



Research Article

Microbiological quality and biochemical composition of water snail (*Pachymelania byronensis*) of lower Niger River basin

Ifeoma Vivian Anyiam, Thomas Ohwofasa Ikpesu

Abstract

Pachymelania byronensis is a mollusk that is very abundant in the Niger Delta ecological zone, Nigeria especially the lower region that has the brackish water. The meat of this gastropod serves as good source of protein to the inhabitants. Hence, its nutritional composition was investigated to ascertain its suitability for human consumption.

The snail samples were randomly hand-picked from their natural environment during August, 2017 to January, 2018 from wet and dry seasons. A total of 144 *P. byronensis* (24 per station) samples were used for this investigation. The total heterotrophic bacteria count was determined using standard pour plates method. Coliform count was determined using MacConkey agar, total Salmonella/Shigella counts was estimated using Salmonella/Shigella agar and total vibrio counts was determined employing Thiosulphate citrate bile salt sucrose agar. The isolates were identified with reference to Bergey's Manual of Determinative Bacteriology. The foot muscle and viscera were used for the proximate estimation of nutritional composition.

The bacteriological load in the *P. byronensis* revealed that there were variations in the occurrence of different groups. Salmonella/Shigella counts was highest $1.96 \pm 3.45 \times 10^3$ (cfu/100mL) followed by Total vibrio counts $1.66 \pm 3.13 \times 10^3$ (cfu/100mL). Total heterotrophic bacteria counts was $1.63 \pm 4.97 \times 10^3$ (cfu/m) and least was Coliform counts $1.30 \pm 3.70 \times 10^3$ (cfu/100mL). The nutritional status showed that the snail is a valuable source of seafood that can meet the nutritional requirements of man.

The findings revealed that human activities might have influenced the microbial load in the Rivers, and the nutritional status of snail revealed that it could be used as food for humans, which would go a long way in maintaining the nutritional balance of human diet.

Keywords biochemical characteristics, microbiological load, *Pachymelania byronensis*, total heterotrophic bacteria

Introduction

The rapid increase in human population and the desire for quality food necessitate the search for new resources to accommodate the challenges. Hence, the need to explore new potential sources of food especially seafood protein and to improve the existing ones becomes inevitable and the demand for fisheries product increases every year [1], and molluscs have been recognized as a high quality nutritious food source and many species are considered as culinary delicacies [2].

Numerous experiments have reported relatively high protein levels in mollusc flesh, including abalone *Haliotis diversicolor* [3] and the oyster

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Crassostrea gigas [4-5]. The nutritional worth of mollusc flesh lies not only within the high quality of protein, but also in its moderately low lipid content and high proportion of PUFAs [6]. Molluscs are known to contain a wide variety of PUFAs, some of which are considered as essential fatty acids that humans cannot synthesize and must be obtained from food [7]. Amongst the PUFAs, long-chain omega-3 fatty acids, such as eicosapentaenoic (EPA), docosapentaenoic (DPA) and docosahexanoic (DHA), are thought to play beneficial roles in a healthy diet. The balanced intake of omega-3 (n-3) and omega-746 (n-6) fatty acid can help prevent cardiovascular diseases [8-9], coronary heart diseases, arthritis [10] and other inflammation [11].

The largest quality component in mollusc flesh is the moisture that can be as high as 80% of the fresh wet weight. The inorganic mineral content (ash weight) ranges between 1 to 17% of the fresh wet weight [12]. Molluscs meat has been recommended in some dietary regimes for its relatively high protein content [5]. Marine gastropods can potentially be used as an alternative to traditional protein sources (such as beef/ chicken/fish) for human consumption.

Pachymelania byronensis is gastropod molluscs and adapted to low salinity. It is a widespread species with no known major threat. It is distributed throughout the world, and predominantly found in West Africa Coast and is cosmopolitan in the entire Niger Delta ecological zone. Till now, there has been little study on *P. byronensis* with regard to its microbial health status, heavy metal composition (detritus feeders) and nutritive value for human consumption. Therefore, the objective of this research is to determine the bacterial contamination status and nutritional composition of *P. byronensis* from brackish water of Niger River basin. As per the available literature, this was the first study to quantify the nutritional composition and health status of this common snail in this region.

