Journal of Science, Technology, and Humanities : 2010, Vol. 8, No. 2, pp. 81-89

BACTERIAL CONTAMINATION IN OYSTER MEAT AND GROW-OUT WATER IN HUANG NAM KAO OYSTER FARMING COMMUNITY, TRAT PROVINCE, THAILAND

Chaninthorn Rattasumpun[*1,4], Subuntith Nimrat[2], Verapong Vuthiphandchai[3], Kashane Chalermwat[3], and Pichan Sawangwong1.

[1]*Center of Excellence on Environmental Health, Toxicology and Management of Chemicals, Burapha University, Chon Buri 20131, Thailand.*
[2]*Department of Microbiology, Faculty of Science, Burapha University, Chon Buri 20131, Thailand.*
[3]*Department of Aquatic Science, Faculty of Science, Burapha University, Chon Buri 20131, Thailand.*
[4]*(Present Address). Department of Environmental Engineering, College of Engineering, Rangsit University, Pathum Thani 12000, Thailand.*

ABSTRACT

The purpose of the study was to investigate *Vibrio parahaemolyticus*, *Escherichia coli*, and fecal coliform contamination in farm raised *Saccostrea cucullata* oyster meat and grow-out water. Oysters and grow-out water samples were collected from Huang Num Khao Village, Trat province, Thailand, from January 2004 to January 2005. The most probable number (MPN) was used to determine *E. coli* and fecal coliform contamination, while the spread plate method was utilized for *V. parahaemolyticus* enumeration. From the study *V. parahaemolyticus*, *E. coli*, and fecal coliform contamination in oyster meat was between 0.00-1.11 CFU/g, 0.00-5.86 MPN/g, and 0.00-69.35 MPN/g, respectively. Meanwhile, the number of *V. parahaemolyticus*, *E. coli*, and fecal coliforms in grow-out water was found to be between 0.00-0.37 CFU/ml, 0.00-2.99 MPN/100 ml, and 0.00-8.18 MPN/100ml, respectively. When indicator bacteria and pathogens were found in the water column, their numbers were amplified in corresponding oyster meat samples. The physicochemical characteristics of farm area grow-out water quality (temperature, salinity, dissolved oxygen, pH, turbidity, transparency, total dissolved solids, and conductivity) was within government standards set for coastal aquaculture. We concluded that although indicator bacteria and *V. parahaemolyticus* numbers were low in water samples, their numbers were consistently higher in corresponding oyster meat samples. This phenomenon demonstrates the ability of oysters to bio-accumulate both indicator bacteria and pathogen. Therefore, water samples alone may not be sufficient for assessment of bacterial contamination in oysters. The inclusion of oyster meat samples and testing is needed to assess the risks posed by bacterial pathogens responsible for human gastrointestinal illness.

Key words: *Vibrio parahaemolyticus*, *E. coli*, coliform bacteria, water quality, oyster.

*Corresponding author. E-mail address: Chaninthorn Rattasumpun<tao232000@yahoo.com>

INTRODUCTION

Trat province is located on the eastern seaboard Thailand and is approximately 315 km southeast from Bangkok. This province is bordered by Cambodia and is well known for its tourism industry that services both international and local tourists. Most tourists come to visit the eastern seaboard provinces' off-shore islands, coastal resorts and seafood restaurants (Szuster et al., 2008). Consequently, a variety of locally raised and harvested seafood products including shellfish are offered in the area. In this province, oysters are mostly grown in small-scale commercial or co-operative farms and are shucked locally for seafood restaurants and food stalls. Oyster aquaculture industry is rapidly increasing during the past several years has resulted in Trat province being one of the leading oyster production areas in terms of both landings and value of farmed oysters (DOF, 2005). Because of the recent expanding tourism industry along the eastern seaboard, the number of farms has increased in conjunction with the development of coastal land into resorts and residential areas. The growing local and tourist population has raised questions pertaining to food safety especially for molluscan shellfish (Chalermwat et al., 2003). The contamination of seafood with human pathogenic organisms has become more of particular interest to fisheries authorities resulting in attempts to create awareness of the potential risks associated with the consumption of bacteria-contaminated seafood that might cause human gastrointestinal illness (Hanak et al., 2002; Utrarachkij et al., 2006).

