Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 08, pp.56304-56308, August, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

MICROBIOLOGICAL QUALITY OF OYSTER (*Crassostrea iredalei*) IN SELECTED PRODUCTION AREAS IN DUMANGAS, ILOILO, PHILIPPINES

[1,*]Jerson C. Sorio and [2]Jose P. Peralta

[1]College of Fisheries and Marine Sciences, Samar State University Mercedes Campus, Catbalogan, Samar, Philippines

[2]Institute of Fish Processing Technology, University of the Philippines Visayas, Miagao, Iloilo, Philippines

ARTICLE INFO

Article History:
Received 27th May, 2017
Received in revised form
14th June, 2017
Accepted 12th July, 2017
Published online 31st August, 2017

Key words:

Oyster,
E. coli,
Vibrio parahaemolyticus,
Vibrio cholera.

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 *E. coli, Vibrio parahaemolyticus, Vibrio cholera* 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 cholera* and *Vibrio parahaemolyticus* were present in all samples. However, *Salmonella* 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.

Citation: Jerson C. Sorio and Jose P. Peralta, 2017. "Microbiological quality of oyster (*Crassostrea iredalei*) in selected production areas in Dumangas, Iloilo, Philippines", *International Journal of Current Research*, 9, (08), 56304-56308.

INTRODUCTION

Bivalves feed on organic and inorganic matter, phytoplankton and particles in suspension present in water by means of filtration. Consequently, if the water in which they are grown is polluted, then the shellfish may concentrate microbes or chemicals which may be injurious to the consumer. The microbiological quality of bivalves is closely related to the aquatic habitat that varies with factors such as environmental conditions, bacterial load of the water, and water temperature (Simental and Martinez, 2008). Bacteria and viruses are naturally present in the environment. Run-off from agricultural areas where manure is either generated or spread on fields can be a source of bacteria and viruses, some of which may be pathogenic, leading to outbreaks of disease. Urban areas can also be a source of pathogenic bacteria and viruses. Rural and urbanized development has also been indicated as a source of fecal coliform contamination along the river system (Campos and Cachola 2007). Oysters are usually consumed raw and in this case the molluscs are ingested as a whole. 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 or are handled under precarious hygiene-sanitary conditions. Contamination of bivalve shellfish occurs mainly because they are suspension feeders that selectively filter small particles of phytoplankton, zooplankton, viruses, bacteria and inorganic matter from the surrounding water (Burkhardt & Calci, 2000; Dunphy *et al*., 2006). Oysters are known to be of poor sanitary quality. The report on quality has remained significant compared to the total exports in the world market, thus, they are usually distributed locally. Measures have not been adopted to ensure safety and wholesomeness of the shellfish products. The consumption of raw and insufficiently cooked bivalve mollusk has frequently caused outbreaks of gastroenteritis or illness associated with typhoid and paratyphoid fevers. 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

******Corresponding author:* **Jerson C. Sorio,**
College of Fisheries and Marine Sciences, Samar State University Mercedes Campus, Catbalogan, Samar, Philippines.

are usually eaten as raw, thus, imposes risks to the health of the consumers.

MATERIALS AND METHODS

Sampling site

Samples were collected in 3 selected production areas in Dumangas, Iloilo, Philippines (see Figure 1). The selection of sampling stations 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. Approximately 15-20 samples were collected in every sampling station for the microbial analysis and at least 30 samples for the morphometric data.

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 ml water sample was transferred into 90 ml 0.1% peptone water. Ten 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 35 ºC. 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.

