fished. The contamination of lake water possibly was due to wastes from households, industries, hospitals and water runoff. This finding is in line with the results reported by Mishra *et al.* (2010) and Rao *et al.* (2014) of contamination of fish with *Salmonella* spp and *E. coli*. At processing level, the *R. argentea* dried on raised racks were not contaminated with *Salmonella* spp and *E. coli*. This is due to the improved handling practices for fresh *R. argentea* and the

processing method employed by processors during drying and the implementation of GMP. However, *R. argentea* dried on ground were cross contaminated with *S. typhimurium*, unidentified *Salmonella* spp and *E. coli*. This was indication of the possible contamination of *R. argentea* with faecal materials of animal origin from the drying areas. Additionally, since birds are among the sources of *Salmonella* spp, possibly their presence in drying areas might cross contaminate the *R. argentea* being dried. This finding is in agreement to those reported previously by David *et al.* (2008); Sifuna *et al.* (2008) and Onyuka *et al.* (2011) in Kenya of contamination of sundried *R. argentea* with enteric bacteria. At market level, the *R. argentea,* which were dried on ground were contaminated with *S. typhimurium*, unidentified *Salmonella* spp and *E. coli*. Possibly, this was due to survival of these bacteria during the drying process and due to the unhygienic storage conditions at the market, which favoured their growth. Also, the presence of waste disposal near the market might contribute to cross contamination of *R. argentea* with *Salmonella* spp and *E. coli* due to proliferation of flies on the products. The contamination of *R. argentea* with *Salmonella* spp and *E. coli* could explain the increased gastroenteritis and typhoid in humans residing along the Lake Victoria basin, in Mwanza as reported by Temu *et al.* (2007) that the presence *Shigella flexneri*, *Shigella dysenteriae* and *Salmonella* spp isolated from patients with bloody diarrhoea in Mwanza.

The results for antibiotic susceptibility testing showed that *S. typhimurium,* unidentified *Salmonella* spp and *E. coli* were resistant to ampicillin, tetracycline and co-trimoxazole. The emergence of *Salmonella* spp and *E. coli* resistant to these antibiotics are related to the frequent use in various treatments in veterinary and human health centres. As a result, the metabolites excreted are deposited in the environment and carried with water runoffs to the lake and cause the emergence of antibiotic resistant bacteria, which in turn contaminate the *R. argentea*. Other possible source of antibiotic resistant bacteria in *R.*

argentea is attributed to the contamination of lake water with wastes from households, hospitals and industries, which may contain low level of antibiotics. The situation is also explained by Bywater (2004) and Okeke *et al.* (2005) on these wastes being the sources of contamination of water bodies with antibiotics. Also, the contamination can be due to improper disposal of expired antibiotics resulting in the contamination of water bodies and aquatic organisms like fish. The presence of antibiotics resistant bacteria is complicating the therapeutic management of severe diseases like salmonellosis, typhoid and gastroenteritis disease in humans because they also encounter antibiotic resistance. This finding is in agreement with Sifuna *et al.* (2008) and Onyuka *et al.* (2011) who reported the presence of *Salmonella* spp and *E. coli* isolated in dried *R. argentea* from Lake Victoria, Kenya, which were resistant to these antibiotics.

Additionally, the results of this study indicated that both *S. typhimurium* and unidentified *Salmonella* spp were sensitive to ciprofloxacin and norfloxacin while *E. coli* were sensitive to chloramphenicol, norfloxacin and ciprofloxacin. This is similar to the findings reported by Mishra *et al.* (2010) that *Salmonella* spp and *E. coli* isolated in fish from different water bodies were sensitive to these antibiotics. Also, Sifuna *et al.* (2008) reported the sensitivity of *E. coli* isolated in *R. argentea* from Lake Victoria, Kenya to chloramphenicol and norfloxacin, Onyuka *et al.* (2011) reported the sensitivity of *Salmonella* spp and *E. coli* to ciprofloxacin. The susceptibility of *Salmonella* spp and *E. coli* to these antibiotics supports the current drugs of choice for the treatment of diseases caused by these bacteria in humans. This antibiotic susceptibility test findings gives valuable information for the selection of drugs for the treatment of diseases caused by *Salmonella* spp and *E. coli* to humans.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the results of the current study, it is concluded that:

i. The sun drying of *R. argentea* using raised racks is more hygienic method compared to ground drying processing method as evidenced by bacteria counts results reported in this study. Though the raised racks processing method provided an acceptable product with low bacteria counts prior to arriving at market, bacteria counts of fish dried via this method increased at the market indicating that more hygienic measures need to be undertaken at the packaging, transportation and storage levels to improve the quality.

