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Morphometric parameters and level of *Salmonella* and *Escherichia coli* contamination of *Tilapia guineensis* and *Sarotherodon melanotheron* in the waterway of Southern Benin

Tossou Jacques Dougnon[1] and Antoine Chikou[2]

1. Laboratory of Research in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 BP 2009 Abomey-Calavi, Benin; 2. Laboratory of Hydrobiology and Aquaculture, Faculty of Agronomic Sciences, University of Abomey-Calavi, 01 BP 526, Cotonou, Benin.

Corresponding author: Tossou Jacques Dougnon, e-mail: dougnonj@yahoo.fr,
AC: chikoua@yahoo.fr

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Abstract

Aim: The present study aims to evaluate the morphometric parameters and level of *Salmonella* and *Escherichia coli* contamination of *Tilapia guineensis* and *Sarotherodon melanotheron* in the waterway of Southern Benin.

Materials and Methods: 183 *T. guineensis* and 195 *S. melanotheron* were collected from June to July 2014 in four waterways: Lake Ahémé, Nokoué Lake, coastal lagoon, and lagoon of Porto-Novo. Weight, total length, and standard length of these fish were evaluated. *E. coli* and *Salmonella* sp. were sought in fresh fish.

Results: The results obtained in this study indicate that *S. melanotheron* presented high-performance of length and weight more than *T. guineensis* in Ahémé Lake and lagoon of Porto-Novo. However, in Nokoué Lake and coastal lagoon, no difference was observed between the two species of fish. As for bacteriological analysis, the population of *T. guineensis* was more contaminated with *E. coli* with respective values of 60% and 59.52% in the Nokoué Lake and coastal lagoon than in the two other streams. Regarding the population of *S. melanotheron,* she was most contaminated in the coastal lagoon with a percentage of 66.66% in Ahémé Lake, Nokoué Lake, and the lagoon of Porto-Novo. However, no *Salmonella* germ was detected in fish analyzed in this study.

Conclusion: It appears that the morphometric parameters and weight of *T. guineensis* are lower than those of *S. melanotheron*. The evaluation of the microbiological quality revealed that *T. guineensis* is more contaminated with *E. coli* that *S. melanotheron.*

Keywords: benin, *Escherichia coli,* morphometric parameters, *Salmonella* sp., *Sarotherodon melanotheron*, *Tilapia guineensis*.

Introduction

Food security is today a challenge for the authorities. People are facing an animal protein deficit. Fishing is one of the very important activities to overcome this problem. Furthermore, fishing is a veritable source of animal protein for the majority of the world population.

In Benin, the national fish production is estimated at 40,363.63 tons in 2012 [1]. Despite this contribution, the supply of fish products is below market needs that are around 8,657,033 tons [1]. In view of the gap between supply and demand plus the decline in fisheries production [2], Benin is dependent on foreign countries for fishery products. This situation resulted in massive imports of frozen fish from neighboring countries and Europe. Fish products are preferred food because they are inexpensive, acceptable, and accessible to the consumers. However, the skin, shell, mucus, gills and gastrointestinal tract of these animals contain a significant number of microbial floras [3]. In the United States, Olsen *et al*. [4] reported that fish was an accessory to 25% of the recorded food poisoning.

The sanitary degradation of the aquatic environment due to the increase in the lacustrine population of Benin affects the aquatic fauna of the Lake [5]. Therefore, it is important to evaluate the morphometric parameters of fish species in some waterways in South Benin. It should also be taken into account the presence of zoonotic gastrointestinal bacteria such as *Salmonella* spp. and *Escherichia coli* in their bodies. *Tilapia guineensis* and *S. melanotheron* are two Indigenous species of cichlid of the Benin water which are strongly consumed. The present study evaluates the morphometric parameters and the presence of *Salmonella* and *E. coli* in two species of cichlid in some streams in Southern Benin.

