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Microbial load of *Auchenoglanis biscutatus* and *Labeo coubie* bought from landings of artisanal fisherfolks at lower river Benue

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Abstract

This research was steered to examine the fungi and bacterial species present in the intestine of *Auchenoglanis biscutatus* and *Labeo coubie* from wadata landing site in Lower River Benue. Ten fish samples were aseptically gotten from artisanal fishermen fortnightly throughout the sampling period. The integrity of fish samples was maintained prior to transportation to the laboratory, Joseph Sarwuan Tarka University Makurdi, for microbiological analysis where the intestine of the fish samples was aseptically swabbed for microbial analysis. There was a significant difference ($p < 0.05$) in total viable count (TVC), total fungal count (TFC). However, no significant difference ($p > 0.05$) was observed for total coliform count (TCC). Total viable count and total fungal count from *Auchenoglanis biscutatus* were 174.70×10^4 cfu/g and 47.30×10^4 cfu/g respectively while total viable count and total fungal count for *Labeo coubie* recorded lowest values of 148.20×10^4 cfu/g and 35.70×10^4 cfu/g respectively. The highest microbial load was observed in *Auchenoglanis biscutatus*. There was variation in the diversity of bacterial and fungi from both species. A total of eight (8) species of fungi and bacteria were isolated from the intestine of both species. The isolated bacteria were gram negative (*E. coli*, *P. fluorescens*, *K. pneumonia*, and *P. vulgaris*) and Gram positive (*S. aureus*, and *B. pumilius*). While the fungi isolates were *Mucor mucedo* and *Rhizopus arrhizus*. The occurrence of these potential pathogenic bacterial and fungi in the intestine of the fish samples calls for public health concern. Therefore, hygienic measures should be employed during the handling and processing of the fishes. To improve the quality of fish before consumption, consumers are advised to ensure proper processing method and cooking before consumption as these will help reduced microbial load which can be of adverse health effect if not properly processed before intake.

Keywords: Microbial load, *Auchenoglanis biscutatus*, *Labeo coubie*, artisanal fisherfolks, river Benue

1. Introduction

The microbial impact on fish quality is one of the major problems faced among fish sellers and consumers. Microbial impact on fish quality is significant, as spoilage bacteria can lead to off-putting odors, flavors and texture changes. Proper handling, storage and hygiene practices are crucial to minimize microbial growth and maintain freshness. The contribution of fisheries resources in combatting food and nutrition insecurity amongst the rural dwellers in most developing countries is highly significant.

The microbial load of fungi and bacteria in fishes is a critical aspects of food safety and quality. Fishes, being highly perishable commodities are susceptible to microbial contamination that can compromise both their edibility and nutritional value. Understanding the dynamics of fungal and bacteria populations in fish is essential for ensuring the safety of seafood products and protecting public health.

Bacteria are ubiquitous in aquatic environments, and their presence in fish can stem from water contamination, handling practices, or processing facilities. Common bacterial species found in fish include *Escherichia coli*, *Salmonella*, and *Vibrio* spp. These microbes can multiply rapidly, especially in conditions conducive to their growth, such as inadequate storage temperatures or improper hygiene during processing. Similarly, fungi contribute to the microbial load in fish. Molds and yeast are prevalent contaminants that can affect the texture, flavor, and overall quality of fish.

As opportunistic microorganisms, they thrive in moist conditions, making fish susceptible to fungal infestations during various stages of handling and distribution.

The significance of monitoring microbial load in fishes extends beyond quality concerns to public health implications. Pathogenic bacteria like *Salmonella* and *Vibrio* can cause foodborne illnesses, posing risks to consumers. Therefore, regulatory bodies enforce strict guidelines to limit microbial contamination in fishery products, ensuring they meet safety standards. To mitigate microbial risks in fishes, proper hygiene practices must be employed throughout the supply chain. From catching and processing to storage and transportation, maintaining a clean environment and adhering to recommended temperatures are crucial.

These bacterial species are facultative pathogenic for both man and fish, and thus could be isolated without apparent signs and symptoms of disease (Novotny *et al.*, 2004) [12]. Qualitative and quantitative investigation of bacterial and fungal contaminants are often used to evaluate the safety level of fish consumption. Therefore, the need to assess the bio integrity of fish and products for public health is of utmost importance.

