

PREVALENCE OF HISTAMINE AND PATHOGENIC MICROORGANISMS IN FISH
CONSUMED IN RURAL MALAWIAN HOUSEHOLDS

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ABSTRACT

PREVALENCE OF HISTAMINE AND PATHOGENIC MICROORGANISMS IN FISH CONSUMED IN RURAL MALAWIAN HOUSEHOLDS

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Fish is a major source of protein in Malawi, contributing about 70% of total animal protein consumed. However, this food resource is typically subjected to poor handling, storage and transportation in Malawi as it changes hands from fishermen through sellers and finally to consumers. Such poor handling exposes the product to contamination by foodborne pathogens and may predispose the fish to formation of histamine. The aim of this study was to determine the prevalence of selected foodborne pathogens and concentration of histamine in common fish species consumed in Malawi and to evaluate handling practices that may be associated with the incidence of these hazards. Raw samples (n=40) collected from Lakeshore and Blantyre markets and cooked samples (n=40) from Mbayani Township households were analyzed for presence of *Salmonella* spp., *Staphylococcus aureus*, *Listeria* spp. and *E. coli* O157:H7. Fish were frequently contaminated with *Salmonella* spp., *Staphylococcus aureus*, or *E. coli* O157:H7 in Lakeshore and Blantyre markets (50% incidence) and Mbayani Township households (63%). Raw/market samples and cooked samples from homes had similar pathogen incidence ($P>0.05$). Sixty-six percent of the samples had histamine concentrations exceeding 50 mg/kg, the guidance level for histamine identified by the US Food and Drug Administration. Preservation method did not influence histamine concentration within fish species ($P>0.05$) No correlations between incidence of food safety hazards and handling practices in homes or after harvest were identified.

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1 INTRODUCTION

In Malawi, cases of foodborne illness are numerous and most of them go unreported. Life expectancy is very low, estimated at 57 years for males and 58 years for females with an under five mortality rate of 112 for every 1000 live births (WHO, 2011; National Statistics Office, 2010). A number of factors contributing to this low life expectancy have been identified such as HIV-AIDS, malaria and diarrheal diseases. Despite diarrheal diseases being one of the major causes of low life expectancy and high infant mortality rate, there is little surveillance to assess impact of food contamination in Malawi.

A 2005 report by the Food and Agriculture Organization (FAO) of the United Nations provided a situation analysis of food safety systems in Malawi. This report noted that cholera and dysentery were the only foodborne diseases that were properly monitored while other potential outbreaks arising from *Salmonella*, *E. coli* and *Staphylococcus aureus* were left undocumented due to the absence of a comprehensive food safety and control program (FAO/WHO, 2005). To the best of my knowledge, to date there have been no major changes to improve the monitoring of other potential foodborne illness outbreaks in Malawi since the 2005 FAO/WHO report. Lack of a surveillance system for foodborne diseases also has been documented as a general problem throughout the African region which limits assessment of food contamination and its impact on public health (De Waal and Robert, 2005).

Personal communication with Mr. Kadziputa and Mr. Katukana, medical officers at Monkey Bay and Ulongwe health centers, respectively, indicated that they handle on average about 300 diarrhea-related illnesses per month. These health centers serve a combined population of over 70,000. These medical officers also mentioned that the causative agents for most of

these cases were not clearly identified and in most cases they assume patients had consumed contaminated water. In addition, other potential causes of foodborne diseases such as toxicity resulting from contaminated food haven't been studied extensively in Malawi.

Eighty percent of Malawi's 14 million people live in rural areas, which are characterized by high prevalence of poverty and food insecurity. In these rural communities, it is a common practice for families to store leftover food at ambient temperature in their homes due to lack of refrigeration equipment (Taulo et al, 2008). Fish cooked for consumption in the home is one of the foods typically stored at ambient temperature. Given the tropical climate in Malawi, storage of perishable foods at ambient temperature is conducive to the rapid growth of microorganisms including bacterial pathogens.

Going by these observations, it appears that most Malawians consuming fish may be at risk of illnesses associated with consumption of foodborne pathogens as well as histamine and other biogenic amines. This research will be the first study in Malawi to address the potential risk of biogenic amines associated with fish consumption, and one of the few studies to assess the incidence of microbial pathogens in fish as consumed in Malawi. The study seeks to determine the incidence of foodborne pathogens and concentrations of histamine in fish consumed by Malawians before cooking and during storage of left-over cooked fish. Incidence of foodborne pathogens and histamine concentrations will be correlated with food handling and storage practices assessed by interviewing Malawian consumers.

2 LITERATURE REVIEW

2.1 Biogenic Amines

Fish is a major source of protein in Malawi, where it is estimated that 60-70% of the protein consumed is provided by fish (Chimatiro, 1998). The development of biogenic amines in fish after capture and growth of microorganisms during post-harvest handling and storage of fish (both raw and cooked) at ambient temperature are potential food safety risks. Biogenic amines can develop in certain foods, particularly certain species of fish, due to contamination by bacteria which produce enzymes that decarboxylate amino acids to form toxic biogenic amines. Nine forms of biogenic amines are primarily formed by bacterial action and are responsible for foodborne illness and toxicity. Histamine, putrescine and cadaverine contamination has been associated with toxicity in human beings. Biogenic amines also have been implicated as precursors for formation of the carcinogenic compound nitrosamine (Shalaby, 1996).

Most technologies used in Malawi to preserve fish, such as open sun drying, smoking, pre-boiling and drying before marketing to the consumer, pose risks of contamination by both pathogenic and biogenic amine forming bacteria. For example, there usually are relatively few hygiene controls in place to limit exposures associated with fomites such as flies and other potential sources of bacterial contamination. Also, extended exposure of fish at ambient temperatures in the market places could facilitate proliferation of pathogenic microorganisms as well as microorganisms responsible for biogenic amine formation. Collectively, these risk factors suggest there is a high likelihood that consumers in Malawi purchase fish that may be contaminated with foodborne pathogens, histamine and other toxic biogenic amines. In addition, poor hygiene and temperature abuse during preservation, marketing and food preparation and handling in the homes pose high risks of further microbial contamination of fish. Work by Taulo

et al (2008) showed high levels of food contamination with microbial pathogens in rural areas of Malawi. This suggests that there could be significant contamination along the preservation and processing and preparation chain before the food is actually consumed. Unlike histamine and other biogenic amines, most pathogenic microorganisms are destroyed by high temperatures during cooking thereby reducing the risk of contracting foodborne illness. However, histamine and other biogenic amines are reportedly stable at high temperatures (Yung-Hsiang et al, 2005).

Refrigeration or freezing effectively controls formation of biogenic amines in food. Veciana-Nogues et al (1997) showed that storage of fresh fish at freezing temperatures can inhibit formation of histamine and other biogenic amines. However, few Malawians can afford refrigeration due to poverty. A study by Taalo et al (2008) revealed that 99% of rural Malawians do not own a refrigerator. In addition, their study revealed that 79% of rural Malawians will typically store leftover food at ambient temperature for a period of 1 to 3 days prior to consumption. This ambient temperature storage of food likely allows proliferation of pathogenic microorganisms in the foods thereby increasing the risk of foodborne illness. Furthermore, it is known that high levels of histamine can be formed before the food appears spoiled or before it is organoleptically unacceptable (Veciana-Nogues et al, 1997). Thus, consumers cannot reliably determine the potential presence of histamine contamination by smell or other senses.

Biogenic amines are formed in foods by the action of bacteria in a process known as amino acid decarboxylation. Consumption of high levels of biogenic amines causes toxicity in both human beings and animals. Histamine, cadaverine, putrescine, tyramine, tryptamine, β -phenylethylamine, spermine and spermidine are the most common biogenic amines formed in food. These biogenic amines may form in a wide range of foods such as fish, meat, eggs, vegetables (fermented) and soya (Shalaby, 1996).

In fish the presence of biogenic amines is known to cause a type of food poisoning termed scombrototoxin poisoning which has been associated with the formation of histamine in *Scombridae* and *Scomberesocidae* families of fish, which include tuna, bonito and mackerel. In addition, some fish species outside the families *Scombridae* and *Scomberesocidae* are known to be susceptible to histamine formation. Although scombrototoxin is characterized by high histamine concentrations, some studies have shown that the presence of cadaverine and putrescine increases the level of reaction to scombrototoxin poisoning (Bjeldanes et al 1978; Lehane and Olley, 2000).

2.2 Histamine Effects in Humans

As a defense mechanism, the human body is able to detoxify low levels of histamines. However, studies indicate that consuming foods containing relatively high levels of histamine (above 200 mg/kg) is responsible for causing illness (Lokuruka, 2009). Once histamine is released in the blood stream various symptoms can be observed affecting the heart, the gastrointestinal system, and the respiratory system. The most common symptoms are tingling, rash, drop in blood pressure, vomiting, headache, dizziness, nausea, vomiting, diarrhea, heart palpitation and respiratory distress (Taylor, 1986; Shalaby 1996; Lehane and Olley, 2000). Documented mortalities associated with scombrototoxin poisoning are rare, with only one death in the US attributed to scombrototoxin poisoning between 1983 and 2008 (CDC, 1990; CDC, 1996; CDC, 2000; CDC, 2013).

Due to this toxicity, different countries have set legal limits for histamine concentration in food for human consumption. Examples of these limits are the 50 mg/kg “guidance level” in the USA (FDA, 1998), 100 mg/kg in Europe (EC, 2003), 200 mg/kg in Australia (Australian

Food Standards Code, 2001) and 100 mg/kg in South Africa (South African Bureau of Standards, 2001).

The mechanism whereby histamine causes illness in humans is not clearly understood. Some studies have proposed that illness due to histamine poisoning is a result of histamine being absorbed in small intestines and released in the blood stream where it binds to cardiovascular and secretory system receptors (Shalaby, 1996; FAO/WHO, 2005). However, studies examining the effects of oral administration of histamine at levels similar to those observed in foods associated with scombroid food poisoning did not cause illness in humans (Taylor, 1986; FAO/WHO, 2005). It has been hypothesized that the presence of other biogenic amines, cadaverine and putrescine, that inhibit histamine-metabolizing enzymes in the small intestine, may be potentiate the toxic effects of histamine (Bjeldanes et al 1978; Lehane and Olley, 2000).

2.3 Histamine Formation in Fish

For histamine to develop in fish three conditions must be met, 1) temperature abuse of the fish, 2) presence of free histidine in fish, and 3) presence of bacteria that produce histidine decarboxylase (Lehane and Olley, 2000). Development of histamine in fish is partly due to temperature abuse of raw fish. Histamine formation is very rapid when raw fish is stored at temperatures above 21.1°C compared to when it is held at temperatures below 7.2°C (FDA, 2011). Therefore, refrigeration is an effective method for controlling histamine formation.

Amino acid decarboxylases of bacterial origin are the enzymes responsible for formation of biogenic amines including histamine. These decarboxylases act on free amino acids present in the food to produce the biogenic amines. Histidine decarboxylase converts free histidine to

histamine. Similarly, cadaverine and putrescine are formed by decarboxylation of lysine and ornithine, respectively (FAO, 2012).

Several bacterial species have been identified to be responsible for histamine formation. In general, formation of histamine and other biogenic amines is a result of the action of mesophilic bacteria rather than psychrotrophic bacteria (Frank et al., 1985). Veciana-Nogues et al. (1997) showed high correlation between histamine formation and presence of Enterobacteriaceae and coliforms. Tsai et al. (2006) isolated *Bacillus coagulans* and *Bacillus megaterium* as weak formers of histamine. Yatsunami and Echigo (1991) found *Staphylococcus* spp., *Vibrio* spp. and *Pseudomonas* spp. as microorganisms capable of forming histamine. Importantly, bacterial species capable of producing the amino acid decarboxylases associated with biogenic amine formation are ubiquitous in the gastrointestinal tract of fish (Dawood, 1988; FAO/WHO, 2005).

