



## MICROBIOLOGICAL QUALITY OF THREE FRESHWATER FISH SPECIES FROM TWO LOCAL MARKETS IN REGION 6, (CORENTYNE, EAST BERBICE) GUYANA

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### ABSTRACT

Present case study was carried out during 2013, to assess the microbiological quality of three freshwater fishes (*Hoplosternum littorale*, *Cichlasoma bimaculatum* and *Hoplias malabaricus*) offered for sale at two local markets in region 6. Fishes were examined for microbial quality and compared to the recommended levels of U.S Food and Drug Association (FDA) and Environmental Protection Agency (EPA) for fish and fisheries product. The results revealed based on the statistical test CHI-SQUARE that the microbial levels for the three freshwater fishes deviated significantly from U.S FDA and EPA expected standard. ANOVA done on the results further showed that there was no significant difference in coliform level among fish species and markets and for *E. coli* level, there was also no significant difference except at  $p < 0.01$ . In conclusion, the three freshwater fishes offered for sale at local markets were not of acceptable standard based on U.S FDA and EPA Acceptable level and thus implores risk to human health.

**Keywords:** Microbiological quality, Freshwater fishes, Coliform, *E. coli*, *S. aureus*.

### Contribution/ Originality

This case study contributes towards the validation of microbial assessment of freshwater fishes in Guyana. The contribution as highly significant in the area of food microbiology.

### 1. INTRODUCTION

Microbial quality of fishes is an essential component during the process of handling. Although it is believed that the freshly caught fish are free of bacteria, several studies have demonstrated many bacterial species encountered in different fishes which are potentially pathogenic under certain conditions as reported for *Pseudomonas anguilliseptica* and *Streptococcus* spp [1-4]. However, most studies which investigated the bacterial flora and its influence on fish spoilage have been confined largely to marine species and little attention has been paid to

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freshwater fishes, particularly in the case of tropical species [4, 5]. *Streptococcus iniae* and *Streptococcus agalactiae* have been shown to cause significant morbidity and mortality among a variety of freshwater and saltwater fish species throughout the world: affecting tilapia, coho salmon, rainbow trout, snapper, yellowtail catfish and mullet [6]. Local markets may have dirty, damp and unhealthy place, poor storage, display and packaging facilities, which encourage microbial contamination from different sources [4, 7]. Freshly caught fish sold in market can be further infected during transportation or storage and people associated with handling them may also become affected. In Guyana freshwater fishes comprise a major source of fish protein and this is evident by the large quantities and varieties sold in market places [8]. With the growing usage of agricultural chemicals, improper sanitary condition and industrial processes, the aquatic environment is exposed and susceptible to many physical, chemical and biological risk factors [9]. This threat to the aquatic environment poses threat not only to the aquatic medium but also to the fishes in these environments. Bacterial contamination of the water can also be transferred to the fish thereby presenting health risks to humans [8, 10]. Like most local markets, markets in Region 6 do not have cold storage facilities for the fish that is offered for sale. As such this places a burden on the vendor to have the fish sold out or suffer spoilage. The absence of cold storage facilities further exposes the fish to bacterial contamination and proliferation since bacteria proliferate under certain specific conditions especially when they are kept out of cold storage facilities [4, 7]. Potential bacterial pathogen that have been found in fishes are *Streptococcus iniae*, *Vibrio cholera*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., and *Vibrio parahaemolyticus*.

Disease outbreaks can cause severe economic losses not only from mortality but also treatment expenses postponement or loss of the opportunity to sell a particular fish. With vast amount of food-borne illness associated with seafood, there is a need to further understand freshwater fishes so as to prevent the spread of diseases. To date, there is no evidence that a bacteriological study of freshwater fishes offered for sale in markets has been conducted in Guyana. Given the importance of such a study for the health and welfare of humans it is vital that this study was conducted. Thus, a study of microbiological quality of fish in the market environments may be opportune given the health concerns and implications. This study aimed at the microbiological assessment of three local freshwater fishes: *Cichlasoma bimaculatum* (Patwa), *Hoplias malabaricus* (Trahira) and *Hoplosternum littorale* (Hassar) at markets in region 6 (Corentyne, East Berbice) Guyana.

