Advanced Journal of Microbiology Research Volume 2014
Available online at http://advancedresearchjournals.org/AJMR

Full Length Research Paper

Bacteriological contamination of the freshwater clam (*Galatea paradoxa*) from the Volta estuary, Ghana

Adjei-Boateng, Daniel[1], Amisah, Stephen[1]* and Quagrainie, K. Kwamena[2]

[1]Department of Fisheries and Watershed Management, Faculty of Renewable Natural Resources, Kwame Nkrumah University of Science and Technology Kumasi, Ghana.
[2]Department of Agricultural Economics, Purdue University, West Lafayette IN, USA

Accepted 5 June, 2013

This study was designed to generate information on the microbiological quality of the clam, *Galatea paradoxa* harvested from the Volta estuary in Ghana. Total Viable Counts (TVC) for heterotrophic bacteria, Total coliforms (TC) and Faecal Coliforms (FC) as indicators of faecal contamination, were evaluated in the rainy season (June - August) and in the dry season (January - February). *G. paradoxa* from the estuary were found to be highly contaminated with the above mentioned micro- flora. There was a significant seasonal variation ($p < 0.03$) in the levels of total heterotrophic bacteria (TVC), total coliforms (TC) and faecal coliforms (FC). Total viable counts of heterotrophic bacteria in clams in the rainy season (June - August) was significantly lower ($p < 0.03$); (June, 1.0×10^7 cfu/g) than for the dry season (February, 7.0×10^{10} cfu/g). Total coliforms (TC) and FC portrayed a similar trend, being significantly higher ($p < 0.01$) in the dry season (1.0×10^{11}) than the rainy season (2.4 x 104 and 1.3 x 104 /g). Considering the importance of the clam fishery as an affordable protein source and a source of livelihood to the riparian communities along the Volta estuary, it is recommended that monitoring and regulatory controls of the fishery and growing waters be enforced whilst public education on the importance of depuration as a means of decontaminating the clams be pursued vigorously.

Key words: *Galatea paradoxa*, coliforms, heterotrophic bacteria, Volta estuary.

INTRODUCTION

In several developing countries in Africa, there is a strong economic incentive derived from a sustained demand for clams as an animal protein source and this is particularly so in Ghana, Nigeria and Cameroon. However, in these countries, harvesting of bivalves has little or no regulatory mechanisms in place and this is further exacerbated by poor sanitary facilities, which require extra attention to curtail the incidence of shellfish-borne diseases. Due to the health hazards inherent with the consumption of bivalves, many developed countries have enacted regulations based on the microbiological analysis of water and/or bivalve flesh. Most of these regulations use coliform counts as an indication of faecal contamination (West and Coleman, 1986; Pujalte et al., 1999; Villalobos and Elguezabal, 2001).

Bivalves are regarded as potentially hazardous foods because of their inherent tendency to bio accumulate pathogenic bacteria and toxic metal through filter feeding (Hatha et al., 2005). The ingestion of bivalves has been frequently associated with food related infectious diseases (Vieira et al., 2003) . It is understood that the inappropriate disposal of raw and partially treated sewage is a principal reason for the increasing incidence of shellfish-borne diseases. Hence strict guidelines are issued by the regulatory authorities of developed countries regarding bacteriological quality of the harvesting waters of the wild caught shell fish (EU SQAP, 1991).

The Volta clam, *Galatea paradoxa* (Born, 1778), *Egeria radiata* (Larmark, 1804), is a filter-feeding bivalve mollusc that is restricted to the lower reaches of a few large rivers

*Corresponding author. E-mail: steveamisah1@yahoo.co.uk

Table 1. Mean total viable counts (TVC), Total coliforms (TC) and faecal coliforms (FC) of the growing waters of clams at the Volta estuary over the wet season (June - August 2008) and dry season (January - February 2009).

Month	TVC(cfu/g)	TC(MPN/g)	FC(MPN/g)
June 2008	$4.5 \times 10^{4} \pm 3.5 \times 10^{4}$	$3.8 \times 10^{4} \pm 5.3 \times 10^{4}$	2.4×10^{4}
July 2008	$5.1 \times 10^{5} \pm 7.0 \times 10^{4}$	$2.0 \times 10^{2} \pm 1.5 \times 10^{1}$	$2.0 \times 10^{1} \pm 6.4 \times 10^{1}$
August 2008	2.0×10^{4}	$2.6 \times 10^{4} \pm 2.9 \times 10^{4}$	$1.7 \times 10^{4} \pm 1.0 \times 10^{4}$
January 2009	$1.7 \times 10^{5} \pm 7.1 \times 10^{1}$	$4.4 \times 10^{5} \pm 5.6 \times 10^{5}$	$2.4 \times 10^{2} \pm 2.7 \times 10^{2}$
February 2009	$2.0 \times 10^{4} \pm 3.4 \times 10^{2}$	$5.3 \times 10^{5} \pm 2.3 \times 10^{3}$	$1.9 \times 10^{3} \pm 2.4 \times 10^{2}$

