Laboratory Studies of Growth Conditions of Vibrio parahaemolyticus in Pacific Oyster (Crassostrea gigas)

with International Considerations to Shellfish-Associated Illnesses in South Korea

By

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OVERVIEW OF INTRODUCTION

This research thesis includes both disciplines of BioResource Research (BRR)

Interdisciplinary and International Degree (ID) Programs at Oregon State University (OSU).

The first section encompasses the BRR research, which is a preliminary study to develop an *in vitro* assay for pathogenic *Vibrio parahaemolyticus* and its adherence to *Crassostrea gigas*, also commonly known as the Pacific Oyster. The second section is the ID research, which covers an exploratory study to examine both economic and cultural impacts due to shellfish-associated illnesses specifically focusing on *Vibrio parahaemolyticus* and other related bacterial issues in the oyster industry of Tong-yeong, South Korea.

Basic information about the bacteria (*Vibrio parahaemolyticus*) and its relation to oysters (*Crassostrea gigas*) is included in the introduction section. For the specific approach to the microbiology study in the chapter one, the general information is as follows: characteristics of *Vibrios*, the habitats for the bacteria and their common physiological relation to the oysters, an introduction to the basic mechanisms of the bacterial pathogenesis, the significance of the *V. parahaemolyticus* epidemics in world-wide, basic concepts to bacterial growth and temperature and salinity as two important environmental parameters for the investigative regime. The introduction of the exploratory study of the ID research in chapter two consists of the general information about the life of *C. gigas* and its production in South Korea, the city of Tong-yeong. The personal observation about the city of Tong-yeong is also part of the discussion.

RATIONALE AND PERSONAL INTERESTS

During my freshman year, I explored various fields of academic interests to decide my current major, BioResource Research (BRR) Interdisciplinary Program. It was a highly attractive major that involved a hands-on scientific research project which includes a development of a thesis document. At that time, I entered college with minimal skills in the English language, and I desired to improve my language skill through the BRR major, which would provide me with intensive communication and writing training in scientific research academia. I also felt strongly that BRR would be a beneficial choice to help me pursue a future career in the field of environmental conservation and natural resources management.

Growing up learning about pollution in the fast growing industrial city of Inchon in South Korea, I often observed dead fish in the creek and the stinky smell that bothered my nose everyday when I walked by my middle school. I was particularly interested in environmental issues concerning water quality and aquatic species especially after my backpacking trip to O'ahu, Hawai'I in 2005. I saw the high diversity of aquatic species in the tropical island through snorkeling and surfing. By interacting with a member of the Hanauma Bay exhibition crew, I saw true evidence that people were trying hard to preserve their natural treasures in the aquatic environment and I wished I could gain more knowledge about environmental issues, particularly related to water quality issues.

As I investigated various research topics of my interests for the BRR program, I interviewed several faculty members at OSU including Dr. Claudia Häse. She kindly explained her research interest in human pathogenic *Vibrios* and their association with a popular shellfish, Pacific Oysters. At that time, I had few laboratory experiences dealing with

mammals and plants, and I preferred to gain laboratory experience with water species. She welcomed me into her research lab and introduced me into the new topics of microbiology. Although it sounded to be very difficult subject in the beginning, I was able to feel more confident about the research project as I knew there was support that I could get from faculty mentors and friends at OSU.

My scientific project through BRR has become highly valuable in my college career as my focused topic was an interest shared among my family members. My mother has been in the food industry for more than a decade, and my brother's future career goal is to become a chef. As I visited my family to Portland, Oregon, we developed many enjoyable dialogues about cooking practices and food safety issues, and it seemed to become beneficial for me to develop my distance relationship throughout the process.

When my family members asked me about my major study, I had a long phrase of explaining the BioResource Research Interdisciplinary Program, yet it did not seem to make sense clearly in their mind. While I was developing a method for an assay to test the adherence of *V. parahaemolyticus* to *C. gigas* in Summer 2007, I had a chance to introduce my research work to my family. Since then, my academic focus has become a lot clearer to my family members.

I remembered how my mother would constantly ignore my advice on the dangerous facts about the pathogenic bacteria residing in raw or undercooked seafood. Instead, both my mom and my brother kept reminding me of how much she would miss the Korean raw oysters and how it should be prepared for authentic tastes. She would express how nice it would be to smell the fresh shucked oysters in the laboratory. I seriously objected to her idea of the oyster

smell for myself, but wondered how it is that dealing with food safety issues would be like to raw oyster lovers like my mother, and perhaps other Koreans.

Up to the point of getting the BRR project, I was ready to focus on aspects in biological science. Beyond my knowledge of scientific study, my interaction with family members helped me recognize that the problems of bacterial infection in food in various social settings. In May 2006, my dad passed away unexpectedly. It was a difficult period especially since I started my academic career at OSU. My dad graduated with a chemistry major from Gunkuk University in Seoul, South Korea, and had a decade of experience working in a laboratory at one of the prestigious chemical industries in South Korea prior to our family's immigration to the U.S. It influenced my academic career highly since I used to have frequent communication about my education and interests about biology and environment to him.

Dealing with a family emergency, I noticed truly that my big motivation in learning science could be related to people and nature. I came across an innovative idea and an opportunity came across during my senior year at OSU, and that was to broadening my worldviews and academic horizon by revisiting my native country, South Korea. The OSU faculty members including Wanda Crannell, the BRR academic advisor and Janet Nishihara, the current interim director of the Educational Opportunity Program (EOP) were truly inspirational in my motivation to make the decision for the study abroad.

I was initially discouraged by several factors, including my family's negative feedback concerning my security, unknown circumstances of finances as well as future plans. Facing the major life challenge in my college career, I recognize my enormous personal growth and strength aided thankfully by friends and mentors from OSU. Balancing my school work and

my health have become important factors to help me motivate my initial plan for the large scale intensive undergraduate senior project in fall 2006.

As I learned about the International Degree (ID) program at OSU and visited with ID faculty members and Dr. Bryan Tilt from the Anthropology Department, my interest turned into tentative and progressive plans and goals focusing towards the topic of my research interests in human pathogen and food safety issues. When I was looking through articles, I skimmed through important facts with significance in pathogenic bacteria in the food industry, and with full consequences of major outbreaks to people, industry, and culture.

I learned that *V. parahaemolyticus* infection has also been a significant problem each year especially through seafood consumption, particularly oysters in South Korea. I started to ponder how Koreans, such as my mother, who eat raw fish and shellfish, might consider the issues with today's growing food safety concern over *V. parahaemolyticus*. Both high numbers of consumers and shellfish productions were in fact occurring in Tong-yeong, South Korea, and I was thrilled to find more information about cultural and economic impacts due to the bacterial infection in their shellfish industry.

In addition to my research interests, my first oversea journey away from my close friends and family in the U.S. was enabled because of my independent work ethic and adventurous personality. By revisiting my native country, I was also very pleased to have learning opportunities in agricultural and environmental issues with "adult eyes" after living in the U.S. for nine years.

Developing a comprehensive undergraduate thesis document, I desired to write a meaningful story that readers would understand how I was able to interconnect the various issues related to the bacterial infection in shellfish farm animals. I also wanted to explain the story of people who might be depended on living with *Crassostrea gigas* for their economic survivals. The story comprises my microbiology work at OSU from October 2005 to June 2006 and research findings which include my special interest and creative assets in the investigation of food safety in the local fishery industries in South Korea from July 2007 to December 2007.