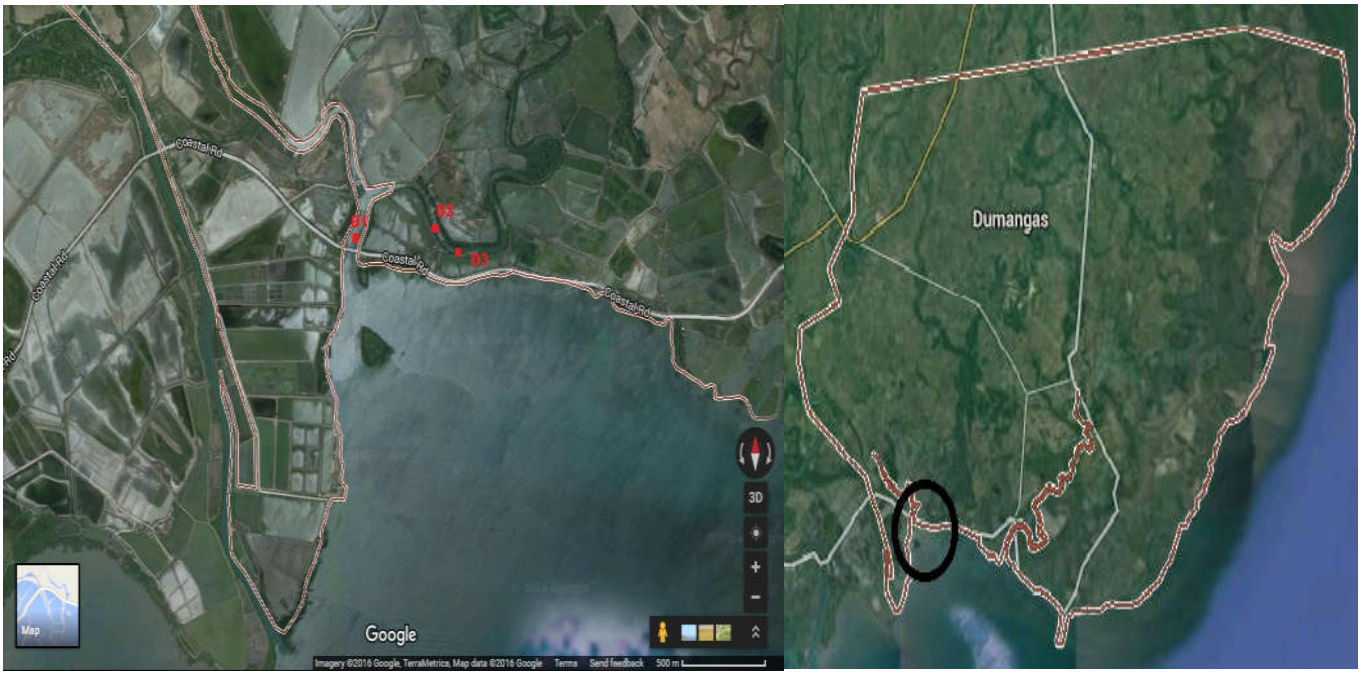

Figure 1. Map of sampling sites in Dumangas, Iloilo

Collection of oyster and water samples

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 5-10 ºC.

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.

E.coli Detection (MPN method)

E. coli in the samples was determined using the conventional five-tube MPN (most probable number) method. Fifty 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 35 ºC. It was then tested for indole production. Quantification was determined using the MPN table and *E. coli* was reported as MPN / 100g sample.

Detection of *Salmonella*

For detection of *Salmonella*, 25 g of the sample was homogenized in 225 mL of pre-enrichment broth and was

incubated at 35°C for 24 hrs. One 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 sites 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 (FAO, 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 oyster decreases with increase in the microbial load in tissue of oyster because of markedly decline the assimilation efficiency of oyster and also oyster would expand considerable energy at high coliform concentration. The condition index (CI) is used to estimate the effect that different environmental factors on oyster meat quality (Harekrishna *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.

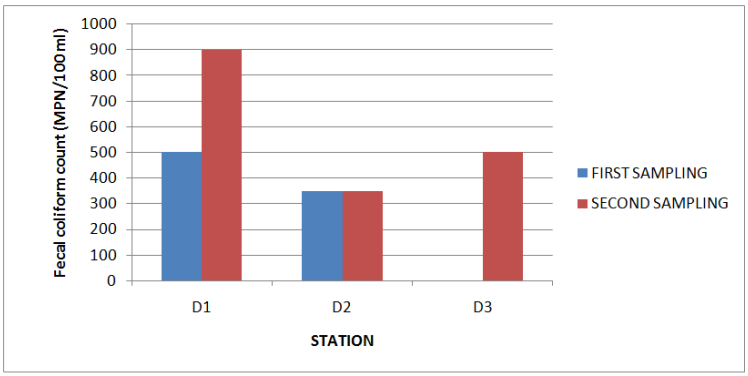

Figure 2. Fecal coliform count of water samples collected from oyster growing areas in Dumangas, Iloilo. Water sample from D3 station was not tested during first sampling