Pathogenic bacteria are natural inhabitants of marine aquatic environment in both temperate and tropical regions in Thailand (Bordalo et al., 2002). Previous studies have reported a positive correlation between water temperature and the number of human pathogenic *Vibrio* and the number of reported infections (Dalsgaard, 1998). However, in many cases the occurrences of pathogens do not generally correlate with traditional bacterial indicator organisms of fecal pollution (Utrarachkij et al., 2006).

This study was initiated as technical aid to a new cooperative oyster farming industry operated by the Huang Nam Khao community in Trat province. Because of the popularity of raw oyster consumption at coastal tourist destinations in this area, we investigate and compare the presence of the pathogens, *Vibrio parahaemolyticus and Escherichia coli* in relation to the presence of traditional water quality indicator bacteria (fecal coliforms) from two sources, oyster tissue and surrounding oyster farm water.

MATERIALS AND METHODS

Collection of oyster and grow-out water samples

A total of 650 live oysters (*Saccostrea cucullata*) were collected from the Huang Nam Kao community in Trat province (Figure 1) from January 2004 to January 2005. Ten oysters were collected from each of five sampling points located at the corners of a rectangular area of approximately 50 x 50 meters and at the center of the plot in the study farm. The oyster samples were then kept in sealed sterile plastic bags, kept on ice, transported back to Burapha University and processed immediately upon arrival. All monthly oyster samples were collected between 11.00-14.00 hours and transported in an ice box to the laboratory within four hours after collection. After arrival at the Department of Microbiology, the oysters were immediately scrubbed with a brush under tap water rinsed with sterile water and shucked aseptically into sterile Stomacher® bags. Each oyster sample containing 10 oysters was then coarsely macerated in a Stomacher® machine for one minute.

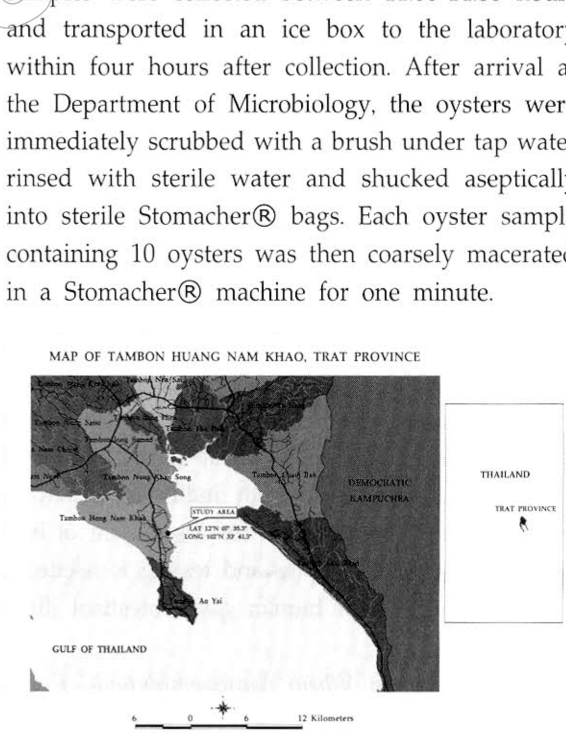

Figure 1. Map of Ban Huang Nam Khao, Trat province.

The in-situ examination of grow-out water quality was conducted using a YSI probe (model 660 Multi-probe, YSI incorporated, Ohio, USA) and to determine temperature, salinity, dissolved oxygen (DO), pH, turbidity, transparency, total dissolved solids (TDS), and conductivity. A Secci disk was used to determine water transparency at the time of collection. At the time of data collection the general weather conditions, air temperature and tide level were recorded. In conjunction with the oyster samples, nine grow-out water samples (five points corresponding to the oyster samples and four additional samples corresponding to the water channel adjacent to the oyster plot) at the same location were collected in sterile glass bottles at a depth of approximately 50 centimeters from the surface. The water samples were kept on ice and transported back to the laboratory in an ice box.