## 2. MATERIALS AND METHOD

The present case study was carried out during 2013 to investigate the microbiological quality of three local freshwater fishes: *Cichlasoma bimaculatum* (Patwa), *Hoplias malabaricus* (Trahira) and *Hoplosternum littorale* (Hassar) in relation to market conditions in region 6. Samples were collected from Skeldon and Rose Hall in region 6. Skeldon and Rose Hall were both large markets with other dynamic activities (goods and services) besides the selling of freshwater fishes. Skeldon

market seemed sometimes to be the main supply of freshwater fishes to other markets of region 6 including Rose Hall –this was observed on collection at 6 am in the morning when some vendor waited for the arrival of fishes from Skeldon. Unlike Rose Hall, Skeldon market's vendors observed sanitary measure for the selling of their freshwater fishes. At Skeldon, Hassar was kept alive in tub or small tank and Patwa and Trahira were stored in non-working (old) refrigerator with ice while at Rose Hall Market fishes were sometimes stored in bucket and on the counter for sale.

Six (6) of each fish (Patwa, Trahira and Hassar) was collected from both markets between 6:55am- 8:25am. These species were collected once within the months of March and April, 2012. Each fish was collected with sterile glove, stored in sterile plastic bag and placed under ice, then transported to the Ministry of Health- Department of Food and Drug for microbiological analysis. Before analysis, each fish was weighed and the length was taken. Overall, there were six (6) samples (three from Skeldon and three from Rose Hall), along with their average weight and length. Samples were prepared by aseptically removing a piece of the tissue layer which included the muscle, skin and scales was removed with a scissors to form a homogeneous sample of 25g, which were placed in sterile plastic bags. Microbiological analysis was carried out based on previously established method recommended by the Department of Food and Drug to determine *S. aureus*, coliform and *E. coli* and *Salmonella* spp. [11-13].

Data obtained between fish species of Skeldon and Rose Hall markets were subjected to Statistical analysis of the *CHI-SQUARE* test and ANOVA Two Factor without replication. Additionally, data from microbiological assessment was compared to the acceptable Standard of United States (U.S.) Food and Drug (FDA) & Environmental Protection Agency (EPA) safety levels in regulation and guidance for fish and fisheries product [14].

### 3. RESULTS AND DISCUSSION

Six (6) of each fish (Patwa, Trahira and Hassar) were collected from both markets between 6:55am- 8:25am. These species were collected once within the months of March and April, 2013. Length, weight and weight-length ratio for Hassar, Patwa and Trahira collected from Skeldon and Rose Hall markets. It was found that Trahira, which is a large freshwater fish among Patwa and Hassar, had the highest weight-length ratio from Rose Hall Market in April while Hassar had the smallest weight-length ratio in March. According to Walker [15] a low standard deviation indicates that the data points tend to be very close to the mean whereas a high standard deviation indicates that the data points are spread out over a large range of values. In the case above, it is observed that the standard deviation of the length and the weight of each fish sample did not deviate from the mean and the data points are within a close range (Table 1).

It is observed in Table 2 that the coliform in April is higher than that of March with Rose Hall (April) having a TNTC value ( $>6.5 \times 10^5$ ). Clearly, this indicates that the freshwater fishes in March were of better quality compared to that of April based on the coliform count. Further, it

was observed that between markets within months, there were no vital differences of the coliform count between fish species. This shows that the fish quality of each species was similar between markets. According to Tortora, et al. [16] TNTC value exceeds Food and Drug Administration convention recommended range for plate count (25 to 250 colonies) (Table 3).

