

**ADDIS ABABA UNIVERSITY  
FACULTY OF VETERINARY MEDICINE**

**STUDY ON BACTERIAL PATHOGENS OF FISH IN SOUTHERN GULF OF LAKE  
TANA WITH SPECIAL REFERENCE TO *AEROMONAS HYDROPHILA* AND  
*EDWARDSIELLA TARDA***

**BY  
ANWAR NURU**

**JUNE 2007  
DEBRE ZEIT, ETHIOPIA**

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Veterinary Science in Tropical Veterinary Microbiology

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**BY  
ANWAR NURU**

**Board of External Examiners:**

**Signature**

1. Prof. Dr. H. J. Schwartz

-----

2. Dr. Tesfaye Kumsa

-----

3. Dr. Adugna Tolera

-----

4. Prof. S. K. Kahr

-----

5. Prof. M. S. Jagannath

-----

6. Dr. Giles Innocent

-----

7. Dr. Filip Claes

-----

8. Dr. Mohammed Abdella

-----

9. Dr. Karim Tounkara

-----

10. Dr. Damen H/ mariam

-----

**Advisors:**

Dr. Bayleyegn Molla

-----

Dr. Eshetu Yimer

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## **DEDICATION**

This work is dedicated to my mother Nurya Seid, father Nuru Arefeayne (Mercy be upon you), wife Alewya Adem and dear child Hamdu Anwar. I love you all.

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## TEBLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS .....	V
LIST OF TABLES .....	VIII
LIST OF FIGERES .....	IX
LIST OF ANNEXES .....	X
ABBREVIATIONS .....	XI
ABSTRACT .....	XII
1. INTRODUCTION.....	1
2. LITRATURE REVIEW .....	5
2.1. Taxonomic classification of bacterial pathogens of fish .....	5
2.2. General characteristics of bacterial pathogens of fish .....	7
2.3. Sources of bacterial infections and predisposing factors.....	7
2.4. Major Gram-negative bacterial pathogens associated with fish and human .....	8
2.4.1. Bacteriology, epidemiology and associated diseases .....	8
2.5. Prevention and control of fish and fish borne diseases.....	16
2.5.1. Prevention and control of fish diseases.....	16
2. 5.2. Prevention and control of fish borne diseases in human .....	18
3. MATERIALS AND METHODS .....	19
3.1. Description of the study area (Lake Tana).....	19
3.2. Study population .....	21
3.3. Study design.....	22
3.3.1. Study type and sample size determination.....	22
3.3.2. Study methodology .....	22
4. RESULTS.....	28
4.1. Study population .....	28
4.2. Clinical and post-mortem findings .....	29
4.3. Isolated bacteria .....	29
4.3.1. Bacteria isolated from the kidney and intestine of fish .....	30
4.3.2 Bacteria isolated among different fish species .....	31
4.3.3. Bacteria isolated from the fish based on different sampling sites .....	32
4.3.4. Bacteria isolated in relation to maturation and sex of fish .....	33

4.3.5. Bacteria isolated from filleted fish.....	35
4.3.6. Isolation of bacteria from water samples.....	37
4.4. Physicochemical make up of the study area.....	38
5. DISCUSSION.....	39
6. CONCLUSION AND RECOMMENDATIONS.....	46
7. REFERENCES.....	48
8. ANNEXES.....	57
9. CURRICULUM VITAE.....	66
10. DECLARATION.....	69

## LIST OF TABLES

Table 1. Bacterial pathogens of fish .....	6
Table 2. Differentiation between <i>A. hydrophila</i> , <i>A. caviae</i> , <i>A. sobria</i> , <i>A. salmonicida</i> , and <i>P. shigelloides</i> .....	27
Table 3. Fish species sampled at Lake Tana based on different parameters .....	28
Table 4. Occurrence and distribution of bacterial species isolated and identified from different sample types.....	30
Table 5. Distribution and frequency of occurrence of bacteria from the kidney and intestine of fish .....	31
Table 6. Bacteria isolated in different fish species .....	32
Table 7. Bacteria isolated from the fish based on sampling sites .....	33
Table 8. Bacteria isolated based on different maturation stages of fish .....	34
Table 9. Bacteria isolated in different sex .....	35
Table 10. Bacteria isolated from water samples collected at different fish habitats .....	37
Table 11. Mean of physicochemical parameters and characteristics of the fish and water sampling stations.....	38

## **LIST OF FIGERES**

Figure 1. Map of the study area .....	19
Figure 2. Flow chart for targeted Gram-negative fish pathogens .....	26
Figure 3. Frequency and percentage of bacteria isolated from filleted fish .....	36

## LIST OF ANNEXES

Annex 1. Sample collection record sheet.....	57
Annex 2. Bacteriological and biochemical test result recording sheet. ....	58
Annex 3. Primary and secondary identification tests. ....	59
Annex 4. List of media utilized .....	64
Annex 5. List of reagents, sugars and diagnostic discs .....	64
Annex 6. Distances of sanitary facilities from Lake Tana.....	65

## ABBREVIATIONS

BHI	Brain Heart Infusion
<i>C. gareipinus</i>	<i>Clarias gariepinus</i>
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
EC	European Commission
FAO	Food and Agricultural Organization of the United Nations
Fr.	Fresh water
H <sub>2</sub> S	Hydrogen Sulphide
HACCP	Hazard Analysis Critical Control Point
IMViC	Indole, Methyl-red, Voges-Proskaur and Citrate test
KOH	Potassium Hydroxide
M	Marine water
MR	Methyl Red
<i>O. niloticus</i>	<i>Oreocromis niloticus</i>
O-F	Oxidation-fermentation test
PBS	Phosphate buffer saline
QA	Quality assurance
TSA	Tryptic Soya Agar
TSI	Triple Sugar Iron Agar
UV	Ultraviolet
<i>V. beso</i>	<i>Varicorhinus beso</i>
VP	Voges-Proskaur

## ABSTRACT

A bacteriological study was conducted from September 2006 to March 2007 to estimate the occurrence and distribution of important Gram-negative bacteria with special reference to *Aeromonas hydrophila* and *Edwardsiella tarda* from the kidney and intestine of apparently healthy fish and their aquatic environment in the southern gulf of Lake Tana and carcasses of filleted fish (from commercial enterprises and local dealers) in Bahir Dar, Ethiopia. From a total of 510 swab samples taken from the kidney and intestine of 255 apparently healthy fish, different bacterial species, which belong to the family Aeromonadaceae, Vibrionaceae and Enterobacteriaceae were isolated and identified.

Among the isolates *Aeromonas (A.) hydrophila* (7.1%), *Aeromonas (A.) caviae* (9.8%), *Aeromonas (A.) sobria* (15%), *Edwardsiella (E.) tarda* (2%), *Vibrio* spp (7.8%), *Yersinia (Y.) ruckeri* (3.1%), *Edwardsiella (E.) ictaluri* (1.2%) and atypical strains of *Aeromonas (A.) salmonicida* (13%) were isolated in both the kidney and intestinal samples. *Proteus (P.) mirabilis* (5.9%), *Enterobacter (E.) aerogenes* (5.1%), *Plesiomonas (P.) shigelloides* (4.3%), *Yersinia (Y.) enterocolitica* (3.1%), *Escherichia (E.) coli* (2.4%), *Klebsiella (K.) pneumoniae* (2.4%), *Proteus (P.) vulgaris* (1.6%), *Shigella (S.) sonnei* (0.8%), *Citrobacter (C.) diversus* (0.4%) and *Citrobacter (C.) freundii* (0.4%) were isolated only from the intestine of fish. From carcasses of filleted fish *A. sobria* constituted 13.2% of the total isolates followed by *A. caviae* (9.6%), atypical strains of *A. salmonicida* (6.4%), *Y. enterocolitica* (4.8%), *A. hydrophila* (3.6%), *E. coli* (2.4%), *P. mirabilis* (1.6%), *C. diversus* (1.2%), *P. shigelloides* (1.2%) and *Vibrio* spp (0.8%).

Out of 62 water samples, 47 (75.8%) were positive for Aeromonadaceae and Enterobacteriaceae. All the bacterial species, which were isolated from the water samples, were also recovered from fish. Indicators of water pollution bacteria such as *A. caviae* and *E. coli* were more frequently isolated from sampling stations found in inshore littoral zone than stations found in sub-littoral and pelagic zones that are situated relatively far from the point source pollutions and hence less likely to be exposed to pollution.

From the results of the present study it was concluded that the intestine of fish have been found to be harboring a large group of bacterial species that may be a resident and/or transient microorganism. Isolation of similar types of bacteria from the fish, filleted fish and their aquatic environment has been recorded during the study period. The detection of similar bacterial species both in the fish and the water samples has some implications on the relationship between the fish and their aquatic environment (even though it requires some molecular characterizations of both isolates). Some of the bacterial isolates including *Aeromonas hydrophila* and *Edwardsiella tarda* are opportunistic pathogens of fish and may cause mortalities when the fish come under stress. Furthermore, the recovery of various organisms, which are potentially pathogenic to humans, in the kidney and alimentary tract of fish, and/or carcasses of filleted fish suggested that fish either improperly handled, undercooked or consumed raw might cause various diseases to susceptible individuals.

**Keywords:** Aeromonadaceae, Vibrionaceae, Enterobacteriaceae, fish, kidney, intestine, filleted fish, water, Lake Tana, Bahir Dar, Ethiopia.

## 1. INTRODUCTION

Fish have certain advantages over land animals in their suitability for farming. Being cold blooded, fish do not have to expend energy in maintaining body temperature. Also they do not have to support their weight, unlike land animals, and should therefore be inherently more efficient at converting food into flesh (Shepherd and Bromage, 1992). Moreover, fish contain as much as 60% first class proteins on a dry matter basis. It is rich in vitamins and other nutrients, variable quantities of fat, calcium, phosphorus and other nutrients important to human health and growth. Fish in many ways is even more nutritious than most meat from warm-blooded animals (Moar *et al.*, 1983).

Aquaculture is growing rapidly worldwide, with fish being the primary source of animal protein in many countries (Dugenci and Candan, 2003). The fishery sector plays a significant role in food security through the supply of food for developing countries as a whole. Fish currently make up about 19% of the total animal protein consumption, or just over 5% of proteins from both plant and animal origin (Dugenci and Candan, 2003).

In Ethiopia, despite favorable physical conditions aquaculture is still virtually non-existent but the country depends on its inland water bodies for fish supply for its population (FAO, 1995). Fishery in Ethiopia has contributed total gross sale of 13.2 millions Ethiopian birr or 1.55 millions USD in the year 2000 at products price averaging 0.82 birr per kg of fresh fish of the common species (Abraham, 2005). The water body covers only 0.7% of the area of the country and comprises 10 lakes in the central highlands, mostly in the rift valley, with a total surface area of 7,500 km<sup>2</sup> (Wudneh, 1998). The largest lake is Lake Tana, located outside the rift valley on the Northwestern plateau, with a surface area of 3,200 km<sup>2</sup>. Minor water bodies such as crater lakes and dam reservoirs occupy a total area of approximately 700 km<sup>2</sup> (Wudneh, 1998). Lake Tana is the biggest and leading lake in potential yield with its yearly estimated potential was about 13,000 to 15,000 tonnes (FAO, 1995; Wudneh, 1998). Lake Tana with its unique hexaploid species flock of large barbs compared to the small diploid African barbs is the only lake having barbs still intact world wide (Nagelkerke, 1997).

The main commercially important fish species in Lake Tana fishery are Nile Tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*), large barbs and Beso (*Varicorhinus beso*) in little amount (Sewmehon, 2003). The water resource from Lake Tana

and all other inland water bodies in the region provide immense potential and playing various roles in the main economic activities. The contribution is for irrigation, electric power generation and fisheries development, as a source of drinking water for both human and animals. The aesthetic value of Lake Tana coupled with the historic ancient monasteries and churches containing various religious icons and manuscripts on its many of the islands, have also added to its value through the development of tourism and the growth of inward investment (Sewmehon, 2003). Lake Tana has also an important value as a source of livelihood to the poor community living around the lake through providing food, employment and income through imploding variety of activities (Sewmehon, 2003).

The southern gulf of Lake Tana which have relatively high fish density (Wudneh, 1998), receives many contaminant inflows from nearby farm lands with intensive fertilizers and untreated industrial, municipal, sewages, as well as leaching from pit latrines and septic tankers remain the most important sources of pollution (Yimenu, 2005). Contamination of the lake water and the surrounding shore lines may result in significant impact upon the lake ecology, specifically it has implication for the fish population and for the sustainability of the livelihood of the local fishermen (Yimenu, 2005).

Like other animals, however, fish are susceptible to microbial disease, and although the range of true pathogens appears to be rather restricted, fish disease is a global problem affecting fresh water and marine fish, cultured and sport fish and even ornamental fish. The problem is of major importance when fish are subjected to intensive culture practices, since it is under these conditions that extremely large numbers of fish can rapidly become diseased and die (Trust, 1986).

Fish in intensive culture are continuously affected by environmental fluctuations and management practices such as handling, crowding, transporting, drug treatments, undernourishment, fluctuating temperatures and poor water quality. All of these factors can impose considerable stress on the homeostatic mechanisms of fish rendering them susceptible to a wide variety of pathogens (Noga, 1996; Woo and Bruno, 1999). There are documented epizootics of diseases among wild fish populations. However, man can also attribute most modern day wild fish losses to environmental alterations. The overcrowded conditions in intensive culture systems are conducive to the movement of pathogens from host to host and

to the development of extremes of water contamination detrimental to the health of each individual fish in the population (Post, 1987).

Fish diseases are very important aspects of modern fish farming given the enormous impact on profitability and also cause of human diseases in many areas of the world (Ruiter, 1995). Although stress appears to play a considerably larger role in causing disease in fish, they are susceptible to the same type of agents that affect warm blood animals, including viruses, bacteria, fungi, parasites, as well as various non-infectious problems (Noga, 1996). The appearance and development of a fish disease is the result of the interaction among pathogens, host and environment. Therefore, only multidisciplinary studies involving the characteristics of potential pathogenic microorganisms for fish, aspects of the biology of the fish hosts as well as a better understanding of the environmental factors affecting such cultures, will allow the application of adequate measures to prevent and control the main diseases limiting fish production (WHO, 1999; Toranzo *et al.*, 2005).

