

Microbiological Evaluation of key body parts of Niger Delta Shell Seafoods, Blood Clam (*Tegillarca Granosa*) and Steamer Clam (*Mya Arenaria*) from Upstream and downstream of Andoni River.

¹Renner Renner Nrior*, ²Chidinma Daokoru-Olukole and
³Awajimerei Migbana Rufus

^{1,3}Department of Microbiology, Faculty of Science, Rivers State University, Port Harcourt, Nigeria

²Department of Environmental Biology, Faculty of Science, Niger Delta University, Bayelsa, Nigeria

*Corresponding author e-mail: renner4nrrior@gmail.com

Abstract: Microbiological evaluation of the key body parts of Niger Delta edible shell seafoods, Blood clam (*Tegillarca granosa*) and Steamer clam (*Mya arenaria*) sampled from Andoni River, Rivers State were investigated. The seafoods (blood clam and steamer clam) were collected, processed and analysed using standard microbiological method to determine the parameters. It was observed from the results that heterotrophic bacterial and fungal count (TBC) varies with region of the body and from location to location. Bacteria count varies from 5.4×10^7 cfu/g to 5.0×10^8 cfu/g and 1.48×10^8 cfu/g for blood clam and steamer clam respectively in the upstream (Station 1). The highest bacteria count (5.00×10^8 cfu/g) were observed in the inner shell surface of blood clam while the lowest count (5.4×10^6 cfu/g) were observed in the intestine while that of steamer clam was just the opposite. Bacteria count also varies between inner shell surface and intestines of both clams. The total bacteria load observed in the blood clam (8.54×10^8 cfu/g) and steamer clam (7.44×10^8 cfu/g) in the upstream were higher than downstream values of 6.00×10^8 cfu/g and 4.90×10^8 cfu/g respectively. Blood clam had higher bacteria count in both stations than the steamer clam. Bacteria count was consistently higher in the inner shell surface of blood clam and in the intestine of the steamer clam. A total of 12 bacteria genera were isolated from upstream and downstream samples which include, *Staphylococcus* spp, *Vibrio* sp, *Escherichia coli*, *Bacillus* sp, *Enterobacter* spp, *Pseudomonas* sp, *Salmonella* spp, *Streptococcus*, *Corynebacterium* spp, *Serratia marcescens*, *Klebsiella* spp, *Enterococcus* spp and *Proteus* spp. Bacterial count in the upstream was higher than that of the downstream. *Proteus* had the highest frequency (23.89%). The frequency (%) trend were: *Proteus* (23.89) > *Enterobacter* spp (18.11) > *Streptococcus* (13.98) > *Escherichia coli* (13.66) > *Staphylococcus* spp (12.71) in the upstream while *Enterobacter* sp (21.91) > *Salmonella* spp (11.43) > *Bacillus* spp (14.29) in the downstream. The Fungal count in blood clam ranged between 2.1×10^7 cfu/g (outer shell surface) and 2.5×10^7 cfu/g (intestine) while that of steamer clam ranged between 1.9×10^7 cfu/g (outer shell surface) and 2.1×10^7 cfu/g (intestine) in the upstream. In the downstream, fungal load in the blood clam ranged between 1.6×10^7 cfu/g (inner shell surface) and 2.3×10^7 cfu/g (intestine) while that of steamer clam ranged between 1.4×10^7 cfu/g (inner shell surface) and 1.7×10^7 cfu/g (intestine). Fungal load in both blood clam and steamer clam were consistently lowest in the outer shell surface and highest in the intestine in the respective stations/location. Fungal load in blood clam was higher than the steamer clam in both the upstream and the downstream. A total of 11 fungi were isolated consisting of *Aspergillus vasicolor*, *A. niger*, *A. flavus*, *A. fumigatus*, *Penicillium cemenberti*, *Alternaria* sp, *Fusarium* sp, *Physarium cinerium*, *Cryptococcus* sp and *Candida* sp. In the blood cockle, *Aspergillus versicolor* had the highest density of 1.0×10^6 cfu followed by *Aspergillus niger* and *A. flavus* with the count of 6.0×10^6 cfu while in the steamer clam *Aspergillus niger* had the highest density of 4.5×10^6 cfu and lowest count/density of 1.5×10^6 cfu in *Alternaria* sp (upstream station 1). It was concluded that the total microbial counts obtained from these organisms in this water body were found to be higher than the specified standard limits (1×10^5 cfu/g for bacteria 1×10^2 cfu/g for coliforms) by ICMSF. There should be adequate creation of awareness especially for consumers of seafoods from this particular area so as to cultivate the habit of proper cooking before consumption; moreover the presence of pathogenic microbes is of great health concern.

Keywords: Edible shell seafood, Blood clam (*Tegillarca granosa*), Steamer clam (*Mya arenaria*), *Vibrio* sp., *Staphylococcus* sp., *Cryptococcus* sp. and *Candida* sp.

Date of Submission: 09-09-2017

Date of acceptance: 06-10-2017

I. Introduction

Seafood is any form of sealife regarded as food by humans. Seafood constitutes an important food component for a large proportion of world population especially those living in coastal areas (John, 2006). The term shellfish covers the bivalve molluscan shellfish (oysters, cockles, clams and mussels), the gastropods (periwinkles, sea-snails) and the crustacean shellfish (crab, lobster, shrimp). A cockle is a small, edible, saltwater clam, a marine bivalve mollusc. Although many small edible bivalves are loosely called cockles, true cockles are species in the family Cardiidae. True cockles live in sandy, sheltered beaches throughout the world. The distinctive rounded shells are bilaterally symmetrical, and are heart-shaped when viewed from the end. Numerous radial evenly spaced ribs are a feature of the shell in most but not all genera (for an exception, see the genus *Laevicardium*, the egg cockles, which have very smooth shells) (Huess *et al.*, 2003).