Materials and Methods

Ethical approval

This study has been approved by the University of Abomey-Calavi Research Ethics Committee. We note that all animals used for this research is with permission of Animal Ethics Committee of University of Abomey-Calavi.

Collection of fish

T. guineensis and *S. melanotheron* were collected in four wild waterways in southern Benin: Ahémé Lake, Nokoué Lake, coastal lagoon, and the lagoon of Porto-Novo. The age of the fish was not established. Collected fish were transported in 1 or 2 h maximum from the site to the Veterinary Clinical Laboratory and Pharmacy Complex of Animal Health Production of the University of Abomey-Calavi for morphometrical and microbiological analyzes. A temperature of 4°C was maintained during transport to avoid deterioration of the specimen.

Morphometrical analyzes

Total length, standard length, and weight measurements

Once at the laboratory, fishes were immediately weighed with a digital scale (KERN®: max 220 g, d=0.1 mg). Figure-1 shows the total length and the standard length of collected fish were taken with an ichtyometer.

Evaluation of the relationship weight-lengt

The type of growth presented by the fish was evaluated through the weight-length relationship. This exponential relationship between weight and length is computed based on Le Cren, (1951); the formula is as follows:

$W = a\ TL^b$

Where: W = Fish weight (g);

TL = Total length of the fish (cm);

a = Coefficient related to the environment of fish (y-intercept of the regression line);

b = The coefficient of relative growth and (b) as the relative growth rate of the mass (slope of the regression line).

The coefficient b varies between 2 and 4. It expresses the relative shape of the body of a fish. When it is equal to 3, fish growth is isometric; below 3, it's allometric, but when coefficient b, is above 3, it indicates better growth in weight and length and *viz*. [6].

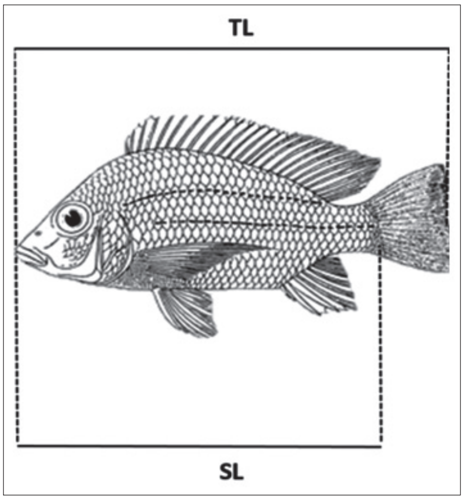

Figure-1: Body measurements taken on each fish (total length (TL), standard length (SL))

Microbiological analyzes

Detection of E. coli

After morphometric analyzes, each fish sample was aseptically dissected to expose the abdominal region. The intestine was removed and cut into small pieces.

A sample of 25 g of intestine was removed. To this sample, 225 ml of buffered peptone water (Oxoid CM0509 lot 1390836, England) was added to obtain a stock solution. Successive dilutions were performed until 10^{-4} from the stock solution. 1 ml of each dilution was inoculated by incorporating on the medium RAPID *E. coli* 2. After incubation at 44°C for 24±2 h, the reading was taken. The colonies of *E. coli* are purple, and those of thermotolerant coliforms are blue.

Detection of Salmonella sp.

For detection of *Salmonella* sp., 225 ml of buffer peptone water (Oxoid CM0509 lot 1390836, England) was added to 25 g of sample intestine. After incubation at 37°C for 18 h, 0.1 ml of this culture was inoculated in 10 ml Rappaport-Vassiliadis broth (Bio-Rad lot 098282, France) and 2 ml in 20 ml of broth Mueller Kaufman. The incubation was performed at 37°C for 24±4 h. The isolation of *Salmonella* was done by seeding xylose lysine decarboxylase media (Oxoid CM0469 lot 1399006, United Kingdom) and Hektoen (Oxoid CM0419 lot 1423494, England). Selecting *Salmonella* characteristic colonies were made after incubation at 37°C for 24±4 h. The discrimination test was performed by inoculating the urea indole medium. *Salmonella* is devoid of urease [7]. The API 20E gallery was finally planted.

