Coliform Content of Shellfish (Anadara antiquata) in Davao Gulf.

TEJADA, ROSIE LYNN P.
Marine Biology Department, Davao del Norte State College, New Visayas, Panabo City
Cell Phone No. 09183699271: email address: oresbio@yahoo.com

Abstract

The study was conducted to determine the coliform content of shellfish Anadara antiquata from three selected areas of Davao Gulf. One shot sampling was employed in the study and it was conducted within the month of February, 2010. Test for coliform and fecal coliform was done using the multiple-tube fermentation technique through the presumptive, confirmed and fecal coliform test. Total Colony Count of coliform in Anadara antiquata was done through plate counting using selective media.

Coliform content in Anadara antiquata from Panabo City and Tagum City were > 1,100 MPN/g of sample. While in Pantukan, the coliform content in Anadara antiquata from the estuary was 460 MPN, 240 MPN near mangrove area. These values exceeded the critical limit 230 MPN set by APHA. Fecal coliform content in Anadara antiquata was high from Panabo City, 240 MPN from areas near estuary and near coastal houses. In Pantukan, Compostela Valley Province, the fecal coliform content in Anadara antiquata was 240 MPN near mangrove area. The fecal coliform content in Anadara antiquata from these areas exceeded the critical limit. Total colony count of coliform in Anadara antiquata from Panabo and Tagum City was higher compared to Pantukan and the total colony count of coliform in Anadara antiquata did not differ in 3 selected habitats.

High coliform content in A. antiquata in three areas of Davao Gulf could pose health risks to the consumers. High content of fecal coliform in shellfish indicates presence of disease causing microorganisms which are harmful to human health. It is recommended to safeguard public health through fecal coliform/bacteriological monitoring of the shellfish and their growing waters since these microorganisms are pathogens or disease causing. Conduct also Information Education Campaign (IEC), informing the Local Government Units and the public that wastes of both fecal and non-fecal in origin can be accumulated by shellfish Anadara antiquata.

Key Words: Coliform, Most Probable Number, Anadara antiquata

Introduction

Shellfish such as oysters, clams and mussels are important protein sources for humans. Their maximum utilization as food is hindered by the public health problems associated with their consumption. Outbreaks of typhoid fever, infectious hepatitis, cholera and paralytic shellfish poisoning (PSP) are among the most commonly reported cases linked to the ingestion of contaminated bivalve meat. These maladies more often results from eating shellfish meat as raw or half-cooked (Toral-Barza, L. and Gomez E., 1985; Tebano, T. and Paulay, G., 2000). Bivalves grow and are commonly grown and harvested in estuarine areas. This is where industry and population growth usually occurs and where stream runoff ultimately flows. Consequently, accumulation of microbial pathogens from sewage pollution, heavy metals, pesticides and other toxic substances very likely exists. As bivalves are filter feeders, feeding on planktons and other micro-flora of the estuary, they are able to ingest pathogenic bacteria, virus, parasites and other dissolved toxic substances present in the rearing waters (Paulay, 1996; Ditschman et al., 1995; Temple, 2007). The presence of Escherichia coli and other coliform bacteria in streams, rivers, and lakes and other bodies of water are possible source of human pathogens. Surface waters may be exposed to numerous sources of bacteria, including domestic and wild animals, agricultural runoff, failed septic systems, combined sewer overflows, and illicit connections of sanitary sewers to storm water sewers (Ditschman et al., 1995; Toral-Barza, L. and Gomez E., 1985).
Bacteria species like *Escherichia coli*, *Enterobacter aerogenes*, and *Klebsiella pneumoniae* comprised the microbial flora of the human intestine while the fecal coliform bacteria are present in large numbers in the feces and intestinal tracts of humans and other warm blooded animals (Kinyanjui, 2008; Greene, 2004). The bacteria can attach to sediment particles, occurs during high run off and grow faster at higher temperatures and high levels of nutrients increases the growth rate of the bacteria (Murphy, 2007).

