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MICROBIAL DIVERSITY OF ECONOMICALLY IMPORTANT UNPROCESSED FRESHWATER FISHES SOLD AT THE MEDINA COURA MARKET IN BAMAKO, MALI

BY:

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DECLARATION

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ABSTRACT

Around the world, millions of people depend on fish for its high protein content, supply of micronutrients and added health benefits. Fish is extremely perishable; its quality changes very quickly after capture unless it is kept cold. Failure to comply with good preservation practices favors the risk of deterioration in market quality and safety, which may lead to foodborne illness. The purpose of this study was to determine the microbial diversity of fresh water fish and evaluate the food safety knowledge and practices of stakeholders of freshwater fish along selected fish value chains in Mali. A structured face-to-face questionnaire on food safety knowledge and practices was designed and administered to 247 respondents comprising 130 fish sellers and 117 fishermen between November 2018 and February 2019. Samples of three fresh water fish species (*Lates niloticus*, *Clarias anguillaris* and *Oreochromis niloticus*) were taken during cold and hot seasons in four fish production areas and at specific points (capture, landing and selling points) in the selected value chains (Niger Central Delta in Mopti, Selingue, Manantali, Markala) and from the Central fish market (Medina Coura market) using standard protocols for microbial analysis. Water samples at capture areas and swabs of fish containers at each of the landing points were also collected for analysis. The following assessments were carried out: enumeration for Aerobic Plate Count (APCs), Total Coliform counts (TCs), Fecal Coliform counts (FCs), and Spores of Sulphite Reducing Bacteria (SRB), *Staphylococcus aureus* and prevalence of *Salmonella spp*. were also determined. Bacterial isolates were identified using biochemical tests (catalase oxidase, API 20E and 20NE). Parasitic infestations of 120 specimens of three freshwater fish species, comprising *Lates niloticus* (44), *Clarias anguillaris* (40) and *Oreochromis niloticus* (36) sold at the Medina Coura market in Mali, were also investigated. The results of the questionnaire survey indicated that respondents had a good level of food safety knowledge but poor food hygiene practices. Most

of the respondents had received no formal education in food safety. It is, therefore, important to put in place a good public health management strategy for food sales services, which will give stakeholders in the fish value chain the knowledge and skills necessary to provide hygienic and safe sales services. Microbial growth was observed in all of the fish species tested. APC ranged from 5.08 to 7.64 Log10 $CFU.g^{-1}$, TC ranged from 4.11 to 7.62 Log10 $CFU.g^{-1}$, FC ranged from 3.64 to 7.5 Log10 $CFU.g^{-1}$ and all had 100% higher counts than the national standard. About 12.7% (17/134) of the samples recorded Sulphite Reducing Bacteria, 20.1% (27/134) had *Staphylococcus aureus*, and 93.3 % (125/134) *Salmonella spp.*. Three species of parasites were detected, namely, *Salmincola edwardsii* (crustacea) at the gills, and *Philonema sp*. and *Raphidascaris sp.* (nematodes) in the abdominal cavity. An overall prevalence of 10% (12/120) parasitic infestation was observed. The study showed that prevalence of parasitic infestation during the cold season was 5.2% and the hot season was 14.5%. The highest prevalence of infestation was observed in *Oreochromis niloticus* (16.7%) followed by *Lates niloticus* (9.1%) and *Clarias anguillaris* (5.0%). A total of 360 isolates (188 in the cold season and 172 in the hot season) were characterized and identified as 24 species of bacteria belonging to 15 families. This study is an important contribution to the knowledge of the bacterial and parasitological flora of fish along the value chain sold on the Medina Coura Market, and contributes, to my knowledge as the first attempt to evaluate the microbiological quality of these freshwater fish along the fish value chains.

DEDICATION

This work is dedicated to my wonderful late father, Mamadou SISSOKO, for giving me an excellent childhood, for always believing in me, and most importantly for teaching me to know God, without whom I am nothing.

May God grant you to Al Jannah.

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Abs/25g	Absence in 25 grams
AFNOR	Association Française de Normalisation
ANOVA	Analysis of Variance
API	Analytical Profile Index
BPW	Buffered Peptone Water
CFA	Communauté Financière Africaine
CFU/g	Colony Formant Unit per gram
DNAER	Direction Nationale de l'Aménagement et de l'Équipement Rural
DRPD	Direction Régionale de la Pêche du District de Bamako
EDTA	Ethylenediaminetetraacetic acid
EHEC	Enterohemorrhagic *E. coli*
EIEC	Entero-invasive *E. coli*
EISMV	Ecole Inter états de Sciences et de Médecine Vétérinaire de Dakar
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
FAO	Organisation des Nations unis pour l'alimentation et l'agriculture
FC	Fecal Coliform
FST	Faculté des Sciences et Techniques de Bamako
GDP	Gross domestic product
H_2S	Hydrogen Sulfide
ICMSF	International Commission for Microbiological Specifications for Foods
IND	Indole

INRSP	National Institute of Research in Public Health
IRD	Institut de Recherche et de Développement
ISFRA	Institut Supérieur de Formation en Recherche Appliquée de Bamako
PADEPECHE	Projet d'Appui au Développement de la Pêche Continentale dans le Delta Central du Niger
PCA	Plate Count Agar
pH	potential Hydrogen
RCM	Reinforced Clostridial Agar
SC:	Selenite Cysteine
SPSS	Statistical Package for the Social Sciences
SRB	Sulfito-Reductor Bacteria
S-S	Salmonella-Shigella
TC	Total Coliform
TMA	TriMethylAmine
TMAO	TriMethylAmine Oxide
TSA	Trypton Soy Agar
UICN	Union International pour la Conservation de la Nature
UV	Ultra-Violet
VRBA	Violet Red Bile Agar
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Around the world, millions of people depend on fish for its high protein content. According to the Food and Agriculture Organization (FAO, 2007), fish is an important source of nutrients because of its protein, vitamin A and D, phosphorus, sulfur and essential amino acids composition. It is also low in cholesterol (Fagbenro *et al.*, 2005; Ayeloja *et al.*, 2013; Aremu *et al.*, 2013; Oladipo & Bankole, 2013 ; Khalili Tilami & Sampels, 2017). Fish can play an important role in improving nutritional status in Africa's food security; as more than 200 million Africans eat fish regularly (Béné & Heck, 2004). Fish, like other agricultural products, is essential for ensuring the food security of the poorest in the country. The nutritional profile of the fish is superior to pork, beef and chicken, providing an excellent source of highly digestible energy and high-quality animal protein (Oladipo & Bankole, 2013). The risk of contracting a cardiovascular disease is rather reduced in people who consume more fish because it is rich in omega-3 fatty acids.

The fishing sub-sector is a key sector of the Malian economy because of its contribution to job creation, food security and increased national wealth (Laë *et al.*, 2004). In normal hydrological period, fish production is approximately 100,000 metric tons/year (FAO, 2007). Gross value added for the entire fishing industry is estimated at more than 90 billion CFA francs ($160,050,637.72), which represents 4.2% of the country's total GDP (FAO, 2007). Fishing also contributes to a large part of the state's budget through taxation. This contribution is estimated at around 10% of the gross value added in the sector (FAO, 2007).

In Mali, fishing activities and practices are common along almost all water bodies in the country. It is the largest freshwater fishing country in the world (FAO, 2007). Fishing is practiced in two River basins (the basins of Niger and Senegal), lakes, ponds and flood plains. The main fishing areas are Central Delta of Niger (about 80% of total freshwater fish production), Selingue Lake, Markala Lake and Manantali Lake. Fish from these areas are sold on the Medina Coura market in Bamako (Sissoko *et al*., 2016). In this market, there are nine (9) different fish families: *Alestidae, Cyprinidae, Osteoglossidae, Gymnarchidae, Centropomidae, Mormyridae, Bagridae, Cichlidae, Mochokidae* (Sissoko *et al.*, 2016).

Fish is, however an extremely perishable food product owing to its biological composition; deteriorating much faster than most other foods. Indeed, in tropical countries, where ambient temperatures range between 25°C and 30 °C, fish are destroyed in less than 12 hours (Degnon *et al*., 2013). After capture and death, fish can normally be stored in chilled brine or crushed ice for preservation., Temperature is the most important environmental factor influencing the composition of the fish microflora (ICMSF, 2005).

Cross-contamination with harmful agents through mishandling and unhygienic practices may expose consumers to foodborne diseases (Kombat *et al.*, 2013). After capture, dead fish undergoes several bacterial and enzymatic alterations. Microbial growth of freshly caught and marketed fish may be caused by unhygienic transportation and handling or lack of appropriate chilling during storage. Dione (2003), also identified that the causes of this rapid spoilage of fish are mainly due to physicochemical and microbiological factors because of poor handling practices, inappropriate storage temperatures and poor packaging methods affecting fish quality. In Mali, the artisanal fisheries sub-sector suffers from a lack of infrastructure for landing and marketing (FAO, 1996). Stakeholders are unaware of the basic hygiene rules and health risks associated with different treatment methods, such as the use of faulty freezers that do not freeze fish. Traditional practices such as sun drying, hot smoking and frying, improper storage can lead to fish degradation. Due to the high content of unsaturated fatty acids in fish, the oxidation of free radicals is a common phenomenon in all fish products (fresh and processed) exposed to the open air (Bourgeois & Leveau, 1991).

Unfortunately, at Medina Coura market, proper fish conservation practices are still not respected. Fresh fish are exposed, in unhygienic places, on mats on the ground, under the sun and often without ice. This can reduce the marketability of fish by reducing the microbiological quality of fresh fish and potentially cause diseases such as gastrointestinal diseases, headaches, diarrhea due to Toxic Collective Infections when toxigenic microbes present on fish exceed infectious doses. A study by Gram & Huss (1996) suggested that lactic acid bacteria and some psychotropic *Enterobacteriaceae* could cause fish spoilage. In another study, freshwater fish at Medina Coura market in Bamako was reported to have prevalence of 51% *Enterobacteriaceae* family, 23% of *Aeromonadaceae* family, 19% of the family *Pseudomonadaceae*, 6% for the family *Moraxellaceae* and 1% for the family *Vibrionaceae* (Sissoko *et al*., 2016). Aboagye (2016) identified *Clostridium perfringens*, enteropathogenic *E. coli*, *Aeromonas sobria*, *Staphylococcus aureus*, and *Listeria spp*., on fresh and processed fish at different stages of the fish value chain in Ghana. These pathogens are among the main microbial cause of foodborne illnesses. Aboagye (2016) also noted the presence of putrefactive microorganisms such as *Proteus vulgaris*, *Proteus mirabilis* and *Pseudomonas spp*. Microorganisms developing at low temperatures and exhibiting proteolytic activity have been presumed to be primarily responsible for the spoilage of fresh fish (Ghaly *et al*., 2010). Potential bacterial pathogens that have been found in fish include *Streptococcus iniae, Vibrio cholera, Staphylococcus aureus, Escherichia coli, Salmonella spp*. and *Vibrio* *parahaemolyticus* (Kombat *et al.*, 2013). Parasites such as nematodes, cestodes and trematodes are found in the aquatic environment. They are associated with freshwater fish and they cause morbidity, mortality and economic losses in Africa (Oniye *et al.*, 2004; Ayanda, 2009).

The body of water which is the source of fish can be the main source of contamination when the water becomes polluted through the discharge of sewage and rain run-offs (Kombat *et al.*, 2013). The presence of pathogenic and spoilage microorganisms in freshwater fishes has both health and economic implications which poses threat to public health. And thus, a threat to public health. There is a high rate of fish consumption in Mali amidst poor aquatic environment, unhygienic handling and storage at fish markets, however, not much studies have been done to assess or characterize the microbiological quality of fish in the fish value chains in the country. This study therefore, sought to assess the microbiological safety of the three fish species (*Lates niloticus*, *Oreochromis niloticus* and *Clarias anguillaris*) abundant in the Medina Coura market and along the value chain to the market.

The study also aimed to map and document the local fish value chain in southern part of Mali (Selingue, Manantali, Markala and Mopti), to identify critical contamination points in the value chain to provide information on the food safety knowledge and practices of stakeholders in the value chain. This research provides useful information for future diversity studies within Mali and even in the sub-region. In addition, to providing information on sociodemographic factors that influence fish handling practices along the value chains as well as information on the food safety knowledge and practices of stakeholders in the fish value chain. It could also influence future policies and interventions aimed at mitigating fish spoilage and addressing food security issues related to artisanal freshwater fish.

1.2 General objective

The main objective of this study was to investigate the microbiological quality and microbial diversity of fresh fishes sold along the fresh fish value chain in Southern Mali (Selingue, Manantali, Markala and Mopti) to Medina Coura market.

1.3 Specific objectives

The specific objectives were to:

- Assess the food safety practices and knowledge of key stakeholders in the identified value chain;
- Determine the effect of seasonal variation on the microbial quality and parasites of freshwater fish;
- Identify the spoilage bacteria associated with freshwater fish using phenotypical methods;
- Identify the pathogenic bacteria associated with fish using phenotypical methods.

CHAPTER TWO

2.0 LITERATURE REVIEW

Mali has a Sudano-Sahelian climate; which is characterized by a strong irregularity in precipitation, which is observed at all timescales (annual, decennial, centennial and millennial). Since the beginning of the 1970s, there have been many periods of drought. During the hydrological year 1994-1995, however, very favorable hydro-climatic conditions were observed, which had a direct and immediate impact on the fishery industry (FAO, 1996). Fishing is carried out on two river basins (Niger river and Senegal river), lakes, ponds and floodplains. Fishing is practiced in practically all the water bodies of the national territory. There are, however, three main production areas: Delta Central of the Niger river, two-artificial lakes (Manantali and Selingue) and Markala dam (FAO, 1996).

2.1.1 Central Delta of Niger River

Located downstream from Segou, the Niger Central Delta is fed by two rivers, Niger and Bani rivers. Nineteen (19) per cent of the water comes from Côte d'Ivoire and Guinea, and the flood occurs from July to August (Laë *et al.*, 2004). The floodplain basins dry up from April to May, but the rivers Bani and Niger and some lakes retain water. In hydrological period, there is a lag of three months between the beginning of the flood in the North (Dire), in the South (Ke Macina) and of the Delta. During the same hydrological cycle, flooded areas can range from 20,000 km^2 at maximum flood to 3,500 km^2 at the end of the low water season (Laë *et al.*, 2004).

The Central Niger Delta is a vast region of about 30,000 km^2 in the centre of Mali. The annual fish production varies between 45,000 and 100,000 tons, according to the extent of the floods (Quensiere, 1994). In general, the deltaic zone can be considered as fully exploited, irrespective of the level of the flood. Fishermen adjust the number of their annual investments according to the extent of the flood (FAO, 1996).

2.1.2 Selingue Lake

At about 140 km South of Bamako, Selingue dam was built in 1980. It provides both hydroelectric power and irrigation, and its management is done by the Office of Exploitation of Hydraulic Resources of the Upper Niger (Laë *et al.*, 2004). The dam is 348 m long and the lake covers 409 km^2 and contains 2.2 billion m^3 of water. However, its depth does not exceed 20 m (Paugy & Leveque, 2006). Every year, the lake produces about 4000 tons of fish (Miller, 2006).

2.1.3 Manantali Lake

Built-in 1987 in the valley of the Bafing river, Manantali Lake provides both irrigation and hydroelectric power (Paugy & Leveque, 2006). It is 208m long and with an area of up to 500 km^2, it contains 11 billion m^3 of water, the depths of the medium and maximum dam are respectively 20 m and 50 m (Paugy & Leveque, 2006). Fish production per year is about 1,500 tons (Miller, 2006). The hydrology of Manantali Lake is particularly characterized by a significant tidal range that reaches more than 8 m between the high-water season and the low water season (Paugy & Leveque, 2006). The shores of the lake are also frequently overlooked by high cliffs, allowing no landing for fishing. The physicochemical characteristics of the waters are similar to those of the Selingue Lake, including waters with satisfactory oxygenation, and enriched with nutrients by the progressive degradation of nearly 12,000 ha of submerged forest (FAO, 1996).

2.1.4 Markala Lake

The "Office du Niger" is an agricultural zone. The Markala dam allows gravitational irrigation.

With 74,000 ha, it is the largest irrigation project in West Africa, was built in 1943, located 250 km downstream from Bamako in Mali to store water for irrigation (Laë *et al.*, 2004). The "Office du Niger" zone, aims to develop agriculture. It allows the production of sugar cane and rice (Laë *et al.*, 2004).

2.2 Fish production in Mali

In Africa, the total fish supply in 2011 was 9 million tons (Kolding *et al.* 2016). Production in landlocked dryland countries, such as Mali, Burkina Faso and Niger, has grown as a result of production from the Senegal and Niger rivers, although the recorded levels of production are much less significant than those of coastal countries (Kolding *et al.* 2016).

The largest in the Sahel is Mali's fisheries production and it represents 40% of West Africa's freshwater fish production (FAO, 2007). Depending on rainfall and flooding annually, production of fishes varies from an estimated 70,000 to 150,000 tons (FAO, 2007). An estimated 10 to 20% of freshwater fish production is exported in the West African sub region (Peterson & Kalende, 2006). According to FAO (2007) there is very little updated data on the fisheries and aquaculture sub-sector, the artisanal type of inland fishing in Mali is carried out on all water bodies: rivers, lakes, ponds, reservoirs of hydroelectric dams and agro-pastoral dams, floodplains, etc.

Mali shares with its neighbors two rivers of major importance: - **The Senegal river** creates in the west a major hydrographic axis 900 km long whose main tributaries are Bafing, Bakoye, Baoulé and Falémé; **The Niger river**, a Sudanian river, is the fourth-longest river in Africa (4,200 km). From the mountains of Fouta Djalon in Guinea where it rises, it flows North- East through Mali where it forms a seasonal floodplain of 20 000km^2 known as the Delta Central. It crosses Nigeria, Niger, and the Republic of Benin where the river enters the Gulf of Guinea (Laë *et al.*, 2004).

The Niger River plays a vital role in the life of a densely populated and vast region of West Africa (FAO, 2007). The drinking water supply of several cities is ensured by the abstraction of the waters of this river operated upstream. It is an important waterway both for navigation associated with active trade (in Mali from Koulikoro in the bend of Niger and Nigeria on lower Niger) and for small canoe traffic throughout its route. Riparian and rural populations benefit from their important fisheries resources (Laë *et al.*, 2004). The flood plains and tributaries of the Inner Delta are used for rice, cotton and wheat cultivation. Besides, flood plains are vital for nomadic pastoralists who use access to water and pastures that are created every year as the water recedes. New developments (hydroelectric dams and irrigation dams) are now likely to give the river a significant economic role where 30% of the watershed is in Mali, which crosses the country from West to East for 1750 km, drawing the Inner Niger Delta to the North and whose main tributaries are Bani and Sankarani (Laë *et al*., 2004).

The basins of the Niger River, the Senegal River and the Volta river (along with the Sourou River) play a very important role in the conservation of biodiversity owing to the vast plains they flood (FAO, 2007). In both lakes, the fish species present are generally the same as those recorded in the Central Delta. In the case of the Selingue Lake, it is because at the time of impoundments of the lake, the same species settled in the environment. For Manantali Lake, which belongs to the Senegal River basin, this could be explained by the proximity of fish population between the waters of Senegal and the middle Niger (FAO, 2007). However, the filling of the lakes has modified the ecological conditions and provoked significant changes in relative abundance favouring some species to the detriment of others (FAO, 1996). In Selingue, the main commercial species belong to the family *Bagridae* (Chrysictis and Auchenoglanis especially), *Cyprinodontidae* (Labeo) and *Cichlidae* (Tilapias), with respectively 21%, 15% and 13.5% of landings (FAO, 1996).

In Manantali, the family *Cichlidae* and *Synodontidae*, on the other hand, account for nearly two-thirds of landings with respectively 48% and 17% (FAO, 1996). In Manantali, the high proportion of tilapia during fishing could be explained by the fact that the fishermen concentrate their effort on the shallows of the water body (FAO, 1996).

2.3 The major categories of fishermen in Mali

Farmers-fishermen (Ethnics groups: *Rimaïbé, Bambara, Soninke* and *Songhai)*: they devote proportionately more time to agricultural work and invest primarily in agriculture, which results in the use of small numbers of gear, of poor quality; they are also fundamentally passive since they create more time for agriculture. Catches are moreover essentially intended for self-consumption at the household level (FAO, 2007).

The sedentary professional fishermen (Bozo and *Somono)*: they consider fishing as a full-fledged economic activity that generates cash income. However, for this second category of fishermen, the links with the land are not broken allowing the households concerned to diversify fishing activities with agriculture, making them less vulnerable to hydrological hazards (FAO, 2007).

The migrant professional fishermen (mainly Bozo): fishing is the only way for them to meet the needs of households since they generally do not have access to land. Migratory fishermen possess a particularly high degree of technicality and minimize the hazards of natural productivity by diversifying techniques and fishing grounds frequented during the annual cycle (FAO, 2007).

2.4 Medina Coura fish market in Bamako

Medina Coura market, also called Dossolo Traore market, is located in Commune II of the District of Bamako. It is limited to the East by Hippodrome, to the West by Modibo Stadium Keita, to the North by the hill of Point G and the South Missira. The market monitoring study carried out by the DRPD (2010) showed that, refueling of fresh fish is made from the fishing centres of Mopti

(34%), Selingue (28%), Senegal (13%), Segou (6%), Mauritania (6%), Manantali (3%), Markala (3%), Macina (3%) and Gao (3%). Fish production converges in Bamako the capital; the Medina market is the place of transit and of marketing fish. It receives the largest amount of fish from other regions. The flow time of fish arriving on the market varies between 2 to 3 days. Fish are exposed on the ground with or without ice. Fish that have not been sold on the same day are kept in non-functional freezers containing ice (Sissoko *et al.*, 2016). The supply for Bamako is made by vehicles (truck or public transports) carrying coolers or baskets with fresh fish on ice from Selingue, Markala and Manantali (Traore, 2014).

2.5 Fisheries value chain in Mali: market organization

The value chain or chain of activities of a product makes it possible to add value to the product. Once caught, the fish is brought back to the landing point where it undergoes a first transformation (sorting, freezing, etc.). The second transformation is the explain this, it is sent to the wholesaler who sends it to the retailer and finally purchased by the consumer (Hempel, 2010). In developing countries, value chain analysis is rare and because it results from different interactions it is difficult to determine when some stages of the chain are missing (Hempel, 2010).

Fish marketing channels are generally organized around three types of markets: Wholesale markets that centralize production (Mopti in the Central Delta of Niger, Faraba and quarry to Selingue, Manantali and Markala); Semi-wholesale markets located in production and consumption centres; and retail markets in towns and villages. Wholesale markets can nevertheless be short-circuited through direct sales to local consumers (Traore, 2014).

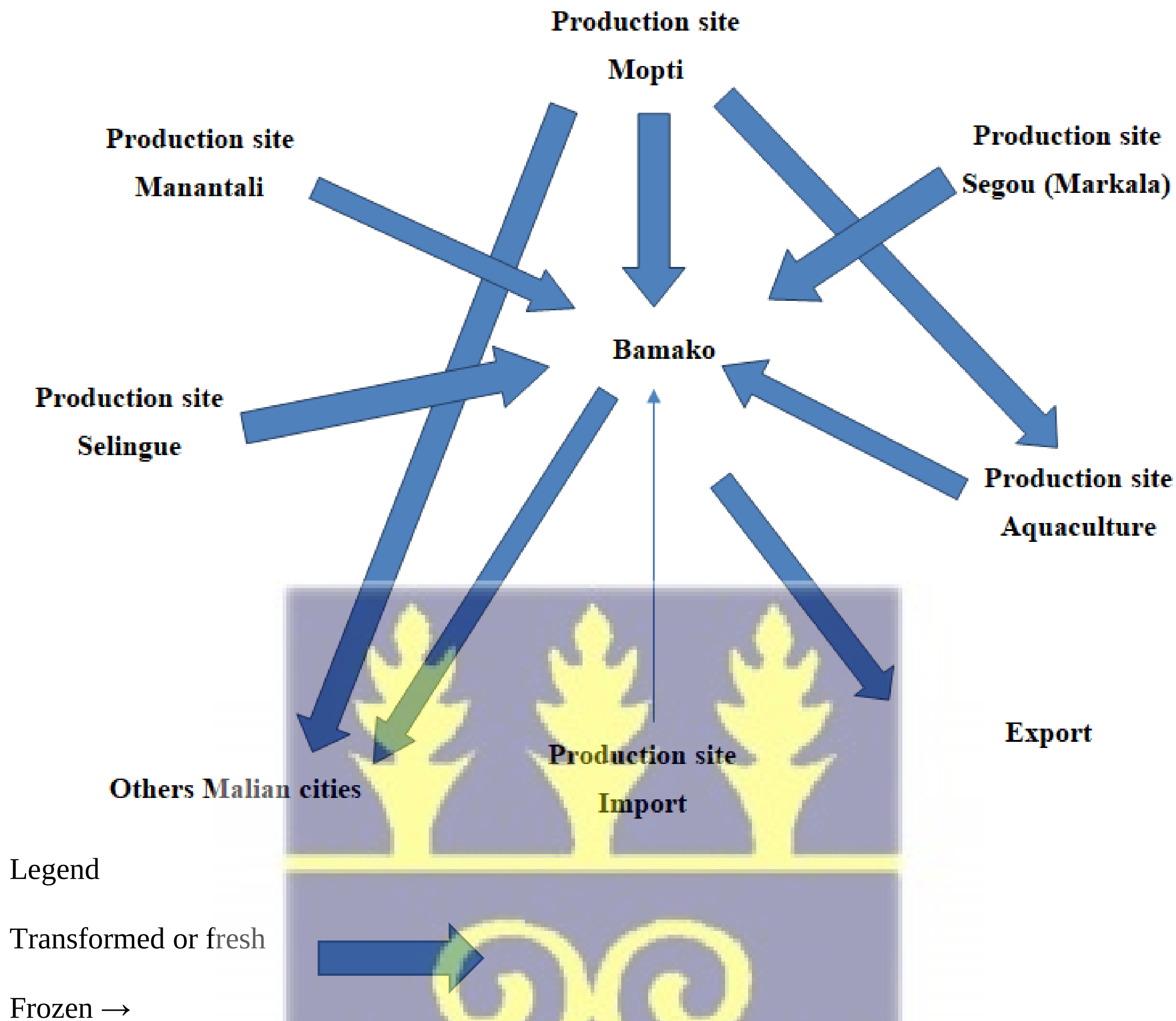

Figure 2.5.1: Fisheries value chain in Mali (Traore, 2014)

2.6 . Artisanal fishing in Mali

According to Peterson & Kalende (2006), Malian fishermen use canoes in nailed boards. These are rarely motorized given the particular fishing conditions. A fisherman's household has on average one to two canoes. In total, taking into account the fishing activities on Malian lakes and rivers, one can reasonably estimate about 25000 fishing canoes in Mali. Most fish are caught from November to March. Day and night fishing is done with local tools, nets, buckets and canoes.

