Magna Scientia Advanced Biology and Pharmacy

eISSN: 2582-8363

Cross Ref DOI: 10.30574/msabp

Journal homepage: https://magnascientiapub.com/journals/msabp/

(RESEARCH ARTICLE)

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The dynamics of microorganisms and their association with clam in fresh water ecosystem

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Magna Scientia Advanced Biology and Pharmacy, 2023, 10(01), 001–018

Publication history: Received on 01 August 2023; revised on 13 September 2023; accepted on 15 September 2023

Article DOI: https://doi.org/10.30574/msabp.2023.10.1.0058

Abstract

Clams are shelled marine or fresh water mullusks belonging to the class Bivalvia. They are invertebrates with shells divided into two pieces called valves. They are rich source of proteins and minerals especially calcium and are recommended in the diet of pregnant women and in protein deficient cases. They inhabit the bottom of fresh water bodies or slow running waters. Fresh water is one which contain less than 0.5 parts per thousand of dissolved salts or other impurities, and are found in fresh water lakes, swamps and some rivers. The deposition of litter, substrates and other faecal materials in water body results in a build-up of pathogenic microorganism (bacteria) in the water which gives high burden on the water inhabitants including clams. The concentration of the bacteria in the water body varies with the season. This research was therefore aimed at accessing the type and density of bacteria and fungi present in fresh water associated with clams, and to determine the effect of the microorganism on the nutritional value of clams over a period of ten months in a fresh water ecosystem. Samples for analysis were water from Itu river, labeled sample A, sample B was water used to rinse the clams, sample C was homogenized clam intestine while sample D was homogenized body of the clam. The microbial load was determined using serial dilution and plating methods. Characterization and identification of microbial isolates was done using different standard biochemical tests, to determine; colonial morphology, Gram staining reaction, spore stain, motility, sugar fermentation, production of indole, coagulase and catalase. The methods as outlined by Association of Official Analytical Chemistry were used for physicochemical and nutritional analysis to test for moisture content, ash content, crude protein, fibre, fats and mineral elements. Results of the various analysis showed that, the total microbial count for the four samples through the ten months sampling period was highest in February with sample C having the highest of 1.2 X 105 cfu/mL, followed by sample D 7.0 X 104 cfu / mL, sample B had 5.8 X 104 cfu / mL while sample A was the lowest with 4.4 X 104 cfu / mL. The count was lowest in the month of September with C having 3.7 X 104 cfu / mL, followed by D with 2.4 X 104 cfu / mL, B had 8.0 X 103 cfu / mL while A the lowest was 4.0 X 103 cfu / mL. Microorganisms present in the fresh water sample and clam were mostly coliform from faecal matter and include; *Staphylococcus aureus*, *Enterobacter aerogenes*, *Spirosoma lingual*, *Bacillus cereus*, *Lactobacillus plantarum*, *Escherichia coli*, *Flavobacterium aquatile* and *Micrococcus varians*. We conclude that microbial load was higher in dry season than in rainy season probably due to dilution of the water and its velocity during rainy season. Results also showed that the nutritional values of the clam varies with season and density of microbial load. We recommend proper sanitation in water where clams are harvested and proper boiling of clams and possible removal of the intestine before consumption especially during the dry season.

Keywords: Dynamics; Microorganism; Clams; Fresh water; Ecosystem

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1. Introduction

Clams are shelled marine or fresh water mullusks belonging to the class Bivalvia. They are invertebrates with shells divided into two pieces called valves. The pieces are joined with a hinge joint, and with two adductor muscles that open and close the shells. Clams have a heart, blood vessels, and kidney [1]. They inhabit the bottom of fresh water body or slow moving water and take water into the shell with an incurrent siphon. The water is circulated throughout the shell by cilia or gills. Oxygen is absorbed from the water by gills inside the shell. Food substances are trapped by sticky mucus and moved through the mouth to the digestive system. Water leaves the shell through ex-current siphon. Mussels have rudimentary sense organs along the edge of the mantle that respond to light and touch [2]. Many bivalves such as clams or oysters are used as food. They have very high nutrient value and are rich in protein and mineral content. Due to their nutritional value, they are recommended for pregnant women and in protein deficiency cases [3].

Fresh water is water containing less than 0.5 parts per thousand of dissolved salts or other impurities. Fresh water may be found in lakes, rivers and bodies of underground water. The ultimate source of fresh water is the precipitation of atmospheric water vapour in the form of rain and snow [4]. Access to unpolluted fresh water is a critical issue for survival of many species including humans who must drink fresh water in order to survive [5]. Only about 3% of water on earth is fresh, and about two thirds of this is frozen in glaciers and polar ice caps. Most of the rest is underground and only about 0.3% is surface water found in lakes, swamps and rivers [4, 5].

Insufficient cleaning of fresh water of substrates materials results in the build-up of bacteria which give high burden on marine habitats including clams. Pathogenic organisms such as *Vibro, Salmonella, Shigella*, and *Coliforms* are particularly increased in number in water due to increase in litter materials, substrates and faecal deposition [6], and this seem to vary with the volume of water and season. This study was aimed at isolation of microorganisms present in fresh water ecosystem, and to determine the effects of the microorganisms on the nutritional value of clams and the dynamics of the microorganisms in the fresh water ecosystem for a duration of ten months.

2. Material and methods

2.1. Chemicals

All chemicals used in this study were of analytical grade. They were purchased from Sigma Aldrich-Germany or BDH Chemical- Poole England through their Nigeria representative. All other materials including autoclave, microscope glass-wares and others were standard equipment used in microbiology laboratory.

2.2. Description of study area

Water sample and clams for the study were collected from Itu river in Akwa Ibom State and taken to the laboratory for analysis.