ii. *Salmonella typhimurium,* unidentified *Salmonella* spp and *E. coli* were found to contaminate fresh *R. argentea* and those dried on the ground. This suggests the contamination of *R. argentea* with faecal materials of human and animal origin in water and in the environment where *R. argentea* were dried. Since *S. typhimurium* is zoonotic bacteria which causes salmonellosis to humans, their contamination in *R. argentea* can be easily transmitted to animals through animal feeds and in turn infect human via contaminated meet and/or meet products.

iii. From this study, it has been noticed that all *Salmonella* spp and *E. coli* were found to be resistant to ampicillin, tetracycline and co-trimoxazole. However, were sensitive to norfloxacin and ciprofloxacin. *Escherichia coli* showed sensitivity to chloramphenicol too.

6.2 Recommendations

Based on findings from this study, it is recommended that:

i. Sun drying of *R. argentea* should be done on raised racks because it improves products quality and safety, which will meet local, regional and international market requirements.

ii. Good Manufacturing Processing for *R. argentea* production and processing should be followed properly and its implementation be monitored along the value chain in Lake Victoria to ensure its quality and safety. Though GMP so far exists, closer monitoring is necessary. Also, Hazard Analysis Critical Control Points needs to be developed and implemented since it does not yet exist in *R. argentea* production. This will improve hygienic fishing, processing, packaging and transportation of *R. argentea* to the markets level so as to minimise food hazards which may be introduced in stages of production along the chain. After buying the fresh *R. argentea* at landing site, the processors should wash them with clean and potable water to minimise contaminants before drying. During drying, birds should be restricted access to the drying area in order to prevent biological and physical contamination of *R. argentea* while being dried.

iii. All landing sites where *R. argentea* are being offloaded should be provided with clean latrines and be utilised in order to minimise the contamination of sardines with enteric bacteria from human waste. Additionally, there is a need to continue with technical supports and policy initiatives by both national and international agencies to monitor quality and safety of *R. argentea* so that it will meet demand and consumer expectation.

iv. There is a need of more studies to be conducted on other biological hazards contamination in *R. argentea* and its environment (water and sediments). So far in Tanzania, there is limited information on the contamination of *R. argentea* with *Vibrio* spp, *Staphyloccocus aureus* and *Listeria monocytogenes* since are of public health importance. Also, a study on the relationship between the moisture content and bacterial loads, particularly during storage of the products is required.

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APPENDICES

Appendix 1: Raw data on bacterial counts of *Rastrineobola argentea* along the value chain

A: Fresh *R. argentea* from the boats at landing sites

S/N	Location	Code No.	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-8	cfu/g	Log cfu/g
1	Kigangama	KIGF1	>300	>300	>300	>300	76	5	0	74000000	7.87
2	Kigangama	KIGF2	>300	>300	>300	>300	51	3	0	49000000	7.69
3	Kigangama	KIGF3	>300	>300	>300	226	62	4	0	60000000	7.78
4	Kigangama	KIGF4	>300	>300	>300	>300	65	7	0	65000000	7.81
5	Kigangama	KIGF5	>300	>300	>300	>300	77	9	0	78000000	7.89
6	Kigangama	KIGF6	>300	>300	132	57	5	0	0	5600000	6.75
7	Kigangama	KIGF7	>300	>300	>300	36	3	0	0	3600000	6.56
8	Kigangama	KIGF8	>300	>300	98	7	1	0	0	950000	5.98
9	Kigangama	KIGF9	>300	>300	123	63	4	0	0	6100000	6.79
10	Kigangama	KIGF10	>300	>300	154	46	7	0	0	4800000	6.68
11	Kijiweni	KIJF1	>300	>300	>300	>300	47	4	0	46000000	7.66
12	Kijiweni	KIJF2	>300	>300	>300	130	17	2	0	17000000	7.23
13	Kijiweni	KIJF3	>300	>300	>300	>300	25	6	0	28000000	7.45
14	Kijiweni	KIJF4	>300	>300	>300	45	5	1	0	4500000	6.65
15	Kijiweni	KIJF5	>300	>300	>300	>300	79	9	0	80000000	7.90
16	Kijiweni	KIJF6	>300	>300	>300	50	4	0	0	4900000	6.69
17	Kijiweni	KIJF7	>300	>300	69	5	0	0	0	670000	5.83