Given seasonal variations in environmental conditions, particularly in the deltaic area, fishermen use a wide range of gear and methods of capture adapted to each type of situation. In the Central Delta, there is a close relationship between the exploited biotopes (watercourse, flooded plains, temporary pools, etc.), the hydrological season (flood, high water, recession, and low water level), the species targeted, and the means of fishing used over the last twenty years (Peterson & Kalende 2006). Production yields per hectare have almost tripled in the Central Delta. On the dam lakes, the gear used is less diversified, the fishermen having gradually adopted those which proved to be the most adapted to lacustrine fisheries (greater depth, presence of immersed strains, weak current). New fishing techniques have also been developed, such as those for gill nets and bell nets, while other techniques have been modified (E.g. durankoro nets used as baited traps for deep-sea fishing).

The main fishing gear used in Malian fisheries can be grouped into six broad categories:

- Gear by injury (harpoons) used in ponds in the process of drying up, in floodplains and during collective fishing;
- Deep-filleted nets (triangular nets used in dam fisheries or collective fisheries, two-handed nets used in depletion fisheries) or thrown nets (hawks used in collective fishing or on river arms in dewatering course);
- The small seine nets of the xubiseu type (80m x 6m) used by one or two fishermen during low water
- The large seine nets of the djoba type (400-1000m x 6-10m) handled by 10 to 20 fishermen and used in the delta mainly during periods of low water and in dam lakes after deforestation of shoreline portions;

- Gill nets used at high water in the plains or the river when the current is not strong, and drifting gillnets (130-450m) at a time when the current is strong (flood and start of the recession);
- The traps of which the most used are Durankoro type or Diene type of larger size;
- Baited or un-baited longlines.

2.7 Different species of freshwater fish in Mali

In Middle Niger, more than 130 species of fish have been identified, which belong to 62 genera and 26 families (Laë et al., 2004). These species have been divided into two groups: migratory species and opportunistic species. The most important and most commercialized species include *Lates niloticus*, *Bagrus bajad*, *Heterotis niloticus*, *Alestes dentes*, *Clarias anguillaris*, *Brycinus leuciscus*, *Hydrocymus brevis*, *Oreochromis niloticus* and *Sarotherodon galilaeus* (Laë et al., 2004).

2.8 Nutritional value of fresh fish

The edible part of the meat makes 40 to 80% of the total depending on the nature of the fish and the requirements of the consumers. It varies according to the food of the species and the season (Ibemenuga & Okeke, 2014). Known for its high nutritional value, fish are relatively low in cholesterol and saturated fat, and high in proteins, polyunsaturated fatty acids, and minerals such as calcium, potassium, phosphorus, sodium and magnesium (Kumolu-Johnson & Ndimele, 2011; Ibemenuga & Okeke, 2014).

In developing countries, a large portion of the population depends on fish for its protein (Ibemenuga & Okeke, 2014) and knowledge of its composition is essential (Ibemenuga & Okeke, 2014). The amount of protein in fish muscle is usually somewhere between 15 and 20 %, but values lower than 15 % or as high as 28 % are occasionally met for some species. Fish also supplies 15 to 20% nitrogenous substances, 3 to 8% lipids (fat), and 1 to 2% mineral substances. The fish is said to be lean when it contains 3% fat (tilapia) and fatty if its flesh contains 8% fat (*Clarias*) (Ibemenuga & Okeke, 2014).

Table 1:Nutritional values of some fish species (Dansoko, 2012)

Fish	Proteins	Lipids	Phosphors	Calcium
Tilapia	26.94%	1.37%	60mg/100g	87mg/100g
Clarias	21.30%	5.10%	21mg/100g	10mg/100g
Hydrocynus	24.33%	8.69%	39mg/100g	25mg/100g
Lates niloticus	25.10%	3.45%	39mg/100g	41mg/100g

Tilapia is the richest fish in terms of nutritional value; *Lates* comes in second, and *Hydrocynus* and *Clarias* in terms of protein, fat, phosphorus and calcium.

The flesh of the fish contains vitamins; A, B12, D; and minerals essential to man: calcium (bone); phosphorus (brain); iodine (growth) etc. The amount of vitamin in fish varies by species; there are fat soluble vitamins such as vitamins a, d, e and k, and water-soluble groups, such as vitamins b and c. (FAO, 2007).

2.9 Consumption of freshwater fish in Mali

Per capita, fish consumption is estimated at 10.5 kg/year (compared to meat consumption of 7.8 kg/year), although fish consumption among fishing families is probably much higher (Laë *et al*., 2004; Peterson & Kalende, 2006). In Africa, fish is widely consumed as a remarkable source of animal protein (Ibemenuga & Okeke, 2014).

2.10 Gender distribution in fishing communities in Mali

Throughout the world, the fishing sector is very important, providing livelihoods for millions of individuals. Fish is an important source of income for many families in developing countries. It has been estimated that the fishing industry supports more than 120 million people around the world (Oladipo & Bankole, 2013; Nwabeze *et al.*, 2013). In terms of employment, fishing activities directly affect nearly 71,000 fishermen, grouped in 32,800 households. Fishing activity is, therefore, a vital resource for about 256, 400 people (Peterson & Kalende, 2006). Applying that figure to Malian rural population, which has about 7.2 million people, it is estimated that fishing activities directly affect nearly 3.6% of the rural population (PADEPECHE, 2004). This average figure, however, masks large disparities encountered at the regional level. Fishing is carried out on almost all the rivers of the Malian territory. There are three main fish production areas, including Lake Selingue, the Central Delta of Niger and Lake Manantali. Thus, in the region of Central Delta of Niger, the largest producing region of the country with almost 80% of the total catch, more than a third of the population is directly affected by fisheries (FAO, 2007).

Taking into account the jobs generated upstream and downstream of the fishing activity, the number of jobs provided by the entire sector can be estimated at 284,000 persons, representing approximately 7.2% of the Malian workforce. The fishing industry could, therefore, employ a total of nearly 8% of the active population (FAO, 2007).

According to FAO (2007), in the fishing sector, men and women complement each other. In most communities, large boats used for fishing, have male crews, while women manage smaller boats and canoes. Land-based activities such as catch processing and marketing and the manufacture and repair of fishing nets are the responsibilities given to women in most communities. In most developing countries, various studies reveal that women in fishing communities work long hours of daily work in household chores, fishing, fish processing, packaging, storage, fish marketing and other non-revenue generating activities (Nwabeze *et al.*, 2013).Women play an important role in fish processing (drying or smoking fish) and marketing (Laë *et al.*, 2004).

2.11 Fish in food security

The role and importance of fish in securing food and nutrition for humans, particularly in developing countries, has frequently been overlooked. There is a growing demand for fisheries resources due to population growth, so it is important to improve fishing techniques. This population growth has an impact on poverty and food security (Limburg *et al.*, 2011). Certain factors such as the uncontrolled practice of fishing as well as the pollution and the degeneration of the fishing environment allow the reduction of aquatic resources. In Africa, severe cyclical drought causes increased desertification, decreases in surface water, huge losses in agriculture and decreases in fish catches (Miller, 2006). These are the difficulties facing Sahelian countries. Alongside these problems are population growth and the people's urgent need for food (Miller, 2006).

2.12 Post-harvest fish losses

‘'Food security is when all people have economic and physical access to the basic food that they need at all times’’ (FAO, 1984). Reducing post-harvest losses and increasing the amount of fish used improves food security by making better use of the fish produced. According to Kumolu-Johnson & Ndimele (2011), the losses caused by the alteration of fish after harvest are of various forms. The method of fishing, lack of regulations governing inadequate handling facilities and time delays between capture, collection and distribution, the quality and standards of fish for sale for human consumption, lack of regular surveillance on the side Government and poor extension services are seen as the main reasons for fish losses (Gebretsadik, 2016).

2.12.1 Physical losses

Physical losses of material are due to poor conservation or handling and the rejection of incidental losses. Physical loss can be caused by insects or others fish (animal predation) eating the fish, by theft, or by bird (Alemu *et al*., 2021). Mechanical damage (e.g. small cut through the sharp edge of the ice and net damage) can help and provide the right conditions for enzyme activities (Sivakumar *et al*., 2018).

2.12.2 Economic losses

Economic losses are caused by the denaturation of the wet fish which causes the loss of the value of the fish; also, the nutritional value of the product is reduced. The exact figures of the total economic losses due to food spoilage is not known, however, the physical damage of fish, obviously leads to the reduction in the price of the fish (Sivakumar *et al*., 2018; Alemu *et al*., 2021).

2.12.3 Nutritional losses

As fish spoils, it loses its nutritional value. The main mechanism of spoilage is protein hydrolysis, which is intended to be the main human food. This is not generally a major factor; as bacterial action generates nitrogenous substances with objectionable smells. The fish will lose its appearance when there is too much nutritional damage (Ghaly *et al*., 2010).

2.13 Fish Spoilage

Food spoilage encompasses any undesirable changes by food spoilage microorganisms and their microbial metabolites that cause repugnant tastes and odors (Veld, 1996). The food becomes both unpleasant smell and sometimes harmful. Food spoilage is mainly due to chemical, enzymatic or microbial activities (Sevindik & Uysal, 2021). A quarter of the world's food supply and 30% of the fish landed are lost due to microbial activity (Ghaly *et al*., 2010). With population growth, it is necessary to store and transport food from one place to another. Good food preservation becomes a necessity to increase shelf life to maintain its nutritional value, flavor and texture (Ghaly *et al*., 2010).

Microbial spoilage is rapid and evident in proteinaceous foods such as meat, poultry, shellfish, fish, milk and some dairy products as changes in flavor, texture and taste. These foods are highly nutritious, possess a neutral or slightly acid pH and a high moisture content and therefore permit the growth of a wide range of microorganisms(Veld, 1996). At temperatures near to 37 °C, most human pathogens grow best whereas most foods are usually stored at temperatures well below the normal body temperature.

The major pathogens associated with post-harvest fish spoilage which can produce toxins and cause food poisoning are *Staphylococcus aureus, Escherichia coli, Klebsiella spp. Salmonella spp., and Pseudomonas spp.* etc. (Awe & Adejo, 2018). Poor handling and washing of the fish in polluted water can result in food poisoning caused by pathogenic bacterial contamination of fresh fish. Fish are extremely perishable (Abiodun, 2020). This is due to the phenomena of post-mortem damage caused by autolytic reactions due to muscular and digestive enzymes and bacteriological changes due to proliferation and microbial invasion. The loss of the fish quality starts as soon as it dies or is caught in the tropics, where the ambient temperature is very high (25 to 30 °C), the fish is altered after 12 to 20 hours depending on the species (Dione, 2003). The cells then initiate new processes characterized by the degradation of glycogen (glycolysis) and energy-rich products. The first autolytic processes occur in the muscle of the fish. The body of healthy fish, alive or freshly caught, carries a very variable microbial load, of the order of 10^2 to 10^5 germs / cm^2 of the skin, 10^3 to 10^7 germs / g of gills and 10^3 to 10^8 germs / g of intestines (Bourgeois & Leveau, 1991).

This great variability reflects the condition of the environment. At death or after capture, when the fish is exposed to a temperature above 8 °C, the bacteria invade the fish flesh through collagen fibers to cause physical and biochemical changes. The softening of the flesh, the weakening of the abdomen and the detachment of the peritoneum is partly due to bacterial activity (Bourgeois & Leveau, 1991; Gram & Huss, 1996).

2.14 Microorganisms involved in fish spoilage

According to Huss (1995), it is important to know that many microorganisms do not propagate on spoilage. The spoilage is the result of fermentative Gram-negative bacteria (such as *Vibrionaceae*), while psychro-tolerant Gram-negative bacteria (such as *Pseudomonas spp*. and *Shewanella spp.)* tend to spoil chilled fish.

Universally, to determine the microbial spoilage of fish by using their trimethylamine level (TMA). Fish use trimethylamine oxide (TMAO) for osmoregulation to avoid dehydration in marine environments and engorgement of tissues in freshwater (Gram & Dalgaard, 2002).

Bacteria such as *Aeromonas spp., Shewanella putrifaciens, Photobacterium phosphoreum, Enterobacteriaceae* psychrotolerant, and *Vibrio spp.* can obtain energy by transforming TMAO into TMA, as well as agents similar to ammonia (Gram & Dalgaard, 2002). Bacteria alteration of the genera *Pseudomonas* such as *Ps. putrifaciens*, *Ps. fluorescens* and others propagate quickly during the first stages of deterioration and produce proteolytic and hydrolytic enzymes (Ghaly *et al.*, 2010). These bacteria are responsible for the sweet and fruity odors of spoilage while *Shewanella putrefaciens* is responsible for the production of H_2S.

The composition of the microbial flora of fish products is generally quite similar to that of their natural environment (Austin, 2006). The primary factors associated with food spoilage are associated with intrinsic food properties and contamination during harvesting, slaughter and processing in combination with temperature abuse (Veld, 1996). The species found in the gut of fish are essentially the same as those isolated from the water in which the fish is caught. The detection of microorganisms in fishery products can have two main origins namely microbial contaminants native to natural freshwater habitats (where fish is sourced) and those associated with water pollution (Bourgeois & Leveau, 1991; Gram & Huss, 1996; Novoslavskij *et al*., 2015). Contamination of the waters of the fishing zones: At this level, the pollution is quite important. The pathogens brought by this route are generally organisms with associated faecal transmission (*Salmonella*, viruses, parasites). The search for faecal indicators will be very useful in this case. In fish, contamination germs are found in the gills in the gut and on the skin. A muscle is free of germs on live fish (Ghaly *et al*., 2010).

–Post-capture contamination: A product that is not originally contaminated may have been contaminated during the various stages preceding its placing on the market. The contamination can already take place on a boat, by contact with dirty material (crate, the ice of bad bacteriological quality). Ice can ensure long conservation of fishing products while preserving their freshness. However, to be fully effective and cost-effective, the hygienic and technological principles of a cold application must be respected. Washing with contaminated water can sometimes introduce pathogens onto the fish.

According to Bourgeois & Leveau (1991), microorganisms isolated from the gills, the intestines and the skin belong mainly to the following genera:

–*Pseudomonas*, *Acinebacter*, (60%),

–*Corynebacterium*, *Flavobacterium*, *Micrococcus* (20%),

–*Bacillus*, *Proteus*, *Serratia* and others (20%).

According to Fane (2011), aquatic environments are generally rich in microorganisms due to favorable environmental conditions for microbial growth. The presence and the number of these microorganisms are largely influenced by several abiotic factors such as temperature, dissolved oxygen, pH, and the presence of dissolved or suspended mineral substances and the availability of organic matter.

Microbial species vary according to the quality and type of water. However, the same species are not found in cold waters, warm waters and polluted waters. Aquatic environments are easily polluted by both wastes from homes, farmlands or industries. This endangers the life of aquatic biota such as fish since fish takes in a large number of bacteria into their alimentary tract from water and food (Ibemenuga & Okeke, 2014). The bacterial flora of living fish reflects the flora of its aquatic ecosystem, but there is a specific flora related to the type of fish. According to Shewan in Fane (2011), microorganisms occur on the entire outer surface of live or freshly caught fish. The microbial load is of the order of 10^2 to 10^7 germs / cm^2 of skin and 10^3 to 10^9 germs / g of gills or intestine.

Thus, the bacterial flora of freshly caught fish depends more on the environment in which it was caught, than the species of fish itself. Fish caught in clean, very cold waters have a lower bacterial load than fish caught in warm waters (FAO, 2007). This bacterial load is 10^7 CFU / cm^2 on fish from polluted water (FAO, 2007). Several species of bacteria are found in fish, psychrotrophs can develop at 0 °C. The microflora of temperate fish, dominated by gram-negative psychrotrophic rod-shaped bacteria, belong to the genera *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Shewanella* and *Flavobacterium*.

Pseudomonas belongs to the family *Pseudomonadaceae*. This genus includes the species *Pseudomonas aeroginosa* which can secrete pigments such as pyocyanin (blue-green), pyoverdine yellow-green fluorescent (Abdulhussien, 2018). Also, the genus *Aeromonas*, belonging to the family of *Aeromonadaceae* are associated with fresh water fish. They are straight bacilli with rounded ends, Gram-negative, catalase-positive, motile and facultative anaerobic. They are ubiquitous in water and many foods. They are psychrotrophic organisms. *Aeromonas* species are human pathogens associated mainly with diarrhea1 symptoms (Hanninen *et al.*, 1997). The optimal growth temperature is 28°C and with a wide growth range and variability in the optimum temperature have been observed. Many strains can grow at 5°C, making *Aeromonas* particularly significant in refrigerated foods (Isonhood & Drake, 2002). This microorganism is an opportunistic pathogen of both aquatic and terrestrial animals (Hanninen *et al*., 1997; Isonhood & Drake, 2002).

2.15 Microbial pathogens in fish

Some harmful microorganisms in food can invade the human body (*Salmonella spp*., *Escherichia coli*, *Listeria monocytogenes*, etc.) and other generators of toxins ingested with a portion of food such as *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum*). Food spoilage is not necessarily due to the growth of pathogenic microorganisms in food. However, pathogenic bacteria such as *Salmonella spp.*, *Shigella spp.*, *Escherichia coli* and *Staphylococcus aureus* are responsible for many foodborne disease outbreaks (Gram & Huss, 1996). The aquatic environment may also contain pathogens such as *Aeromonas spp*., which is a pathogen of both fish and humans (Mhongole, 2009). *Enterobacteriaceae* is one of the most important families of ubiquitous bacteria. These are Gram-negative bacilli of medium size (coccobacillus, often polymorphic), and negative oxidase. The presence of *Enterobacteriaceae* is often used as an indication of poor hygiene, inadequate processing or post-process contamination (Veld, 1996). Moreover, the presence of *Escherichia coli* which is an indicator of fecal contamination suggests the probability of contamination of food produced with wastewater of human or animal origin. On the other hand, certain strains of *E.coli* (Enteropathogenic EPEC; Entero-invasive EECI; Enterotoxigenic ETEC; and Enterohemorrhagic EHEC) are pathogenic and cause serious human disease transmitted by water and food (Mhongole, 2009). *Vibrionaceae* family is gram-negative bacilli with a positive mobile oxidase reaction through a polar ciliate. These include particularly *V. cholera, V. parahaemolyticus* and *V. vulnificus*, which are generally recognized as the leading cause of human gastroenteritis associated with seafood consumption globally (Bonnin-Jusserand *et al.*, 2017). Consumption of raw or undercooked seafood contaminated especially with *V. parahaemolyticus* may lead to the development of acute gastroenteritis characterized by diarrhea, vomiting, headache, nausea, abdominal cramps and low fever (Aboagye, 2016). The source of contamination of cholera is the transmission of the microorganism through water and food and the pollution of the aquatic environment by the excrement of infected people (Mhongole, 2009).

2.16 Parasites

Some major public health problems can be caused by helminth parasites transmitted by fish and shellfish products. Globally, the number of people at risk including those in developed countries is more than half a billion (Rim *et al.*, 1994). The consumption of raw, undercooked fish or inadequately preserved fish infected by the parasites may cause human infection due to their pathogenicity (Rim *et al.*, 1994; Lima dos Santos & Howgate, 2011).

The main cause of diseases is the consumption of raw or undercooked fish in which parasites present are not killed. Approximately 50 helminthic parasites from fish may cause disease in man. The fish borne parasites come from three groups of parasites; namely nematodes (roundworms), trematodes (flukes), and cestodes (tapeworms) (ICMSF, 2005; Lima dos Santos & Howgate, 2011). In addition, a study by Dorny *et al.* (2009) explained that various types of parasites could infect fish and cause zoonotic infections in humans when consumed raw or not properly cooked. Most of the species have lifecycles involving several hosts.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

The study was in three parts:

A cross-sectional survey to assess the food safety knowledge and practices of key players or stakeholders within the identified value chain through questionnaire surveys.

A microbiological survey was also conducted along three value chains for the following fish: *Lates niloticus*, *Oreochromis niloticus* and *Clarias anguillaris* to assess quality and bacterial diversity through various stages of the respective value chains. These fishes were selected based on their availability, their economic importance, popularity and high commercial value in Medina Coura market.

Fish samples were taken during two seasons (cold and hot), three different samples (fishes, water and Storage container) were taken at four areas (Selingue, Manantali, Markala and Mopti) at three sampling points (Capture, landing and selling). Water samples at the Capture points and swabs of storage containers were also taken for microbiological analyses.

3.2 The study areas

The study was carried out at the Medina Coura market and four (4) landing areas (Selingue, Manantali, Markala, and Mopti) from where fish is supplied to the market, between December 2018 -February 2019 (cold season) and April-June 2019 (hot season). Medina Coura market, also called Dossolo TRAORE market is located in Commune II of the District of Bamako. The study areas are depicted in figure 3.2.1.

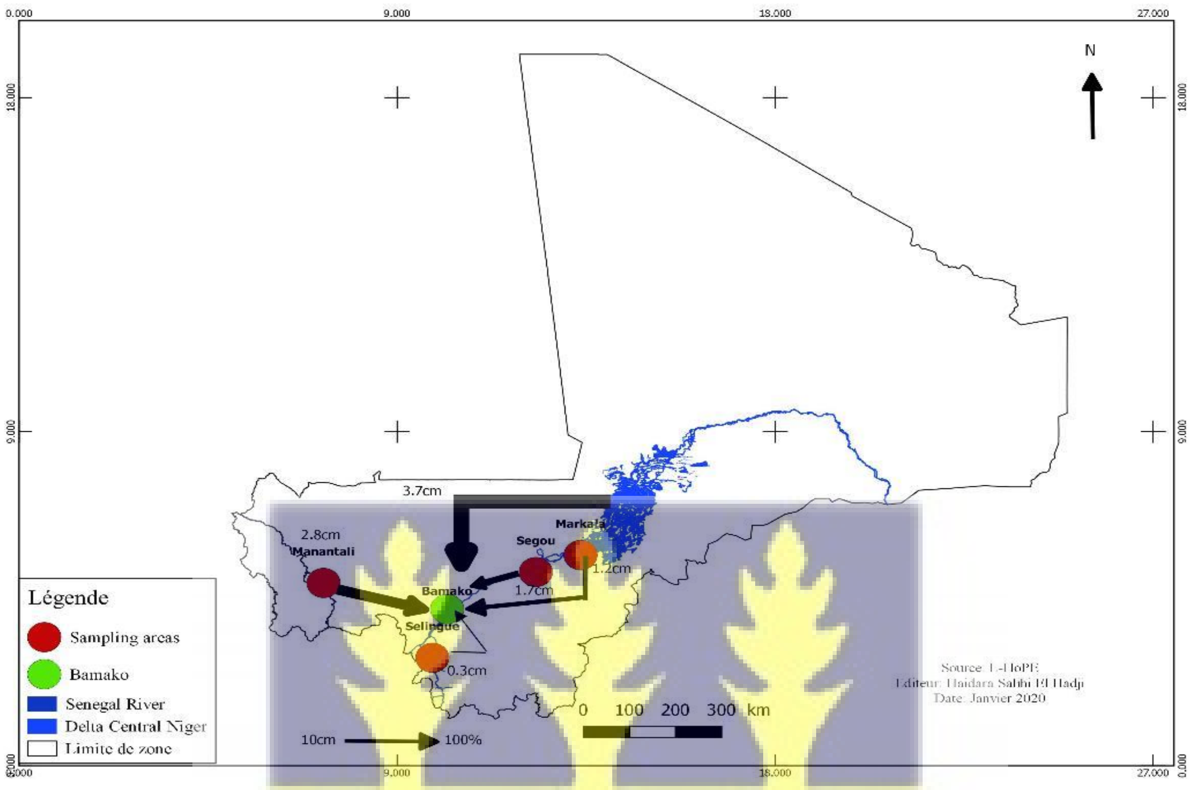

Figure 3.2.1: Map of Mali showing the landing beaches where the study was carried out

3.3 Ethical clearance

Ethical approval was obtained from the Ethics Committee of the National Institute of Research in Public Health (INRSP), Mali. The provisions relating to ethics were observed and respected according to the provisions of Law No. 2013-015 of 21 May 2013 on the Protection of Personal Data in the Republic of Mali and the Declaration of 'Helsinki 5 version 2013 to establish fact sheet, a free and informed consent form. Participation in the survey was on a voluntary basis.

