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Effects of environmental conditions on the microbiological quality of two small marine pelagic fishes landed in Accra and Tema, Ghana

Emmanuel O. Kombat[1*], Francis K. E. Nunoo[2], Joseph A. Ampofo[3] and Phillis G. A. Addo[4]

[1]Department of Applied Biology, University for Development Studies, Tamale, Ghana
[2]Department of Marine and Fisheries Sciences, University of Ghana, Legon, Ghana
[3]Water Research Institute, Council for Scientific and Industrial Research, Accra, Ghana
[4]Department of Animal Experimentation, NMIMR, University of Ghana, Legon, Ghana

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ABSTRACT

This study investigated the effects of environmental conditionson the microbiological quality of the European Anchovy (Engraulisencrasicolus) and the Round Sardinella (Sardinellaaurita) caught along the coast of Accra and Tema, Ghana by artisanal fishermen. A total of approximately, 500 g each of fresh E. encrasicolusand S. auritawith individual weights between 1.15 to 36.56 g and total lengths between 4.9 to 16.0 cm were collected from Accra and Tema at sea immediately after capture, on landing at landing beach, and at local retail markets. Three separate microbiological analyses were conducted to assess the changes in their microbiological qualities. Samples were aseptically macerated and serially diluted, and the pour plate and spread plate methods were used to enumerate total heterotrophic bacteria, total coliform bacteria, yeast and moulds and Bacillus cereus colonies in the samples. The results showed an increase in microbial loads from harvest to market in both fish species collected from both Accra and Tema. There were counts of microorganisms at all stages of production, but samples obtained from retail markets recorded colony counts of total heterotrophic bacteria (> 1.0 x 10^6 cfu/g) and total coliforms (> 1.0 x 10^4 cfu/g) higher than both local and international standards. Counts of yeast and moulds, and Bacillus cereus were recorded for samples of E. encrasicolus and S. aurita from both Tema and Accra, but these counts were within accepted limits (< 1.0 x 10^4 cfu/g) for consumption. It was concluded that, samples of both E. encrasicolus and S. aurita obtained at the retail markets recorded higher counts of microorganisms and therefore were contaminated. Fish handling obviously contributed to the increased microbiological load after harvesting. Education of fishers is required to improve their hygienic practices and should be followed by regular hygiene inspections.

Keywords: Microbiological quality, Small pelagic fishes, Anchovy, Sardinella, Coliform bacteria.

INTRODUCTION

Ghana has a shoreline of about 550 km at the Gulf of Guinea and fishing is the most important activity in the entire coastal zone in terms of the number of people involved in it both directly and indirectly [1]. The fishing sector has been an important contributor to economic development in Ghana and other West African countries. It has been estimated to contribute 3% of the Ghana's Gross Domestic Product (GDP) and 5% of its agriculture GDP [2].The fishing sector is a primary source of income and employment to most people living along the coast of Ghana, providing employment to about 2 million people of which approximately 27% are directly employed [3]. Fish is the most important animal protein food available in the tropics representing about 14% of all animal protein on a global

basis [4]. According to Ashitey and Flake [5], fish is a cheaper and preferred source of animal protein which contributes about 60% of animal protein intake in Ghana. In their report, the annual domestic fish production of Ghana has been fluctuating but generally on a gentle decline since 2000 from 460,000 tons down to 436,000 tons in 2008.

Large quantities of European Anchovy (*Engraulisencrasicolus*, Linnaeus) and the Round Sardinella (*Sardinellaaurita*,Valenciennes) are landed in most landing sites in Ghana, particularly during the season of glut in October to November each year [6]. These species of fish are coastal small pelagics which prefer clear saline water with a minimum temperature below 24°C. They normally swim in large schools from depths of 0 to about 400 m tolerating salinities of 5 to 41 ppt[7, 8].

The major challenge worldwide including Ghana, is the unhygienic environmental conditions in which fish finds itself before and after capture, before it comes to table for consumption [9]. Methods used in handling or processing fish are likely to contribute to contaminating the fish with pathogens. Of much concern in public health is the contamination of fish by faecal coliforms in contaminated waters [10]. In Ghana and other parts of the world, consumption of fish contaminated by pathogens have led to serious health consequences and are responsible for some of the recorded deaths [11, 12]. Shewan[13] discovered that the microbial flora associated with freshly harvested fish is principally a function of the environment in which the fish are caught and not of the fish species, hence, the indigenous microbial populations of fish can vary significantly. Other studies on the microbiological quality of fish raised in contaminated water have shown that, faecal bacteria may penetrate fish flesh when fish is grown in highly contaminated water [14, 15]. The occurrence of pathogens such as *Staphylococcus aureus*, *Salmonella typhimurium*, *Bacillus cereus*, *Vibrio* spp.and *Clostridium botulinium*in fish has raised major concerns among researchers since they are the main causes of food borne illnesses [16].