RESEARCH OBJECTIVE AND GOALS

The preliminary part of the BRR research investigates the specific adherence mechanism of *V. parahaemolyticus* to oysters. The primary research goal of the Claudia Häse Laboratory was to understand bacterial expression of specific proteins for survival and colonization in the host, particularly oysters. The main objective was to develop an *in vitro* assay which could potentially examine optimal growth conditions for these bacteria of several different temperatures and salinity levels. The research focused on two different temperatures: room temperature (21.5°C) and human body temperature (37°C). Different salinity levels were included to examine the correlation with optimal adherence to the gut tissues of oysters. The ultimate goal of this preliminary research was to find the bacterial growth conditions that allow optimal adherence of *V. parahaemolyticus* to oysters. After establishing relevant growth conditions, the next step will look into the molecular mechanism of bacterial adherence to the oyster host in a laboratory setting. Overall, this research could potentially provide the foundation for developing other molecular studies related to *V. parahaemolyticus* and other marine organisms.

The second part of the investigation was an exploratory study to understand how the bacterial outbreak, a particular focus in *V. parahaemolyticus* infection, had influenced the culture and the economy in Tong-yeong, South Korea. The background information included in the introduction section is composed of the production of *C. gigas* in South Korea, *Vibrio* issues in *C. gigas* and geographic feature and population in the city of Tong-yeong. The research questions are as follows: 'How big of a problem was bacterial contamination in the oyster industry in Tong-yeong? What problems did it cause and when did they occur? How significant were the economic

losses due to *V. parahaemolyticus* outbreaks? Also, how had the incidences affected various practices in the shellfish industry?' In addition to these specific research questions, a secondary objective was to understand the general structure of the shellfish industry and its importance for the Korean economy and culture. In order to understand the fate of bacterial contamination in oysters, the general overview of the pre-harvest and the post-harvest conditions were investigated during fall term study abroad in 2007. The major *V. parahaemolyticus* outbreak of South Korea occurred in 2003 was discussed.

Overall, the research aimed to evaluate food safety issues and to practice a holistic approach in undergraduate study. The study demonstrates cross disciplines of biological and social sciences. The laboratory practice of the study will be beneficial to conduct other molecular studies related to human pathogens, such as *Vibrios* and its related marine hosts. The investigation throughout the ID research is in value of linking diverse communities within environmental health issues in a global context.

Preliminary Study on

Developing In Vitro Assay of Pathogenic Vibrio Parahaemolyticus in Pacific Oyster
(Crassostrea gigas)

AN ABSTRACT OF THE THESIS OF

<u>Jeehye Lee</u> for the degree of <u>Bachelor of Science in Bioresource Research</u> presented on <u>March 16th, 2009</u> Title: <u>Preliminary Study on Developing In Vitro Assay of Pathogenic Vibrio parahaemolyticus in Pacific Oyster (Crassostrea gigas)</u>

Abstract approved: _		
	Dr. Claudia Häse	

ABSTRACT

Vibrio parahaemolyticus, a naturally occurring halophilic pathogen, is seasonally abundant in marine and estuarine environments. It is also the leading cause of gastroenteritis in humans world-wide. Numerous outbreak incidences associated with the pathogenic Vibrio parahaemolyticus contaminations in oyster products have raised public-health concerns as well as economic challenges to the shellfish growers since the discovery of the bacteria in 1950s. A lack of molecular understanding in association of the bacteria and the host animal, C. gigas, has been conducted in the Häse lab which aims to find bacterial factors in the host animal interaction and "targeted intervention strategies" to remove the presence of the pathogen. A preliminary study for the *in vitro* assay was initially designed to help the molecular study. It tested bacterial adherence to animal samples in different temperature and salinity levels with a single-factor scheme. The average number of inoculates for each temperature and salinity treatment were $1.65 \times 10^8 \pm 5.39 \times 10^7$ CFU/ml and $2.11 \times 10^8 \pm 2.05$ x 10^7 CFU/ml with the mean animal's gut weight of 6.61 ± 0.09 g and 6.69 ± 0.23 g respectively. From the temperature treatments, the average number of *V. parahaemolyticus* isolates per gram sample was $4.47 \times 10^5 \pm 3.23 \times 10^5$ CFU/ml. From the three different salinity level treatments (1%, 3%, and 6% NaCl), the average number of the bacterial isolates per gram sample was $1.84 \times 10^5 \pm 2.62 \times 10^5$ CFU/ml. The result from the study had shown that the

optimal growth condition for *V. parahaemolyticus* was at 37°C and 3% NaCl. Better bacterial adherence occurred in temperature treatment groups of *V. parahaemolyticus* compared to *E. coli*. Better bacterial adherence occurred in treatment groups of room temperature (21.5°C) and the concentration of 6% NaCl. Yet, high resource variables in the research design and low precisions seemed to create higher margin of errors and limited understanding for the optimal bacterial growth conditions associated with the degree of bacterial adherence to the animal. The research recommends greater sample numbers and replicates in order to reduce the high variability associated with bacterial growth for the future related assay developments.

INTRODUCTION

Characteristics of Vibrios

The genus *Vibrio* belongs to the family *Vibrionaceae* (59). As *Vibrio* spp. were isolated from a hydrothermal vent, researchers claim that historical origination of the bacteria is the deep sea (cited in 8) and adopted its growth under salt water conditions (59). Most *Vibrio* species are aquatic and found either in temperate regions in the deep sea or in estuaries worldwide (23, 34). These gram-negative bacilli are single and straight or rigidly curved rods (38, 51). They are oxidase-positive and highly motile with a single polar flagellum when grown in a liquid medium. The cells are non-spore forming and can grow under facultative anaerobic conditions (38, 51). Their sizes are generally between 0.5nm and 0.8 nm wide and 1.4 nm and 2.6 nm long (45). Pathogenic *Vibrios* have a life cycle divided into a free-swimming phase and a host phase. In the free-swimming phase, they are associated with zooplankton, crustaceans, insects, and water plants in marine and estuarine environments. In the host phase, a complex and incompletely understood regulatory cascade controls virulence (45).

There are 65 species of the genus *Vibrio*. More than 12 species are known to be associated with human diseases (20), and the major human pathogens are *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* (51). The genome sequences of these pathogens have been completely analyzed and fostered active studies on the pathogenic mechanisms of these species. *Vibrio parahaemolyticus* was discovered by Japanese researchers during the outbreak incidences of 1950s in Osaka, Japan (22). The bacterium is a gram negative bacterium occurring in curved or straight rods. It is also enteropathogenic (causing disease in the intestinal tract) and a facultative anaerobic bacterium (19, 52).

Ecology of Vibrio parahaemolyticus

Vibrios spp. can be isolated from near-shore marine waters and estuarine environments, and are typically found in the water column during late spring or early summer and in the sediment during winter. The bacteria are associated with marine organisms including zooplankton and farmed aquatic animals (32, 34, 57). Particularly, there is a strong association with zooplankton. According to Kaneko and Colwell in 1973, close to the total viable count of plankton collected from Cheasapeake Bay in summer contains Vibrio species (32).

Since the discovery of *Vibrio parahaemolyticus*, the complete understanding of host-microbe interactions of *Vibrio parahaemolyticus* has remained unclear. Some studies showed that *Vibrio* species are known to be "free-living chemoheterotrophs" (cited in 10) and the normal bacterial biota in the digestive tract or on the skin surfaces of many aquatic animals, including flatfish, jackmackerel, salmonids, larval and juvenile sea beam, blue crabs, shrimps, and oysters (57). The gut environment of the marine animals such as red seabream and black seabream, and salmonid fish species can provide organic matter and other possible benefits for bacterial growths and will be beneficial for *Vibrios* since the common aquatic environment would contain low bioavailability of nutrients. Therefore, *Vibrios* have adapted to low pH, secretion of bile acids, and micro-or anaerobic conditions (57). Sawabe and colleagues states that these kind of findings represents that the potential of a well-evolved mutualistic or commensal relationship of the bacteria in their hosts (cited in 10, 57).