The inclusion criteria: Fishermen and wholesalers in the study areas above 18 years and who consented to participate in this study.

3.4 Survey on the knowledge and practices of Stakeholders of the freshwater fish value chains to the Medina Coura market

The survey was targeted at fishermen and fish sellers. A total of 247 stakeholders (117 fishermen at the landing site and 130 vendors at the selling point) were interviewed in the value chain. The questionnaire comprised four sections: demographic characteristics, business characterization, food safety knowledge and personal hygiene practices (Appendix 1A, 1B). The declarations on the questionnaire were obtained from previous studies (Kunadu *et al.*, 2016; Aboagye, 2016) and modified to correspond to the objective of the study.

Fishermen were the first point of contact. They were asked about their activities, what types of fishing gear and canoes they used for their activities, what the three species frequently caught were, the period of successful fishing, how much fish they caught per day, how often they went fishing, who provided the resources necessary for their expeditions, to which groups of people they sold their catch and, finally what problems are encountered in their fishing activities (Appendix 1B). The value chain was then followed to identify the next group of participants. These are wholesalers or retailers operating at the Medina market (Appendix 1A). Questions related to the origin of the fish, the means of transport, the quantity of fish traded, how they detected spoiled fish and what they did with them, and difficulties they encountered during their work.

All questionnaires were administered in the local language to the fishermen (Selingue, Manantali, Markala and Mopti) and to the sellers at the market; to determine the different fish handling and storage practices. Data were collected through face-to-face interviews. The interviewer entered responses given. The average completion time for one questionnaire was between 10-15 minutes.

3.5 Physico-chemical and microbiological analysis of samples

3.5.1 Samples and Sampling methods

3.5.1.1 Water sample at the capture point

During this study, it was not possible to collect water samples from the exact location of the capture point in the river in the Selingue, Manantali and Markala areas as women are not allowed to go there. Three samples of river water were collected using a sterile 100 ml bottle (Falcon) at 3 different locations on the surface of the water. Duplicate samples of water were taken at the river's edge for Selingue, Manantali, Markala, and where the fish was taken for Mopti. Immediately, the bottle containing the water sample was properly closed and stored in a cooler with ice and transported to the laboratory.

3.5.1.2 Swab sample from the storage container at the landing point

Four swab samples were taken at each landing site from the basket used to transport fish to the selling sites in Bamako. Samples were taken by rubbing sterilized cotton swab over the inner-surface of the storage container. The swab was inoculated into 9ml of sterile Peptone Buffered Water PBW. The tube was closed and stored in a cooler with ice for transport to the laboratory. The sampling was done before the basket was filled with fish.

3.5.1.3 Fish sample from fishermen at the capture point:

The sampling was done by convenience. Fish samples were purchased at the edge of the river from fishermen coming directly from the fishing point (Selingue, Manantali, Markala and Mopti). Four fish samples of each specimen were collected. The sampled fish were transferred to sterile plastic bags. The bags were sealed and put in the thermos cooler containing ice and transported to the laboratory for analyses.

3.5.1.4 Fish sample from wholesalers at the Landing point

At each landing point, fresh fish samples were purchased from wholesalers before being transported to the Medina Coura market. Sampling of each fish type was done in duplicates and transported as earlier described. The fish samples were transported the same day to the laboratory at Bamako.

3.5.1.5 Fish sample from wholesalers at the selling point

Samples of fresh fish were purchased from wholesalers or retailers at the Medina Coura fish market. In the Medina Coura market, the fish were sampled from retailers who were recipients of the fish coming from the different landing points in each area.

3.5.2 Analysis of samples

3.5.2.1 Physico-chemical analysis of water samples

The sample were collected for the determination of pH, recorded immediately after sample collection using a multi-parametric probe (mobile pH-meter, 604 Metrohm AG, Herisau, Switzerland).

3.5.2.2 Microbiological analysis of samples

The freshwater fish species were sampled from four (4) landing points (Selingue, Manantali, Markala, and Mopti) and the commercial hub, Medina Coura market, where the fish from the four landing points are retailed.

3.5.2.2.1 Sample of fish for analysis

Four (4) specimens of three (3) species of fish were sampled at each sampling point. The fish species were:

- Nile perch: *Lates niloticus*, vernacular name in Bambara (Saalen);

- Tilapia: *Oreochromis niloticus,* vernacular name in Bambara (N'teben fin);
- Catfish: *Clarias anguillaris*, vernacular name in Bambara (Maanogo Blen).

Figure 3.5.1*: Lates niloticus*

Figure 3.5.2: *Oreochromis niloticus*

Figure 3.5.3: *Clarias anguillaris*

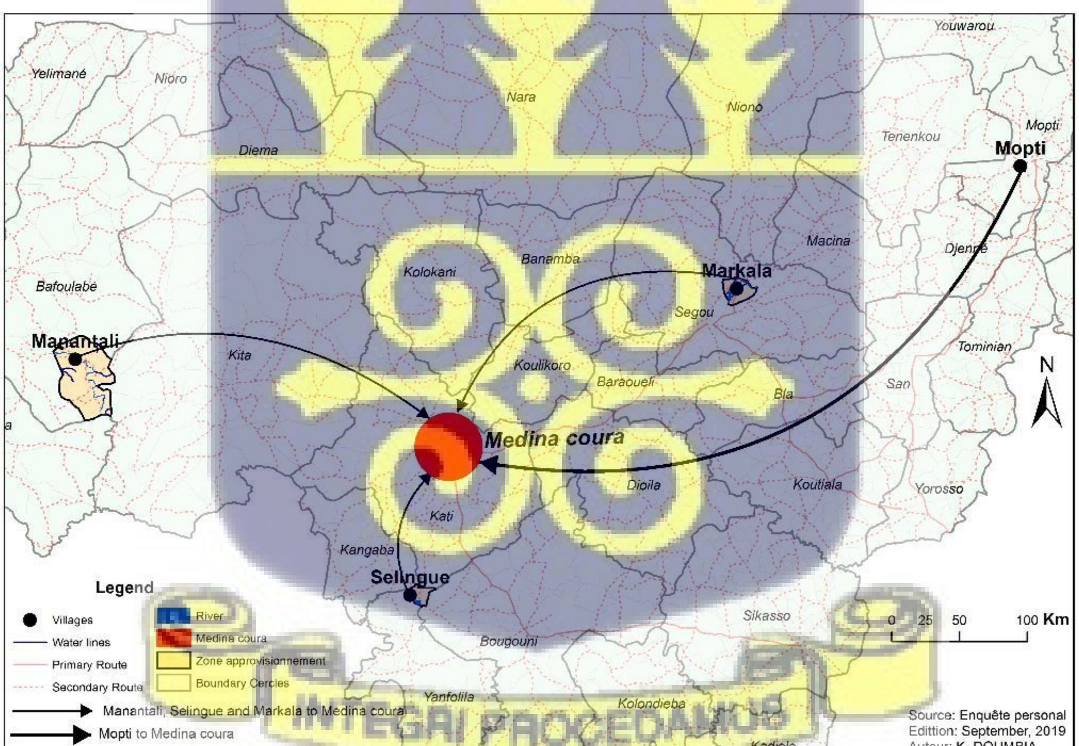

Figure 3.5.4: Map showing the different sampling points

Fish samples were purchased from various points of sampling by convenience sampling then placed in sampling bags. Water samples from the four (4) landing points (Selingue, Manantali, Markala, and Mopti) and swab samples of fish storage container surfaces, were collected as earlier described. A total of 300 samples were taken, 134 fish samples for bacteria and fungi analysis, 120 fish samples for parasite analysis, 24 water samples and 22 swab samples of the fish storage containers (Table 3.5.1).

Table 3.5.1: Number of samples collected during the study

Sampling areas	**Sampling point**	**Number of fish species examined**						**Number of Water sample examined**		**Number container sample examined**	
		L. niloticus		***C. anguillaris***		***O. niloticus***					
		CS	**HS**	**CS**	**HS**	**CS**	**HS**	**CS**	**HS**	**CS**	**HS**
Selingue (64)	Capture	5	4	5	4	5	4	3	3	N/A	N/A
	Landing	5	4	3	4	5	4	N/A	N/A	4	2
	Selling	N/A	4	N/A	4	N/A	4	N/A	N/A	N/A	N/A
Manantali (64)	Capture	4	4	4	4	4	4	3	3	N/A	N/A
	Landing	4	4	4	4	4	4	N/A	N/A	2	3
	Selling	4	4	2	4	2	N/A	N/A	N/A	N/A	N/A
Markala (56)	Capture	4	4	2	4	2	4	3	3	N/A	N/A
	Landing	4	4	4	4	4	4	N/A	N/A	3	2
	Selling	4	N/A	4	N/A	4	N/A	N/A	N/A	N/A	N/A
Mopti (70)	Capture	4	4	4	4	4	2	3	3	N/A	N/A
	Landing	4	4	4	4	4	4	N/A	N/A	3	3
	Selling	4	4	4	4	4	4	N/A	N/A	N/A	N/A
Total		**46**	**44**	**40**	**44**	**42**	**38**	**12**	**12**	**12**	**10**
Total		**254**						**24**		**22**	

N/A: Not Applicable (No sample were taken at this area) **CS:** cold Season**, HS:** Hot Season

The samples were transported in an isolated container with ice chest, to the laboratory for analysis. Bacteria and parasite were determined at the Central Veterinary Laboratory of Bamako.

Figure 3.5.5: Samples in the cooler with ice for laboratory analysis

3.5.2.2.2 Analytical methods

Samples were analyzed for bacteria and parasites. Bacteria characterization was done on spoilage and pathogenic microorganisms associated with fish using phenotypic methods. Prevalence and seasonal distribution of parasites in the sampled fish species were determined.

To analyze the samples, suspensions and decimal dilutions were performed according to standard AFNOR (2004) i.e French Standardization Association. In the samples, the Aerobic Plate Count (APC), Total Coliforms (TC), Thermo-tolerant Coliforms or Fecal Coliforms (FC), spores of Sulphite Reducing Bacteria, presumptive *Staphylococcus aureus* were enumerated, presence of *Salmonella spp.* was also determined.

According to the manufacturer's instructions, all microbiological media were prepared and sterilized (Appendix 2). The overnight incubation was done for each media and diluents for sterility check at their respective temperatures for the required time prior to use for microbiological assessments.

3.5.3 Sample preparation

Samples of the fish were collected. An amount of ten grams of each sample was taken from the skin, flesh, intestines and gills of each fish sample with sterilized scissors and knife and added to a 90 ml of 0.1% Buffered Peptone Water (BPW) to prepare an initial dilution. The samples were transferred to the stomacher model 400 and blended for 1 to 2 min.

One (1) ml of this suspension was transferred aseptically into each tube in dilutions of 10^{-1} to 10^{-5} in sterile Petri dishes. The serial dilutions were carried out for the various microbial counts described above and according to the methods applied by the International Commission for Microbiological Specifications for Foods (ICMSF, 2005).

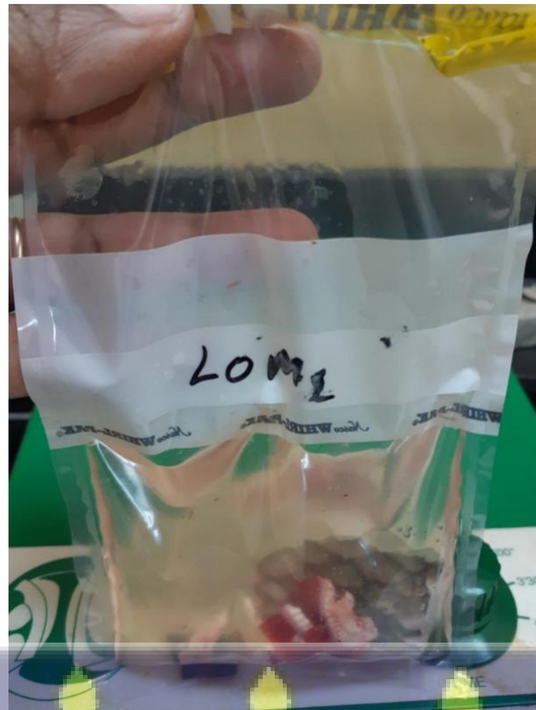

Figure 3.5.6:10 g of the sample in 90 ml of Buffered peptone water

3.5.2 Enumeration of microflora

3.5.2.1 Enumeration of the Aerobic mesophilic (Aerobic Plate Counts)

Plate Count Agar PCA (Sigma- Aldrich) was used for enumeration. One (1) mL inoculum from appropriate serial dilutions were transferred into sterile petri dishes. The sterilized molten PCA was cooled in a water bath to 50°C and poured onto the inoculum using the pour plate technique. Then, rotary movements were done to homogenize the mixture. After solidification, the second layer of 5 to 7 ml of PCA was added to limit the contamination. The inoculated plates were incubated at 30°C for 72 hours. All colonies that grew between the two layers were counted using a colony counter with a magnifying glass. Plates containing 30 to 300 colonies were counted and recorded. All counts were reported as the logarithm to base 10 of colony-forming units per gram (Log10 CFU/g).

3.5.2.2 Enumeration of Total Coliforms

The culture was carried out on Violet Red Bile Agar (VRBA, Sigma- Aldrich) by the double-layer method. The sterilized molten VRBA was cooled in a water bath to 50°C. Appropriate serial dilutions were enumerated by pour plate overlay method as described in 3.6.1.

The incubation was carried out at 30°C for 24 hours. Only very red colonies with a diameter greater than 0.5 mm were enumerated.

3.5.2.3 Enumeration of fecal or thermo-tolerant coliforms

The culture was carried out on VRBA (Sigma- Aldrich) as before by the double-layer method. The medium was cooled in a water bath to 50°C. The enumeration was made at the dilution 1/10; 1 ml of dilution is introduced into a Petri dish to which the VRBA has been added. After solidification, a second layer was poured on the surface as before. The incubation was carried out at 44°C for 48 hours. Only very red colonies with a diameter greater than 0.5 mm and having grown deep were counted.

3.5.2.4 Enumeration Sulphite Reducing Bacteria

Reinforced Clostridial Agar RCM (Becton Dickinson) was used for the enumeration of Sulphite Reducing Bacteria. A quantity of the suspension was boiled at 80°C for 15 minutes to eliminate the vegetative forms. The enumeration was made at the dilution 1/10; 1 ml of dilution from a boiled suspension is introduced into a Petri dish to which the RCM agar has been added. After solidification, a second layer was poured on the surface as before. The incubation was carried out at 37°C for 48 hours in anaerobic jars. The large black colonies were selected and counted.

3.5.2.5 Enumeration of *Staphylococcus aureus*

The cooled, melted Mannitol Salt Agar (Scharlau) was poured into a Petri dish. After solidification, 0.1 ml of the 1/10 solution was spread over the entire surface of the plate and incubated at 37°C for 24 hours. Mannitol salt agar was used to presumptively identify *Staphylococcus aureus* which appear as golden yellow colonies. Colonies were selected and counted. The colony on Mannitol Salt Agar was added to 0.5 ml of reconstituted rabbit plasma (Bactident Coagulase EDTA, Merck KGaA and Darmstadt, Germany). After mixing by gentle rotation, the tubes were incubated at 37°C. Clotting was evaluated at 30 min intervals for the first 4 h of the test and then after 24 h incubation. The reaction was considered positive, if any degree of clotting was visible within the tube when tilted.

3.5.2.6 Detection of *Salmonella spp.*

Twenty-five grams of fish samples were homogenized in 225ml of Peptone Buffered Water (Difco, BBL/USA)

- **The pre-enrichment:** was carried out by incubating the suspension for 24 hours at 37°C.
- **Enrichment**: 1 ml of the pre-enrichment medium was seeded in a tube containing 10 ml of Selenite Cysteine Broth (Difco) and incubated at 37°C for 24 hours.
- **Isolation**: a drop of Selenite Cysteine Broth was streaked on dried plates of *Salmonella-Shigella* Agar (Conda pronadisa) previously poured and solidified.

The inoculated plate was incubated at 37°C for 24 hours. Colorless colonies with a black center are considered presumptive *Salmonella spp*. Presumptive *Salmonella* were purified on nutrient agar. The isolates were further identified biochemically.

3.5.2.7 Detection of Pseudomonas spp.

Pseudomonas was detected on King A and King B medium. For this, two petri dishes containing King A and two petri dishes King B agars were inoculated with the first dilution and incubated at 37°C for 24 hours. The first plate was used to determine fluorescence of bacterial colonies under Ultraviolet light, and the other for purification and biochemical characterization.

The presence of diffusible pigments results in the appearance of a color, which can diffuse throughout the plate:

- The blue color on King A medium (presence of pyocyanin),

- Fluorescent yellow-green color on King B medium (presence of pyoverdin).

3.5.2.8 Detection of other pathogenic bacteria

Suspect *Salmonella* colonies were isolated and purified on Trypton Soy Agar TSA (Liofilchen) for biochemical testing.

Table 3.55.2: Summary of microbiological media and culture conditions for detection and enumeration of bacteria

Germs	Media	References	Incubation Temperatures
Aerobic Plate Count (APC)	Plate Count Agar (PCA)	NF V08-051	30°C for 72H
Total Coliforms	Violet Red Bile **Agar** (VRBA)	NF ISO 4831	30°C for 24H
Thermo-Tolerant Coliforms (Fecals)	Violet Red Bile **Agar** (VRBA)	NF ISO 4831	44°C for 24H
Sulphite Reducing Bacteria	Reinforced Clostridial Agar (RCM)	NF V 08-061	37°C for 24H
Presumed pathogenic staphylococci	Chapman	NF EN ISO 6888-1	37°C for 24H
Salmonella	Peptone water Selenite cysteine Broth *Salmonella- Shigella* Agar	NF V 08-052	37°C for 18H 42°C for 24H 37°C for 24H
Spoilage microorganisms	King A, B	NFV04-505-1988	37°C for 24H

The results of the bacteriological analyses were interpreted according to the 2-class plan regarding the microbiological criteria for fresh animal products (French legislative and regulatory guide, N° 8155 of 12 December 2000).

3.5.3 Isolation, characterization and identification

Representative colonies on the King A and King B Agar for detection of the spoilage microorganisms and *Salmonella- Shigella* Agar for Pathogenic microorganisms were isolated and characterized. Two suspect colonies were taken from each plate. These colonies were purified using a buttoned Pasteur pipette on Trypton Soy Agar TSA (Liofilchen). The incubation was carried out at 37 ° C for 24 hours, and the isolated colonies were repetitively sub-cultured until pure cultures were obtained.

3.5.3.1 Macroscopic characterization

After 24 hours of incubation of the cultures, the colonies were observed. The size, appearance of the surface, the shape of the outline, the color, the consistency and the elevation of the colonies were observed.

3.5.3.2 Microscopic observation

The microscopic examination was used to determine the form of the microbial cells, their gram stain and initial grouping of the isolates (Appendix 3). From a pure culture, the smear produced on a slide was fixed and stained with gentian violet for 1 minute, and then the slide was rinsed with demineralized water. A few drops of Lugol iodin were placed on the slide to strengthen the adhesion of the dye for 20 seconds. The slide was discolored using alcohol for (5-10 seconds). Rinsed under a trickle of demineralized water, it was recolored by adding fuchsin for 1 minute. Finally, the slide was rinsed gently with deionized water and dried. The slide thus prepared was observed at 100X objective (G 1000X) in a drop of immersion oil.

3.5.3.3 Biochemical characterization of isolates

The characterization of the isolates was done using the catalase and oxidase tests as well as API 20NE for the colonies on King A and King B Agar and API 20E for the colonies on S-S Agar. This gallery included 20 micro tubes containing dehydrated substrates.

- **Catalase test**

The catalase test was based on the decomposition of hydrogen peroxide (H_2O_2) into H_2O and O_2, which is released according to the reaction stated below:

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

A few drops of hydrogen peroxide were placed on a slide. Using a sterile buttoned Pasteur pipette, a portion of the colony to be identified was removed and placed in the H_2O_2 solution. The formation of bubbles (release of oxygen resulting from the decomposition of the peroxide) signified a positive reaction (catalase +).

- **Oxidase test**

Oxidase testing involved looking for the presence of a cytochrome (presumably cyt C) present in the respiratory chain. This presence was revealed by the ability of the bacteria to oxidize the reduced colorless form of N-methylated derivatives of paraphenylenediamine. Oxidation leads to the purplish-pink semi-quinone form. For this test the soluble reagent was used. The colony identified was deposited using a buttoned pipette on the blotting paper previously impregnated with the reagent. Oxidation of the reagent was manifested by the appearance of purple coloration.

- ***Coagulase test***

Coagulase test was used to differentiate between the non-coagulase producing Staphylococcus from the coagulase producing staphylococci. Mannitol salt agar was used to presumptively identify *Staphylococcus aureus* which appeared as golden yellow colonies. Plates were incubated aerobically at 37°C for 24 h. Colonies were selected and counted. The colony on Mannitol Salt Agar was added to 0.5 ml of reconstituted rabbit plasma (Bactident Coagulase EDTA, Merck KGaA, and Darmstadt, Germany). After mixing by gentle rotation, the tubes were incubated at 37°C. Clotting was evaluated at 30 min intervals for the first 4 h of the test and then after 24 h incubation. The reaction was considered positive, if any degree of clotting was visible within the tube when tilted.

- **Biochemical identification on API 20NE system**

API 20NE is a standardized system for the identification of non-fastidious, non-enteric Gram-negative rods. A pipette was used to pick up 1-4 colonies of identical morphology from the agar plate. NaCl 0.85 % Medium, 2 ml (Ref. 20 070) was used to make the suspension. Suspension touches were prepared with a turbidity equivalent to 0.5 McFarland Standard (Ref. 70 900). Inoculate test NO_3 to PNPG was done by distributing saline suspension into the tubes using the same pipette.

The tubes and cupules of test GLU and PAC with the suspension. Mineral oil was added to the cupules of the 3 underlined tests (GLU, ADH, URE). The incubation box was closed and incubated at 30° C for 24 hours. The reagents: JAMES (Ref. 70 542) NIT 1 + NIT 2 (Ref. 70 442), Zn (Ref. 70 380) were added. The strip was read by referring to identification software V 7.0.

- **Biochemical identification on API 20E system**

API 20E system is an identification system for *Enterobacteriaceae* and other non-fastidious Gram-negative rods. It has 20 microtubes containing API M Medium (Ref. 50 120) which are dehydrated substrates. Colony of the bacterial strains (isolated colonies) of the 24 hours' cultures was added to the saline solutions of 5.0 ml of 0.85% NaCl (Ref. 20 070) and vortexed to form a bacterial suspension. A bacterial suspension of about 0.5 McFarland Standard (Ref. 70 900) was used for inoculation. Each of the microtubes in the test was filled with the bacterial suspension using a sterile Pasteur pipette. Mineral oil (Ref. 70 100) was used to create anaerobiosis: Interpretation of the test results was made by dropping a media: TDA (Ref. 70 402), JAMES (Ref. 70 542) and VP 1 + VP 2 (Ref. 70 422) - API 20 E identification software (consult bioMérieux) bioMérieux® was used to do the reading.

3.5.4 Parasitic analysis

Two (2) specimen of each of the three (3) species of fish were purchased at the Capture point, at the landing point where the fishes are packed for the market and at Medina Coura market (selling point). The samples were transported directly to the helminthology laboratory of the Central Veterinary Laboratory of Bamako in a cooler, where they were subjected to parasite analyses. Dissection was performed on each fish sample; Skin, flesh, gills and abdominal cavity were observed macroscopically. The macroscopic observation was made by observing the general state of each sample; the level of skin damage, observation of the flesh to see if there was a presence or absence of cyst or larva, state of the gills (pink color, sharp and no attack) the presence of crustacean, observation in the abdominal cavity of a parasite, larva, and cyst, and finally the coprological result was determined. The fish were also examined under the Olympus BO61 binocular magnifier for the presence of cysts.

3.6 Statistical analysis

The results of the analysis were synthesized and processed using the Microsoft Excel 2016 spread sheet. Descriptive statistics such as means, standard deviations and frequencies were used to analyze microbial counts. Analysis of variance (one-way ANOVA) was used to test the multi interaction effect of the two seasons, various areas, different sampling points (capture, landing and selling) and fish species. pH was tested for statistical significance ($p=0.05$).

The means were data from three independent experiments for microbial counts of fresh and processed fish. Frequency calculation, averaging and variance analysis (ANOVA) were performed with SPSS v 23.0 software. Tukey HSD post-hoc analysis was performed to further identify significant differences within sample groups and within value chains.

For the parasitic analysis, the results were analyzed for the following parameters Margolis *et al.(*1982).

Prevalence (%) = number of infected hosts × 100 / total number of hosts examined.

The one-way analysis of variance (ANOVA) was applied to determine the significance of variations in the prevalence of infection between the two seasons of the study period. The percentages of infestation were compared by the chi-square test. The SPSS 23.0 program was used for all statistical tests and the security level chosen was 95%.