Different studies have been done on microbial load of different species of fish within and outside the Nigerian territorial waters by many authors. Some of these works includes that of Ayuba and Ataguba, (2012) [4], on *Clarias gariepinus* in River Benue at Makurdi, Benue State; Ogbonne *et al.*, (2020) [13], on *Tilapia guineensis* in Nigeria; Emikpe *et al.*, (2011) [7] also reported on *Clarias gariepinus* and *Oreochromis niloticus* in Ibadan. However, no work has been done on the microbial load of these two species.

Labeo coubie, commonly known as the African pike or Congo Tetra, is a freshwater fish species native to Africa. It belongs to the family Cyprinidae and is often found in rivers and lakes (Idodo-umeh, 2005; Ayotunde *et al.*, 2007) [11, 3]. It is known for its distinct silver coloration and elongated body. The *Labeo coubie* is popular among aquarium enthusiasts for its unique appearance.

Auchenoglanis biscutatus commonly known as the Giraffe catfish, is a freshwater catfish species native to Africa. This fish is recognized for its distinctive appearance, featuring a long slender body with a pattern resembling the markings on a giraffe. It is found in various African water bodies, including rivers and lakes. In aquarium hobby, *Auchenoglanis biscutatus* is sometimes kept by enthusiasts due to its unique appearance and behaviour.

The microbial load of fungi and bacteria in fishes is a multifaceted aspect that requires careful consideration to ensure the safety and quality of seafood products. Continuous monitoring, adherence to hygiene practices, and the application of advanced technologies are essential components of effective management strategies in the fisheries industry.

As consumer awareness grows and global trade in seafood expands, maintaining stringent control over microbial contamination becomes imperative for safeguarding public health and fostering confidence in the consumption of fish products. Therefore, identification of bacterial isolates and microbial load of fish species is of utmost importance not just to consumers but to fishmonger and also processors as some of these isolates are spoilage organisms. This study aims at determining the microbial load of these two species (*Auchenoglanis biscutatus* and *Labeo coubie*) obtained from lower River Benue landing sites at Makurdi.

2. Materials and Methods

2.1 Description of Study Area

This research work was carried out in the capital city of Benue State, Makurdi. Benues topography is mainly undulating plains with occasional elevations of between 1500 m and 3000 m above sea level. The state main geological formations are sandy-loam shelf basement complex and alluvial plains. Makurdi is located in Benue valley within latitude 7°44'N and longitude 8°54'E, and is drained by river Benue and many tributaries due to the general low relief of the area. A large portion of the area is waterlogged and flooded during heavy rainstorms. River Benue is the second largest River in Nigeria. It extends from the Adamawa mountains of Cameroon 500 Km beyond the Nigeria frontier and flows eastwards through Cameroon 800 Km of Nigeria territory before joining Nigeria at Lokoja in Kogi State. The river has extensive alluvial plain stretching for many kilometers, which covers a distance of approximately 187 kilometers. The highest water level is in August to September and the lowest are in March to April.

2.2 Collection of Samples

Ten samples were collected monthly (November, 2022 – January, 2023) each of *Labeo coubie* and *Auchenoglanis biscutatus*, were gotten from artisanal fishermen at the Wadata landing site of Lower River Benue in Makurdi fortnightly. The fish samples were kept in ice chest bags and were taken to the microbiological laboratory, Federal University of Agriculture Makurdi for Microbiological analysis.

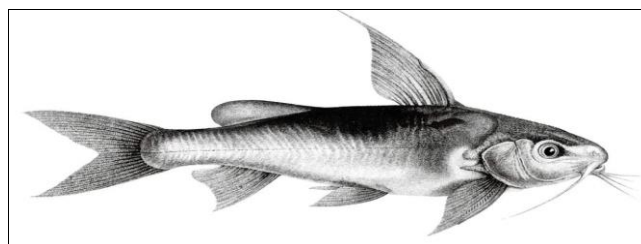


Fig 1: Diagram showing *Auchenoglanis biscutatus*

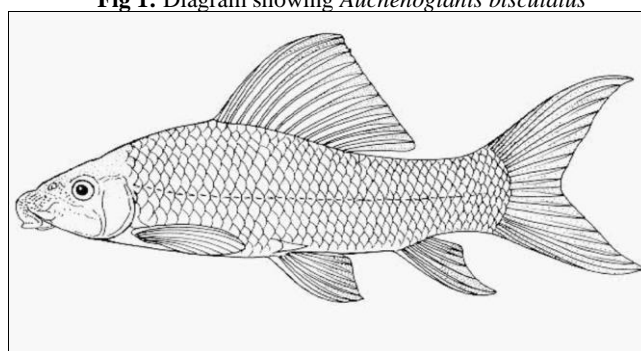


Fig 2: Diagram showing of *Labeo coubie*

2.3 Media Preparation

2.3.1 Preparation of Materials

All the equipment's used were thoroughly clean with ethanol to disinfect them and air-dried after which they were sterilized in hot air oven at 160 °C for 1hour.