During the last few decades a number of studies have been conducted on fish diseases all over the world and a significant amount of parasitic, bacterial, viral and fungal pathogens were identified from different species of fish both in fresh and marine cultures. But research with regard to fish diseases is in its infancy in Ethiopia. So far, very few studies have been conducted on fish parasites at Lake Awassa, Chamo, Tana and Zeway (Amare, 1986; Tefera, 1990; Eshetu, 2000; Eshetu and Mulualem, 2003; Gebeyehu, 2003; Dejen *et al.*, 2006), and a preliminary survey on bacterial pathogens of fish at Lake Zeway and Lake Tana have also been conducted by Eshetu (2000) and Gebeyehu (2003), respectively.

There is an increasing demand for fish and fish products around the world. In Ethiopia the current annual demand for fish is estimated at 65,544 tonnes, equivalent to 1 kg per person (Abraham, 2005). The future demand at the present population growth rate will reach 83483 tones in year 2010, 94526 and 1171586 tones by the year 2015 and 2025, respectively (Abraham, 2005). This is a minimum demand, since factors other than population were not considered. But the country annual total exploitable fish potential is estimated to be 51481 tonnes, which can meet only 79% of the current actual demand, 55% and 44% of the projected demands in year 2010 and 2015, respectively that are based solely on population size (Abraham, 2005). In view of this, the present water bodies as a fish supply source are unable to meet the demand. This calls for an increasing focus on stocking and enhancement of

artificially made water bodies and development of aquaculture, which again urges a closer investigation of the management, feeding and disease conditions of fish and their potential pathogens. Moreover, there is substantial evidence that fish and other seafood are high on the list of foods associated with outbreaks of food borne diseases. Bio-toxins, histamine and viruses cause a large proportion of these outbreaks (WHO, 1998). Although fish may serve as both passive and active carriers of human pathogens, many such pathogens are thought to be introduced into the aquatic environment through human sewage and other sources of water pollutions (WHO, 1998).

There is a need for increasing focus on stocking and enhancement of artificially made water bodies and development of aquaculture, due to the imbalance between current total exploitable fish potential of Ethiopian water bodies and the future demand for fish at the present population growth rate. Since fish diseases are one of the important aspects of modern fish farming, to have information on the occurrence and distribution of fish pathogens will be essential. Furthermore, in Ethiopian water bodies including lakes the only group of pathogens of fish that have been relatively well-studied are helminthes, however less attention have been given to bacterial pathogens of fish including those which have also zoonotic importance. Although a number of eukaryotic microorganisms produce fish diseases, this study was restricted to only selected Gram-negative fish bacterial pathogens, especially this with on few systemic pathogens that are frequently having zoonotic importance. Therefore, the objective of this study was to estimate the occurrence and distribution of important Gram-negative bacteria from:

- The kidney and intestines of apparently healthy fish
- Filleted fish, and
- The environment of fish at Lake Tana with special reference to *Aeromonas (A.) hydrophila* and *Edwardsiella (E.) tarda*

## **2. LITRATURE REVIEW**

### **2.1. Taxonomic classification of bacterial pathogens of fish**

There are members of the true bacteria (Eubacteriales), the ray-bacteria (Actinomycetales) and the gliding bacteria (Cytophagales) associated with diseases of fish (Post, 1987). The taxonomic classification and the occurrence of each organism are summarized as shown in table 1.

Table 1. Bacterial pathogens of fish

Family	Genus	Species	Fr./ M
Cytophagaceae (-)	<i>Flexibacter</i>	<i>F. columnaris</i>	Fr.
		<i>F. psychrophila</i>	Fr.
		<i>F. marinus</i>	M
		Unclassified <i>Myxobacterium</i>	Fr., M
Enterobacteriaceae (-)	<i>Edwardsiella</i>	<i>E. tarda</i>	Fr.
		<i>E. ictaluri</i>	Fr.
Pseudomonadaceae (-)	<i>Pseudomonas</i>	<i>P. fluorescens</i>	Fr., M
	<i>Alteromonas</i>	<i>A. piscicida</i>	M
Vibrionaceae (-)	<i>Aeromonas</i>	<i>A. hydrophila</i>	Fr., M
		<i>A. salmonicida</i>	Fr., M
	<i>Vibrio</i>	<i>V. anguillarum</i>	M, (Fr.)
		<i>V. ordalii</i>	M
		<i>V. vulnificus</i>	M
Uncertian (-)	<i>Flavobacterium</i>	<i>Flavobacterium</i> spp.	Fr., M
	<i>Haemophilus</i>	<i>H. piscicium</i>	Fr.
	<i>Pasteurella</i>	<i>P. piscicida</i>	M, Fr.
Corynebacteriaceae (+)	<i>Renibacterium</i>	<i>R. salmoninarum</i>	Fr., (M)
Streptococcaceae (+)	<i>Streptococcus</i>	<i>S. faecalis</i>	Fr., M
		<i>Streptococcus</i> spp	Fr., M
Bacillaceae (+)	<i>Clostridium</i>	<i>C. botulinum</i>	Fr., M
Nocardiaceae (+; A. F.)	<i>Nocardia</i>	<i>N. asteroides</i>	Fr.
		<i>N. kampachi</i>	M
Mycobacteriaceae (A.F.)	<i>Mycobacterium</i>	<i>M. fortuitum</i>	Fr., M
		<i>M. marinum</i>	Fr., M

Fr. = fresh water; M = marine water; (Fr.) (M) = infrequent occurrence; (-) = Gram-negative; (+) = Gram-positive; A.F. = Acid-fast

Source: (Frerichs, 1984)

## **2.2. General characteristics of bacterial pathogens of fish**

Most bacteria pathogenic to fish are rod-shaped; only a few pathogens, which are spherical in shape, are known and there are no known helical forms (Post, 1987). Many bacteria pathogenic to fish are psychrophilic; only a few species will grow above 35<sup>0</sup>C. Most have a maximum growth temperature of 35<sup>0</sup>C or less. The psychrophilic species have an optimum growth temperature of 10<sup>0</sup>C and continue to replicate at 4<sup>0</sup>C (Frerichs, 1984). Opportunistic pathogens of fish can replicate at a pH range of 5.5 up to 10. The primary pathogens of fish usually have a narrower pH tolerance, generally 6 to 9 (Post, 1987).

Nearly all of the bacteria infecting fish are aerobic or facultative anaerobic. Strictly anaerobic bacteria are rarely found to be associated with fish diseases. All organisms apart from *Clostridium botulinum*, which requires strict anaerobic conditions, can be cultivated aerobically (Fraser *et al.*, 1991).

Fresh or warm-water fish tend to have a biota that is composed of more mesophilic Gram-positive bacteria than cold-water fish, which tends to be largely Gram-negative (Jay, 2000).

Many of the bacteria capable of causing disease in fish are saprophytic. They become pathogens when fish are physiologically unbalanced, nutritionally deficient, and when there are other abnormalities, which allow opportunistic organisms to invade (Post, 1987; Noga, 1996). Some bacterial pathogens of fish are fastidious and require special growth media for laboratory culture. However, the greatest number of bacterial species found as fish pathogens grow well on common laboratory media (Frerichs, 1984; Post, 1987).

## **2.3. Sources of bacterial infections and predisposing factors**

There are essentially two types of pathogenic bacteria: primary or obligate pathogens, which are pathogens that are not part of the normal aquatic flora and are capable of causing disease in healthy individuals, typically *Aeromonas salmonicida* (Post, 1987; Fraser *et al.*, 1991). The other type is opportunistic pathogens; these are normally free living either in the water or on the fish, but are capable of adopting a pathogenic role under circumstances. Many of these are saprophytes, normally living on dead organic matters such as plants and animal remains or

feces, typically *Aeromonas hydrophila*, *Pseudomonas* and *Vibrio* species are the most important (Post, 1987; Fraser *et al.*, 1991; Noga, 1996).

The occurrence of infection seems to be due to damage to the integument (skin) resulting from abrasion, parasite activity or chemical damages like ammonia, nitrite, high or low pH (Woo and Bruno, 1999). Moreover, if the fish normal defenses are depressed as a result of environmental stress or diseases, will enable opportunistic bacteria to take advantage (Post, 1987). Epidemics of bacterial diseases are common in dense population of cultured fish and in some cases of natural water bodies. Predispositions to such outbreaks are frequently associated with poor water quality, organic load of the aquatic environment, marked temperature changes, hypoxia and related stressful conditions (Noga, 1996; Ruth, 1998).

## **2.4. Major Gram-negative bacterial pathogens associated with fish and human**

### 2.4.1. Bacteriology, epidemiology and associated diseases

#### *Aeromonas* species

The genus *Aeromonas* contains two well-separated groups. The first group consists of a single psychrophilic, non-motile species, *A. salmonicida*, which is a strict parasite under natural conditions. *A. salmonicida* is the causative organism of furunculosis, which is economically important in salmon and trout fish, but is not a pathogen of man. The second group consists of mesophilic motile strains. Three species were described (*A. hydrophila*, *A. sobria* and *A. caviae*). Subsequent DNA hybridization studies have shown that there are at least 11 species of mesophilic aeromonads (Varnam and Evans, 1991; Woo and Bruno, 1999). There is evidence to suggest that those strains best adapted to grow at low temperatures are more commonly pathogens of fish and not humans (Bremer *et al.*, 2003). According to Joseph and Carnahan (2000), the genus *Aeromonas* is now classified within the family Aeromonadaceae and consists of 14 different confirmed species, one of which is *A. hydrophila*.

Members of the genus *Aeromonas* are Gram-negative, facultatively anaerobic, non-spore forming, rod-shaped bacteria. *A. hydrophilia* is motile by a single polar flagellum, catalase-positive, oxidase-positive rod, which ferments glucose. It is neither salt (<5%) nor acid (min. pH ~ 6.0) tolerant and grows optimally at around 28<sup>0</sup>C. It has the ability to grow at cold temperatures, reportedly as low as -0.1<sup>0</sup>C for some strains (Daskalov, 2006).

The cells in the genus *Aeromonas* range from straight rods with rounded ends to cocci. They are 1.0 to 4.4 µm long, occasionally forming filaments up to 8 µm long. They occur singly, in pairs, or in chains. *Aeromonas* species break down carbohydrates to acid or acid and gas (CO<sub>2</sub> and H<sub>2</sub>). They ferment glucose, fructose, maltose, and trehalose, but not adonitol, dulcitol, inositol, inulin, melezitose, sorbose, and xylose. Starch, dextrin and glycerol are hydrolysed. Nitrate is reduced to nitrite. Aeromonads are active producers of protease, diastase, lipase, DNase, and lecithinase. After 24 hours of incubation on nutrient agar colonies are 1 to 3 mm in diameter, convex, smooth, and whitish and translucent; they become light beige on further incubation. There is a wide zone of β-hemolysis on blood agar after one day. The growth becomes dark green after 2 to 3 days. *Aeromonas* species share some properties with the members of the family Enterobacteriaceae and with *Plesiomonas* and *Vibrio* species (Khardori and Fainstein, 1988). *A. hydrophila* is resistant to the vibriostatic agent O/129 (phosphate: 2,4-diamino-6,7-di-isopropylpteridine phosphate) 150 µg (Woo and Bruno, 1999) and novobiocin (5 µg) (Woodland, 2006).

*Aeromonas* is most commonly confused with the members of Enterobacteriaceae. The oxidase test is a major distinguishing test and should be routinely performed on all suspected isolates (Khardori and Fainstein, 1988).

*Aeromonas hydrophila* is an emerging aquatic pathogen widely distributed in waters, water habitats, domestic animals and food products (fish, shellfish, poultry and raw meat). The principal reservoir is the aquatic environment such as fresh water lakes and streams and waste water systems (Daskalov, 2006).

The distribution of mesophilic *Aeromonas* species in the aquatic environment may be related to the level of pollution in the water. In less polluted water, either fresh or marine, *A. caviae* and *A. hydrophila* were almost equally distributed. *A. sobria* can be found in unpolluted waters, brackish waters and sewage effluents. Large numbers of aeromonads, especially *A. caviae*, could therefore be considered to be indicative of nutrient-rich conditions of water. The number of mesophilic *Aeromonas* species in some aquatic environments have been reported to show a seasonal variation, with populations greatest in the warmer months of the year for both temperate freshwater lakes and chlorinated drinking water (Basti *et al.*, 2006).

As for all enteropathogenic bacteria, also in *Aeromonas* species, the pathogenicity is mainly related to two mechanisms: tissue adherence and toxin production (Bottarelli and Ossiprandi, 1999). Pathogenicity and virulence of *A. hydrophila* depends on the ability to produce factors associated with gastroenteritis. These properties are exotoxins, cytotoxins, endotoxins, siderophores, invasins, adhesins and flagella (Daskalov, 2006).

*Aeromonas hydrophila* and other motile aeromonads are recognized pathogens of fish, reptiles, amphibians and cattle (Varnam and Evans, 1991). Fish develop furunculosis or hemorrhagic septicemia due to aeromonads. Furunculosis is a septicemic illness caused by *Aeromonas salmonicida* and is manifested as either a rapidly fatal infection or a slow infection with lethargy and focal necrotic lesions in various muscles (Khardori and Fainstein, 1988). Infections caused by *A. hydrophila* are usually opportunistic requiring stress factors for the initiation of the disease. It causes hemorrhagic septicemia in fresh water fish although it can also be encountered in domestic animals and man (Quinn *et al.*, 2002). Epizootics can occur when the water temperature is increased above 12 to 14°C. Large numbers of the organism are shed into the water from acute or subacute cases, those becoming significant sources of bacteria (Post, 1987). The agent is transmitted horizontally and can be transmitted by discharges from the intestinal tract and external lesions on the skin. Parasite damage and fungal infection of the epidermis may allow entry and spread of bacterial infection (Woo and Bruno, 1999). Experience indicates that latent cases and carrier fish become important factors when *A. hydrophila* is involved (Post, 1987).