At the international level there are regulatory and advisory groups such as the World Health Organisation (WHO), the Food and Agriculture Organisation (FAO) and the International Commission on Microbiological Specifications for Food (ICMSF) [17, 18, 19]; and control measures such as the Codex Alimentarius, Sanitary and Phytosanitary (SPS) measures, Hazard Analysis of Critical Control Points (HACCP), to ensure the safety standards of fish and other related food products consumed by humans. Several developing countries have been banned from exporting seafood products because they do not meet these standards [20].It is therefore necessary to find out whether what is produced in Ghana meets those standards for export and for local consumption. This study assumes that Ghana's fish will be wholesome for both local consumption and export given the significant amounts that are exported. The Ghana Standards Authority (GSA) has established safety standards for fish and its products to ensure its wholesomeness. Although these safety standards exist, very little or nothing is done to enforce them at the artisanal fishery level giving room for the production of low quality fish for consumption by local populace and sometimes for export. The industrial fisheries and fish processing companies which dominate the export market, however, experience some level of fish quality inspections from the standards authority. Thestudy sought to assess the effects of sewage pollution in Ghana's coastal waters; and the effects of handling and storage of fish at the landing sites and at the local retail markets on the microbiological quality of the European anchovy (*E. encrasicolus)* and the Round Sardinella (*S. aurita)* caught along the coast of Accra and Tema.

MATERIALS AND METHODS

Study locations

The study was carried out at the Jamestown fish landing site and at canoe basin in Accra and Tema respectively, both in the Greater Accra Region of Ghana.

Accra,located at 5.55°N 0.2°W is the capital and largest city in Ghana with a population of 3,963,264. It has a land area of about 200 km^2 with a population density of 9,816/km^2 [21]. Accra also doubles as the capital of the Greater Accra Region and of the Accra Metropolitan District. It is the most important city in Ghana because it is the administrative, communications and economic centre of Ghana. The Jamestown fish landing site which is one of the largest and important landing site in Accra where large amounts of several species of fish are landed annually is located close to the KorleGonno Beach Liquid Waste Disposal Site.

Tema, located at 5.667°N 0°E is a city on the Atlantic Ocean coast, 25 km east of the capital city of Accra, in the region of Greater Accra, in Ghana. It has a population of 209,000 [21]. Originally, it was a small fishing village, but

it grew after the construction of a large harbour in 1961 and is now the nation's largest sea port. It is home to an oil refinery and is an important centre of manufacturing industries and has a fishing harbour which is situated at the eastern end of the Tema commercial harbour. The fishing harbour comprises the inner fishing harbour, the canoe basin, the outer fishing harbour, and a commercial area with marketing and cold storage facilities. The canoe basin where this work was carried out caters for the artisanal fishermen.

Measurement of Physico-chemical Parameters of the sea

Water quality parameters (Temperature, pH, Dissolved Oxygen (DO), Conductivity, Total Suspended Solids (TSS), Total Dissolved Solids(TDS), and Salinity) were measured using a multi-parameter probe. These parameters were measured *in-situ* in the surface water of the sea both in Accra and Tema where anchovies and sardines were sampled. The procedure was done at two randomly selected locations in the water and a mean of the two measurements was calculated.

Sampling of Sea Water for Microbiological Quality Analysis ofWater

Samples of water were collected from the sea and analyzed for their microbiological quality. These samples were collected at locations where catch was made at a depth of 50 cm from surface of water. The samples were collected into sterile bottles (500 ml) fitted with tight screw caps. Care was taken to avoid accidental contamination of the water during collection and transportation to the laboratory for analysis.

Laboratory Analysis for Microbiological Quality ofWater

E. coli count of the water was used for detecting of microbiological quality of the sampled water using the membrane filtration technique [22].Total coliform counts (TCC) and total faecal coliform counts (TFC) were also enumerated using the membrane filtration technique. In this technique, a 100 ml water sample was filtered through a membrane filter. The membrane was cultured on a pad of a sterile selective medium (HiCrome Coliform Agar) [22].After 16-24 hours of incubation at 37 °C *E. coli* appeared as blue colonies, while total coliforms appeared blue and pink colonies. The standard plate count (SPC) method was used to enumerate total heterotrophic counts of bacteria (THC) using the Standard Plate Count agar (PCA) [23]. All counts of bacteria were detected and quantified with the use of a digital colony counter (Stuart colony counter-SC6+). These counts were expressed as colony forming units per 100 milliliter (cfu/100mL) of the water sample.