Survey on the microbiological quality of shellfishes has shown Shellfishes to harbor pathogens which have been implicated in outbreaks of food -borne diseases in many parts of the world. These illnesses include typhoid fever, hepatitis and similar disorder of the digestive system and neurological disorder (Ukpong and Utuk 1992) In major parts of the world, about 10-19% of food-borne illness involved shellfishes as a vehicle and between 1993 and 1997, 6.8% of the food borne illnesses involved consumption of fish and shellfishes (Huss *et al.*, 2003).

Like any food items, seafood carry a variety of bacteria, viruses, and parasitic pathogens capable of causing disease In consumers (WHO, 2010). These agents are acquired from the three sources, faecal pollution of the aquatic environment, to a lesser extent, the natural aquatic environment and Industry, retail, restaurant, or home processing and preparation.

The quality of our foods is of major concern to food processors and public health authorities. It has been estimated that there are more than 100 million cases per annum of seafood illnesses in the world. This microorganism causing Food borne infection and food intoxication in seafood is a major concern to public health authorities, as a result of this, microbiological standards as being set and are implemented through quality control and quality assurance (Miller and Kvenberg, 2010).

In recent years, the Niger delta, environment have been exposed to organic and inorganic contaminants from industries and domestic wastes, especially oil related activities which are predominant in the region, thereby enhancing the capacity of the ecosystem into harboring a sizeable population of microorganisms [Huess, *et al.*, 2003]. The continuous release of these pollutants has resulted in an enriched microbial community. These microbes often find surfaces or organs of aquatic organisms for colonization Hayat *et al.*, [2003], noted that the number and type of microorganisms found on freshly caught seafood are influenced by location, season and rate of environmental pollution such as crude oil. Due to high level of consumption of seafoods in Riverine area, especially in Andoni, there is need for continuous microbiological analysis of foods from the aquatic environment in order to understand their hazard levels and thus creating awareness to the public on health risks in consuming raw or undercooked and under processed shellfishes.

The microbiological flora in the intestines of sea foods such as finfish, shellfish and cephalopods is quite different being psychotrophic in nature and to some extent believes to be a reflection of general contamination in the aquatic environment. In filter feeding bivalve molluscan shellfish (oyster) and accumulation and concentration of bacteria and viruses from the environment is generally taking place (Adebayo-Tayo *et al.*, 2012a). Consumption of seafoods such as clam, conch, cockle, periwinkle, mullet and snails in the Niger Delta area for food has been of immense importance in maintaining the health status of rural inhabitants over the years through their nutrient contents. However, the microbiological characteristics of the various seafoods with respect to species, body parts and regions or locations were not put into consideration hence the need for this research. More so information on the type and nature of microbes precisely bacteria and fungi species are yet to be documented. The consumption of fresh and marine water shell sea food are on the increase in both rural and urban centres in Nigeria (FDF, 2007; Emikpe *et al.*, 2011). However, there is scare information on the microbial load in the different parts of shell sea food from the Niger Delta environment.

Thus, the present study was designed to give an overview of the risk associated with the consumption of these shell sea food harvested in these oil region where the environment is changing every day. Enumerate the microbial quantity in the seafoods (Clam) from the Andoni River in Niger Delta, determine the species of microbes (bacteria and fungi) present in these organisms with respects to body parts and locations, compare the microbial population in the various seafood organisms (blood clam and steamer clam) from the Andoni area, to assess the microorganisms in seafoods in Andoni River so as to ascertain their health status thereby creating awareness to the general public who will be involved in its consumption.

II. Materials And Methods

Sample Collection and Preparation

Live samples of the blood cockle and Steamer clam were collected from the two locations /stations of Andoni River in Andoni Local Government area of Rivers State. The specimens (blood cockle and steamer clam) were gotten from the fisher women in the respective stations who specialized in the harvest of the particular species of shell fish. The samples after collection were brought to the laboratory for storage in a sterile plastic container. The samples were stored in the refrigerator at 4°C for 24 hours. A preliminary field survey was carried out to identify the samples collected from the Andoni River in Niger Delta. Aseptic procedures were carried out so as to minimize contamination from bacteria not associated with the collected specimens.



Fig. 1: Fresh Shell seafoods Blood Clam (*Tegillarca granosa*) and Steamer Clam (*Mya Arenaria*)

Media and diluents used.

The used media include Nutrient agar (Biomark Laboratory India), MacConkey agar (Micro Master India) and Sabouraud dextrose agar (Biomark Laboratory India). The various media were prepared according to the instructions of the manufacturers. Physiological saline will be prepared by adding 0.85g of sodium chloride (NaCl) to 100ml of sterile distilled water for use as diluents.

Sample preparation for microbiology

Internal and external samples of the cockle (intestine and inner fluid) and the external (outer surface) from the various project sites were prepared according to the methods described by Adedire (2002). The clam and cockle from the various sites were kept in a deep freezer separately for 30 minutes to be immobilized before clipping the body to ease further dissection. 1 gram of the intestine; 1 ml of inner fluid and 1ml of aseptically washed surface of external contents (outer surface/shell) were aseptically transferred into 9ml of diluents (physiological saline) in a 150ml conical flask which will be vigorously shaken to dislodge the microbes (bacteria and fungi) present in each of the parts(outer shell/surface, inner surface/body fluid and intestine). Ten-fold serial dilutions were carried out by adding 1.0ml of the initial dilution to 9ml of fresh diluents according to Harrigan and MaCance(1990) method.

At the end, 0.1ml of an appropriate dilution were inoculated on dry nutrient agar, MacConkey agar and Sabouraud dextrose agar plates in duplicates and evenly spread with a sterile spreader and incubated at 37°C for 24 hours for Nutrient agar and Mac Conkey agar; 28±2°C for 3-5days for Sabouraud dextrose agar.