2.3.2 Preparation of Fish Samples for Microbiological tests

Triplicates samples were collected from the samples obtained from the artisanal fishermen. The intestine was minced

following serial dilution. After which, total viable count and coliform count was done, colonies on the plates were picked and sub-cultured and then identified.

2.3.3 Preparation of Nutrient agar

This was prepared by weighing 28 g (Himedia, India) of the powder in a 1000 ml conical flask, distilled water was added to 500 mls marks, shaken until all the powder agar was suspended in the distilled water and this was made up to 1000 ml, heated over a laboratory hot plate and sterilized using autoclave (Nixon, Germany) at 121 °C, 15lb for 15 minutes. This was cool in a water bath at 42 °C before use.

2.3.4 Preparation of Potato Dextrose Agar

This was prepared by weighing 40 g (Himedia, India) of the powder in a 1000 ml conical flask, distilled water was added to 500 mls marks, shaken until all the powder agar was suspended in the distilled water and this was made up to 1000 ml, heated over a laboratory hot plate and sterilized using autoclave (Nixon, Germany) at 121 °C, 15lb for 15 minutes. This was cool in a water bath at 42 °C before use.

2.3.5 Preparation of MacConkey agar

This was prepared by weighing 51.1g (Himedia, India) of the powder in a 1000ml conical flask, distilled water was added to 500 mls marks, shaken until all the powder agar was suspended in the distilled water and this was made up to 1000 ml, heated over a laboratory hot plate and sterilized using autoclave (Nixon, Germany) at 121 °C, 15lb for 15 minutes. This was cool in a water bath at 42 °C before use.

2.4 Total Aerobic Plate Count

The bacteria load of the fish samples was determined using surface plating method (Spread plate) (El-Mahmod, 2009) [8]. Serial dilution of the fish samples was done by pipetting 1g of the fish samples into 9mls of sterile normal saline. Then 1 ml of stool saline suspension was pipetted into the next test tube carrying 9 ml sterile normal saline in the next test tube carrying 9 ml sterile normal saline in that order in a ten-fold serial dilution up to 10⁻⁵ in order to have countable colonies. One milliliter of the of the final dilution was inoculated in Nutrient, MacConkey, Potato Dextrose agar in a 90mm petri dish using pour plate method. The plates were allowed to set and incubates at 35-37 °C for 24 hours, after which the colonies were counted and plate counts were calculated in colony forming unit per gram of sample using the function.

$$\text{Total Aerobic plate count} = \frac{\text{Number of colonies}}{\text{Volume of Inoculum}} \times \text{Dilution factor}$$

After plate count discrete colonies were picked and streaked on the various media to produce pure cultures. The pure cultures were further sub-cultured on nutrient agar plates for biochemical test and slide preparation.

2.5 Gram staining/ Microscopy

A thin smear of the test isolate was prepared by emulsifying a 24 hours old culture from a pure colony on the culture plate into a drop of sterile water on a clean, grease-free slide. The smear was allowed to air dry and then heat fixed by passing then reverse side of the slide quickly over a flame for 3-4 times. Lugol's iodine was added as a mordant and left for a minute, it was rinsed with excess water and then the stained was decolourized with 95% alcohol for 5seconds and was quickly rinsed with excess water. The slide was the flooded

with excess safranin for a minute, then rinsed with water to remove the secondary stain, the excess water drained and allowed to air-dry. The smear preparation was observed under the light microscope using the oil immersion (x 100) objective.

2.6 Biochemical Test

The following biochemical test were carried out on the pure cultures. They include; catalase, citrate, urease, indole, motility test, methyl red and oxidase.