Collection and Laboratory Analysis of *E. encrasicolus*and *S. aurita* Samples

A total of approximately, 500g of each fresh*E. encrasicolus*and *S. aurita*with individual weights between 1.15 to 36.56 g and total lengths between 4.9 to 16.0 cm were collected from Accra and Temafor their microbiological quality analysis at each of the following stages of fish production: at sea immediately after capture;on landingat landing beach; andat local retail markets during marketing.Sampling was conducted once in each month of the study period (March- May, 2012).All collected samples were placed in well labeled sterile plastic bags (ziplock bags) and immediately delivered to the laboratory on ice in an ice chest under hygienic conditions for analysis. The pour plate method was used to enumerate total heterotrophic bacteria, total coliform bacteria and yeast and moulds colonies, while the spread plate method was used to enumerate *Bacillus cereus* colonies in the fish samples collected [23].

Preparation and Sterilization of Media

All media were prepared and sterilized according to manufacturer's instructions. The media used for this study were obtained from the Oxoid Limited, England. Sterility control plates of each media and diluents were made by incubating them overnight at 37 °C.

Serial Dilution and Platingof*E. encrasicolus*and *S. aurita* samples

Twenty-five grams (25g)each of whole *E. encrasicolus*and *S. aurita*at each production stage (i.e. at harvest, on landing and at the retail market) were aseptically weighed on an electronic balance and macerated in a sterile laboratory mortar and pestle. Each macerated fish was then kept in a 250ml conical flask containing 225ml sterile 0.1% peptone water. The content was vortexed for 60 seconds to homogenize the mixture to obtain a 1:10 (10^{-1}) dilution. Aseptically, 1ml of the 1:10 dilution sample was transferred into a 25ml universal bottle containing 9ml of sterile 0.1% peptone water with a sterile microtitre tip to make a 1:100 or 10^{-2} dilution. This procedure was continued until 10^{-8} dilution was obtained.

One milliliter (1ml) of each dilution was aseptically dispensed into sterile labeled petri dishes in duplicates and mixed thoroughly with molten agar, previously held in a water bath at 50^{o}C.The Standard Plate Count Agar (PCA)

was used for total heterotrophic counts (THC), MacConkey agar (MAC) for total counts of coliform bacteria and Oxtetracycline Glucose Yeast Extract agar (OGYE) was used for counts of yeast and moulds.

0.1ml of the inoculum of each dilution was also placed on the surface of well dried *Bacillus cereus* Selective Agar (BCSA) in duplicates. The inoculums were then spread rapidly and evenly over the entire agar surface using a thin sterile bent glass rod (spreader). A separate sterile spreader was used for each series of the plates representing a single sample, starting from the highest dilution (10^{-8}) to the lowest dilution (10^{-1}). These procedures were performed for all the samples collected at each of the fish production stages considered for this study. All glassware and plates were labeled to correspond with fish species collected.

Incubation and counting of bacterial colonies on plates

Plates of PCA, MAC and BCSA were aerobically incubated in inverted positions at 37^{o}C. Likewise, OGYE plates were also aerobically incubated in inverted positions but at 26^{o}C. Counting of microbial colonies were done after 24 hours and repeated after 48 hours using the colony counter. Colonies on fresh OGYE were counted again after 72 hours. Counting of microbial colonies on plates was conducted using a digital colony counter (Stuart colony counter-SC6+).All colonies were counted as colony forming units per gram of fish sample (cfu/g) according to the Microbiology of food and animal feeding stuffs [22].

Statistical Analysis

Data collected from this study were analysed using the XLSTAT 2012 computer software. First, the data were subjected to a descriptive statistical analysis where it was summarized numerically for easy understanding of the result. In doing this, descriptive statistics such as means, standard deviations and standard errors were computed. A one-way analysis of variance (ANOVA) was used to test the significance difference in levels of retrieved bacteria from fish species at harvest, at landing and at the point of sale in local retail markets. The Tukey's post-hoc test (HSD) was used if the means of two different groups under comparison were significantly different in the normally distributed population from which the samples were drawn. $p< 0.05$ was regarded as statistically significant.

RESULTS

Physico-chemical Parameters of seawater in Tema and Accra

Table 1 shows the results of monthly mean values of physico-chemical characteristics of the seawater in Tema and Accra recorded during study period.