Isolation and Identification of Microbes

Bacteria: A part of 1ml of the 10⁻¹ diluents were inoculated onto nutrient agar, spread out to cover the entire surface and incubated at 37°C for 24 hours. Representative colonies were picked and inoculated onto nutrient agar to obtain pure cultures. Pure cultures were stored as frozen 10% (v/v) glycerol suspensions at -35°C in a refrigerator (Wellington and Williams, 1978). This glycerol served as a means for fresh working cultures.

Identification of the isolates were carried out according to the schemes of Cowan and Steel (1966) and Buchanam and Gibbon(1974). The tests employed include morphological tests, catalase, citrate, motility vp, coagulase, indole and methyl red, growth on MacConkey agar, fermentation/oxidation of glucose, sucrose and mannitol.

Fungi: Identification of fungi isolate was based on the morphological and microscopic characterization such as type of mycelium, pigmentation type of sporulating structures and sexual reproduction (if present). They are examined using hand lens to determine those morphological characteristics.

Microscopy: Several coloured colonies were selected from the incubated plate for identification using the following procedure: a wet mount slide was prepared by transferring a small amount of the culture with a dissecting needle or inoculating loop to make a slide. This was covered with a cover slip and examined under low power (x10) or high power (x100) objective.

III. Results

Microbiology of blood clam and steamer clam

Total Bacteria Count: The total heterotrophic bacteria count of blood clam and steamer clam in the two locations in Andoni River are as shown in figure 1. It is observed from the results that heterotrophic bacteria count (TBC) varies with region of the body and from location to location. In location 1(upstream) bacteria count varies from 5.4×10^7 cfu/g to 5.00×10^8 cfu/g and 1.48×10^8 cfu/g for blood clam and steamer clam respectively. In the blood clam the highest bacteria count (5.00×10^8 cfu/g) were observed in the inner shell surface while the lowest count (5.4×10^7 cfu/g) were observed in the intestine. In steamer cockle the highest bacteria count 3.76×10^8 cfu/g were observed in the intestine while the lowest count (1.48×10^8 cfu/g) were observed in the inner shell surface.

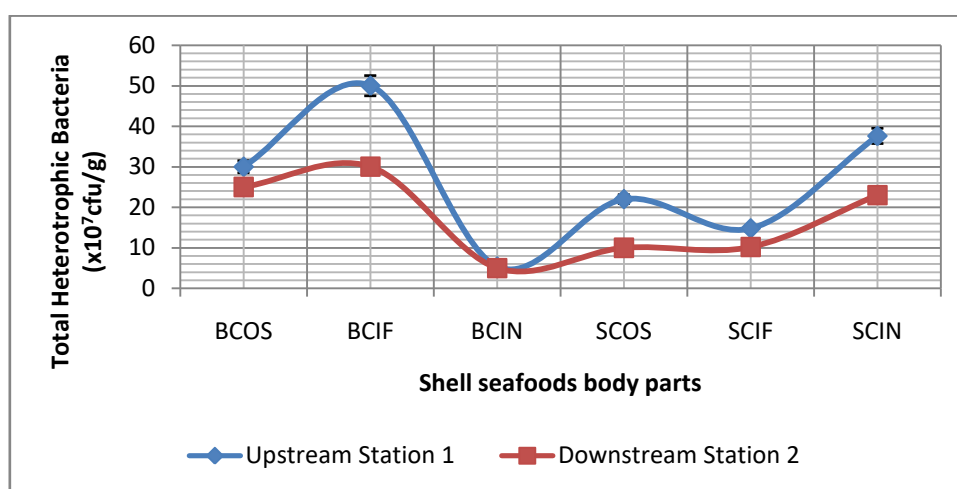


Fig. 2: Bacterial Count of Shell Seafoods Blood clam (*Tegillarca granosa*) and Steamer clam (*Mya arenaria*) in from Upstream and Downstream of Andoni River.

Key: BCOS = Blood Clam outer surface, BCIF = Blood Clam innershell fluid, BCIN = Blood Clam intestine, SCOS = Steamer Clam outer surface, SCIF = Steamer Clam innershell fluid, SCIN = Steamer Clam intestine.

In the second station/location which formed the downstream segment of Andoni River, bacteria count also varies with respect to the body region and organism just as noticed in location 1 which is the upstream. Bacteria count varies from 5.0×10^7 cfu/g (intestine) to 3.00×10^8 cfu/g (inner shell surface) in the blood clam and 100×10^6 cfu/g (inner shell surface) to 2.30×10^8 cfu/g (intestine) in the steamer clam. The total bacteria load observed in the blood clam and steamer clam in the upstream were respectively 8.54×10^8 cfu/g and 7.44×10^8 cfu/g while the downstream were 6.00×10^8 cfu/g and 4.90×10^8 cfu/g respectively. It is therefore observed that blood clam had higher bacteria count in both stations/location than the steamer clam. Also, bacteria count was consistently higher in the inner shell surface of blood cockle but consistently higher in the intestine of the steamer clam just as observed in the blood clam.