Values are the means ± standard deviation (SD) of 40 samples from the two sampling sites.

in West Africa such as the Volta (Ghana), Cross and Nun (Nigeria), and Sanaga (Cameroon), (Etim and Brey, 1994). This clam has high nutritional value and constitutes an important protein source to the riparian human communities where it occurs (King, 2000). It is widely consumed in southern Ghana and serves as a means of livelihood to young men and women in these communities who fish, process and market the clams. It is harvested from the natural growing beds at the Volta estuary, regardless of the level of pollution of the waters. Little or no study has been carried out to assess the microbial load in the clams of the estuary. This study was, therefore, designed to generate information on the extent of bacteriological contamination of *G. paradoxa* harvested from the Volta estuary, Ghana, by assessing the presence and levels of heterotrophic bacteria, Total Viable Counts (TVC) and indicators of Faecal Contamination (Total Coliforms (TC) and Faecal Coliforms (FC).

MATERIALS AND METHODS

Sampling of clam and water samples

Clam and water samples were collected from two active fishing sites, Ada (5°49 10 N, 0°38 38 E) and Aveglo (5°52 54 N, 0°38 55 E) at the Volta estuary in rainy (June - August, 2008) and dry (January - February, 2009) seasons. Water samples were collected in sterile bottles at 30 cm below the surface and 30 cm above the riverbed where the clams live. The samples were tran-sported in thermally insulated boxes to the laboratory for analysis. Clams were washed with a brush and water to remove all material adhering to the shells and allow to air dry. Subsequently, the clams were opened aseptically using a sterile scalpel. Clam flesh weigh-ing 10 g were homogenised in a blender with 90 ml of sterile distil-led water, corresponding to a 10^{-1} dilution (Hatha et al., 2005). The homogenate was serially diluted up to 10^{-12} using 9ml of sterile dilution blanks. Overall 40 samples were collected from the two sites and each replicated 4 times.

Using the pour plate method 1.0 ml of the dilutions were transferred to sterile petri dishes and plated in duplicate in standard plate count agar. The plates were incubated at 37°C for 24 h. After incubation, the plates with 30 - 300 colonies were chosen for counting and the total plate count bacteria expressed as the number of colony forming units (cfu) per gram of shellfish.

Enumeration of total heterotrophic bacteria or total viable count

After counting and estimating total bacteria load, morphologically different colonies were picked up using a sterile inoculation needle and aseptically transferred to a sterile nutrient slants for further characterisation. The isolates were checked for their purity and characterised up to genera following a standard characterisation key (Bodyfelt, 1979) based on Gram staining, spore staining, motility, Kovac's oxidase, oxidation/fermentation (O/F) test and catalase tests.

Enumeration of total and faecal coliforms

A sample homogenate was prepared in the same way as described for the total plate count bacteria and 10^{-1} to 10^{-12} dilutions were used for estimating the coliform bacteria. A standard 3 - tube dilution most- probable number (MPN) method (West, 1989) procedure was used to enumerate the coliform load in the clam samples. Using a sterile pipette 1 ml sample each was inoculated into 10 ml sterile McConkey broth. After inoculation, the tubes were incubated at 37°C for 24 h and checked for gas production. The tubes with gas production were recorded and referred to the MPN table to ascertain the MPN index for the coliforms.

All findings of MPN for FC and TC were log transformed ($\log_{10}$) for the purpose of statistical analysis. Analysis of variance (ANOVA) was performed on the total viable count (TVC), total coliforms and faecal coliform densities of the water and clam over the seasons.

RESULTS

The bacteriological quality of the water and clams in the wet season (June - August 2008) and the ensuing dry season (January - February 2009) at the Volta estuary are presented (Tables 1 and 2). The results indicate significant seasonal variations in the concentration of total heterotrophic bacteria (TVC), total coliforms (TC) and faecal coliforms (FC) (Table 2).