Estuarine environments are naturally known as nitrogen limited environments. More than half of the dizotrophic bacteria collected from *Spartina alterniflora*, a smooth salt marsh cord grass, found in intertidal zones of estuaries, was composed of *Vibrio* spp. in which *V. parahaemolyticus* constituted approximately14% in the earlier research from the Criminger

and coworkers showed (cited in 10). Criminger and coworkers detected *nifH* (a structural gene which encodes the nitrogenase iron protein) sequences of the bacteria allied to the plant's rizosphere using acetylene reduction assay (10). This finding leads to critical evidence of a nitrogen fixation in *Spartina alterniflora* due to *Vibrio parahaemolyticus* and other *Vibrio* spp. interaction with the rhizosphere. The research team explains that "cooler, growth-limiting temperatures and predation" could become beneficial for bacteria to reside under plant root system. The finding of the study has lead to a deeper understanding of the ecological role, played by *Vibrio* spp. (10). Disruption of the rhizosphere due to natural and human activities might then ultimately causes possible distribution of naturally occurring pathogens to fresh or brackish waters. This raises the concerns of potential risk of outbreaks.

Defensive Mechanisms of Oysters

In estuaries or oyster growing bays, numerous suspended or adsorbed microorganisms to phytoplankton could easily enter into Mollusks, particularly oysters, since their organs would filter the water inflow (23). Typically a water inflow runs of 4 to 40 h⁻¹oyster⁻¹ runs from the oyster gill cilia to the oyster pallial (mantle) cavity (2, 23, 50). Some microorganisms might be filtered out by the outflow of water or be destroyed by the defensive mechanisms of oysters, whereas some others could remain through either adhesion or invasion of the tissues of oysters (23), (63).

The two major defensive mechanisms of oysters are known to be humoral and cellular immunity (25). The microorganisms must defeat these defensive mechanisms to survive in the bodies of oysters. Factors of the humoral defensive mechanisms include agglutinins, lysosomal enzymes, and toxic reactive oxygen intermediates which occur during a respiratory rupture in the hemocyte phagocytic process (25). According to the on-line Medline Dictionary

(http://medlineplus.gov/), agglutinins are antibodies of oysters and cause reactions to generate clumps or masses during serological responses to specific antibodies (56). Agglutinins in oysters' cells facilitate bacterial aggregations and further initiate the binding of hemocytes to the bacteria (25). Thus, the humoral mechanism thereby increases the capability of phygocytosis (25).

The mechanism of cellular immunity includes abilities to phagocytize, encapsulate, and destroy microorganisms (25). Genthner and coworkers research implies that the ability of bacteria to respond to bactericidal activities of oyster's hemocytes might vary among bacteria genera, and morphological features of bacteria influence the motility of bacteria might be associated with the result of the seasonal variation to the bactericidal activity. The result from the study suggested that the opaque phenotype of *V. parahaemolyticus* seemed to be less disturbed by the cellular defensive mechanism of oysters compared to the translucent phenotype (25).

Understanding about the Pathogenic Form of V. parahaemolyticus

Both pathogenic and non-pathogenic forms of *V. parahaemolyticus* exist (65). *V. parahaemolyticus* carries *tdh* and *trh* genes which causes toxin mediated gastroenteritis in humans. Thermostable direct hemolysin (TDH) or TDH-related hemolysin (TRH) are found in pathogenic strains of *V. parahaemolyticus*; these hemolysins are the indicators differentiating pathogenic from the non pathogenic strains (57). According to the CDC's *Emerging Infectious Disease*, most clinical strains of *V. parahaemolyticus* have these virulent factors, whereas to only less than 1% of environmental strains of *V. parahaemolyticus* (39). In 2000, DePaola and colleagues stated that the density of *V. parahaemolyticus* in oyster samples was not mostly detected up to the "exceeding the level of concern of 10,000⁻¹g," and relatively low levels

 $(<10^{-1}\text{g})$ of pathogenic strains of *V. parahaemolyticus* were isolated from oyster samples collected from three states in the U.S. coastal regions (14).

Wagatsuma, one of the pioneer researchers of the early studies on *V. parahaemolyticus*, created a blood agar medium in order to test which *V. parahaemolyticus* strains could demonstrate either positive or negative response to hemolytic characteristics. The result of the study showed that strains isolated from the infected patients' stools had positive responses to the hemolytic characteristic test. Later studies termed Kanagawa phenomenon (KP) when the strain causes hemolysis on the Wagatsuma agar media. The TDH-positive strains are betahemolytic on Wagatsuma agar, and are called Kanagawa-positive strains. TRH producing strains are known to be Kanagawa negative, and both TDH and TRH producing strains are known to cause diarrheal illnesses.

The authors of The *BIOLOGY of VIBRIOS* described that there is limited knowledge on how these two virulence factors cause gastroenteritis, and the complete understanding of the overall mechanism of pathogenesis by *V. parahaemolyticus* has remained unclear in present. The recent report of the complete genome sequence of *V. parahaemolyticus* has opened more research opportunities to update unknowns to the pathogenesis mechanism of *V. parahaemolyticus* (57).

Public Health Concern: V. parahaemolyticus outbreak incidences

V. parahaemolyticus has been recognized as a worldwide pathogen occurring in both industrialized and developing countries since the bacterium was initially isolated from Japan in the 1950s (6, 57, 58). In South Korea, a total of 2,242 Koreans were infected due to the consumption of fish contaminated with pathogenic *V. parahaemolyticus* from 2003 to 2006 (35). In the U.S., most occurrences of *V. parahaemolyticus* illnesses have been related to

crustacean products yet they imply to Mollusks (16). Common sources of gastroenteritis outbreaks associated *V. parahaemolyticus* include crabs, shrimps, lobsters, and oysters, and they are typically cooked before eating (57). Most Americans eat oysters that are often stewed or cooked in "half shell" preparation. Raw oysters are popular in coastal regions. Shucked oysters are a frequent term when oysters are mentioned; they are readily available across the U.S. (16).

Since the first report made in 1971 from Maryland, sporadic outbreaks have been reported throughout the U.S. coastal regions mainly including Gulf Coast, Pacific Northwest, and Atlantic Northeast states from July to August in 1998, May to July 1998, and July to September 1998 respectively (3, 4, 14). In 1997, 209 people had gastroenteritis by eating raw oysters that were contaminated with *V. parahaemolyticus*, and these shellfish products were harvested from California, Oregon, and Washington in the U.S. and from British Columbia in Canada. In the later report made in 2006, 177 cases were due to the consumption of molluscan shellfish harvested in the Northwest including Washington in the U.S. and BC, Canada (7).

The Centers for Disease Control (CDC) have listed pathogenic *V. parhaemolyticus* as one of the major microorganisms causing food borne disease outbreaks in the U. S. These strains cause food poisoning generally due to consumption of seafood that is contaminated by the toxin producing by *V. parahaemolyticus* before eating (31). Molecular analysis showed that *V. parahaemolyticus* serotype with particularly known as O3:K6 antigen has been frequently isolated from the past outbreak incidences in South East and East Asia as well as the U.S., and Nishina and colleagues cited that there have been significantly increased cases due to the serotype (42).

The increasing trend of human incidences of gastroenteritis due to seafood contaminated with *V. parahaemolyticus* has gained significant national and worldwide attention. The Food Borne Diseases Active Surveillance Network reports that *Vibrio* infection rate has been the highest, 47%, from 1996 to 2004, compared to other bacterial infections, such as *Salmonella*, *Campylobacter*, *Listeria*, and *E. coli* (5). The significance of *V. parahaemolyticus* infection in humans has continued to rise since 2000 in the U.S. In 2006, the Council of State and Terrestrial Epidemiologists suggested all *Vibrio* illnesses, including non-cholera *Vibrio* illness, should be classified as nationally notifiable diseases (5).