Statistics analysis

Morphometric parameters measured were encoded in Excel and statistically analyzed using SAS [8]. Proc GLM procedure was used for the analysis of variance. The F-test was used to determine the significance of the effect species. Means were compared pairwise by the Student's t-test. The Chi-square test was used to determine the significance of the frequencies of bacterial contamination levels by species and waterways. The test bilateral of Z was used to compare pairwise the frequency.

Results

Morphometric analyzes

Total length, standard length, and weight measurements

In Ahémé Lake, the average values of morphometric parameters were, respectively, 133.58±1.98 and 102.56±1.53 mm for the average total length and the average standard length for *S. melanotheron*. On the other hand, in *T. guineensis*, the average total length was 116.22±2.19 mm with an average standard length of 88.24±1.70 mm.

The average body weight was 48.1±1.62 g in *S. melanotheron* against 32.44±1.79 g in *T. guineensis*.

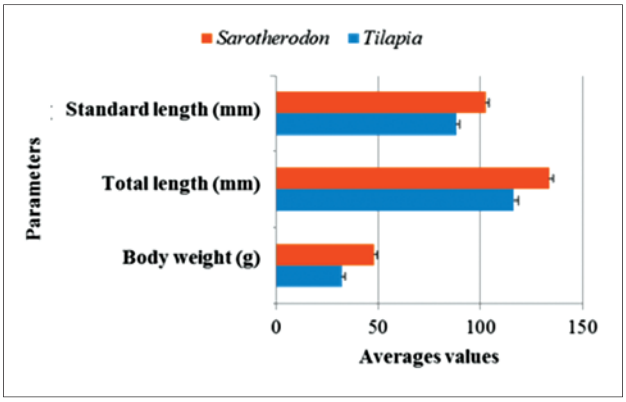

Figure-2: Morphometric parameters of *Tilapia guineensis* and *Sarotherodon melanotheron* in Ahémé Lake

These parameters varied significantly between the two species in the rivers (p<0.001) (Figure-2).

In Nokoué Lake, the average total length and average standard length were, respectively, 161.64±2.83, 123.16±2.19 mm for *S. melanotheron*. These parameters were, respectively, 156.34±2.83 and 119.42±2.19 mm in *T. guineensis*. The average body weight in *S. melanotheron* was 86±4.47 and 80.60±4.47 g in *T. guineensis* (Figure-3). In this stream, both species showed no significant difference between their's morphometric parameters (p>0.05).

In the coastal lagoon, the average total length and average standard length for *S. melanotheron* were, respectively, 131.6±2.96 and 98.87±2.29 mm. In *T. guineensis*, the average total length was 128.31±3.06 mm and the average standard length was 96.64±2.37 mm. As regards the body weight, *S. melanotheron* weighed 52.44±3.13 against 44.93±3.24 g for *T. guineensis* (Figure-4). These parameters have not varied significantly between the two species in the lagoon (p>0.05).

However, in the lagoon of Porto-Novo, the total length of *S. melanotheron* and *T. guineensis* was, respectively, 176.28±3.73 and 161.28±3.73 mm, with respective average standard lengths of 135.54±2.91 and 123.88±2.91 mm. In this stream, the average body weight of *S. melanotheron* was 113±6.66 against 89.70±6.66 g in *T. guineensis*. The statistical analysis showed that there is a significant difference between these parameters in these two species (p<0.01) (Figure-5).

Figure-6 shows the morphometric parameters of *S. melanotheron* and *T. guineensis*. The total length and standard length of *S. melanotheron* were, respectively, 151.27±2.00 and 115.45±1.57 mm against, respectively, 142.27±2.06 and 108.43±1.62 mm in *T. guineensis*.

As regards the weight of *S. melanotheron*, it was 75.46±2.87 against 64.11±2.96 g in *T. guineensis*. For these three parameters, the values were significantly different between the two species (p<0.01).