Once the amino acid decarboxylase enzyme is formed, it continues to produce histamine even if the microorganisms have been deactivated. Once formed, the decarboxylase enzymes maintain activity even at refrigeration temperatures. Freezing is an effective control for histamine formation as it prevents proliferation of the microorganisms responsible for forming the decarboxylase enzymes and also inhibits activity of pre-formed decarboxylase enzymes. Cooking of fish deactivates both the decarboxylase enzyme and the microorganisms responsible for its formation, but it is important to note that cooking does not destroy histamine (FDA, 2011; Yung-Hsiang et al, 2005).

The species of fish has a very strong influence on the potential for scombrotxin formation. Early studies identified *Scombridae* and *Scomberesocidae* as the fish families most commonly responsible for histamine poisoning. The fish in these families includes many species

that are commonly consumed including bonito, mackerel, tuna, saury and wahoo. Subsequent research has indicated that other species of fish outside the *Scombridae* and *Scomberesocidae* are commonly predisposed to scombrototoxin formation. In most cases the species identified as being predisposed to scombrototoxin formation are marine fish having a high free histidine content. A comprehensive list of fish species which are considered high risk for histamine formation was included in a recent FAO report (FAO, 2012). Additional susceptible species include amberjack (yellowtail), anchovy, herring, mahi-mahi, salmon, sardine and other species. Peculiar to these species is the presence of high concentration of free histidine which serves as a substrate for histamine formation.

Over many years, the focus of histamine poisoning has been on marine fish, as these have been predominantly associated with scombrototoxin food poisoning outbreaks. Very little is known about the potential of fresh water fish species to be associated with histamine poisoning. Few published studies have assessed the potential for histamine formation in fresh water fish. Chytiri et al (2004) assessed the formation of biogenic amines in whole and filleted freshwater catfish stored on ice for up to 18 days. They observed that putrescine and spermidine were the major biogenic amines formed under these conditions, with relatively little histamine (< 2 mg/kg) formed even after 18 days of storage on ice. Catfish fillets had significantly greater concentrations of biogenic amines compared to whole, uneviscerated fish held under these conditions.

Rezaei et al (2007) studied the formation of biogenic amines in whole farmed rainbow trout stored in ice for 18 days. They observed significant increases in putrescine, cadavarine and histamine concentration with storage time. However, the maximum histamine concentration

observed after 18 days (1.61 mg/kg) was lower than the FDA 50 mg/kg guidance level for histamine.

Another study by Coban and Patir (2008) observed an average histamine concentration of 12.21 mg/kg in rainbow trout studied between the months of September 2003 to April 2004. Furthermore, histamine concentrations decreased significantly ($P < 0.05$) with decrease in temperature during winter such that they observed a maximum histamine concentration of 16.37 mg/kg in September and the lowest concentration of 7.66 mg/kg in February. Histamine concentration in fresh water fish was lower than that of marine fish (Coban and Patir, 2008).

A study by Chakrabarti (1998) on shelf life and histamine content of ten fish species stored at ambient temperature revealed the ability of two freshwater catfish species to form histamine. Chakrabarti (1998) observed that catfish was organoleptically acceptable when held at tropical ambient temperatures up to 6 hrs. The histamine level in this catfish after 6 h of holding at ambient temperature was below 100 mg/kg which was deemed to be acceptable based on regulatory requirements in most countries. These results suggest that postharvest histamine formation may not be a significant hazard in temperature abused freshwater fish. However, the observation also should be treated with caution as ambient temperature holding times in Malawi may exceed six hours, particularly in rural settings where refrigeration equipment is lacking.

A similar study by Dawood et al. (1988) assessed the effect of holding freshly caught whole rainbow trout (*Salmo irideus*) at different temperatures (10, 20 and 30° C) for 6 h prior to chilling (0° C for up to 14 days). Dawood et al. (1988) observed increased levels of histamine, putrescine and cadavarine with increase in storage time. The observed concentrations of biogenic amines after 14 days of chilled storage did not exceed the safe limit, with maximum observed histamine levels being approximately 13 mg/kg and the other biogenic amines typically being

detected at concentrations less than 10 mg/kg. Eviscerated fish contained lower concentrations of amines than fish that were not eviscerated prior to refrigerated storage. It should be noted again that this study was conducted in controlled refrigeration environments and with minimal contamination which does not simulate conditions in rural areas in Malawi.

The effect of temperature on histamine formation was determined in a study by Veciana-Nogues et al (1997). In their study, tuna samples were stored at 0, 8 and 20° C, and high histamine levels (100 mg/kg) were observed in samples stored at 8 and 20° C. In addition, this study demonstrated that, given a consistent fish source and microbial flora, histamine formation escalates dramatically with increase in storage temperature. This suggests that when cold storage is inadequate for fish susceptible to histamine formation, the potential of a toxicological hazard will be high. This observation could relate very well to a rural Malawi setting where refrigeration facilities are lacking and hygiene practices are relatively poor. Therefore, scombrototoxin food poisoning could be a concern in rural Malawi if the fish typically harvested and consumed are shown to be susceptible to histamine formation.

Lake Malawi and other fresh water lakes in central Africa contain a rich diversity of fish species. Lake Malawi has over 1000 different species and only half have been identified (Weyl and Weyl, 2001). Some of the families identified are *Cichlidae*, *Cyprinidae*, and *Alestiidae* which are also highly consumed both by urban and rural populations. Research available on Malawian fish species has focused on nutrition content mainly protein, fat and minerals. The precursors for biogenic amine formation such as histidine have not been extensively studied in these species.

Cases of histamine poisoning resulting from consumption of fish from Lake Malawi and other fresh water lakes are not commonly reported. Auerswald et al. (2006) noted this underreporting occurs across the African continent, and speculated this underreporting was due to a lack of laboratory facilities to analyze histamine as well as histamine poisoning possibly being mistaken for seafood allergy. For example, as of 2009 there had been no cases of scombrototoxin food poisoning reported in Kenya even though consumption of scombroid fish species is high in that country. This may be due to ignorance by consumers regarding the symptoms of scombrototoxin food poisoning, as the illness has transient symptoms that typically appear 2-8 h after consumption (Lokuruka, 2009).

2.4 Fish handling and risk of contamination

In Malawi, fresh fish is mostly captured by gillnetting (Weyl and Weyl, 2001). A large proportion (90%) of the total catch is preserved. Fifty percent of the fish are preserved by sun drying while 40% are preserved by smoking (Chimatiro, 1998). A small proportion of the catch is sold fresh as observed by Mumba and Jose (2005). Sun dried, smoked parboiled fish are ideal for rural populations in Malawi where refrigeration equipment is often unavailable. The preservation methods for sun drying and smoking are usually done with homemade equipment making the process very slow. This slow preservation process may allow proliferation of both spoilage and pathogenic bacteria. If the fish contain high concentrations of free histidine and come in contact with histidine decarboxylase forming bacteria, there is a significant likelihood of histamine being formed.

Auerswald et al., (2006) observed higher histamine levels in sundried fish and smoked fish compared to fish preserved by canning. Those elevated levels of histamine were a consequence of continued favorable conditions for bacterial growth during the sun drying or smoking processes that allowed more histamine to form before a substantial reduction in water activity was reached and inactivation of both histamine forming enzymes and bacteria occurred. Similarly, Lehane and Olley (2000) observed that in Africa it is common for air drying or sun drying to precede smoking and, in the course of sun drying, bacteria can multiply in the moist interior of the fish (which has higher water activity) causing the inside to spoil while the outside appears satisfactory. Furthermore, whereas the common fish preservation practices (sun drying or smoking) may control growth of microorganisms and activity of decarboxylase enzymes, these practices will not impact the concentrations of histamine already formed prior to preservation.

Poor transportation and marketing facilities also increase the potential for fish spoilage and biogenic amine formation. Temperature and moisture control during transportation and retailing of fish are lacking in Malawi. A number of studies discussed before in this review have stressed the necessity of continuous temperature control of fish starting right after the catch through preparation and consumption (Auerswald et al, 2006; Dawood et al, 1998; Veciana-Nogues et al, 1998). In Malawi, processed and fresh fish are typically transported by public transport. Preserved fish is carried in cartons and baskets with little temperature control (Est.ryukoku, 2001). Fresh fish is typically covered with ice, although sometimes this ice is not of sufficient quantity to maintain a safe temperature for long distances. In addition, there is often a greater chance of pathogen contamination at the market place where fish is exposed openly for

sale at ambient temperature allowing contamination by flies and other potential sources of pathogen contamination.

2.5 Contamination in the home

The way food is prepared and handled in the home may increase the risk of contamination with microbial pathogens. As noted by Unusan (2006), the home has no legal regulations for food preparation and storage. Home food safety is controlled through education of the consumer. In Malawi there have been a number of consumer education campaigns through radio and other media by various Non-Governmental Organizations (NGOs) and the government.

However, a study by Taalo et al. (2008) revealed high incidences and concentrations of bacteria in cooked fish sampled from households near coastal waters of Lake Malawi. Fish samples obtained from these households (n=37) had incidences of nonspecific *E. coli*, *E. coli* O157:H7, *Salmonella* spp. and *Staphylococcus aureus* of 49, 5, 30 and 51%, respectively. Median concentrations of *Staphylococcus aureus*, nonspecific *E. coli*, and *Salmonella* in these fish samples were 3.90, 4.01, and 3.36 log₁₀ CFU/g, respectively. The high microbial loads were thought to be a result of poor handling practices in the homes (Taalo et al., 2008).

Another study by Kapute et al. (2012) also isolated both pathogenic and spoilage microorganisms in fresh *Oreochromis* spp. (tilapia, local name Chambo) sold at local markets and supermarkets in Malawi suggesting that contamination of fish also occurs at the market place. In their study, they observed occurrences of *Corynebacterium* (20%) *Micrococcus* (16%), *Pseudomonas* (15%), *Bacillus* (15%) *Flavobacterium* (15%) and nonspecific *E. coli* (10%). Incidences of *Salmonella*, *Staphylococcus* and *Shigella* were below 5%. However, in some samples, the concentrations of *Salmonella*, *Vibrio* and *E.coli* were observed to be above

10^8 CFU/g. Furthermore, they observed that fish from local markets had significant higher bacterial counts ($P < 0.01$), 9.5×10^8 , and 2.7×10^5 CFU/g respectively.

2.6 Summary

Fish represents a significant source of protein for people living in Malawi. Much of the fish consumed in Malawi is sourced from Lake Malawi and poor post-harvest handling of these fish predisposes them to contamination with spoilage microorganisms and bacterial pathogens. Improper handling practices and lack of refrigeration in the homes of residents in rural Malawi may exacerbate contamination arising during post-harvest handling of fish. Relatively few studies have assessed the incidence of contamination of fish consumed in Malawi households with bacterial pathogens. Those studies that have been conducted (Taulo et al., 2008; Kapute et al., 2012) have indicated that contamination with bacterial pathogens is significant in cooked fish and other foods commonly consumed in Malawi households.

To date, no published studies have assessed the incidence or concentrations of histamine in fish consumed in Malawi households. Recent studies have indicated the potential of fresh water fish and non scombroid fish to form histamine when held under improper conditions such as temperature abuse. It is important to explore the potential of fish commonly consumed in Malawi to cause histamine poisoning. From the ongoing discussion, it appears that conditions permissive for formation of histamine and other biogenic amines are present given the normal fish handling practices in Malawi. The likelihood of histamine poisoning in Malawi could be high despite a lack of clinical data indicating that histamine toxicity is prevalent. Surveillance for foodborne illness in Malawi is limited (FAO/WHO, 2005) and cases of histamine poisoning

could be overlooked due to a lack of recognition of the symptoms of histamine poisoning by health workers or consumers (Auerswald et al., 2006; Lokuruka, 2009). My research aims to fill this data gap by analyzing the incidence and concentrations of histamine in fish commonly consumed in Malawi.

