

## Quality Assessment Of Clupeids Along The Coastal Fisheries Value Chain Of Ogun Waterside Local Government Area, Ogun State, Nigeria

Odebiyi O.C.<sup>1</sup>, George F.O.A.<sup>1</sup>, Odulate D.O.<sup>1</sup>, Akinyemi A.A.<sup>1</sup>, Arowosegbe A.<sup>2</sup>, Oke A.O.<sup>3</sup>

<sup>1</sup> Department of Aquaculture and Fisheries Management, Federal University of Agriculture Abeokuta, P.M.B. 2240, Abeokuta, Ogun State, Nigeria

<sup>2</sup> Department of Microbiology, Federal University of Agriculture Abeokuta P.M.B. 2240, Abeokuta, Ogun State, Nigeria

<sup>3</sup> Department of Fisheries and Aquatic Resources Management, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, P.M.B 7267, Umudike, Abia State, Nigeria  
[funksod4real@yahoo.com](mailto:funksod4real@yahoo.com), [odebiyioc@funaab.edu.ng](mailto:odebiyioc@funaab.edu.ng)

**Abstract:** Quality assessment of three clupeids (*Ethmalosa fimbriata*, *Ilisha africana* and *Sardinella mardenerensis*) fish species was carried out using colonial, morphological and biochemical microbial analyses. Samples of fish were collected from the fishermen, fish processors and fish-marketers along the coastal fisheries value chain (CFVC) from which micro-organisms were identified, characterized and number of colony forming units (CFU) were estimated from the skin, gills and intestines of these fish species. The total bacteria counts ranged from  $0.3 \times 10^6$  CFU/g to  $4.7 \times 10^6$  CFU/g across the entire nodes of the CFVC. According to International Commission on Microbiological Specification for Food (ICMSF, 1986), the maximum recommended bacteria count for good quality fish product was  $5.0 \times 10^5$  (5.7Log cfu/g) and the maximum for marginally acceptable quality fish product was  $1.0 \times 10^7$  (7 log cfu/g). Thus, the bacteria load obtained from the fresh landed fish by the fisherman and preserved fish by the fish-marketers across the CFVC was higher than the marginally acceptable level; but closer to the maximum recommended value which makes the fish samples not to be in their best condition for human consumption. A total of eight bacteria and five fungi species of food importance were isolated along the chain; the Bacteria isolated includes *Escherichia coli*, *Proteus spp*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas sp*, *Micrococci spp* and *Pseudomonas fluorescens* while fungi isolated were *Aspergillus flavus*, *Aspergillus niger*, *Mucor spp*, *Penicillium notatum* and *Fusarium spp*. *Staphylococcus aureus* dominated the value chain with a percentage occurrence of (20.0%, 30.0% and 36.67%) while *Aspergillus niger* occurred most (26.67%, 31.67 and 33.3%) in fish samples from the fishermen, fish processors and fish-marketers respectively. In conclusion, the quality assessment carried out revealed that fish samples at all the chain nodes harboured microorganisms which are either of pathogenic, food poisoning, food spoilage or of epidemiological importance, hence; this study provides the knowledge on the microorganisms associated with each stages of the chain. Thus, consumers who consume these fish without further cooking, washing or heating are at a risk of contracting food borne infections as a result of poor hygiene and poor post harvest handling on the part of the sellers.

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### 1.0 Introduction

Fish is the most important animal protein food available in the tropics, and it represents about 14% of all animal protein on a global basis (Eyo, 2001). It plays an important role in the diet of human beings since it is a good source of animal protein as reported by Immaculate et al., (2012). Fish is a valuable and nutritious food, and an essential source of high quality and cheap animal protein crucial to balance of diets in marginally food secure communities (United nation, 2004). More than half of the world's population depend on fish as their principal source of animal protein. In many countries, people derive

more than 50% of their daily animal protein requirements from fish products (World Bank, 2004).

Fish is a major source of protein and its harvesting, handling, processing and distribution provide livelihood for millions of people (Al-jufaili and Opara, 2006). Coastal fisheries are important and contribute at least 40 percent of fish production from all sources in Nigeria between 1995 and 2008 (FDF, 2011). Clupeids are one of the commercially important fish species in Nigeria (FDF, 2005). Fish are among the most perishable food products known to man and the monitoring and control of fish quality is one of the main goals in the fish industry. Its shelf life is influenced by a number of factors such as

initial microbiological load, season and handling and the limited and variable shelf lives of Fish are major problems for fish quality and assurance (Konstantinos, 2001).