Table-1 shows the morphometric parameters of *T. guineensis* in different streams. The body weight of *T. guineensis* in Nokoué Lake (80.60±4.20 g) and in

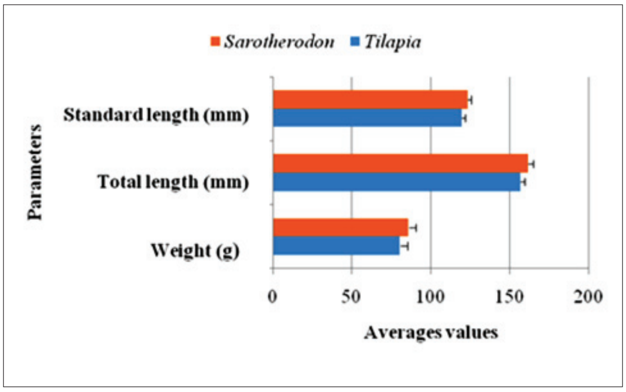

Figure-3: Morphometric parameters of *Tilapia guineensis* and *Sarotherodon melanotheron in NokouéLake*

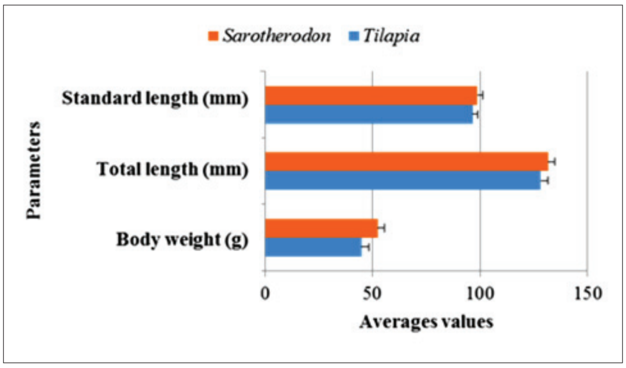

Figure-4: Morphometric parameters of *Tilapia guineensis* and *Sarotherodon melanotheron* in coastal lagoon

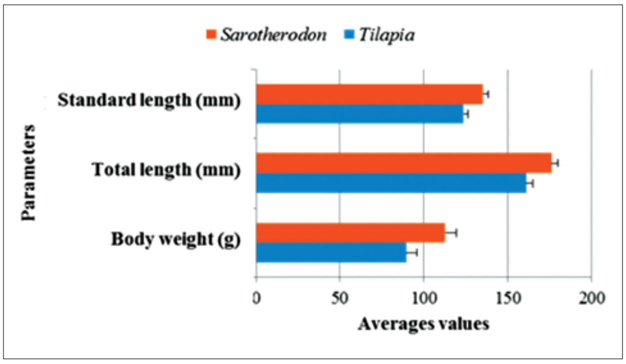

Figure-5: Morphometric parameters of *Tilapia guineensis* and *Sarotherodon melanotheron* in the lagoon of Porto-Novo

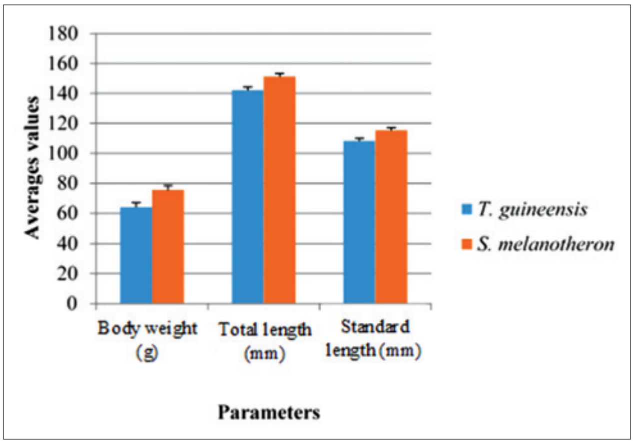

Figure-6: Morphometric parameters of *Tilapia guineensis* and *Sarotherodon melanotheron* compared in different streams

Table-1: Morphometric parameters of *Tilapia guineensis* in different streams.