Total viable counts of heterotrophic bacteria in clams in the rainy season (June -August) was significantly lower ($p < 0.03$) (June, 1.0×10^{7} cfu/g) compared to the dry season (February, 7.0×10^{10} cfu/g). Total coliforms (TC) and FC portrayed a similar trend, being significantly higher ($P < 0.01$) in the dry season (1.0×10^{11}) compared with the rainy season (2.4×10^{4} and 1.3×10^{4} /g).

DISCUSSION

Two groups of bacteria are of public health interest: bacteria naturally present in the environment such as *Aeromonas hydrophila*, *Clostridium botulinum*, *Vibrio* species and enterobacteriaceae such as *Salmonella, Shigella,* and *Escherichia coli*, which originates from contamination

Table 2. Mean total viable counts (TVC), total coliforms (TC) and faecal coliforms (FC) of clams harvested from the Volta estuary over the wet season (June - August 2008) and dry season (January - February 2009).

Month	TVC(cfu/g)	TC(MPN/g)	FC(MPN/g)
June 2008	$1.0 \times 10^{7} \pm 2.0 \times 10^{7a}$	$2.4 \times 10^{4} \pm 2.7 \times 10^{4a}$	$1.3 \times 10^{4} \pm 1.6 \times 10^{4a}$
July 2008	$6.0 \times 10^{8} \pm 7.9 \times 10^{8ab}$	2.4×10^{8ab}	2.4×10^{8bc}
August2008	$1.9 \times 10^{6} \pm 3.4 \times 10^{5a}$	$1.2 \times 10^{5} \pm 4.0 \times 10^{4bc}$	9.3×10^{4a}
January 2009	$4.0 \times 10^{7} \pm 2.8 \times 10^{7ab}$	$7.5 \times 10^{6} \pm 1.1 \times 10^{7ab}$	$2.0 \times 10^{6} \pm 3.0 \times 10^{6ab}$
February 2009	$7.0 \times 10^{10} \pm 4.0 \times 10^{10b}$	$1.0 \times 10^{11} \pm 1.5 \times 10^{11c}$	$1.0 \times 10^{11} \pm 1.5 \times 10^{11c}$

Values are mean ± SD of 4 replicates. Mean values in the same column with $^{a, b, c}$ are different superscripts are significantly different ($P < 0.05$).

of the water with human residue (Vieira et al., 2003; Pereira et al., 2006). The determination of coliforms of faecal origin and *E. coli* provides relevant information regarding the hygiene-sanitary conditions of both the clams and the cultivation water. The results of this study show considerable contamination of the clams with bacteria of the later group. The results are in agreement with Ekanem and Adegoke (1995) who studied the bacteriological quality of a stock of *G. paradoxa* in Cross River, in Nigeria over two dry seasons in the month of January. The present study indicates significantly higher levels of bacteria than earlier studies by Hatha et al., (2005), who studied the bacteriology of the freshwater clam, *Battisa violacea,* in Fiji. Their study observed that less than 5% of the samples had acceptable levels of TVC (5×10^{5} cfu/g) as outlined in guidelines of the centre for food safety and applied nutrition (CFSAN, 2003) of the United States Food and Drug Administration. For the present study, the TVC values recorded both in the rainy and dry season were higher than the acceptable limits given by CFSAN (2003) (Table 2).

Other studies conducted on the microbiological quality of bivalves in Brazil portrayed a significantly lower TC and FC levels compared to this study. Vieira et al. (2003) observed TC and FC ranges between < 1.8 - > 1600 /g and < 1.8 - > 920 /g respectively for *Crassostrea rhizophorae* in the Coco river estuary, Brazil. Additionally, Pereira et al. (2006) reported TC and FC ranges between < 3 - > 1100 /g and < 3 - > 1100 /g respectively for *C. gigas* in Brazil.

According to the European Union Shellfish Quality Assurance Programme (EU SQAP, 1991), shellfish from a Category A area can go for direct human consumption if they contain less than 300 FC/100 g of meat. Shellfish from Category B areas must not exceed, in 90% of the samples, the limit of 6000 FC/100 g of meat. Such shellfish can only be placed on the market after depuration over a specified period, relaying or heat treatment by an approved process in order to meet the Category A standard. If the European standards were applied, all the clams from the Volta estuary cannot be placed directly on the market after harvesting. The clams would have to undergo depuration or purification for a period of at least two months, a practice that is uncommon among clam fishers and marketers in Ghana.