Methodology

Description of the study area

Fish Town Rivers is an offshoot of Sangana River (Figure 1). This river is severely affected by human activities that re-defined its natural habitat. The water is also quite variable as marine tides go high and low, consequently increasing or decreasing the salinity of water. Hence, the living forms that stay in and are dependent on the water must also adapt according to salinity changes and several other physical aspects of water including temperature, water density, pH balances, buoyancy, wave action and changes in nutrients.

The stations chosen (Angalabiri, Better land and Farm camp) along the river were the places where *P. byronensis* was dominant. They were found attached to substrates at the edge or in the shallow region where they experience fluctuating tides.

Hydro-chemical parameters determination

Salinity, temperature, dissolved oxygen, total hardness, pH, turbidity, total dissolved solid, chemical oxygen demand, nitrate and phosphate of different stations within the fish Town Rivers were measured as hydro-chemical parameters. Samples were monthly collected and parameters were followed using standard method [13].

Salinity

Salinity was measured using an electrical conductivity meter (EC meter). The device determined the salinity of a sample by sending an electrical current through the sample, and measured the resistance of the sample towards the flow of the current. For accurate reading, the EC meter that measured up to 19.99mS/cm (19.99ds/m) was used.

Temperature

Mercury-in-glass thermometer was used to measure the temperature of the water. The thermometer consisted of a uniform-diameter glass capillary that opened into a mercury-filled bulb at one end. The assembly was sealed to preserve a partial vacuum in the capillary. Mercury expanded and rise in the capillary on temperature increase. The unit of measurement was $^{\circ}\text{C}$.