CHAPTER FOUR

4.0RESULTS AND DISCUSSION

4.1 Survey on food safety knowledge and practices of freshwater fish value chain stakeholders

4.1.1 Demographic characteristics of stakeholders along the fish value chains

In all 247 respondents participated in this study. 130 respondents worked in the market of Medina Coura as fish sellers and 117 were in the capture point as fishermen in various areas of sampling (Table 4.1.1). Among the respondents in the market, the sale of fresh fish was dominated by women (84.6%), except for fishing areas where the fishermen were all men. Similar studies have also reported a higher proportion of women than men in sales, where both men and women have distinct roles in fish value chains (FAO, 2013; Kunadu *et al.*, 2016; Iwu *et al.*, 2017; Aboagye, 2016; Mgqibandaba *et al.*, 2020). According to FAO (2013), women are dominant in the secondary sector, which is handling, conservation and processing of fishery products, especially in activities, which are generally considered appropriate activities for women given their home tasks and responsibilities related to family care. The predominance of women in the sales sector could be attributed to their traditional responsibilities of housework and childcare. Through these income generating activities, women are able to contribute to the household income and therefore play a key role in poverty mitigation and food security (Omemu & Aderoju, 2008; FAO, 2013).

The majority of respondents that participated in the study were over 40 years of age. This could be explained by the fact that the choice of respondents was made according to the duration and stability in the field. These were also similar to the results of Mgqibandaba *et al*. (2020) whose survey revealed that 40% of their respondents were between 31 and 40 years old and that of Kunadu *et al*. (2016) of which 31% were between 36-50 years old.

The majority of fishermen (78.6%) had no formal education, and sellers had an incomplete basic school education (53.1% middle school and 40.0% primary school); this is in agreement with other studies carried out by Zanin *et al*. (2015) where 51.8% had an incomplete basic school education and that of Aboagye (2016) in Ghana that 32% of fishermen had no formal education. This could be an impact on the safe delivery of fish and result in a change in food handling behavior. Majority of the sellers were Bambara (48.5%) and the fishermen were Bozo (64.1%). This is because in Bamako, Bambara is the main ethnic group and fishing belongs to the Bozo or the sedentary professional fishermen for whom fishing represents a full-time economic activity (Konare, 1977). For the seller study population, 65.4% were wholesaler while 33.8% retailer in Mali. In Mali, the marketing circuit for fresh fish shows that the wholesaler at the same time serves as the retailer or the consumer from which he is most favored. The retailer is the least favored because the fish from this outlet is more expensive (Traore, 2014).

For the fishermen, the level of experience in fishing activities ranged from 0-40 years with a majority (85.5%) having between 31-40 years. According to FAO (2013), family communities that practically monopolize the fishery sector operate in the sector.

Table 2: Demographic characteristics of the study population

Variable		% Stakeholder (n)	
		Sellers (n=130)	**Fishermen (n=117)**
Gender	Male	15.4 (20)	100(117)
	Female	84.6 (110)	0
Age	18-24 yrs	0	9.4(11)
	25-29yrs	1.5(2)	5.1(6)
	30-39yrs	20(26)	24.8(29)
	> 40yrs	78.5(102)	60.7(71)
Ethnic Group	Bozo	29.2(38)	64.1(75)
	Bambara	48.5(63)	18.8(22)
	Malinke	6.2(8)	10.3(12)
	Songhai	2.3(3)	0.9(1)
	Soninke	3.1(4)	2.6(3)
	Fulani	10.8(14)	3.4(4)
Educational Level	Middle School	53.1(69)	0
	Secondary	3.1(4)	0.9(1)
	Primary	40(52)	20.5(24)
	No formal education	3.8(5)	78.6(92)
Stakeholders			
	Wholesaler	65.4(85)	100(117)
	Wholesale-Retailer	33.8(44)	0
	Retailer	0.8(1)	0
Number of years in fishing activity	1 – 10	5.4(7)	4.3(5)
	11-20	24.6(32)	10.3(12)
	21-30	25.4(33)	0
	31-40	44.6(58)	85.5(100)

n: frequency

4.1.2 Business characteristics

Table 4.1.2 shows how business takes place in the fresh fish sales process. In Medina Coura market, fish come from different areas; 8.5% are sourced from Mopti and Markala, 52.3% from Mopti-Segou-Manantali, 25.4% Mopti-Selingue-Gao- Manantali and 13.8% from Mopti-Selingue. These data are in agreement with those obtained by FAO (2007), which assert that the major capture sites are, in order of importance, the Central Delta of Niger at Mopti (80,000 to 90,000 tons of fresh fish), Lake Selingue (4,000 tons of fresh fish) and Lake Manantali (3,000 tons of fresh fish).

The means of transportation of fish to the retail market was mainly by public transport (Car) 99.2%. However, the process of transporting fish to Bamako is trouble laden. The problems include among others, the socio-political crises or uncontrolled or sometimes restrictive administrative and customs procedures, and especially the instability in the northern part of the country, causing the majority of fishermen to abandon their activities. Poor transport could lead to a decrease in the shelf life of the fish, and the degradation of the fish leading to a drop in the price of the product (Diei-Ouadi & Mgawe, 2011). The time taken to sell 80% of fish stock on the market is 2-3 days. Fifty percent of sellers believe that the quantity of spoiled fish is low. Post-harvest losses during fishing activities is a major concern since it is equivalent to the loss of animal protein for consumers and a loss of income for fishermen, processors and traders (FAO, 2007). In Medina Coura market the sellers receive their fish in basket but they sell by kilo.

Table 3: Frequency of fishing businesses doing by the fish sellers

Variable	Categories	% Sellers (n=130)
Origin	Mopti-Markala	8.5 (11)
	Mopti-Segou-Manantali	52.3 (68)
	Mopti-Selingue-Gao-Manantali	25.4 (33)
	Mopti-Selingue	13.8(18) (5)
Means of transport		
	Car (public transport)	99.2 (130)
	Refrigerated truck	0.8(1)
Flow time of fish	2-3 days	46.9 (61)
	7 days	0.8(1)
	1 day	46.2(60)
	3-4 days	4.6(6)
With what measure do you sell your fish in the market?		
	In weight (Kg)	100(130)
	Per basket	0 (0%)
How many kg of fish can one basket contain?		
	300kg	65.4(85)
	600kg	34.6(45)
How many baskets do you take from various areas?		
	4-6 Baskets	64.6(84)
	1-3 Baskets	33.1(43)
	1-7 tons	0.8(1)
	1 ton	1.5(2)
What is the frequency of reception of fish per week?		
	2 -3 days	1.5(2)
	4-5 days	15.4(20)
	6-7 days	83.1(108)
How long does it take before the fish gets to the market?		
	>24hrs	33.1(43)
	11-15 hrs	63.8(83)
	6-10hrs	3.1(4)
Can you estimate the average amount of spoiled fish per batch?		
	1-5 fish	53.8(70)
	6-10 fish	14.6(19)
	11-20 fish	31.5(41)
Have you encountered sick fish in the lot you selling?		
	Yes	100(130)
How do you display the fish for sale?		
	On floor mat	93.8(122)
	On the table	6.2(8)
How do you store fish during the sales period?		
	In a broken fridge with ice	100(130)

n: frequency

The fishing gears used by fishermen vary depending on the area (Table 4.1.3). Malian fishermen use a wide range of gear and catching techniques, adapted to changing conditions, to the environment depending on the season (Kodio & Keita, 1999; FAO, 2007). Exposure of fishing gear-containing fish for long periods could lead to spoilage of the fish, especially at elevated temperatures. In some areas, either the number of canoe owners is low or some fishermen do not have the financial means to have motorized canoes.

According to the fishermen respondents, there is no difficulty in selling freshly caught fish. Once they land in, the women come to take them as wholesalers to sell them locally or send them to Bamako for sale.

Fishermen face a lot of difficulties resulting in a huge loss in their activities. The survey showed that these difficulties include the mining in the area of Selingue, depletion of fish in the river, theft of fishing gear and also insecurity in the Northern part of the country. According to Olagunju (2015), over the past two decades, drought and demographic factors (increase in the fishing population) have contributed to reducing fishermen's income as well as economic returns, technical and economic factors (diversification of fishing gear and increased fishing effort) and commercial factors such as poor valuation of products, reduction in the quantity of marketable fish.

Table 4:Business activities of fishermen in selected fishing areas

Variables	Categories	Areas % (n) Selingue (31)	Manantali (25)	Markala (31)	Mopti (30)
What fishing gear do you use for fishing?					
	Gill net	80.6(25)	40(10)	100(31)	60(18)
	Cast net	6.5(2)	-	-	7(11)
	Fishing net	12.9(4)	4(1)	-	-
	Seine net	-	56(14)	-	3.3(1)
What types of fishing canoes do you use?					
	%Canoes non-motorized	-	-	100(31)	16.7(5)
	%Canoes motorized	100(31)	100(25)		83.3(25)
How do you do your fishing activities?					
	The nets are left at 4 p.m. and collected overnight	100(31)	28(7)	96.8(30)	73.3(22)
	Spend 4 to 5 days in the lake	-	8(2)	-	-
	Spend 5 to 7 days in the lake	-	64(16)	-	-
	Spend less than 2 hours in the river	-	-	3.2(1)	26.6(8)
Which period of the year is fishing most beneficial to you?					
	February - September	100(31)	-	-	-
	June - August	-	100(25)	-	-
	November - January	-	-	100(31)	100(30)
What is a average quantity of fish you harvest by shipment?					
	1-20Kg	96.8(30)	16(4)	74.2(23)	40(12)
	21-40Kg	3.2(1)	8(2)	16.1(5)	13.3(4)
	41-60Kg	-	16(4)	3.2(1)	26.7(8)
	61-80Kg	-	8(2)	-	3.3(1)
	>100Kg	-	52(13)	6.5(2)	16.7(5)
How often do you go fishing?					
	4-5 Days/week	3.2(1)	16(4)	-	6.7(2)
	6-7 Days/week	96.8(30)	81(21)	100(31)	93.3(28)
In average, how much time do you spent on each shipment?					
	> 2hrs	-	-	-	46.7(14)
	2-6hrs	96.8(30)	100(25)	96.8(30)	36.7(11)
	7-12hrs	3.2(1)	-	3.2(1)	16.7(5)
What are the problems you are having?					
	The use of pesticides in water by miners (cyanide, Mercury)	87.1(27)	-	-	-
	Lack of fish in a water	3.2(1)	44(11)	51.6(16)	30(9)
	Lack of ice and storage material	9.7(3)	56(14)	9.7(3)	40(12)
	Theft of fishing gear	-	-	9.7(3)	3.3(1)
	Insecurity in a North of the country	-	-	9.7(3)	26.7(8)

n: Frequency

4.1.3 Food Safety Knowledge

From this study, it is shown that the respondents had a good knowledge of food safety (Table 4.1.4). A similar study done by Grema *et al.* (2018), indicated the importance of food safety knowledge of food handlers. It reported that good knowledge mostly translates into positive behaviour and practices leading to safe food production and handling. All personnel involved in food handling must maintain good hygiene, and a high degree of cleanliness to ensure food safety in food preparation, and to avoid contamination of the diet with pathogenic microorganisms (Howes *et al*., 1996).

The study revealed that the level of knowledge could be significantly associated with the food hygiene practices. This was consistent with the study by Iwu *et al*.(2017) in Nigeria, where knowledge, attitude and training were significantly associated with the practice. On the contrary, a study in Nigeria by OTU (2014), suggested that knowledge was not linked to practice and that this was attributed to the existing socio-cultural context that probably had a greater influence on safe eating practices.

Table 5: Responses to questions on food safety knowledge by fish sellers

Variable	Categories	%Fish sellers (n)
Do you know spoilt fish?	Yes	100(130)
Do you know what causes fish spoilage?	Lack of ice	64.6(84)
	Broken down car	34.6(45)
Insanitary condition of sale 0.8(1)		
Do you think the way the fish is handled contributes to its spoilage?	Yes	100(130)
Have you encountered sick fish in the lot you are selling?	Yes	100(130)
If yes, please describe the state of the spoiled fish	Texture change	47(61)
	Change in eye color	15.4(20)
	Discoloration	10(13)
	Fish swelling	27.2(36)
Do you think the infected fish could cause illness?	No	74.6(97)
	Yes	25.4(33)
If yes, which kind of disease?	Abdominal pain	15.4(20)
	Diarrhea	84.6(110)
What measures could be taken to ensure that the fish in our local markets are healthy and safe for human consumption?		
	Need a refrigerated truck for transport, and a cold room	83.1(108)
	Financial aid	0.8(1)
	Vehicle stop by customs for a long time	9.2(12)
	Unsanitary conditions, lack of evacuation system	6.9(9)

n: frequency

Table 6:Responses to questions on food safety knowledge by fishermen

Variable	Categories	%Areas (n=117)			
		Selingue	Manantali	Markala	Mopti
Are you able to identify fish which is going bad or spoilt?					
	Yes	100(31)	100(25)	100(31)	100(30)
	No	-	-	-	-
Do you think the way the fish are handled could contribute to their spoilage?					
	Yes	100(31)	100(25)	100(31)	20(6)
	No	-	-	-	80(24)

n: frequency

4.1.4 Food Safety practices

Table 4.1.6 shows that all fish sellers received the fish from different areas in baskets with ice. At Medina Coura market, unsanitary conditions of the sales outlets were observed. Fish was stored in broken-down freezers with ice and the fish was displayed on a mat on the floor for sale, sometimes without ice. The breaking of the cold chain following the use of broken-down freezers with their walls covered with rust, in which worn bags were placed to prevent their contact with the products and the escape of cold produced by ice are improper techniques. According to the FAO & WHO, (2012) fish sale areas must be in good condition, durable and easy to maintain and disinfect. Appropriate and adequate facilities must be provided for the storage and / or production of ice.

Table 7: Responses to questions on food safety practices by fish sellers

Variables	Categories	% Sellers (n=130)
How do you store fish during the sales period?	In broken fridge with ice	100(130)
	Cold-store refrigeration	0(0)
In which condition does the fish arrive on the market?	Basket with ice	100(130)
	Basket with wet sand	0(0)
	Basket with wet cloth	0(0)
How do you display the fish for sale?	On floor mat	93.8(122)
	On table	6.2(8)

n: frequency

In all the areas, the majority of the respondents lose their fish after capture due to spoilage (Table 4.1.7). The loss of fresh fish could be due to the inadequate use of ice from capture to sale, in the market the use of freezers without insulation, the practice of exposing fish on the ground at room temperature is often high. During transport, the delay is often due to vehicle breakdown, or stopping of the vehicle by customs officers. At the landing point, the fish are not washed in potable water, contrary to stipulations by the FAO & WHO (2012) that water for cleaning fish must be potable to avoid cross-contamination.

Table 8: Responses to questions on food safety practices by a fishermen

Variable	**Categories**	**% Areas (n=117)**			
		Selingue	**Manantali**	**Markala**	**Mopti**
Do you sometimes lose fish?	Yes	100(31)	100(31)	87.1(27)	66.7(20)
	No	-	-	12.9(4)	20(6)
	Rarely	-	-	-	13.3(4)
If not, please describe how you handle your catch to avoid spoilage?					
	Bringing the ice	93.5(29)	100(25)	-	70(21)
Go fishing very early in the morning		3.2(1)	-	-	-
	Speed	3.2(1)	-	6.5(2)	3.3(1)
	Nothing	-	-	93.5(29)	26.7(8)
What have you done with the spoilt fish?					
Sell it to retailers at low cost		100(31)	4(1)	-	13.3(4)
Sell it to wholesalers at low cost		-	96.0(24)	100(31)	3.3(1)
Self-processing		-	-	-	46.7(11)
Retailer		-	-	-	36.7(11)

n: frequency

4.1.5 Relationship among age, education, and fish safety knowledge and practice of the fish sellers

Table 4.1.8 presents the results of the relationship between age, duration of service, fish safety practice and knowledge. The analysis of the table reveals that fish safety knowledge of the vendors has a significant positive impact on their safety practices. This result implies that the more educated the vendors are the more knowledge they have and the more their fish handling behavior will be positively influenced. This means regular training and workshops will greatly improve their fish safety knowledge and practice. Additionally, the age and educational status of the vendors also have a positive influence on their knowledge; implying that, the older they are and the more educated they are, the better their handling practices of fish along the value chain. The results differ from the findings of Grema *et al*.(2018) in Kaduna State, Nigeria, that indicated that most fish sellers had good knowledge of food hygiene practices such as hand washing; however, knowledge about hygienic handling of fish could not be translated into practice. This study supports the findings of a study done by Amuna (2014) which also reported that food handlers tend to overestimate the frequency with which they carry out safe food handling practices

Table 9: Bivariate correlation of age and education with food safety knowledge and food safety practice using Spearman's rho coefficient

Predictors	Correlation Coefficient	P-Value
Food safety knowledge VRS Food safety practice	0.249**	0.007
Age VRS Food safety knowledge	0.262**	0.004
Education VRS Food safety knowledge	0.384**	0.000

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

4.1.6 Relationship among age, duration of service, and food safety knowledge and practice of the fishermen

Bivariate Spearman's rho correlation coefficient was used to investigate whether or not knowledge has any influence on fishermen's fish safety practice, and also to find out if age and duration of service have any influence on their knowledge. The results displayed in Table 4.1.9 reveal that food safety knowledge significantly influence the food safety practices of the fishermen ($P < 0.05$); however, age and duration of service have no effects on food safety knowledge of the fishermen

(P > 0.05). This means that the more food safety training and workshops are organized for the fishermen to improve their knowledge, the more it will enhance their food safety practices. Besides the food safety knowledge of the fishermen is not influenced by length of service in the fishing business nor their age. This finding is similar to findings of Grema *et al.*,(2018) which found a positive correlation recorded between knowledge and practices (rs = 0.2, P = 0.23) but negative correlation (n) between practices with years of business experience (rs = -0.03, P = 0.830).

Table 10: Bivariate correlation among age, education and food safety practice and knowledge using Spearman's rho coefficient

Predictors	Correlation Coefficient	P-Value
Food safety knowledge VRS Food safety practice	-0.231*	0.012
Age VRS Food safety knowledge	0.053	0.573
Duration of Service VRS Food safety knowledge	-0.043	0.643

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

4.2 Physico-chemical analysis of water and fish in the freshwater fish value chain

4.2.1 Determination of pH of water, and pH of fish species

The pH values of the surface water recorded ranged from 5.05 to 5.6 during the cold season and 5.1 to 5.9 during the hot season (Figure 4.2.1). According to AMANORM (2011) (Malian Agency for Standardization and Promotion of Quality, pH standard of water must be between 5.5 and 9. The average pH value of the water remained slightly acidic, i.e., between 5.05 and 5.9 throughout the study periods and it was found slightly higher during the hot season. This may be due to a higher water temperature during this period, which could cause the conversion of CO_2 to organic carbon by the process of photosynthesis. A similar study done by Merhabi (2020) in Libya, also found the pH of the water to be slightly acidic. The pH of the water can have a significant influence on the toxic action on fish due to the presence of hydrogen sulfide, ammonia, cyanides and also of heavy (Pedrosa-Menabrito & Regenstein, 2007).

The pH values of the fish samples are listed in Table 4.2.1. The values vary from 6.1 ± 0.3 to 6.9 ± 0.6 during the cold season and 6.2 ± 0.2 to 6.9 ± 0.6 during the hot season. The values for the various seasons are not, statistically, significantly different ($p \leq 0.05$). In the cold season, samples of *Oreochromis niloticus* in Mopti had higher pH at the capture point while samples of *Clarias anguillaris* in Markala had lower pH at the landing point. In the hot season, samples of *Lates niloticus* in Manantali had higher pH at the selling point and lower pH at landing point.

The values of pH recorded were close to that of well-preserved harvested fish (pH of 6.2- 6.9). The pH measurements in this study were carried out 4 to 9 hours after the death of the fish, depending on the place of sampling. The low pH encountered a few hours after harvest may also indicate that the fish was not harvested under the right conditions and that they must have been stressed. The glycogen in fish muscle had been metabolized to lactic acid, causing a drop from the typical pH of ≈ 7.0 of live fish muscle. The pH is one of the important parameters for determining the quality of fish flesh during storage (Abbas *et al.*, 2008). According to Degnon *et al.*(2013), after the death of the fish, the initial pH varies from 5.4 to 7.2.Acid pH leads to gill damage while alkaline pH leads to super oxygenation (Ikeogu *et al.*, 2010).

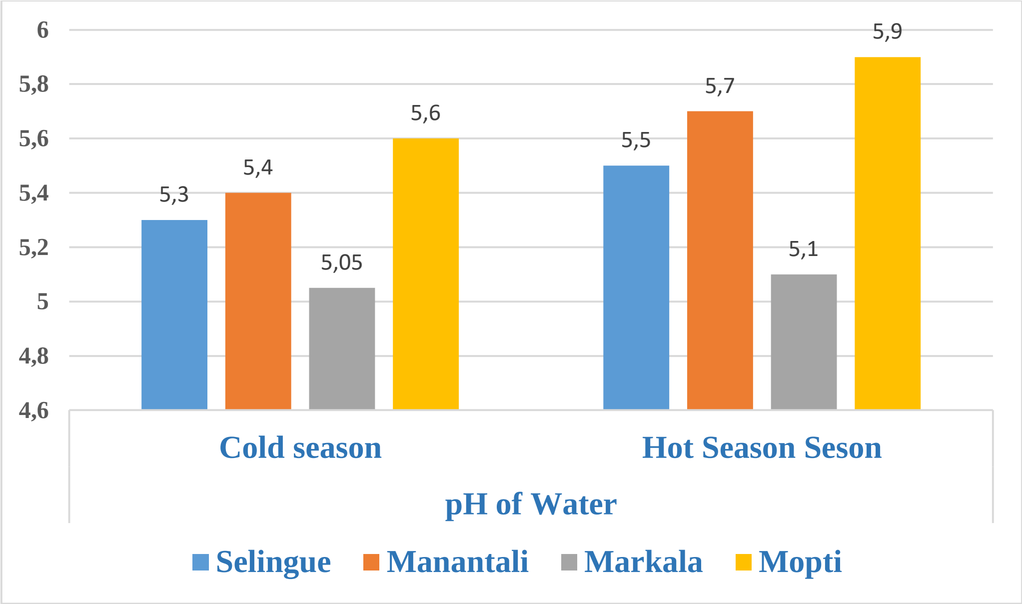

Figure 4.2.1:Mean value of water pH during the 2 seasons

Table 4.2.1: Mean values of pH of fish species sampled in different seasons

Sampling point	Fish species	Selingue		Manantali		Markala		Mopti	
		Cold	Hot	Cold	Hot	Cold	Hot	Cold	Hot
Capture	*L. niloticus*	6.3	6.5	6.4	6.6	6.8	6.6	6.5	6.6
	C. anguillaris	6.6	6.8	6.5	6.7	6.6	6.3	6.4	6.6
	O. niloticus	6.5	6.3	6.3	6.3	6.2	NA	6.9	6.5
Landing	*L. niloticus*	6.6	6.5	6.7	6.7	6.7	6.2	6.5	6.5
	C. anguillaris	6.8	6.5	6.4	6.7	6.1	6.5	6.7	6.5
	O. niloticus	6.2	6.5	6.4	6.7	6.4	6.6	6.6	6.5
Selling	*L. niloticus*	NA	6.6	6.4	6.5	6.3	NA	6.4	6.9
	C. anguillaris	NA	6.6	6.6	6.5	6.4	NA	6.4	6.4
	O. niloticus	NA	6.4	6.5	NA	6.3	NA	6.5	6.3
Overall P value		0.06	0.73	0.17	0.33	0.28	0.41	0.63	0.09

NA: Not applicable

4.3 Microbiological quality of water and fish in the freshwater fish value chain

Microbiological analysis of freshwater fish species was carried out. Fishes were examined for microbial quality. Fishes were examined for microbial quality. The purpose of these tests was to detect pathogenic and spoilage microorganisms on freshwater fishes. Microbial quality of water sample at the capture point.

The mean microbial counts in water samples in Selingue, Manantali, Markala and Mopti at capture in the two seasons are presented as Figure 4.3.1. There were significant differences between Total Coliforms, Fecal Coliforms and Sulphite Reducing Bacteria counts for the four sampling areas. The values were higher than microbiological criteria in international standards (AFNOR, 2004).

Aerobic Plate Counts of the fresh fish species investigated ranged from 5±0.69 to 6.6±0.9 Log10 CFU/ml for samples in Markala in the hot season and Selingue in the cold season, respectively. Total coliforms, ranged from, 3.98±0.33 to 5.77±0.17 Log10 CFU /ml in hot season at Manantali and Selingue. Faecal coliforms of the water samples ranged from 2 to 5.6±0.13Log10 CFU/ml in hot season at Markala and Selingue. For the Sulphite Reducing Bacteria spores in the water samples, Mopti in the cold season had a higher concentration, 1.13±0.0513 Log10 CFU/ml than the standard. The microbiological data obtained from this study clearly showed a high number of Aerobic Plate Counts, Total and Fecal Coliforms and Sulphite Reducing Bacteria. The relatively high counts of total and fecal coliforms may be due to additional contamination from bathing, washing, and swimming activities carried out by the occupants near this point. The contamination levels of water samples analyzed were of relatively within acceptable levels as regards *Staphylococcus aureus*. These results are in agreement with that of Simplice *et al.* (2018) who found a low rate of water contamination by *Staphylococcus aureus*.