2.3. Identification of samples for microbiological and nutritional analysis

Water from the river was taken as sample A. The clams were rinsed with water, and water was taken as sample B. The clams were removed from the shell and further separated into the body and the intestine. The intestine was homogenized and water from the homogenized intestine was taken as sample C and finally the body was homogenized and the water from the homogenized body was taken as sample D. The samples were analyzed microbiological and nutritionally.

2.4. Microbiological analysis

The microbial load of the samples were determined by serial dilution and plating method. For determining the water sample, l.0 mL of water from Itu river was added to 9.0mL of sterile distilled water in a test tube and shaken thoroughly. One milliliter of the suspension was diluted serially to 10^{-3} dilution. One milliliter of the suspension was transferred from the 10^{-3} dilution tubes into sterile labeled Petri dishes. Twenty milliliters of sterile agar was poured into each Petri dish and this was rotated gently to disperse the inoculums in the medium. Total coliform count was determined on MacConkey agar plates. Total bacterial count was determined on Nutrient agar plate and Sabouraud Dextrose Agar (SDA) plates were incubated at room temperature for 5 to 7 days for determining fungi. Mineral salt agar was also used to culture the water samples from the river to check for the presence of bio-degraders. This analysis was carried out monthly for 10 months.

2.5. Characterization and identification of microbial isolates

Different biochemical tests were carried out to characterize and identify microbial isolates.

2.5.1. Colonial Morphology

The cultural/colonial morphologies as described by Cowan [7] was observed. These includes shape, size, elevation, edge, pigmentation, colony, surface and optical characteristics.

2.5.2. Gram Stain Reaction

The methods specified by Harrigan and McCance [8] was used. The test organism was heat-fixed on a clean slide. The slide was flooded with crystal violet and allowed for one minute after which it was washed off with running tap water. The slide was then flooded with Lugol's iodine and allowed for one minute after which it was washed off with running tap water. The slide was then decolourized with 95% ethanol for 30 seconds and washed off with running tap water. It was drained and blotted dry. The slide was further counter stained with safranin for 30 seconds and washed with tap water. It was drained and blotted dry. The slide was viewed under the oil immersion lens of the microscope. Gram positive cells retained blue or purple colour while Gram negative cells took up the counter stain (pink or red) colour.

2.5.3. Spore Stain

Stain technique by Collins and Lyne [9] was used. It was used to identify spore forming organisms such as *Bacillus sp.* A heat-fixed smear of organism was prepared on a clean grease- free slide. This was flooded with Malachite green stain and steamed over a beaker of boiling water for ten minutes and more stain was added to avoid drying. The slide was washed under running tap water and was counter-stained with safranin for twenty seconds. It was viewed under the microscope. The spores stained green and the vegetative cells stained red.

2.5.4. Motility Test (Hanging Drop Method)

This method described by Fawole and Oso [10] was used. The test was employed to determine the flagellated micro-organisms. A little liquid paraffin was placed round the edge of the depression of a cavity slide, a loopful of a young broth culture of 12-24 hours of the test isolate was transferred to the cover slip such that the culture dropped in the center of the slide depression. The slip was pressed down carefully but firmly in position. The slide was then carefully and quickly inverted in such a way that the culture drop appeared "hanging" and the drop was examined under the microscope immediately. The low power objective was used to focus the edge of the drop, and the high power objective was used to observe the motile cells which were seen to start movements in opposite directions.

2.5.5. Indole Production Test

The method used was described by Collins and Lyne [9]. The indole production test was used to demonstrate the ability of certain micro-organisms to degrade the amino acid tryptophan to indole as one of the products. The medium used was tryptone water. A loopful of the test organisms was inoculated into 5mL sterile tryptophan medium. It was incubated for 48 hours at 37°C. Then 0.5mL of Kovac's reagent was added and allowed to stand for some minutes. Indole production was indicated by a red colouration in the alcohol layer.

2.5.6. Coagulase Test

The method used was outlined by Collins and Lyne [9]. The test was used to differentiate between the pathogenic and non- pathogenic strains of *Staphylococcus sp.* The test is based on the ability of the organisms to produce coagulase enzymes which are capable of coagulating blood plasmas. Usually 18-24 hours culture was emulsified with a loopful of normal saline (0.9% NaCI w/v) on a clean grease-free slide. A loopful of human plasma was added and mixed. A positive result to the test was indicated by coagulation or agglutination within 10 seconds.

2.5.7. Catalase Test

This test was carried out as described by Cruickshan [11]. The test was aimed at detecting micro-organisms that were able to produce the catalase enzymes which catalyzed the release of oxygen from hydrogen peroxide. A loopful of 24 hour-old culture was emulsified with a drop of 3% hydrogen peroxide on a clean grease-free slide. The spontaneous liberation of free oxygen as gas bubbles (effervescence) indicated the presence of catalase, i.e. positive reaction.

2.5.8. Oxidase Test

The method outlined by Cruicksharjk [11] was employed, it is used to differentiate between the oxidase enzyme producing organisms and the non-producing ones. A sterile loop was used to transfer a speck of the test organism into the oxidase strip (filter paper moistened with oxidase reagent i.e. 1.0% aqueous tetraethylparaphenylefledjamine dihydrochlorjde) A positive result is indicated by a deep blue or Purple coloration

2.5.9. Methyl Red-Voges Proskauer (MR-VP) Test

The method described by Cruickshank [11] was employed. The organism was inoculated into MR VP medium (glucose peptone phosphate) and incubated at 37°C for 48 hours and then divided into two portions for MR and VP respectively.

MR TEST

To the first portion, two drops of methyl red indicator were added, shaken and examined for the production of a red colour in the medium.

VP TEST

To the second portion 0.6mL of α-naphthol solution and 0.2mL of 40% KOH solutions were added, mixed thoroughly and allowed to stand for 10-20 minutes. The medium was then observed for the development of pink colour on the surface of the medium.