Microbiological pollution in shellfish-growing waters is a common problem due to shellfish-borne infectious diseases that are transmitted through a fecal-oral route (shellfish become contaminated by sewage and are eaten by humans) deposited on land surfaces. Davao Gulf is very rich of marine resources utilized for food consumption or sources of livelihood. Among these resources are the bivalve shellfish including gastropods, clams, oysters, cockles, scallops and geoducks which are filter feeders that accumulates contaminants through the process of filtering food from the water column (Huss et al., 2000). In Pantukan Compostela Valley Province, Tagum City and Panabo City, gleaning of shellfish *Anadara antiquata* is one of the sources of livelihood in fishing community. Thus, in knowing the dangerous effect that these coliform bacteria especially the fecal coliform, there is a need to test the shellfish quality particularly its coliform content in Davao Gulf.

**Methodology**

**Study Area**

The study was conducted in three selected areas of Davao Gulf, particularly in the cities of Tagum and Panabo, Davao del Norte and Pantukan, Compostela Valley (Fig. 1). Three sampling habitats were chosen in each area namely: near mangrove area, near estuary, and near coastal houses. The three areas were the usual sources of *Anadara antiquata* that was studied. One shot sampling was employed in the study and it was conducted within the month of February, 2010.

**Panabo City** is a 4th class city near Davao City in the province of Davao del Norte, Philippines. The city has estimated population of 133,950 people in 27,225 households. Panabo is occupied by agricultural lands utilized for big banana plantation and other agricultural crops. It is also an industrialized city and is known as exporter of banana. There are more than 500 households/families living near the coastal area of Barangay Cagangohan where the *Anadara antiquata* was taken.

**Tagum City** is another city which has popularly become a first class city in the 1st Congressional District of the Province of Davao del Norte, Philippines serving as its provincial capital. It has a total land area of approximately 19,850 hectares which is predominantly occupied by agricultural lands utilized in various kinds of crops like coconut, rice, Cavendish banana, fruit trees and other non-commercial agricultural crops. It has estimated total population of 240,599 inhabitants in 2010 based on census by NSO with an average growth rate of 2.8 percent per annum and this will bring the cityhood to a “highly urbanized” status. Tagum City, from being a purely agricultural city, has become Mindanao’s fastest-rising urban city due to its strategic location, being in the crossroads between the rural areas of Davao del Norte and Compostela Valley and the urban Metro Davao. There are more than 300 households/families living near the coastal area of Barangay Liboganon where the sample *Anadara antiquata* was gleaned.

**Pantukan** on the other hand, is a first class municipality in the province of Compostela Valley, Philippines. It has a population of 61,801 people in 13,311 households. It is subdivided into 13 barangays in which the upper part of the municipality is mostly mining sites. It also has an agricultural area planted by coconut and banana. An estimated of more than 150 households/families are living the coastal area where the sample *Anadara antiquata* was taken.
Sample collection

Collection of shellfish samples was done in three selected areas during lowest low tide. Samples were bought from the gleaners in the sampling area, and was rinsed and drained at the time of sampling. There were 3 batches of samples from (near estuary, near mangrove area, and near coastal houses) that were gathered in every station. A total of 9 batches of samples were tested from three areas. Length of individual shellfish was measured by using a vernier caliper in centimeters. Total weight was also measured by using portable weighing balance in grams. Samples were placed in a plastic bag, properly packed in a cooled box within an hour. Shellfish samples were brought to laboratory of Department of Science and Technology (DOST), Davao City and Davao del Norte State College (DNSC) for analysis. During transportation, the temperature of ice box was maintained at 4°C.

Preparation of Culture Media

All the glasswares were cleansed thoroughly with a tap water mixed with detergent and later rinsed with distilled water, before the culture media preparation. These glasswares were sterilized in dry heat oven for one hour and thirty minutes at 160°C to 170°C. After using the glasswares, it was placed in the autoclave for 30 minutes at 121°C to 125°C in order to avoid spreading of the microorganisms that may be present.

The peptone water at 0.1% peptone (1g/L water) was prepared and dispensed into 9 ml test tubes. The test tubes were sterilized at 15 psi for 15 minutes together with fermentation tubes for coliform culture. Lauryl Tryptose Broth for Presumptive Test was prepared using the Lauryl Tryptose Broth. The amount of 35.60 grams was mixed with 1000 ml of distilled water and later distributed into the test tubes containing inverted durnham tubes at 10 ml each and were sterilized in the autoclave at 15 lbs psi (121 °C) for 15 minutes. Brilliant Green Lactose Bile Broth (BGLBB) for Confirmed Test was prepared by mixing the 37.0 grams powder to 1000 ml of distilled water. The solution was distributed to fermentation test tubes containing inverted durnham tubes and was sterilized in the autoclave at 15 lbs pressure (121 °C) for 15 minutes. EC medium for Fecal Coliform Test was prepared by mixing the 37.0 grams of EC broth powder to 1000 ml of distilled water. The medium was heated to dissolve the EC Broth powder. The prepared broth was dispensed into the fermentation test tubes containing durnham tubes and was sterilized in the autoclave at 15 lbs pressure (121°C) for 15 minutes.