Aeromonads may cause infections following trauma, or after ingestion and the organisms are recognized as the cause of disseminating infections in the immunocompromised, and are also wound pathogens. In recent years, aeromonads have received increased attention as an agent of food borne diarrheal disease in otherwise healthy people. Most human pathogenic strains fall into hybridization groups of *A. hydrophila*, *A. caviae* and *A. sobria*. Other species have also been associated with diarrheal diseases (Varnam and Evans, 1991). Symptoms of *Aeromonas* infections in healthy people usually involve gastroenteritis; disseminating infections are the norm among the immunocompromised and other people of high susceptibility. Gastrointestinal infections are of two distinct types. The first type, which accounts for 75% of cases, is cholera-like illness and the second type of infection is dysentery-like. Enteritis due to *Aeromonas* species is usually mild and self-limiting, but severe and life-threatening cases of both types of infection have been reported (Varnam and

Evans, 1991). In the United Kingdom two outbreaks of *A. hydrophila* infections were reported in association with fish and shellfish in the years between 1965 to 1982 (Gilbut, 1991). *Aeromonas* can also produce a wide range of extraintestinal infections in susceptible persons. Septicaemia and meningitis are most common. Other usual manifestations of extraintestinal *Aeromonas* infections include eye infections, pneumonia, urinary tract infections, osteomyelitis and endocarditis (Khardori and Fainstein, 1988; Varnam and Evans, 1991; Daskalov, 2006). The mortality rate due to extraintestinal *Aeromonas* infections in the immunocompromised may exceed 60% (Varnam and Evans, 1991).

Significant rates of isolation of *A. caviae* and *A. sobria* from stool specimens from patients with gastroenteritis have been reported. Cases of *A. sobria* bacterium and cases of cholera like illness caused by enterotoxigenic *A. sobria* have also been described (Khardori and Fainstein, 1988).

#### *Edwardsiella tarda*

The genus *Edwardsiella* includes two species of bacteria that causes diseases in fish: *Edwardsiella tarda* infects fish and other animals including human beings, and *Edwardsiella ictaluri* infect fish only (Woo and Bruno, 1999).

Species of *Edwardsiella*, a member of the Enterobacteriaceae, are small, straight rods, Gram-negative, usually motile with peritrichous flagella, and are facultative anaerobic. The organism, which frequently found in organically polluted water, grows at temperatures between 25 and 37°C, at pH of 4 to 10 (optimum 7.5 to 8) and at 0 to 4% sodium chloride (optimum 0.5 to 1%) (Woo and Bruno, 1999; Novotny *et al.*, 2004). *Edwardsiella tarda* is catalase-positive, cytochrome oxidase negative and glucose fermentative, reduces nitrate to nitrite, is lactose negative and indole positive, and produces an alkaline slant, acid butt and hydrogen-sulphide (H<sub>2</sub>S) on triple sugar iron (TSI) agar (Woo and Bruno, 1999).

*Edwardsiella tarda*, the causative agent of edwardsiellosis in fish, is responsible for extensive losses in both fresh water and marine aquaculture. *E. tarda* infection of many commercially important cultured and wild fish has been reported. It has a wide host range, thus causing infection in higher vertebrates such as birds, reptiles and mammals including humans (Putanae *et al.*, 2001).

In many instances, *E. tarda* is part of the normal intestinal microflora of aquatic animals. It is a ubiquitous organism, having been isolated from animals or the environment of most continents (Woo and Bruno, 1999). It is a normal inhabitant of the intestine of snakes and is occasionally found in human faeces (Post, 1987). It has also been isolated from domestic animals, rates, birds, frogs, turtles and healthy fish (Dixon *et al.*, 1990; Novotny *et al.*, 2004).

Pathogenicity of *E. tarda* is multifactorial, and many potential virulent factors have been suggested, namely dermatotoxins, antiphagocyte killing, hemolysins, serum resistance and the ability to invade epithelial cells. Although both virulent and avirulent strains were able to invade cultured cells in vitro, only the virulent strain could enter fish in large numbers via mucus, gills and the gastrointestinal tract, and multiplies inside various internal organs, causing death (Putanae *et al.*, 2001).

In fish, *E. tarda* causes septicemia with extensive skin lesions, affecting internal organs such as the liver, kidney, spleen and muscle. These bacteria systematically avoid host defense mechanisms, thereby rapidly proliferating within the host and causing death (Putanae *et al.*, 2001). *Edwardsiella* septicemia, caused by *E. tarda*, is a mild to severe condition, but clinical signs of infection differ slightly between species of fish. The source of *E. tarda* is presumably the intestinal contents of carrier animals, but it may be a common inhabitant of the aquatic environment. In the U.S.A., *E. tarda* was isolated from 75% of water samples, from as high as 88% of fillets of dressed cultured catfish and from 30% of imported fish fillets (Woo and Bruno, 1999).

In humans the bacterium usually causes diarrhea and gastroenteritis, while extraintestinal infections may produce a typhoid-like illness, peritonitis with sepsis, and cellulitis. Clinical conditions like meningitis have also been associated with *E. tarda* infections. There have been significant isolation rates of *E. tarda* from human patients with diarrhea in Mali, where fish are the principal source of dietary protein (WHO, 1998). Risk factors that are associated with *E. tarda* infections include in addition to contact with exotic animals (reptiles or amphibians) or preexisting liver diseases, exposure to aquatic environments and dietary habits (raw fish ingestion) (Novotny *et al.*, 2004).

## *Vibrio* Species

*Vibrio* species can be present in both fresh water and sea water as well as in the alimentary tracts of animals and man. At least five *Vibrio* species are human pathogens including *V. cholerae* and *V. parahaemolyticus*, which cause food poisoning. *V. anguillarum* causes infections in many species of fish especially in salt or brackish water (Noga, 1996; WHO, 1998; Quinn *et al.*, 1999). *V. vulnificus* biotype 1 is an important human pathogen (EC, 2003), whereas the predominant biotype isolated from an eel is *V. vulnificus* biotype 2 (Woo and Bruno, 1999). The risk factors for severe *V. vulnificus* infections are chronic hepatitis or immunodeficiency in patients (Novotny *et al.*, 2004).

*Vibrio* infections are most commonly associated with the consumption of raw and/or improperly cooked fish and shellfish (WHO, 1998). Two outbreaks of *Vibrio cholera* and 11 *V. parahaemolyticus* were reported in the U.S.A. in the years between 1970 and 1978 that were associated with fish and shellfish (Gilbut, 1991) Two clinical syndromes have been described in humans. The first is a primary septicemia, most often associated with consumption. The second clinical syndrome produced is one of wound infections and is characterized by cellulitis, edema, hemorrhage and extensive tissue necrosis (Stoskopf, 1993).

## *Plesiomonas shigelloides*

*Plesiomonas shigelloides* is a motile, oxidase positive, facultatively anaerobic and Gram-negative rod, which is presently classified in the family Enterobacteriaceae (Dulger, 2004). This bacterium is primarily an aquatic organism that occurs in fresh and estuarine waters, and sea waters, especially in tropical and subtropical climates although it may also be found in fresh water environment in temperate and cold climates (Dulger, 2004). The organism has also been isolated from the intestinal tract of tropical fresh water fish (Novotny *et al.*, 2004).

*Plesiomonas shigelloides* has been isolated from wild caught and cultured fish and shellfish and can result in food poisoning in humans when uncooked fish or shellfish are consumed. *P. shigelloides* gastroenteritis is usually a self-limiting disease with fever, chills, abdominal pain, nausea, diarrhea, or vomiting. Most human *P. shigelloides* infections are suspected to be water borne and frequently associated with water contamination by sewage wastes (WHO, 1998).

In humans *P. shigelloides* is recognized as a rare cause of extraintestinal diseases and which have received increasing attention as an agent of gastrointestinal illness ranging from mild to severe, especially in children, aged and immunocompromised individuals, also being related to travel-associated diarrhea. Most of the gastroenteritis cases and outbreaks are associated with consumption of contaminated water, raw or undercooked fish, crustaceans and shellfish (Dulger, 2004). Two epidemics of water borne diarrhoeal disease involving 1000 persons in which no enteropathogens other than *P. shigelloides* were isolated and reported from Japan. Moreover, 81% isolation rate of *P. shigelloides* were reported in bacteriological examinations of stools of ill attendees just after an acute food borne outbreak in Younde, Cameroon, following consumption of cold fish and hard boiled eggs in a private party (Wouafo *et al.*, 2006). This organism was also isolated from several indigenous animals including fish and shellfish (Khardori and Fainstein, 1998).

### *Escherichia coli*

*Escherichia coli* organisms are classic examples of enteric bacteria causing gastroenteritis. The primary risk of infection with *E. coli* appears to be through ingestion of improperly prepared and contaminated fish food products (Stoskopf, 1993). The contamination of food of fish origin with pathogenic *E. coli* probably occurs during handling of fish and the production process (Novotny *et al.*, 2004). *E. coli* isolated from fish have been shown to be capable of producing various virulence factors associated with human pathogenicity. *E. coli* is generally nonpathogenic in the host fish. The presence of these bacteria have led to recommendations for strict hygienic practices during the processing and handling of aquaculture products to prevent the transfer of pathogenic bacteria to humans (WHO, 1998).

Coliform organisms were detected at nearly 43% of skin or gill samples and 100% of intestine and fish flesh samples in a study made on *Tilapia nilotica* caught from Nasser's Lake in Aswan (Zanfaly and Ibrahim, 1982). In Ethiopia *E. coli* was isolated and reported from faecal samples of *Oreochromis niloticus* (Eshetu, 2000) at Lake Zeway and from fillets and liver swab samples taken from fish at Lake Tana (Gebeyehu, 2003).

### *Salmonella* spp

Among the non-saprophytes, the salmonellae have been most studied among the zoonotic agents of poikilotherms and it was isolated from the intestine of fish caught in contaminated waters (Schwabe, 1984). *Salmonella*, because of its prevalence in nearly all-agricultural environments, is an important aquatic zoonotic pathogen. Non-typhoidal *Salmonella* has been isolated from a variety of fish and shellfish (WHO, 1998).

Fish and shellfish appear to be passive carriers and excreter of *Salmonella* species without clinical disease. The contamination of this organism derives from terrestrial sources and fish may serve as a vector for *Salmonella* spp. (Novotny *et al.*, 2004). Risk of human infection is due to ingestion of improperly prepared contaminated fish (Stoskopf, 1993). In Ethiopia, *Salmonella Newport* and *S. Zanzibar* were isolated from meat of Tilapia fish from supermarkets in Addis Ababa (Endrias, 2004).

### *Yersinia* spp

Most *Yersinia* infections are not associated with a seafood vector. However, strains of *Y. enterocolitica* have been identified in fish and shellfish in both wild and aquaculture settings. Additionally *Yersinia* is a psychrotrophic bacterium, which can multiply at low temperatures. This may increase the potential for cold-stored or frozen seafood to become a vector for human illness (Lipp and Rose, 1997).

*Yersinia ruckeri* is the agent of enteric red-mouth disease in salmonids and is considered to be highly pathogenic to infect fish and cases of human infection have also been reported (Stoskopf, 1993).

*Yersinia enterocolitica* was isolated from fillets of *O. niloticus* and *C. gariepinus* fish at Lake Zeway (Eshetu, 2000) and Lake Tana (Gebeyehu, 2003), respectively.

## **2.5. Prevention and control of fish and fish borne diseases**

### **2.5.1. Prevention and control of fish diseases**

Prevention and control require a thorough knowledge of the environment of fish, the etiology of the disease and fish biology (Svobodova and Vykosova, 1991). Good management of fish farms is of primary importance in avoiding disease and the effective preventive measures are to be applied above all in specialized fish culture units (Coche and Edwards, 1988; Svobodova and Vykosova, 1991). Some of the prevention and control measures are listed as follows:

#### **Provision of pathogen-free water**

Underground water is the most suitable water source for free of pathogens (Coche and Edwards, 1988). Chemical treatment of inflow water and disinfections of the water entering fish culture units by UV radiation is considered as the simple method to destroy viruses, bacteria and moulds (Svobodova and Vykosova, 1991), and the water from each pond or reservoir should be drained separately and should not flow into any other (Coche and Edwards, 1988).

#### **Protection from transfer of pathogens**

The transfer of fish with unknown health condition is to be avoided in principle. All transported fish are to be accompanied by veterinary certificate (Svobodova and Vykosova, 1991). Transmissible diseases with no known cure may be controlled by restricting the movement of fish products (Post, 1987).

#### **Control of wild fish**

Wild fish living in the canals and ponds of fish farms are hosts and vectors for pathogens. Placing wire-mesh screens over the water inflows to ponds can hinder their access. If necessary, chemicals can be applied to eradicate them (Coche and Edwards, 1988).

### Stocking density

Avoid over crowding of fish at any time, and particularly during hot weather (Coche and Edwards 1988). High stocking results in stress behavior worsen condition and resistance and make the expansion of the disease easier (Svobodova and Vykosova, 1991).

### Optimalization of environmental conditions

Fish-pond and hatchery water must be provided in sufficient quantity, at a suitable temperature free of pollutants, and rich in oxygen (Coche and Edwards, 1988). Maximum development of natural food resources by the adequate interventions, feeding fish by supplementary feed mixtures in sufficient amount and quality (Svobodova and Vykosova, 1991).

### Disinfection of ponds, fish culture units and equipment

Chemical disinfections is an effective way of prevention from and/or suppressing of fish diseases usually accessible disinfective preparation are used in fish culture like chlorinated lime, potassium permanganate etc. (Svobodova and Vykosova, 1991).

### Regular control of health condition

Health condition of fish is always to be controlled before fishing out, transporting and stocking of fish. Based on the investigational results preventive treatments can be suggested and treatment is performed into the water environment and feeding by medicated feeds (Svobodova and Vykosova, 1991).

### Fish vaccination and therapy

Bacterial infections caused by Gram-negative bacteria such as *Vibrio* and *Yersinia* spp has been effectively controlled by vaccination (Gudding and Lillehug, 1999). The vaccines being developed range from killed whole cell preparations to recombinant and DNA vaccines (Adams, 1999). Significantly higher antigen-specific antibodies were detected in serum,

intestinal mucosa and bile than non-immunized control group after immunization of Common carp (*Cyprinus carpio*) orally with liposome of *A. salmonicida* antigen (Irie *et al.*, 2005).

Drugs used for systemic therapy, disease prophylaxis and disinfection procedures have been of limited use for diseases of fish (Coche and Edwards, 1988). However, therapeutic treatment should be regarded as emergency measure resorted to when prevention has failed (Svobodova and Vykosova, 1991).