2.5.10. Citrate Utilization

The method used was described by Cruickshank [11]. The medium used was Simmon's citrate medium. The medium was dispensed into MacCartney bottles and sterilized in the autoclave. A sterile inoculating wire loop was dipped in each inoculum and then transferred into the medium. This was incubated at 37°C for 24 to 48 hours and observed daily for a colour change from green to bright blue. This test indicates the ability of an organism to utilize citrate as a sole source of carbon and energy and ammonium salt as nitrogen source.

2.5.11. Urease Test

This test was carried out as described by Collins and Lyne [9]. Urea agar base (2.4 g) was dissolved in 100 mL of distilled water and sterilized by autoclaving at 121°C for 15 minutes in test tubes and allowed to set in slanting positions. Using a sterile loop, the test organism was inoculated on the slopes and incubated at 37°C for 2 to 3 days. A change in colour to pink indicated a positive reaction.

2.5.12. Sugar Fermentation Test

The test shows the ability of the isolates to utilize different sugars. The sugars used were glucose, lactose, sucrose, dextrose, maltose, mannitol and galactose. One gram of peptone water was dissolved in 100 mL of distilled water, 1.0 g of sugar was added and stirred to mix. Phenol red was added to the medium, l0 mL of the medium was dispersed into test tubes and the Durham tubes were inverted into the tubes. The medium was sterilized at 121°C for 15 minutes. An 18 to 24 hours broth culture of the organism was inoculated into the medium and incubated at 37°C for 24 hours. After incubation, each of the inoculated tubes was compared with the control of the same medium to determine gas production as well as acid production. The gas was produced in the Durham tubes while positive reaction of acid production was indicated by a colour change of broth medium from pink to yellow [8].

2.6. Physicochemical and nutritional analysis

2.6.1. Moisture content

This was done according to Association of Official Analytical Chemistry (AOAC) method [12]. Two grams of the clam sample was weighed in duplicate into a pre-weighed porcelain crucible, the weight of the sample with the crucible was recorded. The crucibles containing the samples were placed in a pre-heated oven dryer at 110°C for 24 hours, after which they were removed and cooled to room temperature in a dessicator. The final constant weight subtracted from the initial weight gave the moisture content which was expressed in percentage thus.

$$\text{Moisture content (\%)} = \frac{\text{Loss in Weight}}{\text{Initial weight}} \text{x} \frac{100}{1}$$

2.6.2. Ash content

This was done according to AOAC method [12]. Two grams of the oven-dried sample was weighed accurately into a pre-weighed porcelain crucible. The crucibles containing the samples were placed in a pre-heated furnace at 660°C for 6 hours after which they were removed and cooled to room temperature in a dessicator and weighed. The ash content in percentage dry weight was calculated thus:

$$\% \text{ Ash} = \frac{\text{Weight of ash (g)}}{\text{Oven} - \text{dried weight of sample}} \text{x} \frac{100}{1}$$

2.6.3. Determination of crude protein

This was done according to AOAC method [12]. Two grams of clam sample were weighed in duplicate into digestion tubes. Five milliliters 95-97 % sulphuric acid was added into each of the tubes. Two special Kjeldahl tablets 5, 2.5 which act as catalyst were also added into each of the digestion tubes. The samples were digested at 42°C using the digestion system for 30-45 minutes or until they become clear. After digestion, it was cooled and transferred into 100 mL standard flask and made up to the mark with distilled water; 25 mL portion of this digest was pipetted into micro-Kjeldahl distillation apparatus and treated with 40 % NaOH solution.

The ammonia evolved was distilled into a 100 mL conical flask containing 10 mL solution of 5% boric acid into which 2 to 3 drops of a double indicator (methyl red + methyl blue) has been added. The tip of the condenser immersed into the boric acid double indicator solution and the distillation continued until about three times the original volume was obtained. Its tip was then rinsed with few milliliter of distilled water. What remained of the 25 mL digest was discarded.

The distillate was titrated with standard HC1 solutions until a purple-pink end point was reached. Distillation was carried out in triplicate for each digest and the percentage nitrogen content was obtained by appropriate calculation. A blank determination was also carried out in a similar manner as described above except for the omission of the sample in the digestion flask.

Calculation: % Nitrogen = Millilitres of HCI (sample)/millilitres of HCl (blank) x1.4g

The crude protein was obtained by multiplying the percentage nitrogen content by the factor 6.25.

2.6.4. Estimation of fibre content

Acid Digestion

The fat free material (8.l0g) was weighed and quantitatively transferred into a 400 mL beaker which been marked at 200 mL level and 500 mL of 1.25% sulfuric acid was added and the mixture was made up 200 mL with distilled water. The content was boiled for 30 minutes and filtered through a Buchner funnel with the aid of the suction pump. The residue was washed with hot water until it was acid-free.

Base Digestion

The residue left was transferred into the 400 mL beaker. Fifty millilitres of 1.25% (w/v) NaOH were added and made up to the 200 mL mark with distilled water. The mixture was again heated for 30 minutes with constant stirring. The content was filtered through the buchner funnel and washed several times with hot water until it was free from sodium chloride.

Finally, it was washed twice with 95% methanol, transferred to a porcelain crucible and dried at 100°C. The weight of the oven-dried residue was noted and the residue was later ignited in a furnace at 550°C. The weight of the ash after ignition was noted. The crude fibre content was determined from loss in weight of crucible and its contents after ignition [12].

2.6.5. Determination of fat content

Ten grams of ground sample was weighed into a fat extraction timble. Petroleum ether 250 mL was poured into a previously weighed 500mL flask containing anti-bumping chips. Soxhlet extractor into which the timble with its content has been introduced was fitted into the flask and the extraction apparatus was set up with the flask sitting on the heating mantle and was heated. The extraction process lasted six hours. The round bottom flask and the lipid extract were finally

dried in an oven at 100°C and weighed. The amount of lipid, extracted was obtained from the different between the weight of the flask before and after extraction [12].

$$\text{Calculation: Lipid Extract} = \frac{\text{Weight of extract}}{\text{Weight of dry sample}} \mathbf{x} \frac{100}{1}$$

2.7. Determination of mineral elements

2.7.1. Estimation of potassium by flame photometry

Two grams of the samples were digested with mixture of Conc. HNO_3 and perchloric acid. The digest was made up to l00 mL with de-ionized water. Aliquots of the dilute clear digest were taken for flame photometry analysis.