From Table 1, temperature range from 28.5 ± 0.2 to 30.7 ± 0.3°C were recorded in Tema while those recorded in Accra ranged from 29.1±0.2 to 29.9±0.1°C within the study period. pHvalues recorded in Tema ranged from 7.4 ± 0.5 to 8.4 ± 0.3 and from 7.1 ± 0.3 to 7.5 ± 0.3 in Accra. Dissolved oxygen (DO) values recorded for Tema varied from 4.9 ± 0.3 to 5.5 ± 0.2 mg/L while those in Accra varied from 5.4 ± 0.1 to 5.8 ± 0.2 mg/L. Conductivity values ranged from 53.8 ± 0.2 to 54.9 ± 0.4 µ/cm and 54.0 ± 0.2 to 54.7 ± 0.2 µ/cm were recorded in Tema and Accra respectively. Total suspended solids recorded ranged from 9.0 ± 1.5 to 16.3 ± 2.4 mg/L in Tema and 9.7 ± 1.8 to 14.3 ± 1.2 mg/L in Accra, while total dissolved solids ranged from 0.03 ± 0.34 to 0.03 ± 0.01 ppm in Tema and 0.03 ± 0.03 to 0.03 ± 0.03 ppm in Accra. Finally, salinity of seawater recorded in Tema and Accra during the study period varied from 35.4 ± 0.2 to 35.6 ± 0.4 ppt in Tema and 35.6 ± 0.2 to 35.8 ± 0.3 ppt in Accra.

Table 1 Mean monthly values of physico-chemical parameters (± standard error) of seawater in Tema and Accra

SITE/ MONTH	Temp (°C)	pH	DO (mg/L)	Cond (µ/cm)	TSS (mg/L)	TDS (ppm)	Salinity (ppt)
TEMA							
March	28.5±0.2	8.4±0.3	5.2±0.1	53.8±0.2	16.3±2.4	0.03±0.01	35.6±0.4
April	30.7±0.3	7.5±0.4	5.5±0.2	54.9±0.4	14.3±1.5	0.03±0.01	35.4±0.2
May	30.4±0.1	7.4±0.5	4.9±0.3	54.0±0.1	9.0±1.5	0.03±0.34	35.5±0.1
Mean±*se*	**29.9±0.7**	**7.8±0.3**	**5.2±0.2**	**54.3±0.3**	**13.2±2.2**	**0.03±0. 00**	**35.5±0.1**
ACCRA							
March	29.9±0.1	7.1±0.3	5.4±0.1	54.6±0.1	9.7±1.8	0.03±0.01	35.8±0.3
April	29.1±0.1	7.4±0.2	5.8±0.2	54.0±0.2	10.7±1.9	0.03±0.03	35.6±0.2
May	29.1±0.2	7.5±0.3	5.4±0.1	54.7±0.2	14.3±1.2	0.03±0.01	35.8±0.3
Mean±se	**29.4 ±0.3**	**7.3±0.1**	**5.5±0.1**	**54.4±0.2**	**11.6±1.4**	**0.03±0.00**	**35.7±0.1**

Temp: Temperature;DO: Dissolved Oxygen; Cond: Conductivity; TSS: Total Suspended Solids; TDS: Total Dissolved Solids

Microbiological Quality of water obtained from Tema and Accra

Table 2 shows the results of colony counts of bacteria in water samples obtained from the sea at Tema and Accra for the three month sampling period.From the results, Tema recorded an THC ranging from 9.4 X 10^2 to 3.1 X 10^3cfu/100 ml, while in Accra it ranged from 1.1 X 10^3 to 3.9 X 10^3cfu/100 ml. In Tema TCC ranged between 7.4 X 10^2and7.9 X 10^2 cfu/100 ml while in Accra it ranged between 3.4 X 10^2and2.5 X 10^3cfu/100 ml. FCC recorded in Tema was between 2.4 X 10^1 and 2.4 X 10^2cfu/100 ml while in Accra it was between 5.8 X 10^1 and 6.5 X 10^2cfu/100 ml. The ECC in Tema ranged from 3.4 X 10^1 to 1.2 X 10^2 cfu/100 ml while it ranged from 1.4 X 10^1 to 2.4 X 10^2cfu/100 ml in Accra. Both Tema and Accra recorded lower counts for all bacterial counts in March and higher counts in May.