Only those compounds, which have been tested and licensed for fish, can conveniently be considered according to their method of application. In bacterial diseases, furan derivatives (furazolidone, nitrofurazone), sulfonamides sometimes in combination with trimethoprim, or antibiotics (oxytetracycline, chloramphenicol, neomycin), are applied with feed (Coche and Edwards, 1998).

#### 2. 5.2. Prevention and control of fish borne diseases in human

There are a number of management schemes aquaculturists use to reduce the incidence of pathogenic microorganisms, including sanitation, isolated rearing systems, limited access to facilities, and pest control. HACCP programs may be combined with quality assurance (QA) programs at the farm level to identify and mitigate pre-harvest food safety problems (WHO, 1998).

### 3. MATERIALS AND METHODS

#### 3.1. Description of the study area (Lake Tana)

##### Topography

Lake Tana is situated in the northwestern highlands of Ethiopia in the administrative regions of Gojjam and Gondar bordered by low plains in the north (Dembecha), east (Fogera), and southwest (Kunzila) (Nagelkerke, 1997).

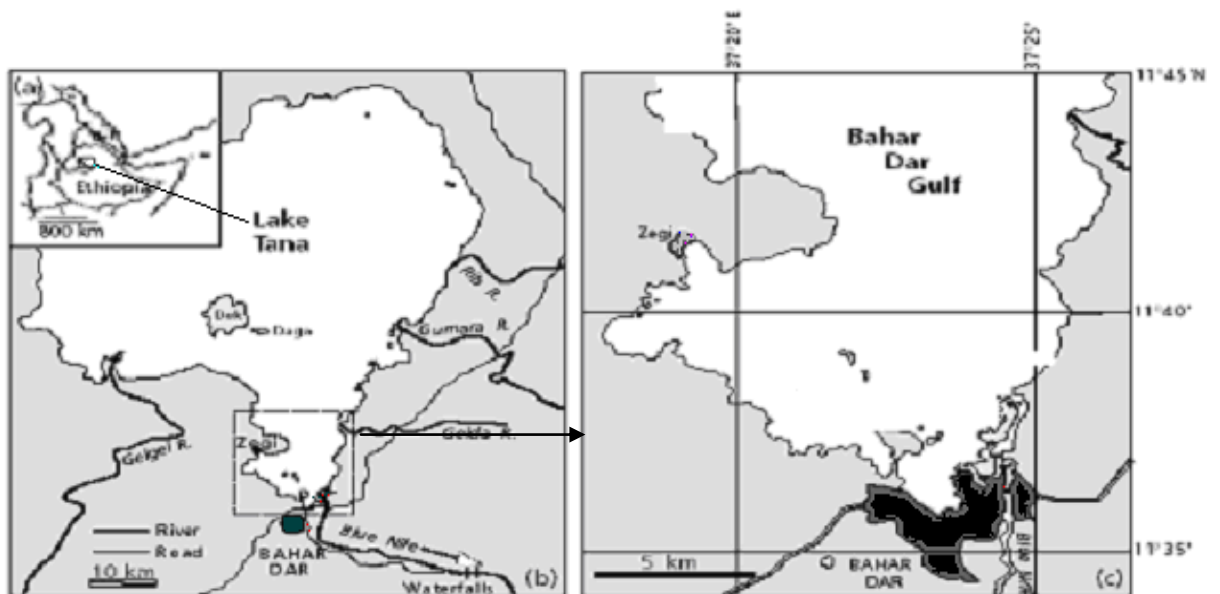


Figure 1 Map of the study area

Lake Tana is an oligo-mesotrophic shallow lake and is well mixed. It is located at 11:4' N and 37:2' E at an altitude of 1830 m and is the source of Blue Nile River (Dejen, 2003).

Four large permanent rivers (Derma, Reb, Gumara and Gelgel Abay) as well as many short seasonal streams tribute to the lake (Nagelkerke, 1997). Lake Tana, which accounts for half of the total freshwater area of the country (Dejen, 2003) covers an area of approximately 3150 km<sup>2</sup>, a maximum length and width of 78 and 68 km respectively, and shallow with an average depth of 8m and a maximum depth of 14 m. Its bottom consists of volcanic basalts, usually covered with a muddy substratum with only little organic matter. At the southern most tip, in the city of Bahir Dar ('sea shore'), the Abay river flows out of the lake, only to drop over

40 m high water falls, 35 km from its origin, at “Tissisat” (the smoking water). This isolates Lake Tana and its tributaries from the rest of the Blue Nile basin and other freshwater systems (Nagelkerke, 1997).

## Climate

The climate of Lake Tana is characterized by a major rainy season with heavy rains, during June to October, and some times a minor rainy season during February to March. The average annual rainfall in the lake area over 1997-2000 was 1418 mm. The water level of the lake fluctuates with rainfall up to 1 m. Maximum water temperature as a monthly average, ranges between 21 and 26°C. Water temperatures vary between narrow limits, with lowest values in January, a sharp increase in February, peak values in May and sharp decline with the big rains in June to July (Dejen, 2003).

## Water

The lake has low chlorophyll A concentration of 3.5 mg m<sup>-3</sup> and low mean biomass (129 mg carbon m<sup>-2</sup>) (Wudneh, 1998). Temperature ranges from 18.3 to 26.2°C (average 22.3°C), conductivity ranges from 136 to 234 µs cm<sup>-1</sup>. The water of the lake is well mixed because of shallow saucer shape of the lake, and fairly strong winds that usually start after sunset (Nagelkerke, 1997).

## Flora and Fauna

Diatoms of the genus *Melosira* dominate the phytoplankton. The eastern and southern shores of Lake Tana are covered with swamps, dominated by papyrus (*Cyperus papyrus*, Cyperaceae). *Typha latifolia* and water lilies are also abundant. Two species of trees often grow close to the shore: *Ficus sycomorus* (Moraceae), the fig tree, or “Warka” and *Millettia ferruginea* (Leguminosae), the barberra (Nagelkerke, 1997).

Most aquatic invertebrates are rotifers, molluscuse, crustaceans and insects (especially their larva). Gastropods are usually found near the littoral vegetation and include the endemic subspecies *Bellamya unicolor abbyssinica* (viviparidae), *Lymnaea natalensis* (Lymnaeidae),

*Anisus natalensis* (Planorbidea), and *Bulinus* spp (Planorbidae), host to *Schistosoma haematobium*, the cause of bilharziasis (Nagelkerke, 1997).

Midges, mosquitoes, mayflies, stoneflies, caddis flies and dragonflies are the most abundant insect larvae. Chadocerans and copepods are the most abundant crustaceans in Lake Tana and are the largest group of pelagic invertebrates (biomass) (Nagelkerke, 1997). Amphibians, especially anurans are present in the lake (marshy-shore areas). The largest Lake Tana reptiles are the Nile monitor and *Python* species. The most diverse vertebrate group is that of aquatic birds (Nagelkerke, 1997).

## Fish

There is one cichlid, *Oreochromis niloticus* (Nile tilapia), which is the most widespread tilapia species in Africa. The Catfish family (Clariidae) is also presented by one species, *Clarias gariepinus* (African catfish), which is the most common member of its genus. The largest fish family in the lake is the Cyprinidae, represented by three genera: *Barbus*, *Garra* and *Varicorhinus*. *Garra* are widespread in the rivers and lakes of the Ethiopian highlands. *Varicorhinus beso* is a very distinct large cyprinid and like *Garra* species, this species is common and generally distributed in the rivers and lakes of the Ethiopian highlands (Dejen, 2003). Recently 15 large barbus morphotypes have been described as species composing a world unique species flock of endemic cyprinid fish (Nagelkerke, 1997). In addition to the above large barbs (up to 85 cm) also three small barbs (< 10 cm) have been reported for Lake Tana: *Barbus tanapelagius*, *Barbus humilis*, and *Barbus pleuogramma*. *Barbus tanapelagius* is endemic in Lake Tana but *Barbus humilis* is a riverian species and more widely distributed in the inflowing rivers of Lake Tana and other parts of Ethiopia (Dejen *et al.*, 2006).

### **3.2. Study population**

The study population consists of two groups. The first group composed of different fish species, which were sampled from the southern gulf of Lake Tana and that were belonging to the family Cyprinidae, Cichlidae and Clariidae. The other group was filleted fish that were sampled from commercial enterprises and local dealers in Bahir Dar and, which composed of commercially important fish species (Large Barbs, *O. niloticus*, *C. gariepinus* and *V. beso*).

### 3.3. Study design

#### 3.3.1. Study type and sample size determination

The study was a cross-sectional and was conducted from September 2006 to March 2007. One sampling day of the week was randomly selected. When the number of fish caught and/or fish filleted per sampling day was more than 20, a simple random sampling technique was employed to select live fish samples, whereas a systemic random sampling was used for filleted fish. However, when the number of fish caught and/or fish filleted per sampling day was less than 20, all were taken and examined accordingly. The sample size was determined according to Thrusfield (2005) as follows:

$$n = \frac{1.96^2 * P_{exp} (1-P_{exp})}{d^2}$$

Where:      n      = required sample size  
                 P<sub>exp</sub> = expected prevalence  
                 d      = desired absolute precision

Therefore, a total of 505 samples were taken for both strata (255 live fish and 250 filleted fish) at 20% expected prevalence (with maximum sample size), 95% confidence interval and 5% desired absolute precision.

#### 3.3.2. Study methodology

##### Data collection

Fish were sampled from southern gulf of Lake Tana of three fish habitats, which were selected with regard to their relative distances from point source pollutions as well as degree of exposure to pollution. The sampling sites are characterized as inshore shallow littoral zone (3 replicates of Gerima station, 3.07 m average depth and which is in the vicinity of point source pollutions), sub-littoral zone (Bahita, Debre-mariam and Airport stations, 5.5 m average depth and about 4.8 km average distance from point source pollutions) and pelagic deep water (Ambobahir, Kibran and Zege stations, about 8.44 m average depth and their average distance from point source pollutions is about 19.3 km).

Samples were obtained using multi-mesh monofilament survey gillnet that is composed of five different randomly distributed mesh size-panels, ranging from 60-120 mm with 50 m total length and 2 m width. Species type, sex and maturity stages were registered (Annex 3) and identified at Fisheries and Other Aquatic Life Research Center (Bahir Dar) using methodologies applied by Nagelkerke (1997) and Nikolsky (1963).

Carcasses of filleted fish from commercial enterprises and local dealers as well as water samples were also collected aseptically for bacteriological analysis. Water samples were collected from Lake Tana of the fish sampling sites about 15 cm down the water surface in 100 ml of sterilized bottle.

The study also examined the physicochemical make up of the water in sampling sites. Water quality measurements were taken simultaneously with fish and water sampling. The core water quality parameters include water temperature and dissolved oxygen levels, pH, conductivity and salinity, turbidity and nitrite concentration that were measured using OxyGuard, pH 315: WTW 82362 Weilheim model, Co 150 model, secchi disc and DREL/2010 laboratory model 26700-01 using cadmium reduction methods, respectively.

#### Clinical and post-mortem examinations

Fish sampled at Lake Tana were thoroughly examined for visible signs of infection and lesion on the external body surface and gills. Following examination of the external body surface and gills, the surfaces of the incision were disinfected with 70% alcohol. The peritoneal cavity was opened aseptically and with care not to puncture any part of the intestinal tract. The internal organs were examined for gross pathological changes. In the absence of any visible lesions swabs from the kidney and intestine were taken for bacteriological examinations just after searing the surface of the organs with a hot scalpel blade to avoid surface contaminants (Carter, 1984). The post-mortem techniques were done according to Noga (1996).

Fish were also inspected for the state of gonad maturity and sex, which was assigned by viewing the gonads. Ovaries were staged in I-V maturity scales according to Nikolsky (1963). Stage I-II were taken as immature stages, stage III-V were considered as mature (Dejen *et al.*, 2006).

## Sample collection

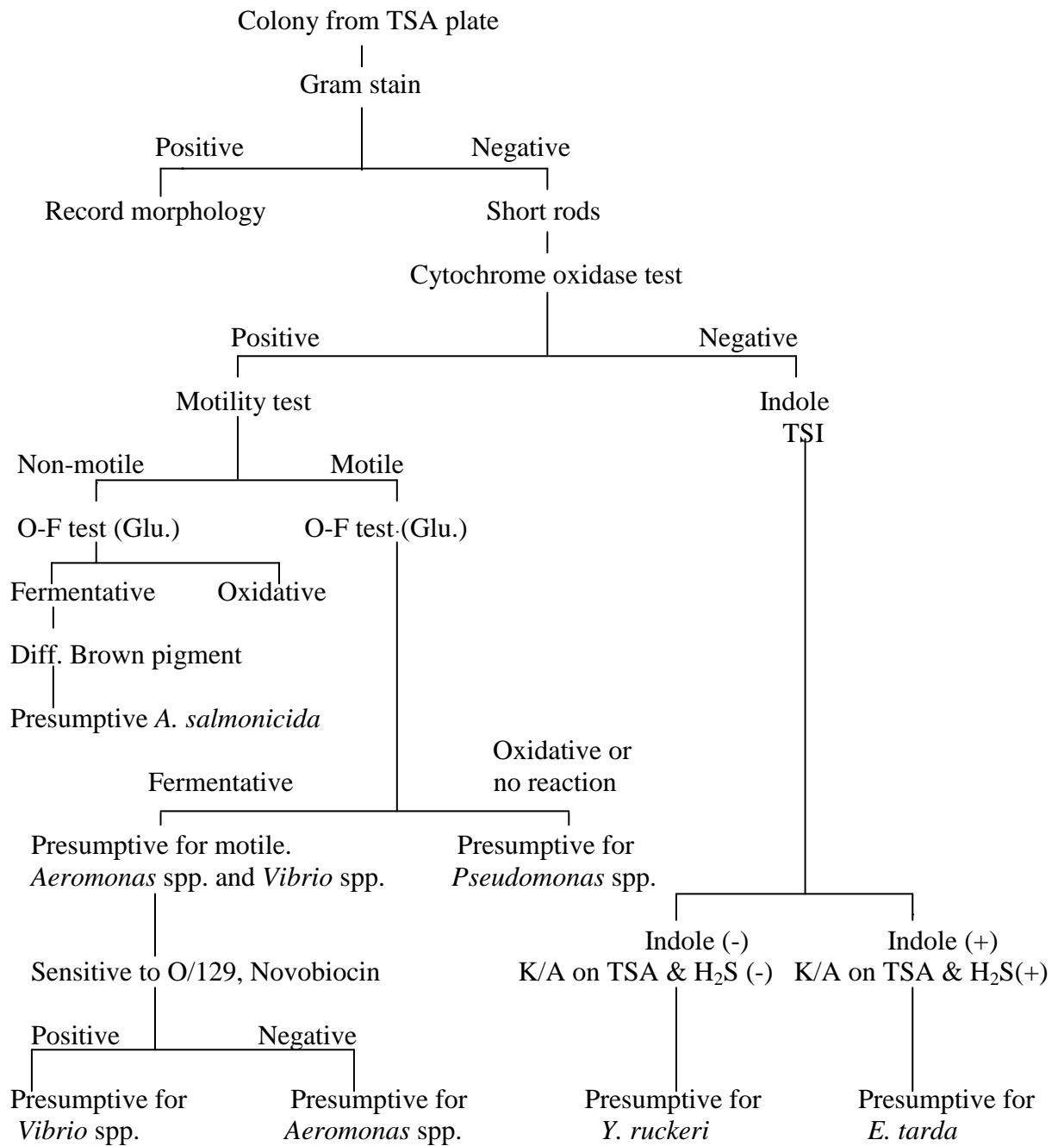
During the study period, swab samples from the intestine (n = 255), kidney (n = 255) and fillets of fish (n = 250) were taken aseptically with sterile cotton swabs moisten with phosphate buffer saline (PBS) and placed in sterile screw-caped tubes, and then transported to Bahir-Dar Regional Veterinary Laboratory (BRVL) for isolation and identification of the bacteria. Moreover, a total of 62 water samples collected simultaneously with fish sampling during the study period (on average 3 samples during each sampling day) from three different sampling sites (littoral, sub-littoral and pelagic). All samples were collected using sterile amber color bottles of 100 ml capacities. Bacteriological analysis was made at Bahir-Dar Regional Veterinary Laboratory (BRVL), whereas measurements for nitrite concentration and water salinity were done at Amhara National Regional State Water Resource Development Bureau (ANRSWRDB).