Microbiological Analysis of Shellfish

Shellfish samples brought to DOST laboratory were prepared for various tests: MPN Presumptive and Confirmed Test for Coliform and Fecal Coliform in Shellfish. The shell of *Anadara antiquata* was thoroughly
washed and scrubbed under running tap water and the surface were sterilized by rinsing in 70% ethanol. It was opened aseptically with a flame sterilized shucking knife. The flesh was weighed before transferring each individual sample to a sterile physiological saline and was crushed using the stomacher.

Using 10-12 shellfish, a 100 g of shellfish liquor and meat was obtained and blended with 100 ml sterile phosphate buffered dilution water or 0.5% peptone water for two minutes to yield a 1:2 dilution of sample. After two minutes of blending of the samples, the analysis of the grounded sample was done. The serial dilutions of samples were prepared in 0.5% sterile peptone water or sterile phosphate buffered dilution water. Lactose Broth or Lauryl Tryptose Broth, at single strength in 10 ml. volume, was inoculated to the samples following the steps: a. To each of 3 tubes, add 2 ml. of the blended homogenate (equivalent to 1 g of shellfish). b. To each of 3 tubes, add 2 ml of 1:10 dilution of homogenate (0.1 g shellfish). c. To each of 3 tubes, add 2 ml. of 1:100 dilution of homogenate (0.01 g shellfish). d. To each of 3 tubes, add 2 ml. of 1:1000 dilution of homogenate (0.001 g shellfish). Test tubes were incubated at 35°C for 24 hours. After incubation, each tube was examined for gas production. If no gas was formed, the tubes were re-incubated for another 24 hours. The presence or absence of gas formation was recorded regardless of the amount gas that each tube produced. Tubes that had gas formation within 48 hours indicated a positive presumptive reaction. Tubes that did not produce gas formation were discarded. Confirmed test was immediately performed after the presumptive test observation was done following the standard procedure of the "Conventional Method for Coliforms, fecal coliforms and E. coli". Finally, the MPN of the coliform was determined. Fecal Coliform Test. To confirm positive tubes, one loopful from gas positive tubes was transferred to EC broth and incubated in a covered circulating waterbath at 44.5°C for 24 hours. Gas production in EC indicated the positive or presence of fecal coliforms. The MPN for fecal coliforms was determined using the MPN table. Total colony count of Coliform was determined using the 15 samples of *Anadara antiquata*. The meat of each shellfish was taken from the shell and was grounded. It was blended to a 10 ml. sterile phosphate buffered dilution water or 0.5% peptone water to yield a 1:2 dilution of sample. Analysis of the grounded samples began after two minutes of blending the meat. Serial dilutions were made in 0.5% sterile peptone water or sterile phosphate buffered dilution water. Then 1 ml. of blended homogenate was transferred to series of 3 tubes contained with Lauryl Tryptose Broth, at single strength in 10 ml volumes and incubated at 35°C for 24 to 48 hours. After the incubation, one ml was again transferred to another series of 3 tubes. After 24 or 48 hours incubation, a loopful of dilution was transferred by streaking it in petri plates. The plates were incubated at 35°C for 24 hours observation. The coliform colony forming unit (cfu) was counted from the dilution using Quebec Colony Counter. Selective media for Coliform (Eosin Methylene Blue –EMB Agar) was used in the preparation of plates for colony counting.

**Data Analysis**

**Determination of the Coliform Content.** The number of positive samples with coliform group of organisms was determined in terms of MPN/g of shellfish sample. The total colony count of coliform in *Anadara antiquata* in three selected stations and its three different habitats were analyzed using the ANOVA while Duncan’s Multiple Range Test was used to test the degree of significant difference. The relationships of the length, total weight, and meat weight of *Anadara antiquata* with total colony count of Coliform from three selected areas of Davao Gulf was determined using the correlation analysis.