Table 2Monthly microbial counts of water obtained from Tema and Accra expressed in cfu/100ml

SITE/ MONTH	Total Heterotrophic Count (THC)	Total Coliform Count (TCC)	Fecal Coliform Count (FCC)	*E. coli* Count (ECC)
		TEMA		
March	9.4 X 10^2	7.4 X 10^2	2.4 X 10^1	3.4 X 10^1
April	1.5 X 10^3	7.9 X 10^2	1.3 X 10^1	5.9 X 10^1
May	3.1 X 10^3	7.4 X 10^2	2.4 X 10^2	1.2 X 10^2
Mean	**1.8 X $10^{3\,b}$**	**8.9 X $10^{2\,ab}$**	**9.1 X $10^{1\,a}$**	**7.1 X $10^{1\,a}$**
		ACCRA		
March	1.1 X 10^3	3.4 X 10^2	5.8 X 10^1	1.4 X 10^1
April	2.8 X 10^3	8.4 X 10^2	4.6 X 10^2	1.4 X 10^2
May	3.9 X 10^3	2.5 X 10^3	6.5 X 10^2	2.4 X 10^2
Mean	**2.6 X $10^{3\,b}$**	**1.2 X $10^{3\,ab}$**	**4.2 X $10^{2\,a}$**	**1.3 X $10^{2\,a}$**

WHO Guidelines: Fecal Coliform count ≤ 1000 cfu/100 ml. Values in a column bearing the same superscript are not significant at 5% (Tukey's post-hoc test, $p < 0.05$).

Microbiological quality of *E. encrasicolus* and *S. aurita* sampled from Tema and Accra

Tables 3, 4, 5 and 6 are the monthly mean counts of microorganisms (Total Heterotrophic Counts (THC), Total Coliform Counts (TCC), Yeast and Moulds Counts (YMC) and *Bacillus cereus* Counts (BCC) expressed in cfu/g fromfresh *E. encrasicolus* and *S. aurita*obtained at various stages of fish production, from harvest to market inTema and Accra.

From Table 3, fresh *E. encrasicolus* obtained at harvest from Tema recorded the lowest counts for all categories of microorganisms cultured in each month, while those obtained from market recorded the highest counts for THC, TCC and YMC for all three months. All samples recorded no counts for BCC in all the three months.

Table3 Monthly mean microbial counts in *E. encrasicolus* obtained from Tema expressed in cfu/g

SAMPLING SITE	MONTH	Total Heterotrophic Count	Total Coliform Count	Yeast and Moulds Count	*Bacillus cereus* Count
HARVEST (FRESH SAMPLES)	March	1.2 X 10^3	6.5 X 10^2	3.0 X 10^1	0
	April	2.1 X 10^3	2.7 X 10^2	7.0 X 10^2	0
	May	1.2 X 10^3	2.3 X 10^2	8.0 X 10^1	0
	Mean	**1.5 X $10^{3\,a}$**	**3.8 X $10^{2\,a}$**	**2.7 X $10^{2\,a}$**	**0 ^{a}**
LANDING (FRESH SAMPLES)	March	3.5 X 10^3	1.0 X 10^3	4.0 X 10^1	0
	April	3.0 X 10^5	3.6 X 10^3	1.8 X 10^3	0
	May	4.3 X 10^4	1.4 X 10^3	4.8 X 10^2	0
	Mean	**1.2 X $10^{5\,a}$**	**2.0 X $10^{3\,a}$**	**7.7 X $10^{2\,a}$**	**0 ^{a}**
MARKET (FRESH SAMPLES)	March	1.8 X 10^4	1.3 X 10^4	5.5 X 10^1	0
	April	5.2 X 10^6	5.7 X 10^4	2.9 X 10^3	0
	May	4.7 X 10^6	4.6 X 10^4	3.5 X 10^3	0
	Mean	**3.3 X $10^{6\,b}$**	**3.9 X $10^{4\,b}$**	**2.2 X $10^{3\,a}$**	**0 ^{a}**

Ghana Standard Authority/ICMSF standards: (Total count: 1 x 10^6 cfu/g; Total coliform count: 1 x 10^4 cfu/g; Bacillus cereus count: 1 x 10^4 cfu/g; Yeast and moulds count: 1 x 10^4 cfu/g). Values in a column bearing the same superscript are not significant at 5% (Tukey's post-hoc test, $p < 0.05$).

From Table 4, *S. aurita* obtained from market in Temarecorded higher counts for THC and TCC. All samples recorded no growth for BCC in April and May. There were counts of yeast and moulds recorded in all collected samples but levels were within 10^1cfu/g and 10^2cfu/g.