A major goal for the food processing industry is to provide safe, whole some and acceptable food to the consumer, control of microorganisms is essential to meet this goal. This control is partly exerted through processing and preservation techniques that eliminate microorganisms or prevent their growth (Prabakaran et al., 2011). It is also required that the basic hygiene level during processing is high and that efficient cleaning and disinfection procedures that eliminate spoilage and pathogenic bacteria (Fonnesbech et al., 2001). It is against this background that this study aims to assess the microbial flora present in fish samples (Clupeids) in the coastal fisheries value chain of Ogun waterside local government area of Ogun State, Nigeria.

## 2.0 Materials and Methods

Samples of Clupeids mainly *Ethmalosa fimbriata*, *Ilisha africana* and *Sardinella maderensis* were taken at each node of the chain; the fishermen, fish processors and the fish-marketers level of the coastal fisheries value chain. Fresh samples from the fishermen was labeled and transported in an ice box at a temperature below 5°C while smoked dried samples were labeled appropriately before transfer to the Microbiological Laboratory of the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria for analysis.

### 2.1 Total bacterial counts (Cfu/g):

The total bacteria count for each sample was determined with the surface plate techniques using nutrient agar. The plates were incubated between 18 and 24 hours at 37°C and all colonies obtained after incubation period were counted and the counts were expressed in colony forming unit per gram [CFU/g] of the sample. Colonies of bacteria growing on the plates were observed, isolated and separated on a fresh media until pure culture was obtained.

### 2.2 Total fungi counts (CFU/g):

The total fungi count from each sample was determined with the surface plate techniques using potato dextrose agar. The plates were incubated at 25°C for 3-7 days to enhance microbial growth. All fungi colonies obtained were counted and the counts were expressed in colony forming unit per gram [CFU/g] of the sample.

#### 2.2.1 Determination of pH:

pH of the fish samples were determined by the method of Waller (1980). 10g of the fish sample were homogenised with 50mls of distilled water and the pH value of the homogenate was measured by means of a pH meter (HANNA, USA).

## 2.3 Microbiological examination:

### a. Skin surface:

Samples of *E. fimbriata*, *I. africana* and *S. maderensis* at the different stages of the chain were taken by using a swab of sterilized cotton to rub the skin of the samples and inoculated in Nutrient agar and the Total Bacteria Count was estimated using the method described by Slaby *et al*, 1981.

### b. Gills:

A part of the gill filament which was exposed with the aid of a pair of forceps was also swabbed with a sterile cotton swab and inoculated in a Nutrient agar as described in Slaby *et al*, 1981.

### C. Intestines:

This was carried out by cutting a small part of the intestine with a sterilized scalpel before inoculating in a nutrient agar.

## 2.4 Isolation and Identification of Bacteria and Fungi:

Morphological characteristics of the various bacterial and fungi isolates were noted in the agar plates and after staining reactions and series of biochemical tests, individual microbial species were identified as described in Slaby *et al*, 1981.

### 2.4.1 Identification of Microorganisms:

Characterization of the organisms was based on colonial, morphological and biochemical characteristics of colonies (Table 5). Macroscopic examination of surface colonies on nutrient agar medium was used to determine the colour, edge, elevation, surface, shape and arrangement of microorganisms. Morphological characteristics were studied under the oil lens immersion microscope after Gram-staining.

## 2.5 Biochemical Tests:

Biochemical tests carried out on the bacterial isolates were Catalase test, Coagulase test, Motility test, Indole production test, Citrate utilization test, Urease test and Sugar Fermentation tests.

## 3.0 Results

Mean Total Bacteria Count (TBC) of the three species of fish at each link of the chain is presented in Table 1. Total Fungi Count (TFC) is presented in

Table 2 while the bacteria and fungi isolates are presented in Tables 3 and 4. The colonial, morphological and biochemical characteristics of the fish samples obtained at the different stages of the value chain are presented in Tables 5 and 6 respectively. Generally the gills had the highest bacterial load, followed by the intestine, while the skin had the lowest bacterial load in the samples at the fishermen and fish-marketers stages of the chain. The bacterial load of the samples collected at the fish processors stage showed a different trend, with the highest bacterial load recorded in the intestine followed by the gill and the least in the skin. There was a significant decrease in the bacteria load at the different parts of the fish sample at the processor stage; while a sharp increase was observed at the fish-marketers stage of the chain (Table 1).

The average bacterial count of the different sections of the fish samples from different stages of

the chain ranged from  $0.3 \times 10^6$ CFU/g from the gill of the smoke-dried *Sardinella marderensis* and *Ilisha africana* at the fish processor level to  $4.70 \times 10^6$ CFU/g in the gill of the *Ethmalosa fimbriata* at the first stage of the chain.