In order to detect the presence of *V. parahaemolyticus*, in oyster tissue and grow-out water, we followed protocols outlined in the Standard Methods for Examination of Water and Wastewater manual (APHA, 1992). The values for most probable number (MPN) of bacteria of fecal coliforms and *E. coli* in oyster samples were determined using the multiple tube fermentation method following protocols detailed by Hitchins et al. (1998). Fecal coliform numbers were determined using lactose broth as the presumptive medium, and confirmed by lactose fermentation with gas production at 44.5 °C in *E. coli* (EC) broth. Five-tube series each of five dilutions ranging from 10^{-1} to 10^{-5} were assayed. The MPN for fecal coliforms was calculated from the number of EC broth Durham tubes that contained gas with the assistance of an MPN table. The presence of *E. coli* in EC broth tubes was examined by sub-cultivation on MacConkey (MC) agar. The suspected colonies on MC agar were picked up, and tested for several biochemical reactions, i.e., reactions on Triple Sugar Ion (TSI), indole tests, methyl red-Voges Proskauer medium (MR-VP test) and citrate utility medium. The MPN for *E. coli* was interpreted from the number of EC broth tubes that contained *E. coli* by means of an MPN table.

Statistical analysis

The number of *V. parahaemolyticus, E. coli,* and fecal coliforms contamination in oyster meat and grow-out water was presented by using frequency and the mean. The physiochemical characteristics of farm area grow-out water quality comprised of temperature, salinity, dissolved oxygen, pH, turbidity, transparency, total dissolved solids, and conductivity were also presented by frequency and the mean.

RESULTS

Bacterial contamination in oyster meat and grow-out water

The average amount of bacterial contamination consisted of *V. parahaemolyticus, E. coli*, and fecal coliforms in each month from January 2004 to January 2005 (13 months) is shown in Figure 2. The bacteria numbers are averaged numbers from five sample points for oyster meat and nine sample points for grow-out water. *V. parahaemolyticus, E. coli*, and fecal coliforms were found in oyster meat and in grow-out water throughout the experimental period, whereas *V. parahaemolyticus* was found in oyster meat in May, July, and October 2004 (O.37 CFU/g, 1.11 CFU/g, and 0.90 CFU/g, respectively) and in grow-out water only in May and October 2004 (0.40 CFU/ml and 0.37 CFU/ml, respectively). The rest of the oyster and grow-out water samples were not contaminated with *V. parahaemolyticus*. The highest average number of *V. parahaemolyticus* in oyster meat was 1.11 CFU/g in July 2004 and the corresponding value for grow-out water was 0.37 CFU/ml in October 2004.

The presence of *E. coli* was recorded in July 2004 both for oyster meat and in grow-out water, with average values of 5.86 MPN/g and 2.99 MPN/100 ml, respectively. The highest average number of fecal coliforms was found in May 2004 in both oyster meat and grow-out water with values recorded as 69.35 MPN/g and 8.18 MPN/100 ml, respectively.