A model for risk characterization based on the historical/surveillance data was developed by CDC's program Cholera and Other *Vibrio* Illness Surveillance System (COVISS). In the study conducted from 1998 to 2002, 62% of *V. parahaemolyticus* illnesses was due to contaminated oyster consumption and wound associated (58).

The Pacific Coast States were the site of the highest number of reported *V*.

parahaemolyticus by the state of residence from a report from the CDC (58); however, there was no direct relation to the oyster harvesting sites. As a result, residents of the Pacific Coast states, such as Washington and Oregon, consumed oysters harvested from various sites in nation as well as other states. This study also found that most oyster-linked *V*.

parahaemolyticus illnesses were associated with harvesting areas in the following order: Gulf Coast oysters, Pacific Northwest oysters, Atlantic oysters, and other states (58). Elston from Aqua Technics in Sequim argues that the consequence of warming of the ocean water due to El Niño effect might be correlated with the sudden growth of bacteria near shore and possible increase of *Vibrio* contamination should be highly considered (18). Food safety concerns are raised since various microorganisms would be contained while the digestive organ of the

shellfish filters the seawater, and entire raw or lightly cooked animals are often consumed by people (16).

The general symptoms due to consumed raw or inadequately cooked seafood infected with *V. parahaemolyticus* are watery diarrhea, abdominal cramps, nausea, vomiting, fever and chills lasting one to three days with onset often within twenty four hours (5, 65). According to the Center for Disease Control and Prevention, although there is no treatment necessary in most cases of *V. parahaemolyticus* infections, it is recommended to patients for drinking lots of liquids in order to restore the lost fluids from diarrhea symptoms. In harsh cases, antibiotics that are susceptible to the microorganisms are used, and they are tetracylcline or ciprofloxacin (6).

Raw or improperly cooked seafood products during warmer seasons lead to higher rates of the world outbreaks of *V. parahaemolyticus*. According to Kaysner, the bacterial contamination could be possibly eliminated by proper heating and cooking practice in dealing with seafood (33). Research conducted in 1970 by Vanderzant and colleagues focused on an isolation of *V. parahaemolyticus* from the shrimps harvested from the Gulf Coast and found that after heat treatment of a shrimp homogenate containing *V. parahaemolyticus* for a minute at 100 °C, no survival of bacteria was found after an hour (60). In live crabs, the bacteria were destroyed after exposure to steam for 15 minutes between 72 °C to 75 °C (26).

In the shellfish industry, depuration is commonly used for managing sanitation and safety of shellfish products to eliminate bacterial growth in host animals, particularly oysters, after the shellfishes are harvested. Common methods of shellfish depurations are the use of relaying, ultraviolet light, chlorine, and ozone. According to the National Shellfish Sanitation Program (NSSP), the process of depuration as "the process of using a controlled aquatic

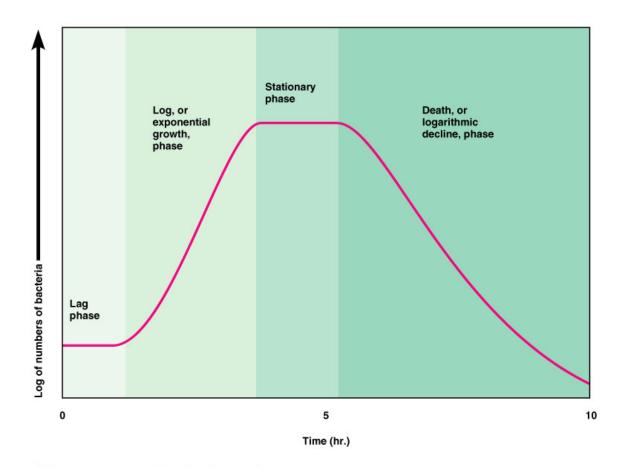
environment to reduce the level of bacteria and viruses that may be present in [live] shellfish harvested from moderately polluted [restricted] waters to such levels that the shellfish will be acceptable for human consumption without further processing (43)."

One of the most common problematic concerns is that all microorganisms are not equally eliminated with a common method such as depuration. Some enteric bacteria such as *E. coli*, fecal coliforms, and *Salmonella* tend to be eliminated more effectively while viruses and naturally occurring *Vibrios* could be left behind (62). At least five known *Vibrio* species including *V. cholerae*, *V. parahaemolyticus*, *V. mimicus*, *V. hollisae*, *V. fluvialis*, *and V. vulnificus* have been linked to human illness after the ingestion of the contaminants in shellfish products (43). Variability exists among oysters. Health conditions and the origins of animals might vary. These factors could become problematic in the depuration treatment. The depuration method might not always work for every animal whose levels of growing condition such as temperature, turbidity, and salinity are different from one another (16). The most common method is the purification method using chlorine seawater; however, ineffective dose level of chlorine based- depuration can harm the bivalve's filtering capability and minimize the overall food quality (43).

General Concepts to Bacterial Growth

Both liquid and solid media can be used to grow bacteria. The use of solid media enables bacterial colonies to be easily counted. A viable cell count is the number of living cells under given incubation conditions using spread plate/surface plate and pour plate methods. The major difference between the two methods is that for the pour-plates inocula (culture mixed with molten agar) are poured onto the surface of dry media, whereas the spread plate method requires a small amount (0.05 to 0.1 ml) of culture to be spread on the surface of the plate (53).

Growth curves display the number of cells against time under given conditions. Four major stages of the life cycles of bacteria in batch culture are lag phase, logarithmic phase or exponential phase, stationary phase, and death phase (53). Batch culture means "a closed system culture of microorganisms with specific nutrient types, temperature, pressure, aeration, and other environmental conditions, where only a few generations are allowed to grow before all nutrients are used up (11)." During the lag phase, cells hardly undergo divisions and cell number remains relatively constant. Once cells adapted to the new environment, cell growth happens rapidly, and active growth and division follows throughout the logarithmic phase or exponential phase. At this stage, the number of cells doubles at a constant rate. Population growth eventually ceases and the growth curve becomes flat (stationary phase). The bacterial growth then enters death phase (53).



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FIG. 1. Microbial growth curve in a closed system

Environmental Parameters: Temperature & Salinity

In each stages of the bacteria's life cycle, environmental factors are crucial for the cells to grow, and survive. The growth of bacteria commonly depends on factors including nutrients, sources of energy, water, temperature, pH, oxygen, and dissolved inorganic solids, particularly for halophilic *Vibrio* spp. such as *Vibrio parahaemolyticus* (53). These factors may not be available steadily nor work independently. Bacterial growth could potentially occur in pre-harvesting, as well as remain in post-harvesting, conditions of shellfish, particularly oysters.

Much research has examined the effect of temperature and salinity on bacterial growth and seasonal abundance of bacteria in ocean water. Those two factors are especially emphasized since they control sea water densities (49). Changes in the level of salinity in water may also be related to temperature changes, since water can evaporate more rapidly during the summer compared to winter. Therefore, the level of salinity was taken in account in order to understand the optimal growth of *V. parahaemolyticus* in order to adhere to oysters throughout my research.

The wild strain of *V. parahaemolyticus* can be isolated in temperate regions either in the deep sea or in estuaries worldwide. An estuary is a semi-enclosed coastal body of water that is also a junction between rivers, streams and open ocean (46). High rates of biological productivity arise in this system and the sea. Both temperature and salinity fluctuate seasonally in estuaries. The national leading production areas for shellfish, particularly oysters, are located in estuaries including Chesapeake Bay, Long Island, Oyster Bay Cove, Gulf of

Mexico, Humbald Bay, and Puget Sound (2, 9, 21, 47). Water quality issues related to coliform and naturally occurring *Vibrio* spp. occur seasonally in these areas.