Appendix 1 continue

S/N	Location	Code No.	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-8	cfu/g	Log cfu/g
18	Kijiweni	KIJF8	>300	>300	138	23	2	0	0	2300000	6.36
19	Kijiweni	KIJF9	>300	>300	114	96	10	0	0	9600000	6.98
20	Kijiweni	KIJF10	>300	>300	>300	>300	43	2	0	41000000	7.61
21	Bwiru	BWF1	>300	>300	222	22	2	0	0	2200000	6.34
22	Bwiru	BWF2	>300	>300	181	16	5	2	0	1900000	6.28
23	Bwiru	BWF3	>300	>300	>300	156	26	2	0	17000000	7.23
24	Bwiru	BWF4	>300	>300	216	36	6	1	0	3800000	6.58
25	Bwiru	BWF5	>300	>300	107	8	0	0	0	1000000	6.00
26	Bwiru	BWF6	>300	>300	237	28	2	0	0	2700000	6.43
27	Bwiru	BWF7	>300	>300	>300	67	15	3	0	7500000	6.88
28	Bwiru	BWF8	>300	>300	75	9	2	0	0	760000	5.88
29	Bwiru	BWF9	>300	>300	>300	40	9	0	0	4500000	6.65
30	Bwiru	BWF10	>300	>300	199	32	1	1	0	3000000	6.48
31	Igombe	IGF1	>300	>300	41	2	0	0	0	390000	5.59
32	Igombe	IGF2	>300	>300	132	21	6	0	0	2500000	6.40
33	Igombe	IGF3	>300	126	34	6	2	0	0	360000	5.56
34	Igombe	IGF4	>300	>300	101	18	1	1	0	1100000	6.04
35	Igombe	IGF5	>300	>300	151	42	3	0	0	4100000	6.61
36	Igombe	IGF6	>300	>300	47	3	0	0	0	450000	5.65
37	Igombe	IGF7	>300	>300	51	7	0	0	0	530000	5.72
38	Igombe	IGF8	>300	>300	84	6	0	0	0	820000	5.91
39	Igombe	IGF9	>300	>300	69	3	1	0	0	650000	5.81
40	Igombe	IGF10	>300	>300	>300	53	4	0	0	5200000	6.72

Cfu/g mean colony forming unity per gram

B: *Rastrineobola argentea* dried on raised racks at processing level (Appendix 1 continue)

S/N	Location site	Code No.	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-8	cfu/g	Log cfu/g
41	Igombe	IGR 1	>300	28	4	0	0	0	0	29000	4.46
42	Igombe	IGR 2	>300	51	4	0	0	0	0	36000	4.56
43	Igombe	IGR 3	>300	61	5	1	0	0	0	60000	4.78
44	Igombe	IGR 4	77	2	1	0	0	0	0	7200	3.86
45	Igombe	IGR 5	37	3	0	0	0	0	0	3600	3.56
46	Kigangama	KIGGR 1	>300	46	4	0	0	0	0	45000	4.65
47	Kigangama	KIGGR 2	>300	23	1	1	0	0	0	22000	4.34
48	Kigangama	KIGGR 3	237	50	6	0	0	0	0	51000	4.71
49	Kigangama	KIGGR 4	16	2	0	0	0	0	0	1600	3.20
50	Kigangama	KIGGR 5	>300	28	2	0	0	0	0	27000	4.43
51	Bwiru	BWR 1	>300	76	3	0	0	0	0	72000	4.86
52	Bwiru	BWR 2	>300	59	4	0	0	0	0	57000	4.76
53	Bwiru	BWR 3	24	1	1	0	0	0	0	2300	3.36
54	Bwiru	BWR 4	>300	46	2	0	0	0	0	44000	4.64
55	Bwiru	BWR 5	61	4	0	0	0	0	0	5900	3.77
56	Kijiweni	KIJR 1	94	9	0	0	0	0	0	9400	3.97
57	Kijiweni	KIJR 2	104	72	5	1	0	0	0	70000	4.85
58	Kijiweni	KIJR 3	35	1	0	0	0	0	0	3300	3.52
59	Kijiweni	KIJR 4	13	2	0	0	0	0	0	1400	3.15
60	Kijiweni	KIJR 5	116	22	4	0	0	0	0	24000	4.38

