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

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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 x 10⁷ cfu/g) than for the dry season (February, 7.0 x 10¹⁰ cfu/g). Total coliforms (TC) and FC portrayed a similar trend, being significantly higher (p < 0.01) in the dry season (1.0 x 10¹¹) 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 Elguézabal, 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.
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' N, 0°38' E) and Aveuglo (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 transported 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 weighing 10 g were homogenised in a blender with 90 ml of sterile distilled water, corresponding to a 10⁻¹ dilution (Hatha et al., 2005). The homogenate was serially diluted up to 10⁻¹² using 9 ml 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 (Boddyfelt, 1979) based on Gram staining, spore staining, motility, Kovac’s oxidase, oxidation/fermentation (O/F) test and catalase tests.

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).

<table>
<thead>
<tr>
<th>Month</th>
<th>TVC(cfu/g)</th>
<th>TC(MPN/g)</th>
<th>FC(MPN/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 2008</td>
<td>4.5 x 10⁴± 3.5 x 10⁴</td>
<td>3.8 x 10¹ ± 5.3 x 10⁴</td>
<td>2.4 x 10⁰</td>
</tr>
<tr>
<td>July 2008</td>
<td>5.1 x 10⁴± 7.0 x 10⁴</td>
<td>2.0 x 10² ± 1.5 x 10⁴</td>
<td>2.0 x 10⁰ ± 6.4 x 10⁰</td>
</tr>
<tr>
<td>August 2008</td>
<td>2.0 x 10⁵</td>
<td>2.6 x 10⁵ ± 2.9 x 10⁴</td>
<td>1.7 x 10⁰ ± 1.0 x 10⁰</td>
</tr>
<tr>
<td>January 2009</td>
<td>1.7 x 10⁸± 7.1 x 10⁷</td>
<td>4.4 x 10⁶ ± 5.6 x 10⁷</td>
<td>2.4 x 10⁵ ± 2.7 x 10⁵</td>
</tr>
<tr>
<td>February 2009</td>
<td>2.0 x 10⁶± 3.4 x 10⁵</td>
<td>5.3x10⁶ ± 2.3 x 10⁵</td>
<td>1.9 x 10⁵ ± 2.4 x 10⁵</td>
</tr>
</tbody>
</table>

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

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 x 10³ cfu/g) compared to the dry season (February, 7.0 x 10⁰ cfu/g). Total coliforms (TC) and FC portrayed a similar trend, being significantly higher (p < 0.01) in the dry season (1.0 x 10³ cfu/g) compared with the rainy season (2.4 x 10³ and 1.3 x 10⁴/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
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 Ekamen 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 x 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 TVC 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 TVC 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

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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).

<table>
<thead>
<tr>
<th>Month</th>
<th>TVC (cfu/g)</th>
<th>TC (MPN/g)</th>
<th>FC (MPN/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 2008</td>
<td>1.0 x 10^7 ± 2.0 x 10^5a</td>
<td>2.4 x 10^5 ± 2.7 x 10^4a</td>
<td>1.3 x 10^5 ± 1.6 x 10^4a</td>
</tr>
<tr>
<td>July 2008</td>
<td>6.0 x 10^6 ± 7.9 x 10^6ab</td>
<td>2.4 x 10^6ab</td>
<td>2.4 x 10^6bc</td>
</tr>
<tr>
<td>August 2008</td>
<td>1.9 x 10^6 ± 3.4 x 10^6a</td>
<td>1.2 x 10^6 ± 4.0 x 10^6ab</td>
<td>9.3 x 10^6a</td>
</tr>
<tr>
<td>January 2009</td>
<td>4.0 x 10^7 ± 2.8 x 10^7ab</td>
<td>7.5 x 10^6 ± 1.1 x 10^7ab</td>
<td>2.0 x 10^6 ± 3.0 x 10^6ab</td>
</tr>
<tr>
<td>February 2009</td>
<td>7.0 x 10^10 ± 4.0 x 10^10b</td>
<td>1.0 x 10^11 ± 1.5 x 10^11c</td>
<td>1.0 x 10^10 ± 1.5 x 10^11c</td>
</tr>
</tbody>
</table>

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).
REFERENCES