Crassostrea gigas is commonly known as Pacific Oyster and Japanese Oyster. *C. gigas* is typically found in China and the Korean peninsula. Since the species was introduced to the U.S., its natural range along the Pacific coast is from northern Vancouver Island to California (16). A number of oyster farms have recently been established in Alaska. In Oregon, a total of twenty- two estuaries are along the Pacific coast, including the famous cultivation sites for *C. gigas* in bays of Tillamook, Newport, and Yaquina (15).

The surface water temperature in oceans varies from -5 °C to 30 °C. "Radiation income" and "prevailing winds" are influential to the temperature in estuarine waters (49). Kaneko and Colwell found that the water temperature in which the culturable cells of *V. parahaemolyticus* were isolated was between 14 °C and 19 °C during the late spring and early summer in Chesapeake Bay (31). The study showed that water temperature is positively correlated to the abundance of *V. parahaemolyticus*. Later research conducted by DePaola and colleagues has reported that water temperatures of oyster growing bays in nine states of the U.S. are the major factor which contributes to the geological and seasonal distribution of *V. parahaemolyticus* (13). The high abundance of *V. parahaemolyticus* has occurred during the warmer seasons in the temperate regions.

According to researchers, ocean water temperature was comprehensively sampled in two oyster-growing bays, Yaquina and Tillamook Bays, from November 2002 to October 2003 in Oregon. The water temperatures at sampling sites in Tillamook and Yaquina Bay, Oregon were recorded between 5.6 °C to 21.4 °C from November 2002 to October 2003, and

the annual mean water temperature was 12.3 °C (17). The study suggested that the occurrence of *V. parahaemolyticus* was positively correlated with water temperature, and the low detection of pathogenic strains of *V. parahaemolyticus* were detected in sampling sites in these two bays (11, 17). Approximately 75% of the total bacteria detected from the study were collected in the summer season from June to September when the temperature was above 15°C (17).

Salinity is a measure of inorganic salts dissolved in water. Salinity is commonly expressed in the field of oceanography as parts per thousand (ppt or denoted by ‰). The average salinity of the surface water is approximately from 3.3% (33 ppt) to 3.7% (37 ppt) (24). Reddy discussed how salinity levels change with factors including "evaporation, freezing and the melting of ice, advection of more or less saline water, mixing with more or less saline water, mixing with more or less saline water, mixing with more or less saline deep water, solution of salt deposits and inflow of freshwater." Out of these factors, the most important are evaporation, precipitation, and freshwater run-off from continents which depends on numbers and sizes of rivers (49).

In Oregon, the Columbia River has an estuary that contains a well-mixed salinity. Typically, low salinity occurs near coastal waters and polar regions. The highest salinity normally occurs in places where frequent evaporation occurs. Out of four geographical locations in the world's oceans, the Pacific Northwest is considered the least saline (49). In estuaries, changes in salinity levels fluctuate from sudden addition of precipitation due to seasonal climate patterns.

In Oregon's major oysters growing beds, located in Yaquina and Tillamook Bay, the salinity of the seawater varies considerably throughout the year and ranges from 1.5 ppt to

33.3 ppt (17). The climate pattern in the coastal area of Oregon is wet during the winter including highest precipitation months from November to January, and dry during the summer season. Rivers such as the Umpqua, Coquille, and Yaquina dissect the coastal range and drain freshwater into the Pacific Ocean. Snowfall and occasional strong winds which can exceed hurricane force may also impact temperature and salinity of coastal waters. The normal annual precipitation is between 65" and 90" and the maximum amount can reach up to 200" with heavy rainfall. The level of salinity is directly associated with the mean seasonal rainfall (55). Tillamook and Yaquina bays, salinity was significantly higher from June to November, compared to December to May (17). Oysters grow best in 16 to 26 ppt, and they normally can only grow in 10 to 32 ppt (28).

Salinity levels may fluctuate seasonally and many studies have shown lack of correlation between density of *Vibrio* spp. and salinity (cited in 48). In the early stage of the *V. parahaemolyticus* study, Kaneko and Colwell concluded that there was a lack of correlation between *V. parahaemolyticus* and salinity (31). No correlation of the density of *V. parahaemolyticus* to the levels of salinity has been found in virtually all the U.S. coastal regions including Pacific Northwest (17). However, higher bacterial density was found in lower salinity level in sample sites in Galveston Bay, Texas which showed that a slightly negative correlation was occurred between the bacterial density and salinity (14).

Certain environmental parameters after harvesting of animals could provide potentially favorable growth conditions for *V. parahaemolyticus* to proliferate and cause food borne illnesses (65). The risk assessment of *V. parahaemolyticus*-caused illness by the U.S. Food and Drug Administration with regards to the outbreak from 1997 to 1998 indicated that the

bacteria multiplied to an infective dose of greater than one million organisms when contaminated oysters were exposed to elevated temperatures before consumption (61, 65).

The U.S. FDA's Bacteriological Analytical Manual to *Vibrios* suggests growing *V.* parahaemolyticus in media containing with NaCl concentrations at 2% or 3% (58). The rapid growth of various strains of pathogenic *V. parahaemolyticus* seemed to occur when levels of NaCl at 1% and 3% were added to incubation media; however, bacteria in 7% NaCl were grown inconsistently according to the bacterial growth kinetics study from Nishina and colleagues (42). The study also included the best suitable temperature condition for the bacterial growth was occurred in order of incubation temperatures at 25°C and 20°C. The FDA's guideline suggests that the optimal growth temperature for the *V. parahaemolyticus* growth is at 37 °C. In the past research studies have shown a minimum of 8 °C in the lab and 10°C in natural setting, and a maximum of 44.5°C (1, 30). Approximately 90% of viable cells of *V. parahaemolyticus* became inactive in 48°C within an hour (31).

Research Design

According to *Stats Data and Models*, an experiment means to "manipulate the factor levels to create treatments, randomly assign subjects to these treatment levels and compare the responses of the subject groups across treatment levels (12)." The research established experiments which were based on two environmental factors with multiple parameters that examine effects on the bacterial adherence of *V. parahaemolyticus* and *E.coli* to *C.gigas*. It is also a preliminary study that potentially seeks the future development of the in-vitro assay.

The LB media was used in addition to appropriate temperature and/or salinity for growing bacteria. Two temperature levels, 37°C and a room temperature of 21.5°C, the lowest temperature achievable in the circumstances of the research design, were selected. Prior to the assay development, the preliminary tests showed the bacterial growth curves up to logarithmic phases within a couple of hours. The bacteria were incubated at 37°C in Luria-Bertani (LB) media with salinity levels of 1%, 3%, 6%, and 8% NaCl. Yet, the treatment group of 8% NaCl had a very slow bacterial growth. Therefore, considering the realistic time frame for the research design, only three representative salinity treatment groups including 1%, 3%, and 6% NaCl were selected.

Designing an experiment includes four major principles: controlling sources of variation, randomizing sample surveys, replicating experiments, and setting blocking variables (12). The source of variation is factors other than what the test expects. The controllable sources of variation were identified by various preliminary tests prior to the research experiment. Some of the examples of the variation were occurred from the imbalanced mixing of homogenate, the finding of mutant strains and undesirable colony development due to

improper spread of the inoculums onto agar media. To control the imbalanced mixing of homogenates, the time and rotations of the blender were carefully managed for balancing the homogenized mixture. After reaching certain levels of optical density measures ranging from 0.65 to 0.70, the culture was inoculated on to dried agar plates and carefully spread. For controlling mutant strains, it was discussed among the Häse laboratory assistants. Controlling the source of variation is critical in the experimental design since it minimize unequal conditions throughout all treatments. Eliminating the source variations is necessary for making better overall quality of the result by minimizing biases.

