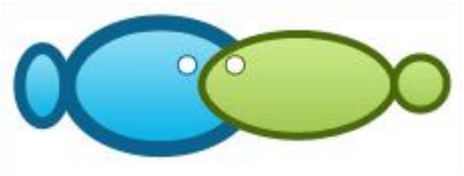

Microbiological quality of oyster (*Crassostrea iredalei*) in selected production areas in Dumangas, Iloilo, Philippines

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Abstract. The microbiological quality of shellfish is dependent on the condition of the surrounding environment. Oysters are filter feeders that can accumulate pathogens from its surrounding waters, which can impose health risks once they are consumed. Microbiological monitoring of this bivalve is essential to ensure the quality and safety of these products. In this study, the microbiological quality of the oyster and the water growing areas in Dumangas, Iloilo, Philippines, was evaluated. The study was limited to the detection of pathogenic bacteria in oyster meat such as *Escherichia coli, Vibrio parahaemolyticus, Vibrio cholerae* and fecal coliforms in water. The result of the study revealed that the count of fecal coliforms present in the growing areas were beyond the standard limit. Likeiwse, *Vibrio cholerae* and *Vibrio parahaemolyticus* were present in all samples. However, *Salmonella* sp. was not detected. This result could be attributed with the condition of the growing environment since the production areas were surrounded by residential houses and fishpond. Based on the findings, it is therefore recommended that the oysters produced in the area should be subjected to depuration and relaying to ensure quality and safety.
Key Words: oyster, *E. coli, Vibrio parahaemolyticus, Vibrio cholerae*.

Introduction. Bivalves feed on organic and inorganic matter, phytoplankton and particles in suspension present in water by means of filtration. Therefore, if the water in the growing areas is contaminated, then the shellfish may accumulate pathogenic microorganisms which may be harmful to the consumer. Environmental factors such as bacterial load of the water and water temperature is closely related to the microbiological quality of bivalves (Simental & Martinez-Urtaza 2008). Bacteria and viruses are naturally present in the environment. Run-off from agricultural areas could be source of pathogenic bacteria and viruses, which would lead to outbreaks of diseases. Urban areas can also be a source of harmful microorganisms. Rural and urbanized development has been identified as a source of fecal coliform contamination along the river system (Campos & Cachola 2007).

Oysters are the second most commercially important marine organism since its consumption has increased considerably over the past decades (FAO 2012). Approximately 4 million tons of oysters are consumed annually and half of them are eaten as raw and ingested as a whole (Fang et al 2015). This leads to the transmission of potentially pathogenic microorganisms to the consumer, a fact that increases the risk of food-borne diseases especially when these molluscs originate from contaminated areas (Pereira et al 2006). Shellfish contamination occurs mainly due to the fact that they are suspension feeders that selectively filter small particles from the surrounding water including pathogenic bacteria (Burkhardt & Calci 2000; Dunphy et al 2006).

In the Philippines, oysters are known to be of poor sanitary quality, hence, they are usually distributed locally (Clague & Almario 1950; Ticar 2015). Studies on depuration had been conducted to resolve issues on the microbiological quality of oyster in the Philippine setting (Gacutan et al 1986; Sorio & Peralta 2017). However, measures

on ensuring safe and wholesome shellfish products have not been adopted. Further, it is important to assess the microbiological quality of shellfish produced in a certain production area. Microbiological evaluation is one of the most significant parameters in determining the viability of an oyster production system that would address public health issues and ecological concerns (Silva Neta et al 2015).

The objective of this study was to assess the microbiological quality of oyster in selected production areas in Dumangas, Iloilo, Philippines and to evaluate the quality of the water-growing area in the selected sampling sites. The study provides baseline information on the microbiological quality of oyster produced in selected production area in Dumangas, Iloilo. The result of the study could be a basis for further studies such as depuration or relaying of the oyster and product development. Information on the quality of oyster is important since these commodities are usually eaten as raw, thus, impose risks to the health of the consumers.