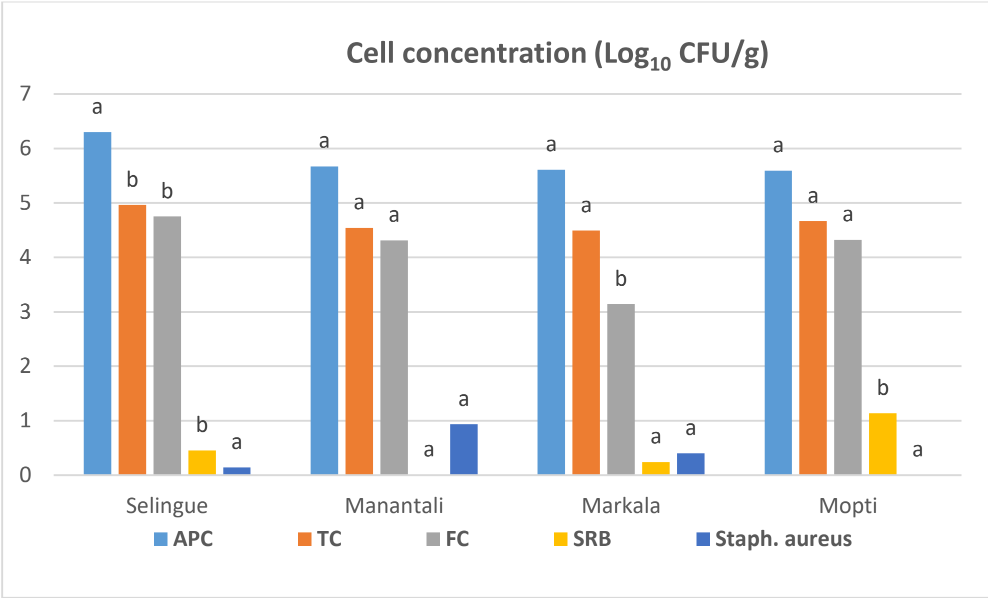

Figure 4.3.1: Microbial count Log10 CFU/ ml in the water at the capture point

Aerobic Plate Counts (P=0.36); Total coliforms count (P=0.39); Fecal coliforms count (P=0.25); Sulphite Reducing Bacteriacount (P=0.18); *Staphylococcus aureus* (P=0.53) across sampling areas. For each microorganism, mean counts (bars) with different alphabets across sampling locations showed statistically significant differences (P < 0.05). Error bars refer to standard error of the mean.

4.3.1 Microbial quality of fish containers at landing

The mean microbial counts and prevalence of *Salmonella* on container surfaces in Selingue, Manantali, Markala and Mopti areas at the landing point in the hot and cold seasons are presented in Figure 4.3.2. There were no significant differences in the Aerobic Plate Counts (P = 0.15), Total coliforms (P = 0.46), Fecal coliforms (P = 0.37), Sulphite reducing bacteria (P = 0.23) and *Staphylococcus aureus* (P = 0.21) on containers surfaces. Aerobic Plate Counts ranged from 6.46±0.1 to 8.71±0.3 Log10 CFU /cm^2 in Manantali in the cold season and Selingue in the hot season, respectively. In different areas, the results showed Total Coliforms, ranged from, 3.98±0.33 to 5.77±0.17 Log10 CFU /cm^2 at Manantali in the cold season and Selingue in the hot season. Fecal Coliforms ranged from 4.3±0.42 to 8.29±0.2 Log10 CFU /cm^2 in hot season at Markala and Selingue, respectively. The analysis of Sulphite Reducing Bacteria from the container swap samples taken was higher (5.02±0.04 Log10 CFU /cm^2) in the cold season in Markala. Prevalence of *Staphylococcus aureus* was higher in Manantali in the cold season (3.49±0.27 Log10 CFU /cm^2).

86. 4 % representing of the container samples had *Salmonella spp*. The concentration of microorganisms in the container samples might be due to how the fish are off-loaded from the canoes, washed in water at the landing site and thrown into a container.

The hygiene and cleanliness of these containers are important in the contamination of fish. The duration of the transport of the fish from the original landing site to the next landing, which is the Medina Coura market can take from 3 to 9 hours depending on the distance from the different areas in Bamako, the engine speed and also the customs stop time. These different conditions of transportation of the fish are likely to affect its quality.

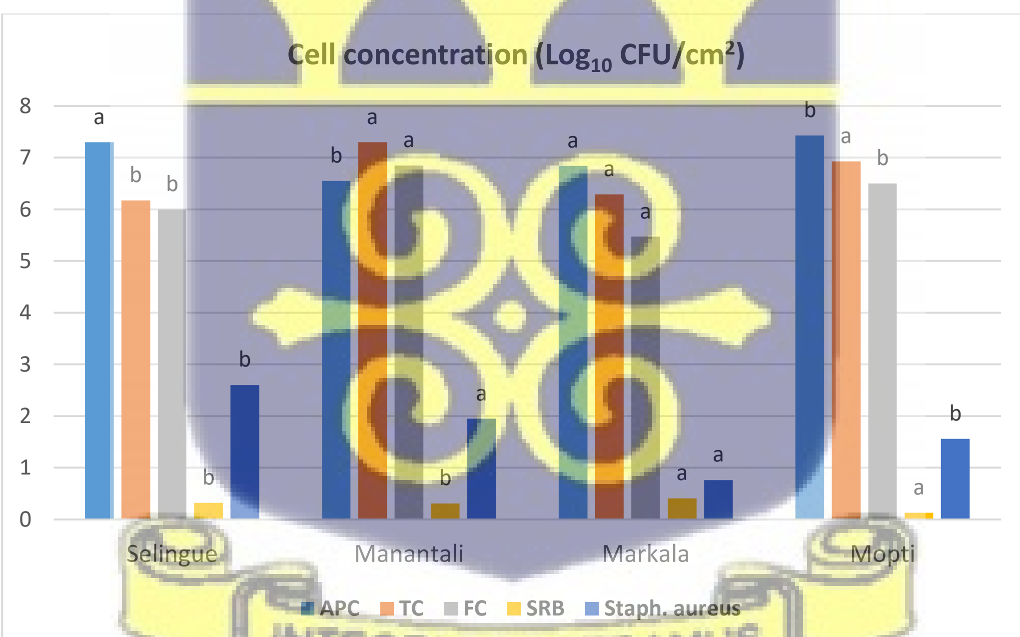

Figure 4.3.2: Microbial counts in fish containers at various landing points.

Aerobic Plate Count (APC) (P=0.15); TC: Total coliforms count (P=0.46); FC: Fecal coliforms count (P=0.37); SRB: Sulphite Reducing Bacteria count (P=0.23); STAPH: *Staphylococcus aureus* (P=0.21)

Error bars refer to standard error of the mean.
1: Selingue, 2: Manantali, 3: Markala, 4: Mopti.

4.3.2 Microbial quality of fish samples along the fresh fish value chain

The value chain of the fishing sector in Mali from capture to sale is generally characterized by the off-loading of unrefrigerated fish at the landing sites and an unhygienic transfer of fish into the loading container intended to be sold at Medina Coura market of Bamako. These different practices affect and reduce the quality of the fish, thus accelerating spoilage and promoting a reduction in the shelf life of the fish. Fish are typically harvested and stored with ice in the transport vehicles to the final destination. Depending on the collection areas, some stakeholders send their fish on the same day to Bamako (Selingue); the other areas (Manantali, Markala and Mopti) store fish for a minimum of 2 days in the broken-down fridge with ice until transportation to the point of sale at Medina Coura market. As soon as it arrives in Bamako, the fish is sold immediately. The rest of the fish not sold the same day is kept in the melting ice and stored in a broken-down fridge until they are completely sold (Figure 4.3.3).

Figure 4.3.3: Fish stored in a broken-down fridge

The microbiological analysis of the freshwater fish species was carried out to assess the quality of the fish during the cold and hot seasons along the value chain. In this study there was no significant seasonal fluctuation in the quality of fish in the 4 areas.

In Selingue, there was no significant difference between the same group of microorganisms in the cold and hot seasons. In cold season: Aerobic Plate Counts (P=0.28); Total coliforms (P=0.26); Fecal coliforms (P=0.40); Sulphite Reducing Bacteria (P=0.24); *Staphylococcus aureus* (P=0.29). In hot season: Aerobic Plate Counts (P=0.28); Total coliforms (P=0.39); Fecal coliforms (P=0.43); Sulphite Reducing Bacteria (P=0.36); *Staphylococcus aureus* (P=0.18) (Figure 4.3.4)

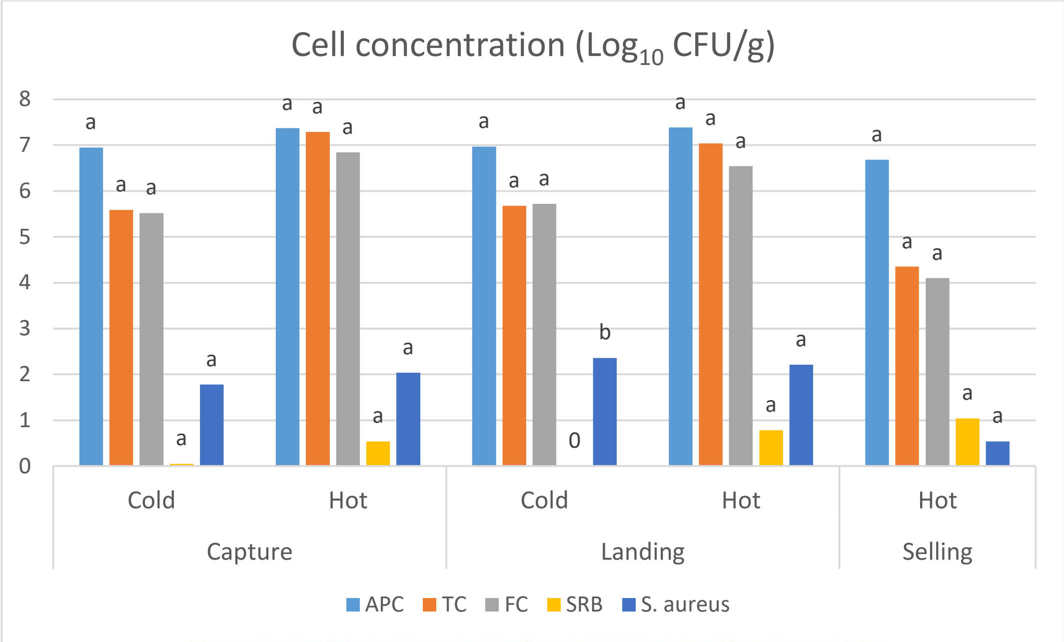

Figure 4.3.4: Microbial mean Log10 CFU/ g of fish in various sampling point in Selingue

In cold season: Aerobic Plate Counts (P=0.28); Total coliforms (P=0.26); Fecal coliforms (P=0.40); Sulphite Reducing Bacteria (P=0.24); *Staphylococcus spp*. (P=0.29). In hot season: Aerobic Plate Counts (P=0.28); Total coliforms (P=0.39); Fecal coliforms (P=0.43); Sulphite Reducing Bacteria (P=0.36); *Staphylococcus aureus* (P=0.18). For each microorganism, mean counts (bars) with different alphabets showed statistically significant differences (P < 0.05). Error bars refer to the standard error of the mean.

Figure 4.3.5 shows that in Manantali there were no significant differences between the same microorganism between the two seasons. In cold season: Aerobic Plate Counts (P=0.23); Total coliforms (P=0.42); Fecal coliforms (P=0.66); Sulphite Reducing Bacteria (P=0.15); *Staphylococcus aureus*. (P=0.74). In hot season: Aerobic Plate Counts (P=0.23); Total coliforms (P=0.20); Fecal coliforms (P=0.29); Sulphite Reducing Bacteria (P=0.18); *Staphylococcus aureus* (P=0.21).

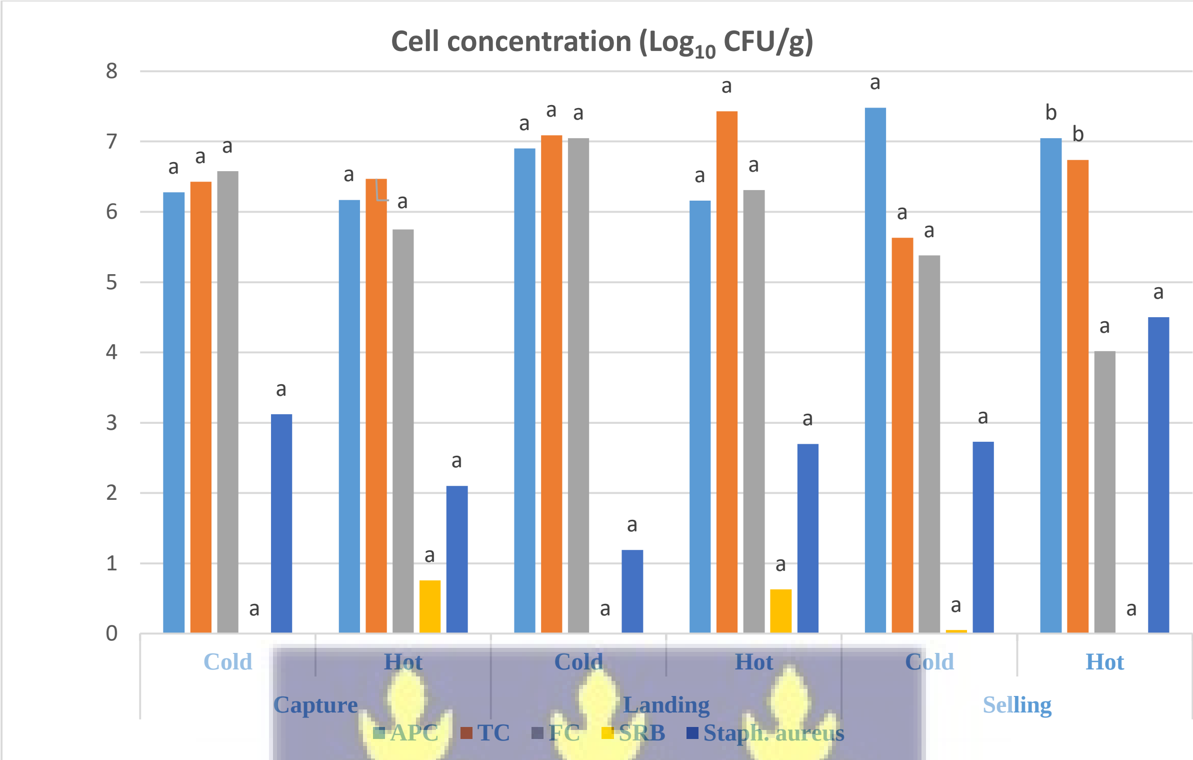

Figure 4.3.5: Microbial mean Log10 CFU / g of fish in in various sampling point in Manantali

In cold season: Aerobic Plate Counts (P=0.23); Total coliforms (P=0.42); Fecal coliforms (P=0.66); Sulphite Reducing Bacteria (P=0.15); *Staphylococcus spp.* (P=0.74). In hot season: Aerobic Plate Counts (P=0.23); Total coliforms (P=0.20); Fecal coliforms (P=0.29); Sulphite Reducing Bacteria (P=0.18); *Staphylococcus aureus* (P=0.21). For each microorganism, mean counts (bars) with different alphabets showed statistically significant differences ($P < 0.05$). Error bars refer to standard error of the mean.

Figure 4.3.6 shows that in Markala the microbial counts were not significantly different same between a same group of microorganisms in the cold and hot seasons.

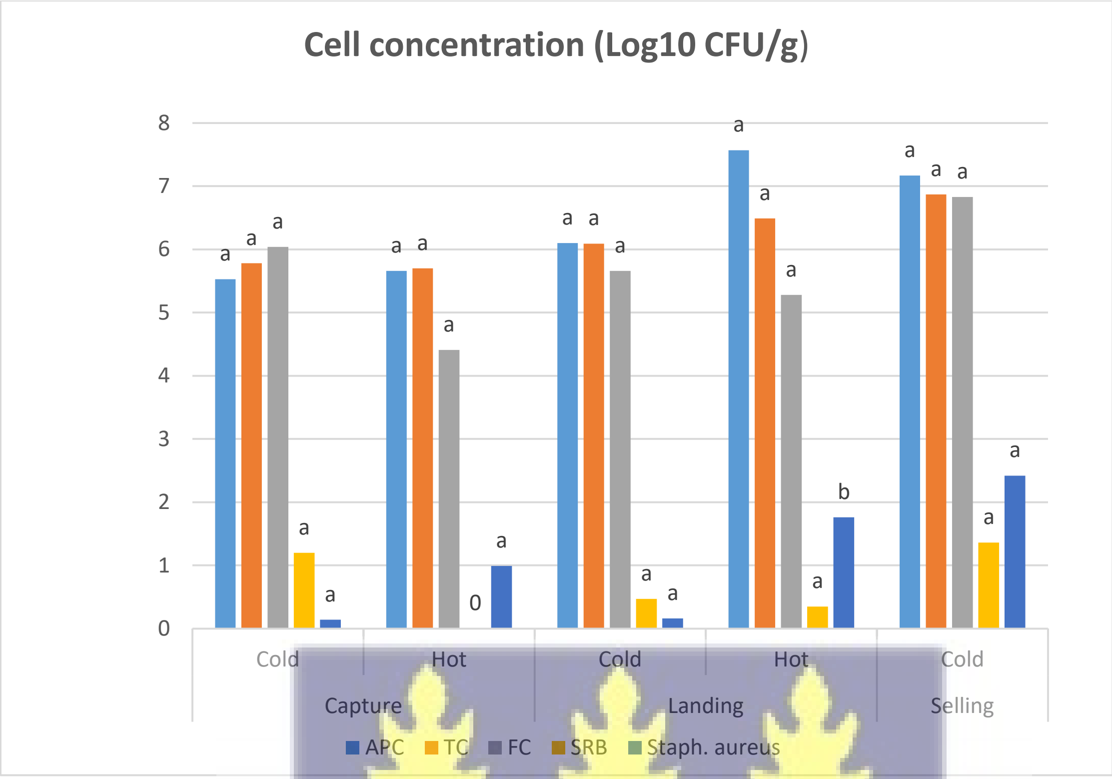

Figure 4.3.6: Microbial count Log10 CFU / g of fish in in various sampling point in Markala

In cold season: Aerobic Plate Counts (P=0.57); Total coliforms (P=0.57); Fecal coliforms (P=0.44); Sulphite Reducing Bacteria (P=0.349); *Staphylococcus aureus* (P=0.17). In hot season: Aerobic Plate Counts (P=0.14); Total coliforms (P=0.18); Fecal coliforms (P=0.33); Sulphite Reducing Bacteria (P=0.15); *Staphylococcus aureus* (P=0.079). For each microorganism, mean counts (bars) with different alphabets showed statistically significant differences (P < 0.05). Error bars refer to standard error of the mean.

In Mopti, the counts of the various microorganisms were also not statistically significantly different as shown in (Figure 4.3.7).

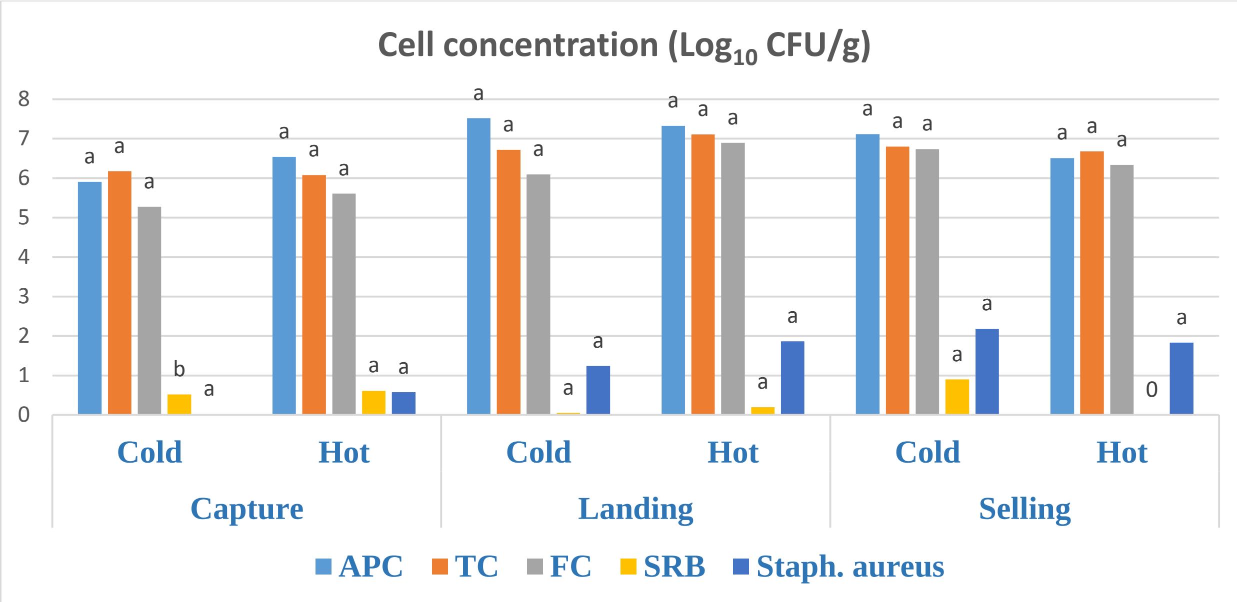

Figure 4.3.7: Microbial count Log10 CFU / g of fish in various sampling point in Mopti.

In cold season: Aerobic Plate Count (P=0.46); Total coliforms (P=0.37); Fecal coliforms (P=0.46); Sulphite Reducing Bacteria (P=0.23); *Staphylococcus spp*. (P=0.69). In hot season: Aerobic Plate Counts (P=0.39); Total coliforms (P=0.51); Fecal coliforms (P=0.39); Sulphite Reducing Bacteria (P=0.11); *Staphylococcus spp*. (P=0.35). For each group of microorganisms, mean counts (bars) with different alphabets showed statistically significant differences ($P < 0.05$). Error bars refer to standard error of the mean.

Among the different fish species investigated, mean Aerobic Plate Counts ranged from 5.23 to 7.83 Log10 CFU g^{-1}. *O. niloticus* at the landing point in Mopti recorded the highest mean counts (Appendix 7), while Nile perch at the capture point in Markala had the lowest. (Appendix 7). All the fish samples tested had mean contamination levels of ≥5.0 Log10 CFU g^{-1}. The high Aerobic Plate Counts recorded for freshwater fishes in this study could be due to a lack of integrity of the cold chain from dispatch, transportation, storage, and cross-contamination from handling by stakeholders. It could also be due to the use of broken refrigerators as containers in the landing area and on the market in the Medina Coura (Sissoko *et al.*, 2016). APC indicates the state of decomposition of the product and therefore constitutes an index of the quality of fish (Kunadu *et al.*, 2018). According to International Standards AFNOR (2004) related to fresh fish, 100% or the entire sample of the fish are non-compliant. The fish in the value chain are likely to have shorter shelf-life.

Among different fish species investigated Total Coliforms (TC), ranged from, 4.11 to 7.49 Log10 CFU g^{-1}. The highest TC count was recorded in *O. niloticus* at the landing point in Manantali (Appendix 5), and the lowest in *O. niloticus* at the selling point in Selingue. Total Coliform counts of the freshwater fish were above the International Commission on Microbiological Specifications for Foods (ICMSF) criteria of ≤ 1 Log10 CFU g^{-1}. Coliforms are not among the normal bacterial flora of fish and their presence demonstrates the level of pollution of their environment (Sichewo *et al.*, 2014).

Among the different fish species investigated, Fecal Coliforms ranged from 3.64 to 7.5 Log10 CFU g^{-1}. The highest number of Fecal Coliforms was recorded in *C. anguillaris* at the selling point at Markala (Appendix 6), and the lowest in *O. niloticus* at the selling point in Selingue (Appendix 5). Fecal coliform bacteria or thermotolerant coliforms are abundant in the aquatic environment, in the soil and on vegetation, also in warm-blooded animal species. These are indicator organisms whose presence in food in large quantities may indicate the presence of pathogenic bacteria. Terentjeva *et al.* (2015) had a similar results in a study in Latvia, where many *Enterobacteriaceae* in gills ranged from 0.7 up to 5.78 Log10 CFU g^{-1}, with the highest *Enterobacteriaceae* count in European perch. Degnon *et al.* (2013) also recorded high rate of thermotolerant coliforms in all samples analyzed in a study; which was explained to be due to the lack of good hygienic practice and the break in cold chain Bornert (2000), indicated that thermotolerant coliforms constitute germs of faecal contamination and are therefore indicators of poor hygienic conditions when handling foodstuffs. Sulphite Reducing Bacteria spores were detected in 11% (4/36) of samples at Selingue, 5.9% (2/34) of samples at Manantali 25% (7/28) of samples at Markala and 11.1% (4/36) of samples at Mopti. Nile perch had a concentration of Sulphite Reducing Bacteria above the standard 1.05±1.48 Log10 CFU g^{-1}in both seasons at the landing point. From the results, in Mopti, Nile perch had a higher concentration of Sulphite Reducing Bacteria at a selling point 6.05 Log10 CFU g^{-1} (Appendix 7) Nile perch had a concentration of Sulphite Reducing Bacteria above the standard of 1.05±1.48 Log10 CFU g^{-1}in both seasons at the landing point. There were no statistical differences between microbial counts at the sampling points in Selingue, Manantali and Markala ($p \leq 5\%$) for all samples of fish taken. However, at Mopti, there was significant difference between the samples at the capture point between a same group of microorganisms in the cold and hot seasons.