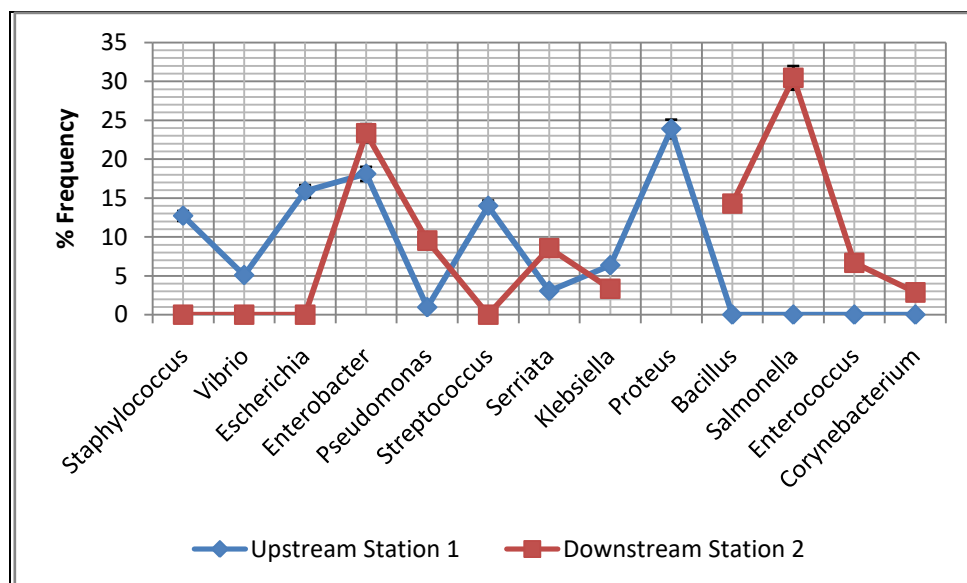


Fig. 3: Bacteria Frequency of Seafoods (Blood and Steamer Clam) in the Downstream of Andoni River.

Table 1 and 2 showed the Biochemical and morphological identification of bacteria isolates. A total of 12 bacteria were isolated from the isolates consisting 3 from blood cockle from the upstream (*Bacillus spp*, *Staphylococcus spp*, and *Escherichia coli*), 3 from downstream blood cockle (*Bacillus sp*, *Enterobacta spp* and *Serratia marcesans*), 3 from the upstream steamer clam (*Pseudomonas sp*, *Bacillus spp* and *Escherichia coli*) and 3 from downstream steamer isolates (*Corynebacterium spp*, *Serratia marcesans*, *Enterobacta spp*. Figure 3 shows the bacteria frequency of blood and steamer clams with respect to location and parts/regions of the body. The table showed that *Proteus species* in the upstream (station1) had the highest frequency of (23.89%) followed by *Enterobacter sp* with the frequency of 18.11%. In the downstream, *Enterobacter species* also appeared highest with the frequency of 21.91%. Others are as in Figure 3. The total bacterial load in both clams in the upstream is higher than that of the downstream. The bacterial load also varies from region to region of the body of the two clams.

Total Fungal Count (TFC) Figure 4 showed the total fungal count per region of the body and location with respect to the two organisms (blood clam and steamer clam) sampled. The Fungal load/count in blood cockle ranged between 2.1×10^7 cfu/g (outer shell surface) and 2.5×10^7 cfu/g (intestine) while that of steamer clam ranged between 1.9×10^7 cfu/g (outer shell surface) and 2.1×10^7 cfu/g (intestine) in the upstream. In the downstream, fungal load in the blood cockle ranged between 1.6×10^7 cfu/g (inner shell surface) and 23×10^7 cfu/g (intestine) while that of steamer clam ranged between 1.4×10^7 cfu/g (inner shell surface) and 1.7×10^7 cfu/g (intestine).

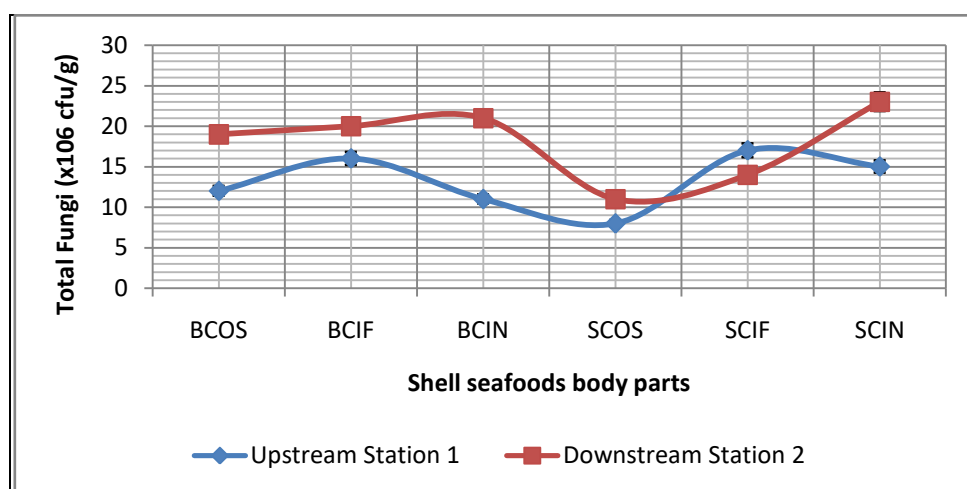


Fig. 4: Total fungi Count of Shell Seafoods Blood clam (*Tegillarca granosa*) and Steamer clam (*Mya arenaria*) in from Upstream and Downstream of Andoni River.