## Isolation and identification of bacteria

The swab samples were streaked directly on Tryptic Soya Agar (TSA) plates by quadrant streaking method and incubated aerobically at 28<sup>0</sup>C for 24 to 48 hours (Woo and Bruno, 1999). After 24 to 48 hours of incubation, the plates were observed for growth of bacterial colonies. Representative colonies of different morphological types present in each plate were subjected to Gram stain reactions for the differentiation of Gram-negative from Gram-positive bacteria. Only colonies of Gram-negative bacteria were isolated and re-streaked on new TSA plates. Pure cultures of isolated bacteria were subjected to standard morphological and biochemical tests for identification (Frerichs, 1984; Rose and Okrend, 1998; Quinn *et al.*, 1999; Woo and Bruno, 1999 and Quinn *et al.*, 2002). All the tests were done according to the flow chart outlined by Fisheries and Oceans Canada (2004) (Figure 2), and was supplemented with IMViC and different sugar tests (Annex 2 and 3) for further identification. Moreover, *A. hydrophila* and other species and subspecies of the genus *Aeromonas* and *Plesiomonas* were characterized according to Khardori and Fainstein, 1988; Varnam and Evans, 1991 and Woo and Bruno, 1999 based up on the different reactions shown in Table 2. Each single colony of interest was subcultured on BHI agar plate and incubated for 24-48 hours at 28<sup>0</sup>C and transferred on to slant agar. These slants were preserved at refrigerator temperature for further use.

## Data management and analysis

Microsoft excel was employed for raw data entry and graphical presentation of the data. Intercooled Stata 7.0 was used for descriptive statistics. Chi-square test were used to determine the significance differences or variations of bacteria isolated among the different parameters considered in live fish and water samples collected at Lake Tana using SPSS 11.5. A statistically significant association between variables was determined at P-value  $<0.05$ , i. e. a 5% significant level.



(TSA= Tryptic Soya Agar; K/A = Alkaline/Acid; H<sub>2</sub>S = Hydrogen sulfide; + = Positive reaction; - = Negative reaction).

Source: Fisheries and Oceans Canada, 2004

Figure 2. Flow chart for targeted Gram-negative fish pathogens

Table 2. Differentiation between *A. hydrophila*, *A. caviae*, *A. sobria*, *A. salmonicida*, and *P. shigelloides*.

Characteristics	<i>A. hydrophila</i>	<i>A. caviae</i>	<i>A. sobria</i>	<i>A. salmonicida</i> subsp			<i>P. shigelloides</i>
				<i>salmonicida</i>	<i>achromogenes</i>	<i>masoucida</i>	
Motility	+	+	+	-	-	-	+
Brown diffusible pigment	-	-	-	+	-	-	-
Gas from glucose	+	-	+	+	-	+	-
Arabinose utilisation	+	+	-	+	-	+	-
Break down of inositol	-	-	-	-	-	-	+

Source: Khardori and Fainstein, 1988; Varnam and Evans, 1991; Woo and Bruno, 1999.

## 4. RESULTS

### 4.1. Study population

The study was conducted between September 2006 and March 2007 in southern gulf of Lake Tana, Bahir Dar, Ethiopia. A total of 510 swab samples were collected equally from the kidney and intestine of 255 apparently health fish. The species of fish, sex, maturation stages and different fish sampling habitats, are shown in Table 3.

Additional 250 swab samples from carcasses of filleted fish that were sampled from commercial enterprises and local dealers, and 62 water samples that were sampled simultaneously with live fish at Lake Tana were collected and cultured for bacterial isolation and identification.

Among the fish samples caught for bacteriological examination, the majority of the fish were *Barbs* species (40.4%) followed by *O. niloticus* (19.6%), *C. gariepinus* (15.3%), *V. beso* (12.9%) and *Garra* species (11.8%) (Table 3).

Table 3. Fish species sampled at Lake Tana based on different parameters

Fish species	No.	Sex		Maturity		Sampling habitats		
		M	F	Immature	Mature	Littoral	Sub-littoral	Pelagic
Barbs spp	103	29	74	66	37	55	30	18
<i>C. gariepinus</i>	39	13	26	33	6	27	0	12
<i>Garra</i> spp	30	11	19	10	20	10	8	12
<i>O. niloticus</i>	50	16	34	31	19	44	3	3
<i>V. beso</i>	33	7	26	26	7	18	9	6
Total	255	76	179	166	89	154	50	51

#### **4.2. Clinical and post-mortem findings**

During the clinical and post-mortem examinations, none of the fish were found to be showing typical signs and lesions of a disease except that some were having bruising and petechial hemorrhage on the external ventral body surface, around their mouth and operculum.

#### **4.3. Isolated bacteria**

Different bacterial species were isolated from the kidney and intestine of live fish, in the carcasses of filleted fish and water samples (Table 4).

Table 4. Occurrence and distribution of bacterial species isolated and identified from different sample types.

Bacteria spp	Live fish		Filletted fish	Water samples
	Kidney	Intestine		
<i>Aeromonas (A.) hydrophila</i>	+	+	+	+
<i>Aeromonas (A.) caviae</i>	+	+	+	+
<i>Aeromonas (A.) sobria</i>	+	+	+	+
<i>Citrobacter (C.) diversus</i>	-	+	+	-
<i>Citrobacter (C.) freundii</i>	-	+	-	-
<i>Enterobacter(E.) aerogenes</i>	-	+	-	+
<i>Escherichia (E.) coli</i>	-	+	+	+
<i>Edwardsiella (E.) tarda</i>	+	+	-	-
<i>Klebsiella (K.) pneumoniae</i>	-	+	-	-
<i>Proteus (P.) mirabilis</i>	-	+	+	+
<i>Proteus (P.) vulgaris</i>	-	+	-	-
<i>Plesiomonas (P.) shigelloides</i>	-	+	+	+
<i>Vibrio spp</i>	+	+	+	-
<i>Yersinia (Y.) enterocolitica</i>	-	+	+	+
<i>Yersinia (Y.) ruckeri</i>	+	+	-	-
<i>Aeromonas (A.) salmonicida</i> (atypical)	+	+	+	+
<i>Edwardsiella (E.) ictaluri</i>	+	+	-	-
<i>Shigella (S.) sonnei</i>	-	+	-	-

+ = isolated; - = non-isolated

#### 4.3.1. Bacteria isolated from the kidney and intestine of fish

Table 5 shows the distribution and frequency of occurrence of bacteria from the kidney and intestine of fish. The most frequently isolated bacteria were *A. sobria* (3.1%) in the kidney and (12%) in the intestine. Except for *C. diversus*, *C. freundii*, *E. tarda*, *P. vulgaris*, *Y. ruckeri*, *E. ictaluri* and *S. sonnei*, the other bacteria isolates were found to be having statistically significant differences ( $P < 0.05$ ) between intestine and kidney.

Table 5. Distribution and frequency of occurrence of bacteria from the kidney and intestine of fish (n = 255)

Isolated bacteria	Kidney	Intestine	Total	P-Value
<i>A. hydrophila</i>	2 (0.8%)	16 (6.3%)	18 (7.1%)	0.000*
<i>A. caviae</i>	4 (1.6%)	21 (8.2%)	25 (9.8%)	0.000*
<i>A. sobria</i>	8 (3.1%)	31 (12%)	39 (15%)	0.001*
<i>C. diversus</i>	-	1 (0.4%)	1 (0.4%)	0.502
<i>C. freundii</i>	-	1 (0.4%)	1 (0.4%)	1.000
<i>E. aerogenes</i>	-	13 (5.1%)	13 (5.1%)	0.000*
<i>E. coli</i>	-	6 (2.4%)	6 (2.4%)	0.015*
<i>E. tarda</i>	1 (0.4%)	4 (1.6%)	5 (1.9%)	0.372
<i>K. pneumoniae</i>	-	6 (2.4%)	6 (2.4%)	0.015*
<i>P. mirabilis</i>	-	15 (5.9%)	15 (5.9%)	0.000*
<i>P. vulgaris</i>	-	4 (1.6%)	4 (1.6%)	0.061
<i>P. shigelloides</i>	-	11 (4.3%)	11 (4.3%)	0.000*
<i>Vibrio</i> spp	7 (2.7%)	13 (5.1%)	20 (7.8%)	0.010*
<i>Y. enterocolitica</i>	-	8 (3.1%)	8 (3.1%)	0.007*
<i>Y. ruckeri</i>	3 (1.2%)	5 (2%)	8 (3.1%)	0.221
<i>A. salmonicida</i> (atypical)	5 (1.2%)	27 (11%)	32 (13%)	0.000*
<i>E. ictaluri</i>	1 (0.4%)	2 (0.8%)	3 (1.2%)	0.623
<i>S. sonnei</i>	-	2 (0.8%)	2 (0.8%)	0.502
Total	31 (12%)	186 (73%)	217 (85%)	

\* means significant at 5% level.

#### 4.3.2 Bacteria isolated among different fish species

Even though differences in frequency and percentage of each particular bacteria species were found among different fish species, stastically significant differences ( $P < 0.05$ ) were recorded only in *A. hydrophila*, *A. caviae*, *A. sobria*, *E. aerogenes*, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris* and *S. sonnei* (Table 6). The percentage and distribution of the bacteria isolates among the different fish species were found to be more pronounced in Aeromonadaceae and Vibrionaceae than each particular species of the Enterobacteriaceae (Table 6).

Table 6. Bacteria isolated in different fish species

Isolated bacteria	<i>Barbs</i> spp n = 103	<i>C. gariepinus</i> n = 39	<i>Garra</i> spp n = 30	<i>O. niloticus</i> n = 50	<i>V. beso</i> n = 33	Total n = 255	P-Value
<i>A. hydrophila</i>	3 (2.9%)	12 (31%)	1 (3.3%)	1 (2%)	1 (3%)	18 (7.1%)	0.000*
<i>A. caviae</i>	10 (9.7%)	8 (21%)	-	6 (12%)	-	24 (9.4%)	0.004*
<i>A. sobria</i>	12 (12%)	12 (31%)	4 (13%)	6 (12%)	5 (15%)	39 (15%)	0.000*
<i>C. diversus</i>	1 (1%)	-	-	-	-	1 (0.4%)	0.830
<i>C. freundii</i>	1 (1%)	-	-	-	-	1 (0.4%)	0.830
<i>E. aerogenes</i>	7 (6.8%)	-	2 (6.7%)	-	4 (12%)	13 (5.1%)	0.001*
<i>E. coli</i>	2 (1.9%)	-	2 (6.7%)	2 (4%)	-	6 (2.4%)	0.305
<i>E. tarda</i>	4 (3.9%)	-	-	1 (2%)	-	5 (2%)	0.403
<i>K. pneumoniae</i>	-	-	-	1 (2%)	5 (15%)	6 (2.4%)	0.000*
<i>P. mirabilis</i>	4 (3.9%)	-	-	3 (6%)	8 (24%)	15 (5.9%)	0.000*
<i>P. vulgaris</i>	-	-	2 (6.7%)	2 (4%)	-	4 (1.6%)	0.045*
<i>P. shigelloides</i>	8 (7.8%)	-	-	3 (6%)	-	11 (4.3%)	0.095
<i>Vibrio</i> spp	6 (5.8%)	2 (5.1%)	4 (13%)	5 (10%)	3 (9%)	20 (7.8%)	0.395
<i>Y. enterocolitica</i>	3 (2.9%)	-	1 (3.3%)	2 (4%)	2 (6.1%)	8 (3.1%)	0.183
<i>Y. ruckeri</i>	3 (2.9%)	-	2 (6.7%)	1 (2%)	2 (6.1%)	8 (3.1%)	0.455
<i>A. salmonicida</i> (atypical)	16 (16%)	5 (13%)	4 (13%)	4 (8%)	3 (9.1%)	32 (13%)	0.515
<i>E. ictaluri</i>	2 (1.9%)	-	1 (3.3%)	-	-	3 (1.2%)	0.530
<i>S. sonnei</i>	-	-	2 (6.7%)	-	-	2 (0.8%)	0.004*
Total	82 (80%)	39 (100%)	25 (83%)	37 (74%)	33 (100%)	216 (85%)	

\* means significant at 5% level.