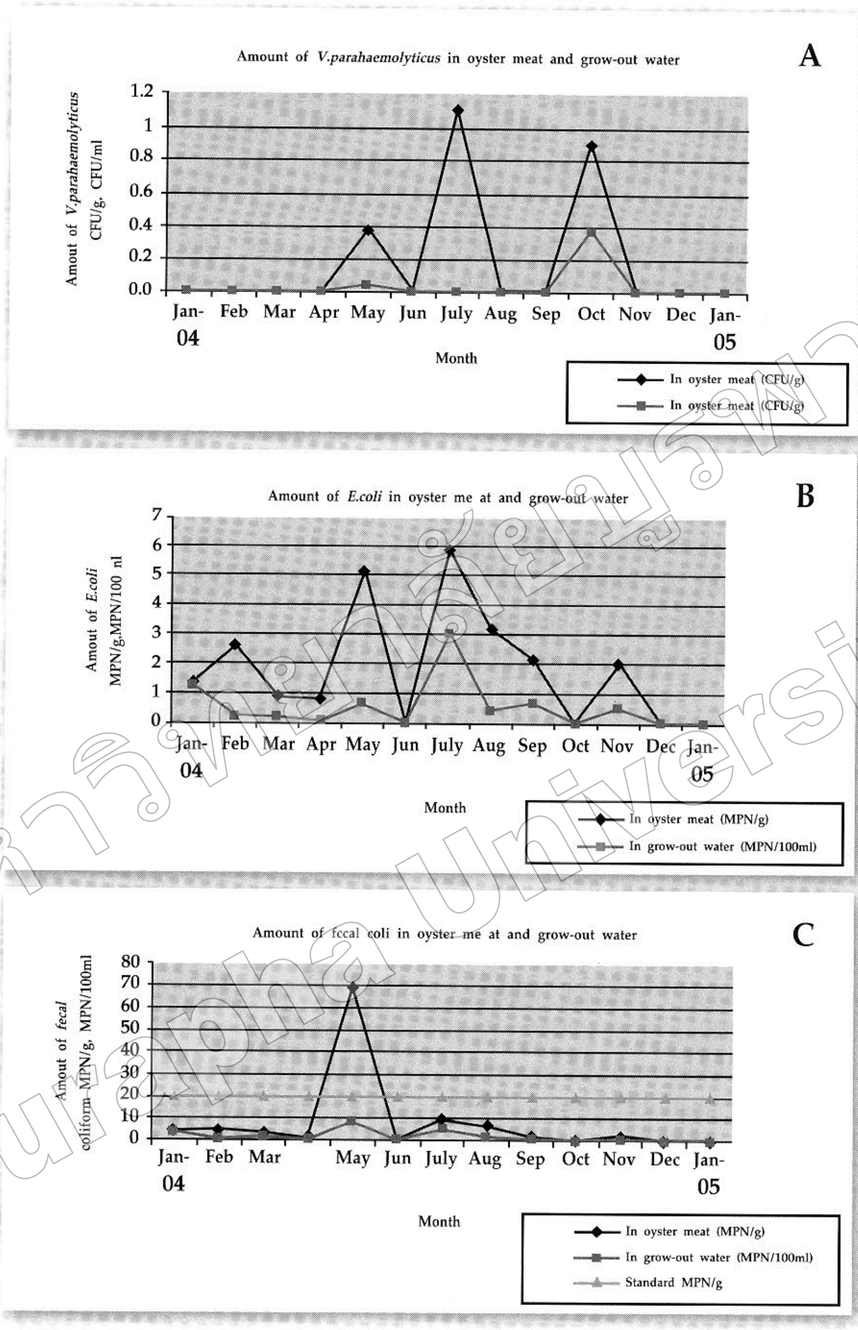

Figure 2. Bacteriological analysis and detection rates of *Vibrio parahaemolyticus*, *E. coli*, and fecal coliforms in oyster meat and in grow-out water from January 2004 to January 2005

Grow-out water quality

Grow-out water quality measurements are summarized in Figure 3. Temperature of grow-out water ranged from 24.4- 34.5 °C (Mean = 30.3°C). The lowest temperature was in December 2004 and the highest temperature was in April 2004. The salinity of grow-out water ranged from 3.5-29.5 psu (Mean = 21.9 psu). The lowest recorded salinity was observed in September 2004 and the highest salinity was found in April 2004. The DO values of grow-out water ranged from of 4.33-9.68 mg/L (Mean = 6.96 mg/L). The lowest recorded DO value was in April 2004and the highest was in March 2004. The pH of grow-out water ranged from 7.49-8.40 (Mean

= 8.00). The lowest pH was recorded in January 2005 and the highest pH was recorded in September 2004. The turbidity of grow-out water ranged from 9.81-57.99 NTU (Mean = 22.89 NTU). The lowest turbidity was found in January 2004 and the highest turbidity was in August 2004. The transparency of grow-out water ranged from 0.45 - 1.03 m. (Mean = 0.70 m.). The lowest transparency was in August 2004 and the highest transparency was in December 2004. The TDS of grow-out water ranged from 0.001-0.248 mg/L (Mean = 0.039 mg/L). The lowest TDS value was in August 2004 and the highest TDS value was in November 2004. Conductivity of grow-out water ranged from 1.70-53.82 ms/cm (Mean = 37.89 ms/cm). The lowest conductivity was recorded in August 2004 and the highest conductivity was recorded in April 2004.