Table 1: Frequency of fungal isolates from body parts of Shell seafoods Blood Clam and Steamer Clam from Upstream Station 1

Isolate	<i>Aspergillus Niger</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus vasicular</i>	<i>Penicillium cemenberti</i>	<i>Alternaria</i>	<i>Fusarium sp</i>	<i>Physarium Cinerium</i>	<i>Aspergillus flavus</i>	<i>Cryptococcus sp</i>	<i>Candida sp</i>
BLOOD CLAM upstream Station 1										
BC _{OS}	-	-	4 x 10 ⁶	3.0 x 10 ⁶	-	-	2.0x10 ⁶	1.0 x 10 ⁶	-	-
BC _{IF}	2.0 x 10 ⁶	2.0 x 10 ⁶	3.0 x 10 ⁶	1.0 x 10 ⁶	3x10 ⁶	-	-	2.0 x 10 ⁶	-	1.0 x 10 ⁶
BC _{IN}	4.0 x 10 ⁶	-	3.5 x 10 ⁶	3.0 x 10 ⁶	1.2x10 ⁶	-	-	3.0 x 10 ⁶	3.1 x 10 ⁶	-
Total(cfu/g)	6.0 x10⁶	2.0x10⁶	1.0x10⁷	4.2X10⁶	4.2x10⁶	2.0x10⁶	2.0x10⁶	6.0X10⁶	3.1 x 10⁶	1.0 x10⁶
STEAMER CLAM upstream Station 1										
SC _{OS}	3.0 x 10 ⁶	-	3.5x10 ⁶	-	-	1.0x10 ⁶	-	-	2.0x10 ⁶	-
SC _{IF}	1.5 x 10 ⁶	-	-	-	1.5 x 10 ⁶	2.5x10 ⁶	-	1.0 x 10 ⁶	-	2.0 x 10 ⁶
SC _{IN}	-	3.0 x 10 ⁶	-	2.0 x 10 ⁶	-	-	3.5x10 ⁶	2.5x10 ⁶	-	1.0 x 10 ⁶
Total (cfu/g)	4.5x10⁶	3.0x10⁶	3.5x10⁶	2.0x10⁶	1.5x10⁶	3.5x10⁶	3.5x10⁶	3.5x10⁶	2.0x10⁶	3.0x10⁶

KEY: BC= Blood clam, SC= Steamer clam, OS= Outer surface, IF= Inner fluid, IT= Intestine

Key: BCOS = Blood Clam outer surface, BCIF = Blood Clam innershell fluid, BCIN = Blood Clam intestine, SCOS = Steamer Clam outer surface, SCIF = Steamer Clam innershell fluid, SCIN = Steamer Clam intestine.

The result shows that fungal load in both blood clam and steamer clam were consistently lowest in the outer shell surface and highest in the intestine in the respective stations/location. Also, the fungal load in blood cockle was higher than the steamer clam in both the upstream and the downstream. In similar manner, fungal load was consistently increasing from the outer shell surface to the intestine in both blood cockle and steamer clam except in the downstream where there is fluctuation in fungal load with respect to the body region of steamer clam.

The table 1-2 shows that blood clam and steamer clam in the upstream had total of 11 different species of fungi but with variations in load/count with respect to the body part and the station/location. The various fungi were *Aspergillus niger*, *Aspergillus fumigates*, *A.vasicular*, *Pennisetum cemenberti*, *Alternaria sp*, *Fusarium sp*, *physarium cinerium*, *Aspergillus flavus*, *Cryptococcus sp* and *candida sp*. In the blood cockle, *Aspergillus vasicular* had the highest density of 1.0x10⁷cfu followed by *Aspergillus niger* and *A. flavus* with the count of 6.0x10⁶cfu while in the steamer clam *Aspergillus niger* had the highest density of 4.5x10⁶cfu and lowest count/density of 1.5x10⁶cfu in *Alternaria sp* (in station 1(upstream). In the downstream, blood cockle fungal count ranged between 1.0x10⁶cfu (*Candida sp*) and 4.5x 10⁶cfu but ranged between 0 to 5.5x10⁶cfu *Fusarium spp* was not present in blood cockle in the upstream (station1) but very high in the downstream (station 2)

Table 2: Frequency of fungal isolates from body parts of Shell seafoods Blood Clam and Steamer Clam from Downstream Station 2

Isolate	<i>Aspergillus Niger</i>	<i>Aspergillus Fumigates</i>	<i>Aspergillus vasicular</i>	<i>Penicillium cemenberti</i>	<i>Alternaria sp</i>	<i>Fusarium sp</i>	<i>Physarium cinerium</i>	<i>Aspergillus flavus</i>	<i>Cryptococcus sp</i>	<i>Candida sp</i>
BLOOD CLAM Downstream Station 2										
BC _{OS}	2.5x10 ⁶	-	-	1.0 x 10 ⁶	-	-	2.0x10 ⁶	1.0 x 10 ⁶	1.2x10 ⁶	-
BC _{IF}	-	1.0 x 10 ⁶	1.5 x 10 ⁶	-	-	-	1.2x10 ⁶	-	-	1.0 x 10 ⁶
BC _{IN}	2.5 x 10 ⁶	2.0x10 ⁶	2.0 x 10 ⁶	-	1.5x10 ⁶	-	-	-	-	-
Total(cfu/g)	4.5x10⁶	3.0x10⁶	3.5x10⁶	1.0X10⁶	1.5x10⁶	2.0x10⁶	3.2x10⁶	1.1X10⁶	1.2x10⁶	1.0x10⁶
STEAMER CLAM Downstream Station 2										
SC _{OS}	1.0 x 10 ⁶	-	-	1.5x10 ⁶	-	1.0x10 ⁶	1.2x10 ⁶	-	2.0x10 ⁶	1.5x10 ⁶
SC _{IF}	2.0 x 10 ⁶	-	1.5x10 ⁶	1.0x10 ⁶	-	0.5x10 ⁶	1.0x10 ⁶	-	1.5x10 ⁶	-
SC _{IN}	2.5x10 ⁶	1.0 x 10 ⁶	2.0x10 ⁶	-	-	2.5x10 ⁶	-	1.2x10 ⁶	2.0x10 ⁶	1.3 x 10 ⁶
Total(cfu/g)	5.5x10⁶	1.0x10⁶	3.5x10⁶	2.5x10⁶	2.0x10⁶	4.0x10⁶	2.2x10⁶	1.2x10⁶	5.5x10⁶	2.8x10⁶

KEY: BC= Blood clam, SC= Steamer clam, OS= Outer surface, IF= Inner fluid, IT= Intestine