Resource variability associated with unknown or uncontrollable were also considered, yet possibly caused margin of error in the study. They are the fluctuation of room temperatures throughout the period of the experiments, the nutrient contents and physical property such as temperature, moisture, pH, and salinity levels of media, and inconsistent animal samples. To manage the unknown or uncontrollable variability, the room temperature of each experiment day was monitored and recorded in the journal notebook. The media was prepared under aseptic technique. A couple of treatment groups were tested on a hot summer day when the room temperature was recorded to be as high as 26°C, and the data result from these treatment groups are not included in this document. Prior to each treatment, animals were purchased in their shells from the local seafood vendor in Corvallis, Oregon, and their appearance, such as smell, color, moisture level, and the seal of the shell were checked for freshness.

Effective temperature and time management were considered in developing the assay scheme. The U.S. FDA suggested that *V. parahaemolyticus* are capable of multiplying to an infective dose in human consumption if the bacterial contaminated shellfish products were kept in the elevated temperatures. The room temperature was maintained throughout infecting,

washing, and homogenizing stages. Since it could allow the condition for bacterial growth, effective time managements were required in order to control the source of variability and avoid any replication of the bacterial cells. An equal number of inoculates for treatments were expected and determined by pour plate methods. Once the selected optical density ranging between 0.65 and 0.70, the bacteria were immediately inoculated to animal samples. The fresh animal samples were shucked immediately in advance of use. Bacteria samples were suspended on the animal-mixture solution for thirty minutes and a total of six ten-minute washes with sterile seawater were implemented.

MATERIALS AND METHODS

According to Stats Data and Models, written by De Veaux, Velleman, and Bock (12), sources of variation should be controlled for all treatment groups. Prior to the assay development, source variables associated with temperature during the culture incubation, optical density spectrometers measures, sizes of animal samples, incubation media, amounts of sterile seawater, time for homogenization, vortex and pipeting techniques were considered and preliminary tests were conduct to minimize the source variability for each process. The major steps of the bacterial adherence assay were included measuring bacterial growth in different factorial treatments, bacterial infection to host animals, washing the infected animals, and homogenization and spread-plate counts. The length of time for each stage was developed to avoid having further bacterial growth. Two explanatory variables were temperature and salinity tested various levels in single factorial scheme. The response variable was to evaluate optimal growth condition for bacterial adherence to host animals. E.coli were tested and compared to *V. parahaemolyticus* since some *Escherichia coli* are also waterborne pathogens that reside in the colon of "humans and other warm-blooded animals." The bacteria species is often found in drinking water contaminated by fecal matters and causes illnesses such as gastroenteritis, urinary tract and respiratory diseases in human worldwide (44, 64, 66). According to Stats Data and Models, treatment refers to a "combination of specific levels from all the factors that an experimental unit receives." A total of four treatment groups were applied for testing V. parahaemolyticus, and a total of two treatment groups were applied for testing E. coli. Each treatment group was repeated three to five times.

Strains. Strains were used included the Invitrogen TOP 10 *Escherichia coli* and *Vibrio parahaemolyticus* (American Type Culture Collection 17802), which was originally isolated in

1951 during the food poisoning incidence in Japan. The frozen stocks were initially prepared by using streak plate technique, and cultures were kept in a liquid mixture of glycerol with streptomycin in a -80°F freezer for maximum of three months. Aseptic technique was used to control non-sterile environment when dealing with culture samples. According to Singleton, "Aseptic technique involves the pre-use sterilization of all instruments, vessels, media etc., and avoidance of their subsequent contact with non-sterile objects-such as fingers, or the bench top etc (53)." The strains were grown overnight at 37°C in Luria-Bertani (LB) agars with NaCl concentration of 3%, which is recommended from the U.S. Food and Drug Administration Bacteriological Analytical Manual. The powdered nutrient agar medium was added into sterile water, and streptomycin was added into the Luria-Bertani (LB) media to control the selected strains of the research interest. All media were autoclave and stored in refrigerators prior to experiments.

Logarithmic phase. The overnight cultures were transferred into the 5mL of sterile LB broth containing 5μl of streptomycin followed by an aseptic technique. Growths of *V. parahaemolyticus* and *E. coli* strains under five different treatment conditions are measured. *V. parahaemolyticus* was grown in LB media at 21.5°C and 37°C containing 3% NaCl and various concentrations of NaCl adjusted to 1%, 3% and 6% at 37°C. *E. coli* were grown at two different temperatures of 21.5°C and 37°C and NaCl was not added for the bacterial growth. A fixed thermo incubator of 37°C was used, and 21.5°C was average room temperature in which rotators were place. Optical densities (OD) were measured at 600 nm using a spectrophotometer. The spread plate method was occasionally used once or twice per hour during the bacterial growth for obtaining number of bacterial colonies. Strains were grown up to the OD levels between 0.65 and 0.70.

Animal samples. Five to ten *Crassostrea gigas*, commonly known as Pacific Oyster were purchased from a local seafood vendor in Corvallis, Oregon for each treatment performed and 25 animals were used from February to June, 2007. These animals were kept in their shells and maintained on a sterile tray in a refrigerator for the freshness before each treatment. Animals were shucked prior to inoculations using a dissecting tray and shellfish knife. The stomach samples of animals were infected with selected dose amount of inoculates. Stomach portions of animals were dissected on a sterile tray and a sterile oyster knife. The color of the stomach is dark, located in the center toward the hinge and surrounded by the mouth, adductor muscles, gills, mantles, and digestive glands/ liver and intestine (2, 54). The stomach samples were weighed after shucking. The samples were then, suspended in 20 ml of sterile seawater prior to the bacterial infection in a 50 ml conical tube. The sea water was provided from Hatfield Marine Science Center (HHSC), Newport, OR.

Bacterial infection. The bacterial growth was terminated after the optical density (OD) reached between 0.65 and 0.70. A 200μl of the culture was inoculated into a 20 ml of the sterile seawater where the animals' stomach sample was suspended. The solution mixture was rotated with a constant rate for 30 minutes at an average room temperature, 21.5°C. Serial dilutions and the spread-plate method were immediately followed once the bacterial growth was terminated in order to count the number of bacteria. To minimize further bacterial growth, 30 minutes were applied since the growth of *V. parahaemolyticus* or *E. coli* was remained in lag phases. This was examined from the preliminary test of the assay development.

Wash. A total of 6 washes per hour with 20 mL of sterile seawater per wash was applied. The solution mixtures were kept on a constant rotation at an average room temperature at 21.5 °C.

Homogenization. The mixture samples were homogenized in an 1:1 ratio of the weight of the animal sample and the sterile seawater for 30 seconds in a sterile metal container (MCI mini 12m-37ml containers 700S, 700G, manufactured by Warning Co.) designed for 0.4 HP and 22,000 no load RPM. For detecting the number of bacterial isolates, serial dilutions and spread-plate count methods were followed immediately when the homogenization was completed. The culture was incubated overnight at 37°C, and the countable number of colonies was measured in the following day.

Statistical analyses. The data result and research design were critically evaluated by Cliff Pereria, Carole Abourached and Aimee Taylor from the OSU statistical department in page 107. The result of the number of colony forming units (CFU) for both inoculates and bacterial isolates was analyzed with a TIBCO Software, S-PLUS 7.0 Professional Developer. The single factor analysis of variance (ANOVA) was conducted separately on the data collected from January, 2007 to June, 2007. The mean values of the inoculates and the bacterial isolates were compared with P-values calculated from the ANOVA test. Results were estimated significant at a P value of \leq 0.05. Testing for the 95% confidence interval of the slope between the number of inoculate and bacterial isolates was approached by an online statistic guideline, Bernard Liengme's Regression Analysis - Confidence Interval of the Slope, and was initially recommended by Taylor in her statistical analysis report (36).