According to Degnon *et al.*(2013) in Benin, Sulphite Reducing Bacteria spores is one of the major causes of food poisoning in humans. The bacterium has been found in many fish. Contamination with sewage is usually the main source of this organism in fishery products.

Prevalence of *Staphylococcus aureus* was 22.2% (8/36) at Selingue, at 29, 4% (10/34) Manantali, 21, 4% (6/28) at Markala and 8.3% (3/36) at Mopti. In Manantali, Nile perch fish samples had higher concentration of *Staphylococcus aureus*, 5.01Log10 CFU g^{-1}, at a selling point (Appendix 6). About 20% (27/134) of the samples recorded *Staphylococcus aureus* above international standard. Degnon *et al.* (2013) reported the absence of *Staphylococcus aureus* in 100% of the samples, *Staphylococcus aureus* are ubiquitous pathogens, widely distributed in nature; but the main source of contamination is humans. *Staphylococcus aureus* can cause severe food poisoning. The presence of this bacteria indicates unhygienic conditions because the product contamination could be the result of a combination of improper handling, improper storage and cross contamination. The ice used for transport may be contaminated with *Staphylococcus aureus*. According to Bujjamma & Padmavathi (2015), the cumulative effect of these practices coupled with unhygienic handling during transportation can lead to high level of *Staphylococcus aureus* in food fish. The use of contaminated water for cleaning and processing fish in the fish market is probably the cause of the secondary contamination. A heavy infestation of flies in this market also promotes tertiary contamination to a large extent due to the lack of adequate drainage of the facilities.

4.3.3 Prevalence of *Salmonella spp*. in the various samples

Fifty percent (50%) of water samples are positive for *Salmonella spp*. These results are not in agreement with that of Simplice *et al.* (2018) who found a complete absence of *Salmonella spp.* . In this study, *Salmonella spp.* were prevalent at 100% of samples from Manantali in a cold season, Markala and Mopti in the hot season. Surface waters are vital and vulnerable freshwater systems that are critical for the sustenance of all life.

The prevalence of *Salmonella spp.* in the fish samples was 8.9% at Selingue (32/36), 91.2% (31/34) at Manantali, 100% (28/28) at Markala and 94.4% (34/36) at Mopti. *Salmonella spp*. was not detected in any of the samples taken in the hot season; which were Nile tilapia at the capture point, Nile perch, and African catfish at the landing point and Nile tilapia at the selling point. *Salmonella spp*. was detected in 50% of samples from Manantali in in the cold season, which were African catfish and Nile tilapia at capture point. Nile perch had a higher concentration at the landing point *Salmonella spp*. was also detected in all samples (100%) from Markala (both seasons). The prevalence of *Salmonella spp* in fish samples in Mopti at selling point, 50% for both African catfish and Nile tilapia in the cold season. The results indicate that 93.3% (125/134) of samples had *Staphylococcus spp*. above the international microbiological criteria. The results of the present study showed higher prevalence of Salmonella compared to those obtained by Sissoko *et al.* (2016) which is 25% *Salmonella spp*. According to Dione (2003), this pathogen is common to animal species and can contaminate fishery products through contaminated instruments, sick workers or healthy carriers, insects and other animals, and water polluted by waste water, and poor hygiene control (Gamane Kaffine *et al.*, 2018). *Salmonella spp.* can tolerate NaCl concentrations of 2 to 8% depending on the pH of the medium and the temperature, but they are destroyed by normal cooking of food. This pathogen is responsible for the deterioration of the sanitary and food safety status of the product and can cause gastroenteritis, septicemia and typhoid fever when a fish product is not adequately cooked prior to consumption.

4.4 Parasites in Freshwater Fish along the Value Chain of Medina Coura Market

4.4.1 Prevalence of infested fish species

About 120 fish samples were analyzed for parasite infestation, parasites were present in fishes at the capture points, the landing point at Selingue, Manantali, Markala, and Mopti and at the selling point Medina Coura market. Parasite infestation varied from one species to another. A low infestation rate was generally observed (10%) in the fish samples. The highest parasite infestation was observed in Nile tilapia with an infestation rate of 16.7% followed by Nile perch, 9.1% and African catfish, 5.0%, during both seasons (Table 4.4.1). No significant difference in prevalence among infestation rates of the fish species examined was observed (χ^2= 0.87). The study revealed an overall rate of parasite infestation of 10% in Nile perch, African catfish, Nile tilapia species. The preliminary investigation of the parasitofauna of fish in Okhuo River in Benin City, Nigeria by Edema *et al.* (2008) indicated 6.94% infestation rate. These were rather very low as compared to other similar studies, such as (Amare *et al.*, 2014) who reported 47.8% prevalence of parasites, in Lake Lugo (Hayke), Northeast Ethiopia and Olofintoye (2006) who observed 62.6% in some freshwater fish species in Ekiti States, Nigeria. These suggest that the distribution of parasites can vary from one habitat to the other due to host parasite relationship and abiotic factors like dissolved oxygen, temperature and pH; and also, the nature of the water, which reflects human use, and the endemicity of infection in the area. The prevalence of parasites was higher in Nile tilapia 16.7% followed by *Lates niloticus* 9.1% and *Clarias anguillaris* 5%. These are lower than rates reported by Amare *et al*. (2014) of the species in Lake Lugo (Hayke), Northeast Ethiopia, which was 50.22%.

Table 11: Prevalence of parasites by fish species

Fish species	Number examined	Number infected	Prevalence (%)
Lates niloticus	44	4	9,1
Clarias anguillaris	40	2	5
Oreochromis niloticus	36	6	16,66
Total	120	12	10

4.4.2 Variation of parasites per infected organs of the fish and areas of sampling

The skin, flesh, gills and abdominal cavity were observed macroscopically. The most affected internal organs of the fish were the gills with the presence of *Salmincola edwardsii* (crustaceans) and the abdominal cavity with the presence of nematodes *Rhaphidascaris spp*. and *Philonema spp.* These parasites belonging to different taxonomic groups were recovered from 12 positive sampled fish. The frequency distribution of parasites among per infected organs per sampling areas is presented in Table 4.4.2. *Philonema spp.* (12.5%) were the most frequently detected parasite in Markala, followed by *Raphidascaris spp*. which were recovered from the abdominal cavity in 10% and 8.82% respectively, of fishes sampled in Selingue and Mopti. *Salmincola edwardsii* was found in the gills of 4.16% of the fish sampled at Markala. There was no significant difference between parasite variation and prevalence at the sampling areas ($\chi^2 = 0.821$).

The study showed that, the prevalence of nematodes was higher than crustacea. The higher rate of nematode infestation may be due to the low host specificity of the adult stage of these parasites, or the ability of different genera and species of nematodes to infect the fish species studied, and the availability of the different host required for the completion of the life cycle of these parasites (Yamaguti, 1963).

The prevalence of *Salmincola edwardsii* was 4.16%; and was observed only in Manantali on *Oreochromis niloticus* in the gills. *Salmincola edwardsii* belongs to the Arthropoda Phylum, subphylum Crustacea, family *Lernaeopodidae*, and are commonly called "gill lice" or "gill fly" (Alteen, 2009). Boucenna *et al.* (2015) also detected the presence of this parasite in the gills. They reported that this parasite can cause swelling around the site of attachment of the bubble, with heavy parasite loads. The presence of this crustacean reduces the feeding activity of the host fish by weakening the host fish and negatively affecting its weight gain, reproduction, and therefore growth. According to Gamane Kaffine *et al.* (2018) the frequency of gill infestation by these parasites may be because gills have a close relationship with the aquatic environment where fish lives. It was observed that *Oreochromis niloticus* collected at the point of sale in the Mopti area during the hot season has a much higher prevalence (50%) than other species. These parasites are capable of absorbing nutrients, vitamins, micro and macronutrients in the digestive tract of fish. According to Bichi & Dawaki (2010) these parasites develop metabolic wastes that have a negative impact on the physiology and reproduction of fish.

Table 12: Prevalence of parasites per infected organs and areas

Parasites	**Infected organs**	**Selingue**		**Manantali**		**Markala**		**Mopti**	
		Number of positive	**Prevalence (%)**	**Number of positive**	**Prevalence (%)**	**Number of positive**	**Prevalence (%)**	**Number of positive**	**Prevalence (%)**
Raphidascaris spp.	**Abdominal cavity**	**3**	**10**	**1**	**3.57**	**0**	**0**	**3**	**8.82**
Philonema sp.	**Abdominal cavity**	**0**	**0**	**0**	**0**	**3**	**12.5**	**2**	**5.88**
Salmincola edwardsii	**Gill**	**0**	**0**	**1**	**4.16**	**0**	**0**	**0**	**0**

4.4.3 Seasonal variation of fish parasites per sampling areas

To assess the seasonal prevalence of parasites in the cold and hot season, 58 and 62 samples were examined for hot and cold seasons respectively of which 3 (5.17%) and 9 (14.52%) were infested respectively (Table 4.4.3).

The parasite frequency distribution per season and per location shows that the highest parasite infestation was observed during the hot season in Selingue with 25% prevalence; followed by Mopti, 20%, Manantali, 16.66% and Medina Coura, 1.7%. In the cold season fish sampled from the selling points had parasite infestation rate of 10% in Selingue, followed by Manantali and Markala with 8.33% at the landing site. There was no significant difference ($\chi 2= 0.363$, $p> 0.05$) of infestation rates during the different seasons.

Table 13: Prevalence of fish infested with parasites per location and season

Seasons	Areas of sampling	Number examined	Number infected	Prevalence (%)
Cold season	Selingue	12	0	0
	Manantali	12	1	8.33
	Markala	12	1	8.33
	Mopti	12	0	0
	Medina Coura Market	10	1	10
	Total	**58**	**3**	**5.17**
Hot season	Selingue	12	3	25
	Manantali	12	2	16.7
	Markala	12	0	0
	Mopti	10	2	20
	Medina Coura Market	16	1	1.60
	Total	**62**	**9**	**14.52**
TOTAL		**120**	**12**	**10**

4.4.4 Prevalence of infected fish species at each sampling points

Table 4.4.4 shows the prevalence of each species of fish in each location of sampling. Parasite prevalence varied from one fish species to another. The highest prevalence of the parasite in *Oreochromis niloticus* was observed at Mopti at the selling point, 50%; followed by *Lates niloticus*, 25%. The other species of fish had a prevalence of 0% at the same point of sale.

Table 14: Prevalence of infected fish species at each sampling point

Areas of sampling	Fish species	Capture point			Landing point			Selling point		
		Number examined	Number infected	Prevalence %P	Number examined	Number infected	Prevalence %P	**Number examined**	**Number infected**	Prevalence %P
Selingue	*L. niloticus*	**4**	**0**	**0**	**4**	**1**	**25**	**2**	**0**	**0**
	C. anguillaris	**4**	**0**	**0**	**4**	**0**	**0**	**2**	**0**	**0**
	O. niloticus	**4**	**1**	**25**	**4**	**1**	**25**	**2**	**0**	**0**
Manantali	*L. niloticus*	**4**	**0**	**0**	**4**	**0**	**0**	**4**	**0**	**0**
	C. anguillaris	**4**	**0**	**0**	**4**	**0**	**0**	**2**	**0**	**0**
	O. niloticus	**4**	**0**	**0**	**4**	**1**	**25**	**0**	**0**	**0**
Markala	*L. niloticus*	**4**	**0**	**0**	**4**	**0**	**0**	**2**	**0**	**0**
	C. anguillaris	**4**	**1**	**25**	**4**	**1**	**25**	**0**	**0**	**0**
	O. niloticus	**4**	**1**	**25**	**4**	**0**	**0**	**0**	**0**	**0**
Mopti	*L. niloticus*	**4**	**1**	**25**	**4**	**1**	**25**	**4**	**1**	**25**
	C. anguillaris	**4**	**0**	**0**	**4**	**0**	**0**	**4**	**0**	**0**
	O. niloticus	**2**	**0**	**0**	**4**	**0**	**0**	**4**	**2**	**50**
Total		42	**4**	**9.52**	**44**	**5**	**11.36**	**26**	**3**	**11.54**

4.5 Identification of the spoilage microorganisms associated with fish using phenotypical methods

4.5.1 Isolation of spoilage microorganisms on fish samples at the various sampling points

The parameters used for macroscopic observations of colonies on King A and King B media were the relief, the colour, and the diameter (Appendix 8). The study showed that the bacterial flora of the samples collected was very dense and very diverse. For microscopic observation (Appendix 9), the bacteria which grew on King medium were all Gram-negative. Two bacterial types were observed for all of the isolates, including 264 rods; 92 cocci. The grouping method was able to show groupings in pairs or diplococcus (146 isolates), in a chains (25 isolates) and in clusters (166 isolates).

4.5.2 Biochemical tests

The Biochemical test showed that 100% of the bacteria were catalase-positive and oxidase-positive (Appendix 9).

A total of 360 isolates (188 in a cold season and 172 in a hot season) were identified and were distributed among 24 species of bacteria. These 24 species of bacteria belonged to 20 genera (Table 4.5.1).

Table 4.5.1: Erreur ! Il n'y a pas de texte répondant à ce style dans ce document.

Family	Bacteria species	Cold season			Hot season		
		Capture	Landing	Selling	Capture	Landing	Selling
Enterobacteriaceae	*Proteus vulgaris*	0	0	0	0	0	1
	Citrobacter freundii	0	0	0	0	0	1
	Enterobacter cloacae	0	0	0	0	0	1
	Providencia rettgeri	0	0	0	0	0	1
Vibrionaceae	*Vibrio alginolyticus*	1	1	0	0	0	0
	Vibrio parahaemolyticus	2	4	0	0	0	0
	Photobacterium damsellae	1	0	1	1	0	0
	Plesiomonas shigelloides	1	3	1	0	0	1
Moraxellaceae	*Psychrob. Phynyl pyrivicus*	8	1	2	3	3	0
Brucellaceae	*Ochrobactrum anthropi*	1	1	2	5	5	7
Pseudomonadaceae	*Ps. Fluorescens*	1	0	0	3	1	0
	Ps. aeroginosa	7	13	2	3	0	1
	Ps. luteola	2	0	3	1	5	1
	Ps. putida	0	0	0	0	0	2
Aeromonadaceae	*Aeromonas hydrophilae/ caviae*	26	13	15	38	23	25
	Aeromonas salmonica spp salmonicoda	1	7	0	0	0	0
	Aeromonas sobria	1	3	0	0	0	0
Flavobacteriaceae	*Chryseobacterium indologenes*	1	2	0	0	0	1
	Chryseobacterium meningosepticum	0	0	1	0	0	1
Rhizobiaceae	*Agrobacterium radiobacter*	1	1	1	2	4	2
Burkholderiaceae	*Burkholderia cepacia*	10	11	3	2	0	0
Comamonadaceae	*Comamonas testosteroni*	4	1	1	1	0	0
Methylobacteriaceae	*Methylobacterium mesophilicum*	3	9	0	2	5	2
Sphingobacteriaceae	*Sphingobacterium multivorum*	1	4	1	0	0	0
Caulobacteraceae	*Brevundimonas vesicularis*	3	5	1	2	4	4

Alcaligenaceae	*Oligella ureolytica*	0	0	0	0	0	1
Neisseriaceae	*Chromo. Violaceum*	0	0	0	3	0	2
TOTAL		75	69	34	66	51	55

These bacteria also belong to 15 families as shown in Figure 4.5.1. In the cold season samples, 35.1% were *Aeromonadaceae*, 14.9% *Pseudomonadaceae*; 12.8% *Burkholderiaceae*; 7.9% *Vibrionaceae*; 6.4% *Methylobacteriaceae;* 5.8% *Moraxellaceae*, 4.9% *Caulobacteraceae*; 3.2% *Sphingobacteriaceae*; 3.2% *Comamonadaceae*; 2.1% *Brucellaceae*; 2.1% *Flavobacteriaceae* and 1.6% *Rhizobiaceae*. Sissoko (2014) reported only 5 families (*Pseudomonadaceae, Aeromonadaceae, Enterobacteriaceae, Vibrionaceae* and *Moraxellaceae*) in fish samples from Medina Coura market. The present results show a large diversity of families, which may be explained by the native flora of the freshly caught fish and the flora of contaminants added along the value chain (capture, landing and sale). This difference could be explained by length of the time between the capture of the fish and the sampling of the fish at the sale point in the market and also by the number of samples taken i.e 134 fresh fish samples in this study as against 20 samples in the study by Sissoko *et al*. (2016) sold on the Medina Coura market. The samples collected during the hot season recorded 50% prevalence of *Aeromonadaceae* as the highest, followed by *Brucellaceae* (10.5%) *Pseudomonadaceae* (9.9%) families with *Comamonadaceae* (0.6%) as the least prevalent family (Figure 4.5.2). Fane (2011) worked on "Diversity of the microbial flora of fish and waters of southern rivers in Mali)" recorded three bacterial families: *Pseudomonadaceae, Enterobacteriaceae* and *Bacillaceae* from 43 samples of 8 species of fish (*Brycinus nurse, Sarotherodon galilaeus, Synodontis membranaceus, Clarias anguillaris, zillii Tilapia, Oreochromis aureus, Oreochromis niloticus* and *Heterotis niloticus*) from rivers in Mali southern.

A study done by Kluga *et al.*(2017) in Nigeria on identification of microflora of freshwater fish caught in the Driksna river and pond in Latvia reported that the majority belonged to bacteria with *Pseudomonas spp*. (55%). In that study, *Pseudomonas spp*. were isolated from the River and the pond. *Pseudomonadaceae* families are well-known fish specific spoilage microorganisms and their abundance in fish may led to rapid fish spoilage processes that causes changes in fish meat quality and makes fish unfit for human consumption. The presence of the spoilage microorganisms potentially may cause consumer health concerns.

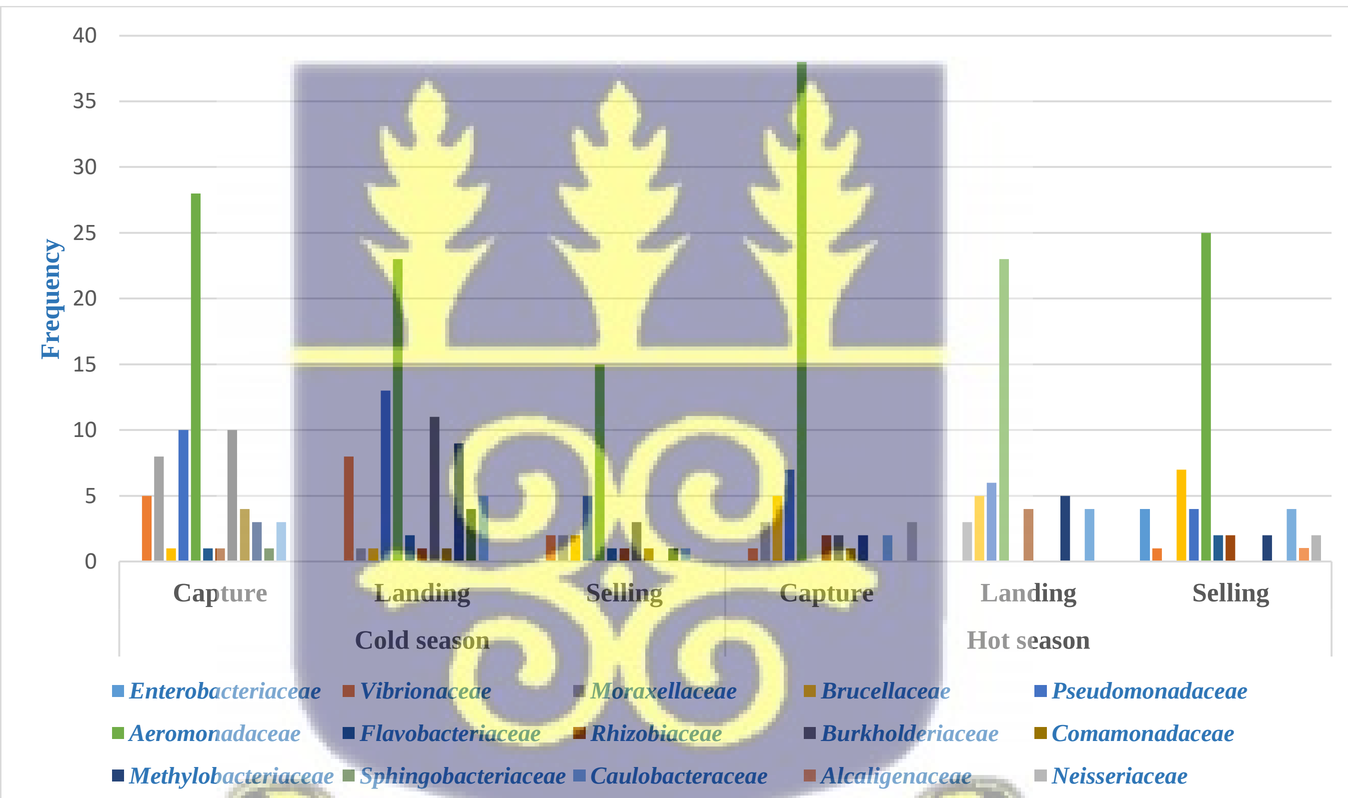

Figure4.5.1:The frequency of the different families of spoilage microorganisms during the cold and hot seasons

4.6 Phenotypic identification of pathogenic microorganisms associated with fish

4.6.1 Biochemical test

All the bacteria isolates were catalase-positive and oxidase-positive. A total of 117 (59 in a cold season and 58 in a hot season) isolates were identified (Table 4.61).

Table 4.6 1:Frequency of isolation of pathogenic microorganisms on fish on various sampling point

Family	Bacteria species	Cold season			Hot season		
		Capture	Landing	Selling	Capture	Landing	Selling
Enterobacteriaceae	*Proteus vulgaris*	3	1	4	2	3	2
	Proteus mirabilis	0	0	3	3	3	0
	Salm. Choler. Arizonae	0	0	0	0	0	0
	Salmonella. Spp	2	1	4	1	2	1
	Citrobacter youngae	0	1	0	1	1	0
	Citrobacter koseri/ amalonaticus	0	0	0	0	2	0
	Citrobacter freundii	1	1	1	1	2	0
	Enterobacter cloacae	1	0	1	0	1	0
	Enterobacter sakazaki	1	0	0	0	0	0
	Ser. marcescens	1	0	0	0	0	0
	E. coli	0	0	0	0	1	0
	Klebsiella ocytoca	1	0	0	1	0	0
	Edwardsiella tarda	0	0	0	1	0	0
	Providencia stuartii	6	0	0	1	0	0
	Providencia alcalifaciens	0	0	0	0	1	0
	Providencia rettgeri	0	0	0	1	0	0
Vibrionaceae	*Grimonthia hollisae*	1	1	0	1	0	1
	Vibrio alginolyticus	0	2	0	0	1	0
	Photobacterium damsellae	0	0	0	2	5	0
Morganellaceae	*Morganella morganii*	2	1	2	2	0	0
Moraxellaceae	*Moraxella spp.*	0	2	0	0	0	0
Brucellaceae	*Pasteurella multocida*	0	0	0	1	0	0
	Ochrobactrum anthropi	4	4	0	0	0	0
Pseudomonadaceae	*Ps. Fluorescens*	3	0	0	0	0	0
	Ps. aeroginosa	0	0	0	2	1	0
Shewarnellaceae	*Shewarnella putrefaciens*	0	3	0	1	1	0
Aeromonadaceae	*Aeromonas hydrophilae/ caviae*	0	0	0	3	1	0

Flavobacteriaceae	*Chryseobacterium indologenes*	0	0	0	1	1	0
Burkholderiaceae	*Burkholderia cepacia*	0	0	0	1	0	0
Alcaligenaceae	*Bordetella/Alca/M. spp.*	0	1	0	0	0	0
Neisseriaceae	*Chromo. Violaceum*	0	0	0	0	0	1
Total		26	18	15	25	27	5

They were made up of 31 species and belonged to 23 genera and 12 families (Figure 4.6.1). In the cold season, *Enterobacteriaceae* was most prevalent (54.4**%**), followed by *Brucellaceae* (13.5 **%**, *Alcaligenaceae* and least of all (1.7%). In the hot season, *Enterobacteriaceae* was most prevalent (55.9%) followed by *Vibrionaceae* (17.5%) and then *Pseudomonadaceae* (5.3%) and *Brucellaceae* the least (1.7 %). The genus *Enterobacteriaceae* is an important indicator of the hygienic state of the environment and products, and it suggests the contamination of the environment (Terentjeva *et al.*, 2015). After capture, the microflora of fish surfaces and skin is continuously affected by the aquatic environment, including bacteria present in water, sediments and contamination by polluted wastewater (Terentjeva *et al.*, 2015).