**Results**

**Most Probable Number (MPN) of Coliform**

Coliform content of *Anadara antiquata* taken from all the three habitats of Panabo City and Tagum City was >1,100 MPN/g of sample respectively, while in Pantukan, the coliform level in *A. antiquata* near the estuary was 460 MPN/g, near the mangrove area with 240 MPN/g while the lowest level was noted near the coastal houses with 93 MPN/g (Table 1).

**Fecal Coliform Count**

For the fecal coliform content of *A. antiquata* in Panabo City, 240 MPN/g of sample was noted near the estuary and near the coastal houses, while in the area near the mangrove, only 150 MPN/g of sample was observed. In Tagum City, the fecal coliform level in the shellfish was 23 MPN near the estuary, followed by 9.2 MPN near the coastal houses and < 3.0 MPN was observed near the mangrove area. In Pantukan, the fecal coliform content in shellfish near the coastal residential area was < 3.0 MPN, 93 MPN near the estuary, and 240 MPN levels near mangrove area respectively (Table 1).

Table 1. Coliform and Fecal coliform content of *Anadara antiquata* in three stations of Davao Gulf. February, 2010.
Station | Shellfish samples from different habitats | Coliform (MPN/g) | Fecal Coliform (MPN/g)
---|---|---|---
Panabo City | S1S1 (near estuary) | > 1,100 | 240 |
| S1S2 (near mangrove area) | > 1,100 | 150 |
| S1S3 (near coastal houses) | > 1,100 | 240 |
Tagum City | S2S1 (near estuary) | > 1,100 | 23 |
| S2S2 (near mangrove area) | > 1,100 | < 3.0 |
| S2S3 (near coastal houses) | > 1,100 | 9.2 |
Pantukan | S3S1 (near estuary) | 460 | 93 |
| S3S2 (near mangrove area) | 240 | 240 |
| S3S3 (near coastal houses) | 93 | < 3.0 |

| a | 100 g of samples of shellfish used per habitat |

| b | The coliform content was taken from the meat of shellfish per microbial analyses; adopted from Bacteriological Analytical Manual. Eighth Edition. 1998. Chapter 4. pages 4.01-4.06. Appendix 2.07. |

Total Colony Count in three Selected Areas of Davao Gulf
The total colony count of coliform in *A. antiquata* sampled in the coastal waters of Davao Gulf is reflected in Figure 2. High counts were noted in shellfish from Panabo City with 2,688 and from Tagum City with 2,642, while found lowest (1,590) in shellfish gleaned from Pantukan. Result showed a significant difference on the total colony count of coliform in *A. antiquata* in 3 selected stations of Davao Gulf. Result of the Duncan’s Multiple Range Test revealed that Panabo and Tagum were not significantly different in total colony count of coliform in shellfish while in Pantukan, a significant difference in the total colony count of coliform was noted.

![Figure 2. Total Colony Count of coliform in *A. antiquata* in three stations of Davao Gulf.](image)

**Total Colony Count in 3 Selected Habitats**
The comparison of total colony count in shellfish gleaned from the three stations of Davao Gulf and in the three habitats is presented in Figure 3. The total colony count recorded in the three habitats did not significantly differ based on ANOVA result.

Figure 3. Comparison of total colony count in three stations of Davao Gulf and in the three habitats.

Length, Weight, Meat Weight and Total Colony Count of Coliform Relationship

Table 2. shows the relationship of length, weight, meat weight and total colony count of coliform in *Anadara antiquata*.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>R values</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length and Total Colony Count</td>
<td>-0.55954</td>
<td>Moderate Negative Correlation</td>
</tr>
<tr>
<td>Weight and Total Colony Count</td>
<td>-0.21935</td>
<td>Low or Slight Negative Correlation</td>
</tr>
<tr>
<td>Meat Weight and Total Colony Count</td>
<td>-0.70739</td>
<td>High Negative Correlation</td>
</tr>
</tbody>
</table>