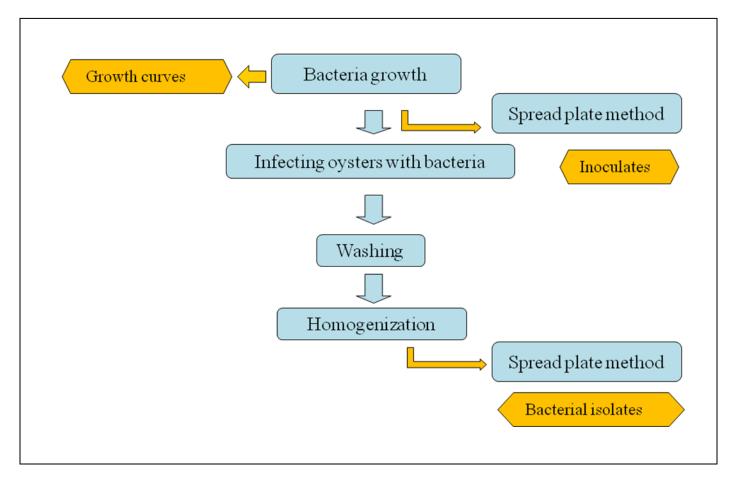


FIG. 2. Assay scheme

DATA

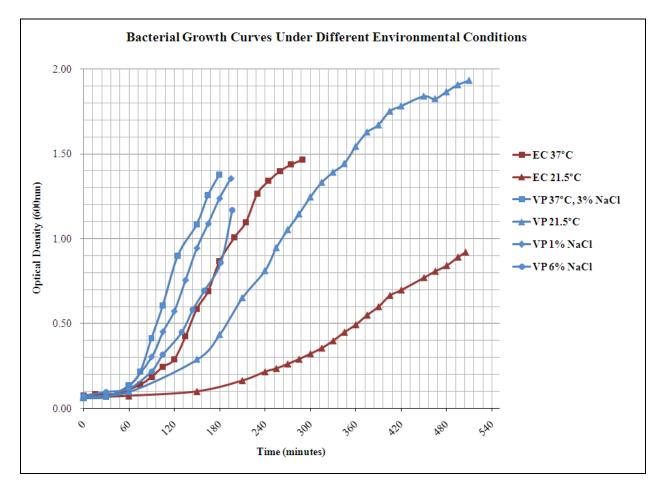


FIG. 3.The bacteria growth under different environmental conditions The growth curves were measured under six different growth conditions and different time sequences with optical density (OD) at 600nm. The logarithmic phases between OD of 0.65 and 0.70 were detected in each measure.

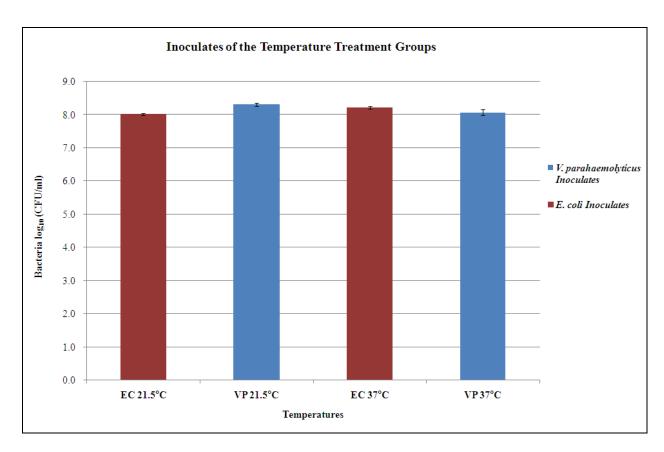


FIG. 4. Comparison of the number of *E. coli* and *V. parahaemolyticus* inoculates in *Crassostrea* gigas from the temperature treatments The mean values of the initial uptake with standard errors (error bars) from temperature treatments are listed. The number of *V. parahaemolyticus* inoculate is not significantly different at a *P* value of 0.062 and *E.coli* is significantly different at a *P* value of ≤ 0.05 . Comparing *V. parahaemolyticus* inoculates and *E.coli* inoculates from temperature treatments, it is not significantly different a *P* value of ≥ 0.05 .

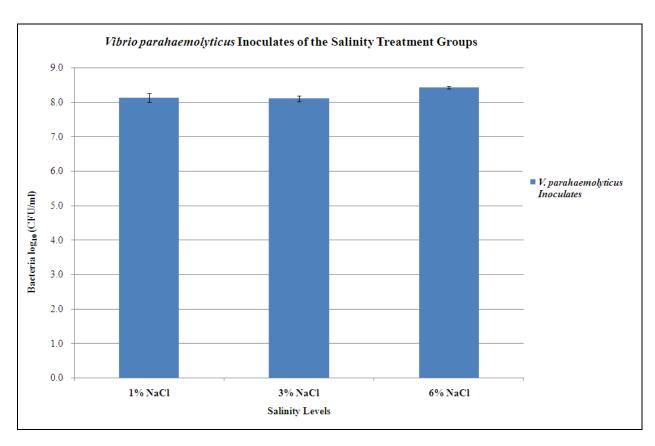


FIG. 5. Comparison of the number of V. parahaemolyticus inoculates in Crassostrea gigas from the salinity treatments The mean values of the initial uptake with standard errors (error bars) from salinity treatments are listed. In salinity treatment, the number of V. parahaemolyticus inoculate is not significantly different among the results from three different levels at a P value of ≥ 0.05 .

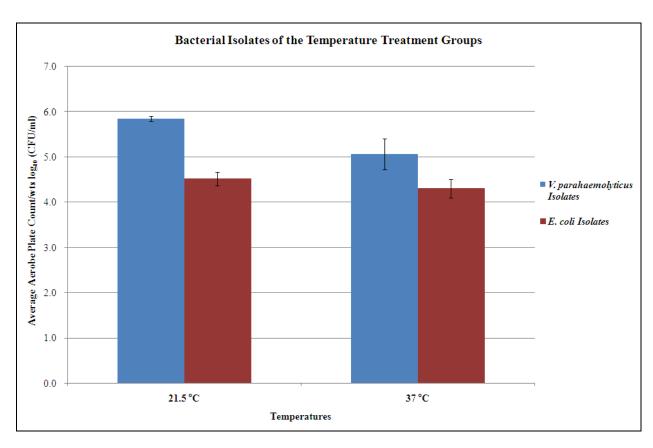


FIG. 6. Comparision of the number of bacterial isolates as a result of the different temperature treatments In temperature treatment groups, the number of V. parahaemolyticus isolates is not significantly different at a P value of ≥ 0.05 and E.coli is not significantly different at a P value of ≥ 0.05 . Comparing V. parahaemolyticus inoculates and E.coli inoculates from temperature treatments, it is significantly different a P value of ≤ 0.05 .

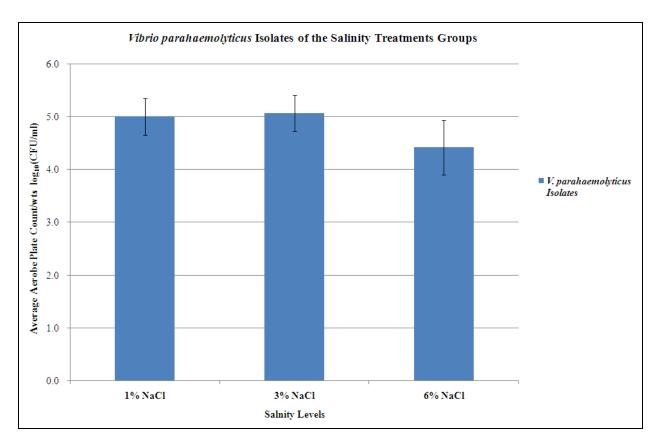


FIG. 7. Comparision of the number of *V. parahaemolyticus* isolates as a result of the different salnity treatments. In salinity treatment, the number of *V. parahaemolyticus* inoculate is not significantly different among the results from three different levels at a *P* value of ≥ 0.05 .

