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Bacteriological and Proximate Analysis of Periwinkles from Two Diffrent Creeks in Nigeria

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Abstract: Hundred pieces each of two genera of freshly harvested Nigerian Periwinkles-*Tympanotonus fuscatus var radula* (a brackish water habitat) and *Pachymelania aurita* (a fresh water species) collected from lshiet and Oron creeks in Akwa Ibom State, Nigeria were evaluated for bacteriological quality, proximate nutrient and mineral composition. The results showed that all the periwinkle contain unacceptable levels of bacteria with *P. aurita* from Oron creeks carrying up to 1.46 x 10^5 cfμ g^{-1}. The level of coliform was generally high ranging from 1.5 x 10^5 cfμ g^{-1} of the total load in *T. fuscatus* from Oron creek to 2.8 x 10^5 cfμ g^{-1} in *P. aurita* (from Oron creeks). The Salmonella count ranged from 8 x 10^5 cfμ g^{-1} in *T. fuscatus t*o 1.9x10^6 cfu g^{-1} in *P. aurita* (from Oron creeks). The organisms isolated from the periwinkle samples include *Escherichia coli, Proteus vulgaris*, *Salmonella paratyphi*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Micrococcus varians* and *Enterobacter aerogenes. Salmonella paratyphi* and *Pseudomonas aeruginosa* from Oron and Ishiet creeks respectively had the highest rate of occurrence among the isolated bacteria, while *Proteus vulgaris* was the least encountered. Proximate analysis revealed that periwinkle samples from Oron creek were nutritionally richer than that of Ishiet creek Protein content ranged from 40.273% in *T. fuscatus* from Ishiet creek to 49.540% in *P. aurita* from Oron creeks. The Ca, K and P content ranged from 1537.04-6753.09, 370.00-508.20 and 236.80-440.00 μg g^{-1}, respectively.

Key words: Periwinkles · *Pachymelania aurita,* · *Tympanotonus fuscatus* · nutritional composition · Bacteria

INTRODUCTION

Periwinkles are mass-consumer products [1]. Which constitute relatively cheap sources of animal protein in Akwa Ibom State, South East Nigeria. They are invertebrates and they belong to the phylum molluscea and class gastropoda [2]. The phylum mollusks is known to radiate successfully into a variety of habitats, the great majority of which are aquatic, Some are found mostly in shallow waters and sometimes in inter-tidal zones where they burrow into the mud in the beds of the river which serves as their habitat [3]. Survey on the microbiological quality of shellfishes has shown shellfishes to harbor pathogenic organisms [4]. These pathogenic organisms have been implicated in outbreaks of food-borne diseases in many parts of the world; these illnesses which include typhoid fever, hepatitis and similar disorders of the digestive system [5, 6] are due to the pollution of the waters in which the shellfish grow [7, 8]. Since shellfishes are found in bodies of water containing untreated human and industrial waste, there is a tendency that they may concentrate and accumulate high levels of pathogens and toxic contaminants which can pose a significant health hazard to consumers [9, 10].

Considering the enormous commercial, nutritional and industrial importance of periwinkle, the fishing industry cannot continue to remain neglected. Hence, there is need to create awareness to the public on the health risks of consuming raw or inadequately cooked periwinkles, as this could be a channel of ingesting pathogenic micro-organisms into the body. This study was undertaken to comparatively evaluate the bacteriological quality as well as the proximate composition of *T. fascatus* and *P. aurita* from the two creeks studied.

MATERIALS AND METHODS

Collection of samples: Periwinkle (freshly harvested) samples (*T. fuscatus* and *P. aurita*) used in this study were collected from Ishiet and Oron creeks, both of which

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are famous periwinkle-producing areas in Akwa Ibom State, Nigeria. 100 pieces of each sample were collected in sterile plastic containers and taken to the laboratory for analysis within 3 h. The periwinkles were scrubbed, rinsed and the meat aseptically extracted as described by APHA [11].

Bacteriological analyses were carried out in triplicate on 50 g raw samples which were blended with 450 ml of sterile 0.1% peptone water as describe in the Bacteriological Analytical Manual [12]. Pour plates were prepared from 10-fold dilutions in nutrient agar (oxoid) for total bacteria count, MacConkey agar (oxoid) for total coliform counts and Salmonella/Shigella agar (oxoid) for Salmonella/Shigella counts were made after incubation at 37°C for 24 h. Colonies were selected randomly. Bacteria cultures were characterized and identified using various morphological and biochemical tests such as gram stain, spore stain, motility, catalase, coagulase, indole, MR-VP, urease, citrate, oxidase and sugar fermentation tests The isolates were identified with reference to Cowan and Steel's Manual for the identification of Medical Bacteria [13] and Fawole and Oso's laboratory manual [14]. Proximate composition were carried out according to the method of A.O.A.C. [15]. This includes determination of pH, moisture content, ash content, crude protein and fiber, fat, total carbohydrate contents. Mineral content such as potassium, calcium, phosphorus and iron.