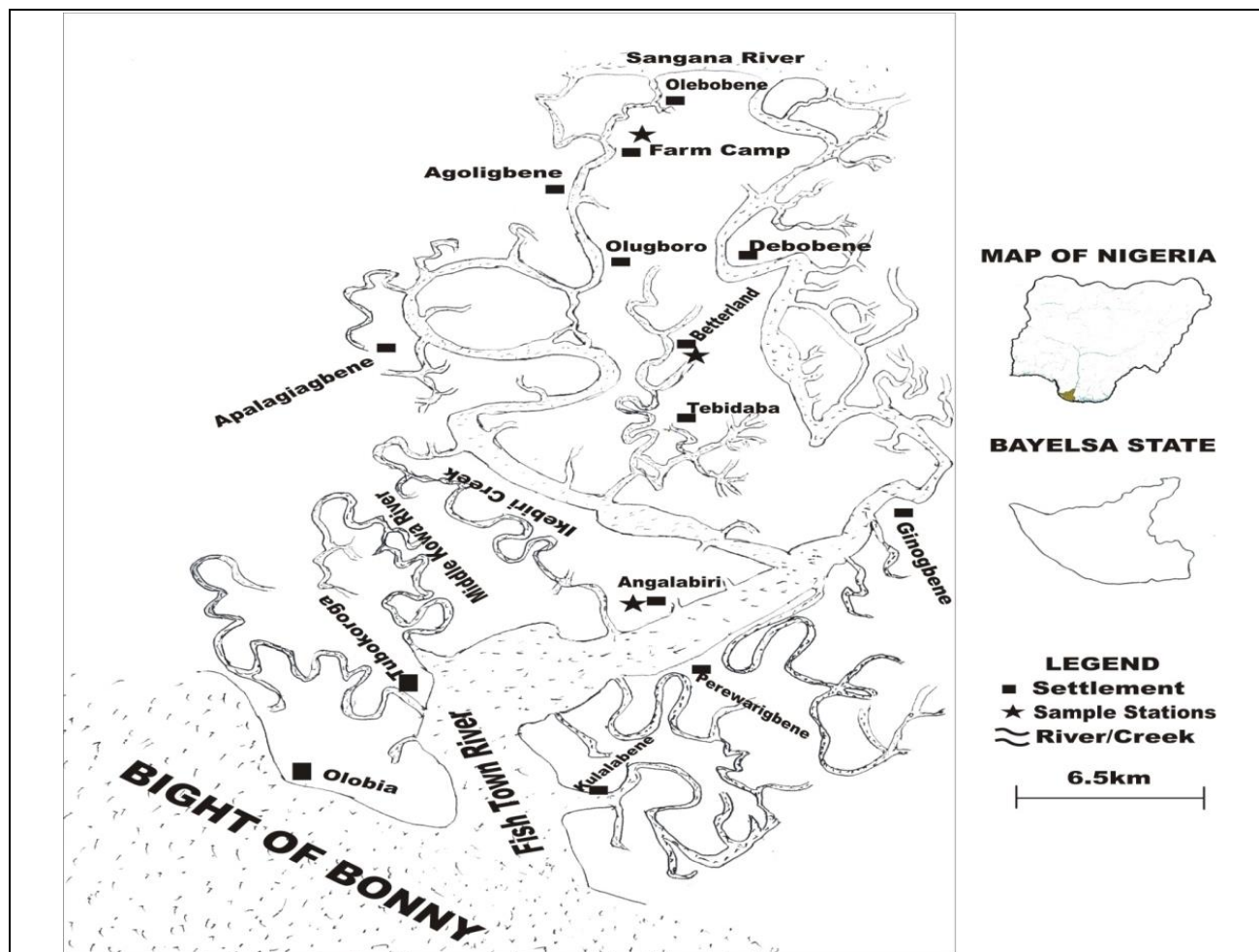


Figure 1. Map of the study River showing the sampling stations

Dissolved oxygen

DO was determined using a “Mettler Toledo” (DO model 90.71). The probe was inserted into the sample container containing the different treatments and the readings were taken. The unit of measurement was mg/L

Total hardness

Total hardness was measured by titration using 0.02N H₂SO₄ (aq) against 25ml of test solution using two drops of methyl orange as indicator. It was calculated by substituting the titre value into the equation below.

$$\text{Total alkalinity} = \frac{\text{Titre value (ml)} \times N \times 50 \times 1000}{25 \text{ ml (volume of test solution used)}}$$

(The unit of measurement is mg/L)

pH

The water pH was determined *in situ* in each of the aquarium with a pH / conductivity / TDS meter (Hanna Products, Portugal). This was achieved by dipping the end of the electrode into the test solution and the mode button was selected to give reading for the desired parameter.



Turbidity

Turbidity values were measured using hand held turbidity meter. The meter was calibrated using standard samples from the meter manufacturer.

Total dissolve solid

TDS combines the sum of all ion particles that are smaller than 2 microns (0.0002cm). It was measured by gravimetry method.

Chemical oxygen demand

The COD was calculated using the formula-

$$\text{COD (mg/L)} = ((B-A)*N* 800) / D$$

Where

B = amount of ammonium sulphate used for titration of the blank

A = is the amount of the ammonium sulfate used for the sample for COD determination

N = the normality of the ferrous sulphate

D = volume of sample used in ml

Nitrate

Nitrate was measured using an Ozonation technique where nitrite was quantitatively oxidized to nitrate. Nitrite was determined using sulfanilic acid and alpha-naphthylamine. The color developed was read in a Beckman DU spectrophotometer at a wavelength of 520 mμ. One-centimeter cells were used for concentrations greater than 10 mμ and 10 cm cells for lower concentrations. Nitrate was reduced by hydrazine and analyzed for the nitrite produced using a wavelength of 524 mμ and 1-cm cells for concentrations greater than 5 mμ. Within the accuracy of the method, there was a linear relationship between optical density and added nitrite or nitrate up to concentrations of 60 mμ.

Phosphate

Phosphate was determined using the Hanna HI 736 pocket spectrophotometer. The Hanna Instruments HI 736 ultra-low range phosphorus checker has a range from 0 to 0.20 mg/L (0 to 200 ppb). It is a battery-operated fixed wavelength (525 nm) spectrophotometer that uses a light-emitting diode light source and a silicon photocell light detector.