#### 4.3.3. Bacteria isolated from the fish based on different sampling sites

*Aeromonas sobria* were the most frequently isolated bacteria in fish sampled in both sub-littoral and pelagic zones (25% and 20 %, respectively), whereas bacteria species such as *A. caviae* and *E. coli* that are indicators of pollution were isolated in greater percentage in fish sampled in inshore littoral zone than the other sampling sites which are relatively far from point source pollutions. The percentage of *A. hydrophila*, *A. sobria*, *P. mirabilis* and *Y. enterocolitica* varied significantly ( $P < 0.05$ ) among the fish sampling sites (Table 7).

Table 7. Bacteria isolated from the fish based on sampling sites

Isolated bacteria	Littoral n = 154	Sub-littoral n = 51	Pelagic n = 50	Total n = 255	P-Value
<i>A. hydrophila</i>	10 (6.5%)	8 (16%)	-	18 (7.1%)	0.002*
<i>A. caviae</i>	17 (11%)	5 (10%)	2 (4%)	24 (9.4%)	0.129
<i>A. sobria</i>	16 (10%)	13 (25%)	10 (20%)	39 (15%)	0.020*
<i>C. diversus</i>	1 (0.6%)	-	-	1 (0.4%)	0.719
<i>C. freundii</i>	1 (0.6%)	-	-	1 (0.4%)	0.134
<i>E. aerogenes</i>	4 (2.6%)	5 (10%)	4 (8%)	13 (5.1%)	0.074
<i>E. coli</i>	5 (3.2%)	1 (2%)	-	6 (2.4%)	0.130
<i>E. tarda</i>	4 (2.6%)	1 (2%)	-	5 (2%)	0.516
<i>K. pneumoniae</i>	6 (3.9%)	-	-	6 (2.4%)	0.133
<i>P. mirabilis</i>	6 (3.9%)	1 (2%)	8 (16%)	15 (5.9%)	0.003*
<i>P. vulgaris</i>	4 (2.6%)	-	-	4 (1.6%)	0.264
<i>P. shigelloides</i>	8 (5.2%)	1 (2%)	2 (4%)	11 (4.3%)	0.827
<i>Vibrio</i> spp	11 (7.1%)	5 (10%)	4 (8%)	20 (7.8%)	0.193
<i>Y. enterocolitica</i>	3 (1.9%)	-	5 (10%)	8 (3.1%)	0.006*
<i>Y. ruckeri</i>	4 (2.6%)	2 (4%)	2 (4%)	8 (3.1%)	0.349
<i>A. salmonicida</i> (atypical)	21 (14%)	5 (10%)	6 (12%)	32 (13%)	0.824
<i>E. ictaluri</i>	3 (1.9%)	-	-	3 (1.2%)	0.370
<i>S. sonnei</i>	2 (1.3%)	-	-	2 (0.8%)	0.516
Total	126 (82%)	47 (92%)	43 (86%)	216 (85%)	

\* means significant at 5% level.

#### 4.3.4. Bacteria isolated in relation to maturation and sex of fish

The frequency and percentage of each particular isolates of bacteria species with regard to maturation and sex differentiation are shown in table 8 and 9. The overall percentage of isolated bacterial species was found to be higher in immature and female than matured and male fish, respectively. Eventhough, the frequency and percentage of each particular isolated bacteria species varied among maturation stages and sex groups, statistically significant

differences ( $P < 0.05$ ) were recorded only in *A. hydrophila* and *E. coli* between the two maturation stages and in *Vibrio* species in case of sex differentiations.

Table 8. Bacteria isolated based on different maturation stages of fish

Isolated bacteria	Immature (I-II) n = 166	Mature (III-V) n = 89	Total n = 255	P-Value
<i>A. hydrophila</i>	16 (9.6%)	2 (2.2%)	18 (7.1%)	0.020*
<i>A. caviae</i>	18 (11%)	6 (6.7%)	24 (9.4%)	0.201
<i>A. sobria</i>	27 (16%)	12 (13%)	39 (15%)	0.347
<i>C. diversus</i>	1 (0.6%)	-	1 (0.4%)	0.651
<i>C. freundii</i>	-	1 (1.1%)	1 (0.4%)	0.349
<i>E. aerogenes</i>	10 (6%)	3 (3.4%)	13 (5.1%)	0.275
<i>E. coli</i>	1 (0.6%)	5 (5.6%)	6 (2.4%)	0.021*
<i>E. tarda</i>	1 (0.6%)	4 (4.5%)	5 (2%)	0.052
<i>K. pneumoniae</i>	5 (3%)	1 (1.1%)	6 (2.4%)	0.318
<i>P. mirabilis</i>	11 (6.6%)	4 (4.5%)	15 (5.9%)	0.349
<i>P. vulgaris</i>	1 (0.6%)	3 (3.4%)	4 (1.6%)	0.124
<i>P. shigelloides</i>	6 (3.6%)	5 (5.6%)	11 (4.3%)	0.326
<i>Vibrio</i> spp	16 (9.6%)	4 (4.5%)	20 (7.8%)	0.110
<i>Y. enterocolitica</i>	6 (3.6%)	2 (2.2%)	8 (3.1%)	0.428
<i>Y. ruckeri</i>	5 (3%)	3 (3.4%)	8 (3.1%)	0.572
<i>A. salmonicida</i> (atypical)	22 (13%)	10 (11%)	32 (13%)	0.401
<i>E. ictaluri</i>	2 (1.2%)	1 (1.1%)	3 (1.2%)	0.720
<i>S. sonnei</i>	2 (1.2%)	-	2 (0.8%)	0.423
Total	150 (90%)	66 (74.2%)	216 (85%)	

\* means significant at 5% level.

Table 9. Bacteria isolated in different sex

Bacteria isolates	Males n = 76	Females n = 179	Total n = 255	P-Value
<i>A. hydrophila</i>	5 (6.6%)	13 (7.3%)	18 (7.1%)	0.541
<i>A. caviae</i>	8 (11%)	16 (8.9%)	24 (9.4%)	0.426
<i>A. sobria</i>	13 (17%)	26 (15%)	39 (15%)	0.364
<i>C. diversus</i>	-	1 (0.6%)	1 (0.4%)	0.702
<i>C. freundii</i>	-	1 (0.6%)	1 (0.4%)	0.702
<i>E. aerogenes</i>	3 (3.9%)	10 (6%)	13 (5.1%)	0.423
<i>E. coli</i>	-	6 (3.4%)	6 (2.4%)	0.117
<i>E. tarda</i>	3 (3.9%)	2 (1.1%)	5 (2%)	0.158
<i>K. pneumoniae</i>	2 (2.6%)	4 (2.2%)	6 (2.4%)	0.578
<i>P. mirabilis</i>	3 (3.9%)	12 (6.7%)	15 (5.9%)	0.296
<i>P. vulgaris</i>	1 (1.3%)	3 (1.7%)	4 (1.6%)	0.656
<i>P. shigelloides</i>	2 (2.6%)	9 (5%)	11 (4.3%)	0.313
<i>Vibrio</i> spp	2 (2.6%)	18 (10%)	20 (7.8%)	0.032*
<i>Y. enterocolitica</i>	1 (1.3%)	7 (3.9%)	8 (3.1%)	0.255
<i>Y. ruckeri</i>	2 (2.6%)	6 (3.4%)	8 (3.1%)	0.556
<i>A. salmonicida</i> (atypical)	11 (14%)	21 (12%)	32 (13%)	0.339
<i>E. ictaluri</i>	-	3 (1.7%)	3 (1.2%)	0.344
<i>S. sonnei</i>	-	2 (1.1%)	2 (0.8%)	0.492
Total	56 (74%)	160 (89%)	216 (85%)	

\* means significant at 5% level.

#### 4.3.5. Bacteria isolated from filleted fish

The bacterial species isolated from carcasses of filleted fish are presented in figure 3.

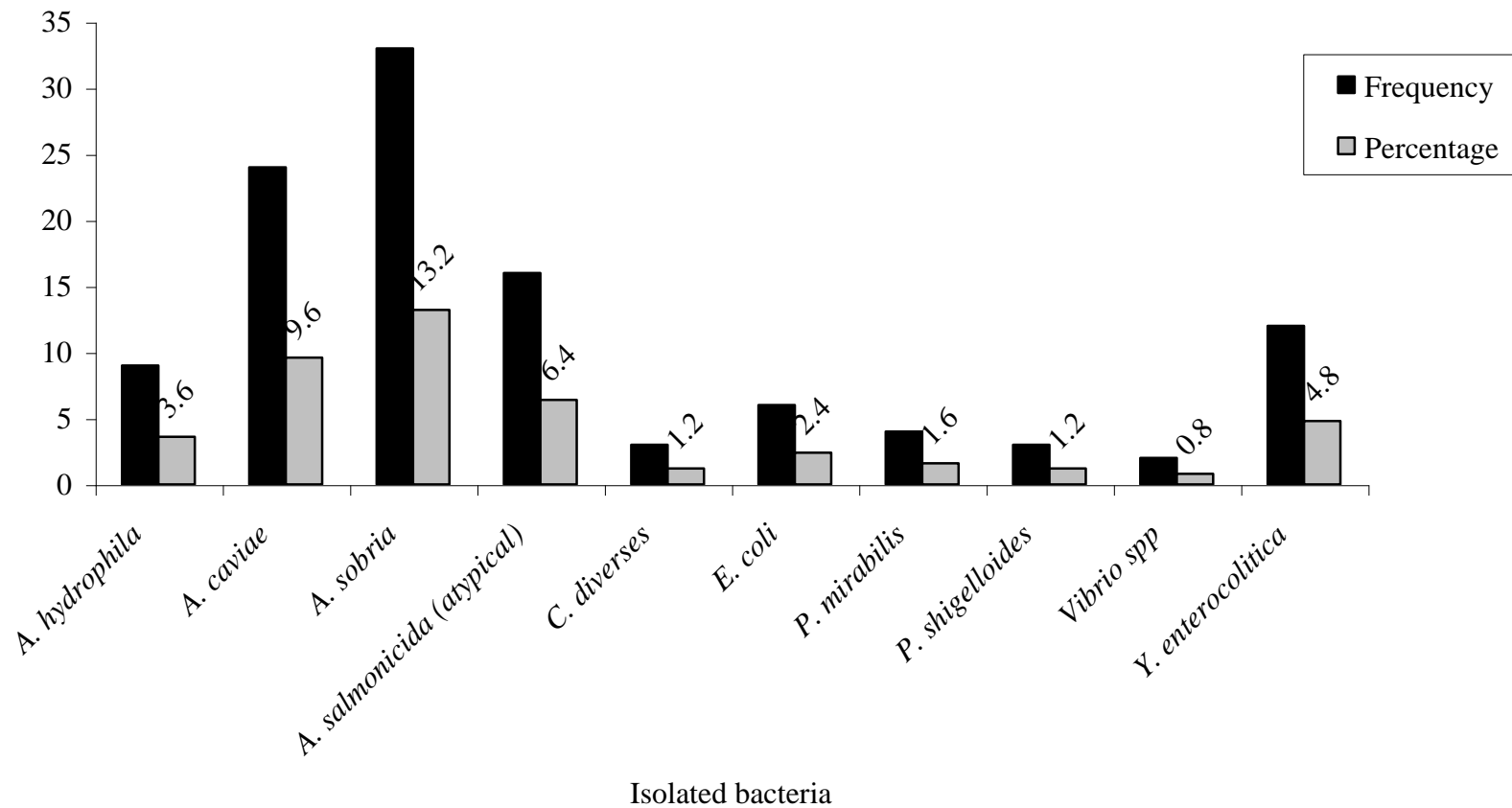


Figure 3. Frequency and percentage of bacteria isolated from filleted fish

#### 4.3.6. Isolation of bacteria from water samples

Among the total bacteria isolated in water samples, indicators of water pollution bacteria were found higher in frequency and percentage in water samples collected in littoral zone than the other fish habitats (Table 10).

Eventhough differences in frequency and percentage were recorded at any particular bacterial isolates among the three sampling sites; statistically significant difference was only recorded in *A. caviae* ( $P < 0.05$ ). The most frequently recovered bacteria among the total isolates in littoral and sub-littoral was *E. coli*, (22%) in littoral and (18%) in sub-littoral and no *E. coli* was isolated in water samples collected in pelagic zone (Table 10).

Table 10. Bacteria isolated from water samples collected at different fish habitats

Bacteria isolated	Littoral n = 23	Sub-littoral n = 17	Pelagic n = 22	Total n = 62	P-Value
<i>A. hydrophila</i>	2 (8.7%)	1 (5.9%)	2 (9.1%)	5 (8.1%)	0.318
<i>A. caviae</i>	4 (17%)	-	-	4 (6.5%)	0.027*
<i>A. sobria</i>	4 (17%)	2 (12%)	3 (14%)	9 (15%)	0.645
<i>A. salmonicida</i> (atypical)	-	3 (18%)	2 (9.1%)	5 (8.1%)	0.125
<i>E. aerogenes</i>	4 (17%)	1 (5.9%)	-	5 (8.1%)	0.094
<i>E. coli</i>	5 (22%)	3 (18%)	-	8 (13%)	0.068
<i>P. mirabilis</i>	-	-	1 (4.5%)	1 (1.6%)	0.397
<i>P. shigelloides</i>	2 (8.7%)	1 (5.9%)	3 (14%)	6 (9.7%)	0.705
<i>Y. enterocolitica</i>	2 (8.7%)	2 (12%)	-	4 (6.5%)	0.286
Total	23 (100%)	13 (76%)	11 (50%)	47 (76%)	

\* means significant at 5% level

#### 4.4. Physicochemical make up of the study area

Results of the measurements of the core water quality parameters for the study sites at Lake Tana are summarized in Table 11. Based on the findings all the measurements were found to be almost uniform and within the normal range.

Table 11. Mean of physicochemical parameters and characteristics of the fish and water sampling stations

Sampling stations	pH	Water T <sup>o</sup> (°C)	DO (mg/l)	Nitrite (ppt)	Salinity (mg/l)	Conductivity (µs/cm)	Secchi depth (Cm)
<u>Littoral</u>							
Gerima	7.9	21.7	7.7	<14.5	0.05	143.8	44.0
<u>Sub-littoral</u>							
Bahita	8.2	22.1	6.4	14.8	0	142.0	40.0
D/ mariam	8.5	21.7	8.1	<2.4	0	145.4	41.0
Airport	7.8	22.5	6.3	<19.6	0	145.0	44.0
<u>Pelagic</u>							
Ambobahir	8.9	19.6	8.1	2.4	0	144.0	42.0
Kibran-Zege	8.1	22.2	7.2	<6.2	0	138.3	35.7

## 5. DISCUSSION

A total of 18 different bacteria species were isolated from the kidney and intestine of fish, 10 from carcasses of filleted fish and 9 from water samples. The bacteria were belongs to the family Aeromonadaceae, Vibrionaceae and Enterobacteriaceae. The majority of the pathogens isolated from the fish were originated from the intestines of fish.