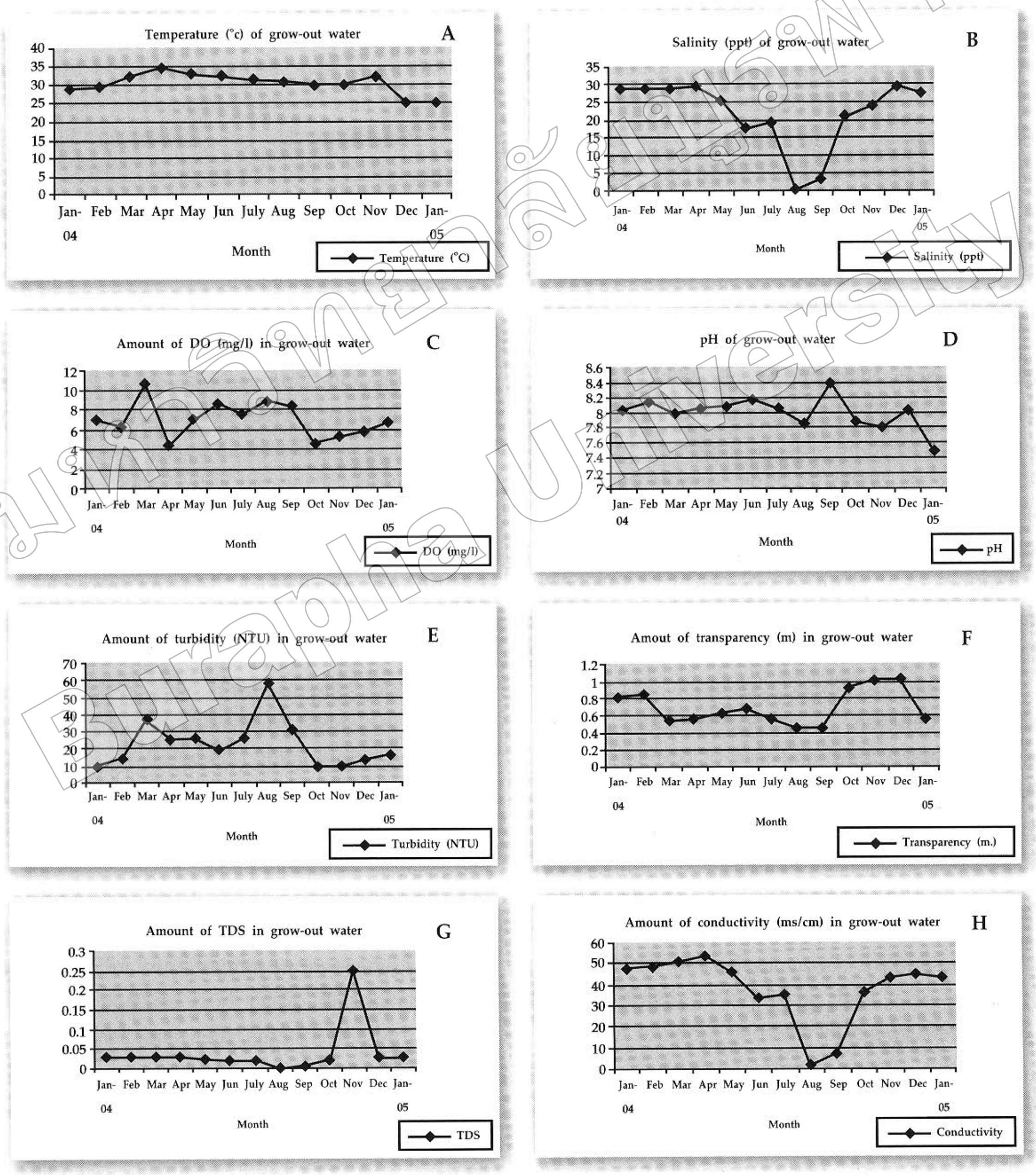

Figure 3. Grow-out water quality from January 2004 to January 2005.