Rastrineobola argentea dried on ground processing level (Appendix 1 continue)

S/N	Location site	Code No.	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-8	cfu/g	Log cfu/g
61	Igombe	IGG 1	>300	>300	>300	>300	92	2	0	85000000	7.93
62	Igombe	IGG 2	>300	>300	>300	42	2	0	0	4000000	6.60
63	Igombe	IGG 3	>300	>300	>300	>300	24	3	0	25000000	7.40
64	Igombe	IGG 4	>300	>300	>300	15	3	0	0	1600000	6.20
65	Igombe	IGG 5	>300	>300	>300	49	6	0	0	5000000	6.70
66	Kigangama	KIGG 1	>300	>300	149	16	2	0	0	1600000	6.20
67	Kigangama	KIGG 2	>300	>300	>300	16	2	0	0	1600000	6.20
68	Kigangama	KIGG 3	>300	>300	>300	42	4	0	0	4200000	6.62
69	Kigangama	KIGG 4	>300	>300	162	21	1	0	0	910000	5.96
70	Kigangama	KIGG 5	>300	>300	>300	113	34	2	0	33000000	7.52
71	Bwiru	BWG 1	>300	>300	56	6	0	0	0	560000	5.75
72	Bwiru	BWG 2	>300	>300	98	10	1	0	0	980000	5.99
73	Bwiru	BWG 3	>300	>300	>300	63	4	0	0	6100000	6.79
74	Bwiru	BWG 4	>300	>300	24	2	0	0	0	240000	5.38
75	Bwiru	BWG 5	>300	>300	103	9	1	0	0	1000000	6.00
76	Kijiweni	KIJG 1	>300	64	5	1	0	0	0	63000	4.80
77	Kijiweni	KIJG 2	>300	>300	>300	26	5	0	0	2800000	6.45
78	Kijiweni	KIJG 3	>300	>300	75	10	1	0	0	770000	5.89
79	Kijiweni	KIJG 4	>300	>300	>300	88	6	0	0	8500000	6.93
80	Kijiweni	KIJG 5	>300	>300	93	5	0	0	0	890000	5.95

C: *Rastrineobola argentea* dried on raised racks at market level (Appendix 1 continue)

S/N	Market	Code No.	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-8	cfu/g	Log cfu/g
81	Mwanza city	MCR 1	>300	71	6	0	0	0	0	70000	4.85
82	Mwanza city	MCR 2	97	8	0	0	0	0	0	9500	3.98
83	Mwanza city	MCR 3	>300	67	6	0	0	0	0	66000	4.82
84	Mwanza city	MCR 4	>300	>300	73	9	0	0	0	750000	5.88
85	Mwanza city	MCR 5	>300	86	2	0	0	0	0	80000	4.90
86	Kirumba	KIR 1	88	10	2	0	0	0	0	8900	3.95
87	Kirumba	KIR 2	>300	>300	27	2	0	0	0	260000	5.41
88	Kirumba	KIR 3	>300	92	6	1	0	0	0	89000	4.95
89	Kirumba	KIR 4	>300	>300	36	4	0	0	0	360000	5.56
90	Kirumba	KIR 5	>300	109	31	2	1	0	0	300000	5.48
91	Nyakato	NYR 1	82	4	1	0	0	0	0	7800	3.89
92	Nyakato	NYR 2	>300	54	3	0	0	0	0	52000	4.72
93	Nyakato	NYR 3	>300	103	22	1	1	0	0	210000	5.32
94	Nyakato	NYR 4	>300	67	5	0	0	0	0	65000	4.81
95	Nyakato	NYR 5	155	44	2	0	0	0	0	42000	4.62
96	Mkuyuni	MKR 1	>300	>300	17	2	0	0	0	170000	5.23
97	Mkuyuni	MKR 2	>300	>300	42	4	0	0	0	420000	5.62
98	Mkuyuni	MKR 3	>300	105	27	3	1	0	0	270000	5.43
99	Mkuyuni	MKR 4	>300	35	5	1	0	0	0	36000	4.56
100	Mkuyuni	MKR 5	>300	102	23	1	0	0	0	220000	5.34