2.7.2. Estimation of calcium, phosphorus and iron by atomic absorption spectrophotometry

Aliquots of the digest as prepared for flame photometry were also prepared for atomic absorption spectrophotometry. The concentrations of these elements were determined by measuring their absorption and reading their actual concentration from a standard curve prepared by using standard solutions of various elements [12].

3. Results

3.1. Microbial counts

The total bacterial counts for the four samples through the l0-month sampling period is shown in Table 1. The microbial count shows that sample C (homogenized intestine of the clam had the highest aerobic bacterial count, followed by sample D (homogenized body of the clam, sample B (water gotten from the washed open shell of the clam) and sample A (water from Itu river) had the least microbial count. It was also observed that more aerobic bacterial counts were obtained in the dry season and less in the rainy season. The bacterial counts for the samples are shown in Figures. 1 to 4.

Table 1 Bacterial Load of Water and Clam Samples from Itu River

Month	A (cfu/mL)	B(cfu/mL	C (cfu/mL	D (cfu/mL)
January	3.6×10^4	4.2×10^4	10.0×10^4	6.0×10^4
February	4.4×10^4	5.8×10^4	12.0×10^4	7.0×10^4
March	4.2×10^4	5.2×10^4	8.4×10^4	6.8×10^4
April	3.6×10^4	4.4×10^4	7.0×10^4	5.5×10^4
May	1.5×10^4	3.6×10^4	5.5×10^4	3.6×10^4
June	1.0×10^4	2.5×10^4	4.3×10^4	3.3×10^4
July	0.7×10^4	1.7×10^4	4.0×10^4	3.0×10^4
August	0.5×10^4	1.0×10^4	3.9×10^4	2.9×10^4
September	0.4×10^4	0.8×10^4	3.7×10^4	2.4×10^4
October	1.5×10^4	2.5×10^4	3.5×10^4	3.0×10^4