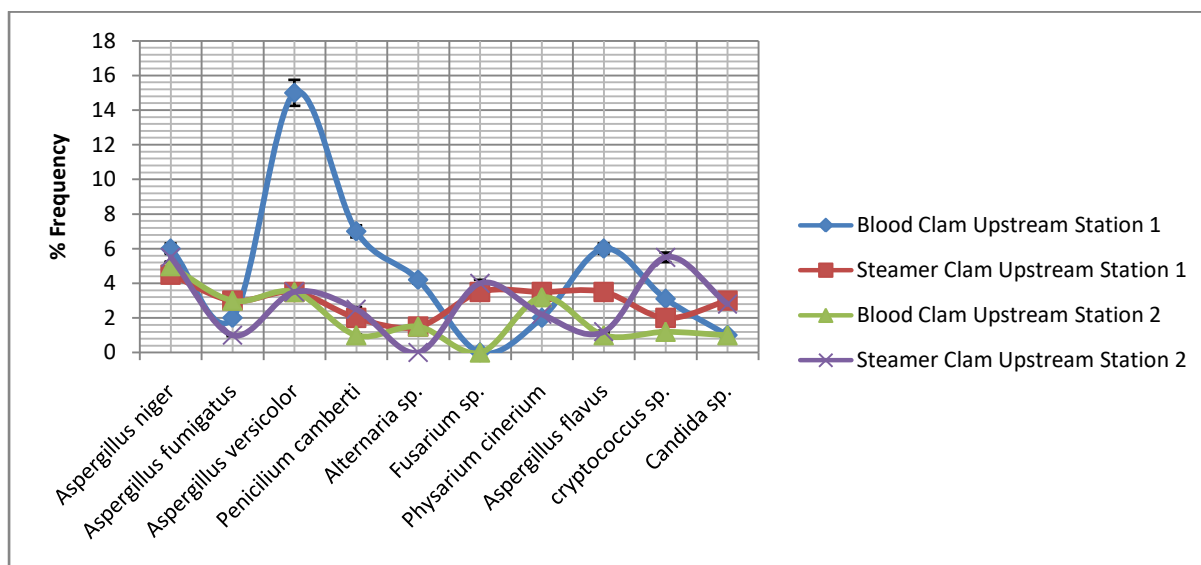


Figure 4: Frequency of fungal isolates from body parts of Shell seafoods Blood Clam and Steamer Clam from Upstream and Downstream Stations

IV. Discussion

The results showed that heterotrophic bacteria count (TBC) varies with region of the body and from location to location such that in location 1(upstream) bacteria count varies from 5.4×10^7 cfu/g to 5.00×10^8 cfu/g and 1.48×10^8 cfu/g for blood clam and steamer clam respectively. This observation is in line with the findings of Bassey *et al* (2014) who disclosed that microbial load or count differs with respect to the part of the body of the organism (molluscs and arthropods) and also with environmental difference or geographical location and the state of the organism. Etim (2015) reported a total bacterial count of 2.6×10^7 cfu/ml, 5.1×10^7 cfu/ml and 3.1×10^7 cfu/ml respectively from different abattoir wastes introduced into the water bodies and was attributed to difference in location. The results showed that bacteria count was consistently higher in the inner shell surface of blood cockle but consistently higher in the intestine of the steamer clam just as observed in the blood clam. The higher bacterial load observed in the inner shell surface and especially the intestine of the organisms in this study is in agreement with the reported count of Ogbalu and Williams (2015) who opined that observed microbial or bacteria load in the gastrointestinal tract (gut) of the *Rhynchophorus phoenicis* (maggot) especially *Staphylococcus* species was similar to that reported by (Wachukwu *et al* 2002) which was attributed to the feeding habit or life style of the organism in the area. According to Wachukwu *et al* (2002) the species such as *Bacillus* and *Staphylococcus* are known enterotoxin producers which therefore portray danger especially for people who may want to eat the organism in raw or partially cooked form.

According to the results of this study a total of 12 bacteria were isolated from the isolates consisting 3 from blood cockle from the upstream (*Bacillus spp.*, *Staphylococcus spp.*, and *Escherichia coli*), 3 from downstream blood cockle (*Bacillus sp.*, *Enterobacta spp* and *Serratia marcesans*), 3 from the upstream steamer clam (*Pseudomonas sp.*, *Bacillus spp* and *Escherichia coli*) and 3 from downstream steamer isolates (*Corynebacterium spp.*, *Serratia marcesans*, *Enterobacta spp*). This result is in disconformity with the finding of Bukola *et al.*,(2011) who reported 16 bacteria isolates of freshwater snail samples from Itu creek, Niger Delta Nigeria to include *Proteus sp.*, *Streptococcus pyrogens*, *Shigella flexneri*, *Staphylococcus aureus*, *E. coli*, *Klebsiella aerogenes*, *Citrobacter*, *Bacillus subtilis*, *Bacillus cereus*, *Aeromonas sp.*, *Micrococcus luteus*, *Streptococcus salivarius*, *Salmonella typhi*, *Vibrio parahaemolyticus*, *Vibrio sp.* and *Vibrio cholera* with *Proteus sp.*, *Aeromonas sp.* and *Micrococcus luteus* reported to have the highest frequency of occurrence (10.25%). According to Bukola (2011) the total heterotrophic count of the sample (water snail) studied ranged from 4.0×10^7 - 1.42×10^8 cfu/g and that the coliform levels were generally high ranging from 2.2 - 6.4×10^7 cfu/g in which the highest was recorded from *P. canaliculata*. The total microbial counts obtained from the work were found to be higher than the specified standard limits (1×10^5 cfu/g) for bacteria 1×10^2 cfu/g for coliforms) by ICMSF (1982) and USFDA (1991).