Rastrineobola argentea dried on ground at market level (Appendix 1 continue)

S/N	Market	Code No.	10^-2	10^-3	10^-4	10^-5	10^-6	10^-7	10^-8	cfu/g	Log cfu/g
101	Mwanza city	MCG 1	>300	>300	>300	>300	33	2	0	32000000	7.51
102	Mwanza city	MCG 2	>300	>300	>300	>300	26	4	0	27000000	7.43
103	Mwanza city	MCG 3	>300	>300	>300	115	86	3	0	81000000	7.91
104	Mwanza city	MCG 4	>300	>300	>300	>300	42	6	1	44000000	7.64
105	Mwanza city	MCG 5	>300	>300	>300	113	10	2	0	11000000	7.04
106	Kirumba	KIG 1	>300	>300	>300	129	31	7	0	35000000	7.54
107	Kirumba	KIG 2	>300	>300	121	13	2	0	0	1800000	6.26
108	Kirumba	KIG 3	>300	>300	>300	240	18	1	0	17000000	7.23
109	Kirumba	KIG 4	>300	>300	>300	>300	35	5	1	36000000	7.56
110	Kirumba	KIG 5	>300	>300	>300	>300	41	1	0	38000000	7.58
111	Nyakato	NYG 1	>300	>300	109	51	3	0	0	4900000	6.69
112	Nyakato	NYG 2	>300	>300	>300	26	2	1	0	2500000	6.40
113	Nyakato	NYG 3	>300	>300	>300	>300	47	3	1	45000000	7.65
114	Nyakato	NYG 4	>300	>300	54	6	1	0	0	550000	5.74
115	Nyakato	NYG 5	>300	>300	114	32	2	0	0	3100000	6.49
116	Mkuyuni	MKG 1	>300	>300	122	65	7	1	0	6500000	6.81
117	Mkuyuni	MKG 2	>300	>300	>300	>300	53	3	0	51000000	7.71
118	Mkuyuni	MKG 3	>300	>300	>300	>300	21	2	0	21000000	7.32
119	Mkuyuni	MKG 4	>300	>300	152	33	2	0	0	3200000	6.51
120	Mkuyuni	MKG 5	>300	>300	127	29	3	0	0	2900000	6.46

Appendix 2: Raw data for enumeration of *Escherichia coli* in *Rastrineobola argentea* along value chain Most Probable Number method

A: *Escherichia coli* in fresh *R. argentea*

S/N	Location	Code No.	10^0	10^-1	10^-2	10^-3	10^-4	10^-5	10^-6	1-0^-7	mpn/g	*E. coli*	QNT *E.coli*	Log mpn/g
1	Kigangama	KIGF1	3	3	3	3	2	0	0	0	9300	P	9.3×10^3	3.97
2	Kigangama	KIGF2	3	3	3	3	3	0	0	0	24000	P	2.4×10^4	4.38
3	Kigangama	KIGF3	3	3	3	3	2	0	0	0	9300	P	9.3×10^3	3.97
4	Kigangama	KIGF4	3	3	3	3	3	1	0	0	43000	P	4.3×10^4	4.63
5	Kigangama	KIGF5	3	3	3	3	3	3	0	0	240000	P	2.4×10^5	5.38
6	Kigangama	KIGF6	3	3	3	2	0	0	0	0	930	A	$<0.3 \times 10^1$	0.30
7	Kigangama	KIGF7	3	3	3	1	1	0	0	0	750	A	$<0.3 \times 10^1$	0.30
8	Kigangama	KIGF8	3	3	3	3	2	0	0	0	9300	P	9.3×10^3	3.97
9	Kigangama	KIGF9	3	3	3	2	2	1	0	0	2800	P	2.8×10^3	3.45
10	Kigangama	KIGF10	3	3	3	0	0	0	0	0	240	A	$<0.3 \times 10^1$	0.30
11	Kijiweni	KIJF1	3	3	3	3	2	0	0	0	9300	A	$<0.3 \times 10^1$	0.30
12	Kijiweni	KIJF2	3	3	3	3	3	0	0	0	24000	P	2.4×10^4	4.38
13	Kijiweni	KIJF3	3	3	3	3	2	2	0	0	21000	P	2.1×10^4	4.32
14	Kijiweni	KIJF4	3	3	3	3	0	0	0	0	240	A	$<0.3 \times 10^1$	0.30
15	Kijiweni	KIJF5	3	3	3	3	3	1	1	0	75000	P	7.5×10^4	4.88
16	Kijiweni	KIJF6	3	3	3	2	0	0	0	0	930	P	9.3×10^2	2.97
17	Kijiweni	KIJF7	3	3	3	3	1	0	0	0	4300	P	4.3×10^3	3.63
18	Kijiweni	KIJF8	3	3	2	0	0	0	0	0	93	A	$<0.3 \times 10^1$	0.30
19	Kijiweni	KIJF9	3	3	3	0	0	0	0	0	240	P	2.4×10^2	2.38