The high TVC and coliforms (TC and FC) in *G. paradoxa* harvested from the Volta estuary is a direct reflection of the quality of the shellfish harvesting waters, which are directly influenced by the rainfall patterns and many anthropogenic activities in the basin. The onset of the rainy season is characterised by heavy runoff from the surrounding villages. The runoff carries raw sewage from human habitations and leachate from waste dumping sites in the catchment area. Bacteria concentration increases to a peak in July but declines with increasing dilution of the estuarine waters by rainfall. The clams are filter-feeders and are able to accumulate the bacteria in their tissues to levels four to seven times higher than the surrounding water (Villalobos and Elguezabal, 2001; Hatha et al., 2005).

This study has provided considerable information on the prevalence and levels of bacteria in the clam, *G. paradoxa,* harvested from the Volta estuary. The results indicated that both the clams and their aquatic habitat carry considerably high and unacceptable levels of pathogenic bacteria. Considering the importance of the clams as an affordable protein source and means of livelihood for the riparian communities, it is recommended that regulatory authorities of the Volta basin put control mechanisms in place to avert the sustained pollution of the river environment and the clams. In addition, adequate and suitable sanitation facilities should be provided for communities of the riparian communities along the Volta basin. Education on the importance of depuration as a means of decontaminating the clams should be incorporated into the general clam fishery management.

ACKNOWLEDGEMENTS

The authors are grateful to the International Foundation for Science (IFS A/4421-1) and AquaFish Collaborative Research Support Program (AquaFish CRSP) for providing financial support to conduct this research work. The authors are also grateful to the Department of Fisheries and Watershed Management of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana for logistical support.

REFEERENCES

Bodyfelt FW (1979). Sensory, shelf -life, microbial and chemical evaluation of creamed cottage cheese treated with sorbates. J. Food Protection. In: *Buchanan*, *R.E.* and *Gibbons*, *N.E.*, Editors, 1974. Bergey's Manual of Determinative Bacteriology *(*8th edn.), The Williams and Wilkins Co*.,* Baltimore pp*.* 217–243.

Centre for Food Safety and Applied Nutrition (2003). National Shellfish Sanitation Program. Guide for the control of molluscan shellfish pp. 357-359

Ekanem EO, Adegoke GO (1995). Bacteriological study of West African clam (*Egeria radiata* Lamarch) and their overlying waters. Food Microbiol. 12**:** 381-385.

Etim L, Brey T (1994). Growth, productivity, and significance for fishery of the bivalve Egeria radiata (Donacidae) in the Cross River, Nigeria. Arch. Fish. Mar. Res. 42(1): 63-75.

EU SQAP (1991). COUNCIL DIRECTIVE 91/492/EEC of 15 July 1991 laying down the health conditions for the production and the placing on the market of live bivalve molluscs pp. 1-19.

Hatha AAM, Christi KC, Singh R, Kumar S (2005). Bacteriology of the fresh water bivalve clam *Batissa violacea* (Kai) sold in the Suva market. The South pacific J. Nat. Sci. 23: 48-50.

King RP (2000). Population structure, growth performance and mortality rates of the freshwater clam *G. paradoxa* Born 1778, in Nun River, Nigeria. Archive Fish. Mar. Res. 48(1) 21-30.

Pereira MA, Nunes MM, Nuernberg L, Schulz D, Batista CRV (2006). Microbiological quality of oysters (*Crassostrea gigas)* produced and commercialised in the coastal region of Florianopolis – Brazil. Br. J. Microbiol. 3: 159 -163.

Pujalte MJ, Ortigosa M, Macian MC, Garay E (1999). Aerobic and Facultative anaerobic heterotrophic bacteria associated to Mediterranean oyster and seawater. Int. Microbiol. 2: 259-266.

Vieira RHSF, Ménages FGR, Fonteles-Filho AA, Torres RCO, Ernani ASS (2003). Bacteria of fecal origin in mangrove oysters (*Crassostrea rhizophorae*) in the Coco River estuary, Ceara State, Brazil. Br. J. Microbiol. 34: 126-130.

Villalobos LB, Elguezabal LA, (2001). Microbiological quality of the bivalve Pinctada imbricate commercialised in Cumana, Venezuela. Food Technology. Acta Cientifica Venezolana 52: 55-61.

West PA, Coleman MR (1986). A tentative national reference procedure for isolation and enumeration of *Escherichia coli* from bivalve molluscan shellfish by most probable number method. J. Appl. Microbiol. 61: 505 – 516.

West PA (1989). Human pathogens and public health indicator organisms in shellfish. In**:** B. Austin and DA Austin (Ed). *Methods for the microbiological examination of fish and shellfish.* Ellis Harwood. Chichester, West Sussex, England pp.273-308.