RESULTS AND DISCUSSION

Table 1 shows the levels of bacterial load in the periwinkles samples from the two different creeks. Total bacterial populations of the samples from Ishiet and Oron creeks varied from 1.12 x 10^8 - 1.38 x 10^8 cfu g^{-1} for *T. fuscatus* and 1.27 x 10^8 - 1.46 x 10^8 cfu g^{-1} for *P. aurita*, respectively. *P. aurita* from Oron creeks, therefore had the highest level of bacteria contamination. Coliform density was also highest in *P. aurita* (2.80 x 10^5 cfu g^{-1}) from Oron creeks. The Samonella/Shigella counts varied from 8.0 x 10^5 - 1.5 x 10^6 cfu g^{-1} for *T. fuscatus* and 1.1 x 10^6 - 1.9 x 10^6cfu g^{-1} for *P. aurita* from Ishiet and Oron creeks respectively. In general, Oron creeks were found to be more contaminated than Ishiet creeks. The higher level of bacteria in *P. aurita* could be due to the fact that it grows in fresh water, which supports the proliferation of a broader spectrum of microorganisms as opposed to brackish water in which the *Tympanotonus* species live [16]. Furthermore, there are greater pollution-causing activities like bathing, washing and sewage discharge in the fresh water environment where *P. aurita* is harvested than in brackish water environment. Jay [17] and Ekanem and Adegoke [18] have reported that the level of contamination of shellfish depends on the extent of pollution in the growing waters. Bacterial contamination level in the two periwinkle species from the two creeks evaluated exceeded the acceptable limits for shellfish. The International Commission on Microbiological Specifications for foods [18] and the US Food and Drug Administration [12] have suggested a maximum microbial count IPC of not greater than 1 x 10^5 cfu g^{-1} and coliform level of not greater than 1 x 10^2 cfu g^{-1} of shellfishes for consumer safety. The result of the present study agrees with the report of Ekanem *et al.* [19] and Ekanem and Adegoke [8] who observed unacceptable levels of bacterial contaminants (including pathogens) in clams. The incidence of micro organisms in bivalves and other shellfishes depends on the quality of the water from which animals are obtained [8]. The bacteria isolates identified in the periwinkle species from the two creeks includes *E. coli*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Micrococus various*, *Salmonella paratyphi* and *Bacillus cereus*. *Salmonella paratyphi* and *Pseudomonas aeruginosa* from Oron and Ishiet creeks respectively had the highest rate of occurrence among the isolated bacteria, while *Proteus vulgaris* was the least encountered (Fig. 1). The occurrence of enteric organisms in the periwinkles was an indication of the pollution of their overlaying water with untreated faecal waste and sewage. All the organisms isolated have health implications for man except *M. varians*, which has not been associated with human infections. It has occasionally been isolated from human clinical specimen where it usually represents contaminants from the skin or mucous membrane surfaces or from the environment [20]. *Escherichia coli* is implicated in newborn meningitis and infantile diarrhea, *Enterobacter aerogenes* causes septicemia and neonatal meningitis, *Salmonella paratyphi* is the causative agent

Table 1: Comparative total count of microbial groups in the two periwinkle samples

Source	Sample	Total Bacterial count (cfμ g^{-1})	Coliform count (cfμ g^{-1})	Salmonellae count (cfμ g^{-1})
Ishiet creek	A_1	1.12x10^8	1.5x10^5	8x10^5
	B_1	1.27x10^8	1.8x10^5	1.1x10^6
Oron creek	A_2	1.38x10^8	2.7x10^5	1.5x10^6
	B_2	1.46x10^8	2.8x10^5	1.9x10^6

Key: A_1 and A_2 =Tympanotonus fuscatus, B_1 and B_2 = Pachymelinia aurita

Table 2: physico-chemical and nutritional composition of the two perinwinkle samples

Source	Samples	pH	Moisture content %	Ash content %	Crude protein %	Crude fibre %	Lipid content %	Total carbohydrate %	Ca (µg g^{-1})	K (µg g^{-1})	P (µg g^{-1})	Fe (µg g^{-1})
Oron creek	A_1	7.23	78.0	3.40	40.27	0.32	5.60	50.40	1537.04	508.20	236.80	126.0
	B_1	7.35	74.0	4.80	41.58	0.30	5.68	47.64	2018.52	620.40	240.40	126.0
Ishiet creek	A_2	8.88	77.0	3.40	47.80	0.29	1.68	46.83	5086.42	370.00	396.00	152.0
	B_2	9.93	74.0	4.72	49.54	0.28	1.80	43.66	6753.09	442.60	440.00	152.6