**Table-1.** Length and weight dynamics of three fish species collected from from Skeldon and Rose Hall markets

Fish species	Sampling Sites						Sampling Sites					
	Skeldon (March)		Weight: length ratio	Rose Hall (March)		Weight: length ratio	Skeldon (April)			Rose Hall (April)		
	Length*	Weight*		Length*	Weight*		Length*	Weight*	Weight: length ratio	Length*	Weight*	Weight: length ratio
<i>Hoplosternum littorale</i> (Hassar)	9.97 ± 0.82	42.92 ± 3.17	4:1	8.78 ± 0.74	29.21 ± 7.51	3:1	11.62 ± 0.36	48.30 ± 5.60	4:1	11.27 ± 0.37	49.95 ± 7.39	4:1
<i>Cichlasoma bimaculatum</i> (Patwa)	9.08 ± 1.05	56.75 ± 6.92	6:1	10.15 ± 0.57	82.38 ± 15.70	8:1	10.0 ± 0.71	51.16 ± 7.64	5:1	10.85 ± 0.92	64.59 ± 14.99	6:1
<i>Hoplias malabaricus</i> (Trahira)	20.6 ± 1.19	139.58 ± 6.92	8:1	16.67 ± 0.52	87.19 ± 28.02	5:2	18.8 ± 1.25	108.84 ± 16.65	6:1	20.53 ± 1.98	193.76 ± 71.03	9:1

\*weight was measured in gram and length was measured in cm.

**Table-2.** Total coliform count for three fish species from Skeldon and Rose Hall markets

Fish species	Total Coliform (TC) on fish samples			
	Sampling Sites		Sampling Sites	
	Skeldon (March)	Rose Hall (March)	Skeldon (April)	Rose Hall (April)
<i>Hoplosternum littorale</i> (Hassar)	0.0039*	0.030*	0.570*	>6.50*
<i>Cichlasoma bimaculatum</i> (Patwa)	0.017*	0.0205*	0.395*	0.510*
<i>Hoplias malabaricus</i> (Trahira)	0.00215*	0.0027*	0.355*	0.720*

\*X10<sup>5</sup>cfu/25g, each value is a mean of duplicated readings of 6 fish sample, >6.5x10<sup>5</sup> represents the Too Numerous to Count (TNTC) value or threshold.

A comparison between the U.S FDA & EPA for good quality of fish or fisheries product along with a comparison of the bacterial load of coliform between fish species and Markets in March and April. It is found that the coliform count for all freshwater fishes in March and April of both markets exceeded U.S. FDA & EPA acceptable limit ( $\leq 330/g \equiv \leq 0.000825 \times 10^5 / 25g$ ). Therefore, U.S. FDA & EPA is suggesting that these freshwater fishes from Skeldon and Rose Hall markets are not of good quality and are unsafe for consumption. The results correlates with earlier works [7, 17]. The contamination of fishes may be due to environmental conditions during transportation to landing centers and whole sale markets through handling process that may be associated with infection [7, 17, 18].

**Table-3.** *E. coli* count for three fish species from Skeldon and Rose Hall Markets

Fish species	Total <i>Escherichia coli</i> (T.E.C) on fish sample			
	Sampling Sites		Sampling Sites	
	Skeldon (March)	Rose Hall (March)	Skeldon (April)	Rose Hall (April)
<i>Hoplosternum littorale</i> (Hassar)	0.001*	Nil	0.1*	3.0*
<i>Cichlasoma bimaculatum</i> (Patwa)	0.01*	0.01*	0.35*	2.05*
<i>Hoplias malabaricus</i> (Trahira)	Nil	0.0015*	0.15*	0.95*

\* X10<sup>3</sup>cfu/25g, each value is a mean of duplicated readings of 6 fish sample.

\*data exceeded U.S. FDA & EPA recommended level ( $\leq 330/g \equiv \leq 0.00825 \times 10^4/25g$ )

The bacterial count in April revealed that *E. coli* was higher on freshwater fishes when compared to March. This result in Table 3 is similar to the result obtained for coliform and also indicates that the above fishes were of better quality in March than April based on *E. coli* count. It was also observed that within months between markets showed no vital differences of the *E. coli* count between fish species. This infers that the fish quality of each species was similar between markets. However, it is important to note that in March, Trahira was free from *E. coli* at Skeldon market and Hassar was free from *E. coli* at Rose Hall market. This may be attributed to improved market situations in March compared to other times indicating inconsistency in hygienic conditions in developing country like Guyana with dirty, damp, poor storage, display and packaging facilities which encourage microbial contamination from different sources [7].