Table 2. Classification of shellfish harvesting areas (PNS-BAFPS, 2011; EC, 1991)

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* 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

Table 3. Microbiological quality of oyster collected from sites in Dumangas, Iloilo

Station	Date of sampling		SHELLFISH MEAT							
			E. coli (MPN/100g)		Salmonella		*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	< 20 MPN/g 16 cfu/ g 11 MPN/g 230 MPN / 100g <300 / 100 ml 4.0 cfu/ g	Singapore Guideline, 1995 ICMSF, 1986 FAO No 211 sec 8, BFAR USFDA Guideline, 1996 Council Directive 79/923/EEC SEAFDEC,1998
Salmonella	Absent in 25 g	ICMSF, 1986; FAO No 211 sec 8, BFAR ; USFDA Guideline, 1996
V. cholera	Absent	USFDA, Guideline, 1996
V. parahaemolyticus	<100 MPN /g 100 cfu/g	Singapore Guideline, 1995; ICMSF, 1986

Water Quality Analysis

The counts of fecal coliforms in the water samples are shown in Figure 1. 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/100ml (see Table 3). 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.

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 (see Table 4), *E. coli* in bivalves should be equal or less than 230 MPN/100g. All samples collected from three stations from 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. *E. coli* is commonly found in human feces and has the characteristics to survive in water which made *E. coli* as an indicator of faecal contamination (Duncan *et al*., 2009). The most important enteric pathogens from water polluted with human and/or animal residues include *Salmonella spp*. According to the International Commission on Microbiological Specifications for Foods (Kfir *et al*, 1993), a single oyster can filter up to 10 liters of water/hour, thus removing microorganisms and pollutants from the water into the mollusk leading to infectious diseases including *Salmonellosis* (Halliday *et al*, 1991). Based on standards for shellfish, *Salmonella* should be absent in 25 g sample. It is expected that *Salmonella* may be present in waters with high level of fecal coliforms. However, *Salmonella* 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 contradicted to the report of Hood *et al*. (2004), which they have studied the relation among fecal coliforms and *Salmonella spp*. The results from that study indicated that samples negative for fecal coliforms might be a good indicator of the absence of *Salmonella spp*. The presence of *Vibrio 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, *Vibrio 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.

Conclusion

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.

REFERENCES

American Public Health Association (APHA). Compendium of methods for the microbiological examination of foods. C. V anderzant and D.F. Splittstoesser eds. Washington D.C. 1992.

Bastardo Luz Betina Villalobos and Luis Elguezabal Aristizabal2. Microbiological quality of the bivalve *Pinctada imbricata* commercialized in Cumana, Venezuela. *Acta Científica Venezolana, 52: 55–61, 2001*

Boehs. 2015. Microbiological quality and bacterial diversity of the tropical oyster *Crassostrea rhizophorae* in a monitored farming system and from natural stocks. *Genetics and Molecular Research,* 14 (4): 15754-15768.

Brandão, M.A.R., Lopes, A.T.S., Silva Neta, M.T., Albuquerque, G.R; Boehs, G, Maciel, B.M. Microbiological quality of oysters (*crassostrea rhizophorae*) intended for human consumption from south coast of bahia. Programa de Pós-graduação em Ciência Animal, UESC – Universidade Estadual de Santa Cruz

(Campus Soane Nazaré de Andrade, Rodovia Jorge Amado, Km 16, Bairro Salobrinho, Ilhéus – BA).

Breisch, L. and V.S. Kennedy. 1980. A selected bibliography of worldwide oyster literature. University of Maryland Sea Grant Publ. No. UM-SG-TS-80-11.

Bureau of Agricultural Statistics Fisheries Situationer January-December 2012.

Burkhardt,W., III, & Calci, K. R. 2000. Selective accumulation may account for shellfishassociated viral illness. Applied and Environmental Microbiology, 66(4), 1375e1378.

Campos CJ, Cachola R. 2007. "Faecal Coliforms in Bivalve Harvesting Areas of the Alvor Lagoon (Southern Portugal): Influence of Seasonal Variability and Urban Development". *Environmental Monitoring and Assessment* 133(1-3), 314.