In addition, the poor post-harvest handling conditions common in Malawi are also permissive for contamination and proliferation of pathogenic microorganisms on fish. Pathogenic bacteria and biogenic amines both have a high likelihood to cause foodborne illness if the fish is subjected to contamination and temperature abuse after harvest. Preservation methods such as smoking and cooking may deactivate pathogenic bacteria while histamine and other biogenic amines remain stable under these conditions and may cause foodborne illness. In addition, poor food handling practices in the distribution chain and in the home may result in recontamination of the fish by pathogenic bacteria.

3 RATIONALE AND SPECIFIC AIMS

Exposure to histamine and other biogenic amines and pathogenic microorganisms is a health risk. Adverse health effects resulting from histamine exposure range from skin rashes to cardiovascular effects. Foodborne illness outbreaks associated with histamine poisoning have been reported globally for several decades. As a consequence of these outbreaks, various government regulatory bodies have established safety limits for histamine in fish. For instance, the US FDA has established 50 mg/kg histamine as the guidance level.

While it is generally known that consumption of histamine contaminated fish is a health concern in developing countries where fish is consumed in large quantities and hygiene practices are typically poor, clinical data are scanty in Malawi and levels of biogenic amines in fish are not known. In addition, most studies have concentrated on histamine formation in marine fish while little is known concerning whether freshwater fish have the capacity to form histamine and other biogenic amines of medical importance. It is believed that most cases of histamine poisoning are either misdiagnosed or are unreported, meaning patients suffer in silence or may receive improper treatments. In addition, studies have indicated that histamine is resistant to heat treatment and its presence is not readily detectable by sight or smell. Therefore, it is important to know the prevalence and levels of exposure to biogenic amines in Malawi from fish commonly consumed in the diet.

Similarly, since studies have shown frequent contamination of fish by bacterial pathogens it is important to explore the prevalence of these pathogens in the fish supply chain and to document handling and processing practices that may increase contamination. This knowledge will help in planning low-cost intervention strategies to reduce the risks both during handling and

consumption of fish. As recommended by Auerswald et al. (2006), such knowledge will help form a basis for setting legal limits for foodborne hazards in fish.

My long term objective is to determine the prevalence and concentrations of food safety hazards (chemical and microbiological) in foods commonly consumed by persons in rural Malawi, and to develop practical guidance and interventions to minimize exposure of Malawian consumers to these food safety hazards. The objectives of this research were to determine the prevalence and concentrations of histamine and selected bacterial pathogens (*Salmonella* spp. *Staphylococcus aureus*, *Listeria* spp. and *E. coli* O157:H7) in raw and cooked fish consumed by residents in rural Malawi, and to correlate the prevalence and concentrations of these hazards with food handling, preparation, and storage practices by fish markets, processors, and consumers in rural Malawi. I pursued these objectives by completing the following specific aims:

1. Quantify the concentration of histamine in raw and preserved fish sampled in fish markets. The working hypothesis is that fish commonly consumed in rural Malawi is subject to temperature abuse and improper handling, and therefore is at high risk of contamination with histamine.
2. Determine the prevalence of pathogenic bacteria in raw and preserved fish sampled in fish markets and in the homes of rural Malawians. The working hypothesis is that fish commonly consumed in rural Malawi is subject to temperature abuse and improper handling, and therefore is at high risk of contamination with bacterial pathogens.
3. Correlate food handling, processing and storage practices with the prevalence and concentrations of histamine and bacterial pathogens in raw and preserved fish consumed in rural Malawi. The working hypothesis is that the prevalence and concentrations food

safety hazards in fish is associated with specific food handling practices during post-harvest handling, preservation, distribution, or home preparation of fish.

By completing these specific aims, I will have completed an initial survey of the prevalence and concentrations of key food safety hazards in fish commonly consumed in Malawi and also identified major factors associated with the presence of these hazards. This information will be essential for determining the potential public health impact of food safety hazards in fish on Malawian citizens as well as for guiding the development of appropriate intervention strategies to minimize risk from these hazards.

4 EXPERIMENTAL DESIGN AND METHODS

4.1 Fish sample collection

Samples of raw and cooked fish were obtained from the lakeshore, markets and homes for this research. The plan used to collect fish samples is presented in Table 1. The sampling plan was balanced with the exception that Kampango was not sampled from Mbayani homes and Mlamba was not sampled from the Lakeshore or Blantyre markets.

Table 1. Numbers of fish samples obtained from various sources for foodborne pathogen surveillance.

Fish type and form	Lakeshore (source)	Blantyre Markets ¹	Mbayani homes
Usipa fresh	2	2	4
Usipa sundried	2	2	4
Usipa smoked	2	2	4
Utaka fresh	2	2	4
Utaka smoked	2	2	4
Utaka sundried	2	2	4
Chambo fresh	2	2	4
Chambo smoked	2	2	4
Kampango fresh	2	2	
Mlamba fresh			4
Mlamba smoked	2	2	4
Total	20	20	40

¹ An additional four fresh fish samples were obtained from Limbe (Blantyre) market and were analyzed for histamine content.

The sampled fish were representative of the major species consumed from Lake Malawi fisheries. Raw samples included “fresh” (fish preserved under ice), smoked and sundried species

of “Utaka” (*Copadichromis spp*), “Usipa” (*Engraulicypris sardella*; alternative common name: Lake Sardine), “Kampango” (*Bagrus meridionalis*; a type of catfish), “Chambo” (*Oreochromis lidole*; a type of tilapia) and “Mlamba” (*Clarias gariepinus*; alternative common name: African Sharptooth Catfish).

Sampling of raw fish was done in two stages. First stage samples were collected from lakeshore areas and second stage samples were from Blantyre markets. Lakeshore markets included Chidzale fishing village, Monkey Bay market in Monkey Bay, Maldeco area and Mangochi Boma market. Blantyre markets included Limbe and Mbayani markets. Fish samples weighing about 500 g were collected into sterile Whirl-Pak sampling bags (Nasco; Fort Atkinson, Wisconsin, USA) and were stored in cooler boxes loaded with ice and transferred to Blantyre for histamine and microbiological analyses. Samples were collected from fishermen, fish processors (smoking and drying) and vendors.

The sampling sites were identified with recommendations from the Fisheries Department in Monkey Bay and are active fish markets in the area. Sampling was done randomly depending on fish species and form that was available on the market. Consumers selected to obtain cooked fish were also sampled randomly though this was difficult since houses/homes are not properly planned in Mbayani Township. Therefore, depending on the willingness of participant at least every fifth house was targeted.

Cooked fish samples for all species studied were collected from 40 homes of Mbayani Township around Kabula and Msikawanjala areas. The cooked fish included those which were originally purchased as fresh, smoked or sundried. Collected samples were fish that were “ready to be consumed” at meal times. Individuals were asked to pick samples from their plates into

sterile Whirl-Pak sampling bags using their utensils. Samples were transported in cooler boxes under ice to the Polytechnic microbiology lab for microbiological analyses. Samples were analyzed on the same day they were collected.

4.2 Objective 1: Determination of prevalence and levels of exposure to histamine

Of 44 raw fish samples, 40 were subjected to microbial testing (Table 1) and an additional 4 samples collected from Limbe market were tested for prevalence of histamine using a Neogen Histamine Veratox ELISA Kit (Neogen Corporation; Lansing, Michigan, USA).

4.2.1 Sample preparation

Smoked and fresh samples of “Kampango” (*Bagrus meridionalis*), “Chambo” (*Oreochromis lidole*) and “Mlamba” (*Clarias gariepinus*) were deboned and the head section was removed before being homogenized by a Waring food blender for 5 minutes while samples of “Utaka” (*Copadichromis* spp) and “Usipa” (*Engraulicypris sardella*) were homogenized without deboning because of their smaller sizes. All fresh samples of Kampango and Chambo were eviscerated before blending. Other species were not eviscerated prior to homogenization as

these small fish species are commonly consumed without evisceration. The blender was washed and sterilized by flame before another sample was analyzed.

4.2.2 Sample extraction and histamine quantification

Samples were prepared and tested according to the manufacturer's instructions. Ten grams of each homogenized fish sample was added to 90 mls of distilled water in a plastic extraction bottle. The mixture was vigorously shaken for about 15 to 20 seconds and allowed to stand for 5 minutes. The mixture was shaken again for 15 to 20 seconds and allowed to stand for another 5 minutes. The mixture was then filtered through glass wool in a syringe and the filtrate was collected into a plastic collection tube. Samples were diluted up to 20 times following preliminary results. Histamine was quantified using the Neogen Veratox ELISA assay with quantification by measuring absorbance at 630 nm using a BioTek ELx800 96 microwell plate reader (BioTek Instruments, Inc.; Winooski, Vermont, USA). Histamine quantification calculations were conducted using Neogen Veratox Software where a standard curve was produced as reference for sample histamine concentrations.

4.3 Objective 2: Determination of prevalence of pathogenic microorganisms in raw and cooked fish

A total of 40 raw and 40 cooked fish samples were tested for presence of *Salmonella* spp., *E. coli* O157:H7, *Listeria* spp. and *Staphylococcus aureus* using Neogen reveal 2.0 for *Salmonella* ELISA Kit, Neogen Reveal for *E. coli* O157:H7 ELISA Kit, Neogen Reveal for *Listeria* spp. (Neogen Corporation; Lansing, Michigan, USA) and the StaphTEX Blue Kit

(Hardy Diagnostics; Santa Maria, California, USA), respectively. The distribution of samples analyzed for microbiological contamination is presented in Table 1.

Samples were stored in a refrigerator (2 to 6^o C) between collection and analysis. Samples were tested within 48 h of collection. Prior to analysis, fish samples were homogenized as whole (without deboning nor removal of head section) using a Waring Food Blender for two minutes. The blender was washed and flame sterilized before processing subsequent samples. These homogenates were then tested for the various bacterial species using the test kits listed above.

4.3.1 Neogen Reveal for *Salmonella*

Enrichment media was prepared following the manufacturer's instructions. Twenty-five grams of homogenized sample was added to 200 ml media and incubated at 42^o C for 24 h. The enriched samples were tested for *Salmonella* presence using the *Salmonella* test devices after incubation at ambient temperature for 15 minutes. The test is sensitive to 1 CFU/g or 10⁶ CFU/ml post enrichment.

4.3.2 Neogen Reveal for *Listeria*

Twenty-five grams of homogenized sample was added to 225 ml of media prepared using the manufacturer's instructions. The mix was tested for presence of *Listeria* spp. following incubation at 30^o C for 30 h using test devices provided by the manufacturer. Results were read from the test device. A double line in both control and test zone after 20 minutes was considered positive while a single line in control area only indicated a negative result. The test is sensitive to detect 1 CFU per g or 10⁶ CFU/ml post enrichment.

4.3.3 Neogen Reveal for *E.coli* O157:H7

Powdered enrichment media (10.7 g; Neogen Corporation) was weighed and added to 325 ml of sterilized water held at 42^o C. Sixty-five grams of homogenized sample was added to the media and the mix was incubated at 42^o C for 20 h. The enriched samples were tested for the presence of *E. coli* O157:H7 using the test devices provided. The test is designed to detect as low as 1 CFU per 375g of sample or 10⁴ CFU/ml post enrichment.

4.3.4 Test for *Staphylococcus aureus*

Twenty-five grams of sample was added to 225 ml sterilized water. Dilutions from the mix were cultured on Plate Count Agar (Biolab; UK) supplied by Merck, South Africa. The composition of the Plate Count Agar in g/l was 5.0, 2.5, 1.0 and 14.5 for tryptone, yeast extract, dextrose and agar respectively). Plates were incubated at 37^o C for 24 h. Each sample was cultured on a separate plate. Multiple colonies from the culture were tested for agglutination following the manufacturer's procedure. Agglutinated colonies were read as positive and non-agglutinating colonies were interpreted as negative. The test has 98.8% sensitivity and 99.5% specificity.