Sample Collection

The water snail (*P. byronensis*) is one of the dominant aquatic organisms in this River; hence, collection was quite less rigorous. Sampling was done in such a way that the entire brackish water that were the snail habituates were considered for collection. Hence, three stations within the river were sampled; Angalabiri (station A), Betterland (station B) and Farm camp (station C). At the end of sampling periods, the average were taken and comparison were made. New latex gloves were worn when the snails were collected for processing. Snails with mean weight (5.00 + 0.10 g) and length (2.10 + 0.13 cm) were hand-picked from the brackish water environment from August, 2017 to January, 2018, depicting wet season (August - October) and dry season (November - January). A total of 144 *P. byronensis* (24 per station) were sampled for this investigation. At the end of each sampling period, they were collected in a sterile bag placed in an ice cooler to maintain a temperature of approximately 4°C, and transported to the biology laboratory in the Department of Biological Sciences at Federal University Otuoke, where they were washed with potable water and sterilized (70% ethanol). They were randomly divided into two portions (with proper identification for different examination) and kept in well ventilated aquaria for each analysis.



Microbiological analysis

The microbiological profile was determined according to APHA method [13] as modified by Maturin and Peeler [14]. The foot muscle and viscera were mechanically extracted. Ten gram of meat was mixed with 90 ml of Ringer solution and well homogenized for 5 minutes.

Total heterotrophic bacteria counts was determined using standard pour plates method. Coliform count, total Salmonella /Shigella count and total vibrio counts was determined using MacConkey agar, Salmonella/Shigella agar and Thiosulphate citrate bile salt sucrose agar, respectively. The bacterial plates were incubated at 37°C for 36-48 hours. The isolates were identified with reference to Bergey’s Manual of Determinative Bacteriology [15].

Proximate Analysis

The foot muscle and viscera were mechanically removed from the shell and weighed using an analytical balance with precision of ± 0.0001 g (model ML 204/01; Mettler Toledo, Plainview, NY., U.S.A.). Briefly, the snail meat was digested in a mixture of HNO₃ (25%) and HCl (75%) (1:3, v/v) subjected to hot-block. Elemental concentrations were determined on a wet weight basis and analyzed on an inductively-coupled plasma (ICP) spectrometer (NexION 300 D series) with ESI SC-FAST Auto Sampler (Perkin Elmer, Waltham, Massachusetts, U.S.A.). The digested meat was portioned and three replicates were analyzed; one portion was used for protein analysis, another for lipid and fatty acid analysis, and third for moisture and ash analysis. The protein content was determined using Lowry et al. [16], fat content was evaluated using Folch et al. [17], while the ash and moisture were determined using the standard AOAC procedure [18]. Total carbohydrate was obtained by subtracting the per cent amounts of crude protein, crude fat, moisture, and ash from 100%.

Statistical Analysis

The data has been expressed as means ± standard error. The Student’s t-test and analysis of variance (ANOVA) were used to test for significance at the 0.05 and 0.01 levels of probability for the seasons and the stations, respectively.

Results and Discussion

Physicochemical parameters of the fish Town Rivers

The impact of human activities on natural ecosystem has changed the balance between biotic and abiotic factors that culminate into imbalance in the natural composition. The average physicochemical parameters of the Rivers sampled for both seasons showed 33.80 ± 1.20‰ salinity; that was within the limit for the estuarine environment (35ppt) [19]. The temperature (25 ± 0.60) °C was fluctuating and less than the recommendation limit set by the various regulatory bodies [19].

Table 1. Range of the concentrations of the hydro-chemical parameters at three sites along the fish Town Rivers, Nigeria; sampled monthly from August, 2017 to January, 2018

Parameters	Angalabiri	Better land	Farm camp	DPR/FMEnv Recommendation
Salinity (%)	28.60-33.20	24.40-38.10	25.80-31.10	35
Water temperature (°C)	23.10-26.2 30	21.50-25.20	18.20-23.20	30
Total dissolve solid (mg/l)	890-1140	830-1110	1100–1550	2000 (USEPA; 100–1000)
Dissolve oxygen (mg/l)	3-6	4-6	4-7	5
Chemical oxygen demand (COD)	32-43	30-41	30-43	40
pH	6.0-7.5	7.0-7.8	6.1-7.2	6.5-9.2
Turbidity	4.2-11	4.0-9.0	6.1-10.0	10
Phosphate (mg/l)	4-7	3-6	5-7	5
Nitrate	17-21	19-21	17-23	20
Total hardness	300-450	320-470	350-508	100-500