Carreon, J.A. 1969. The malacology of Philippine oysters of the genus 'Crassostrea' and a review of their shell characters. Proc. Nat. Shellfish. Assoc. 59: 104-115.

Duncan, P. F., Andalecio, M. N., Peralta, E., Laureta, L., Hidalgo, A. and Napata, R. 2009. Evaluation of production technology, product quality and market potential for the development of bivalve mollusk aquaculture in the Philippines . ACIAR GPO Box 1571 Canberra ACT 2601 Australia. ISBN: 978 921615412.

Dunphy, B. J., Hall, J. A., Jeffs, A. G., &Wells, R. M. G. (2006). Selective particle

EC (European Commision) Council Directive 79/923/EEC of July 1991 as cited by Huss, H *et al*. 2003. In; Assessment and Management of Seafood Safety and Quality FAO Fisheries Technical Paper No 444, 230 p

FAO. 1984. Yearbook of fishery statistics, catches and landings, 1983. FA0 Fish. Ser. V. 56.

Feeding by the Chilean oyster, Ostrea chilensis; implications for nursery culture and broodstock conditioning. Aquaculture, 261(2), 594e602.

Food and Agriculture Organization of the United Nations. 2008. FAO Fisheries and Aquaculture Species Fact Sheets

Galtsoff, P.S. 1964. The American oyster. U.S. Fish Wildl. Serv. Fish Bull. V. 64.

Goel AK, Jain M, Kumar P, Jiang SC. 2010. Molecular characterization of *Vibrio cholerae* outbreak strains with altered El Tor biotype from southern India. *World J. Microbiol. Biotechnol*., 26(2):281-287.

Huss, H.H.; Ababouch, L.; and Gram, L. 2003. Assessment and management of seafood quality and safety. FAO fisheries technical paper 444. Food and Agricultural Organization (FAO) of the United Nations (UN), Rome, Italy.

ICMSF 1986. Microorganisms in Foods 5: Microbiological Specifications of Food Pathogens Toronto: University of Toronto Press

Kfir, R., Burger, J. S. and Idema, G. K., 1993. Detection of *Salmonella* in shellfish grown in polluted sea water. Water Science and Technology, 27 (3-4): 41-44.

KOLM Hedda Elisabeth and ABSHER, Theresinha Monteiro. Bacterial density and coliform organisms in waters and oysters of paranaguá estuarine complex, Paraná, Brazil. *B. Inst. Pesca*, São Paulo, *34*(1): 49 - 59, 2008

Lee, J-K., Jung, D-W, Eom, S-Y, Oh, S-W., Kim, Y., Kwak, H-S, Kim. 2008. Occurrence of Vibrio parahaemolyticus in oysters from Korean retail outlets. Food Control 19: 990–994.

Neta M.T. Silva, B.M. Maciel, A.T.S. Lopes, E.L.S. Marques, R.P. Rezende and G.

Peralta Ernestina M. and Andalecio Merlina N. 2011. Microbiological Quality of Oyster (*Crassostrea sp*) and Mussel (*Perna viridis*) in Selected Growing Areas in Western Visayas, Philippines. *Phil J. of Nat. Sci., 16 1-8.*

Quayle, D.Q 1975. Tropical oyster culture--a selected bibliography. IDRC052e. International Development Research Centre, Ottawa, Canada.

Samonte, G. 1993. Socio-economics Study of oyster and mussel farming in Western Visayas, Philippines. ICLARM, Makati, Metro Manila, Philippines.204pp.

Scott G.I. 1976. Oyster condition index as a monitor of biological pollution in South Carolina coastal waters: A pilot study, University of South Carolina, Marine Science Program, p 101.

Simental L, Martinez-Urtaza J. 2008. "Climate patterns governing the presence and permance of salmonella in coastal areas of Bahia de Todos Santos, Mexico" *Appl Environ Microbiol* 74(19), 5918-24.

Ukpong, E.; and Utuk, O. 1992. Microbiological quality of Egaria radiate in Cross River System. Book of Abstracts, Nigerian Institute of Food Science and Technology, Lagos State, Nigeria, pp. 15-6.

Utrarachkij F, Intalapaporn K, Kumkrong K, Kittigul L. 2006. "Microbiological Quality of Raw Oysters from Local Markets in Bangkok" *Journal of Public Health,* 36(2), 112-122

****