DISCUSSION

Shellfish are filter feeders that may accumulate human pathogens from unsanitary aquatic environments (Kueh and Chan, 1985; Rippey, 1994; Sincero et al., 2006). For seafood consumers the transmission of microbes causing gastrointestinal tract infections may result from the consumption of fecal contaminated shellfish. In Thailand, gastrointestinal illness caused by enteric bacteria has increasingly become an important public health concern particularly with consumers of raw oysters (Utrarachkij et al., 2006). Globally, gastroenteritis outbreaks associated to oyster consumption have been reported in many countries (Lozano-Leon et al., 2003; Terajima et al., 2004; McLaughlin et al., 2005), and in many cases the outbreaks can also be linked to the consumption of raw oysters, which were contaminated by *V. parahaemolyticus* (Lozano-Leon et al., 2003).

V. parahaemolyticus is a gram-negative, halophilic bacterium that occurs naturally in estuarine environment worldwide. Its densities in the environment and seafood can vary greatly by season (DePaola et al., 2003), location, sample type, fecal pollution, and analytical methodology (Lee et al., 2006). In the United States, seasonal and geographical distributions of *V. parahaemolyticus* were related to water temperature, with highest densities in samples collected in the spring and the summer (DePaola et al., 2003). They also found that there was positive correlation between *V. parahaemolyticus* density and water temperature and lack of correlation with salinity, aerobic plate count, and fecal coliform density. The correlation between *V. parahaemolyticus* density and temperature in the study parallels that observed in clinical investigations on the timing of *Vibrio* infection in patients (Kelly and Stroh, 1988). Temperature seems to be the major factor in both seasonal and geographical distribution of *V. parahaemolyticus* in shellfish-growing areas.

In this study, *V. parahaemolyticus, E. coli*, and fecal coliforms in oyster meat and in grow-out water were determined in several months during the 13 month study period. Generally, when *V. parahaemolyticus, E. coli*, and fecal coliforms were found in oyster meat, they would be also found in grow-out water. The amounts of *V. parahaemolyticus, E. coli* and fecal coliforms found in oyster meat were higher than those in grow-out water demonstrating the ability of *S. cucullata* to accumulate pathogens that may be present in their environment. DePaola et al. (1990) reported that the mean *V. parahaemolyticus* density was more than 100 times greater in oyster meat than in grow-out water, and that the density of fecal coliforms was approximately 10 times higher than in sea water. Microorganisms ingested by oysters are not necessarily digested or killed and may remain viable for extended periods within the oyster gut (Rowse and Fleet 1982, Timoney et al., 1984; Wang et al., 2010).

Fecal coliforms in oyster meat levels in certain months were higher than the standard level (< 20 MPN/g). When comparing the amount of bacterial contamination in oyster meat and grow-out water with earlier studies in other parts of Thailand (Gannarong and Rattanachote, 1997) the numbers reported in Trat are substantially lower. The high number of fecal coliforms in oyster samples collected in May might correspond with bioaccumulation and the beginning of rainfall following a long dry season. Fecal coliforms levels are generally required for the classification of shellfish growing areas in order to protect humans from consuming contaminated shellfish.

Lower salinities during such periods may also favor the prolonged survival of *V. parahaemolyticus* in marine environments (Lee et al., 2006). However, on some occasions, levels of *V. parahaemolyticus* appear to fluctuate independently of temperature and salinity (DePaola et al., 2003). It is possible that the oyster, as a living host, may have some contribution to the variability in *V. parahaemolyticus* counts independent of temperature and salinity due to fluctuations in oyster host physiology resulting from reproductive status, diet, and health (DePaola et al., 2000)

The present study shows that *E. coli* counts in oyster meat was occasionally higher than the standard level (<2.3 MPN/g) in May and July (5.86 MPN/g and 5.11 MPN/g, respectively). Whereas, fecal coliforms counts in oyster meat was highest in May and was also higher than the standard level (< 20 MPN/g). Bacterial contamination in grow-out water was consistently lower than the standard level of less than 1000 MPN/100ml.

During periods of high rainfall and first seasonal flushing that begin in May in Thailand, the number of bacterial contaminants in the water column may become elevated. Kirby-Smith and White (2006) stated that rainfall and water level was positively correlated with elevated fecal coliforms contamination. Turbidity, temperature, salinity, and DO have also been associated with fecal coliforms contamination. Bordalo et al. (2002) studied the survival of fecal indicator bacteria in the Bangpakong River estuary in Thailand and found that overall survival was higher in low salinities.