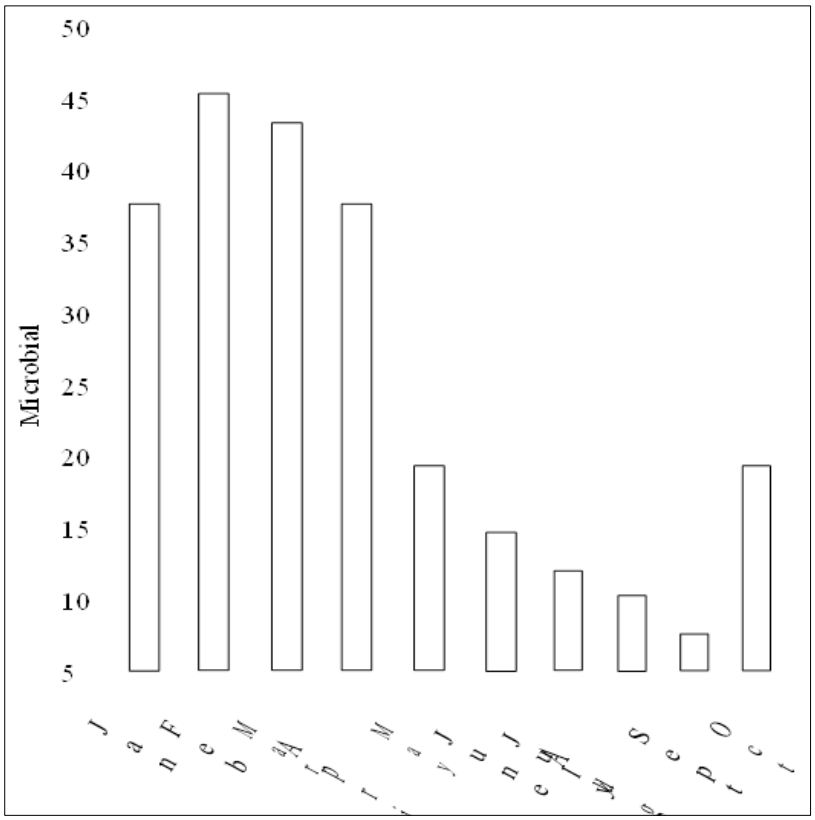

Figure 1 Bar Charts of Microbial Counts (x10⁴ cfu / mL) of sample A

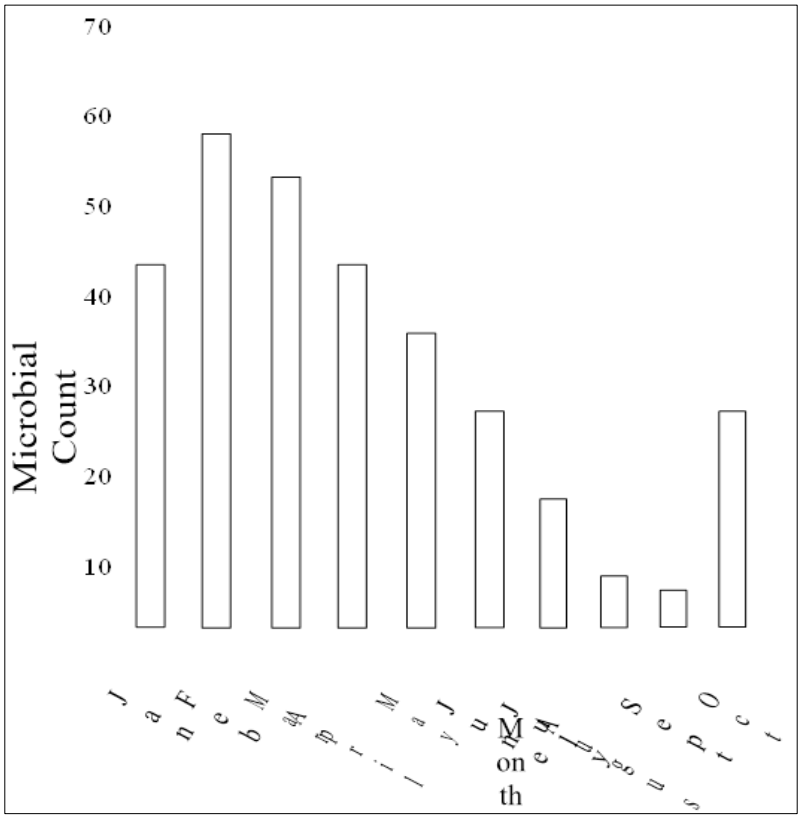

Figure 2 Bar Charts of Microbial Counts (x10⁴ cfu / mL) of sample B

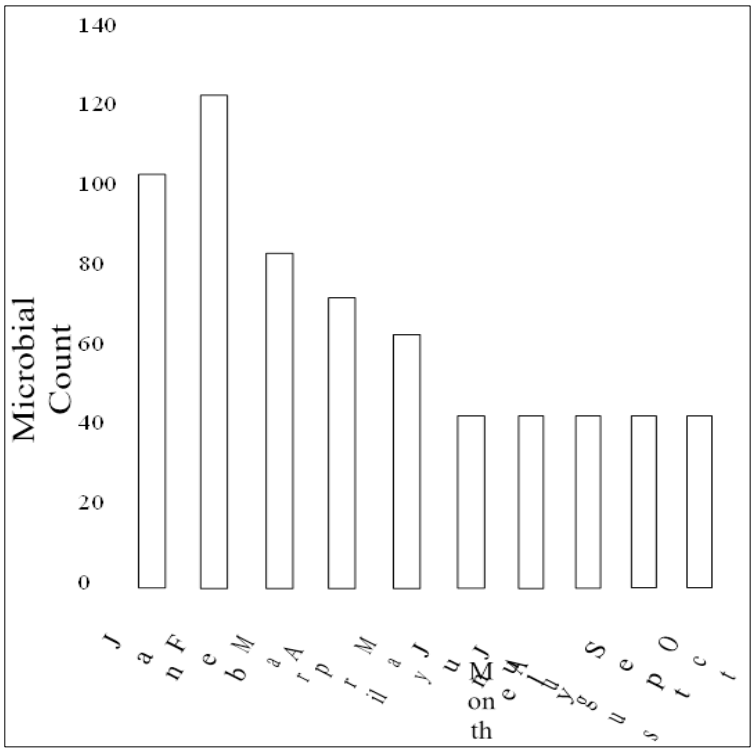

Figure 3 Bar Charts of Microbial Counts (x10⁴ cfu / mL) of sample C

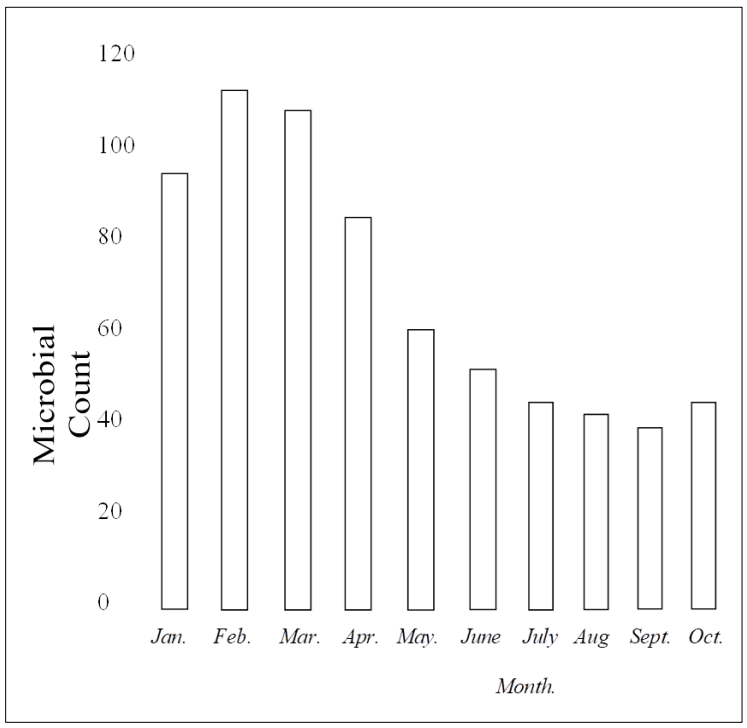

Figure 4 Bar Charts of Microbial Counts (x10⁴ cfu / mL) of sample D

3.2. Characterization and identification of microorganism

Bacteria and fungi associated with fresh water from the river and clam body samples were isolated. Bacterial isolates were subjected to various morphological and biochemical tests for their characterization and identification. The isolates were identified in accordance with Cowan [7] and Fawole and Oso [10]. Detailed results of the cultural and biochemical characteristics of the isolates are shown in Tables 2 to 5.