A comparison between the U.S FDA & EPA for good quality of fish/fish product along with a comparison of the bacterial load of *E. coli* between fish species and Markets in March and April. It is found that the *E. coli* count for all freshwater fishes exceeded the U.S. FDA & EPA acceptable limit ( $\leq 330/100g \equiv \leq 0.00825 \times 10^4/25g$ ) except for Hassar and Trahira in Skeldon (March) and Rose Hall (March). Hence, U.S. FDA & EPA is suggesting that these freshwater fishes from Skeldon and Rose Hall markets are not of acceptable standard except for Hassar and Trahira in the month of March. The present findings are in agreement with reports of Shewan [19]; Shewan [20] and Begum, et al. [7].

**Table-4.** *S. aureus* count for the three fish species from Skeldon and Rose Hall Markets

<i>S. aureus</i> on fish samples					
		Sampling Sites (1 <sup>st</sup> batch)		Sampling Sites (2 <sup>nd</sup> batch)	
		Skeldon (March)	Rose Hall (March)	Skeldon(April)	Rose Hall (April)
Fish species	Samples				
<i>Hoplosternum littorale</i> (Hassar)	A	>6.5	>6.5*	>6.5*	>6.5*
<i>Cichlasoma bimaculatum</i> (Patwa)	B	>6.5*	>6.5*	>6.5*	>6.5*
<i>Hoplias malabaricus</i> (Trahira)	C	>6.5*	>6.5*	>6.5*	>6.5*

\*  $10^6cfu/25g$ , each value is a mean of duplicated readings of 6 fish sample,  $>6.5 \times 10^5$  represents the too numerous to count (TNTC) value or threshold.

It is observed (Table 4) that there was no difference of the bacterial count between Markets and fish species. However, this was a result of the TNTC values ( $>6.5 \times 10^5$ ) that exceed Food and Drug Administration convention recommend range (25-250 colonies). A comparison between the U.S FDA and EPA for good quality of fish/fish product along with a comparison of the bacterial load of *S. aureus* between fish species and Markets in March and April. It was found that the bacterial count for *S. aureus* exceeded U.S FDA and EPA recommended level ( $\leq 1 \times 10^4/g \equiv \leq 2.5 \times 10^5/25g$ ) and as a result, these freshwater fishes from Skeldon and Rose Hall markets are not of good quality and may be potentially unsafe for consumption based on U.S. FDA and EPA acceptable Standard.

**Table-5.** Qualitative analysis for *Salmonella* spp., for the three fish species from Skeldon and Rose Hall markets

Qualitative analysis for <i>Salmonella</i> spp*				
Fish species	Sampling Sites		Sampling Sites	
	Skeldon (March)	Rose Hall (March)	Skeldon (April)	Rose Hall (April)
	Salmonella Occurrence		Salmonella Occurrence	
Hoplosternum littorale (Hassar)	+	+	+	+
Cichlasoma bimaculatum (Patwa)	+	+	+	-
Hoplias malabaricus (Trahira)	+	+	+	+

\* Each data was a triplicated qualitative reading obtained from different agar plates from 6 fish sample (25g of samples), + = positive for *Salmonella* spp., - = negative for salmonella spp.

The higher values may suggest that hygienic conditions are not suitable in local markets that indicate the substantial presence of such microbial flora and are in agreement with the works of [21-24].

The results in Table 5 indicated that all freshwater fishes tested positive for *Salmonella* spp., except for Patwa which tested negative only once in April for Rose Hall market. Therefore, infers that these freshwater fishes are of poor quality except Patwa in Rose Hall (April) based on U.S FDA and EPA recommended level [14] which suggests that *Salmonella* spp., is highly pathogenic and should not be present on fish/fish products at all and as such, these freshwater fishes, according to U.S. FDA and EPA acceptable limit can impose high risk to public health since *Salmonella* spp., is of major public health concern [4]. The present research correlates with the earlier works of Begum, et al. [7] where the tests were positive for the local markets. On the other hand, samples from super markets tested negative that may be attributed to good processing, handling and storage conditions [7].