Mpn/g is most probable number per gram and QNT is Quantification

Appendix 2 continue

S/N	Location	Code No.	10^0	10^-1	10^-2	10^-3	10^-4	10^-5	10^-6	1-0^-7	mpn/g	*E. coli*	QNT *E.coli*	Log mpn/g
20	Kijiweni	KIJF10	3	3	2	0	0	0	0	0	93	A	$<0.3 \times 10^1$	0.30
21	Bwiru	BWF1	2	1	0	0	0	0	0	0	4	A	$<0.3 \times 10^1$	0.30
22	Bwiru	BWF2	3	3	3	2	0	0	0	0	930	P	9.3×10^2	2.97
23	Bwiru	BWF3	3	3	3	0	0	0	0	0	240	A	$<0.3 \times 10^1$	0.30
24	Bwiru	BWF4	3	3	3	3	2	0	0	0	9300	P	9.3×10^3	3.97
25	Bwiru	BWF5	3	3	3	2	2	0	0	0	2100	A	$<0.3 \times 10^1$	0.30
26	Bwiru	BWF6	3	3	3	1	0	0	0	0	430	A	$<0.3 \times 10^1$	0.30
27	Bwiru	BWF7	3	3	3	3	2	0	0	0	9300	P	9.3×10^3	3.97
28	Bwiru	BWF8	3	3	3	2	1	0	0	0	1500	P	1.5×10^3	3.18
29	Bwiru	BWF9	3	3	3	0	0	0	0	0	240	A	$<0.3 \times 10^1$	0.30
30	Bwiru	BWF10	3	3	3	3	2	0	0	0	9300	P	9.3×10^3	3.97
31	Igombe	IGF1	3	3	3	3	1	0	0	0	4300	P	4.3×10^3	3.63
32	Igombe	IGF2	3	3	3	3	2	0	0	0	9300	P	9.3×10^3	3.97
33	Igombe	IGF3	3	3	3	2	1	0	0	0	1500	P	1.5×10^3	3.18
34	Igombe	IGF4	1	0	0	0	0	0	0	0	5	A	$<0.3 \times 10^1$	0.30
35	Igombe	IGF5	2	0	0	0	0	0	0	0	10	A	$<0.3 \times 10^1$	0.30
36	Igombe	IGF6	3	3	3	2	1	0	0	0	1500	P	1.5×10^3	3.18
37	Igombe	IGF7	3	3	2	2	0	0	0	0	210	A	$<0.3 \times 10^1$	0.30
38	Igombe	IGF8	3	3	3	1	0	0	0	0	430	A	$<0.3 \times 10^1$	0.30
39	Igombe	IGF9	3	3	3	3	2	0	0	0	9300	P	9.3×10^3	3.97
40	Igombe	IGF10	3	3	3	1	1	0	0	0	750	P	7.5×10^2	2.88

B: *Rastrineobola argentea* dried on raised racks at processing level (Appendix 2 continue)