4.4 Objective 3: Documentation of food handling and storage practices

Two questionnaires (Appendices A and B) with multiple and structured questions were used to document food handling and storage practices by consumers in their homes and by the fishermen, fish processors and fish marketers. The instruments were translated to Chichewa, a

local language, and were initially pretested before use. The questionnaire in Appendix A targeted homes while the questionnaire in Appendix B was used for fish processors, fishermen and vendors in markets. In general, the questions in both tools were based on similar practice questionnaires used by Taulo et al. (2008) and Redmond and Griffith (2003) with some modification.

For homes, three areas of interest were assessed - food preparation and handling practices, food storage practices, and the general environment of the home. Hand washing practices, equipment used for preparation of food, and the source of water for both cleaning utensils and cooking were considered under food preparation and handling. Storage of leftover food and storage length were considered under storage practices. The condition of the home was evaluated based on exposure to domesticated animals, the presence of children with diapers in the home, and assessing the persons responsible for cooking and cleaning of utensils. The questionnaire in Appendix B assessed fish handling practices at the market place and fish handling during processing.

Questionnaires were administered by direct one-to-one interviews after obtaining informed consent. An interview followed after collection of sample from the seller or home owner. A total of forty interviews were completed for the markets and forty for the homes. To guarantee anonymity of responses and protection of identity of respondents, randomly selected numbers were assigned to each questionnaire. Survey procedures were conducted with the approval of the MSU Human Subjects Institutional Review Board.

4.5 Statistical Analyses

Histamine concentrations were calculated using Neogen's Veratox Software version 3.3. Questionnaire and foodborne pathogen data were analyzed using SPSS (Version 20.0). Descriptive statistics were conducted for prevalence of both histamine and pathogenic microorganisms. Difference in prevalence of foodborne pathogens with location and fish form were conducted using a Chi-Square test. Analysis of variance was conducted to test differences in histamine concentration with form of fish species. Significant differences were observed at ($P < 0.05$).

5 RESULTS

For presentation of results the samples were coded as shown in Table 2 below.

Table 2. Sample codes.

Scientific Name	Common Name	Form	Sample Code
<i>Engraulicypris sardella</i>	Usipa	Fresh	ESF
<i>Engraulicypris sardella</i>	Usipa	Sundried	ESD
<i>Engraulicypris sardella</i>	Usipa	Smoked	ESS
<i>Copadichromis</i> spp.	Utaka	Fresh	CSF
<i>Copadichromis</i> spp.	Utaka	Sundried	CSD
<i>Copadichromis</i> spp.	Utaka	Smoked	CSS
<i>Oreochromis</i> spp.	Chambo	Fresh	OSF
<i>Oreochromis</i> spp.	Chambo	Smoked	OSS
<i>Bagrus meridionalis</i>	Kampango	Fresh	BMF
<i>Clarias gariepinus</i>	Mlamba	Smoked	CGS

5.1 Prevalence of histamine in raw fish

Histamine concentrations (mg/kg) observed in raw fish samples for the various species studied are presented in Table 3

Table 3. Least square mean concentration of histamine in selected fish species in Malawi.

Specie Name	Code	LS Mean	SEM
Usipa - fresh	ESF	^A 344	67
Usipa - sundried	ESD	^A 382	60
Usipa - smoked	ESS	^{AB} 300	60
Utaka - fresh	CSF	^{CD} 95	77
Utaka - sundried	CSD	^{ABC} 281	67
Utaka - smoked	CSS	^{BCD} 126	67
Chambo - fresh	OSF	^D 80	60
Chambo - smoked	OSS	^D 71	60
Kampango - fresh	BMF	^D 74	60
Mlamba - smoked	CGS	^D 17	67

Average histamine concentrations for samples (n=44) ranged from 17 to 382 mg/kg. The lowest average concentration was observed in smoked Mlamba while highest average concentration was observed in sundried Usipa. Sixty-six percent of the fish samples obtained from fishermen or markets in this study had histamine concentrations that exceeded the FDA guidance level of 50 mg/kg. It is of interest to note that all smoked Mlamba samples contained histamine concentrations below the FDA guidance level while all fresh Usipa and sundried Usipa samples contained histamine concentrations exceeding the FDA guidance level. Three fish samples (7%), all of which were Usipa, had histamine concentrations exceeding 500 mg/kg.

A one-way ANOVA revealed that there were no significant differences in histamine concentrations for fish preserved by different preservation methods, (ice, sundrying or smoking) within the samples ($P>0.05$).

5.2 Prevalence of pathogenic microorganisms in raw and cooked fish

The prevalence of *Salmonella* spp., *Staphylococcus* spp., *E. coli* O157:H7 and *Listeria* spp. was assessed in both raw and cooked fish samples ($n=80$). Data are summarized in Table 4. *Salmonella* spp. was detected in 28% of all samples, *Staphylococcus aureus* was observed in 36% of all samples, and *E. coli* O157:H7 was present in one sample (1%). *Listeria* spp. was not detected in any samples.

Table 4. Prevalence of *Salmonella* spp., *Staphylococcus aureus*, *Listeria* spp. and *E. coli* O157:H7 in all fish samples.

Pathogen	Numbers Present/Absent		Percentage
<i>Salmonella</i> spp.	Absent	58	72.5%
	Present	22	27.5%
<i>Staphylococcus aureus</i>	Absent	51	63.7%
	Present	29	36.2%
<i>Listeria</i> spp.	Absent	80	100.0%
	Present	0	0.0%
<i>E. coli</i> O157:H7	Absent	79	98.8%
	Present	1	1.2%

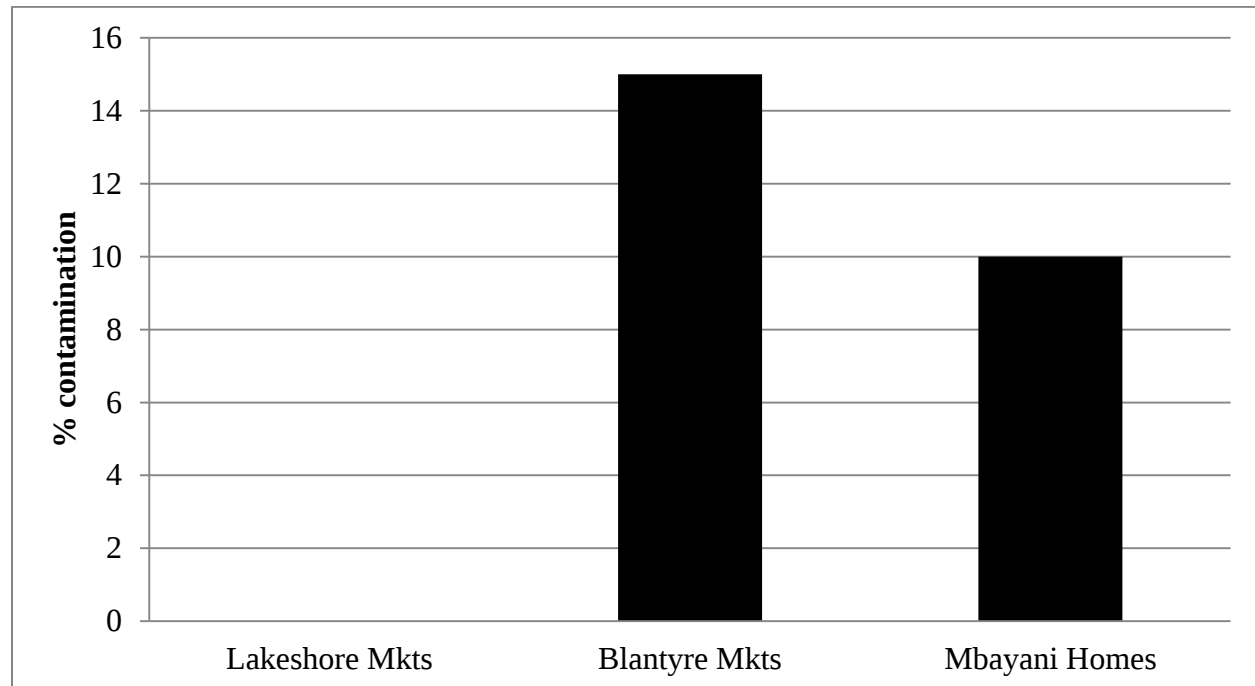
5.2.1 Prevalence of foodborne pathogens in raw samples

Fifty percent (50%) of raw samples (lakeshore samples and Blantyre markets samples) (n=40) showed the presence of *Salmonella* spp., *Staphylococcus aureus*, or both pathogens. *Salmonella* spp. was detected in 23% of the samples while *Staphylococcus aureus* was detected in 35% of the samples. *Listeria* spp. and *E. coli* O157:H7 were not detected in any of the raw samples.

Samples which were obtained at the Lakeshore (n=20) had lower detection rates for microbial pathogens compared to the stage two samples obtained from Blantyre markets. Thirty-five percent of Lakeshore samples were contaminated with either *Salmonella* spp. (5%) or *Staphylococcus aureus* (30%). Alternatively, in samples obtained from Blantyre markets (Limbe market and Mbayani Market; n=20) contamination was detected in 65% of all samples with 40% being contaminated with *Salmonella* spp. and 40% being contaminated with *Staphylococcus aureus*. As stated previously, no contamination by *Listeria* or *E. coli* O157:H7 was detected in any samples procured from the Lakeshore or Blantyre markets.

Contamination with more than one pathogen (Figure 1) was observed in 9% of all samples (7/80). One sample was contaminated by both *Salmonella* and *E. coli* O157:H7 while six samples (8% of the total) involved contamination by both *Salmonella* and *Staphylococcus aureus*. In this research, no sample obtained from Lakeshore areas was contaminated with more than one pathogen. However, double contamination was observed in 15% of Blantyre market samples (3/20) and in 10% of cooked samples obtained from Mbayani households (4/40).

Figure 1. Percentage of fish samples obtained from various sites that were contaminated with more than one bacterial pathogen.



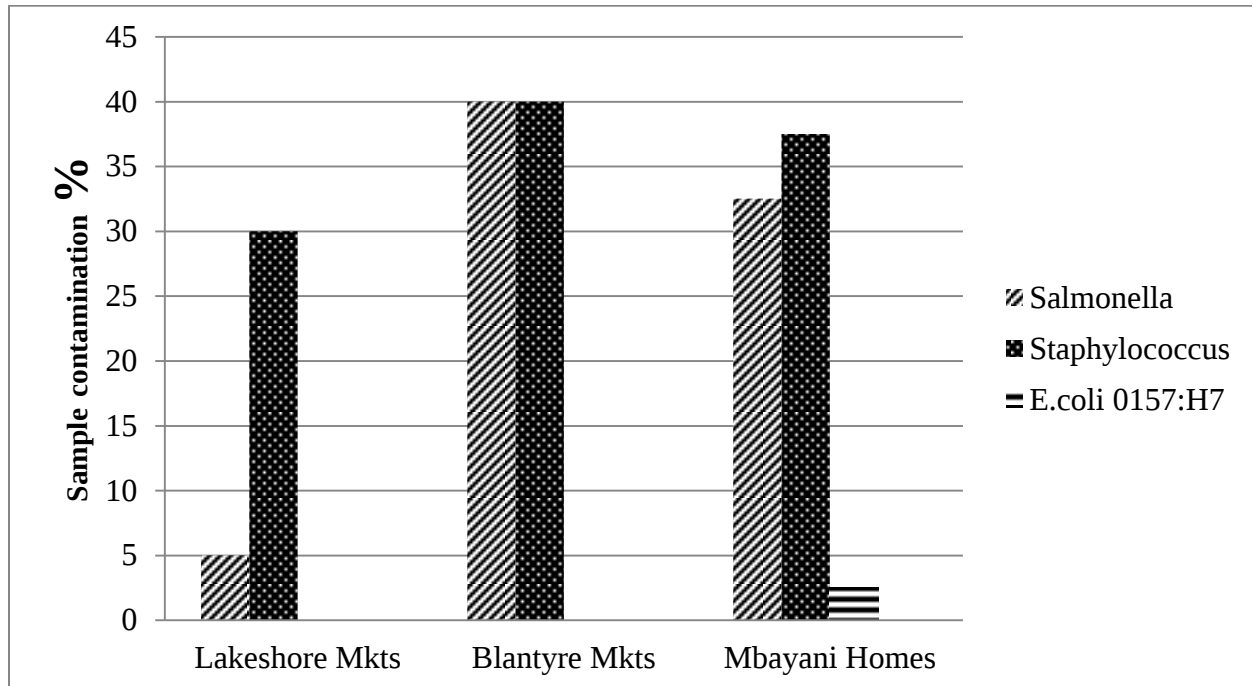
5.2.2 Prevalence of foodborne pathogens in cooked samples

For cooked samples collected from Mbayani Township in Blantyre, pathogen contamination was in 63% of samples (25/40). Thirty-three percent of samples were contaminated with *Salmonella*, 38% with *Staphylococcus* and 3% with *E. coli* O157:H7 (Figure 2). Furthermore, while *E. coli* O157:H7 was detected in this sample group, no *Listeria* was detected.