Total dissolve solid, total dissolve oxygen, chemical oxygen demand, phosphate, pH, turbidity, nitrate and the total hardness were within the limit for aquatic life [20] (total dissolve solid; 1003 ± 3.20 mg/l total dissolve oxygen; 5.70 ± 0.20 mg/l, chemical oxygen demand; 39.20 ± 0.50 pH; 6.80 ± 0.20 , turbidity; 7.20 ± 1.12 , phosphate mg/l; 5.30 ± 0.30 , nitrate; 19.20 ± 0.20 and total hardness; 475 ± 4.80) (Table 1).

Microbiological Assessment

The bacteriological load in the *P. byronensis* sampled from Fish Town River, lower Niger River Nigeria showed that there were variations in the occurrence of the different groups. Salmonella /Shigella count was highest $1.96 \pm 3.45 \times 10^3$ (cfu/100mL) followed by Total vibrio counts $1.66 \pm 3.13 \times 10^3$ (cfu/100mL). Total heterotrophic bacteria count was $1.63 \pm 4.97 \times 10^3$ (cfu/m) and with least Coliform count, $1.30 \pm 3.70 \times 10^3$ (cfu/100mL) (Table 2 and 3).

Table 2. Bacteriological load in *P. byronensis* sampled from fish Town River, lower Niger River Nigeria during the wet season

Samples stations	Total heterotrophic bacteria counts (cfu/ml) ($\times 10^3$)	Coliform counts (cfu/100ml) ($\times 10^3$)	Salmonella/ Shigella counts (cfu/100ml) ($\times 10^3$)	Total vibrio counts (cfu/100ml) ($\times 10^3$)
Station A	2.01 ± 7.45	1.20 ± 5.20	1.40 ± 2.45	1.10 ± 3.20
Station B	1.70 ± 2.68	1.30 ± 3.10	1.80 ± 4.70	2.30 ± 1.10
Station C	1.20 ± 1.40	1.40 ± 2.10	2.70 ± 3.10	1.60 ± 2.10
Mean \pm SE	1.63 ± 4.97	1.30 ± 3.70	1.96 ± 3.45	1.66 ± 3.13

The high Salmonella / Shigella species reported in this investigation is an indication of sewage contamination. Besides, they were higher than the EPA water standard for drinking water, which states that these pathogenic organisms must not be present in water. As they are of public health concerns, they have been linked with various infections such as new born meningitis and infantile diarrhea [19]. It is important to note that *Salmonella* species is food-borne pathogen and apart from human, it is also pathogenic to reptiles [21].

Total vibrio count (TVC) that was prevalent during the wet season in the Fish Town River could be attributed to the runoff from the seepage of toilets and the impact of the environment. This was due to the higher influence of the brackish environment that provokes the inactivation of the allochthonous microbiota from sewage discharges except for the halotolerant microorganisms' vibrio and staphylococci [22].

Table 3. Bacteriological load in *P. byronensis* sampled from fish Town River, lower Niger River Nigeria during the dry season

Samples stations	Total heterotrophic bacteria counts (cfu/ml) ($\times 10^3$)	Coliform counts (cfu/100ml) ($\times 10^3$)	Salmonella/ Shigella counts (cfu/100ml) ($\times 10^3$)	Total vibrio counts (cfu/100ml) ($\times 10^3$)
Station A	1.30 ± 2.20	1.20 ± 1.10	0.60 ± 0.20	0.50 ± 3.20
Station B	1.10 ± 1.40	1.10 ± 0.50	0.10 ± 0.13	ND
Station C	1.50 ± 2.70	ND	ND	ND
Mean \pm SE	1.30 ± 2.1	0.76 ± 0.53	0.23 ± 0.11	0.17 ± 0.06

The total heterotrophic bacteria counts were higher than the recommendation limit set by various regulatory bodies [23]. This is an indication of the existence of organic matter and dissolved minerals in the Rivers. These may be attributed to the faecal contamination from human settlement along the coastal

stretch. Similarly, open defecation in rarely available land spaces in the riverine communities is a common practice, hence possibly due to this, high HPC was reported in this investigation.