Table 4Monthly mean microbial counts of *S. aurita*obtainedfrom Tema expressed in cfu/g

SAMPLING SITE	MONTH	Total Heterotrophic Count	Total Coliform Count	Yeast and Moulds Count	*Bacillus cereus* Count
HARVEST (FRESH SAMPLES)	March	3.6×10^3	5.3×10^2	3.0×10^1	5.0×10^1
	April	1.9×10^3	8.5×10^2	7.5×10^1	0
	May	8.8×10^2	2.6×10^2	5.4×10^1	0
	Mean	**$2.1 \times 10^{3\,a}$**	**$5.5 \times 10^{2\,a}$**	**$5.3 \times 10^{1\,a}$**	**$1.7 \times 10^{1\,a}$**
LANDING (FRESH SAMPLES)	March	5.2×10^5	8.2×10^3	5.5×10^1	5.0×10^2
	April	5.7×10^4	5.8×10^3	1.7×10^2	0
	May	4.9×10^4	4.9×10^3	8.0×10^2	0
	Mean	**$2.0 \times 10^{5\,a}$**	**$6.3 \times 10^{3\,a}$**	**$3.4 \times 10^{2\,a}$**	**$1.7 \times 10^{2\,a}$**
MARKET (FRESH SAMPLES)	March	9.0×10^5	2.7×10^4	1.4×10^2	1.8×10^3
	April	8.7×10^6	8.7×10^4	2.2×10^2	0
	May	7.9×10^6	9.8×10^4	3.3×10^2	0
	Mean	**$5.8 \times 10^{6\,b}$**	**$7.1 \times 10^{4\,b}$**	**$2.3 \times 10^{2\,a}$**	**$6.0 \times 10^{2\,a}$**

Ghana Standard Authority/ICMSF standards: (Total count: 1 x 10^6 cfu/g; Total coliform count: 1 x 10^4 cfu/g; Bacillus cereus count: 1 x 10^4 cfu/g; Yeast and moulds count: 1 x 10^4 cfu/g). Values in a column bearing the same superscript are not significant at 5% (Tukey's post-hoc test, $p < 0.05$).

From Table 5, fresh *E. encrasicolus* obtained at harvest from Accra recorded the lowest counts of THC and TCC in each month, while those obtained from market recorded the highest counts for THC and TCC for all three months. Fresh samples of *E. encrasicolus* recorded no growths for BCC in the three months except for those obtained at market.

Table5 Monthly mean microbial counts of *E. encrasicolus*obtained from Accra expressed in cfu/g

SAMPLING SITE	MONTH	Total Heterotrophic Count	Total Coliform Count	Yeast and Moulds Count	*Bacillus cereus* Count
HARVEST (FRESH SAMPLES)	March	7.0×10^3	2.7×10^3	0	0
	April	1.9×10^3	9.6×10^2	2.5×10^1	0
	May	2.9×10^3	3.8×10^2	3.5×10^1	0
	Mean	**$3.9 \times 10^{3\,a}$**	**$1.3 \times 10^{3\,a}$**	**$2.0 \times 10^{1\,a}$**	**$0^{\,a}$**
LANDING (FRESH SAMPLES)	March	2.5×10^5	4.2×10^4	0	0
	April	2.4×10^5	1.1×10^3	3.0×10^1	0
	May	3.4×10^5	2.3×10^3	4.7×10^1	0
	Mean	**$2.8 \times 10^{5\,a}$**	**$1.5 \times 10^{4\,ab}$**	**$2.6 \times 10^{1\,a}$**	**$0^{\,a}$**
MARKET (FRESH SAMPLES)	March	8.5×10^6	8.8×10^4	4.4×10^2	0
	April	8.2×10^6	1.5×10^4	2.2×10^2	4.5×10^3
	May	5.2×10^6	5.1×10^4	3.4×10^2	6.5×10^2
	Mean	**$7.3 \times 10^{6\,b}$**	**$5.1 \times 10^{4\,b}$**	**$3.3 \times 10^{2\,b}$**	**$1.7 \times 10^{3\,a}$**

Ghana Standard Authority/ICMSF standards: (Total count: 1 x 10^6 cfu/g; Total coliform count: 1 x 10^4 cfu/g; Bacillus cereus count: 1 x 10^4 cfu/g; Yeast and moulds count: 1 x 10^4 cfu/g). Values in a column bearing the same superscript are not significant at 5% (Tukey's post-hoc test, $p < 0.05$).