The fungal count was found to be highest in the gills at all the stages of the value chain except in *Sardinella marderensis* species where the highest was recorded in the intestines (Table 3). Bacteria isolates identified across the chain nodes included: *E. coli*, *Proteus spp*, *Bacillus subtilis*, *S. aureus*, *S. epididemis*, *Pseudomonas spp*, *Micrococci spp* and *P. fluorescens* while the Fungi isolated included *A. flavus*, *A. niger*, *Mucor spp*, *P. notatum* and *Fusarium spp* (Table 3 and 4). The moulds observed on fish specimens were all raised in elevation with the mycelia varying in colour.

Table 1: Microbial load and pH range of fish samples from the nodes of the Ogun Waterside coastal fish value chain

Fish species	Body part	Average Bacteria Count (X $10^6$ CFU/g)			pH
		Fishermen	Fish Processor	Fish-marketers	
<i>Ethmalosa fimbriata</i>	Gill	4.7	2.1	4.35	7.6 $\pm$ 0.01
	Intestine	3.3	3.0	3.02	
	Skin	3.1	1.9	2.97	
<i>Ilisha africana</i>	Gill	1.6	0.8	1.36	8.1 $\pm$ 0.05
	Intestine	1.4	1.0	1.32	
	Skin	0.3	0.4	0.41	
<i>Sardinella marderensis</i>	Gill	1.2	0.8	1.23	6.6 $\pm$ 0.03
	Intestine	0.8	1.0	0.73	
	Skin	0.5	0.3	0.47	

Source: Field Survey: 2013

Key: CFU/g= colony forming unit per gram.

Table 2: Average fungal count in the fish samples along the Ogun Waterside coastal fish value chain

S/N	Fish Species	Body parts	Average Fungi Count ( x $10^6$ CFU/g)		
			Fisherman	Fish Processors	Fish-marketers
1.	<i>Ethmalosa fimbriata</i>	Gill	0.2	0.2	0.5
		Intestine	0.0	0.0	0.2
		Skin	1.1	0.3	0.4
2.	<i>Ilisha africana</i>	Gill	0.0	0.0	0.1
		Intestine	0.2	0.0	0.3
		Skin	0.1	0.7	0.9
3.	<i>Sardinella marderensis</i>	Gill	0.4	0.3	0.6
		Intestine	0.1	0.4	0.7
		Skin	0.0	0.3	0.4

Source: Field Survey: 2013

Key: CFU/g= colony forming unit per gram.

Table 3: Bacteria isolates from fish samples in the Ogun Waterside coastal fish value chain

S/N	Bacterial isolates	Fishermen	Fish Processors	Fish-marketers
1.	<i>Escherichia coli</i>	6(10.0%)	8(13.3%)	12(20.01%)
2.	<i>Proteus spp</i>	4(6.67%)	7(11.67%)	4(6.67%)
3.	<i>Bacillus subtilis</i>	11(18.3%)	4(6.67%)	7(11.67%)
4.	<i>Staphylococcus aureus</i>	12(20.0%)	18(30.0%)	22(36.67%)
5.	<i>Staphylococcus epididemis</i>	8(13.3%)	10(16.67%)	8(13.3%)
6.	<i>Pseudomonas spp</i>	7(11.7%)	10 (16.67%)	3(5.0%)
7.	<i>Micrococci spp</i>	9(15.0%)	6(10.0%)	2(3.33%)
8.	<i>Pseudomonas fluorescens</i>	3 (5.0%)	5(8.3%)	2(3.33%)

Source: Field Survey: 2013

Table 4: Fungal isolates from Clupeids at different stages along the Ogun Waterside coastal fish value chain

S/N	Fungal Isolates	Percentage Occurrence		
		Fisherman	Fish Processor	Fish-marketers
1.	<i>Aspergillus flavus</i>	16(26.67)	19(31.67)	20(33.3%)
2.	<i>Aspergillus niger</i>	21(35.0%)	24(40.0%)	26(43.3%)
3.	<i>Mucor spp</i>	8(13.3%)	5(8.3%)	2(3.3%)
4.	<i>Penicillium notatum</i>	11(18.3%)	9(15.0%)	10(16.67%)
5.	<i>Fusarium spp</i>	6(10.0%)	3(5.0%)	2(3.3%)

Source: Field Survey: 2013

Table 5: Colonial, cultural and morphological characteristics of bacteria isolated from different parts of fish samples from the Ogun Waterside coastal fish value chain