When Data subjected to ANOVA Two Factor without replication for the coliform and *E. coli* count of Hassar, Patwa and Trahira of both markets, the p values (0.39 and 0.29) were greater than 0.01 between species and within markets-months for bacterial count of coliform respectively. For *E. coli* count, p value (0.44) was greater than 0.01 between species but less than 0.01 within markets-months. As a result, there was significant difference of fish quality within markets-months (especially April) based on *E. coli* count but no significant differences based on the other p values between fish species of both coliform and *E. coli* and no difference within markets-months for coliform count. Overall, this infers basically that Hassar, Patwa and Trahira from Skeldon and Rose Hall markets were of the same quality based on the bacterial count of coliform and *E. coli* except for when  $p < 0.01$  because of *E. coli* count in April of the three freshwater species. The *S. aureus* count and qualitative analysis of *Salmonella* also gave inference to this same notion of Skeldon and Rose Hall markets since no vital differences of these two microbes observed between species and within market-months.

It has been well known that both fresh and brackish water fishes can harbor human pathogenic bacteria particularly the coliform group [4]. Pathogenic bacterial isolates like *Salmonella* spp., *S. aureus* and *E. coli* presence on Hassar, Patwa and Trahira from Skeldon and

Rose Hall markets are organisms of public health concerns. They are likely an indication of possible contamination by Food handlers (fish vendors) or storage facilities. Adedeji, et al. [4] found that apart from the microorganisms that fishes have at the time of capture more is added through unhygienic practices and contaminated equipment such as storage facilities. At the time of sample collection (6:55 am- 8:25 am), it was observed that Skeldon market kept Hassar alive while Trahira and Patwa were kept in an old Refrigerator under ice if dead (not FRESH) for sale. Unlike Skeldon market, all three species (Hassar, Patwa and Trahira) were sold dead at Rose Hall market whether store in Buckets or kept on market counters. Vendors did not observed any hygienic quality (gloves) when handling dead fishes at both markets. It is therefore clear that the sanitary measures did not play any major role on the effects of bacterial load present on Hassar, Patwa and Trahira from both markets since there were no significant differences between any of the microbes. Although the *E. coli* count in the month of April may have supported the fact that freshwater fishes from Skeldon had better Sanitary measures for handling and storing of fish than Rose Hall, the data is not sufficient. Of such, the definite input of bacterial contamination of the freshwater fishes at the markets in region 6 is unknown and may be due the time of capture, market conditions (sanitary and hygienic practices), or during the time of transportation of fishes to markets.

The *CHI-SQUARE* value (186.0) is greater than the critical *CHI-SQUARE* value (10.83), therefore, there is significant difference of the coliform count compare to the U.S FDA & EPA expected Standard. The *CHI-SQUARE* values (119.5) is greater than critical *CHI-SQUARE* value (10.83), therefore, there is significant difference of the *E. coli* count compared to the U.S FDA & EPA expected Standard. The *CHI-SQUARE* values (40.96) is greater than critical *CHI-SQUARE* value (10.83), therefore, there is significant difference of the *S. aureus* count compare to the U.S FDA & EPA expected Standard.

Many indices have been used for the assessment of fish quality during storage [4]. In the current study, U.S Food and Drug & Environmental Protection Agency safety levels in regulations and guidance was used. Although, it was found that the observed microbial counts of *Hoplosternum littorale* (Hassar), *Cichlasoma bimaculatum* (Patwa) and *Hoplias malabaricus* (Trahira) often exceeded U.S FDA and EPA microbiological criteria, the *CHI-SQUARE* test revealed that the observed microbial counts (Coliform, *E. coli* and *S. aureus*) deviated significantly from U.S FDA and EPA expected standard. Based on statistical test, the null hypothesis of this study was rejected. The fishes sold in local markets are not of standard for consumption due to high microbial levels [7]. To control such grave situations, it is important to follow code of practices concerning handling of catch, icing, post-harvest procedures and storage and hygienic measures [7].

#### 4. CONCLUSION

The results indicate bacteriological contamination of *Hoplosternum littorale* (Hassar), *Cichlasoma bimaculatum* (Patwa) and *Hoplias malabaricus* (Trahira) from region 6 (Corentyne, East Berbice) Guyana at Skeldon and Rose Hall market. Based on the recommended levels of the U.S Food and Drug Association (FDA) and Environmental Protection Agency (EPA), the three freshwater fishes (Hassar, Patwa and Trahira) sold at the two local markets in region 6 are not within the safe standards for consumption.

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