The present study shows that several oyster meat samples were contaminated with *V. parahaemolyticus; E. coli;* and fecal coliforms. The level of bacterial contamination was generally highest during the monsoon season (May – October). Consumers should avoid eating raw oysters in those months due to the ability of oysters to bioaccumulate bacteria and pathogens (Kittigul et al., 2010). Apart from bioaccumulation, these bacteria may also increase in numbers within the oyster during harvesting, storage, transportation, and processing (Gooch et al., 2002). This possibility poses a serious health risk for consumers and might be responsible for outbreaks of gastrointestinal illness for consumers of raw or marginally cooked oysters.

Results from this study show that the amount of indicator-bacteria (fecal coliforms) contamination in Huang Nam Khao oyster farm grow-out water was lower than those proposed for consumer safety according to government guidelines throughout the year. However, corresponding fecal coliforms counts in oyster meat was higher than the standard value (<20 MPN/L) in May, 2004. The same discrepancy between grow-out water and oyster meat was also observed for *E. coli* and *V. parahaemolyticus* where water quality standards have not yet been set. Although bacterial indicator numbers may be acceptable in the water column, the corresponding bacterial contamination levels in oyster meat may be significantly higher and pose a risk of gastrointestinal disease to the consumer. Therefore, although time consuming and more expensive, routine testing of oyster meat for pathogen contamination is recommended. Presently, sensitive and less time consuming methods for detecting pathogens, directly, are available. Timely detection of *V. parahaemolyticus* or other pathogens in oysters will help the Thai shellfish industry prevent gastrointestinal disease outbreaks, protect consumer health and strengthen local tourism. The oyster consumer in tropical regions should avoid eating raw oysters during the first rains due to elevated risks of bacterial contamination that can occur through bioaccumulation.

ACKNOWLEDGEMENTS

This study was jointly funded by the Center of Excellence on Environmental Health, Toxicology and Management of Chemicals and the Canadian International Development Agency (CIDA; UPCD Tier 2 Project # 098/ S47074-323). We thank Prof. Mark Flaherty, Department of Geography, Victoria University, British Columbia, Canada for comments and suggestions throughout the study. We also thank Mr. Sanoh Sungworn, Huang Nam Kao community leader and the graduate students who assisted in our field work.

REFERENCES

APHA. 1992. *Standard Methods for Examination of Water and Wastewater. 15th* ed. Water Pollution Control Federation, New York.

Bordalo, A.A., Onrassami, R. and Dechsakulwatana, C., 2002. Survival of fecal indicator bacteria in tropical estuarine waters (Bangpakong River, Thailand). *Journal of Applied Microbiology* 93: 864-871.

DOF. 2005. *Thailand Fishery Statistics.* Department of Fisheries, Ministry of Agriculture and Co-operatives, Bangkok, Thailand.

Chalermwat, K., Szuster, B. and Flaherty, M. 2003. Shellfish aquaculture in Thailand. *Aquaculture Economics and Management* 7: 249-261.

Dalsgaard, A. 1998. The occurrence of human pathogenic

Vibrio spp. and *Salmonella* in aquaculture. *International Journal of Food Science and Technology* 33: 127-138.

DePaola, A., Hopkins, L.H. and Peeler, J.T. 1990. Incidence of *V. parahaemolyticus* in U.S. coastal waters and oyster. *Applied and Environmental Microbiology* 56: 2299-2302.

DePaola, A., Kaysner, C.A. and Bowers, J.C. et al. 2000. Environmental investigations of *V. parahaemolyticus* in oyster following outbreaks in Washington, Texas, and New York (1997, 1998). *Applied and Environmental Microbiology* 66: 4649-4654.

DePaola, A., Nordstrom, J.L. and Bowers, J.C. 2003. Seasonal abundance of total and pathogenic *V. parahaemolyticus* in Alabama oyster. *Applied and Environmental Microbiology* 69: 1521-1526.

Gannarong, M. and Rattanachote, A. 1997. *Bacterial Contamination of Oysters (Crassostrea belcheri), Seawater and Sediment in Ban Don Bay, Suratthani Province. Suratthani: Suratthani Coastal Aquaculture.* Development Center, Coastal Aquaculture Division, Department of Fisheries, Thailand (in Thai).