Parameters	Aheme Lake (n=41)	Nokoue Lake (n=50)	coastal lagoon (n=49)	Porto-Novo lagoon (n=50)	Significance
Total length (mm)	116.22±2.94[c]	156.34±2.66[a]	128.31±2.91[b]	161.28±2.66[a]	***
Standard length (mm)	88.24±2.35[c]	119.42±2.13[a]	96.64±2.33[b]	123.88±2.13[a]	***
Body weight (g)	32.44±4.64[b]	80.60±4.20[a]	44.93±4.58[b]	89.70±4.20[a]	***

Averages in the same row followed by different letters are significantly different at the 5%; ***p<0,001

the lagoon of Porto-Novo (89.70±4.20 g) did not differ significantly (p>0.05). However, the body weight of this species in these two waterways is higher than the coastal lagoon and Ahémé Lake where the respective values of this parameter were 44.93±4.58 and 32.44±4.64 g (p<0.05). As for the average total length, *T. guineensis* from Nokoué Lake and Porto-Novo lagoon showed the highest values (156.34±2.66 and 161.28±2.66 mm, respectively) compared to those living in the Ahémé Lake and coastal lagoon. Furthermore, *T. guineensis* had a higher total length in the coastal lagoon than Lake Ahémé with respective values of 128.31±2.91 and 116.22±2.94 mm (p<0.05). Regarding the standard length, it was higher in *T. guineensis* living in Nokoué Lake and Porto-Novo lagoon than the ones living in the coastal lagoon (p<0.05).

In addition, *T. guineensis* of the coastal lagoon was longer than the ones of Ahémé Lake. In sum, *T. guineensis* of Nokoué Lake and Porto-Novo lagoon had the highest morphometric parameters (p<0.001).

Morphometric parameters in *S. melanotheron* compared in different waterways

In the lagoon of Porto-Novo, the body weight, the total length, and standard length were, respectively, 113.00±4.65 g, 176.28±3.15, and 135.54±2.4 mm for *S. melanotheron*. The values of these parameters were lower for those living in the Nokoué Lake with respective values of 86±4.65 g, 161.64±3.15, and 123.16±2.4 mm (p<0.05). Furthermore, the values for these parameters in the Nokoué Lake were higher than those obtained in the Ahémé Lake and in the coastal lagoon (p<0.05) (Table-2).

Body weight and total length relationship in *T. guineensis* and in *S. melanotheron*

In *T. guineensis*, the values of growth rate ranged from 2.55 to 3.15 in Ahémé Lake, Nokoué Lake, the coastal lagoon, and the lagoon of Porto-Novo. In Ahémé Lake and coastal lagoon, the growth of *T. guineensis* was negative allometric (b<3) while in the lagoon of Porto-Novo, this species was positively allometric growth (b>3). By cons, *T. guineensis* of Nokoué Lake presented an isometric growth (b=2.92) (Table-3). Regarding *S. melanotheron*, the values of growth coefficient in the Ahémé Lake, coastal lagoon, and Porto-Novo lagoon were, respectively, 2.07, 2.76, and 2.53. In these waterways, *S. melanotheron* presented a negative allometric growth. By cons, in Nokoué Lake the growth of these fish was isometric (b=2.92) (Table-3). The body weight and total length relationship of *T. guineensis* and *S. melanotheron* were highly significant in Ahémé Lake, coastal lagoon, and lagoon of Porto-Novo (p<0.001).