S/N	Location	Code No.	10^0	10^-1	10^-2	10^-3	10^-4	10^-5	10^-6	mpn/g	*E.coli*	QNT *E.coli*	Log mpn/g
41	Igombe	IGR 1	3	1	1	0	0	0	0	7	A	$<0.3 \times 10^1$	0.30
42	Igombe	IGR 2	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
43	Igombe	IGR 3	3	1	0	0	0	0	0	4	A	$<0.3 \times 10^1$	0.30
44	Igombe	IGR 4	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
45	Igombe	IGR 5	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
46	Kigangama	KIGR 1	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
47	Kigangama	KIGR 2	3	1	0	0	0	0	0	4	A	$<0.3 \times 10^1$	0.30
48	Kigangama	KIGR 3	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
49	Kigangama	KIGR 4	3	1	0	0	0	0	0	4	A	$<0.3 \times 10^1$	0.30
50	Kigangama	KIGR 5	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
51	Bwiru	BWR 1	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
52	Bwiru	BWR 2	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
53	Bwiru	BWR 3	3	1	0	0	0	0	0	4	A	$<0.3 \times 10^1$	0.30
54	Bwiru	BWR 4	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
55	Bwiru	BWR 5	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
56	Kijiweni	KIJR 1	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
57	Kijiweni	KIJR 2	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
58	Kijiweni	KIJR 3	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
59	Kijiweni	KIJR 4	2	0	0	0	0	0	0	2	A	$<0.3 \times 10^1$	0.30
60	Kijiweni	KIJR 5	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30

Rastrineobola argentea dried on ground at processing level (Appendix 2 continue)

S/N	Location	Code No.	10^0	10^-1	10^-2	10^-3	10^-4	10^-5	10^-6	mpn/g	*E.coli*	QNT *E.coli*	Log mpn/g
61	Igombe	IGG 1	3	3	3	2	2	0	0	2100	P	2.1×10^3	3.32
62	Igombe	IGG 2	3	3	3	2	0	0	0	930	P	9.3×10^2	2.97
63	Igombe	IGG 3	3	2	1	0	0	0	0	15	A	$<0.3 \times 10^1$	0.30
64	Igombe	IGG 4	3	3	3	1	0	0	0	430	P	4.3×10^2	2.63
65	Igombe	IGG 5	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
66	Kigangama	KIGG 1	3	3	3	2	2	0	0	2100	P	2.1×10^3	3.32
67	Kigangama	KIGG 2	3	3	3	2	1	0	0	1500	P	1.5×10^3	3.18
68	Kigangama	KIGG 3	3	3	3	2	2	0	0	2100	P	2.1×10^3	3.32
69	Kigangama	KIGG 4	1	0	0	0	0	0	0	1	A	$<0.3 \times 10^1$	0.30
70	Kigangama	KIGG 5	3	3	3	2	2	0	0	2100	P	2.1×10^3	3.32
71	Bwiru	BWG 1	3	1	0	0	0	0	0	4	A	$<0.3 \times 10^1$	0.30
72	Bwiru	BWG 2	3	2	1	0	0	0	0	15	P	1.5×10^1	1.18
73	Bwiru	BWG 3	2	1	0	0	0	0	0	4	A	$<0.3 \times 10^1$	0.30
74	Bwiru	BWG 4	3	2	0	0	0	0	0	9	A	$<0.3 \times 10^1$	0.30
75	Bwiru	BWG 5	3	1	1	0	0	0	0	7	A	$<0.3 \times 10^1$	0.30
76	Kijiweni	KIJG 1	3	1	0	0	0	0	0	4	A	$<0.3 \times 10^1$	0.30
77	Kijiweni	KIJG 2	3	2	2	0	0	0	0	21	A	$<0.3 \times 10^1$	0.30
78	Kijiweni	KIJG 3	3	3	2	1	0	0	0	150	P	1.5×10^2	2.18
79	Kijiweni	KIJG 4	3	3	3	1	1	0	0	740	P	7.4×10^2	2.87
80	Kijiweni	KIJG 5	3	3	3	2	2	0	0	2100	P	2.1×10^3	3.32

C: *Rastrineobola argentea* dried on raised racks at market level (Appendix 2 continue)