Discussions

The coliform content in the shellfish samples taken from Panabo City and Tagum City were notably higher as compared to the shellfish samples taken from Pantukan. The coliform level in the three habitats of Panabo City and Tagum City, and in the two habitats of Pantukan exceeded the standards set by the American Public Health Association (APHA) which is 230 MPN of coliform. Only shellfish samples taken in the coastal houses of Pantukan with coliform content of 93 MPN had passed the standards of APHA. The high coliform content in samples from Panabo City could be attributed to the heavy rains experienced prior to sample collection (three consecutive weeks in the month of January, 2010). The flushing of domestic wastes towards the mouth of the Lasang River, Panabo Creek and from the adjacent Panabo City Mariculture Park where more than 500 fish cages were constructed must have an effect on the shellfish. Besides, shellfish samples were collected during low tide and the water was observed as to be turbid. According to Murphy (2007), fast-running water can carry more sediment, so higher level of bacteria can occur during high run-off events. It was observed that the coastal area of Panabo City was densely populated with more than 500 households/families. Recreational cottages for beach goers were also constructed along the shoreline. The coastal area was also used as a navigational lane for commercial ships transporting banana and products domestically and to other countries as well. It was also further noted that the residents do not dispose their garbage properly. Many of their wastes were thrown directly to the sea as evidently seen in their surroundings. In Tagum City, high coliform content
can be attributed to runoff from Bincungan and Tuganay rivers. Fish ponds located in the upstream could have contributed to the high level of coliform. It was also observed that water from fishponds was being released into the river. The high coliform content in the area could be attributed to the residential (of more than 300 households/families living near the coastal area) and commercial activities along the shoreline. Besides, many cottages and small restaurants were found to be operating around the area. Just like in Panabo, heavy rains experienced from previous days (for three consecutive weeks in the month of January, 2010) could have also contributed to the high coliform content in most of the habitats. In addition, residents in the vicinity were observed that they did not dispose their garbage properly. According to some residents being interviewed, no garbage truck were collecting garbage. Thus, garbage were just scattered everywhere in the area. Many plastics and other forms of wastes were seen in the mangrove area, including the area near estuary. In this study, the high content of coliform indicated a high degree of coliform contamination in the area. In Pantukan, coliform level was found to be lower considering that there were only 100 households/families residing in the area. Also, the area of beach resort was not as big as that of Panabo City and Tagum City. Though heavy rains occurred prior to sampling collection in three consecutive weeks, the Barangay Captain had initiated a coastal clean up (which is done once a month) in the whole shoreline area of the Barangay. This could be the reason for the minimal garbage floating in the water near the shore. As observed, no floating plastics could be seen in the mangrove area unlike in Panabo City and Tagum City where much garbage were deposited in the area. In the study of Duncan, et. al (2009) on the microbiological quality of shellfish and shellfish growing waters in Panay, Dumangas (Iloilo Province), the high coliform content of >2,400 MPN/g on shellfish meat in the two sampling sites of the area could be attributed to the residential and commercial activities along the shoreline. Several restaurants were operating around the area. Bacterial contents on the second sampling (usually wet season) increased dramatically in 2 stations. Samples were collected during low tide. In this condition, water movement from upstream carrying domestic and agricultural wastes towards the outlet or mouth of the river could be an explanation for the high coliform content. Fish ponds located in the upstream could have contributed to the high coliform content. It was also observed during sampling that water from the fishponds was being released into the river. Rain experiences from previous days could have also contributed to the high coliform content. In a similar study conducted in Batan, Aklan (Iloilo Province) where the shellfish farms were mostly located along Batan Bay, the coliform in the shellfish was high > 2,400.00 MPN/g. The sampling site was observed to be too close to the shore where residential houses were present, which had absolutely contributed to its high level of coliform content. Further, it was noted that E. coli may be present, or even multiple, in tropical waters not subject to human fecal contamination suggesting that source could have been from wild animals, including birds. Salmonella and Vibrio cholerae were also present, but were only analyzed during the second sampling. Vibrio parahaemolyticus content was at <1000 MPN/g for most of the shellfish, and was a naturally occurring bacterium in sub/tropical marine waters. However, favorable conditions, e.g. warm temperatures, could increase numbers and can subsequently increase coliform contents in the shellfish meat (WHO, 1997). The fecal coliform level in areas near the estuary and those near the coastal houses in Panabo City were 240 MPN and it exceeded the standard set by the American Public Health Association (APHA) which was 230 Most Probable Number (MPN) of coliform. The two areas were contaminated with feces of warm-blooded animals. As per observation, not all residents in the coastal area of Panabo had toilets. Some were found to have been raising domestic animals like pigs, chicken, ducks and dogs in which the wastes are directly thrown into the seawater. Higher level of fecal coliform indicated that the shellfish samples contained pathogens which posed health risk to the consumers. In the mangrove area, the coliform content was 150 MPN which was lower than the set standard of APHA. The wastes present in the mangrove area were not however, of fecal origin, though it was observed that there was much garbage thrown or trapped in the mangrove roots, stem and branches.