Material and Method

Sampling site. Samples were collected in 3 selected production areas in Dumangas, Iloilo, Philippines (10°47'52.8"N 122°40'25.9"E). The selection of sampling stations (Figure 1) was based on the interview conducted with the Municipal Agriculture Officer in the Municipality of Dumangas, where the area was identified as one of the major producers of oyster in the locality. Ease of transportation was also considered in order to immediately process the samples within 6 hours following the standard sampling protocol for the collection of bivalve and water for microbiological analysis (APHA 1992). All sampling stations are located in a river and are congested with residential houses. Sampling was done every month of June and July 2016. Approximately 15-20 samples were collected in every sampling station for the microbial analysis and at least 30 samples from each sampling station for the morphometric data.

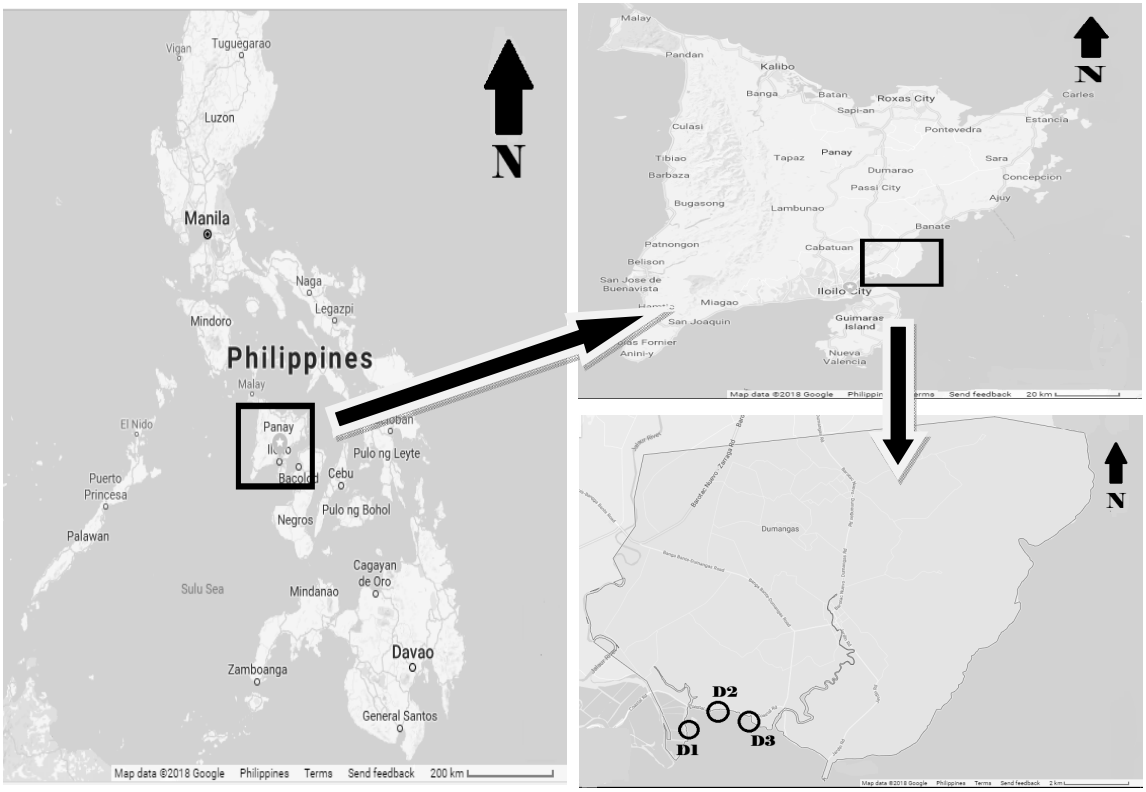

Figure 1. Map of sampling sites in Dumangas, Iloilo, Philippines.

Collection of oyster and water samples. The oysters were randomly collected in the production area. After harvest, the oysters were cleaned and rinsed with distilled water and packed in plastic bag and stored in cooler box with ice. It was then transported from

the site to University of the Philippines Visayas - IFPT laboratory for analysis within 3 hours. The temperature was monitored during transport and maintained at 5-10ºC.

Bivalve morphometrics. Samples of 30 oysters were obtained from each sampling site. The oysters were cleaned and morphometric data were collected: total shell length, width, height, whole weight, flesh weight and the whole weight-flesh weight ratio. The meat was removed from the shell using a knife. Length measurements were taken to the nearest centimeter using Vernier calipers and weights in grams using electronic balance.