The coliform count reported in the study may be an indication that the Rivers are contaminated with fecal materials [23]. Similar to the HPC, the total coliform counts which were remarkably present and above the EPA maximum contamination level (MCL) for coliform bacteria in water of zero total coliform per 100ml of water [23]. However, it was below the upper threshold for European Union class B classification (4,600 *Escherichia coli* CFU/100 g).

All the groups of investigated bacteria were detected during the wet season only as Coliform and Salmonella /Shigella were not observed in station C and Vibrio were not observed in station B and C during the dry season (Table 3). Similarly, the occurrence of the bacteria was more in wet season than in the dry season, with the highest occurrence of $1.96 \pm 3.45 \times 10^3$ (cfu/100mL) recorded in Salmonella /Shigella count (wet season), while the least of $0.17 \pm 0.06 \times 10^3$ (cfu/100MI) recorded in total vibrio counts and was observed in dry season. The wet season (August-October) contained more bacteria loads than the dry season (November-January), which may be attributed to the anthropogenic factors and physico-chemical properties of the aquatic environment. It is well documented that human activities and oil pollution effects play a significant role in the influence the microbial qualities of aquatic environment. Benka-Coker and Ohimian [24] observed that increased human activities can easily influence the level of organic matter which can cause high microbial load. Besides, the introduction of waste water into water body also increased the amount of organic matter and essential nutrients, causing changes in the microflora [25].

Proximate analysis

The food shortage situation is more extreme with protein deficit when compared to the availability

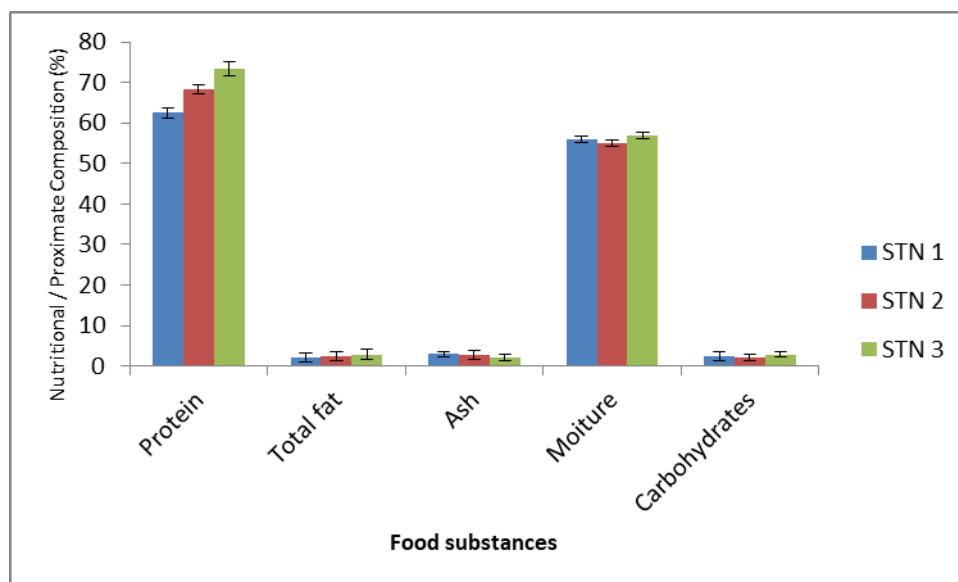


Figure 1. (A) The nutritional quality of *P.byronensis* tissue (edible part) from three stations within the fish Town Rivers Niger Delta Nigeria during the wet season (Means \pm SE)