Pathogen prevalence in samples obtained from the lakeshore markets, Blantyre markets and Mbayani homes was compared, as there appeared to be a general increase in prevalence of pathogen contamination as the fish traveled from the Lake shore to the markets and homes (Figure 2). A Chi-square test for prevalence of *Salmonella* indicated a significant increase in prevalence of *Salmonella* with location ($P < 0.05$), with samples obtained from the lakeshore

being less likely to be contaminated with *Salmonella* compared to those obtained from Blantyre or Mbayani. However, no statistically significant differences were noted for prevalence of *Staphylococcus* and *E. coli* O157:H7 in fish samples obtained from the different locations.

Figure 2. Prevalence of *Salmonella* spp., *Staphylococcus aureus*, *E. coli* O157:H7 in lakeshore markets, Blantyre markets and Mbayani households.



The prevalence of *Salmonella* spp. and *Staphylococcus aureus* in raw/market samples and cooked fish samples is summarized in Figures 3 and 4, respectively. The prevalence of *Salmonella* spp. and *Staphylococcus aureus* did not differ between raw and cooked samples.

Figure 3. Prevalence of *Salmonella* spp. in raw and cooked fish samples.

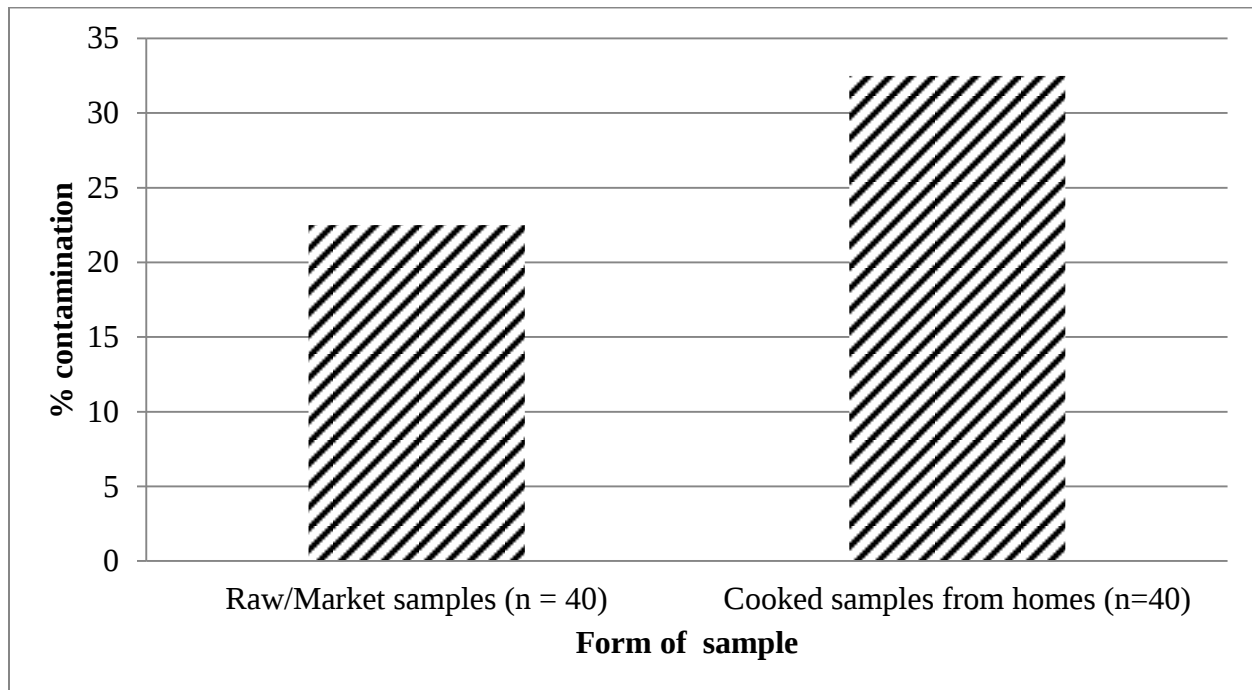
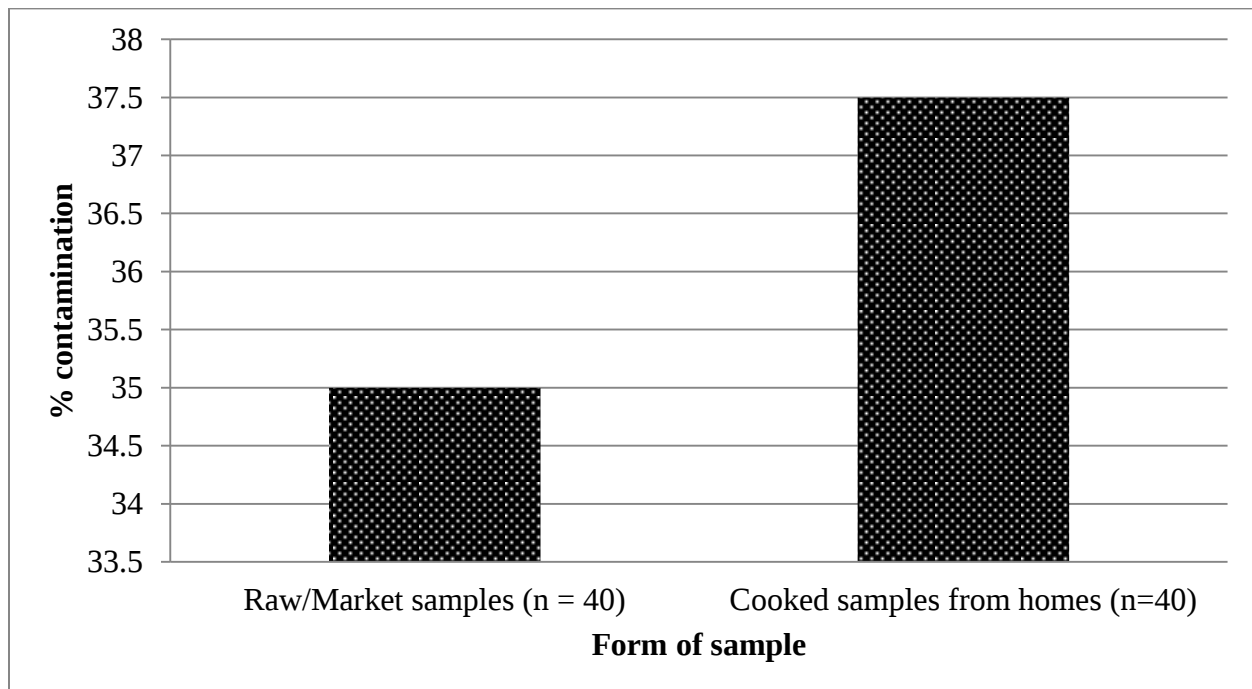


Figure 4. Prevalence of *S. aureus* in raw and cooked fish samples.

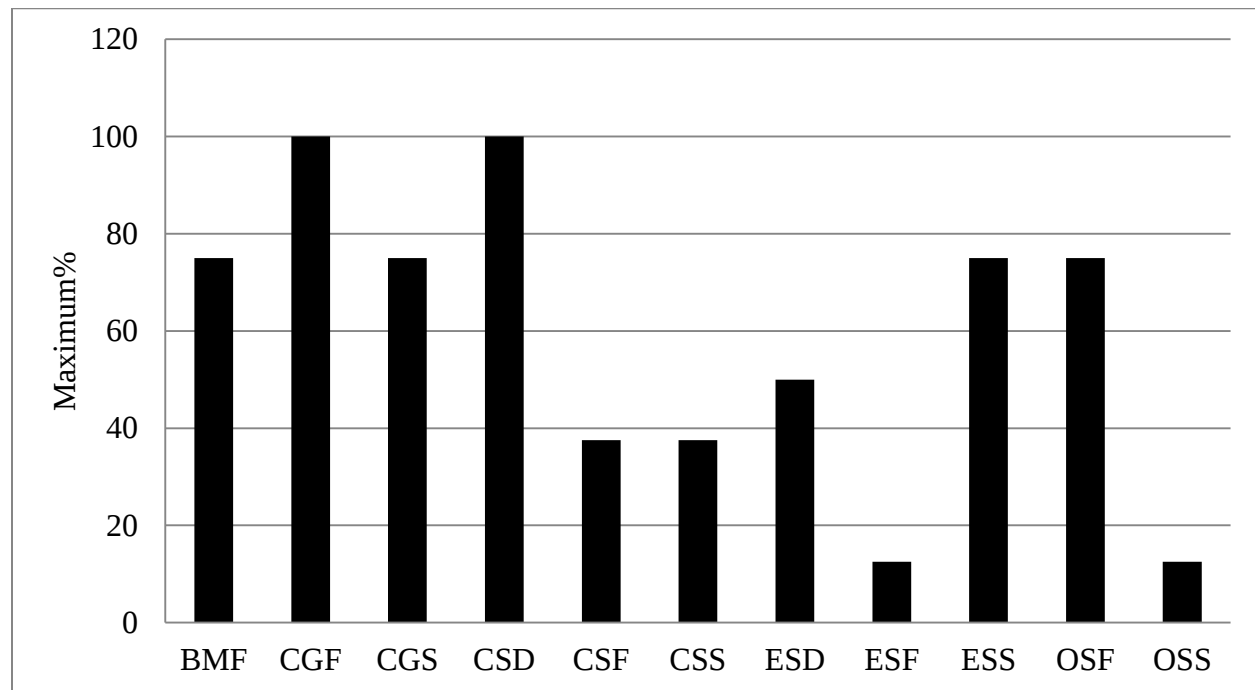


The percentages of fish samples within each species and treatment group that were contaminated with pathogens are presented in Table 5 and Figure 5. In summary, CGF and CSD were the most contaminated form with 100% of samples contaminated while OSS and ESF had the lowest levels of contamination (13%).

Table 5. Summary of contamination in all samples.

ID	no of samples	<i>Salmonella</i>	<i>Staphylococcus</i>	<i>E. coli</i> 0157:H7	Double contamination	% contamination
BMF	4	2	1	0		75
CGF	4	2	3	0	1	100
CGS	8	4	3	0	1	75
CSD	8	5	4	0	1	100
CSF	8	1	2	0		38
CSS	8	1	2	0		38
ESD	8	1	3	0		50
ESF	8	0	1	0		13
ESS	8	1	6	0	1	75
OSF	8	4	3	1	2	75
OSS	8	1	1	0	1	13

Figure 5. Percentage of fish samples (by species and preservation method) containing bacterial pathogens.