Evaluation of the level of contamination by *Salmonella* and *E. coli* in *T. guineensis* and *S. melanotheron* taken from Lakes Ahémé, Nokoué; coastal lagoon, and the lagoon of Porto-Novo

The level of contamination of *Salmonella* and *E. coli* in *T. guineensis* and *S. melanotheron* taken from different waterways were presented in Table-4. In the coastal lagoon, 66.66% of the population of *S. melanotheron* were contaminated with *E. coli* against 59.52% of the population of *T. guineensis* (p<0.05). In Nokoué Lake, populations of *T. guineensis* were more contaminated with 60% against 20% in *S. melanotheron*. In the lagoon of Porto-Novo, we note the same registration with 30% in populations of *T. guineensis* and 10% in *S. melanotheron* (p<0.001). About the level of contamination of *E. coli* in *T. guineensis* and *S. melanotheron* in Ahémé Lake, no significant difference was observed. However, microbiological analyzes revealed no sample contamination by *Salmonella* sp.

Discussion

Morphometric parameters of *T. guineensis* and *S. melanotheron*

The results obtained in this study are in accordance with those obtained by Fousséni [9] in these same waterways in Southern Benin. In Nigeria, it reports that the morphometric parameters of *S. melanotheron* are higher than those of *T. guineensis* [10]. Other authors report that the quality and quantity of food, as well as environmental variables, affect fish growth [11]. This can explain the difference in morphometric parameters and body weight between *S. melanotheron* and *T. guineensis*. *S. melanotheron* is particularly suitable in estuaries and brackish water. It tolerates a very wide range of salinity and low dissolved oxygen levels. It is an omnivorous species whose food resources vary in accordance with the environment [12]. According to Halvorsen and Svenning [13], a temperature change causes a difference in growth. In the lagoon of Porto-Novo and Nokoué Lake, the temperature is lower than in the Lake Ahémé and coastal lagoon [9]. This also explains the low morphometric parameter of *T. guineensis* in these two rivers. The same authors relate that *T. guineensis* presents low morphometric parameters and a relatively low weight gain when the salinity and dissolved oxygen levels are high. In their natural environment,

Table-2: Morphometric parameters of *Sarotherodon melanotheron* in different streams.

Parameters	Aheme Lake (n=41)	Nokoue Lake (n=50)	coastal lagoon (n=49)	Porto-Novo lagoon (n=50)	Significance
Total length (mm)	133.58±3.15[c]	161.64±3.15[b]	131.6±3.32[c]	176.28±3.15[a]	***
Standard length (mm)	102.56±2.4[c]	123.16±2.4[b]	98.87±2.53[c]	135.54±2.4[a]	***
Body weight (g)	48.1±4.65[c]	86±4.65[b]	52.44±4.90[c]	113.00±4.65[a]	***

Averages in the same row followed by different letters are significantly different at the 5%; ***p<0,001

Table-3: Body weight and total length relationship in *Tilapia guineensis* and *Sarotherodon melanotheron* in streams in southern Benin.

Waterways	Body weight (g)	Total length (cm)	*T. guineensis*			*S. melanotheron*		
			a	b	r^2	a	b	r^2
Aheme Lake	[15-75]	[8,6-15,8]	0,06	2,55*	0,83	0,22	2,07*	0,78
Nokoue Lake	[20-170]	[11,2-20,5]	0,02	2,92	0,8	0,02	2,92	0,82
coastal logon	[15-90]	[8,3-17,2]	0,03	2,88*	0,83	0,04	2,76*	0,92
Lagon of Porto-Novo	[35-245]	[13,8-22,2]	0,01	3,15*	0,95	0,08	2,53*	0,95

The sign (*) indicates that the value is significantly different from 3; a is ordered to the origin of the regression line; b or regression coefficient is the gradient of the regression line; r^2 is the coefficient of determination

Table-4: Contamination level of *salmonella* and *E. coli* in *Tilapia guineensis* and *Sarotherodon melanotheron.*