Sample preparation for microbial analysis. The meat of the bivalve was removed from its shell using a sterile knife. The intravalvar fluid and meat were transferred aseptically to a sterile Erlenmeyer flask and homogenized using a sterile homogenizer.

Fecal coliforms determination. The water samples were collected in a sterile bottle. Ten (10) mL water sample was transferred into 90 mL 0.1% peptone water. Ten (10) mL of the dilution was transferred into a 5-tube set with double strength lactose broth (LB); 1.0 mL and 0.1 mL portions for 5-tube set of single strength LB and were incubated for 24-48 hours at 35ºC. Durham tubes were contained to all single and double strength LB tubes. Positive tubes that showed gas production were inoculated to EC broth and were incubated for 24-48 hrs in waterbath at 44.5°C. Quantification was determined using the MPN table and was reported as MPN/100 mL sample.

E. coli detection (MPN method). *E. coli* in the samples was determined using the conventional five-tube MPN (most probable number) method. Fifty (50) grams oyster meat sample was homogenized in 100 mL 0.1% peptone. Dilution tubes (up to 10^3) were prepared and 2 mL of each dilution was inoculated into each tube of lauryl tryptose broth. Each tube contained inverted Durham tubes. It was incubated at 35°C for 24 hrs. All tubes that showed turbidity and gas production were inoculated to EC broth and were incubated at 44.5°C in waterbath for 24 hrs. A loopful of sample from positive EC broth were inoculated in Tryptone and incubated for 24 hours at 35ºC. It was then tested for indole production. Quantification was determined using the MPN table and *E. coli* was reported as MPN/100 g sample.

Detection of Salmonella sp. For detection of *Salmonella* sp., 25 g of the sample was homogenized in 225 mL of pre-enrichment broth and was incubated at 35°C for 24 hrs. One (1) mL of the pre-enrichment broth was transferred to tetrathionate broth (TTB) and was incubated for 24 hrs at 35°C. The selective enrichment cultures were streaked on xylose lysine deoxycholate (XLD) agar and were incubated at 35ºC for 24 hours. Typical *Salmonella* sp. colonies were submitted to biochemical screening on triple sugar iron agar (TSI) and IMViC test.

Results and Discussion

Bivalve morphometric data. Morphometric data of the bivalve samples collected from culture sites in Dumangas, Iloilo is shown in Table 1. There were at least 30 samples of oyster collected in every site on the first and second sampling. Bivalves were gathered randomly from each sampling site.

Based on the results, it was observed that the average values in each size parameters greatly vary in every site and sampling period. Also, sample sizes were relatively small compared to the reported measurement of oysters. *C. iredalei* are usually 6-9 cm long and the moderately sized *C. malabonensis* are usually 4-5 cm long (Lovatelli 1988). This could be attributed to the high level of bacterial load in the oyster meat and fecal coliform count in their growing water areas. Condition index of the oyster decreases with increasing microbial load in its tissue. This is due to the decline of the assimilation efficiency of the oyster and it expands considerable energy at high coliform concentration (Jana et al 2014). The condition index (CI) is used to estimate the effect of the different environmental factors on oyster meat quality (Jana et al 2014). Moreover, oyster

condition and gonadal indices decline in areas receiving high levels of bacterial pollution, adjacent to known pollution sources (Scott 1976).

Table 1

Morphometric data of oyster collected from sites in Dumangas, Iloilo

Sampling period	*Sampling site*	*Length (cm)*	*Width (cm)*	*Height (cm)*	*Whole wt. (g)*	*Flesh wt. (g)*	*Flesh yield (%)*
June	D1 (n = 30)	4.77± 0.17	0.96± 0.08	5.87± 0.23	45.67± 2.51	6.67± 0.36	15.13± 0.78
	D2 (n = 30)	4.89± 0.10	0.71± 0.07	5.42± 0.20	54.10± 3.58	7.00± 0.52	13.53± 0.86
	D3 (NT)	NT	NT	NT	NT	NT	NT
July	D1 (n = 30)	3.39± 0.14	0.57± 0.05	5.62± 0.16	48.05± 1.62	7.92± 0.34	16.80± 0.78
	D2 (n = 30)	3.21± 0.13	0.63± 0.07	5.66± 0.17	51.66± 2.41	7.97± 0.49	15.62± 0.75
	D3 (n = 30)	2.70± 0.18	0.71± 0.06	5.50± 0.16	49.25± 2.32	7.88± 0.46	16.16± 0.73

Legend: NT = not tested, n = number of samples.