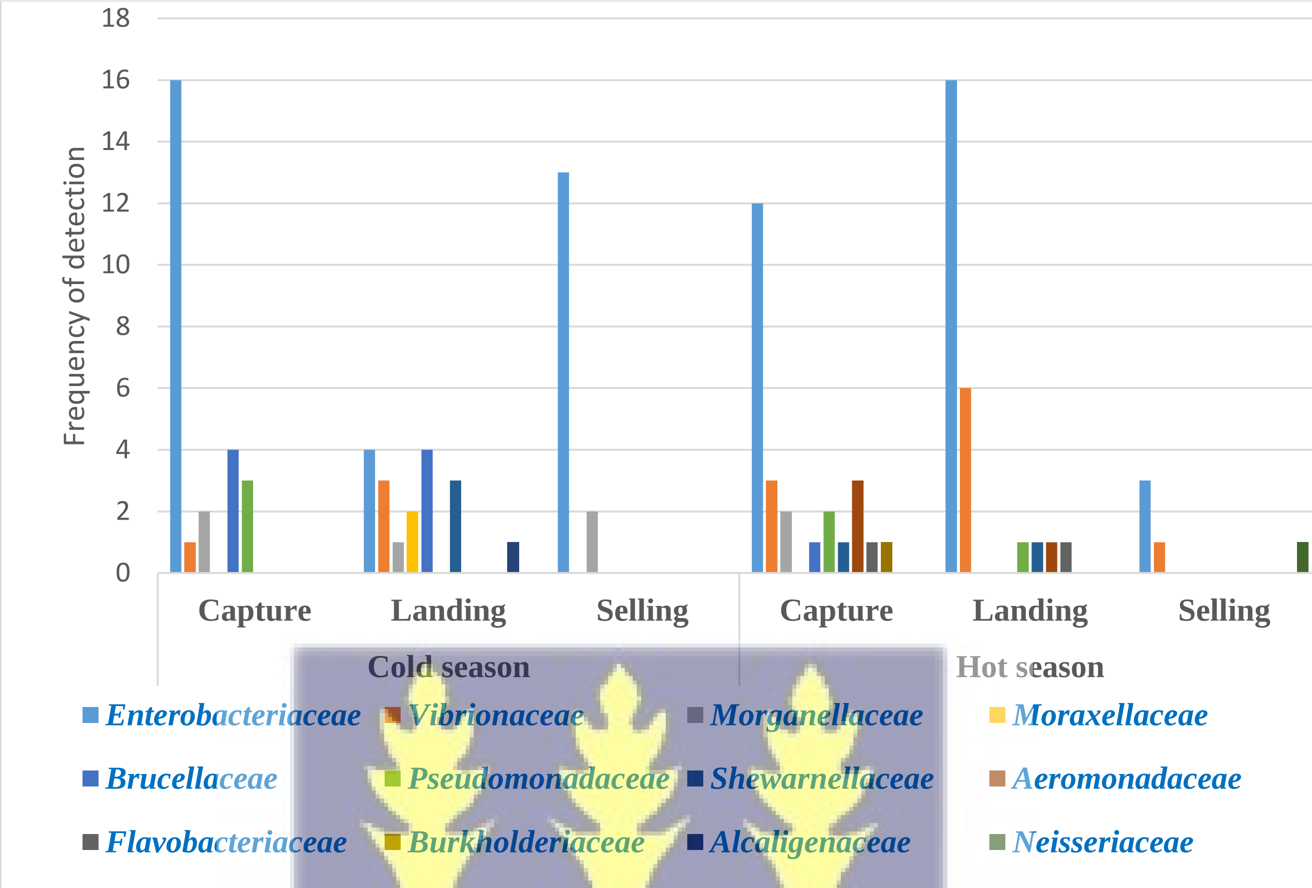

Figure 4.6.1: Percentage distribution of the different families of pathogenic microorganisms in fish during from the cold and hot seasons

4.6.2 Isolation of pathogenic microorganisms on fish samples at the various sampling points

Salmonella- Shigella agar medium was used to isolate the pathogenic microorganism from the samples collected for the study and the results are recorded in Appendix 13.

Macroscopic and microscopic characteristics of the microbial flora of fish species.

The parameters used for macroscopic observations were the color, and the diameter (Appendix 10). The study showed that the bacterial flora of the samples collected was very dense and very diverse.

For microscopic observation in Appendix 11, the bacteria which grew on S-S medium were all Gram-negative. Two bacterial types were identified, including 117 rods; 8 cocci.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study found that although respondents have a certain level of knowledge that does not translate into food safety practices in the artisanal fish value chain. Poor handling practices by workers, in particular, non-compliance with hygiene rules and storage at an inappropriate temperature could be associated with the fish losses. Insufficient knowledge and practices of respondents in food safety lead to poor microbial quality of fish and self-reported fish losses by respondents.

The bacteriological analysis has shown that fresh freshwater fish sold along the value chain in the Medina Coura market has high counts of Aerobic Plate Counts, Total coliform, Fecal coliform, Sulphite Reducing Bacteria spores, *Staphylococcus aureus*. and *Salmonella spp*. However, the prevalence of parasites was low. Microorganisms isolated from fish samples analyzed were diverse and were made up of 24 species of bacteria (spoilage and pathogenic flora) belonging to15 families for a spoilage bacteria and 31 species of bacteria belonging to 23 genera for the pathogenic bacteria. These bacteria are divided into 12 families. An overall prevalence of 10% (12/120) parasitic infestation was observed. Prevalence was lower during the cold season, (5.2% of the fish) than in the hot season (14.5%). The highest prevalence of infestation was observed in *Oreochromis niloticus* (Tilapia) at 16.7% followed by *Lates niloticus* (Nile Perch) (9.1%) and *Clarias anguillaris (Catfish)* (5.0%). Research finding showed that infection prevalence and diversity of parasites were relatively low.

Overall, the study is an important contribution to the knowledge of the bacterial and parasitological flora of fish along the value chain of fish sold on the Medina Coura Market, It is the first study to elucidate the microbiological quality of these freshwater fish along studied value chain.

5.2 Recommendations

Given the importance of fish in human food and the rapid increase the consumers it is necessary to strengthen vigilance and control of the practices of the actors of the application of regulations, good hygiene practices and food security control. Fish marketing systems should be clean with improvements in handling and processing to minimize the prevalence of pathogenic bacteria. Measures must be taken by local authorities, who are empowered to exercise their functions without constraints, to prevent and control the potential risk and spread of the disease. In the interest of public health, the government and the regulatory bodies, good management of the fishing sector must be implemented, starting with formal training in hygienic practices and regular controls of the personal and environmental hygiene of all actors in the artisanal fish value chain. The landing site must be constructed and well equipped with cold storage facilities, which would allow stricter regulations by the appropriate bodies to ensure proper sanitation and safety of the fish. Parasites should not be detected in fish because they reduce the market value of fish and may cause human infections when live cysts or matured parasites are ingested. This study shows that microbial contaminants in fish samples sold along the value chain are high but further investigation is needed to determine the levels and extent of microbial contamination. Further studies on identification driven by molecular biology could involve a quantitative evaluation of samples of fish contaminated with these pathogens, as this would provide a clear indication of the levels of contamination of fish sold in the Medina Coura market. Follow up studies could be carried out to determine the economic, public health and social implications of high contamination levels on the fishes studied and the efficacy of targeted interventions to improve microbiological quality and safety of fish.

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APPENDICES

DEPARTMENT OF NUTRITION AND FOOD SCIENCE

UNIVERSITY OF GHANA, LEGON

MICROBIAL DIVERSITY OF ECONOMICALLY IMPORTANT UNPROCESSED FRESHWATER FISHES SOLD AT THE MEDINA COURA MARKET IN BAMAKO, MALI

Dear respondent, this questionnaire aims to collect information on the origin of the fish, The flow time, the handling, storage, knowledge of food quality and safety of the fish on Medina Coura market in Bamako and various area of sampling. It is part of a PhD food science thesis, microbiology option. The information you provide in this document will be treated as confidential and used for academic purposes only. Thank you.

Appendix 1A: Fish sellers questionnaire at Medina Coura Market

Date: ______________________

Location: ___________________

Respondent's Code

Kindly tick (√) the responses that apply to you. Where appropriate, write out your own responses in the spaces provided.

BACKGROUND INFORMATION RESPONSE

[For interviewer use only]

1. Sex:

    1=Male

    2=Female

2.Ethnic Group

    1=Fulani

    2=Bozo

    3=Bambara

    4= Malinke

    5= Songhai

    6=Soninke

3. Age

    1=30 – 40 years

    2=41– 50 years

    3=51 – 60 years

    4=61 – 70years

4. How long (year) have you been doing this fishing activity?

    1=1-10 years

    2=11-20years

    3=21-30 years

    4=31-40 years

    5=41-50 years

    6=51-60 years

5. Education level

1= Primary

2=Secondary

3=High school

4=No educated

6. How will you define your retail business?

1=Wholesaler

2=Middleman

3=Retailer

7. Where does your fish come from?

1=Mopti -Markala

2=Mopti-Selingue

3=Mopti- Segou- Manantali

4=Mopti- Selingue –Gao-Manantali

8. What is your means of transporting fish?

1=Car

2=Refrigerated truck

9. How to display the fish for sale?

1= On a mat on the floor

2= On a table

10. How long does the fish last?

1= 1 day

2= 2-3 days

3= 4-5 days

4= 6-7 days

11. How do you store fish?

1= With ice

2= Without ice

12.What measurement do you use to weigh your fish?

1= In weight (Kg)

2= In 300 Kg basket]

13. In what condition the fish arrives on the market.

1=In the basket with ice

2= In the basket without

14. How much fish do you receive?

1= 1-3 Baskets

2= 4-6 Baskets

3= 1-7 Tones

15. How often do you receive fish per week?

1= 2-3 days

2= 4-5 days

3= 6- 7 days

16. Do you know a spoiling fish?

1= Yes

2= No

17. What is the cause of the spoiling?

1= Lack of ice

2= Broken down vehicle

18. Do you think handling can contribute to the spoilage of fish?

1= Yes

2=No

19.How do you recognize a spoiled fish?

1= Texture change

2= Change in eye color

3= Change in texture, swelling

4= Change in eye color, darkening of gills

20. Do you often encounter spoiled fish?

1= Yes

2= No

21.What do you do with spoiled fish?

1= Loss sale to processors

2= Transform yourself

22. In your opinion, do spoiled fish cause health problems?

1= Yes

2= No

23. What do you suggest to avoid losing the fish?

1=Clean-up workplaces

2= Need financial assistance

3. Need refrigerated truck for transportation

4. Vehicle stop by customs for long time

5. Need a factory to manufacture ice, cold room

Appendix 1B: Fishermen questionnaire at the four capture point

Date: ______________________

Location: ___________________

Respondent's Code

Kindly tick (√) the responses that apply to you. Where appropriate, write out your own responses in the spaces provided.

BACKGROUND INFORMATION RESPONSE

1. Sex:

1=Male

2=Female

2.Ethnic Group

1= Fulani

2= Bozo

3=Bambara

4= Malinke

5= Songhai

6=Soninke

3. Age

1=30 – 40 years

2=41– 50 years

3=51 – 60 years

4=61 – 70years

4. How long (year) have you been doing this fishing activity?

1=1-10 years

2=11-20

3=21-30

4=31-40

5=41-50

6=51-60

5. Education level

1= Primairy

2=Secondary

3=High school

4=No educate

6. What fishing gear do you use for fishing?

1= Gill nets

2= Fishing net

3=Cast net

4=Seine net

7. What kinds of canoe do you use for fishing?

1= Motorized canoe

2=Non-motorized canoe

8. How does your fishing activity take place?

1=The nets are left at 4 p.m. and collected overnight (1 a.m.)

2= Spend 4 to 5 days in the lake

3= Spend 5 to 7 days in the lake

4=Going in the lake and at the same time return

5= Going on the lake every hour

9. What species (3 species more caught) of fish are you Caught in this period?

1=*Oreochromis niloticus,Hydrocynus brevis,Lates niloticus*

2=*Lates niloticus,Oreochromis niloticus,Synodontis schall*

3=*Brycinus leuciscus,Lates niloticus,Oreochromis niloticus*

4=*Brycinus nurse, Brycinus leuciscus, Oreochromis niloticus*

5=*Brycinus leuciscus, Oreochromis niloticus,Clarotes laticeps*

10. What is the most successful fishing period?

1= February to September

2= June to August

3= November to January

11. What is your average harvest in an expedition?

1= 1-20 Kg

2=21-40 Kg

3= 41-60 Kg

4= 61-80 Kg

5=>100 Kg

12.How often do you go fishing?

1= 2 days

2= 3-5 days

3= 7 days

13. On average, how much time do you spend on each shipment?

1= Less than 2 hours

2= 2 to 6 hours

14. Do you have refrigeration systems on the canoe?

1= Yes

2= No

15. If not, please describe how you handle your catch to avoid spoilage?

1= Speed

2= Ice]

3= Nothing

16. Do you sometimes lose fish?

1= Yes

2= No

17.How do you market your catch?

1=Our women come to take to sell

2= Wholesalers come to buy to resell

3= Retailers come to buy to resell

4= Wholesalers and Retailers come to buy to resell

18. How long do you take to market your catch?

1= Less than 2 hours

2= 2 to 6 hours

19. What do you do with fish that are deteriorating?

1= Dried

2= Others

20. What are the difficulties you encounter in your work?

1= Pesticide in water by miners (cyanide, Mercury)]

2= Lack of fish in the water

3= Lack of means of preservation (cold room, ice)

4= Theft of fishing gear

5= Insecurity in northern Mali

6= No problem

7= Difficulty in transport

Appendix 2: Chemical composition of the culture media used

Peptone Buffered Water (formula: g/l)

Disodium Hydrogen Phosphate Dehydrate……………………………….7.2

Sodium Chloride……………………………………..…………………..,3

Potassium Dihydrogen phosphate……………………………………..…..3.6

Pancreatic Digest of caseine……………………………………………….1

pH: …………………………………………………………….7.0±0.2 à 25°C

Plate Count Agar (formula: g/l)

Casein peptone………………………………………………………………5.00

Yeast extract…………………………………………………..……………2.50

Dextrose………………………………………………………………...……..1.00

Agar……………………………………………………………………..……...15.00

pH: ……………………………………………………………7±0,2 à 25°C

Violet Red Bile Lactose Agar (VRBA) (formula: g/l)

Peptone……………………………………………………………………...7

Yeast extract ………………………………………………………………..3

Lactose ……………………………………………………………………..…10

Sodium chloride ……………………………………………..…………5

Bile Salts No.3 ……………………………………………………0.5

Cristal violet ……………………………………………………...0.002

Neutral red ………………………………….………………...0.03

Agar-agar ………………………………………….…………….....15

pH: ………………………………………………………7±0.2 - 25°C

King A Agar(formula: g/l)

Magnesium Chloride …………………………………………….…...1.4

Potassium Sulfate ……………………………………..…………10.0

Agar …………………………………….……………....13.6

pH…………………………………………………………7.0±0.2 - 25°C

King B Agar (Formula: g/l)

Peptone ……..…………………………………………………..20.0

Proteose peptone No. 3 …………………………………………..20.0

Dipotassium phosphate………………………………..…………15.0

Magnesium sulfate…………………………………………………1.5

Agar ……………………………………..……………………………14

pH : …………………………………..…..……………..7.0±0.2 - 25°C

Reinforced Clostridial MediumRCM(Formule: g/l)

Yeast extract…………………………………………………… 13.0

Peptone………………………………………………………….. 10

Glucose……………………………………………………………..5.0

Soluble starch………………………………………………………1.0

Sodium chloride………………………………………………...5.0

Sodium acetate………………………………………………… 3.0

Cysteine hydrochloride………………………………………… 0.5

Agar……………………………………………………………….13.5

pH :……………………………………………………6.8±0.2 C

Mannitol Salt Agar (Chapman Agar) (formula: g/l)

Meat extract……………………………………………………………………..1

Caseine pepton……………………………………………………………….5

Meat pepton…………………………………………………………………5

Sodium chloride……………………………………………………………..75

D mannitol……………………………………………………………………10

Phenol red……………………………………………………………….0.025

Agar……………………………………………………………………………15

pH: …………………………………………………………………7.0±0.2 -25°C

*Salmonella- Shigella*S-S Agar(formula: g/l)

Lactose ……………………………………………………………………10

Bile Salts mixture………………………………………………………………8.5

Sodium citrate ……………………………………………………………………8.5

Sodium Thiosulfate…………………………………………………… 8.5

Beef extract………………………………………………………………………5.0

Peptone mixture……………………………………………………………………5.0

Ferric citrate………………………………………………………………………1

Neutral red………………………………………………………………………0.025

pH: ……………………………………………………………………7.0±0.2 -25°C

Trypton Soy Agar TSA(formula: g/l)

Pancreatic digest of casein…………………………………………………17.0

Papaic digest of soyabean meal……………………………………………..3.0

Sodium chloride…………………………………………………………………5.0

Dextrose (Glucose)………………………………………………………………2.5

Dipotassium hydrogen phosphate………………………………………………2.5

Agar………………………………………………………………………………15.0

pH: ……………………………………………………………………7.0±0.2 -25°C

Brain Heart Infusion (BHI) Agar(formula: g/l)

HM infusion powder……………………………………………………………12.5

BHI powder…………………………………………………………………..5.0

Proteose peptone……………………………………………………………10.0

Dextrose (Glucose)……………………………………………………...2.0

Sodium chloride……………………………………………………………….5.0

Disodium phosphate…………………………………………………………2.5

Agar……………………………………………………………………………….15.0

pH: ………………………………………………………………7.0±0.2 -25°C

Appendix 3: Gram Staining

It was developed by Danes Christian Gram in 1881, it is the most important and most used coloration of bacteria, and it allows to divide the bacterial world into two subgroups:

Gram-positive bacteria: these organisms retain the primary dye, the crystal violet, after discoloration and appear dark blue when viewed under light microscopy since the counter-dye (safranin) cannot displace the primary dye.

Gram-negative bacteria: where the primary dye is removed by washing with ethanol and replaced by the last dye used safranin. These bacteria will appear red to us under light microscopy.

The variation in the ability to retain the primary dye (crystal-violet) is linked to differences in the chemical composition of the cell wall.

Gram staining has four steps as shown in the following Gram staining table

Réactif	**Effet**	**Gram +**	**Gram -**
Cristal violet	Primary staining	Blue	Blue
Lugol (iodine)	Mordant *	Blue	Blue
95% Ethanol	Differentiation agent	Blue	Incolore
Safranine	Against dye	Blue	Red

*Iodine acts as a mordant because iodine forms with crystal violet an iodine-violet crystal complex, which is more easily retained by Gram + bacteria.

Make the Gram preferably on young cultures (24 to 48 hours), because bacteria often show a Gram + only when they are actively growing: they lose the ability to retain the crystal-violet-iodine complex when active growth stopped. This is particularly true for sporulated strains.

Method:

- Prepare a smear from each bacterial species and fix it as described in the simple staining section.
- Cover the smear with Hucker's purple crystal. Leave in contact for one minute before washing with water. At this stage it is necessary to avoid over washing because there may be discoloration, no more than 2-3 seconds.
- Cover the smear with the iodine gram solution and allow to settle for one minute before washing with water. (At this stage crystal violet is complexified and cannot be removed by washing).
- Keep the blade in an oblique position and bleach with 95% alcohol until the alcohol remains colorless, the duration of the bleaching depends on the thickness of the smear: 5-15 seconds for a thin smear, 15- 30 seconds for a thicker smear, rinse with water.
- Apply the counter-dye (safranine) and leave in contact for one minute. Wash with water, lightly, because saffron can be washed out by excessive washing, and dry with blotting paper (without rubbing) before observing.

Appendix 4: Microbial mean Log10 CFU g^{-1}prevalence of *Salmonella and pH of fish* in sampling point in Selingue

Sampling point	Fish species	Cell concentration (Log10 CFU g^{-1})					% Prevalence (N=134)	pH of fish
		Total Viable Bacteria	Total Coliforms	Fecal Coliforms	Sulphite Reducing Bacteria	*Staphylococcus aureus*	*Salmonella spp.*	
Capture	*L. niloticus*	6.85	6.38	6.3	0.39	2.71	100	6.44
	C. anguillaris	7.52	6.53	5.96	0.5	1.97	100	6.66
	O. niloticus	7.13	6.41	6.28	0	1.05	75	6.39
Landing	*L. niloticus*	6.76	5.71	5.63	0.53	3.7	75	6.65
	C. anguillaris	7.3	6.72	6.33	0	1.17	75	6.77
	O. niloticus	7.16	6.5	6.25	0.64	2.72	100	6.76
Selling	*L. niloticus*	6.65	4.57	4.31	1	1.64	100	6.56
	C. anguillaris	6.88	4.39	4.42	0.62	0	100	6.63
	O. niloticus	6.53	4.11	3.64	1.47	0	50	6.64
Standard		5	1	1	1	2	Absence/25g	6.8 - 7.6

Appendix 5: Microbial count, prevalence of Salmonella and pH of fish in sampling point in Manantali

Sampling point	Fish species	Cell concentration (Log10 CFU g-1)						pH of fish
		Total Viable Bacteria	Total Coliforms	Fecal Coliforms	Sulphite Reducing Bacteria	*Staphylococcus aureus.*	*Salmonella spp.*	
Capture	*L. niloticus*	5.82	6.75	7.04	0.51	3.07	100	6.47
	C. anguillaris	7.03	6.89	6.28	0.49	2.61	75	6.42
	O. niloticus	5.83	5.71	5.16	0.15	2.15	75	6.55
Landing	*L. niloticus*	6.8	7.29	6.52	0.26	2.43	75	6.48
	C. anguillaris	6.55	7.49	6.62	0.68	1.82	100	6.44
	O. niloticus	6.28	7	6.91	0	1.58	100	6.48
Selling	*L. niloticus*	7.36	6.47	4.95	0	5.01	100	6.66
	C. anguillaris	7.5	6.23	5.85	0.075	2.17	100	6.52
	O. niloticus	7.5	6.23	5.85	0	2.85	100	6.51
Standard		5	1	1	1	2	Absence/25g	6.8 - 7.6

Appendix 6: Microbial count, Prevalence of Salmonella and pH of fish in sampling point in Markala

Sampling point	Fish species	Cell concentration (Log10 CFU g-1)						pH of fish
		Total Viable Bacteria	Total Coliforms	Fecal Coliforms	Sulphite Reducing Bacteria	*Staphylococcus aureus*	*Salmonella spp.*	
Capture	*L. niloticus*	5.23	5.56	4.9	0.69	0.22	100	6.69
	C. anguillaris	5.93	6.2	5.37	0.26	0.41	100	6.59
	O. niloticus	5.6	6.36	6.4	1.74	0.12	100	6.25
Landing	*L. niloticus*	6.46	6	5.68	1.05	0	100	6.63
	C. anguillaris	7.7	6.39	5.5	0	1.74	100	6.22
	O. niloticus	6.35	6.49	5.23	0.19	1.13	100	6.43
Selling	*L. niloticus*	7.03	6.5	6.18	1.34	1.71	100	6.28
	C. anguillaris	7.33	7.1	7.5	1.65	2.65	100	6.41
	O. niloticus	7.17	7.01	6.82	1.1	2.9	100	6.32
Standard		5	1	1	1	2	Absence/25g	6.8 - 7.6

Appendix 7: Microbial count, Prevalence of Salmonella and pH of fish in sampling point in Mopti

Sampling point	Fish species	Cell concentration (Log10 CFU g-1)						pH of fish
		Total Viable Bacteria	Total Coliforms	Fecal Coliforms	Sulphite Reducing Bacteria	*Staphylococcus aureus.*	*Salmonella spp.*	
Capture	*L. niloticus*	6.06	5.92	5.5	0	0	100	6.53
	C. anguillaris	6.25	6.2	5.05	0	0	100	6.6
	O. niloticus	6.36	6.43	6.22	1.51	0.1	100	6.72
Landing	*L. niloticus*	7.01	6.75	6.41	0.2	1.65	100	6.65
	C. anguillaris	7.42	7.23	6.8	0.1	1.67	100	6.58
	O. niloticus	7.83	6.76	6.28	0.075	1.33	100	6.64
Selling	*L. niloticus*	6.72	6.54	6.47	6.05	2.33	100	6.54
	C. anguillaris	6.83	6.95	6.4	0.56	1.83	75	6.4
	O. niloticus	6.91	6.76	6.73	0.19	1.87	75	6.5
Standard		5	1	1	1	2	Absence/25g	6.8 - 7.6

Appendix 8: Macroscopy observation for the spoilage microorganisms

Areas	Season	Sampling point	Macroscopy observation							
			Relief		Diameter			Color		
			Flat	Convex	Large	Small	Tiny	Colorless	Yellow	White
Selingue	Cold	Capture	21	6	9	8	10	15	4	8
		Landing	26	8	6	11	7	11	19	4
	Hot	Capture	8	12	1	10	7	13	1	6
		Landing	10	6	4	5	7	5	5	6
		Selling	5	7	2	5	5	5	-	7
Manantali	Cold	Capture	9	6	3	5	7	6	1	8
		Landing	11	-	3	4	4	8	1	2
		Selling	11	1	1	8	3	5	1	6
	Hot	Capture	6	12	4	5	9	12	1	5
		Landing	6	12	2	4	12	5	1	12
		Selling	3	9	2	2	8	6	2	4
Markala	Cold	Capture	5	13	4	5	9	5	5	8
		Landing	8	9	1	6	10	2	6	9
		Selling	5	5	2	4	4	7	1	2
	Hot	Capture	8	5	2	3	8	7	-	6
		Landing	7	13	1	5	10	5	1	10
Mopti	Cold	Capture	8	8	5	2	9	10	1	5
		Landing	9	8	6	1	10	6	5	6
		Selling	4	8	1	5	6	6	1	5
	Hot	Capture	6	12	6	7	5	6	4	8
		Landing	6	11	2	4	11	8	1	8
		Selling	7	5	2	6	6	8	2	2
Total			189	176	69	115	167	161	63	137