Table6 Monthly mean microbial counts of *S. aurita*obtainedfrom Accra expressed in cfu/g

SAMPLING SITE	MONTH	Total Heterotrophic Count	Total Coliform Count	Yeast and Moulds Count	*Bacillus cereus* Count
HARVEST (FRESH SAMPLES)	March	3.7×10^3	1.5×10^3	2.4×10^1	0
	April	8.5×10^2	1.7×10^2	6.5×10^1	0
	May	6.4×10^3	3.4×10^2	2.7×10^1	0
	Mean	**$3.7 \times 10^{3\,a}$**	**$6.7 \times 10^{2\,a}$**	**$3.9 \times 10^{1\,a}$**	**$0^{\,a}$**
LANDING (FRESH SAMPLES)	March	4.5×10^5	4.9×10^3	3.7×10^3	0
	April	1.1×10^4	2.3×10^2	2.3×10^2	0
	May	4.1×10^5	1.6×10^3	3.2×10^2	0
	Mean	**$2.9 \times 10^{5\,ab}$**	**$2.2 \times 10^{3\,a}$**	**$1.4 \times 10^{3\,a}$**	**$0^{\,a}$**
MARKET (FRESH SAMPLES)	March	6.4×10^5	6.8×10^4	5.0×10^3	6.5×10^2
	April	5.1×10^6	6.4×10^3	4.5×10^2	0
	May	8.2×10^6	5.9×10^3	5.5×10^2	0
	Mean	**$4.6 \times 10^{6\,b}$**	**$4.6 \times 10^{4\,b}$**	**$2.0 \times 10^{3\,a}$**	**$2.2 \times 10^{2\,a}$**

Ghana Standard Authority/ICMSF standards: (Total count: 1 x 10^6 cfu/g; Total coliform count: 1 x 10^4 cfu/g; Bacillus cereus count: 1 x 10^4 cfu/g; Yeast and moulds count: 1 x 10^4 cfu/g). Values in a column bearing the same superscript are not significant at 5% (Tukey's post-hoc test, $p < 0.05$).

From Table 6, fresh *S. aurita* obtained at harvest from Accra recorded the lowest counts for THC while those obtained from the retail market recorded the highest counts for THC. Samples of *S. aurita* recorded no growths for BCC in all three months except for those obtained at market where growth was recorded in March. There were counts of yeast and moulds in all collected samples but counts were within 10^1 and 10^3 cfu/g.

In general, there was an increase in trend of microbiological quality changes from harvest to market for the samples. However, only samples collected at the market recorded THC and TCC values above the GSA/ICMSF standards (1.0×10^6 and 1.0×10^4 cfu/g respectively). This was observed in both species from both study sites.

DISCUSSION

Environmental conditions do not only influence the quality of fish, but also influence fish distributions, communities and seasonal movements. To minimize energy expended for survival, organisms typically favor areas that optimize their physiological processes [24]. Water quality is therefore, an important factor that determines the environmental conditions of fish. It is an indicator of excellent and poor living conditions of any fish. Good and optimum water conditions promote growth and reproduction of fish and reduce their susceptibility to diseases or stress in general [25].

The mean temperatures of 29.9 ± 0.7 °C and 29.4 ± 0.7 °C for Tema and Accra respectively were within the optimumtemperatues for both *E. encrasicolus*and *S. aurita* because both species prefer a minimum temperature of 24 °C which conformed to that reported by[7]. These species prefer a pH of almost neutral which was the case in both Tema and Accra.The mean dissolved oxygen concentrations of seawater in Tema and Accra were at considerably good levels since they were relatively higher than the lethal level of less than 1 mg/L. Salinity in both areas was also within optimum range (5 to 41 ppt) for both fish species as reported by[7].

Faecal coliform counts of the seawater were below the WHO guideline of ≤ 1000 cfu/100 ml of water [26]. This suggested that, the idea that the assimilation capacity or carrying capacity of the ocean is high enough to naturally deal with pollutants is a fact.Continuous dumping of sewage at the KorleGonno Beach Disposal Site did not affect the microbiological quality of the water considering the closeness of the study sites to the dumping site. Apart from the volume of the water, this could have also been related to the salt composition of the sea water since most pathogens do not thrive well in saline environment.

Fresh fish samples obtained at sea immediately after harvest were of good quality because all microbial colony counts were below the Ghana Standard Authority (GSA) and the International Commission on Microbiological Specifications for Foods (ICMSF) standards (1.0×10^6cfu/g for THC; and 1.0×10^4cfu/g for TCC, YMC, and BCC). This further confirmed the fact that, the seawater was not polluted and that the water did not have any adverse effects on the quality of fish.