Colour	Shape	Elevation	Surface	Arrangement	Isolated Organism
Cream to opaque	Irregular	Raised	Smooth	Short rod in singles	<i>Escherichia coli</i>
Cream	Irregular	Flat	Smooth	Rod	<i>Proteus spp</i>
Cream to brown	Irregular	Slightly raised	Wrinkled	Rod in chains	<i>Bacillus subtilis</i>
Yellow	Round	Raised	Glossy	Cocci in clusters	<i>Staphylococcus aureus</i>
Creamy	Round	Raised	Glossy	Cocci in clusters	<i>Staphylococcus epididemis</i>
Translucent	Irregular	Raised	Mucoid	Rod	<i>Pseudomonas spp</i>
Creamy		Raised	Convex	Rod	<i>Micrococci spp</i>
Translucent	Irregular	Slightly raised	Mucoid	Cocci	<i>Pseudomonas fluorescens</i>

Source: Field Survey: 2013

Table 6: Biochemical test of the bacteria isolated from the fish samples

Organism	Gram	Motility	Catalase	Coagulase	Oxidase	Indole	Citruse Utilization	Sugar Fermentation
<i>E. coli</i>	-	+	+	-	-	+	-	N/A
<i>Proteus spp</i>	-	+	+	-	-	-	-	+
<i>Bacillus subtilis</i>	+	+	+	-	-	-	-	+
<i>Staphylococcus aureus</i>	+	-	+	+	-	-	-	N/A
<i>Staphylococcus epididemis</i>	+	N/A	+	-	-	-	+	+
<i>Pseudomonas spp</i>	-	+	+	+	+	-	-	N/A
<i>Micrococci spp</i>	+	N/A	+	-	+	N/A	-	+
<i>Pseudomonas fluorescens</i>	-	+	+	N/A	+	-	-	N/A

Source: Field Survey, 2013

Table 7: Morphological and cultural characteristics of moulds isolated from the fish samples from the Ogun Waterside coastal fish value chain

Fungal Isolates	Shape	Surface	Elevation	Colour of Spore	Mycellium Type	Reproduction	Septation	Appearance on plate
<i>Aspergillus flavus</i>	Circular	Powdery	Raised	Parrot green	Conidiospore	Sexual	Septate	
<i>Aspergillus niger</i>	Filamentous	Powdery	Raised	Black	Conidiospore	Sexual	Septate	Footcell
<i>Mucor spp</i>		Smooth	Raised		Sporangiospore	Sexual	Non-Septate	
<i>Penicillium notatum</i>	Circular	Fluff	Raised	Greenish blue	Conidiospore	Sexual	Septate	
<i>Fusarium spp</i>	Filamentous	Powdery	Raised	Yellowish brown	Conidiospore	Sexual	Septate	Creamy white

Source: Field Survey, 2013

#### 4.0 Discussion

##### Microbial analysis

Being the most abundant fish species in the coastal fisheries value chain, the Clupeids, *Sardinella maderensis*, *Ethmalosa fimbriata* and *Ilisha africana* were assessed for microbial load and microbial composition at each node of the fish value chain. The total count (in CFU/g) of bacteria and fungi present in the fish samples analyzed were high. The total bacterial counts for all the samples ranged from  $0.3 \times 10^6$  to  $4.7 \times 10^6$ , while the total fungi counts ranged from  $0.0 \times 10^6$  to  $1.1 \times 10^6$ . This findings agreed with the report of Ola and Oladipo (2004) and Ibrahim and Samia (2011) who reported higher bacteria load on studies conducted on fresh Croaker and Tilapia respectively. According to International Commission on Microbiological Specification for Food (ICMSF, 1986), the maximum recommended bacteria count for good quality fish product was  $5.0 \times 10^5$  ( $5.7 \log \text{ cfu/g}$ ) and the maximum for marginally acceptable quality fish product was  $1.0 \times 10^7$  ( $7 \log \text{ cfu/g}$ ). The bacteria load obtained from the fresh landed fish by the fisherman was higher than the marginally acceptable level; but closer to the maximum recommended value. Therefore, the experimental fish samples were not in their best condition for human consumption. This finding corroborated with the observations made during the survey at the landing sites with various unsanitary conditions observed at the coastal fishing communities.

The pH of the fresh fish samples (from the fisherman) ranged from slightly acidic to close to neutral. These pH ranges are known to generally favour the growth of microorganisms and may further explain the higher bacterial load in the fresh fish samples.

The total bacteria and fungi counts varied across the entire chain due to many factors including:

variations in processing operations and conditions, employments of different handling techniques, variation in length of time after processing and before sale, post processing exposure and storage time among others.