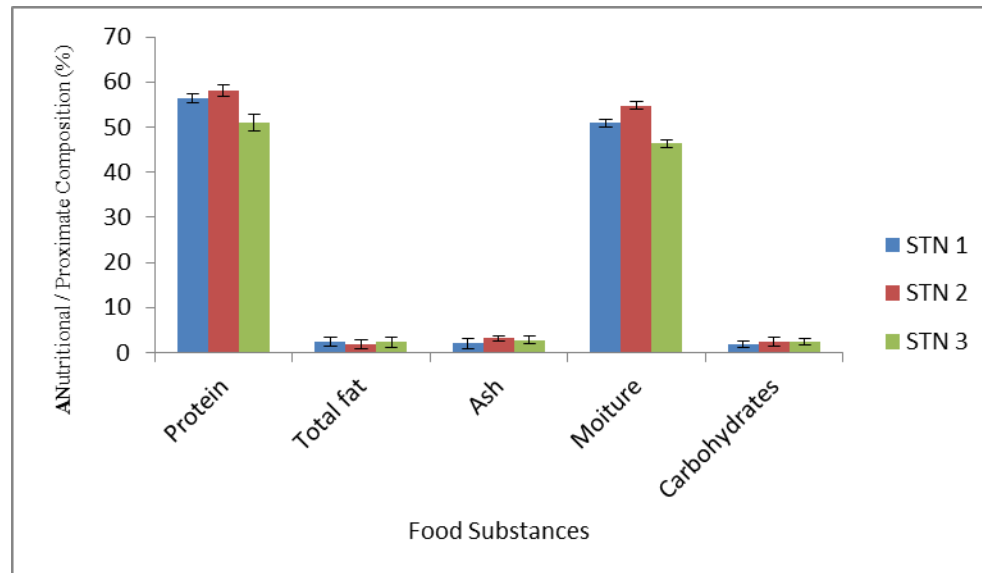


Figure 3. The nutritional quality of *P.byronensis* tissue (edible part) from three stations within the fish Town Rivers Niger Delta Nigeria during the dry season (Means \pm SE)

of carbohydrate and micro elements [26]. The proximate composition of *P.byronensis* from three selected stations within the Fish Town Rivers during the wet and dry season is shown in Figure 2 and 3.

No significant difference ($P > 0.05$) in the nutritional composition between the stations was investigated. The large proportion of protein in the snail's meat was neither varies significantly with seasons nor in spatial distribution ($p > 0.05$, $F = 9.70$). These results were in agreement with the study of [27] with 88.37 percent crude protein, that was little higher than this investigation. Similarly, lower crude protein (18.3%) was reported by [28] and (25.7%) by [29] for *Archachatina maginata* snail.

Comparing with the other meat, snail meat had higher protein level than (18.3%) crude protein value obtained from chicken meat and (19.6) from beef [30]. This is an indication that the protein content in this snail is higher than those found in the meat of other conventional livestock. This supports the assertion that snail meat contains protein, vitamin A, B6, B12, K, foliates, fat, iron, calcium, magnesium, phosphorus, copper and zinc [31]. The meat also has amino acids arginine and lysine at higher levels than in whole egg, plus essential fatty acids such as linoleic and linolenic acids, required for normal tissue development and maintenance [32].

The snail meat was high in moisture content (49%), though lower than in beef (63.90%) and chicken (69.40%), but low in; carbohydrates (2.1%), total fat (2.6%) and ash (2.6%). This indicates that the snail is a valuable source of sea food that will meet the balance nutritional needs of man.

Conclusion

The results of this study provided valuable information about *P. byronensis*, a dominant aquatic snail in the Niger Delta environment. The findings revealed that human activities may have influenced microbial load in the Rivers, and the nutritional status of snail revealed that it could be used as food for humans, which would go a long way in enhancing nutritional value of diet as the gastropod is very rich in protein and other necessary food requirements of man.



Contribution of Authors

Ifeoma Vivian Anyiam, develop the concept and participate in the samplings and analysis of the data. Thomas Ohwofasa Ikpesu, liaised with the communities where samples were collected, and supported in the analysis, interpretation of data and take care of the correspondence.

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