Table 2 Cultural and Biochemical Characteristics of Bacterial Isolate from Sample A

ISOLATE NO	CELL SHAPPE	GRAM RECTION	MOTILITY	SPORE	CATALASE	OXIDASE	COAGULASE	UREASE	CITRATE	INDOLE	MR	VP	GLUCOSE	SUCROSE	DEXTROSE	MANNITOL	MALTOSE	LACTOSE	XYLOSE	PROBABLES ORGANISMS
A1	Cocci in clusters	+	-	-	+	-	+	+	+	-	-	+	AG	AG	AG	AG	AG	A	A	*Staphylococcus aureus*
A2	Short Rods	+	-	-	+	-	+	+	+	+	-	+	AG	AG	A	AG	A	AG	A	*Enterobacter aerogenes*
A3	Long rods	+	-	-	-	-	-	+	+	+	+	-	A	A	A	A	A	A	A	*Spirosoma lingual*

Table 3 Cultural and biochemical, characteristics of bacterial isolates from sample B

ISOLATE NO	CELL SHAPPE	GRAM RECTION	MOTILITY	SPORE	CATALASE	OXIDASE	COAGULASE	UREASE	CITRATE	INDOLE	MR	VP	GLUCOSE	SUCROSE	DEXTROSE	MANNITOL	MALTOSE	LACTOSE	XYLOSE	PROBABLES ORGANISMS
C1	Short Rods	-	+	-	+	-	-	-	-	+	+	-	AG	A	A	AG	AG	A	A	*Escherichia coli*
C2	Cocci in clusters	+	-	-	+	-	+	+	+	-	-	+	AG	AG	AG	AG	AG	A	A	*Staphylococcus aureus*
C3	Slender shots rods	-	-	-	+	+	+	-	+	+	-	+	A	A	-	A	A	A	A	*Flavobacterium aquatile*
C4	Long rods	+	-	-	-	-	-	+	+	+	+	-	AG	AG	AG	AG	AG	AG	AG	*Lactobaccillus plantarum*

Table 4 Cultural and Biochemical Characteristics of Bacterial Isolates from Sample C

ISOLATE NO	CELL SHAPPE	GRAM RECTION	MOTILITY	SPORE	CATALASE	OXIDASE	COAGULASE	UREASE	CITRATE	INDOLE	MR	VP	GLUCOSE	SUCROSE	DEXTROSE	MANNITOL	MALTOSE	LACTOSE	XYLOSE	PROBABLES ORGANISMS
D1	Cocci in clusters	+	-	-	+	-	-	+	-	-	+	-	AG	AG	AG	AG	AG	-	A	*Micrococcus varians*
D2	Short Rods	-	+	-	+	-	-	-	-	+	+	-	AG	A	A	AG	AG	AG	AG	*Escherichia coli*
D3	Slender shots rods	-	-	-	+	+	+	-	+	+	-	+	A	A	A	A	A	A	A	*Flavobacterium aquatile*
D4	Long rods	+	-	-	-	-	-	+	+	+	+	-	AG	AG	AG	AG	AG	AG	AG	*Lactobaccillus plantarum*

Table 5 Cultural and Biochemical Characteristics of Bacterial Isolates from Sample D

ISOLATE NO	CELL SHAPPE	GRAM RECTION	MOTILITY	SPORE	CATALASE	OXIDASE	COAGULASE	UREASE	CITRATE	INDOLE	MR	VP	GLUCOSE	SUCROSE	DEXTROSE	MANNITOL	MALTOSE	LACTOSE	XYLOSE	PROBABLES ORGANISMS
D1	Cocci in clusters	+	-	-	+	-	-	+	-	-	+	-	AG	AG	AG	AG	AG	-	A	*Micrococcus varians*
D2	Short Rods	-	+	-	+	-	-	-	-	+	+	-	AG	A	A	AG	AG	AG	AG	*Escherichia coli*
D3	Slender shots rods	-	-	-	+	+	+	-	+	+	-	+	A	A	A	A	A	A	A	*Flavobacterium aquatile*
D4	Long rods	+	-	-	-	-	-	+	+	+	+	-	AG	AG	AG	AG	AG	AG	AG	*Lactobaccillus plantarum*

Table 6A Cultural and Morphological Characteristics of Filamentious Fungi from Sample A

Isolate code	**Colony colour**	**Type of some**	**Nature of hyphae**	**Special vegetative Structure**	**Asexual spore**	**Special reproductive structure**	**Conidial head**	**Vesicle shape**	**Probable organism**
EBSM20	Dense felt grey-green spreading colony	Filamentous	Septate	Broom- like appearance	Globose conidia produced in long columns	Erect conidiophores terminating in whorl of phialides	-	-	*Penicillium frequentum*

Table 6b Cultural and morphological characteristics of yeasts from sample a

Isolate code	**Colony colour**	**Type of soma**	**Nature of hyphae**	**Pseudomyce lium**	**Asexual reproductive spores**	**Growth on naci**	**Sugar fermentation Sugar Assimilation**											**Probable organism**
							Glu	**Lac**	**Mal**	**Suc**	**Raf**	**Gal**	**Glu**	**Gal**	**Lac**	**Mal**	**Raf**	**Suc**	
EBSY 1	Moist milky colony	Large Single Globos		Rudimentary pseudomyce hum	Budding cells	+ (10%)	+	0	+	+	v	+	+	+	0	+	+	+	*Saccharo myces cerevisiae*
EBSY6	Greamy milky White colony	Pseudo hyae at 28± 2^{o}C	Septate	Well developed	Blastoconida	+ (15%)	+	v	0	0	v	+	+	+	v	0	+	0	*Candida marina*

Table 7A Cultural and morphological characteristics of filamentous fungi from sample B

Isolate code	Colony colour	Type of soma	Nature of hyphae	Special vegetative structure	Asexual spore	Special reproductive structure	Conidial head	Vesicle shape	Probable organism
ESM_4	Gray-green wrinkled spreading colony	Filamentous	Septate	-	Ovate conidia	Ascoma	Radiating condidia	Subglobes	*Eurotium*
$EBSM_{14}$	Smoky or gray-green colony	Filamentous	Septate	Foot cell	Globose conidia	Short conidiaphores	Typically columnar	Dome shaped broadly clavate	*Aspergillus fumigatus*
$EBSM_{11}$	Compact white or yellow basal dark colony	Filamentous	Septate	Foot cell	Globose conidia	Smooth walled erect condidophores	Globose	Globose	*Aspergillus niger*
ESM_3	Dense felt yellow green colony	Filamentous	Septate	Foot cell	Globose conidia	Phialides borne directly on the vesicle sclerotia	Radiate	Subglobose	*Aspergillus flavus*
ESM_9	White becomingpale green	Filamentous	septate	Broom –like appearance	Globose chained conidia	Dense felt brush- like conidiophores	-	-	*Penicillium nalgiovense*
ESM_6	White often with peech tinge	Filamentous	septate	-	Foot celled conidia, chlamy dospores	Sporodochia absent	-	-	*Pusarium semitectum*