Again, microbial counts in fresh fish samples collected on landing in both Tema and Accra were below local and international standards. This may be due to the fact that the fishers stored and transported harvested fish to the shores under good and hygienic conditions, or the time spent to get to the shores was too short to have allowed spoilage of the fresh fish to begin. The difference between microbial counts in samples obtained at harvest and on landing were not significant ($p < 0.05$) in both Tema and Accra.

With respect to both local and international standards, samples obtained from retail markets from both Tema and Accra were of poor quality and hence were unwholesome for consumption (thus,consuming without adequate hygienic processing such as properly cooking them). This was because the density of total heterotrophic bacteria and coliform bacteria counted in fresh samples obtained from the market were all above the GSA and ICMSF standards (see Tables 3, 4, 5 and 6.). The THC and TCC recorded for fresh samples from the markets however, were significantly different from those recorded at harvest and on landing ($p < 0.05$). This result may have been due to the poor handling methods used by fishers. Example, scooping fish out of the boats with all sorts of containers, such as buckets and pans which bruise the surface of the fish making it more susceptible to bacterial attack. This result conforms to results reported by Lan*et al.* [27], who recorded low amounts of microbial load in common carp, silver carp and Nile tilapia at harvest from both wastewater-fed ponds and non-wastewater-fed ponds in Hanoi, Vietnam, but recorded high amounts of microbial loads in fish sampled at the point of sale in retail markets. Lan*et al.* [27]attributed their result to poor handling practices used by the local fishmongers in processing fish. Colakoglu*et*

al.[28] also recorded a similar result where seafood (finfish and shellfish) sampled from retail markets recorded high bacteria counts than those sampled at wholesale markets in the Dardanelles, Turkey. Abou-Elela and Farag[29]; Topic *et al*. [30]; and Abolagba and Igbinevbo[4] all reported similar results where fresh fish samples at retail markets recorded higher levels of bacterial loads that did not meet international standards.Debrah, *et al*.[9] observed higher counts above the GSA standard of coliforms and *E. coli* loads in fresh Yellow Fin Tuna (*Thunnusalbacares)* landed and marketed at the Dixcove Beach in Ghana. This was a clear indication that fishmonger education is required on good handling and processing methods to improve their hygienic practices at landing sites and markets in Ghana.

Coliform bacteria are indicator organisms whose presence in food in large quantity indicates the probability of presence of pathogenic bacteria.Coliforms are abundant in the feces of warm-blooded animals, but can also be found in the aquatic environment, in soil and on vegetation [22]. Their presence in the fresh fish samples obtained at harvest was an indication that they were present in the fish's environment and that there is a probability that there may be pathogenic bacteria in the fish or its environment or both. There were counts of yeast and moulds recorded for all collected samples of *E. encrasicolus* and *S. aurita* from both Tema and Accra, but these counts were not significantly different ($p < 0.05$) from each other. The values recorded were within 0 and 1.5 x 10^3 cfu/g with the highest counts found in samples from the retail market. This is also an indication that the samples in the market were more contaminated than those at harvest and on landing. Though these microbes were present, their levels were significantly below ($p< 0.05$) the GSA and the ICMSF standards (1.0 x 10^4 cfu/g).

The recording of higher counts of total heterotrophic bacteria and coliform bacteria in both *E. encrasicolus*and *S. aurita* samples from the retail markets in both Tema and Accra suggests that there are potential human pathogens present in fish sold in local retail markets in Ghana. This implies that fish and fish products from these markets could pose serious health threats to humans when they are consumed without adequate hygienic processing such as properly cooking them, as consumption of potential human pathogens in large quantities could cause serious food-borne illnesses or poisoning [31].Inglis*et al*. [32] reported that the consumption of fish contaminated with pathogens which are stored at temperatures conducive for bacterial multiplication may result in gastroenteritis, typhoid fever, diarrhoea and emesis. These infections may only occur if fish is consumed without any further treatment, therefore proper treatment of fish before consumption is highly recommended.

CONCLUSION

The study revealed that, fresh samples sold at the local retail markets had the highest loads of microbial counts up to levels above local and the International standards, and therefore were unwholesome for immediate consumption without adequate hygienic processing. Microbial loads in samples obtained from the local retail markets were significantly higher than those obtained at harvest and on landing. This was attributed to poor handling and storage methods used by fishers and fish mongers at the landing beaches or the retail markets.The results indicated that, the poor insanitary conditions of landing beaches should be dealt with through continuous sensitization of the fisher folk in the artisanal fishery on the need to keep the landing sites or beaches clean and on the need for good personal hygiene. The safety standards established by the Ghana Standards Authority (GSA) for ensuring the wholesomeness of fish and its products should be enforced.

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