5.3 Fish handling and storage practices in markets and food processors

Fish handling and storage practices in markets and by food processors were documented by using a questionnaire tool (Appendix B) and by observations targeting fish sellers, processors and fishermen in their respective places of business. The questionnaires were completed by conducting direct interviews of the subjects and noting practices as described by the subjects or as observed by the researcher. The respondents also provided samples for microbiological analysis.

Twenty interviews were completed for the lakeshore markets and twenty interviews for Blantyre markets. It should also be noted that for the lakeshore, there was interrelation of activities such as fishermen being involved in fish drying and smoking and furthermore others would transport their products from Lakeshore to Blantyre markets (especially Limbe market),

an activity that is also done by middlemen. When fish arrived at Limbe market it was typically sold to Blantyre market fish retailers who would finally sell to the consumer.

At the lakeshore, it was observed that fish was generally caught during the night and fish landing times were mainly in the morning around 5:30 am to 7:00 am. Once the fish was caught, a portion was often sold to middlemen who would preserve it in ice and transport to inland markets including Blantyre. Alternatively the fisherman would carry out the preservation themselves and sell to inland markets. Some fresh fish would enter the preservation process (sundrying or smoking) and this was also done by either the fishermen who caught the fish or by middlemen who could purchase fresh fish from fishermen. Preservation by sundrying would take about 2 to 3 days while smoking was completed within 1- 2 h. However, the smoking process would continue for 1-3 days until the processor had adequate volumes for sale. Once enough volume was achieved for both dried and smoked fish the processor would either transport and sell the fish to inland markets themselves or he might decide to sell it to a middleman who would transport it to inland markets including Blantyre.

Fishermen, processors and sellers (n =20) were asked to provide information on the time required for the fish (fresh, smoked, and dried) to be transported from the lakeshore market to Blantyre market (Limbe) once the fish was made ready. Sixty-five percent of smoked fish was transported to Limbe market on the same day (Table 6). Responses for sundried fish indicated that 65% transported the fish to Limbe market at least two days after drying (Table 7).

For fresh fish, 95% of the respondents indicated that they take their produce to the lakeshore market on the same day while 5% sold their produce on the second day (Table 8).

Table 6. Time taken for smoked fish to reach market.

Location		Number of days taken for smoked fish to reach market			Total
		1	2	4	
Lakeshore markets	Respondents	13	6	1	20
	% of respondent	65.00%	30.00%	5.00%	100.00%
Blantyre markets	Respondents	17	3	0	20
	% of respondent	85.00%	15.00%	0.00%	100.00%

Table 7. Time taken for sundried fish to reach market.

Location of respondent		Number of days taken for sundried fish to reach market				Total
		1	2	3	7	
Lakeshore markets	Respondents	8	5	4	3	20
	% respondents	40.0%	25.0%	20.0%	15.0%	100.0%
Blantyre markets	Respondents	14	6	0	0	20
	% of respondents	70.0%	30.0%	0.0%	0.0%	100.0%

Table 8. Time taken for fresh fish to reach market.

Location of respondent		Number of days taken to get fresh fish to the market		Total
		1	2	
Lakeshore markets	Respondents	19	1	20
	% of respondents	95.0%	5.0%	100.0%
Blantyre markets	Respondents	17	3	20
	% of respondents	85.0%	15.0%	100.0%

The responses from survey participants in Blantyre were slightly different from those of lakeshore sellers. Eighty-five percent of Blantyre sellers (n=20) brought their smoked fish to the market on the same day while the remaining 15% waited until the following day. For fresh fish, 85% of the respondents indicated that they begin selling their produce on the same day they purchase it while 15% didn't begin to sell until the second day. Sale of sundried fish began on the same day for 70% of the sellers, while 30% waited until the following day.

The time it took for all procured fish products to be sold from the market was also documented for both lakeshore sellers (n=20) and Blantyre sellers (n=20). Sixty-five percent of smoked fish for lakeshore sellers was sold on the same day it was procured while interviews revealed that the remaining 35% would take up to 5 days to be completely sold. Fresh fish sellers indicated that 95% of fish would be sold within 24 h whereas 5% indicated that sales of fresh fish could continue for up to 48 h. Sundried fish was sold less quickly, with only 10% sold on the same day it was procured while the rest would remain on the market for up to 5 days.

Interviews with Blantyre sellers indicated that same day sale occurred for 85% of fresh and smoked fish sold in Blantyre markets and for 70% of sundried fish. All fresh fish was sold within 2 days. Smoked fish could stay on the market for up to 5 days. The volumes sold by Blantyre sellers were observed to be smaller as compared to their lakeshore market counterparts, which likely contributed to the reduced time required to sell all products.

A summary of the maximum time taken for fish to reach consumers in Blantyre are presented in Table 9. Fresh fish took a maximum of 4 days while sundried and smoked fish took a maximum of 13 and 14 days, respectively.

Table 9. Summary of maximum time taken for each fish form to reach consumer once caught.

Form	Fishing hours	Processing (drying / smoking)	Lakeshore Markets	Blantyre Markets	Max time to reach Consumer
Fresh	12		1day	1 -2 days	4 days
Smoked	12	1-2days	1-5days	1- 5 days	13 days
Sundried	12	2-3days	1-5days	1-5days	14 days

5.3.1 Storage practices

Common storage utensils for Lakeshore and Blantyre sellers were also analyzed. The summary is presented in Table 10. Canoes and basins were common for fishermen who would leave their fish in canoes waiting for buyers. Lakeshore middle men mainly used cartons (45%) for smoked and dried fish, and wooden or plastic cooler boxes (20%) for fresh fish. It was also noteworthy that 20% of respondents who stored sundried fish indicated that they store their products on the floor.

Fresh fish in Blantyre was commonly stored in Styrofoam or plastic cooler boxes (40%). Respondents for sundried and smoked fish mainly mentioned cartons (30%) and plastic bags (30%). Moreover, it was discovered that Limbe market fish sellers had special lockable cupboards within the market where they would store their product after the market day while their equivalents in Mbayani would store the remaining product at their homes.

Table 10. Common fish storage utensils in markets.

Location of respondent		Fish storage aftermarket day					Total
		Cartons	Plastic bags	Cooler boxes	Sale all	Floor	
Lakeshore markets	Count	9	0	4	3	4	20
	% of respondents	45%	0%	20%	15%	20%	100%
Blantyre markets	Count	6	6	8	0	0	20
	% of respondents	30%	30%	40%	0%	0%	100%
	% of Total	15%	15%	20%	0%	0%	50%

5.3.2 General market conditions

The general market conditions which included source of water at the market and use of proper bench (e.g. a table or other elevated display for the fish) were also documented and are summarized in Tables 11 and 12.

Table 11. Sources of water at markets.

Location of respondent		Source of water at the market			Total
		Borehole	River or Lake	Potable water	
Lakeshore markets	Count	11	9	0	20
	% of respondents	55%	45%	0%	100%
Blantyre markets	Count	0	0	20	20
	% of respondents	0%	0%	100%	100%

Table 12. Use of proper bench at markets.

Location of respondents		Use of proper display bench		Total
		Yes	No	
Lakeshore markets	Count	13	7	20
	% of respondents	65%	35%	100%
Blantyre markets	Count	11	9	20
	% of respondents	55%	45%	100%

Analysis of the source of water (Table 11) for lakeshore market sellers (n=20) revealed that 55% used borehole water while 45% used water from the lake to clean their utensils and fishing equipment. On the other hand, all respondents from Blantyre indicated that they used potable water for cleaning of their utensils.

General assessment of the market conditions indicated that all markets observed in this study, either at the lakeshore or Blantyre, had a separate fish section where fish was sold separately from other products. All markets observed were exposed to animals. In addition it was observed that in all markets the sellers would use their hands to display fish or arrange fish. Sixty five percent of lakeshore sellers used proper benches to display their products while 35% used a mat on the floor (Table 12). In Blantyre, 55% of the sellers used proper benches while the remaining 45% used a mat on the floor.

5.4 Food preparation and handling practice in the home

Common food preparation and handling practices in Mbayani homes and their association with pathogen contamination were documented and are summarized in Table 13.

Table 13. Distribution of food preparation and handling practices in the homes and incidence of all pathogens.

Kitchen utensils	Frequency	Percent	Incidence of all pathogens/frequency
Winnowing basket and knife	12	30	7 (58)
Plastic basin and knife	14	35	11 (78)
winnowing basket, basin and knife	14	35	11 (78)
Washing of hands			
Before preparation of food	40	100	29 (73)
After visiting toilet	40	100	29 (73)
After changing a child's diaper	40	100	29 (73)
Washing with soap	40	100	29 (73)
Sources of water			
Water kiosk	29	72	22 (76)
Taps within reach	11	28	7 (64)
Method of cleaning utensils			
Cold water and soap	35	88	24 (68)
Hot water and soap	3	8	3 (100)
Cold water only	2	5	2 (100)

The common kitchen utensils used for food preparation by survey respondents in Mbayani Township homes (n=40) were distributed in this order (Table 13); 35% used plastic basins and a knife, 35% used plastic basins, winnowing baskets and knives, and the remaining 30% used winnowing baskets and a knife. All respondents (100%) indicated that they wash their hands with soap before food preparation, after visiting a toilet, and after changing a child's diapers.

When questioned about sources of water for cleaning utensils and cooking, all respondents (n=40) used portable water supplied by the Blantyre Water Board. However, it is revealing to note that 73% used strategically positioned water kiosks while 27% had water taps within their reach. For cleaning utensils, 87% indicated that they used cold water and soap while 5% used only cold water with no soap.

5.4.1 Food storage practice

Interviews with consumers about their food storage practices (Table 14) revealed that 95% of the homes stored leftover fish for 1 to 2 days with the majority (95%) keeping it for only one day. It was further revealed that 87% of the respondents stored their leftover food at ambient temperature while the remaining 13% had refrigerators.

Table 14. Distribution of food storage practice and incidence of all pathogens in the homes.

Storage of left over fish	Frequency	Percent	Incidence of all pathogens/frequency
Yes	38	95	27 (71)
No	2	5	2 (100)
Time food storage (days)			
One day	36	95	26 (72)
Two days	2	5	1 (50)
Place of food storage			
Ambient	33	87	24 (73)
Refrigerator	5	13	3 (60)

5.4.2 General condition of the home

Assessment of the place of food preparation (Table 15) showed that only 22% (9/40) of the homes had a designated kitchen while 78% (31/40) did not. When those without a designated kitchen were questioned further, consumers in 60% of homes mentioned using either the verandah or preparing food in the open and 18% mentioned using the living room. Assessment of animal exposures found that 35% of the homes kept either chickens, dogs or cats. Some respondents mentioned keeping chickens in their living rooms or store rooms.

Table 15. General condition of the home.

	Frequency	Percent	Incidence of pathogens /frequency
Place of food preparation			
Designated kitchen	9	22	6 (67)
Verandah	21	52	15 (71)
In the open	3	8	3 (100)
In the living room	7	18	5 (71)
Exposure to animals			
Yes	14	35	10 (71)
No	26	65	19 (73)
Exposure to children with diapers			
Yes	22	55	16 (73)
No	18	45	13 (72)
Person responsible for cooking			
Adult	40	100	29 (73)
Person responsible for cleaning cooking utensils			
Adult	39	98	28 (72)
Child	1	2	1 (100)
Illness associated with fish for past 3 months			
Yes	13	32	9 (69)
No	27	68	20 (74)

In all the homes visited (n=40), food preparation was conducted by female adults, while only one household involved children in cleaning of kitchen utensils. It was also noted that 55% of the homes visited had children in diapers. When consumers were questioned about previous

foodborne illnesses that they associated with fish consumption, 33% of the interviewees (13/40) indicated that persons in their homes had been affected by such an illness in the previous 3 months.