The results of this study showed the presence of microbes across all the stages of the value chain in the coastal area of Ogun state, Nigeria. The presence of microbes at the fisherman level was not surprising as the artisanal fisherman who went fishing for long periods of up to 24 hours had nothing on board to preserve the keeping quality of the fish. At their landing sites fishermen further increased the microbial load of the fish by employing different forms of handling techniques for sorting, grading and selling on the sandy soil before the fresh fish moved to the next stage of the chain. Moreover, the gills had the highest bacteria count compared to the skin and intestines at the fishermen's node due to the continuous contact of the gill with water. This explained the higher bacterial load in the gills of the fresh fish samples. The higher bacterial load found at the fish-marketers level may have resulted from post processing contamination and low sanitary compliance of the fish handlers. Other sources of contamination may be attributed to airborne microbes whose spores could easily settle on the exposed parts of the fish including the gill and skin.

Different genera of bacteria were isolated and identified from the fish samples obtained from 3 different nodes of the chain. Bacteria families included: *Escherichia coli*, *Bacillus subtilis*, *Micrococcus spp*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Proteus spp*, and *Pseudomonas fluorescens*.

*Staphylococcus aureus* and *Staphylococcus epidermidis* were isolated from all the stages of the chain and in all the species. This is in line with expectations as these two species naturally inhabit the

human body (Jay, 1978) and *S. epididemis* is part of the normal non-pathogenic flora of the human body. However, *S. aureus* inhabits the human skin and could be pathogenic, causing pneumonia, pimples, meningitis and arthritis, while some strains also cause food poisoning (Thomas, 1974).

*Micrococcus spp* were also present in the fish samples probably as a result of the presence of these microbes in plants and air. Its presence in fish samples may have resulted from fish's to air or post-processing contamination. Presence of Micrococci in fish for consumption is not encouraged as it could be pathogenic causing infection in the blood circulator system and central nervous system as reported by Young *et al* (1984) in Akinyemi (2000).

*Escherichia coli*, a food poisoning agent was observed. The presence of *E. coli* was an indication of faecal contamination. Its presence at the fishermen level may be as a result of its presence in the intestine of fish during consumption in the wild as the coastal water in the study area is the medium through which they defecate. Its presence at the other stages might have been from the soil, flies and hand or nails of the food handlers especially the nursing mothers who might not wash their hand well before turning fish during processing or marketing.

*Pseudomonas spp* were also isolated from the fish samples, it is a well known fish spoilage microorganism. It is found mostly in soil and water which is primary habitat of the fish before capture and its presence after processing might be due to unhygienic processing or handling activities of the various actors of the fish value chain.

*Proteus spp* was also isolated and it's of high spoilage importance in fish.

The occurrence of *Staphylococcus aureus*, *Penicillium sp* (moulds) and *Aspergillus niger* in the smoked-dried fish samples were in accordance with Martin (1994) when he stated that these organisms were the commonest microorganisms associated with smoked fish. The presence of *Staphylococcus aureus* in fish samples according to Okonko *et al.* (2008) might have been through contamination by handling.

The fungi isolated were mainly moulds (*Aspergillus spp*, *Mucor spp*, *Penicillium notatum* and *Fusarium spp*). They were harboured by almost all the fish samples used for the analysis. This may be due to the fact they possess spores that often survive unfavourable condition that may be provided by the only value addition activity carried out in the study area which was smoking. *Aspergillus niger* dominated the samples with a percentage occurrence of 37%, 43% and 46% at the fisherman, fish processors and fish marketers of the value chain this was observed in a similar study by Oduneye in 2010 who reported over 35% of *Aspergillus* dominance. The occurrence of

*Aspergillus sp*, *Rhizopus sp*, and *Penicillium sp* could be due to the fact that during storage, the fish sample reabsorbed moisture from the environment which then supported the growth of the microorganisms in addition to the contamination during processing, handling and unhygienic display on the market stalls (Christianah and Fagade, 2010).

## 5.0 Conclusion

The quality assessment carried out revealed that fish samples at all the chain nodes harboured microorganisms which are either of pathogenic, food poisoning, food spoilage or of epidemiological importance, hence; this study provides the knowledge on the microorganisms associated with each stages of the chain. Thus, consumers who consume these fish species in the CFVC without further cooking, washing or heating are at a risk of contracting food borne infections as a result of poor hygiene and poor post harvest handling on the part of the sellers.

## Correspondence to:

Odebiyi Oluwaseun Christianah  
Department of Aquaculture and Fisheries  
Management  
Federal University of Agriculture, Abeokuta  
P.M.B. 2240, Ogun State, Nigeria  
[Funksod4real@yahoo.com](mailto:Funksod4real@yahoo.com) [odebiyioc@funaab.edu.ng](mailto:odebiyioc@funaab.edu.ng)

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6/18/2013