Although the internal organs of apparently healthy fish should be sterile (Sutin *et al.*, 2007) and the microbial biota is found generally only in the outer slime, gills and the intestines of feeding fish (Jay, 2000), in this study bacteria like *A. hydrophila*, *A. caviae*, *A. sobria*, *E. tarda*, *Vibrio* spp, atypical strain of *A. salmonicida* and *E. ictaluri* were isolated from the kidney. The isolation of bacteria from the kidney, liver and spleen of apparently healthy fish have frequently been reported (Cahill, 1990; Sousa and Silva-souza, 2001; Eshetu, 2000; Gebeyehu, 2003; Apun *et al.*, 1999). The presence of the microorganisms in internal fish organs could indicate the breakdown of immunological defense mechanisms (Cahill, 1990; Magnadottir, 2006). McVicar (1997) pointed out that the occurrence of an infection in a fish could not be necessarily an abnormal event nor will it lead to a disease situation. Moreover, under natural conditions, most infectious agents coexist with their host without causing significant disease. However, stress factors are frequently blamed for the dissemination of infections and also for the occurrence of many disease outbreaks (McVicar, 1997).

The percentage of some bacteria isolates was found to vary significantly among different fish species, fish sampling sites, maturation stages and sex groups. In this study the differences in the frequency of each bacteria isolates between groups of the different variables were more pronounced due to the variation of the frequency of bacteria isolates in the intestine of fish, which were sampled during the study period. It is accepted that fish posses a specific intestinal microbiota, but the bacterial composition may change with age, nutritional status, species (attribute to complexity of the fish digestive system) and the environmental conditions (salinity of the habitats and the bacterial load in the water) (Cahill, 1990; Ringoa *et al.*, 2003). Characteristics of the microenvironment at various locations through the alimentary tract of each fish species also influence the taxonomic composition as well as the numerical abundance of bacteria present (Horsley, 1997).

During the study period differences were also observed in the bacterial composition and frequency across the sampling sites of both fish and water, this may be generally attributed to the relative distance and degree of exposure to the nearby point source pollution around the study area (Annex 6). Differences were also recorded between the two maturation stages; this may also be related to the change in the microenvironment of the intestine at different age levels of the fish (Cahill, 1990; Ringoa *et al.*, 20003). *E. coli* (2.4%) were isolated during this study period in the intestine of fish. The occurrence of faecal coliforms in fish intestine reflects the warm-blooded animal-pollution level of the water and also indicate that the organism can probably survive and multiply when fish and water temperatures are 20<sup>0</sup>C or higher (Geldreich and Clarke, 1966).

The bacterial species isolated from carcasses of filleted fish were also recovered from the water samples (except *C. diversus*) and in the intestine of fish. The microbial status of filleted fish after catch may be closely related to the microbial conditions of the water and the intestine of the fish. The factors include distance between localization of the catch and polluted area (containing human and animal faeces), natural occurrence of bacteria in the water, ingestion of food by fish, method of catch and post harvest handling or processing conditions.

Among the water samples examined bacteriologically during the study period, bacteria species such as *A. hydrophila*, *A. caviae*, *A. sobria*, atypical strains of *A. salmonicida*, *E. aerogenes*, *E. coli*, *P. mirabilis*, *P. shigelloides*, and *Y. enterocolitica* were isolated. The presence of these bacteria both in the aquatic environment and in the fish has been often observed elsewhere (Cahill, 1990; Apun *et al.*, 1999).

Even though statistically significant differences were recorded only in *A. caviae*, most of the bacteria species isolated in water samples collected from inshore littoral zones that were near to point source pollutions, were found to be higher in frequency and isolation rate than the other sampling sites. The differences in the frequency and percentage of bacteria isolates, especially those bacteria associated with water pollutions like *E. coli*, *E. aerogenes* and *A. caviae* might be attributed to their distances from the point source pollutions. This was in agreement with Fogarty *et al.* (2003), who reported that pollution level decreases with an increase in distances from the source of pollutions. The recovery of *E. coli* also indicated that the presence of water pollution in areas corresponding to the local fishing grounds, which are

extensively exposed to raw sewage discharged into the lake from the nearby municipal pit latrines and septic tankers. This idea was supported by WHO (1984) that the presence of total coliforms, including *E. coli* and *E. aerogenes* are biological parameters, which indicate that pollution of the water by all types of organisms being human and/or animals. This was consistent with the study conducted by Yimenu (2005). The same author reported that from the total water samples collected from shorelines of Lake Tana for bacteriological analysis majority of the samples showed bacterial growth, of which more than 80% were, faecal coliforms.

In general except *C. diversus* (only isolated in fish and filleted fish), *Vibrio* spp and *E. aerogenes* (in fish and water samples) other bacterial isolates such as *A. hydrophila*, *A. caviae*, *A. sobria*, *E. coli*, *P. mirabilis*, *P. shigelloides*, and *Y. enterocolitica* were the common bacterial species recovered in the intestine of fish, water samples and carcasses of filleted fish. Therefore the recovery of these bacteria species and others from the intestine of fish is the cause for concern as most of those organisms are potential enteropathogens, and any mishandling of the fish can lead to the transmission of the pathogens to humans.

#### *Aeromonas* species

Members of the mesophilic *Aeromonas* species, namely *A. hydrophila*, *A. caviae*, *A. sobria* were isolated in the kidney and intestine of apparently healthy fish, carcasses of filleted fish and their aquatic environment (Lake Tana). The isolation of motile aeromonads in the mucosal surface and internal organs of clinically healthy freshwater fish were also documented by Noga, 1996; Gebeyehu, 2003; Popovic *et al.*, 2000; Arbaciauskiene, 2005 and. Yucel *et al.*, 2005. Apun *et al.*, 1999 also reported *A. hydrophila* from the fish intestine and samples of water from their aquatic environment.

The distribution of mesophilic *Aeromonas* species in the aquatic environment may be related to the level of pollutions in the water. It has been reported by several authors including Stecchini and Domenis (1994) that *A. caviae* predominates in waters with a high degree of fecal pollution. In less polluted water, either fresh or marine, *A. caviae* and *A. hydrophila* were almost equally distributed. *A. sobria* can be found in unpolluted waters, brackish waters and sewage effluents. Large numbers of aeromonads, especially *A. caviae*, could therefore be considered to be indicative of nutrient-rich conditions of water. The numbers of mesophilic

*Aeromonas* species in some aquatic environments have been reported to show a seasonal variation, with populations greatest in the warmer months of the year for both temperate freshwater lakes and chlorinated drinking water (Basti *et al.*, 2006).

#### *Citrobacter* species

*Citrobacter diversus* (from the intestine and filleted fish) and *C. freundii* from the intestine of fish were isolated during the study period. Eshetu (2000) and Apun *et al.* (1999) also reported the isolation of *Citrobacter* species from the intestine of apparently healthy fish. *C. diversus* inhabits faeces of man, animal, soil and sewage. Meningitis in human neonates and mastitis in cattle have been reported due to *C. diversus*. It is also capable of opportunistic pathogens in other animals (Quinn *et al.*, 1999). The bacterium *C. freundii* has been isolated from naturally infected aquarium fish species. The infection causes grave clinical symptoms (skin ulceration, distended abdomen, pale liver, darkened spleen, watery kidney and bloody exudates in the gastrointestinal tract) (Aydin, 1997).

#### *Enterobacter aerogenes*

During the study period 5.1% isolates from the intestine of fish and 8.1% isolates from water samples were recovered. The same isolate from the intestine of fish were reported by Apun *et al.* (1999). Gebeyehu (2003) were also reported *E. cloaca* from liver and kidney of fish at Lake Tana.

#### *Escherichia coli*

*Escherichia coli* were also isolated in all types of sample. Similar isolates were reported by Eshetu (2000), Apun *et al.* (1999), Rodriguez *et al.* (1997) and Gebeyehu (2003) from the fish intestine and kidney. Thampuran (2005) and Gebeyehu (2003) also isolated *E. coli* from filleted fish, and Yimenu (2005) from water samples collected at Lake Tana. *E. coli* are generally non pathogenic in the host fish. The presence of these bacteria has lead to recommendations for strict hygienic practices during the processing and handling of aquaculture products to prevent the infection of human beings. In addition, its presence in water alerts public health officials to know possible presence of other human and animal intestinal pathogens (WHO, 1998).

### *Edwardsiella* species

The genus *Edwardsiella* includes two species of bacteria, *E. tarda* infects fish and other animals including human beings and *E. ictaluri* infects fish only (Woo and Bruno, 1999). In this study *E. tarda* were isolated both in the kidney and intestine of fish. Similar isolation from the fish intestine with sporadic cases of tropical diarrhoea in humans was traced to contact with or consumption of fresh water fish was reported by Damme and Vandepitte (1980). *E. tarda* were also isolated from dressed fish samples by Wyatt *et al.* (1979) and Noga (1996), and from water samples by Sechter *et al.* (1983).

### *Klebsiella Pneumoniae*

In this study *K. pneumoniae* was isolated only from the intestine of fish (2.4%). Apun *et al.* (1999) reported this organism in the intestine and Eshetu (2000) also reported *K. oxytoca* in similar organ of fish. Since *K. pneumoniae* inhabits intestinal tract of animals and man, soil and sawdust accounts for wide distribution of the organism (Quinn *et al.*, 1999). The strains responsible are nearly always biochemically typical members of *K. pneumoniae*. In humans, the strains of *Klebsiella* occasionally causes severe bronchopneumonia, and also more chronic destructive lesions with multiple abscess formation in the lungs (Colleier *et al.*, 1998).

### *Proteus* species

Generally in this study *Proteus* species were isolated from fish intestine, carcasses of filleted fish and water samples. Faeces of mammals and environment are inhabitants of these bacterial species. In addition to their wide saprophytic existence, strains of *Proteus* cause septic infections in man and animals (Colleier *et al.*, 1998).

### *Plesiomonas shigelloides*

In this study *P. shigelloides* was isolated from fish intestine, carcasses of filleted fish and water samples. This result was also supported by the reports of Damme and Vandepitte (1980) and Arai *et al.* (1980) from fresh water fish, Gonzalez *et al.* (2003) and Arai *et al.* (1980) from lake and river water samples.

### *Vibrio* species

During the study period non-specified *vibrio* species were also recovered from fish kidney and intestine as well as carcasses of filleted fish. Isolation of *V. anguillarum* from fish intestine was reported by Apun *et al.* (1999). Noga (1996) also indicated that *vibrio* are commonly isolated from the mucosal surfaces and internal organs of clinically healthy fish as well as the water bodies, with highest environmental prevalence in organically polluted water and salinity.

### *Yersinia* species

In this study *Y. enterocolitica* have been isolated in the intestine, filleted fish and water samples. Whereas *Yersinia ruckeri* isolated only in the kidney and intestine. Isolation of *Y. enterocolitica* also reported from filleted fish (Eshetu, 2000) and (Gebeyehu, 2003). *Yersinia enterocolitica* and other *yersinia* species are ubiquitous in the natural environment, and may be recovered from water, soil, animals and food. The presence of pathogenic *Y. enterocolitica* produces a special concern since those organisms are significant causes of human diseases and are capable of growth at refrigerator temperature (Johnson, 1998). *Y. enterocolitica* is mainly a human pathogen that usually affects children (Acha and Szyfers, 2001).

### *Shigella* species

Only two isolates of *Shigella sonnei* were recovered in the intestine of fish during the study period. Isolation of *Shigella* species from the spleen of *O. niloticus* by Eshetu (2000) and *Shigella dysenteriae* by Gebeyehu (2003) were also reported. *Shigella* species inhabits the intestinal tract of man and other primates and the bacterium causes dysentery or diarrhoea in man and other primates (Quinn *et al.*, 1999). Shigellosis is often a disease of children occurring by faecal-oral-route. Adults can also be infected from children, although it can be transmitted by infected adult food handlers who contaminate the food (Fox, 2002).

As to the physicochemical water parameters mean values of pH, water temperature, nitrite, salinity and others in all the three sampling sites were found to be rather uniform and within the normal range at which most freshwater fish species become non-stressed. The mean

dissolved oxygen was high (7.4 mg/l) indicating that there was a good aeration during the study period, which may be attributed to lower temperature and good flow rate (Buttner *et al.*, 1993; Sutin *et al.*, 2007).

The impact of stress on fish depends on the severity of the stress, its duration and the physiological state of the fish. The absence of fish diseases with grossly visible pathological changes might be attributed relatively to the quality of the lake that was observed during the study period.

## 6. CONCLUSION AND RECOMMENDATIONS

In the present study different bacterial species were isolated from the kidney and intestine of fish, carcasses of filleted fish and their aquatic environment, which belong to the family Aeromonadaceae, Vibrionaceae and Enterobacteriaceae. The findings have shown that the intestine of fish have been found to be harboring a large group of bacterial species that may be resident and/or transient microorganisms. Isolation of similar types of bacteria from the fish, filleted fish and their habitat has been recorded during the study period. The detection of similar bacterial species both in the fish and the water samples has some implications on the relationship between the fish and their aquatic environment (even though it requires some molecular characterizations of both isolates). Some of the bacterial isolates including *Aeromonas hydrophila* and *Edwardsiella tarda* are opportunistic pathogens of fish and may cause mortalities when the fish come under stress. Furthermore, the recovery of various organisms, which are potentially pathogenic to humans, in the kidney and alimentary tracts of fish, and/or carcasses of filleted fish suggest that fish either improperly handled, undercooked or consumed raw may cause various diseases in susceptible individuals.

Based on the observations made during the study period, the following recommendations are forwarded:

The isolation of *E. coli* and other enteropathogens from the fish, filleted fish and their aquatic environment during the study period indicates a need for strict hygienic practices during the processing and handling of fish and fish products to prevent the spread of pathogenic bacteria to human beings.