Appendix 9: Microscopy observation and biochemical test for the spoilage microorganisms

Areas	Season	Sampling point	Microscopy observation							Biochemical Test			
			Shape		Mode of grouping			Gram		Catalase		Oxydase	
			Rods	Cocci	In pair	Chain	Cluster	Neg	Pos	Neg	Pos	Neg	Pos
Selingue	Cold	Capture	12	12	18	4	2	24	-	-	24		24
		Landing	10	23	27	4	2	33	-	-	33	-	33
	Hot	Capture	14	6	2	-	18	20	-	-	20	-	20
		Landing	7	9	3	1	12	16	-	-	16	-	16
		Selling	11	1	4	3	5	12	-	-	12	-	12
Manantali	Cold	Capture	15	-	14	1		15	-	-	15	-	15
		Landing	8	3	9	1	1	11	-	-	11	-	11
		Selling	10	2	5	4	3	12	-	-	12	-	12
	Hot	Capture	12	0	3	-	9	12	-	-	12	-	12
		Landing	14	4	5	3	10	18	-	-	18	-	18
Markala	Cold	Capture	19	4	8	1	14	23	-	-	23	-	23
		Landing	9	8	9	-	8	17	-	-	17	-	17
		Selling	9	1	8	-	2	10	-	-	10	-	10
	Hot	Capture	10	3	6	2	5	13	-	-	13	-	13
		Landing	5	11	1	-	15	16	-	-	16	-	16
Mopti	Cold	Capture	9	1	7	-	3	10	-	-	10	-	10
		Landing	16	1	9	1	7	17	-	-	17	-	17
		Selling	11	1	8	-	3	12	-	-	12	-	12
	Hot	Capture	18	-	-	-	18	18	-	-	18	-	18
		Landing	16	1	-	-	17	17	-	-	17	-	17
		Selling	11	1	-	-	12	12	-	-	12	-	12
Total			246	92	146	25	166	338			338		338

Appendix 10: Macroscopy observation for the pathogenic microorganisms

Areas	Season	Sampling point	Macroscopy observation								
			Relief		Diameter		Color				
			Flat	Convex	Small	Tiny	Colorless	CBC	Yellow	Black	Pink
Selingue	Cold	Capture	10	5	3	7	6	-	-	2	2
		Landing	8	-	5	3	7	-	-	1	
	Hot	Capture	-	8	-	8	1	2	1	-	4
		Landing	1	5	3	3	2	-	-	-	4
		Selling	-	5	-	5	3	2	-	-	-
Manantali	Cold	Capture	3	-	-	3	2	1	-	-	-
		Selling	6	-	-	5	4	2	-	-	-
	Hot	Capture	-	9	2	7	4	2	-	-	3
		Landing	-	9	1	8	3	4	1	-	2
Markala	Cold	Capture	1	3	-	4	1	3	-	-	-
		Landing	1	3	-	4	1	1	-	-	2
		Selling	4	2	1	5	3	2	-	-	1
	Hot	Capture	-	2	-	2	-		1	-	1
		Landing	1	4	-	4	-	2	1	-	1
Mopti	Cold	Capture	1	8	3	6	4		2	-	3
		Landing	1	6	-	7	-	4	2	1	-
		Selling	-	3	-	3	1	1	-	-	1
	Hot	Capture	3	4	-	7	1	2	-	-	4
		Landing	5	4	1	8	-	1	4	2	2
		Selling	-	2	-	2	-	1	-	-	1
Total			44	76	19	101	43	30	12	6	31

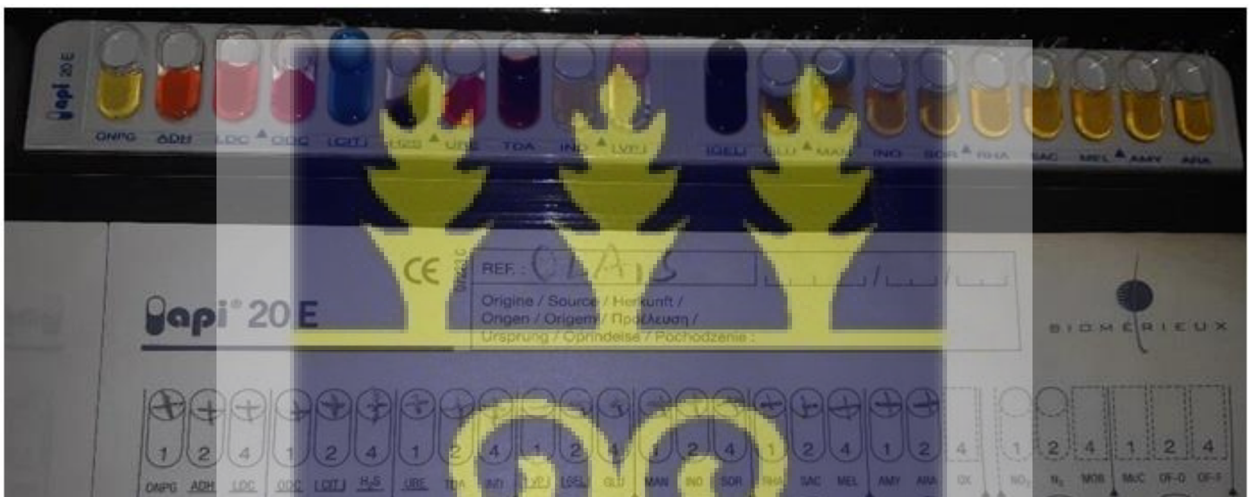
CBC: Colorless with black center

Appendix 11: Microscopy observation and biochemical test for the pathogenic microorganisms

Areas	Season	Sampling point	Microscopy observation							Biochemical Test			
			Shape		Mode of grouping			Gram		Catalase		Oxydase	
			Rods	Cocci	In pair	Chain	Cluster	Neg	Pos	Neg	Pos	Neg	Pos
Selingue	Cold	Capture	9	1	5	3	2	10	-	-	6	-	10
		Landing	6	2	3	1	4	8	-	-	8	-	8
	Hot	Capture	8	-	1	-	7	8	-	-	8	-	8
		Landing	5	1	-	2	4	6	-	-	6	-	6
		Selling	5	-	1	-	4	5	-	-	5	-	5
Manantali	Cold	Capture	3	-	2	-	1	3	-	-	3	-	3
		Selling	5	1	-	-	-	6	-	-	6	-	6
	Hot	Capture	9	-	-	1	8	9	-	-	9	-	9
		Landing	9	-	3	-	6	9	-	-	6	-	9
Markala	Cold	Capture	4	-	3	-	1	4	-	-	4	-	4
		Landing	4	-	-	-	4	4	-	-	4	-	4
		Selling	6	-	2	-	4	6	-	-	6	-	6
	Hot	Capture	2	-	1	-	2	2	-	-	2	-	2
		Landing	4	-	-	2	2	4	-	-	4	-	4
Mopti	Cold	Capture	7	2	7	-	2	9	-	-	9	-	9
		Landing	7	-	3	-	4	7	-	-	7	-	7
		Selling	3	-	-	-	3	3	-	-	3	-	3
	Hot	Capture	7	-	-	2	5	7	-	-	7	-	-
		Landing	9	-	-	-	9	9	-	-	9	-	9
		Selling	5	-	2	-	3	5	-	-	5	-	5
Total			117	8	32	11	75	124			117		117

Appendix 12: API 20NE reading

Appendix 13: API 20E reading



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PARASITES IN FRESHWATER FISH ALONG VALUE CHAIN OF MEDINA COURA MARKET IN BAMAKO, MALI

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ABSTRACT
Parasitic infestations of 120 specimens of three freshwater fish species, comprising of *Lates niloticus* (44), *Clarias anguillaris* (40) and *Oreochromis niloticus* (36) sold at the Medina Coura market in Mali, were investigated. The fresh fish samples were taken during two seasons (cold and hot) in four fish production areas (Niger Central Delta in Mopti, Selingue, Manantali, Markala) and from the Medina Coura market. In each area, the samples were taken at three points (capture, landing and sale), using standard methods and procedures. A dissection was performed on each fish sample and the conditions of the skin, flesh, gills and abdominal cavity were observed for the detection of infestations. Three species of parasites were detected, namely, *Salmincola edwardsii* (crustacea) at the gills, and *Philonema sp.* and *Raphidascaris sp.* (Nematodes) in the abdominal cavity. An overall prevalence of 10% (12/120) parasitic infestation was observed. Prevalence was lower during the cold season, (5.2% of the fish) than in the hot season (14.5%). The highest prevalence of infestation was observed in *Oreochromis niloticus* (Tilapia) at 16.7% followed by *Lates niloticus* (Nile Perch) (9.1%) and *Clarias anguillaris* (Catfish) (5.0%). Research finding showed that infection prevalence and diversity of parasites were relatively low.

Keywords: Parasites, fresh fish, freshwater, Niger river, Senegal river.

1. INTRODUCTION
Fish is one of the best sources of animal protein for human health. According to the Food and Agriculture Organization (Djiré, 2007), fish is an important source of nutrients, because of its protein, vitamin A and D, phosphorus, lysine, sulphur and essential amino acids composition. Cholesterol level is also low in fish meat (Fagbenro *et al.*, 2005; Aremu *et al.*, 2013; Ayeloja *et al.*, 2013; Oladipo and Bankole, 2013; Khalili Tilami & Sampels, 2017).
All fish species are vulnerable to various parasitic infections depending on the species of fish and the type of watercourses inhabited (Oniye *et al.*, 2004). Various parasites are associated with freshwater fish; they cause morbidity, mortality and economic losses in Africa (Oniye *et al.*, 2004; Ayanda, 2009). When the parasite infects the fish, they usually cause chronic damage that can be tolerated by the fish (Bamidele, 2015). Factors favoring parasitic infection in fish include reduction of the oxygen content of water, an increase of organic matter and poor environmental

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conditions (Edema *et al.*, 2008). Fresh or undercooked infected fish can cause zoonotic infections, which are a real public health problem (Dorny *et al.*, 2009; Bouchriti *et al*., 2014).

In Mali, food safety risks associated with the presence of parasites in fishery products are poorly known by professionals and consumers. Parasitic fish must be rejected, if it is not sufficiently cooked since its penetration into the upper digestive tract may be a threat to the health of the consumer. Therefore, it is important to sensitize the population about fish hygiene practices to minimize the impact of the parasite on public health.

The present study was conceived to determine the prevalence and seasonal distribution of parasites in economically important and popular freshwater fish species: *Lates niloticus*, *Clarias anguillaris*, and *Oreochromis niloticus* sold on the Medina Coura market in Bamako.

2. MATERIAL AND METHODS

Study Area

In Mali, fishing is practiced in almost all water bodies in the country. It is the largest freshwater fishing country in the world. Fishing is practiced in two river basins lakes, ponds and flood plains (Quensiere, 1994). The main fishing areas are Central Delta of Niger (about 80% of total freshwater fish production), Selingue Lake, Markala Lake and Manantali Lake (Figure1).

The Central Niger Delta is a vast region of about 30 000 km^2, in the Center of Mali. The annual production varies between 45 000 and 100 000 tones according to the importance of the floods (Quensiere, 1994). About 140 km South of Bamako, Selingue The dam is 348 m long and the lake covers 409 km^2 and contains 2.2 billion m^3 of water. However, its depth does not exceed 20 m (Paugy & Leveque, 2006). By year, the lake produces about 4000 tons of fish (Miller, 2006). Manantali Lake provides both irrigation and hydroelectric power. 208m long with an area of up to 500 km2, it contains 11 billion m^3 of water. The depths of the medium and maximum dam are respectively 20 m and 50 m (Miller, 2006). Per year, fish production is about 1,500 tons, Markala dam was built in 1943, located 250 km downstream from Bamako in Mali to store water for irrigation (Miller, 2006). The "Office du Niger" zone, aims to develop agriculture.

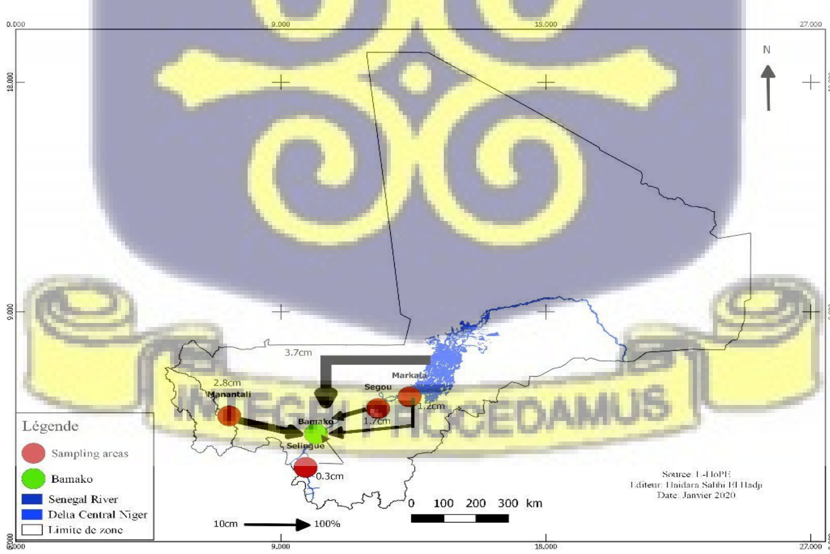

Figure 1: Map showing the different sampling points

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Sampling procedure

Three freshwater fish species sold on the Medina Coura market, a major fish market in Bamako were sampled for this study. These were Nile perch: *Lates niloticus*, vernacular name in Bambara (Saalen); Catfish: *Clarias anguillaris*, vernacular name in Bambara (Maanogo Blen) and Tilapia: *Oreochromis niloticus*, vernacular name in Bambara (N'teben fin). These fishes were selected because of their economic importance and popularity in Mali. All samples were obtained from harvest and landing sites, from four (4) areas (Selingue, Manantali, Markala, Mopti) and a selling site in Medina Coura market.

Two (2) specimens of each of the three (3) species of fish were sampled for parasitic assessments.

Sample size

The Samples were taken during the cold season (December - February) and the hot season (March-May) (table1). 120 randomly selected fresh specimens of 44 *Lates niloticus*, 40 *Clarias anguillaris*, and 36 *Oreochromis niloticus*, were purchased at capture point, at the landing point where the fishes are packed for the market and at Medina Coura market, which is selling point. The samples were transported directly to the helminthology laboratory of the Central Veterinary Laboratory of Bamako in a cooler, where they were subjected to parasite analyses.

Table 1: Distribution of fish species collected by sampling site

Areas	Seasons	*L. niloticus*	*C. anguillaris*	*O. niloticus*	Total
Selingue	Cold	4	4	4	30
	Hot	6	6	6	
Manantali	Cold	6	4	4	30
	Hot	6	6	4	
Markala	Cold	6	4	4	26
	Hot	4	4	4	
Mopti	Cold	6	6	6	34
	Hot	6	6	4	
Total		44	40	36	120

The parasitological examination was made on fish by its lateral decoupage through the mouth. The alimentary canals were removed and cut into different parts, such as the stomach, large intestine, and small intestine. This was done on a petri dish with normal saline for parasite recovery. The intestines were carefully cut to promote the emergence of parasites.

The macroscopic examination involved observation of the general state of each sample, the level of deterioration of the skin, the state of the gills by the appreciation of the color, and the signs of attack. The fish were also examined under the Olympus BO61 binocular magnifier for cysts, larvae in the flesh and the abdominal cavity. The results were analysed for prevalence according to Margolis *et al.*, 1982:

Prevalence (%) = number of infected hosts × 100 / total number of hosts examined

Crustacea identification was based on morphological features according to Yamaguti, 1963.

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Data analysis method

The one-way analysis of variance (ANOVA) was applied to determine the significance of changes in the prevalence of infection between the two seasons in 4 areas of the study period. The percentages of infestation were compared by the chi-square χ^2 test.

SPSS 23.0 Software was used for data analysis with a confidence interval of 95%.

3. RESULTS

Of the 120 fish samples analyzed, parasites were present in fishes at the capture points, the landing point at Selingue, Manantali, Markala, Mopti and at the selling point Medina Coura market.

Prevalence of infected fish species

Parasite infestation has varied from one species to another. A low infestation rate was observed (10%) in our fish samples. The highest parasite infestation was observed in *Oreochomis niloticus* with an infestation rate of 16.7% followed by *Lates niloticus* 9.1% and *Clarias anguillaris* 5.0% during both seasons. A no significant difference in prevalence between fish species examined was observed chi-square χ^2 = 0.87, (p> 0.05). It showed a lower variation of prevalence of parasites among the fish species in this study

Table 2: Prevalence of parasites by fish species

Fish species	Number examined	Number infected	Prevalence (%)
Lates niloticus	44	4	9,1
Clarias anguillaris	40	2	5
Oreochromis niloticus	36	6	16,7
Total	120	12	10

Variation of parasitism according to the organs of the fish and areas of sampling

The skin, flesh, gills, and abdominal cavity were observed macroscopically. The most affected internal organs of the fish were the gills with the presence of *Salmincola edwardsii* (crustaceans) and the abdominal cavity with the presence of nematodes *Rhaphidascaris sp*. and *Philonema sp.*. These parasites belonging to different taxonomic groups were recovered from 12 positives sampled fish.

The frequency distribution of parasites among the three fish species (*Lates niloticus, Clarias anguillaris*, and *Oreochromis niloticus*) per sampling area is presented in Table 3. *Philonema sp.* (12.5%) was the most frequently found parasite in Markala followed by *Raphidascaris sp*. Which were recovered from the abdominal cavity in 10% and 8.82% respectively of fishes sampled in Selingue and Mopti. *Salmincola edwardsii* was found in the gills at 4.16% of the fish sampled at Markala. These results show that there is no significant difference between parasite variation and prevalence at the sampling areas (χ^2 = 0.821, p> 0.05).

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Table 3: Prevalence of parasites per infected organs and areas

Parasites	**Inf. Organs**	**Selingue**		**Manantali**		**Markala**		**Mopti**	
		NOP	P(%)	NOP	P(%)	NOP	P(%)	NOP	P(%)
Raphidascaris sp.	Abd. cavity	3	10	1	3.57	0	0	3	8.82
Philonema sp.	Abd. cavity	0	0	0	0	3	12.5	2	5.88
Salmincolaedwardsii	Gill	0	0	1	4.16	0	0	0	0

Key: NOP – Number of positive; P – Prevalence; Inf. – Infected; Abd– Abdominal

Seasonal variation offish parasites per sampling areas

To assess the seasonal prevalence of parasites in the cold and hot seasons, 58 and 62 samples were examined for hot and cold seasons respectively of which 3 (5.17%) and 9 (14.52%) were infested respectively.

The parasite frequency distribution per season and the per area have shown that the high parasite infestation was observed during the hot season in Selingue with 25% prevalence followed by Mopti 20%, Manantali 16.66%, and Medina Coura 1.66%. In the cold season fish sampled from the selling point had parasite infestation rate of 10%, followed by Manantali and Markala with 8.33% in a landing point. The seasonal variation on parasites of fish species samples per area was not significantly different (χ^2= 0.363, p> 0.05) from each other during the different seasons.

Table 4: Prevalence of fish infested with parasites per areas and season

Seasons	Sampling Site	No. Examined	No. Infected	Prevalence (%)
Cold season	Selingue	12	0	0
	Manantali	12	1	8.33
	Markala	12	1	8.33
	Mopti	12	0	0
	MCM	10	1	10
	Total	**58**	**3**	**5.17**
Hot season	Selingue	12	3	25
	Manantali	12	2	16.66
	Markala	12	0	0
	Mopti	10	2	20
	MCM	16	1	1.60
	Total	**62**	**9**	**14.52**
TOTAL		120	12	10

Key: Medina Coura Market

3.4 Prevalence of infected fish species at each sampling point

Table 5 shows the prevalence of each species of fish in each area at the sampling points. Parasite prevalence varied from one fish species to another. The highest prevalence of the parasite in *Oreochromis niloticus* was observed at Mopti at the selling point (50%) followed by

Lates niloticus 25% while the other species of fish have a prevalence of 0% at the same point of sale.

Table 5: Prevalence of infected fish species at each sampling point

Sampling Site	Fish Species	Capture point			Landing point			Selling point		
		NE	NI	P(%)	NE	NI	P(%)	NE	NI	P(%)
Selingue	*L. niloticus*	4	0	0	4	1	25	2	0	0
	C. anguillaris	4	0	0	4	0	0	2	0	0
	O. niloticus	4	1	25	4	1	25	2	0	0
Manantali	*L. niloticus*	4	0	0	4	0	0	4	0	0
	C. anguillaris	4	0	0	4	0	0	2	0	0
	O. niloticus	4	0	0	4	1	25	0	0	0
Markala	*L. niloticus*	4	0	0	4	0	0	2	0	0
	C. anguillaris	4	1	25	4	1	25	0	0	0
	O. niloticus	4	1	25	4	0	0	0	0	0
Mopti	*L. niloticus*	4	1	25	4	1	25	4	1	25
	C. anguillaris	4	0	0	4	0	0	4	0	0
	O. niloticus	2	0	0	4	0	0	4	2	50
Total		42	4	9.5	44	5	11.4	26	3	11.5

Key: *NE – Number examined; NI – Number infected; P – Prevalence*

4. DISCUSSION

Our study revealed an overall rate of parasite infestation of 10% in *Lates niloticus, Clarias anguilaris, Oreochromis niloticus* species. The preliminary investigation of the parasite fauna of fish in Okhuo River in Benin City, Nigeria done by obtained 6.94% infestation rate. These were rather very low as compared to other similar work such as(Amare *et al.*, 2014a)who reported 47.8%prevalence of parasites, in Lake Lugo (Hayke), Northeast Ethiopia, and Olofintoye (2006) who observed 62.6% in his study on a parasitofauna in some freshwater fish species in Ekiti States, Nigeria suggesting that the distribution of parasites can be varied from one habitat to the other due to host parasite relationship and abiotic factors like dissolved oxygen, temperature and pH. This depends on a number of factors which include among other things, the nature of the water which is reflected in the human use and the endemicity of infection in the area.

The prevalence of parasites was higher in *Oreochromis niloticus 16.7%* followed by *Lates niloticus 9.1%* and *Clarias anguillaris 5%*. In the present study prevalence finding are lower than Amare et al., 2014b in Lake Lugo (Hayke), Northeast Ethiopia who found a prevalence rate of 50.22% in *Oreochromis* species.

The study showed that, the prevalence of nematodes was higher than crustacea. The higher rate of nematode infestation may be due to the low host specificity of the adult stage of these parasites, or the ability of different genera and species of the nematode to infect the fish species studied, and the availability of the different host required for the completion of the life cycle of these parasites Yamaguti, 1963.

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The prevalence of *Salmincola edwardsii* is 4.16% observed only in Manantali on *Oreochromis niloticus* in the gills. *Salmincola edwardsii* belong to the Arthropoda Phylum, subphylum Crustacea, family *Lernaeopodidae*, and are commonly called "gill lice" or "gill fly" Alteen, 2009 and Boucenna *et al.*, 2015 also detected the presence of this parasite in the gills. They inferred that this parasite can cause swelling around the site of attachment of the bubble, with heavy parasite loads. The presence of this crustacean reduces the feeding activity of the host fish by weakening the host fish and negatively affecting its weight gain, reproduction, and therefore growth. According to Folefack *et al.*,(2019a) the frequency of gill infestation by these parasites is may be because gills have a close relationship with the aquatic environment where fish live. It is observed that *Oreochromis niloticus* collected at the point of sale in the Mopti area during the hot season has a much higher prevalence (50%) than other species. These parasites are capable of absorbing nutrients, vitamins, micro and macronutrients in the digestive tract of fish. According to Bichi & Dawaki (2010), these parasites develop metabolic wastes that have a negative impact on the physiology and reproduction of fish.

5. CONCLUSION

The study allowed us to determine the parasites present in the 5 areas (Selingue, Manantali, Markala, Mopti, and Medina Coura market) for freshwater fresh fishes from harvest capture to sale. The prevalence of parasites among fish in rivers in Mali was low.
However, parasites should not be neglected in fish, they are important enough to reduce the number of fish per death, reduce their market value, and may cause human infections when live cysts or matured parasites are ingested.

6. DECLARATION

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Conflict of interest: Not applicable

Ethical approval: Ethical clearance was obtained from the Ethics Committee of the National Institute of Research in Public Health (INRSP). The provisions relating to ethics have been observed and respected according to the provisions of Law No. 2013-015 of 21 May 2013 on the Protection of Personal Data in the Republic of Mali and the Declaration of 'Helsinki 5 version 2013 to establish fact sheet, a free and informed consent form.

Consent for publication: Not applicable

Consent to participate: Written informed consent was obtained from the participants.

Availability of data and material: All available data supporting the study have been included in the write-up

Code of availability: Not applicable

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AUTHOR CONTRIBUTION

Aminata Sissoko designed the topic, collected data, and wrote the manuscript, while Fassé Samaké, Tano Debrah Kwaku, and Angela Parry-Hanson Kunadu approved the topic, supervised and reviewed the manuscript, Felix Kwashie Madilo and Boubacar Madio dit Aladiogo Maïga[1]conducted the data analysis, and formatted the manuscript. Ibrahima Mariko conducted the laboratory analysis.

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