S/N	Market	Code No.	10^0	10^-1	10^-2	10^-3	10^-4	10^-5	10^-6	mpn/g	*E.coli*	QNT *E.coli*	Log mpn/g
81	Mwanza city	MCR 1	3	2	1	0	0	0	0	15	A	$<0.3 \times 10^1$	0.30
82	Mwanza city	MCR 2	3	2	0	0	0	0	0	9.2	A	$<0.3 \times 10^1$	0.30
83	Mwanza city	MCR 3	2	0	0	0	0	0	0	2	A	$<0.3 \times 10^1$	0.30
84	Mwanza city	MCR 4	3	2	2	0	0	0	0	21	A	$<0.3 \times 10^1$	0.30
85	Mwanza city	MCR 5	3	3	1	2	0	0	0	120	P	1.2×10^2	2.08
86	Kirumba	KIR 1	3	2	2	1	0	0	0	28	P	2.8×10^1	1.45
87	Kirumba	KIR 2	3	1	1	0	0	0	0	7.4	A	$<0.3 \times 10^1$	0.30
88	Kirumba	KIR 3	3	2	0	0	0	0	0	9.2	A	$<0.3 \times 10^1$	0.30
89	Kirumba	KIR 4	3	2	1	0	0	0	0	15	A	$<0.3 \times 10^1$	0.30
90	Kirumba	KIR 5	3	3	2	0	0	0	0	93	A	$<0.3 \times 10^1$	0.30
91	Nyakato	NYR 1	3	2	1	0	0	0	0	15	A	$<0.3 \times 10^1$	0.30
92	Nyakato	NYR 2	3	3	1	0	0	0	0	43	P	4.3×10^1	1.63
93	Nyakato	NYR 3	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
94	Nyakato	NYR 4	3	1	1	0	0	0	0	7	A	$<0.3 \times 10^1$	0.30
95	Nyakato	NYR 5	3	2	2	0	0	0	0	21	A	$<0.3 \times 10^1$	0.30
96	Mkuyuni	MKR 1	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
97	Mkuyuni	MKR 2	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
98	Mkuyuni	MKR 3	3	2	1	0	0	0	0	15	A	$<0.3 \times 10^1$	0.30
99	Mkuyuni	MKR 4	0	0	0	0	0	0	0	0	A	$<0.3 \times 10^1$	0.30
100	Mkuyuni	MKR 5	3	1	1	0	0	0	0	7	A	$<0.3 \times 10^1$	0.30

Ratrineobola argentea dried on ground at market level (Appendix 2 continue)

S/N	Market	Code No.	10^0	10^-1	10^-2	10^-3	10^-4	10^-5	10^-6	mpn/g	*E.coli*	QNT *E.coli*	Log mpn/g
101	Mwanza city	MCG 1	3	3	3	0	0	0	0	240	P	2.4×10^2	2.38
102	Mwanza city	MCG 2	3	3	3	2	0	0	0	930	P	9.3×10^2	2.97
103	Mwanza city	MCG 3	3	3	2	0	0	0	0	93	A	$<0.3 \times 10^1$	0.30
104	Mwanza city	MCG 4	3	3	1	0	0	0	0	43	A	$<0.3 \times 10^1$	0.30
105	Mwanza city	MCG 5	3	3	2	0	0	0	0	93	A	$<0.3 \times 10^1$	0.30
106	Kirumba	KIG 1	3	3	2	1	0	0	0	150	P	1.5×10^2	2.18
107	Kirumba	KIG 2	3	3	3	1	0	0	0	430	P	4.3×10^2	2.63
108	Kirumba	KIG 3	3	3	2	0	0	0	0	93	P	9.3×10^1	1.97
109	Kirumba	KIG 4	3	2	1	0	0	0	0	15	A	$<0.3 \times 10^1$	0.30
110	Kirumba	KIG 5	3	2	2	1	0	0	0	150	P	1.5×10^2	2.18
111	Nyakato	NYG 1	3	3	1	1	0	0	0	74	A	$<0.3 \times 10^1$	0.30
112	Nyakato	NYG 2	3	3	2	1	0	0	0	150	P	1.5×10^2	2.18
113	Nyakato	NYG 3	3	3	3	3	1	0	0	4300	P	4.3×10^3	3.63
114	Nyakato	NYG 4	3	3	3	2	2	0	0	2100	P	2.1×10^3	3.32
115	Nyakato	NYG 5	3	3	3	2	1	0	0	1500	P	1.5×10^3	3.18
116	Mkuyuni	MKG 1	2	1	1	0	0	0	0	7	A	$<0.3 \times 10^1$	0.30
117	Mkuyuni	MKG 2	3	3	2	2	0	0	0	210	P	2.1×10^2	2.32
118	Mkuyuni	MKG 3	3	2	1	1	0	0	0	74	P	7.4×10^1	1.87
119	Mkuyuni	MKG 4	3	3	3	1	1	0	0	740	P	7.4×10^2	2.87
120	Mkuyuni	MKG 5	3	3	3	2	2	0	0	2100	P	2.1×10^3	3.32