In the study of Duncan, et. al (2009) in Negros, another island of Western Visayas, two sites were selected (Hinigaran and Himamayan). Water samples from Hinigaran contained relatively very high fecal coliform during the first and second sampling. Identified stations were located along the river, adjacent to residential houses, which were major sources of domestic wastes such as human sewage and animal feces. Frequent supply of fresh wastes into the river greatly affected the quality of the water. Aside from this, the river was basically crowded with aquaculture operations such as fish pens and traps, which hindered the efficient flow and exchange of water. Land-based aquaculture (fish ponds) was also one of the businesses in the municipality and its waste water was usually discharged into the river during low tide. These activities greatly contributed to the degradation of microbial water quality. The high levels E. coli obtained in shellfish samples could be attributed to the high fecal coliform content in water. It was also observed that the shellfish were exposed to heat (sunlight) during low tide for most shellfish sites. Although the heat may have affected the survival of the organism (shellfish), such condition may also be favorable for the multiplication of bacteria, especially during post-harvest, when subsequent flushing would not be possible. Bacterial contents in the second sampling period for some samples were relatively low and below the recommended safety standards of APHA which is 230 MPN. However, shellfish from the 2 sites consistently showed high E. coli contents. The station was a few meters away from the river bank with a pig cage close by. It was observed that when the cage was cleansed, the waste water
flowed directly into the river. This was aside from the observation that houses were built very close to, even over the river, at this station. *Vibrio cholerae* was present in all samples in the first sampling and had been introduced into the water column through discharges from humans, who in turn may have been affected with a disease brought about by the organism. Many of the species of fecal coliform bacteria are harmful to human health. Their presence in water, however, indicates contamination by the fecal material of humans or other warm blooded organisms. This fecal material may contain pathogens including: Norwalk virus, typhoid, Hepatitis A, cholera, bacterial dysentery. Many pathogens associated with fecal material are discharged into coastal waters. Fecal coliform lives in the intestinal tract of warm-blooded animals, and each person excretes approximately two billion of these bacteria per day. (Tortora, Funke and Case, 2001, Harley, Klein and Prescott, 1996). There are several potential sources of fecal coliform bacteria contamination in estuarine waters. Some of these sources are: improperly maintained septic systems, domestic and wild animals, illegal discharge from boats, sewer overflows (Tortora, Funke and Case, 2001; Harley, Klein and Prescott, 1996). Microbiological pollution in shellfish-growing waters is a common problem in almost all the coastal areas of developing countries. Shellfish-borne infectious diseases are generally transmitted through a fecal-oral route (the shellfish become contaminated by sewage and are eaten by humans). The pathway can be quite circuitous. The cycle usually begins with fecal contamination of the growing waters. Feces deposited on land surfaces can release pathogens into surface waters via storm water runoff or collected wastes can be discharged directly into a waterway. The runoff or discharge may go directly into the growing area or indirectly as is the case with wastes transported by freshwater streams to estuarine or marine waters (Stegeman *et al.*, 2002). In the study of Chun-zi and Huang (1983) on fecal coliform contamination of intertidal bivalves from Hong Kong, they found out that as the ocean becomes a man’s dumping ground, the presence of fecal coliform bacteria in seawater is indicative of pollution by human and domestic effluents. Examining contamination in bivalve species, researchers from the National Bureau of Oceanography, China, collected six species of marine bivalves from six stations in Hong Kong. Among the selected bivalve species were *Perna viridis, Barbatia virescens, Isognomon ephippium, Saccostrea cucullata, S. echinata, and Alectryonella radix*. Bacteriological examination of the samples was accomplished utilizing the ‘Most Probable Number’ (MPN) test. Results indicated a strong relationship between total fecal coliform numbers in bivalves and seawater. The authors also reported higher numbers of coliforms in water samples and bivalves at low tide than those obtained at high tide, evidenced that bivalves concentrated their food to a maximum at ebb tide. The authors concluded that fecal coliform levels in bivalves reliably reflected pollution levels in surrounding seawater.