Table 7B Cultural and Morphological Characteristics of Yeasts from Sample B

Isolate code	Colony colour	Type of soma	Nature of hyphae	Pseudomycelium	Asexual reproductive spores	Growth on naci	Sugar fermentation					Sugar assimilation						Probable organism
							Glu	Lac	Mal	Suc	Raf	Gal	Glu	Gal	Lac	Mal	Raf	Suc	
EBSY 11	white smooth membranous colony	Filamentous	Septate dichotomously branched	Absent	*Cylindrical conidia*	+(5%)	0	0	0	0	0	0	+	+	0	0	0	0	*Geotrichum candidum*

Table 8A Cultural and Morphological Characteristics of Filamentous Fungi from Sample C

Isolate code	**Colony colour**	**Type of soma**	**Nature of hyphae**	**Special vegetative Structure**	**Asexual spore**	**Special reproductive structure**	**Conidial head**	**Vesicle shape**	**Probable organism**
$EBSM_{11}$	Compact white or yellow basal dark colony	Filamentous	Septate	Foot cell	Globose conidia	Smooth walled erect conidiophores	Globose	Globose	*Aspergillus niger*

Table 8b Cultural and Morphological Characteristics of Yeasts from Sample C

Isolate code	**Colony colour**	**Type of soma**	**Nature of hyphae**	**Pseudomycelium**	**Asexual reproductive spores**	**Growth on naci**	**Sugar fermentation**			**Sugar assimilation**								**Probable organism**
							Glu	**Lac**	**Mal**	**Suc**	**Raf**	**Gal**	**Glu**	**Gal**	**Lac**	**Mal**	**Raf**	**Suc**	
EBSY 8	creamy white smooth unextensive colony	Pseudo hyphae at 28 ± 2^0C	Septate	Well developed	Blastoconidia	+(10%)	+	+	0	+	+	+	+	+	+	0	+	+	*Candida pseudotropicalis*
EBSY7	creamy white milky colony	Pseudo phyae at 28 ± ^{20}C	Septate	Well developed	Blastoconida	+(10%)	+	0	+	+	+	+	+	+	0	+	+	+	*Candia tropicelis*
EBSY5	Moist milky colony	Single oval cells	-	Rudimentary pseudmycelium	Budding cells	+(10%)	+	0	+	+	+	+	+	+	0	+	+	+	*Saccharo myces estuari*

Table 9A Cultural and Morphological Characteristic of Filamentous Fungi from Sample D

Isolate code	**Colony colour**	**Type of soma**	**Nature of hyphae**	**Special vegetative structure**	**Asexual spore**	**Special reproductive structure**	**Conidial head**	**Vesicle shape**	**Probable organism**
ESM18	Leathery Blue- Green Colony With Red Pigments	Filamentous	Septate	Broom-like appearance	Globose conidia produced in columns	1 stage branched conidophonres	-	-	*Penicillium citrinum*
EBSM16	Floccose felty whitish colony with a purple tinge	Filamentous	Septate		Microconidia	Short branched conidiophores sporodochia	-	-	*Fusarium oxysporum*
EBSM11	Compact white or yellow basal dark colony	Filamentous	Septate	Foot cell	Globose conidia	Smooth walled erect condidophores	Globose	Globose	*Aspergillus niger*

Table 9b Cultural and Morphological Characteristic of Yeasts from Sample D

Isolate code	**Colony colour**	**Type of soma**	**Nature of hyphae**	**Pseudomycelium**	**Asexual reproductive spores**	**Growth on naci**	**Sugar fermentation**			**Sugar assimilation**							**Probable Organism**
							Glu	**Lac**	**Mal**	**Suc**	**Raf**	**Gal**	**Glu**	**Gal**	**Lac**	**Mal**	**Raf**	**Suc**	
EBSY8	Creamy White Smooth Unextensive Colony	Pseudo Hyphae At 28 +2^{0}C	Septate	Well developed	Blastoconidia	+(10%)	+	+	0	+	+	+	+	+	+	0	+	+	*Candida pseudotropicalos*

Fungi and yeasts were isolated from the different samples after being subjected to various cultural and morphological tests. Detail results of the cultural and biochemical characteristics of the fungal and yeast isolates are shown in tables 6 to 9.

There were no bio-degraders present in the water sample cultured from the river as there was no growth seen in the mineral salt agar.

KEY:

Raf = raffinose
Glu = glucose
Gal = galactose
Suc = sucrose
Mal = maltose
+ = positive reaction
O = negative reaction
V = reaction variable

- **Sample A:** Water from Itu River
- **Sample B:** Water from the washed opened clam shell
- **Sample C:** Homogenized intestine of the clam
- Sample D: Homogenized body of the clam

3.3. Physicochemical and nutritional characteristics

The physicochemical and nutritional composition of the clam were taken for ten months; January to October. The results obtained showed that the seasons and the microbial loads affected the nutritional values as shown in carbohydrate, crude protein, moisture, lipid and ash contents. There was no change in the fibre content and the elements calcium, iron and phosphorus. These results are shown in Table 10.