5.4.3 Relationship between practice and level of contamination

The relationship between level of pathogen contamination with practices (incidence of pathogens/frequency of respondents) was calculated for the homes (refer to Tables 13, 14 and 15). For food preparation and handling practices, the common use of plastic basin and knife and use of winnowing baskets, plastic basin and knife contributed 11 (78%) incidences each. Despite all respondents mentioning that they wash hands regularly before food preparation, after visiting the toilet, and after changing a child's diapers, 29 (73%) incidences were observed in this category. Use of water kiosks was associated to 22 (76%) incidences compared to water taps within reach which was associated to 7 (64%) incidences. Mode of cleaning of kitchen utensils revealed use of hot water and soap and use of cold water only to be associated to 3 (100%) and 2 (100%) incidences respectively compared to use of cold water and soap which was associated to 24 (68%) incidences.

For food storage practice, storage of left over fish was associated to 27 (71%) incidences. Storage of leftover food at ambient temperature was associated to 24 (73%) incidences compared to use of refrigeration with 3 (60%) incidences.

A general assessment of the homes revealed that observed practices generally were not significantly associated with incidences of pathogens. Homes that prepared food in a designated kitchen had a 67% incidence of pathogen contamination in the fish that was sampled, whereas homes preparing food on the verandah, in the open, or in the living room had pathogen

incidences of 71, 100 and 71%, respectively. For homes which were exposed to animals, 10 (71%) incidences were observed though homes with no animals also revealed high incidences of pathogens (13 incidences; 73%). Homes with children in diapers were associated to 16 (73%) incidences which was not different compared to homes which did not have children in diapers (13 incidences; 72%). Overall, few observed practices in the homes were correlated with increased incidence of bacterial pathogens in fish sampled from the homes.

The relationship between practices and incidence of pathogens for both lakeshore and Blantyre markets is summarized in Table 16. Lack of proper benches/display cases at lakeshore markets contributed to 4 (57%) incidences of pathogens while in Blantyre markets it contributed to 6 (57%) pathogen incidences. In Blantyre markets presence of a proper display bench did not seem to reduce the incidences as this was associated to 10 (91%) incidences.

Table 16. Relationship between practice and incidence of pathogens in lakeshore and Blantyre Markets.

		Frequency	Percentage	Incidence of pathogens/frequency (%)
Sources of water				
Lakeshore	Borehole	11	55	4 (36)
	River or lake	9	45	3 (33)
Blantyre	Potable water	20	100	16 (80)
Use of proper bench				
Lakeshore	Yes	13	65	3 (23)
	No	7	35	4 (57)
Blantyre	Yes	11	55	10 (91)
	No	9	45	6 (67)
Fish storage utensils				
Lakeshore	Carton (boxes)	9	45	5 (56)
	Cooler boxes	4	20	0 (0)
	Sale all	3	15	0 (0)
	floor	4	20	1 (25)
Blantyre	Carton (boxes)	6	30	5 (83)
	Plastic bags	6	30	3 (50)
	Cooler boxes	8	40	8 (100)

For fish storage utensils, use of carton boxes at lakeshore markets was associated with 5 incidences. On the other hand at Blantyre markets cooler boxes were associated with 8 (100%) incidences followed by carton boxes (5 incidences; 83%) and plastic bags (3 incidences; 50%).

6 DISCUSSION

It is noteworthy that fish species in this study showed significant concentrations of histamine despite the species sampled in this study being generally considered as not susceptible to scombrototoxin formation. A study by Auerswald et al. (2006) found 399 mg/kg histamine in yellowtail, a non scombroid fish. Most studies have correlated increased storage time and higher temperatures with increased histamine concentrations (Dawood et al., 1988; FDA, 1998; Auerswald et al., 2006). Thus, the high histamine values observed in this study are indicative of temperature abuse of raw fish during the initial stages of storage and preservation. In the area under study, it was observed that most fresh fish were sold at ambient temperatures. While fresh fish could be transported on ice, it was exposed at ambient temperature for many hours during sale. This observation may explain the higher histamine values observed in ESF in this study.

Furthermore, it was observed that some fish processors opted to preserve their catch by either sun drying or smoking as a second option if fresh fish did not seem to fetch good money in the market or if they had unsold fish by close of the market day. This suggests that high histamine concentrations observed in processed fish could have developed during initial exposure during presentation for sale as fresh fish. Secondly, preservation by sun drying is a slow process, is weather dependent, and can require two to three days even in ideal weather conditions in Malawi. Thus, high histamine levels observed in ESD, ESS and CSD likely could have developed during their preservation processes. Long transit times from the catch, through preservation to the market could also influence changes in histamine concentrations in the fish. Histamine concentrations greater than 500 mg/kg which were observed in ESD are of concern as such concentrations are reported to cause sickness (Taylor, 1989; FDA, 1998). Since this study did not look at the other parameters that influence histamine development in fish, such as free

histidine content or presence of microorganisms responsible for histamine formation, there is a need to carry out these tests for conclusive results in the species studied.

The presence of *Salmonella* spp., *Staphylococcus aureus* and *E. coli* O157:H7 in the species studied is of concern to human health. These microorganisms enter the food chain by cross contamination during handling, processing, and sale. *Salmonella* spp. is commonly associated with poultry (Adams and Moss, 2008; Parry et al., 2002). The presence of *Salmonella* spp. suggests possible cross contamination between poultry and food products. All the markets visited for this study had a separate section for sale of fish, but the poultry section in these markets where live chickens were sold were often close enough (about 3 to 5 meters) for cross contamination to occur (insects, wind, etc). Furthermore, cross contamination could have been caused by buyers in the market who would visit both the poultry and fish sections. In this study it was observed that both sellers and buyers frequently handled fish with bare hands, so this could be a source of cross contamination especially for those buyers who have touched poultry and fish products. Buyers were frequently seen using hands to select fish while sellers were also using bare hands to sort their product. In addition, poor sanitary handling of fish could be another factor of concern. Kapute et al., (2013) observed that unsanitary handling practices and conditions at the local markets were the reasons for occurrences of both spoilage and disease causing microorganisms in fresh Chambo sold in Malawian markets.

The significantly lower prevalence of *Salmonella* spp. observed in lakeshore markets compared to those in Blantyre suggest that cross contamination between poultry (or other *Salmonella* sources) and fish was less common in lakeshore areas. It was observed in this study that most fish products were sold in bulk in lakeshore markets and at this stage of sampling the fish were only handled by fishermen and processors. For instance, fresh fish was sold directly

from the canoe to the dealers and traders. In comparison, fish sold in Blantyre had been subjected to handling by middlemen and exposed during transportation from the lakeshore to Blantyre. The retailing practices used by traders in Blantyre also allowed exposure to contamination by flies and other fomites that could be a source of bacterial pathogens. Lack of proper benches in Blantyre markets could also be another factor contributing to higher pathogen prevalence as it was observed that 45% of fish sellers did not have an elevated bench (i.e. at least a table) compared to 35% observed in lakeshore markets. Those persons who lacked benches typically kept their fish on the floor in the market.

Observation of food handling practices in homes found common usage of winnowing baskets (68%) which are made of wood, keeping of chickens in the homes (35%), keeping of food at ambient temperature (87%), and use of contaminated raw products from the market as some of the risk factors potentially leading to the high prevalence of *Salmonella* in the home. Use of wooden food contact materials such as winnowing baskets and common usage of plastic basins are considered potential risk factors as wooden and scratched plastic basins are reported to absorb and retain viable bacteria (Kohl et al., 2002). Other studies (Parry et al., 2002; Kohl et al, 2002; Mitakatis et al, 2004) identified handling of free range chickens as significant risk factors associated with *Salmonella*. Furthermore, keeping contaminated food at ambient temperatures would likely result in rapid proliferation of the microorganisms resulting in the high prevalence of *Salmonella* observed in this study.

High prevalence of *Staphylococcus aureus* in markets and homes is indicative of poor personal hygiene and temperature abuse of fish and fish products (Le Loir et al., 2003). *Staphylococcus* spp. is known to inhabit the nose and skin of human beings (Adams and Moss, 2008). Common use of hands at the market places and improper washing of hands by individuals

involved in preparation of food in the homes are the likely factors for the observed prevalence of *Staphylococcus aureus*. Despite all respondents mentioning that they wash hands regularly, it could be that hands were not sufficiently washed to reduce bacterial populations. In addition, sneezing and coughing during food preparation and storage of food at ambient temperature (87%) in homes could also be sources of contamination as observed by Sudershan et al. (2012) in studying microbiological hazard identification and exposure assessment of poultry products sold in various localities of Hyderabad, India.

The occurrence of *E. coli* O157:H7 in one cooked fish sample from a home in Mbayani suggests further compromise in sanitation practices.

The high prevalence of *Salmonella* and *Staphylococcus* observed in CGF (fresh Mlamba), CSD (dried Utaka), OSF (fresh Chambo), and ESS (smoked Usipa) is striking. In this study, the higher prevalence observed in the “dried” products (sundried and smoked) is supported by considering the time taken for those products to be ready for the market. Because of reduced perishability, processed fish were kept relatively longer in the market (up to 14 days before sale) while fresh fish were sold within a maximum of 4 days. It is therefore suggested that a long processing and storage period increased chances for contamination for CSD and ESS.

Since the study found no significant difference in prevalence of *Salmonella*, *Staphylococcus* and *E. coli* O157:H7 between raw fish (from the markets) and cooked fish (from homes), this suggests either that the fish was not cooked properly to destroy pathogens and/or that cooked fish was rapidly re-contaminated after the cooking process in most homes. Further study is necessary to assess the specific reasons for the very high frequency of pathogen contamination in cooked fish products in this study.

Correlations between handling/processing practices and the incidence of pathogens in homes showed no clear distinctions between practices and their associated contribution to incidence of pathogens. However, it is clear that some practices identified in this study such as keeping of animals, lack of designated kitchens, distant water supplies, and storage of food at ambient temperature could contribute to cross contamination and proliferation of pathogenic microorganisms. Similarly, for the lakeshore and Blantyre markets, lack of proper benches, sale of fish in the open at ambient temperature, and poor storage utensils/facilities could be contributing factors for the incidences observed despite no distinct association with pathogen incidence being identified with these practices.

7 SUMMARY AND CONCLUSIONS

In this study, it has been demonstrated that there is a possibility of histamine being present in fresh, smoked and sundried fish species under study and that temperature abuse during sale, handling, preservation and processing are likely causes of the observed high histamine concentrations. Histamine levels observed in ESD are alarming as they have a potential to cause human illness. Future research should assess other parameters associated with histamine formation such as free histidine concentrations and presence of histamine forming bacteria in the fresh fish species that were most susceptible to histamine contamination.

This study has demonstrated that contamination of fish sold at lakeshore markets and Blantyre markets with *Staphylococcus* and *Salmonella* is common. The prevalence of *Salmonella* was observed to be significantly higher in Blantyre markets compared to lakeshore markets. In addition, the fish appeared to be more contaminated as it moved from the source (Lake Malawi) to homes. Based on the high prevalence of pathogens in cooked fish sampled from Mbayani Township homes, we consider recontamination of cooked fish to be common.

In this study no clear correlations between practices and incidence of pathogens could be identified. However, the study has revealed some practices which were generally associated with cross contamination and rapid growth of microorganisms in both the markets and homes.

APPENDICES

APPENDIX A

QUESTIONNAIRE FOR ASSESSING HOME FOOD HANDLING AND PREPARATION PRACTICES AND STORAGE PRACTICES IN RURAL HOMES OF MALAWI

Part A: Food handling practice

Q1. What type of utensils do you use for food preparation?

		Check here
A	Winnowing baskets and knife	
B	Plastic basin and knife	
C	Metallic basin and knife	
D	Hands and knife	
E	Hands only	

Q2. Do you wash your hands at the following times? Please check all that apply.

		Yes	No
A	Before food preparation?		
B	After using the toilet?		
C	After changing child's diapers?		
D	With soap?		