In Tagum City, the fecal coliform levels in *A. antiquata* examined from the three selected habitats were 23 MPN, >3.0MPN and 9.2 MPN. These values were lower than the standard set by the American Public Health Association (APHA) which was 230 Most Probable Number (MPN) of coliform. These mean that the wastes in the area were not fecal in origin. It was observed that residents in the coastal area have toilets. Some have animals like pigs, chicken, ducks and dogs and they drained their waste not directly into the seawater. They have compost pit as drainage of the waste of their animals like pigs. Low fecal coliform content could have also been attributed to the presence of heavy metal like mercury in Tagum City. In Pantukan, the fecal coliform levels in the shellfish near the estuary and near coastal houses were 93 MPN and >3.0MPN respectively and these values were lower than the standard set by the American Public Health Association (APHA). It was observed that residents in the coastal area also have toilets. Just like in Tagum City the low fecal coliform content could have also been attributed to the presence of heavy metal like mercury. In the mangrove area of Pantukan, the fecal coliform was 240 MPN and was relatively higher than the set standard of APHA. It was observed that pigs and piglets were raised in the mangrove area. Pigs were tied in the mangrove tree and stayed there for the whole day and sometimes during night time. According to one resident, fishermen sometimes deposited their fecal wastes upon passing by the mangrove area before going to fishing. On the other hand, the presence of a heavy metal like mercury in Tagum City and Pantukan could be partly responsible for the low bacterial count of fecal coliform and other bacterial pathogens. Dr. Abarquez (2009) found out that there was a high mercury concentration in *Anadara sp.* from coastal waters of Liboganon Tagum City and Pantukan, Compostela Valley, and that it exceeded the maximum recommended limit of 0.500 ppm commonly allowed for fisheries products in the European community. The mercury concentration in *Anadara sp.* from Cagangohan Panabo City did not exceed the allowable limit of 0.500 ppm. It was also found out in her study that the high mercury concentration in the water samples from Pantukan, Compostela Valley exceeded the DENR Administrative Order (DAO) series (1990) critical standard for the marine water. The mercury concentration in the marine water from Liboganon, Tagum City, did not exceed this critical limit, while the mercury concentration in the marine water from Cagangohan, Panabo City was not detected. The high mercury concentration in the sediments from Pantukan, Compostela Valley exceeded also the critical standard of Environment Canada Toxic Effects Threshold for the Protection of Aquatic life (1.0 mg/kg) while the mercury concentration in the sediments from Cagangohan, Panabo City and that from Liboganon, Tagum City did not exceed this critical standard.

In a study conducted by Pengson (2001), total mercury levels of oysters from Naic and Bacoor (Cavite) were <0.05 ppm and 0.138 ppm, respectively. Fabia (2001) determined mercury levels in mussels from Naic and
Bacoor (Cavite) and found them out to contain <0.05ppm (wet wt) and 0.185ppm (dry wt), respectively. Although the levels were below the permissible level set by WHO (<0.3 ppm total mercury), shellfish has the ability to accumulate it through time and eventually can hazard the consumers. On the other hand, the presence of heavy metals such as mercury and lead in Manila Bay could have been partly responsible for the low bacterial count of fecal coliforms and other bacterial pathogens. Research has shown that microbial inactivation appears to be associated with the action of heavy metals and other substances aside from other biological and physical factors (USFDA-CFSAN, 2001).

Total Colony Count
The total colony count of coliform in Panabo and Tagum City was higher compared to Pantukan. Results mean that the total colony count in A. antiquata specifically in Panabo and Tagum did not differ while in Pantukan, the total colony count was lesser. It was observed that the coastal areas of Panabo City and Tagum City were densely populated of more than 500 and 300 households/families respectively. Beach resorts and cottages existed along the shoreline. Fish cages and fishponds also abound in the area and it was noted that the residents did not dispose their garbage properly. They also raised animals like pigs, dogs, and ducks. All of these most likely have contributed to the higher coliform count in the shellfish obtained from Panabo City and Tagum City. According to Hann (2006), the actual concentration of pathogenic microorganisms in the tissues is dependent upon the rate at which bivalves pump water through the gills; the concentration and physical characteristics of the microbe in the ambient sea water; and sea water temperature, salinity and turbidity. The study of physico-chemical parameters is also important as these factors determine the distribution and composition of bacterial community. The concentrations may also vary temporarily and spatially within habitat (Lindstrom, 1998; Dominik and Hofle, 2002) as well as between habitats (Yannarell and Triplett, 2004; Wu and Hahn, 2006). Environmental parameters do not only influence the size and composition of microbial communities but also the activity and viability of these bacteria. Hence, it is very important to study the distribution of the microbes and try to determine the range of physico-chemical parameters in order to understand the dynamic of microbial communities available in aquatic organism (Jalal et al., 2009). According to Tortora, et al. (2001), there are several potential sources of fecal coliform bacteria contamination of estuarine waters. Some of these sources are: improperly maintained septic systems, domestic and wild animals, illegal discharge from boats, sewer overflows.