Waterways	*Escherichia coli* (%)		Significance	*Salmonella* sp. (%)		Significance
	Sarotherodon melanotheron	*Tilapia guineensis*		*Sarotherodon melanotheron*	*Tilapia guineensis*	
Aheme Lake	30[a]	26.83[a]	NS	0	0	NS
Nokoue Lake	20[a]	60[b]	***	0	0	NS
coastal logon	66.66[b]	59.52[a]	*	0	0	NS
Lagon of Porto-Novo	10[b]	30[a]	***	0	0	NS

Averages in the same row followed by different letters are significantly different at the 5%; ***p<0.001; *p<0.05 and [NS]p>0.05

the average total weight obtained in *T. guineensis* (64.11 g) is higher than that obtained in fish breeding (44.34 g) by Toko-Imorou *et al*. [6]. In the same study, the population of *S. melanotheron* with highest morphometric parameters in the natural environment is found in Porto-Novo lagoon with an average weight of 113 g and a total length of 176.28 mm. It showed that *S. melanotheron* is less efficient growth when it is bread in cages than when it is bread Acadja in lagoon environment [14]. Similarly, morphometric parameters, growth indicators, are influenced by the waterway effect in *T. guineensis* [15].

Weight-length relationship

In this study, the results obtained in *T. guineensis* in Ahémé Lake, coastal lagoon, and the lagoon of Porto-Novo are in accordance with those of Lalèyè *et al*. [16] at the Ahémé Lake. On the other hand, the research conducted by Fontaine *et al*. [15] in Ahémé Lake, the lagoon of Porto-Novo and Nokoué Lake from August to October 2013, reveal isometric growth in the first two waterways and allometric growth in the latter. These types of variations are also observed in *Labeo barbus batesii* during its growth [17]. These changes can be explained by the fact that the growth factor is related to biotic factors, abiotic and availability of food as well as to habitat type [18].

In this work, regarding *S. melanotheron*, allometric coefficients obtained, respectively, in the Ahémé Lake, the coastal lagoon, and the lagoon of Porto-Novo are in accordance with those obtained by Fagnon [19] who reveals an allometric coefficient of 2.75 in the coastal lagoon and 2.73 in the lagoon of Porto-Novo.

Bacteriological analyzes in *T. guineensis* and *S. melanotheron*

The presence of *E. coli* in the intestine of *T. guineensis* and *S. melanotheron* is linked to unhealthy waterways [20]. The results obtained in this study are in accordance with those obtained by Sèdogbo [21] and Dègnon *et al*. [20] on shrimp in the Nokoué Lake and the Ahémé Lake. The poor quality of the captured fish species is due to the high level of bacterial contamination of streams [22,23]. Similarly, Dègnon *et al*. [24] reported high bacterial contamination of *Trachurus trachurus* by coliforms in Benin. The absence of *Salmonella* in samples analyzed may be explained by his absence in waterways or the influence of resistance of fish in relation to the bacteria. These results are consistent with those obtained by N'diaye [25] on fishery products in 1998 in Dakar.

Conclusion

This study allows understanding the morphometric parameters and weight of *S. melanotheron* and *T. guineensis* in some stream in Southern Benin. It appears that the morphometric parameters and weight of *T. guineensis* are lower than those of

S. melanotheron. The evaluation of the microbiological quality revealed that *T. guineensis* is more contaminated with *E. coli* that *S. melanotheron*. Since these two species are very important in the commercial market, particular attention should be paid to different cooking techniques to prevent bacterial infections that can result from the consumption of these fish species. It would be also interesting to perform the analysis of bacterial contamination of edible parts of the studied fish since intestines are probably not consumed. In addition to state that there is a risk for the final consumer of those fish a quantification of the amount of the colony-forming unit of *E. coli* should be performed.

Authors' Contributions

The present study was a part of original research work by TJD. He conceptualized the aim of the study, designed, planned, and supervised the experiment. Collection of samples and execution of experimental study were done by him and AC. Analysis of data, interpretation of the results, and drafting of the manuscript were done by AC. TJD helped in the analysis, draft and revision of the manuscript. All authors read and approved the manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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