Q3. How do you clean your kitchen utensils?

		Check here
A	Cold water and soap	
B	Hot water and soap	
C	Cold water only	
D	Hot water only	
E	Wiping only	

E)Other.....

.....
.....

Q4. What is the source of water for cleaning of your utensils?

		Check here
A	Protected well	
B	Unprotected well	
C	Borehole	
D	River or Lake	

Q5. What is the source of water for cooking?

		Check here
A	Protected well	
B	Unprotected well	
C	Borehole	
D	River or Lake	

Part B: Storage Practice

Q6. Do you store left over fish and other foods?	Yes	No
--	-----	----

If no skip question 7 and 8

Q7. How long is that food stored?

		Check here
A	1 day	
B	2 days	
C	More than 2 days	

Q8. How is the food stored?	Answer here
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Part C: Condition of the home

Q9. Where do you prepare your food?

		Check here
A	Separate kitchen	
B	Verandah	
C	In the open	

Q10. Are you exposed to the following?

		Yes	No
A	Domesticated animals		
B	Children with diapers		

Q11. Have you or your family member got sick suspected to be due to consumption of fish?	Yes	No
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Q12. Who is responsible for cooking of food in your home?	Answer here
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Q13. Who is responsible for cleaning of cooking utensils in your home?	Answer here
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APPENDIX B

QUESTIONNAIRE FOR ASSESSING FISH HANDLING AND STORAGE PRACTICES IN RURAL FISH MARKETS AND PROCESSORS

Part A: Fish handling at the market

Q1. How long does it take to get the fish to the market on average?

	Hours or days (Fill below)
A. Smoked fish	
B. Fresh fish	
C. Dried fish	

Q2. On average, how long does the fish stay on the market before being sold to a consumer?

	Number of storage hours or days (Fill below)
A. Fresh fish	
B. Smoked fish	
C. Dried fish	

Q3. How is the fish stored after market-day?

.....

.....

.....

Q4. What is the source of water for cleaning of your utensils at market?

		Check here
A	Protected well	
B	Unprotected well	
C	Borehole	
D	River or Lake	

E)Other.....

.....

.....

Q5. General market conditions: please check appropriately.

		Check below	
A.	Separate fish section	Yes	No
B.	Exposure to flies/ rodents or other animals	Yes	No
C.	Use of hands to display, arrange/select fish	Yes	No
D.	Proper display bench	Yes	No

E)Other.....

.....

.....

Part B: Fish handling during processing

Q6. How long does it take to sell the fish after catch or preservation?

	Time
A. Fresh	
B. Smoked	
C. Dried	

Q7. How do you store the fish awaiting buyers?

.....

.....

.....

Q8. What is the source of water for cleaning your utensils during processing or preservation?

		Check here
A.	Protected well	
B.	Unprotected well	
C.	Borehole	
D.	River or Lake	

E. Other.....

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REFERENCES

REFERENCES

Adams.M.R.and Moss.M.O. 2008. Food Microbiology. Third edition. Cambridge. Royal Society of Chemistry. Pg 235-257.

Auerswald L., Morren C., and Lopata L.A. 2006. Histamine levels in seventeen species of fresh and processed South African sea food. Journal of Food Chemistry 98:231-239.

Australian Food Standards Code. 2001. Part D: Fish and fish products. Standards D1 and D2. Version 18.

Bjeldanes L.F., Schultz M.M., and Morris M.M. 1978. On the aetiology of scombroid food poisoning: Cadavarine potentiation of histamine toxicity in the guinea pig. Food cosmetic toxicology.16:157-159.

CDC. 1990. Foodborne disease outbreaks, 5-year summary, 1983-1987. (Accessed 2013 December 8). Available from: www.cdc.gov/mmwr/preview/mmwrhtml/00001597.htm

CDC.1996. Surveillance for Foodborne-Disease Outbreaks -- United States, 1988-1992 (Accessed 2013 December 8). Available from: www.cdc.gov/mmwr/preview/mmwrhtml/00044241.htm

CDC.2000. Surveillance for Foodborne Disease Outbreaks --United States, 1993-1997. Accessed 2013 December 8). Available from: www.cdc.gov/mmwr/preview/mmwrhtml/ss4901a1.htm

CDC.2013. Surveillance for foodborne outbreaks – United States 1998 -2008. (Accessed 2013 December 8). Available from: www.cdc.gov/mmwr/preview/mmwrhtml/ss6202a1.htm?s_cid=ss6202a1_w

Chakrabarti R. 1998. Shelf-life and histamine content of ten species of fish stored at tropical ambient temperature. J. Food Sci. Technol. 35:62-5.

Chimatiro S.K. 1998. Aquaculture production and potential for food safety hazard in sub Saharan Africa: with a special reference to Malawi. *Int. J. Food Sci. Technol.* 33:169-176.

Chytiri S., Paleologos E., Savvaidis I., and Kontominas M.G. 2004. Relation of biogenic amines with microbial and sensory changes of whole and filleted freshwater rainbow trout (*Onchorynchus mykiss*) stored on ice. *J. Food Protect.* 67:960-965.

Çoban O.E., and Patir B. 2008. Investigation of histamine level and some chemical quality parameters in fish consumed in Elazig City. *Int. J. Sci. Technol.* 3:59-65.

Dawood A., Karkalas J., Williams S.C., and Roy N.R. 1988. The occurrence of non-volatile amines in chilled stored Rainbow Trout (*Salmo irideus*). *J. Food Chem.* 27:33-35.

De Waal C., and Robert N. 2005. Food safety around the world. Center for science in the public interest. Washington, DC. Pg 23-29.

Doyle, M. 1991. *Escherichia coli* O157 H7 and its significance in foods. *Int. J. Food Microbiol.* 12:289-301.

EC 2003. Commision recommendation of 10 January 2003 concerning a coordinated programme for the official control of foodstuffs for 2003 (2003/10/EC). Official Journal of the European Commission 7:76-81.

Est.ryukoku [internet].ryukoku 2001. [Accessed 2012 May 31]. Available from:www.est.ryukoku.ac.jp/est/yuhma/FLM-pamph

FAO. 2012. Meeting Report: Joint FAO/WHO Expert Meeting on the Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products. 23-27 July 2012. FAO Headquarters, Rome, Italy.

FAO/WHO. 2005. Conference Proceedings on Food Safety for Africa. Harare, Zimbabwe, 3-6 October 2005. Situation analysis of food safety systems in Malawi. Agenda 1

FDA. 1998. FDA and EPA guidance levels. In fish and fishery products hazards and controls guide (pp. 245–248, Appendix 5). Department of Human Health and Human Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, Washington, DC.

FDA. 2011. Fish and fishery products hazards and controls guidance. (Accessed 2012 May 31). Available from: www.usfda.gov

Frank H.A., Yoshinaga D.H., and Nip W. 1981. Histamine formation and honeycombing during decomposition of skip jack tuna, *Katsuwonus pelamis* at elevated temperatures. *Marine Fisheries Review* 43:9-14.

Hwang D.F., Chang S.H., Shiua C.Y., and Chai T. 1997. High-performance liquid chromatographic determination of biogenic amines in fish implicated in food poisoning. *Journal of Chromatography B*. 69:23-30.

Kapute, F., Likongwe, J., Kang'ombe, J., Kiiyukia, C., and Mpeketula, P. 2012. Quality assessment of fresh Lake Malawi tilapia (Chambo) collected from selected local and super markets in Malawi. *Internet Journal of Food Safety*.14: 112-120

Kohl, K.S., Rietberg, K., Wilson, S., and Farley, T.A. 2002. Relationship between home food-handling practices and sporadic salmonellosis in adults in Louisiana, United States. *Epidemiology and Infection* 129:267-276.

Le Loir, Y., Baron, F., and Gautier, M. 2003. *Staphylococcus aureus* and food poisoning. *Genet Mol. Res.* 2:63-76.

Lehane L., and Olley J. 2000. Histamine food poisoning revisited. *J. of Food Microbiol.* 58:1-37.

Lokuruka M.N. 2009. Scrombotoxicosis in African fisheries-its implications for international fish trade: An overview. *African Journal of Agriculture Nutrition and Development* 9:1617-1634.

Mitakakis T.Z., Wolfe R., Sinclair M. I., Fairley C.K., Leder K., and Hellard M.E. 2004. Dietary intake and domestic food preparation and handling as risk factors for gastroenteritis: a case-control study. *Epidemiology and Infection*, 132:601-606.

Mumba P.P., and Jose M. 2005. Nutrient composition of selected fresh and processed fish species from Lake Malawi: A nutritional possibility for people living with HIV/Aids. *Journal of Consumer Studies* 29:72-77.

National Statistics Office, 2011. Malawi Health and Demographic Survey 2010 Preliminary Report . Pg 15-24.

Parry S.M., Palmer S.R., Slader J., and Humphrey T. 2002. Risk factors for *Salmonella* food poisoning in the domestic kitchen-a case control study. *Epidemiology and Infection*, 129:277-285.

Redmond C.E. and Griffith C.J. 2003. Consumer food handling in the home: A review of food safety studies. *J. Food Protect.* 66:130-161.

Rezaei M., Montazeri N., Langrudi H.E., Mokhayer B., Parviz M., and Nazarinia A. 2007. The biogenic amines and bacterial changes of farmed rainbow trout (*Oncorhynchus mykiss*) stored in ice. *Food Chemistry* 103:150-154.

Shalaby A. 1996. Significance of biogenic amines to food and human health. *Food Research International* 29:675-691.

South African Bureau of Standards. 2001. Regulations governing microbiological standards for foodstuffs and related matters. Government Notice No. R 490.

Tsai, Y. H., Lin, C. Y., Chien, L. T., Lee, T. M., Wei, C. I., and Hwang, D. F. (2006). Histamine contents of fermented fish products in Taiwan and isolation of histamine-forming bacteria. *Food Chemistry* 98(1): 64-70.

Sudershan R.V., Naveen Kumar R., Kashinath L., Bhaskar V., and Polasa K. 2012. Microbiological hazard identification and exposure assessment of poultry products sold in various localities of Hyderabad, India. *The Scientific World Journal*.2012:1-7

Taulo S., Wetslen A., Abrahamsen R., Kululanga G., Mkakosya R., and Grimason A. 2008. Microbiological hazard identification and exposure assessment of food prepared and served in the rural households of Lungwenya, Malawi. *Int. J. Food Microbiol.* 125:111-116.

Taylor S.L. 1986. Histamine food poisoning: Toxicology and clinical aspects. *Critical Reviews in Toxicology* 17:91-128.

Unusan N. 2007. Consumer food safety and practice in the home in Turkey. *Journal of Food Control* 18:45-51.

Veciana-Nogues M.T., Marine-Font A., and Vadal-Carou M.C. 1997. Biogenic amines as hygienic quality indicators of tuna. Relationships with microbial counts, ATP-related compounds, volatile amines, and organoleptic changes. *J. Agric. Food Chem.* 45:2036-2041.

Weyl O.L.F., and Weyl M.V. (editors). 2001. Lake Malawi fisheries management symposium proceedings; 2001 June 4-9; Lilongwe, Malawi. Organizing Committee of the Lake Malawi Fisheries Management Symposium, Lilongwe, Malawi, pg 95-104.

WHO. 2013. Countries statistics: Malawi. (Accessed 2012 May 31). Available from: www.who.int/countries/mwi/en/

Yatsunami, K., and Echigo, T. (1991). Isolation of salt tolerant histamine-forming bacteria from commercial rice-bran pickle sardine. *Bulletin of the Japanese Society of Scientific Fisheries.* 57, 1723–1728.

Yung-Hsiang T., Chueh-Yueh L., Liang-Tan C., Tsong-Ming L., Cheng-I W., and Deng-Fwu H. 2006. Histamine contents of fermented fish products in Taiwan and isolation of histamine-forming bacteria. *Journal of Food Chemistry* 98:64-70.