Water quality analysis. The counts of fecal coliforms in the water samples are shown in Figure 2. During the first sampling, the counts of fecal coliforms in water samples obtained in D1 station were higher than in samples from D2. This result could be due to the fact that D1 station is located in a river near a bridge and is surrounded by many residential houses which may have contributed to the high counts. D2 station is also located in a river surrounded by few residential houses and a fishpond. During the second sampling, fecal coliforms count in water samples obtained from D1 station increased significantly while water samples from D2 showed no changes in value. The microbiological limit for fecal coliforms established by PNS-BAFPS and European Commission for shellfish growing waters is < 230 MPN/100 mL (Table 2). The counts of fecal coliforms in water samples collected in the three sampling stations were much higher than the established limit. The results suggest that the shellfish collected from these oyster growing areas should be depurated or re-laid until they meet category A standard.

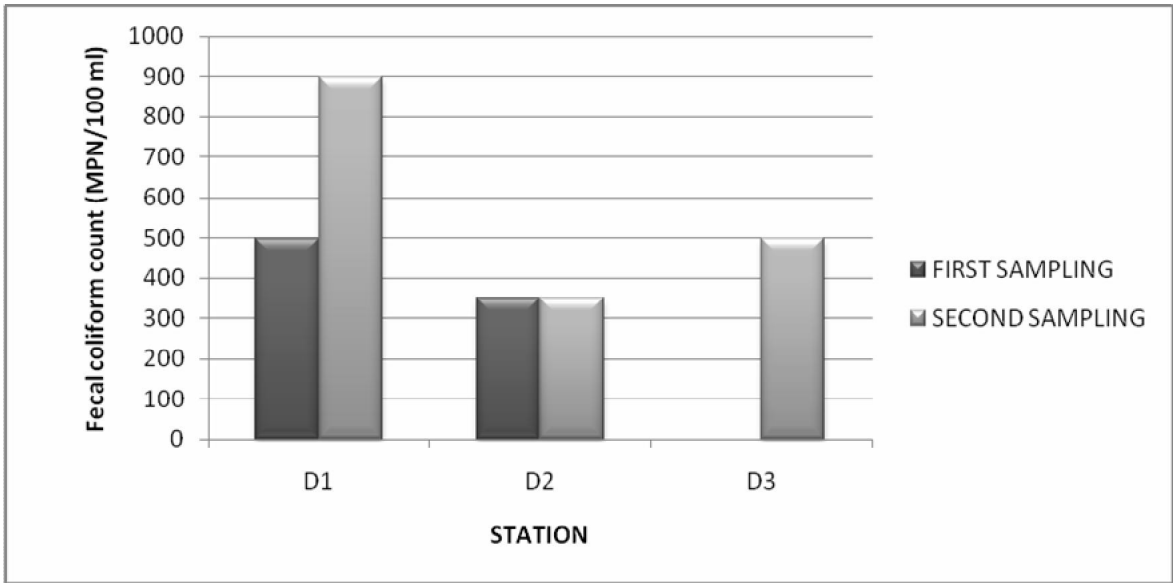

Figure 2. Fecal coliform count of water samples collected from oyster growing areas in Dumangas, Iloilo. Water sample from D3 station was not tested during first sampling.

Table 2

Classification of shellfish harvesting areas (EC 1991; PNS-BAFPS 2011)

	Classification	*Microbiological criteria (cfu/100 g shellfish)*	*Method*
A	No restriction. Shellfish acceptable for immediate consumption.	< 230 *E. coli* or < 300 faecal coliforms; No *Salmonella* sp. in 25 g	5 tube 3 dilutions MPN-test
B	Shellfish must be depurated or re-laid until they meet category A standard.	< 4600 *E. coli* or < 6000 faecal coliforms in 90% samples	5 tube 3 dilutions MPN-test
C	Shellfish must be re-laid over a long period (> 2 months) until they meet category A standard.	< 60,000 faecal coliforms	5 tube 3 dilutions MPN-test

Microbiological quality of oyster. The microbiological quality of oyster collected from three sites in Dumangas, Iloilo is shown in Table 3. Based on microbiological standards for shellfish (Table 4), *E. coli* in bivalves should be equal or less than 230 MPN/100 g. All samples collected from three stations during the first and second sampling contained high *E. coli* count and exceeded the standard limit. The high level of *E. coli* could be due to the high count of fecal coliforms in their growing water areas. It was observed that culture areas were surrounded by residential houses and some of them have no toilet facilities. Duncan et al (2009) reported that *E. coli* is commonly found in human feces and can survive in water, which made *E. coli* as an indicator of faecal contamination. Furthermore, the microbiological quality of oysters is closely related to the sanitary conditions of water, and is affected by untreated wastes from urban or rural areas (Souza et al 2012).