Table 10 Nutritional characteristics of clam samples

Month	Carbohydrate (%)	moisture	Crude protein (%)	Lipid content	Ash content (%)	Fibre content (%)	Calcium µg/g	Iron µg/g	Phosphor µg/g
January	11.32	86.73	65.63	15.50	5.83	1.72	15.310.00	782.40	46.67
February	8.64	88.38	67.43	16.20	6.01	1.73	15.310.00	782.40	46.67
March	10.56	80.20	66.23	15.90	5.60	1.71	15.310.00	782.40	46.67
April	12.88	79.00	65.00	15.20	5.20	1.72	15,310.00	782.40	46.67
May	13.99	78.73	64.66	14.90	4.73	1.72	15.310.00	782.40	46.67
June	17.33	6259	63.15	13.49	4.31	1.72	15.310.00	782.40	46.67
July	19.01	6063	62.19	13.00	4.09	1.71	15.310.00	782.40	46.67
August	21.21	59.11	60.25	12.99	3.84	1.71	15.310.00	782.40	46.67
September	23.72	58.02	59.73	11.53	11.53	3.29	1.73	15.310.00	782.40
October	17.44	60.79	62.25	12.40	4.18	1.73	15,310.00	782.40	46.67

4. Discussion

Studies on the dynamics of microorganisms and their association with clam in freshwater ecosystem were carried out. The effect of season and microorganisms on the nutritional value of the clam and the dynamics of the organisms in different parts of the clam in a freshwater ecosystem was also evaluated. The samples analyzed exhibited changes in microbial count depending on the season. More counts were observed in the dry season. Sample C had the highest microbial count. Its microbial count was high through the whole season, when compared with other samples. This is in

agreement with the report of Loosanoff and Nomejko [13] that shellfish contamination occurs through the feeding activities of the animals. Ingested bacteria, viruses and protozoa may pass through the stomach wall and enter the blood and tissues of the animal. According to Schwab *et al.,* [14] and Dore and Less [15], majority of microorganisms are retained in the gastrointestinal tract of the animal.

A numerical scale from low to high rate of microbial count in the clam was developed in this study to grade the load intensity of the microorganism based on the occurrence of this parasite in clam tissues. Previous studies on aquatic animals indicated that the gill is the portal for microorganisms based on the findings that microorganisms are limited to gill tissues in the early phase [16, 17, 18]. As the infection advances, microorganisms spread from the gill to other tissues using host hemolymph as a medium of dispersal. At a higher level of infection, microorganisms are found in all tissue, including adductor muscle and gonadal connective tissues [17,18, 19, 20]. In this study, microorganisms were confined to the intestinal tissues in most of the clams, suggesting that the level of infection in clams at Itu river is high at the late stage.

It was observed that during the rainy season, between April and September, microbial load was low in the clam and this could be associated with dilution effect of the water body in the clam density during the rainy season [21].

There was also seasonal variation in the chemical composition. The clam samples showed seasonal variation in their chemical composition and nutritional value. Such variation may be linked with the reproductive cycles of the organism, and may be so large as to render the animal unsuitable as food during certain season as found with crab after moulting in summer [22]. From the results it was observed that the moisture content of the clam was very high from January to May and reduces from July to October, the crude protein also increased during this period, while the lipid content was high in June to October and slightly higher in February to May. This type of seasonal variation in chemical composition was reported in Galatea by Etim [23] who linked the variation with the cycles of reproduction of the animal, having observed an increase in tissue moisture content during spawning. This investigator concluded that the organism absorbs more water during spawning probably to fill the lumen created following the release of the gamete.

Microorganisms were isolated from the different samples used for the analysis. Bacteria, fungi and yeasts were isolated from each of the samples. This is in agreement with the work of Lees [24], who reported that bivalve molluscs are filter-feeding shellfish, which can cause health problems because they concentrate and retain human pathogen found in the areas where they grow. Water quality monitoring indicates that portion of this creek system are impaired with too much bacteria as measured by fecal *coliform* bacteria counts in the water. These bacteria live in the intestinal tracts of warm blooded animals and are mostly pathogenic [25]. According to Berg *et al.*[26] intensive environmental investigation revealed that, every two of the three outbreaks, was caused by disposal of sewage by the clam harvesters directly into the clam water bed which was the most likely source of contamination.

5. Conclusion

This study highlights the dynamics of microorganism and their association with clam in the fresh *water* ecosystem. The need to determine the *microorganisms* present in freshwater microorganisms present in the clam, the effect of microorganisms in the nutrition value of the clam and the dynamic of microorganisms and clam in the fresh water ecosystem for the duration of ten months.

Several parameters were considered including microbial counts, different species isolated, the nutritional quality of the clam samples. Changes did occur in all parameters considered in all the samples for the ten months. The degree of changes was a function of the season in which the samples were taken. The most significant changes occur in the dry season, and in the intestine of the clam, while the least was at the rainy season and also in the water sample collected from the river.

After careful study, analysis and observations, the following conclusions were drawn from the research:

- Microorganisms present in freshwater sample and clam are mostly *coliforms* from faecal matter deposited by human and other animals.
- The study has shown that higher microbial loads were obtained in the dry season than in, the rainy season. This is attributed to the dilution effect of the water body by rain and decrease microbial density in the clam during the rainy Season.

- Nutritional value of the clam varies with season and with microbial load. There is increase in nutritional value and microbial growth during the dry season. These are due to variation with the cycles of reproduction of the animal, having observed an increase in tissue moisture content during spawning.

Recommendations

Ecological survey of the clam in other aquatic environments is recommended in order to compare data, and ascertain the pattern of nutritional values and seasonal microbial growth in relation to that done in this study so that consumers could be properly advised. Also, there is need for proper boiling of the animal before consumption and to discard the intestine while preparing the clam for consumption especially during the dry season

Compliance with ethical standards

Acknowledgments

The authors wish to appreciate the divers who went to the river bed to harvest the clams. We are also grateful to staff of microbiology Laboratory University of Uyo who assisted in the microbial analysis. We are also grateful to staff of pharmaceutical chemistry laboratory Madonna University who assisted in the nutritional and elemental analysis.

Disclosure of conflict of interest

The authors have declared that no competing of interest exist in this research.

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