2.6.1 Catalase Test

A smear of 24-hour old culture was made on a grease-free clean microscopic slide and three drops of 3% hydrogen peroxide (H₂O₂) was added to the smear on the slide. The production of effervescence was indicative of the production of catalase enzyme that breaks down the H₂O₂ to release oxygen (Cheesbrough, 2005) [6].

2.6.2 Motility Test.

The test was carried out to determine the presence of or absence of flagella an organelle of movement in the bacteria isolates. A drop of peptone water was dropped on a grease-free microscopic slide into which a colony of 24 hours of the test organism was mixed and covered with a cover slip after a minute. Then, it was viewed microscopically with high power objective lens of x 40.

2.6.3 Indole Test

Several organisms possess tryptophanase enzyme which aids them to hydrolyze the amino acid tryptophan. This test is useful in the identification of bacteria. The experimental animal was inoculated into bijoux bottles containing 5ml of sterile peptone water and incubated at 37 °C for forty-eight hours; 0.5 ml of Kovac's reagent was then added and shake gently and then observed. (Cheesbrough, 2005) [6].

2.6.4 Citrate utilisation test

This test was carried out to check the ability of organisms to utilize citrate as a sole source of carbon using Simmon's citrate medium. The isolates were inoculated aseptically using a sterile wire loop into vials containing the Simmon's citrate medium and incubated at 37 °C and incubated for 48 hours and observation made and recorded. This test is commonly used to differentiate *Klebsiella* which is positive (+) from *Escherichia coli* which is negative (-) (Cheesbrough, 2005) [6].

2.6.5 Urease test

This is a common test used in the identification of several genera and species of bacterial, including *Proteus*, *Klebsiella*, and some *Yersinia* and *Citrobacter* species. The development of ammonia alters the pH medium to alkaline, and the pH shift is observed by the color change of phenol red (light orange at pH 6.8 to pink at pH 8.1). Rapid urease-positive organisms undergo a spontaneous change within 24 hours to medium pink while weak-positive organisms may take several days. Negative organisms do not produce any colour change, but in rare cases may change to yellow due to acid production. The Christensen urease medium was used. The 24-hour old test isolate was streaked onto the surface of a urea agar slant which was loosely capped and incubated t at 37 °C in ambient air for 48 hours to 7 days. (Cheesbrough, 2005) [6].

2.7 Identification of bacteria Isolates.

Bacteria were identified using cultural (colony colour, shape

and elevation,) morphological (grams reaction, morphology and motility) and biochemical characteristics (Catalase, citrate, urease, indole, hydrogen sulphide production) characteristics. Fungi on the other hand were identified using their microscopic and macroscopic characteristics.

2.8 Data Analysis

The results on microbial load were subjected to students T-test using the statistical package for social science (SPSS) version 21.0. The separation of individual mean was done using LSD method. Results are presented in charts and tables.

3. Result

3.1 Total Viable Count, Total Coliform Count and Total Fungi Count of The Isolated Bacteria

Considering the parameters, it was found that the means value of Total viable count (TVC), Total coliform count (TCC) and total fungi count (TFC) of *Labeo coubie* and *Auchenoglanis*

biscutatus, as shown in table 1 were significantly different ($p < 0.05$) throughout the sampling months (November, 2022 - January, 2023) exception of TFC which showed no significant difference in both species in January, 2023.

The overall results showed no significant difference ($p > 0.05$) in total coliform count (TCC) of both species. However, the Total viable count (TVC) and Total fungi count (TFC) of both species shows significant difference ($p < 0.05$). The result revealed that *Auchenoglanis biscutatus* had the highest total viable count (TVC) and total fungi count (TFC) of 174.70×10^4 cfu/g and 47.30×10^4 cfu/g respectively. However, *Labeo coubie* had the lowest TVC and TFC of 148.20×10^4 cfu/g and 35.70×10^4 cfu/g respectively compared to that of *Auchenoglanis biscutatus*. The results revealed that microbial loads were significantly higher in *Auchenoglanis biscutatus* as compared to *Labeo coubie* obtained from the same study location.