According to the finding of Baros (2007) this results showed that occurrence of enteric organisms like *Escherichia coli*, *Enterobacter aerogenes*, *Shigella* and *Salmonella sp* which belong to the group Enterobacteriaceae, is indicative of faecal contaminations as they are normal inhabitants of intestinal tracts of human and other animals. Most of the isolated bacteria are pathogens; a good example is *Staphylococcus aureus* which causes food poisoning, skin infections and urinary tract infection as well as gastroenteric and gastroenteric cases. *Bacillus sp* and *Escherichia coli* are responsible for the gastrointestinal tract disease such as diarrhea.

These could be regarded as destabilization of the water ecological balance arising from contamination depriving people of the usage of the seafoods receiving such wastes. Benka-Coker and Ojior [1995] associated human health with the possibility of accumulation of pathogenic enteric microorganisms by aquatic organisms. Although these seafoods (steamer clam and blood clam) were rich sources of protein but the presence of *Staphylococcus aureus* is of public health significance.

The absence of the bacteria species such as yeast or lactic acid bacterium in this study is an important finding since the samples were not gotten from palm wine. According to Omotoso and Adedire (2007), high bacterial load and protein content of a particular food indicates high nutritional quality and susceptibility to spoilage microorganisms. This statement is in agreement with this finding since the maggot has high microbial load and therefore susceptible to quick microbial deterioration.

The Fungal load/count in blood clam in this study ranged between 21×10^6 cfu/g (outer shell surface) and 2.5×10^7 cfu/g (intestine) while that of steamer clam ranged between 1.9×10^7 cfu/g (outer shell surface) and 2.1×10^7 cfu/g (intestine) in the upstream. This is in total agreement with the finding of Bukola (2011) who reported fungal count to range from $1.7-3.5 \times 10^7$ cfu/g. The result of this study also shows that fungal count/load varies with region of the body. This observation is in line with the findings of Bassey *et al* (2014) who disclosed that microbial load or count differs with respect to the part of the body of the organism (molluscs and arthropods) and also with environmental difference or geographical location and the state of the organism. The result shows that fungal load in both blood clam and steamer clam were consistently lowest in the outer shell surface and highest in the intestine in the respective stations/location. This result also corroborates with the finding of Ogbalu and Williams (2015) who opined that observed microbial or fungal load in the gastrointestinal tract (gut) was similar to that reported by (Wachukwu *et al* 2002) which was attributed to the feeding habit or life style of the organism in the area.

The different species of fungi observed in this study were *Aspergillus niger*, *Aspergillus fumigates*, *A. versicolor*, *Penicillium cemenberti*, *Alternaria sp*, *Fusarium sp*, *Physarium cinerium*, *Aspergillus flavus*, *Cryptococcus sp* and *Candida sp*. This is similar to the fungi isolates reported by Bukola (2011) which include *Aspergillus terreus*, *Cladosporium sp*, *Fusarium oxysporum*, *Cryptococcus sp*, *Aspergillus flavus*, *Aspergillus glaucus* and *Aspergillus niger* in which *A. niger*, *A. terreus* and *F. oxysporum* had the highest occurrence (16.67%). *Aspergillus* species observed in this study has been reported by Ogbalu and Williams (2014) in a study and was said to be responsible for aflatoxins which may be implicated in hepato-cellular carcinoma. Ogbalu and Williams (2014) also reported the *Penicillium* species present in this study to be attributed to ochratoxin-A production which is a potent nephrotoxin and causes damage in the body system. Most of the isolated fungal genera contain species that are potential pathogens or opportunistic pathogens. The main hazardous species belong to *Aspergillus*, *Penicillium*, *Candida* and *Fusarium*. Various strains of these families of molds have been implicated in being causative agents in asthma, hypersensitivity, pneumonitis and pulmonary mycosis. The total fungal counts were up to 10^2 cfu/ml. *Candida* was predominantly present in all the stations and in all the organisms sampled. This high occurrence of *Candida* is of considerable concern as the genera can cause candidiasis, endocarditis, septicemia, protracted urinary tract infections, kidney and lung infections, esophagitis and other soft tissue infections. *Fusarium* species are common plant pathogens and causative agents of superficial and systemic infections in humans (Mayayo, 1999). The presence of these organisms is a clear indication of the need to constantly monitor the water quality of the Andoni River to prevent excessive bioaccumulation of organic wastes by seafoods especially the clams studied. According to Akamatsu (1983) the purpose of microbiological tests is to detect the presence or absence of pathogenic bacteria (*Salmonella*, *Staphylococcus aureus*, *E. coli*) or indicator organisms of fecal pollution (*fecal coliforms*, *fecal streptococci*) or other types of general contamination or poor handling practices (coliform bacteria, *faecal streptococci*, *total viable count*) so as to prevent further contamination of seafood.

V. Conclusion And Recommendations

It is of pertinent to note that these highly desired and consumed seafoods have high microbial (fungi and bacteria) load or population in different parts of the bodies that are consumable. The microbial flora such as *Bacillus* species, *Staphylococcus* species, *Enterobacter* species and host of other fungi such as *Penicillium* species, *Candida* species, *Fusarium* species, *Aspergillus nidulans* etc are present in these seafoods. The inner shell surface and the intestines were observed to have higher load of the microbes than the outer shell. Therefore, it is also of interest to note that microbial load is highest in the clams obtained from upstream which could be attributed to their unhygienic practices and some environmental factors which must have made the environment suitable or favourable for microbial growth. The bacterial load of the seafoods (blood clam and steamer clam) in the areas studied is higher than the fungal load. Therefore adequate care should be taken in the process of consumption.