Key: A_1 and A_2 = *Tympanotonus fuscatus* B_1 and B_2 = *Pachymelinia aurita*

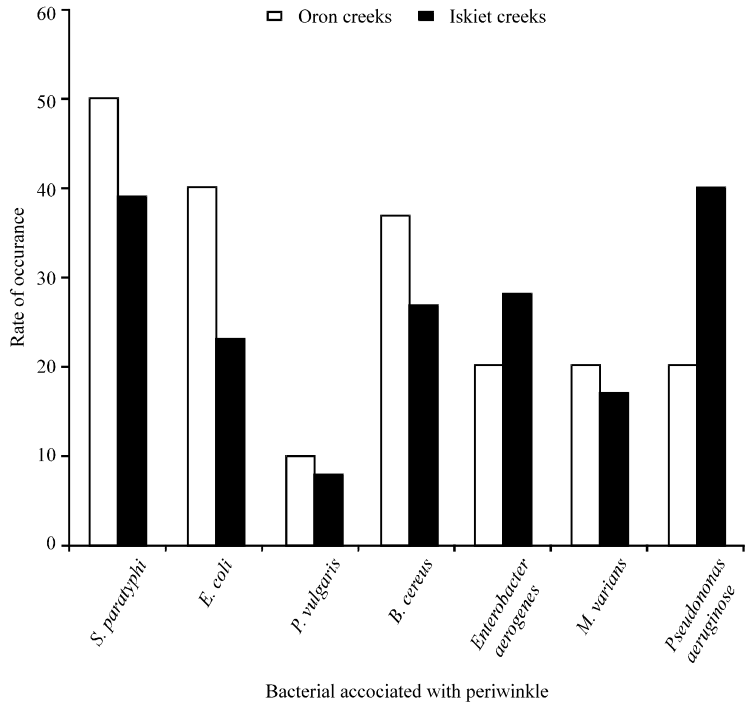

Fig. 1: The rate of occurnece of bacteria associated with periwinkle from two different creeks

of paratyphoid fever in humans, who are the only reservoir of this organism [21]. *B. cereus* causes a toxin-medicated disease rather than infection [22]. *P. aeruginosa* on the other hand is prevalent among patients with wounds, burns, cystic fibrosis and some blood stream infections.These organisms are likely to have been introduced into the environment by swimmers and infected individuals who use these creeks for recreational purposes

P. aurita from Oron creek was found to have the highest content of protein followed by *T. fuscatus* from the Oron creek. *P. aurita* from Oron creek had the highest Ca, P and Fe content (6753.09, 440.00, 152.60 µg g^{-1}), respectively while *P.aurita* from lshiet creek had the highest K content (620.40 µg g^{-1}). Mollusk shellfishes have been reported to serve as good source of protein and mineral elements [23]. Since it is high in dietary ingredient such as protein, it helps in the repair of worn out tissue and bodybuilding. In some Western food cultures, clams are used in the preparation of some taste-tempting products like soufflés, fritters, clip and chowders. Dehydrated clam flavor powder has been produced from clam and is used in formulated foods like dips and snacks [24]. The protein values of mollusks exceed the amounts in many food legumes (20-40%, dry basis) and compared favorably with the levels in the best of these pulses [25]. It constitutes an important group of low unit value marine products that supply cheap food of high quality [26].

Results obtained from this work revealed that though periwinkles are cheap/good sources of protein, they harbor a lot of pathogenic microorganisms that pose

serious health risks to man. The bodies of water in which the periwinkles were harvested contains untreated human and industrial wastes and since they are filter feeders there is a tendency that they will accumulate high level of pathogens and toxic contaminants as a result of cross contamination from the water body.

CONCLUSIONS

A comparative study of the samples from the two creeks microbiologically and nutritionally have revealed Oron creek samples to be more contaminated and nutritionally richer than Ishiet creek with *P. aurita* carrying the highest level of contaminants and nutritional value in both creeks. Samples from both creeks contained unacceptable levels of bacteria. Considering the public health implications of the poor bacteriological quality of these shellfishes, particular attention should be paid to their safety through proper processing, storage and handling procedures. The introduction of enforceable microbiological guidelines as a way of protecting consumers appears to be highly desirable.

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