Table 1: Mean Variations in the Microbial Load of two Freshwater Fish *Labeo coubie* and *Auchenoglanis biscutatus* bought from Artisanal Fisherfolk at lower River Benue

Variable/Month	Fish species		Df	T-Value	P-Value
	L. Coubie	A. Biscutatus			
November					
TVC	158.50±9.50	214.00±2.00	2	5.72	0.01*
TCC	110.00±6.00	116.00±4.00	1	0.83	0.05*
TFC	37.50±5.50	58.00±2.00	1	3.50	0.03*
December					
TVC	128.00±4.00	180.00±8.00	2	5.81	0.01*
TCC	80.00±2.00	94.00±2.00	1	1.41	0.02*
TFC	23.50±2.50	36.00±2.00	1	3.90	0.04*
January					
TVC	158.00±6.00	130.00±6.00	2	3.30	0.01*
TCC	100.00±4.00	68.50±0.50	1	7.81	0.03*
TFC	46.00±2.00	48.00±4.00	1	0.45	0.06
Overall (Nov, '22 – Jan, '23)					
TVC	148.20±7.10	174.70±1.60	6	1.54	0.02*
TCC	100.00±4.10	92.70±8.80	7	0.75	0.07
TFC	35.70±4.50	47.30±4.20	9	1.90	0.05*

*Indicates statistical significance at 95% CL

3.2 Biochemical, Morphological and Cultural Characterization of Bacteria Isolates

Both gram negative and positive bacteria were observed.

Bacteria isolates were characterized by biochemical test. These tests were catalase, indole, citrate, urease, oxidase and hydrogen sulphide (H₂S) test (Table 2).

Table 2: Biochemical, Morphological and Cultural Characterization of Bacteria Isolates of Two Freshwater Fish *Labeo coubie* and *Auchenoglanis biscutatus* bought from Artisanal Fisherfolk at Lower River Benue

Colony colour	Colony shape	Morphology	Gram Reaction	Catalase	Citrate	Urease	H ₂ S	Indole	oxidase	Bacteria Species
Cream Nutrient Agar	Circular	Cocci	+	+	+	-	-	-	-	<i>Staphylococcus</i> Species
Pale Macconkey Agar	Circular	Rod	-	+	+	+	+	-	-	<i>Proteus</i> Species
White on Nutrient Agar	Irregular	Rod	+	+	+	-	-	-	-	<i>Bacillus</i> Species
Green Nutrient Agar	Circular	Rod	-	+	+	-	-	-	+	<i>Pseudomonas</i> Species
Pink on Macconkey Agar	Circular	Rod	-	+	-	-	-	+	-	<i>Escherichia coli</i>

3.3 Percentage Prevalence of Bacteria and Fungi Isolate in *Auchenoglanis Biscutatus* and *Labeo Coubie*

Table 3 shows the Percentage Prevalence of bacteria and fungi isolates in both species. Eight bacteria and fungi isolate namely, *Pseudomonas fluorescens*, *Bacillus pumilius*, *Staphylococcus aureus*, *Proteus vulgaris*, *Mucor mucedo*, *Escherichia coli*, *Klebsiella pneumonia* and *Rhizopus arrhizus*, were identified in both species using their cultural, morphological and biochemical characteristics (Table 2). Bacterial isolates such as *Proteus*, *Bacillus* and *Klebsiella*

were more prevalent in *Labeo coubie* while the likes of *Mucor*, *Escherichia coli*, *Rhizopus species*, *Pseudomonas* and *Staphylococcus aureus* were more prevalent in *Auchenoglanis biscutatus*. However, *Bacillus pumilius* has the highest percentage prevalence of 18.54 in *Labeo coubie* while *Pseudomonas fluorescens* recorded the lowest value, 5.79. Also, in *Auchenoglanis biscutatus*, *Rhizopus arrhizus* recorded the highest percentage prevalence of 19.23 while *Bacillus pumilius*, and *Klebsiella pneumoniae* recorded lowest value of 6.60 for each.

Table 3: Percentage Prevalence of Microbes in two Freshwater Fish *Labeo coubie* and *Auchenoglanis biscutatus*

Microbes	Percentage Prevalence of the Microbes Species Isolated	
	<i>L. coubie</i>	<i>A. biscutatus</i>
<i>Staphylococcus aureus</i>	11.57	13.21
<i>Proteus vulgaris</i>	17.98	14.10
<i>Bacillus pumilus</i>	18.54	6.60
<i>Escherichia coli</i>	11.80	14.10
<i>Mucor mucedo</i>	11.24	13.21
<i>Rhizopus arrhizus</i>	11.57	19.23
<i>Klebsiella pneumoniae</i>	11.24	6.60
<i>Pseudomonas fluorescens</i>	5.79	13.21