Based on the result of this finding, the following recommendations were made:

The seafoods were though considered nutritional but contains huge microbial (Bacteria and fungi) load, hence adequate hygienic practices and proper processing are therefore required before their consumption. There should be adequate creation of awareness especially for users of the seafood from this particular area to cultivate the habit of proper cooking before consumption. Also, there should be implementation of laws forbidding unnecessary dumping of wastes into the water bodies to avoid further contamination of the water body. There should be regular research in the water body to monitor the level of wastes and pollution. Nevertheless, the vast microbial loads in these species of seafoods (blood clam and steamer clam) could serve as a ready source of microbes in some processing and pharmaceutical industries.

References

- [1]. Brousseau, D.J., 1978a. Spawning cycles, fecundity, and recruitment in a population of soft-shell clam, *Mya arenaria*, from Cape Ann, Massachusetts. Fishery Bulletin. Fish and Wildlife Service. United States Department of the Interior, 76, 155-166.
- [2]. Brousseau, D.J., 1978b. Population dynamics of the soft-shell clam *Mya arenaria*. Marine Biology, 50, 67-71.
- [3]. Clay, E., 1966. Literature survey of the common fauna of estuaries. 12. *Mya arenaria* L., *Mya truncata* L. Imperial Chemical Industries Limited, Brixham Laboratory, BL/A/707.
- [4]. Commito, J.A., 1982. Effects of *Lunatia heros* predation on the population dynamics of *Mya arenaria* and *Macoma balthica* in Maine, USA. Marine Biology, 69, 187-193.
- [5]. Cutting, C.L.; and Spencer, R. 1968. Fish and fishery products. In: S.M. Herschdoerfer (ed.). Quality control in the food industry. Vol. 2. Academic Press, London England, UK, pp. 303-52.
- [6]. Eno, N.C., Clark, R.A. & Sanderson, W.G. (ed.) 1997. Non-native marine species in British waters: a review and directory. Peterborough: Joint Nature Conservation Committee.
- [7]. FNB/NRC (Food and Nutrition Board, National Research Council, USA) 1985. An evaluation of the role of microbiological criteria for foods and food ingredients (Sub-committee on Microbiological Criteria, Committee on Food Protection). National Academy Press, Washington D.C., USA.
- [8]. Fujioka, R.S., K. Tenno and S. Kansako 1988. Naturally occurring fecal coliforms and fecal streptococci in Hawaii's freshwater streams. Toxic Assess. 3, 613-630.
- [9]. Hazen, T. 1988. Fecal coliforms as indicators in tropical waters: A review. Toxic Assess. 3, 461-477.
- [10]. Huss, H.H.; Ababouch, L.; and Gram, L. 2003. Assessment and management of seafood quality and safety. FAO fisheries technical paper 444. Food and Agricultural Organization (FAO) of the United Nations (UN), Rome, Italy.
- [11]. John M. Lawrence(2006). "Sea Urchin Roe Cuisine" in John M. Lawrence, Edible sea urchins: biology and ecology
- [12]. Loosanoff, V.L. & Davis, H.C., 1963. Rearing of bivalve mollusks. Advances in Marine Biology, 1, 1-136.
- [13]. Metcalf, T.G.; Slanetz, L.W.; and Bartley, C.H. 1973. Enteric pathogens in estuary waters and shellfish. In: C.O. Chichester; and H.D. Graham (eds.). Microbial safety of fishery products. Academic Press, London, England, UK, pp. 215-34.
- [14]. Mossel, D.A.A. 1967. Ecological principles and methodological aspects of the examination of foods and feeds for indicator microorganisms. J. Assoc. Agric. Chem. 50, 91-104.
- [15]. Nelson, T.C., 1928. On the distribution of critical temperatures for spawning and for ciliary activity in bivalve molluscs. Science, 67, 220-221.
- [16]. Newell, C.R. & Hidu, H., 1986. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (North Atlantic). Softshell clam. <http://www.nwrc.usgs.gov/wdb/pub/0168.pdf>, 2000-10-02.
- [17]. Pfitzenmeyer, H.T. & Drobeck, K.G., 1967. Some factors influencing reburrowing activity of soft-shell clam, *Mya arenaria*. Chesapeake Science, 8, 193-199.
- [18]. Rice R. (2004). Seafood - an essential part of 21st century eating patterns. The Fish Foundation.
- [19]. Silliker, J.H. and D.A. Gabis 1976. ICMSF method studies VII. Indicator tests as substitutes for direct testing of dried foods and feeds for *Salmonella*. Can. J. Microbiol. 22, 971-974.
- [20]. Strasser, M., 1999. *Mya arenaria* - an ancient invader of the North Sea coast. Helgoländer Meeresuntersuchungen, 52, 309-324.
- [21]. Strasser, M., Walensky, M. & Reise, K., 1999. Juvenile-adult distribution of the bivalve *Mya arenaria* on intertidal flats in the Wadden Sea: why are there so few year classes. Helgoland Marine Research, 53, 45-55.
- [22]. Toranzos, G.A., C.P. Gerba and H. Hansen 1988. Enteric viruses and coliphages in Latin America. Toxic Assess. 3, 491-510.
- [23]. Ukpogon, E.; and Utuk, O. 1992. Microbiological quality of *Egeria radiata* in Cross River System. Book of Abstracts, Nigerian Institute of Food Science and Technology, Lagos State, Nigeria, pp. 15-6.
- [24]. Warwick, R.M. & Price, R., 1976. Macrofauna production in an estuarine mud-flat. journal of the Marine Biological Association of the United Kingdom, 55, 1-18

Renner Renner Nrior. "Microbiological Evaluation of key body parts of Niger Delta Shell Seafoods, Blood Clam (*Tegillarca Granosa*) and Steamer Clam (*Mya Arenaria*) from Upstream and downstream of Andoni River. ." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), vol. 11, no. 10, 2017, pp. 40-48.