Table 3

Microbiological quality of oyster collected from sites in Dumangas, Iloilo

Station	*Date of sampling*		*Shellfish meat*							
			E. coli (MPN/100 g)		*Salmonella* sp.		*V. cholerae*		*V. parahaemolyticus*	
	(S1)	(S2)	(S1)	(S2)	(S1)	(S2)	(S1)	(S2)	(S1)	(S2)
D1	June 21, 2016	July 11, 2016	700	940	-	-	+	+	>1100	>1100
D2	June 21, 2016	July 11, 2016	460	630	-	-	+	+	>1100	4600
D3	June 21, 2016	July 11, 2016	NT	700	NT	-	NT	+	NT	4600

Legend: NT= not tested, (S1) = first sampling, (S2) = second sampling.

Table 4

Microbiological standards for shellfish

Organism	*Microbial limit*	*Reference*
E. coli	230 MPN/100 g; < 300/100 mL	ICMSF (1986); PNS-BAFPS (2011); EC (2007); FDA (2009)
Salmonella	Absent in 25 g	ICMSF (1986); PNS-BAFPS (2011)
V. cholerae	Absent	ICMSF (1986); FDA (2009)
V. parahaemolyticus	< 100 MPN/g; 100 cfu/g	ICMSF (1986)

The most important enteric pathogens from water polluted with human and/or animal residues include *Salmonella* sp. Oyster can filter seawater at a rate of 10 liters/hour, thus removing microorganisms from the surrounding water into the mollusk leading to infectious diseases such as *Salmonellosis* (Kfir et al 1993). Based on standards for shellfish, *Salmonella* sp. should be absent in 25 g sample. It is expected that *Salmonella*

sp. may be present in waters with high level of fecal coliforms. However, *Salmonella* sp. were absent in all samples during first and second sampling, although the fecal coliforms in the growing waters were recorded higher than the standard limit. The result of this study conforms to the report of Brands et al (2005) that there is no correlation between the number of fecal coliforms and *Salmonella* isolates. However, the result of this study contradicts to the report of Muniain-Mujika et al (2003) showing no evidence of *Salmonella* if fecal coliforms were absent.

The presence of *V. cholerae* in shellfish was also tested. The result revealed that all samples from three stations during first and second sampling were positive in *V. cholerae*. This bacterium inhabits aquatic environments and water play a significant role in its transmission and epidemiology of this disease leading to outbreaks at endemic, epidemic, and pandemic levels (Goel et al 2010). Likewise, *V. parahaemolyticus* was also present in all samples. This bacterium is naturally present in marine environment. According to microbiological standards for shellfish, level of *V. parahaemolyticus* should be < 100 MPN/g or 100 cfu/g. Levels of *V. parahaemolyticus* of > 1000 cfu/g are considered potentially hazardous (ICMSF 1986). Based on the results, the level of *V. parahaemolyticus* in all samples was beyond the standard limit. It is therefore suggested that the shellfish collected in the site should first undergo relaying or depuration.

Conclusions. The microbiological quality of the oyster produced in the study area was generally low. The presence of pathogenic bacteria in the meat was beyond the standard limit. Fecal coliform counts in growing waters were also in an unacceptable range. Bivalve molluscan shellfish are effective carrier of pathogenic bacteria from its surrounding waters. Since oysters are frequently consumed as raw, the consumption of these bivalves may impose health risks to the consumers. As such, controlling fecal pollution in the area is a must in order to produce clean and safe oysters. It is important to employ safety measures such as depuration or relaying to minimize the bacterial load in oysters prior to consumption. Overall, the study provided baseline information on the quality of oyster produced in the area which may be needed for further studies.

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