Although incidence of zoonotic infections with fish pathogens around the study area was not well known, as has been observed around the lake during the study period the public health risks might be associated with puncture or open wound or possibly due to the consumption of raw or undercooked fish products. Therefore, public health risk awareness should be created among fish consumers and those who have contact with fish, fish products and their aquatic environment.

Even though the ultimate effects of pollution in the study area is not noticeable yet, the findings of this and other studies conducted around Lake Tana indicate the existence of

bacterial contaminants that have both public and veterinary importance. Therefore, to safeguard both the public and the fish, sanitary measures around Lake Tana should be taken by the concerned authorities and organization.

Further detailed studies should be conducted on the epidemiology and molecular characteristics of the various bacterial pathogens of fish to further understand their ecology, cross-similarity and possible health hazards to human either through contact or ingestion of fish and fish products.

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## 8. ANNEXES

### Annex 1. Sample collection record sheet

Sampling date \_\_\_\_\_

Sampling site \_\_\_\_\_

Type of habitat \_\_\_\_\_

Sample ID . No.	Type of fish species	Sex and maturity stage	Clinical and postmortem findings

Annex 2. Bacteriological and biochemical test result recording sheet.

Sample ID No.	No. of colonies identified	Gram stain reaction	Cytochrom oxidase	Oxidase negative					Oxidase positive								Presumptive isolates			
				Indole	TSI	MR	VP	Citrate	Motile				None-motile							
									OF	Novobiocin sensitivity	Gas from glucose	Arabinos utilization	Acid from inositol	Diff. brown pigment	OF	Gas from glucose		Arabinos utilization		

### Annex 3. Primary and secondary identification tests.

#### 1. Gram stain (Quinn *et al.*, 1999).

##### Procedure:

- Prepare a bacterial smear from a pure culture.
- Flood the slide with crystal violet, and allow remaining on the slide for 60 seconds.
- Wash of the crystal violet with running tab water.
- Flood the slide with Gram's iodine, and allow remaining on the slide for 60 seconds.
- Wash off with running tab water.
- Decolorize with 95% alcohol and 5% acetone solution.
- Rinse immediately with running tab water.
- Counter stain with diluted carbol fuchsin for 60 seconds.
- Rinse with running tab water and allow to air dray.

##### Results:

- Gram-negative = Pink to Red color; Gram-positive = Purple to Dark color.

#### 2. Cytochrome oxidase test (Koneman *et al.*, 1988).

This test determines the presence of cytochrome oxidase enzymes

##### Procedures:

- 2 to 3 drops of the reagent are directly added to bacterial colonies growing on plate medium

##### Results:

- Positive: deep blue color at the inoculation site within 10 seconds.

#### 3. Motility test (Woodland, 2006).

This test determines if a bacterial isolate is motile by means of flagella.

##### Procedure:

- Stab the motility test medium with a small amount of inoculums.
- Incubate overnight at room temperature.

Result:

- If the bacterial species is motile, the medium will become turbid with growth that radiates from the line of inoculums. If the bacterial species is non-motile, only the stab line will have visible bacterial growth.

#### 4. Indole test (Koneman *et al.*, 1988).

A test to determine bacterial ability to split indole from the tryptophan molecule.

Procedure:

- Inoculate tryptone broth with light inoculums from an 18 to 24 hours pure culture.
- Incubate 24 to 48 hours at 20 to 24°C
- After 24 hours of incubation aseptically remove 2 ml of media and place in an empty sterile test tube. Save extra tube for 48 hours incubation, if necessary.
- Add about 5 drops of Kovac's reagent to one of the tubes and agitate the tube.

Result:

- Positive: -Within 1 to 2 minutes, a cherry red ring will form at the surface of the media.
- Negative: -No color formation is observed at the surface; the color remains that of the reagent-yellow.
- Variable:-An orange color may develop. This indicates the presence of skatole, which may be a precursor of indole formation.

#### 5. OF-test (Glucose fermentation) (Woodland, 2006).

Metabolism of the carbohydrate dextrose by either an aerobic or anaerobic pathway results in acid production. The resulting acidic environment causes bromthymol blue pH indicator in the medium to turn from green to yellow. The presence of bubbles in the tube indicates gas production (aerobic). If no reaction occurs, the medium can remain unchanged or become alkaline (blue at the surface).

Procedure:

- With a sterile needle take a small inoculums from an isolated colony and stab to the bottom of the tube of the glucose fermentation medium.
- Incubate at 20 to 24°C for 24 to 48 hours for acid and/ or gas production.

Result:

A = acid (yellow); AG = acid + gas; N = no change or alkaline.

	Top of tube	Bottom of tube
Oxidative	A	N
Fermentative	AG or A	AG or A
Non-reactive	N	N

#### 6. TSI-test (Woodland, 2006).

This medium can determine the ability of an organism to utilize specific carbohydrates incorporated in a basal growth medium, with or without production of gas, along with the determination of hydrogen sulfide (H<sub>2</sub>S) production.

Procedure:

- Inoculate the TSI slant by stabbing to the bottom of the tube and then streaking the surface of the slant as the needle is drawn out of the tube. Screw the cap on loosely.
- Incubate at 20 to 24°C. Read after 18 to 24 hours.

Result:

A = acid; K = alkaline; H<sub>2</sub>S = Hydrogen sulfide produced; N = no change

Slant/Butt	Color/Reaction	Interpretation
K/N or K/A	red/orange (oxidative) or red/yellow (fermentative)	only peptone utilized or only glucose-fermented
A/A	yellow/yellow	glucose, plus lactose and/or sucrose-fermented
Gas	splitting or bubbles	gas production
H <sub>2</sub> S	black butt	Hydrogen sulfide produced

#### 7. Novobiocin sensitivity test (Woodland, 2006).

This test determines the sensitivity of a bacterial organism to novobiocin

Procedure:

- A # 0.5 McFarland standard suspension is prepared

- Streak suspension on plate in three planes with a cotton swab.
- Place a 5 µg novobiocin disc in the center of the inoculums.
- Incubate at 20 to 24°C for 24 hours.

Result:

- Sensitive: Zone of inhibition around the disc.
- Resistant: Growth adjacent to the disc.

#### 8. Carbohydrate fermentations (glucose, arabinose and inositol) (Quinn *et al.*, 1999).

- Test: Peptone water sugars
- Medium: 1% sugar in peptone water
- Product tested for: Enzymatic attack on sugar with acid and gas (Durham tube) production
- Test reagent (pH indicator): Phenol red (red at pH 7.4)
- Result: Yellow (acid produced)

#### 9. Methyl Red (MR) (Koneman *et al.*, 1988).

This test is a quantitative test for acid production, requiring positive organisms to produce strong acids (lactic, acetic, formic) from glucose through the mixed acid fermentation pathway.

Procedure:

- Inoculate the MR-VP broth with a pure culture of the test organism.
- Incubate the broth at 28°C for 48 to 72 hours (no fewer than 48 hours).
- At the end of incubation add 5 drops of the MR-reagent directly to the broth

Result:

The development of a stable red color in the surface of the medium indicates sufficient acid production to lower the pH to 4.4 and constitutes a positive test.

#### 10. Voges-Proskauer (VP) (Koneman *et al.*, 1988).

Pyruvic acid, the pivotal compound formed in the fermentative degradation of glucose, is further metabolized through a number of metabolic pathways, depending upon the enzyme

possessed by different bacteria. One such pathway results in the production of acetoin (acetyl-methyl carbinol). In the presence of atmospheric oxygen and 40% Potassium hydroxide, acetoin is converted to diacetyl, and  $\alpha$ -naphthol serves as a catalyst to bring out a red color complex.

Procedure:

- Inoculate a tube of MR-VP broth with a pure culture of the test organism.
- Incubate for 24 hours at 35<sup>0</sup>C.
- Aliquot 1 ml. of broth to a clean test tube.
- Add 0.6 ml of 5%  $\alpha$ -naphthol, followed by 0.2 ml of 40% KOH.
- Shake the tube gently to expose the medium to atmospheric oxygen.
- Allow the tube to remain undisturbed for 10 to 15 minutes.

Result:

- A positive test is represented by the development of a red color 15 minutes or more after the addition of the reagents.
- False-positive interpretation resulted if the test is read after standing for over one hour.

#### 11. Citrate utilization (Koneman *et al.*, 1988).

The utilization of citrate by a test bacterium is detected in citrate medium by the production of alkaline by-products. Bromthymol blue is the indicator.

Procedure:

- A well-isolated colony is picked from the surface of a primary isolation medium.
- Inoculate as a single streak on the slant surface of the citrate agar tube.
- Incubate the tube at 28<sup>0</sup>C for 24 to 48 hours.

Result:

- A positive test is represented by the development of a deep blue color within 24 to 48 hours.

#### Annex 4. List of media utilized

1. MR-VP Medium (Oxoid, Hampshire, England)
2. Simmons Citrate Medium (Oxoid, Hampshire, England)
3. Mueller-Hinton agar (Oxoid, Hampshire, England)
4. Tryptic Soya Agar (TSA) (Oxoid, Hampshire, England)
5. Tryptone Soya Broth (Difco, Becton Dickinson, U.S.A.)
6. Buffer Pepton Water (Difco, Becton Dickinson, U.S.A.)
7. OF Basal Medium (Difco, Detroit, U.S.A.)
8. Triple Sugar Iron (TSI) Agar (Oxoid, Hampshire, England)
9. Brain Heart Infusion (Oxoid, Hampshire, England)

#### Annex 5. List of reagents, sugars and diagnostic discs

1. Oxidase reagent (0.5% N, N, N', N'-tetramethyl-p-phenylenediamine)
2. Kovac's Indole Reagents
3. Phenol-Red Indicator
4.  $\alpha$ -naphthol
5. 40% KOH.
6. Glucose
7. Arabinose
8. Inositol
9. Gram stain reagents (Crystal violet, Gram's iodine, 95% Ethanol and carbol fuchsin).
10. Novobiocin discs (5  $\mu$ g, BBL Microbiology system)

## Annex 6. Distances of sanitary facilities from Lake Tana

Site	Distance (m)	Type of sanitary facilities
Medhinealem Orphanage	10	Pit latrine, septic tanker
Water Works	20	Pit latrine, septic tanker
Lake Side Recreation	25	Pit latrine, septic tanker
Tana Hotel	35	Pit latrine, septic tanker
Regional Prison	50	Pit latrine, septic tanker
Gust House	70	Pit latrine, septic tanker
Ghion Hotel	135	Pit latrine, septic tanker
Alma Office	150	Pit latrine, septic tanker
Shume Abo Recreation	215	Pit latrine, septic tanker
Hospital	500	Pit latrine, septic tanker

Source: yimenu, 2005

## **9. CURRICULUM VITAE**

### **A. Biographic data**

Name	Anwar Nuru
Date of birth	12 February 1969
Place of birth	Gondar
Marital status	Married
Sex	Male
Nationality	Ethiopian
Profession	Veterinarian
Occupation	Woreda Veterinary Officer
Address	Woreta Office of Agriculture and Rural Development South Gondar, Ethiopia Tel. 0911-39 75 16 or 058-220 46 78 E-mail: anwaruwa@yahoo.com

### **B. Educational background**

1976-1981	Tsadiku Yohannis Elementary School, Gondar (Grade 1-6).
1982-1987	Fassiledes Comprehensive Secondary School, Gondar (Grade 7-12).
1987	Achievement: Ethiopian school leaving certificate examination.
1988-1993	Undergraduate program: Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.
1993	Achievement: Doctor of Veterinary Medicine
2006-2007	Postgraduate study: Addis Ababa University, faculty of Veterinary Medicine, Debre Zeit, Ethiopia.
2007	Achievement: MSc. in Tropical Veterinary Microbiology.

### **C. Work experience**

1995-2005	Field Veterinarian (Waghimira and South Gondar Administrative Zones)
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1998 (3 month)	} Teaching experience at Kombolcha Agricultural Training Center for trainees of animal health technicians (Course titles: Pathology and Vet. Medicine).
1999 (3 month)	
2002 (3 month)	
2001-2002	Part time service at Debre Tabor Child Care Center (Dairy Farm).
2003 (3 month)	} Part time teaching experience at Woreta Agricultural TVET College (Course title: Animal Health).
2004 (3 month)	

#### **D. Research output/ Technical paper**

“Major Constraints of Livestock Production in Ethiopia.”	Seminar paper, 1992.
“Comparison of Prevalence Rate of Bovine Trypanosomoses in Tsese Controlled Area of Limushye and Non-Controlled Galle Settlement, Illubabure, Ethiopia.”	DVM thesis, 1993.
“Review on the Major Bacterial Pathogens and Bacterial Zoonoses of Fish”	MSc. Seminar paper, 2006
“Study on Bacterial Pathogens of Fish in Southern Gulf of Lake Tana with special reference to <i>Aeromonas hydrophila</i> and <i>Edwardsiella tarda</i> , Bahir Dar, Ethiopia.”	MSc. Thesis, 2007.

#### **E. Membership**

Member of Ethiopian Veterinary Association

#### **F. Language**

Amharic	Mother tongue
English	Writing and speaking
Tigreigna	Writing and speaking

**G. Other skills**

Computer literate

**H. Hobbies**

Reading, indoor games and tour

**I. References****1. Bayleyegn Molla (DVM, MSc., PhD)**

Associate Professor

Department of Microbiology and Veterinary Public Health

Faculty of Veterinary Medicine

Addis Ababa University

Debre Zeit, Ethiopia

P. O. Box 34

Fax: +433 99 33

Tel.: +433 85 33

E-mail: bayleyegn\_molla@yahoo.com

**2. Eshetu Yimer Ahmed (DVM, MSc.)**

Zoonoses Research Team

Ethiopian Health and Nutrition Research Institute (EHNRI)

Associate Professor of Aquatic Veterinary Medicine, FVM, AAU

Addis Ababa, Ethiopia

P.O. Box 181689

Mobile: 0911652711

E-mail: esyima\_n@yahoo.com

## 10. DECLARATION

This thesis is my original work, has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

Name -----

Signature -----

Date of submission -----

This thesis has been submitted for examination with my approval as university advisor.

Dr. Bayleyegn Molla -----  
(Associate Professor)

Dr. Eshetu Yimer Ahmed -----  
(Associate Professor)