Gooch, J.A., DePaola, A., Bowers, J., and Marshall, D. L. 2002. Growth and survival of *Vibrio parahaemolyticus* in post harvest American oyster. *Journal of Food Protection* 65(6): 970-974.

Hanak, E., Boutrif, P., Fabre, P. and Pineiro, M. 2002. Food Safety Management in Developing Countries. *In* Proceedings of the International Workshop, CIRAD-FAO, 11-13 December 2000, Montpellier, France, CIRAD-FAO, CIRAD CD-ROM, Montpellier, France.

Hitchins, A.D., Feng, P. and Watkins, W.D. 1998. *Escherichia coli* and the coliform bacteria. In *Food and Drug Administration, Bacteriological Analytical Manual.* (8th edition), AOAC International, Gaitherburg, Maryland, USA.

Kelly, M.T. and Stroh, M.D. 1988. Temporal relationship of *V. parahaemolyticus* in patients and environment. *Journal of Clinical Microbiology* 26: 1754-1756.

Kirby-Smith, W.W. and White, N.M. 2006. Bacterial contamination associated with estuarine shoreline development. *Journal of Applied Microbiology* 100: 648-657.

Kittigul, L., Pombubpa, K., Sukonthalux, S., Rattanatham, T., Ultrarachkij, F., and Diraphat, P. 2010. Detection of Hepatitis A and bacterial contamination in raw oysters in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* 41: 105-113.

Kueh, C.S.W. and Chan, K-Y. 1985. Bacteria in bivalve shellfish with special reference to the oyster. *Journal of Applied Microbiology* 59: 41-47.

Lee, J.k., Jung, D.W., Yoon, K. and Yoon, S.K. 2006. Effect of diluent salt concentration and pH on the enumeration of *V. parahaemolyticus* by direct plating on selective agar. *Food Science and Biotechnology* 15: 866-870.

Lozano-Leon, A., Torres, J. and Osorio, C.R. 2003. Identification of tdh-positive *V. parahaemolyticus* from an outbreak associated with raw oyster consumption in Spain. *FEMS Microbiology Letter* 226: 281-284.

McLaughlin, J.B., DePaola, A. and Bopp, C. A. 2005. Outbreak of *V. parahaemolyticus* gastroenteritis associated with Alaskan oyster. *New England Journal of Medicine* 353: 1463-1470.

Rippey, S.E. 1994. Infectious diseases associated with molluscan shellfish consumption. *Clinical Microbiology Reviews* 7: 419-425.

Rowse, A. and Fleet, G. 1982. Viability and release of *Salmonella charity* and *Escherichia coli* from oyster feces. *Applied and Environmental Microbiology* 44: 544-548.

Sincero, T.C.M., Levin, D.B., Simoes, C.M.O., and Barardi, C.R.M. 2006. Detection of hepatistis A virus (HAV) in oysters *(Crassostrea gigas). Water Research* 40: 895-902.

Szuster, B.W., Chalermwat, K., Flaherty, M., and Intacharoen, P. 2008. Peri-urban oyster farming in the upper Gulf of Thailand. *Aquaculture Economics and Management* 12: 268-288.

Terajima, J., Tamura, K., and Hirose, K. 2004. A multi-prefectural outbreak of *Shigella sonnei* infections associated with eating oysters in Japan. *Microbiology Immunology* 48: 49-52.

Timoney,J.F., and Abston, A. 1984. Accumulation and elimination of *Escherichia coli* and *Salmonella typhimurium* by hard clams in an in vitro system. *Applied and Environmental Microbiology* 47: 986-988.

Utrarachkij, F., Intalapaporn, K., Kumkrong, K., and Kittigul, L. 2006. Microbiological quality of raw oysters from local markets in Bangkok. *Journal of Public Health* 36: 112-121.

Wang, D., Yu, S., Chen, W., Zhang, D., and Shi, X. 2010. Enumeration of *Vibrio parahaemolyticus* in oyster tissues following artificial contamination and depuration. *Letters in Applied Microbiology* 55: 104-108.