4. Discussion, Conclusion and Recommendation

4.1 Discussions

In this study, the micro-organisms found to be associated with both species includes, *Pseudomonas*, *Bacillus pumilus*, *Staphylococcus aureus*, *Proteus vulgaris*, *Mucor mucedo*, *Escherichia coli*, *Klebsiella pneumoniae* and *Rhizopus arrhizus*. The fungi and bacteria isolates identified from this study were similar to earlier assertion reported by Tivkaa and Sampson (2013) [16] and Osungbemiro *et al.*, (2014) [15]. Similarly, the result of the present study is in consonance with the work of Olugbojo and Ayoola (2015) [14]. The microbial load of fungi and bacteria isolated in the studied species is a multifaceted aspect that requires careful consideration to ensure the safety and quality of these products. Some of these microorganisms are known to be specific spoilage organisms example includes *Pseudomonas*, *Bacillus*, *E. coli*. These organisms have the potential of deteriorating the quality of the fish which gives it an unpleasant odour, thereby affecting the taste of the fish. The presence of these microbes encountered during the study may be attributed to the discharge of poultry waste and sewage directly into the water from which both species were caught.

However, it was observed that higher loads of the microbes isolated were recorded for *Auchenoglanis biscutatus* compared to *Labeo coubie*. This may be attributed to species preference and the individual immunity of each species.

According to Eze *et al.*, (2011) [10], consuming fish with a high microbial count can pose health risks, leading to foodborne illness. Bacterial contamination may cause symptoms like nausea, vomiting, diarrhea or more severe issues. The result of microbial population of 10^4 cfu/g recorded in this study agrees with the findings of Osungbemiro *et al.* (2014) [15], that reported similar counts of 10^4 cfu/g for *C. gariepinus* in fresh and brackish water habitats of Ondo State. Similarly, Adedeji *et al.* (2011) [2] reported lower bacterial population in a related study. Based on the values of microbial load (TVC, TCC, and TFC) recorded for *Auchenoglanis biscutatus* and *Labeo coubie* during the study which were lower than the recommended 10^6 cfu/g, hence, both species were considered suitable for consumption.

Moreover, results from this study showed that fish can be infected with different species of bacterial, which is a reflection of microbes found in their habitat (Abidemi and Fofah, 2016) [1]. Occurrence of the coliform group such as *klebsiella* and *E. coli* can constitute a public health hazard. The manifestation of *Staphylococcus* spp. observed from both species can be attributed to improper hygiene practices from catching and processing to storage and transportation this assertion is in consonance with the findings of Eze *et al.*

(2011) [10]. The identification of *E. coli* in the fish samples is an evidenced of organic pollution mainly due to faecal contamination (Carla and Maria, 2016) [5]. *E. coli* was identified in both *Auchenoglanis biscutatus* and *Labeo coubie* during the study duration. Therefore, we can deduce that the lower River Benue Makurdi is severely impacted by anthropogenic activities notably sewage, domestic wastes and direct open defecation. Osungbemiro *et al.* (2014) [15] in a related studies also isolated *E. coli* which he attributed to evidence of organic pollution.

However, the presence of bacteria isolated from the studied species fell within acceptable range which does not pose a threat to consumer health. To minimize bacterial risks when consuming fish, ensure it is properly cooked to kill the bacteria present. Freshness, proper storage, maintaining a clean environment and adhering to recommended temperatures for storage are crucial to prevent bacteria contamination.

4.2 Conclusion

Excessive microbial load in fish can lead to various health issues, including infections, disease and compromised immune systems. It may result from poor water quality, inadequate hygiene in handling or stressful environmental conditions. Maintaining optimal water quality and hygiene during handling and processing is crucial for minimizing the impact of microbial load on fish health. Based on the result of findings, it can be concluded that microbial loads in *Auchenoglanis biscutatus* is higher than that of *Labeo coubie* obtained from landings of fisherfolks at the Wadata landing site of lower River Benue in Makurdi which makes them unsafe to consume even though the examined microbial load are within the recommended levels. Qualitative and quantitative investigation of bacterial and fungal contaminants are often used to evaluate the safety level of fish. Therefore, to improve the quality of fish before consumption, consumers are advised to ensure proper processing method and cooking before consumption as these will help reduced microbial load which can be of adverse effect if not proper processed before intake. Also, hygienic measures should be ensured when harvesting and handling to help reduced infestation by microbes.

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