Total Colony Count in 3 Selected Habitats
The coliform content in the three habitats of the different stations of Davao Gulf were the same as reflected in the total colony count. “Non-point pollution” was found to be the source of the coliform bacteria in these areas. This was caused by one or many activities that took place near shore. These activities included human habits and lifestyles, and natural events. High numbers of colonies were formed because of the high coliform count in shellfish. Coliform contaminated the seawater as an effect of storm water runoff, poor farming management, waste from pets and wildlife, leaking sewage and failing septic systems (Ditschman et al., 1995).

Length, Weight, Meat Weight ad Total Colony Count of Coliform Relationship
It was found out that length shows a moderate negative correlation to the total colony count of coliform. This explains that the length may not influence the total colony count of coliform. Thus, it does not mean that as the length increases the total number count may also increase or vice versa. The weight and total colony count shows a low or slight negative correlation. This implies that as the weight increases, the total colony count may increase minimally or not at all. Meat weight and total colony count shows a high negative correlation. This means that although Anadara antiquata is having a less meat weight but its total colony count of coliform is high or vice versa. This also implies that small Anadara antiquata can accumulate more microorganisms compared to larger ones. According to Duncan (2009) bivalve mollusks are dependent for growth and condition on phytoplankton and hence upon aquatic nutrient inputs. The primary ‘plant’ nutrients are nitrogen and phosphorus, which are commonly associated with both agricultural and anthropogenic-derived sewage or runoff inputs. Bivalves are well known to mobilize somatic resources in response to breeding cycles or environmental changes, and therefore it is possible that calcification processes are boosted during the wet season, with somatic growth more stable or increased in the dry season. Changes in the size, shape, and ornamentation of the bivalve Anadara from the Kettleman Hills of California were compared with the environmental variables temperature, salinity, and substrate. Geographically within the Pecten zone, the size of Anadara varies directly with salinity gradients. Stratigraphically, the size variations of Anadara correspond directly to salinity and temperature fluctuations through the Pliocene. Populations of smaller-sized Anadara with high juvenile mortality rates are associated with sediments containing a high percentage of fines (JSTOR Journal, 2000).
Conclusion
Coliform content in *Anadara antiquata* from Panabo, Tagum and from two habitats of Pantukan are high and they could pose health risk to the consumers. At present fecal coliform are high in *A. antiquata* from two habitats of Panabo and one habitat of Pantukan. These imply that shellfish from these areas contain the disease causing microorganisms which are harmful to human health.

Recommendations
Safeguard public health through fecal coliform/bacteriological monitoring of the shellfish and their growing waters since these microorganisms are pathogens or disease causing. Conduct Information Education Campaign (IEC), informing the Local Government Units and the public that wastes of both fecal and non-fecal in origin can be accumulated by shellfish *Anadara antiquata*. Conduct another study on the mercury content of *A. antiquata* from Pantukan, Tagum and Panabo. Consumers of *A. antiquata* must employ safety measures (like relaying and depuration) to reduce the microbial load of the shellfish prior to consumption.

References
Abarquez, J. 2009. Mercury Concentration in Selected Fish Species from Davao Gulf. (A Dissertation Study)
ANZECC/ARMCANZ (October 2000) *Australian and New Zealand Guidelines for Fresh and Marine Water Quality.*
Fabia, T.B. 2001. Mercury level determination of green mussels (Perna viridis) from Cavite using flameless atomic adsorption spectrophotometry. BSFT Thesis UPCHE,Diliman Quezon City 52 pp