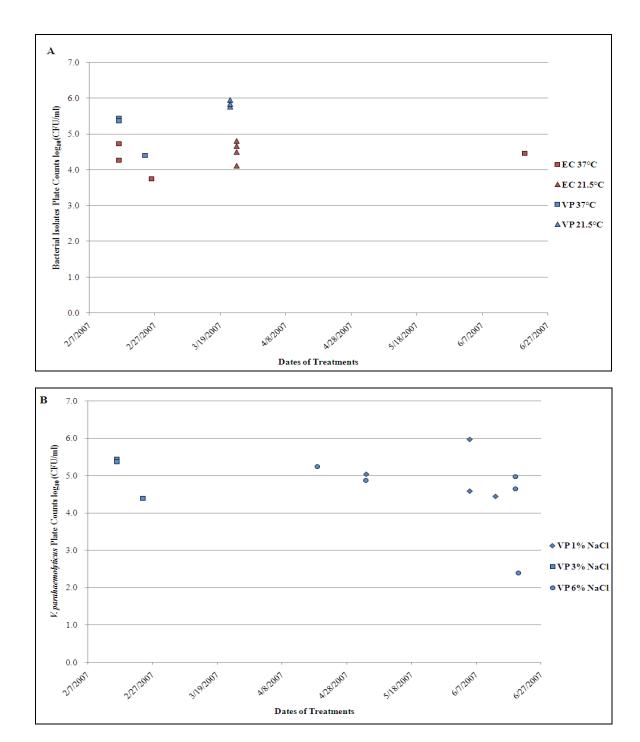


FIG. 8. (A,B) Distribution of aerobe plate counts of bacterial isolates over different dates of temperature and salinity treatments The number of bacterial isolates from different dates were calculated after temperature treatment in figure A and salinity treatment in figure B.

TABLE 1. Total average time spent for the logarithmic phase detection between Optical Density (OD) 0.65 and 0.70

Bacterial Growth Factors —		Average time (minutes)		
		Vibrio parahaemolyticus	Escherichia coli	
Tomporoturas	37°C	105 - 117	153 - 171	
Temperatures	21.5°C	204 - 228	390 - 436	
Salinity Levels	1% NaCl	123 - 135	N/A	
	3% NaCl	105 - 117	N/A	
	6% NaCl	147 - 165	N/A	

TABLE 2. Aerobe plate counts of inoculates and bacterial isolates

	Total aerobe plate count (CFU/ml)				
Inoculates of different					
treatments	Inoculates before infection	Isolates after infection			
E.coli 37°C	$1.04 \times 10^8 \pm 1.60 \times 10^7$	$1.79 \times 10^5 \pm 1.19 \times 10^5$			
E.coli 21.5°C	$1.69 \times 10^8 \pm 2.67 \times 10^7$	$2.52 \times 10^5 \pm 1.24 \times 10^5$			
V. parahaemolyticus 37°C	$1.18 \times 10^8 \pm 3.63 \times 10^7$	$1.12 \times 10^6 \pm 8.57 \times 10^5$			
V. parahaemolyticus 21.5°C	$1.60 \times 10^8 \pm 2.88 \times 10^7$	$4.80 \times 10^6 \pm 1.08 \times 10^6$			
V. parahaemolyticus NaCl 1%	$2.17 \times 10^8 \pm 1.51 \times 10^8$	$1.90 \times 10^6 \pm 2.61 \times 10^6$			
V. parahaemolyticus NaCl 3%	$1.18 \times 10^8 \pm 3.63 \times 10^7$	$1.12 \times 10^6 \pm 8.57 \times 10^5$			
V. parahaemolyticus NaCl 6%	$2.83 \times 10^8 \pm 2.69 \times 10^8$	$5.20 \times 10^5 \pm 3.88 \times 10^5$			

TABLE 3. The 95% confidence interval tests for the slopes of bacterial isolates and inoculates

Bacteria	Temperature Treatments		Salinity Treatments		
Dacteria	37°C	21.5°C	1% NaCl	3% NaCl	6% NaCl
V nanaha omobiti ora	No ^a	No ^a	No ^a	No ^a	Yes ^b
V. parahaemolyticus	3.89 ± 0.95^{c}	-0.70 ± 0.70^{c}	-0.94 ± 1.49^{c}	3.89 ± 0.95^{c}	-0.59 ± 1.64^{c}
E. coli	Yes ^b	No ^a	N/A	N/A	N/A
E. cott	0.64 ± 0.90^{c}	3.13 ± 0.64^{c}			

^a The slope of 1 is not in the 95% confidence interval

^b The slope of 1 is in the 95% confidence interval

^c The slope of the 95% confidence interval

^{*95%} of the values are within two standard deviations

RESULTS

A total of 6 bacterial growth curves were taken up to selected ranges of OD from 0.65 to 0.70. The serial dilution was taken immediately for measuring the number of inoculates applied to animal samples. In the result from the bacteria growth in six different treatments (Fig 3), *E.coli* generally grew slower than *V. parahaemolyticus*. The average time for determining the optical density between 0.65 and 0.70 started as early as 105 minutes for *V. parahaemolyticus* grown at 37°C and as late as 436 minutes for *E. coli* grown at 21.5°C (Table 1). The number of inoculates and bacterial isolates were collected from a total of 25 treatment groups between February 1st and June 20th in 2007 (Figure 4 and Figure 5). The average numbers of inoculates and bacterial isolates per gram stomach sample of animals were calculated with base 10 logarithm.

Out of a total of 13 temperature treatment groups, each 21.5°C and 37°C treatments for *V. parahaemolyticus* comprised 3 tests and *E.coli* had 4 tests per temperature treatment. The number of bacterial isolates per gram sample from each treatment was calculated with base 10 logarithms. A total of 12 treatment groups were based on three different salinities. Four tests salinity treatments group of 1% NaCl, 3 tests for the concentration of 3% NaCl and 5 tests were for the concentration of 6% NaCl. The data of the 3% NaCl treatment group of *V. parahaemolyticus* was repeated from the result from the 37°C temperature treatment since bacterial growth conditions for both treatment were identical.

The number of logarithmic phase bacteria was initially detected and listed in figure 4 and figure 5. For all temperature treatment groups, on average, $1.57 \times 10^8 \pm 4.16 \times 10^7 \text{CFU/ml}$ of *V. parahaemolyticus* inoculates and $1.36 \times 10^8 \pm 5.35 \times 10^7 \text{CFU/ml}$ of *E. coli* inoculates were exposed to a mean weights of 6.53 ± 0.19 g and 6.61 ± 0.09 g host animals respectively.

The number of *V. parahaemolyticus* inoculates was not significantly different at a *P* value of 0.062; however, the number of *E.coli* inoculates were significantly different at a *P* value of ≤ 0.05 . In the salinity treatment group, the average number of *V. parahaemolyticus* inoculates was $1.92 \times 10^8 \pm 5.09 \times 10^7$ CFU/ml and the mean weight of host animal was 6.69 ± 0.23 g (Table 2). There was no significant difference among data results from different salinity treatments of *V. parahaemolyticus* at a *P* value of ≥ 0.05 .

The average aerobe plate counts of *V. parahaemolyticus* isolates per gram sample with logarithm to the base of 10 including different temperatures and salinities treatments are compared in figure 4 and figure 5. Average number of *V. parahaemolyticus* isolates per gram samples of base 10 logarithm were $4.86 \times 10^6 \pm 1.08 \times 10^6$ CFU/ml from the 21.5° C treatment, $1.12 \times 10^6 \pm 8.57 \times 10^5$ from the 37° C treatment. The average number of *E.coli* isolates from 21.5° C and 37° C treatments were $2.52 \times 10^5 \pm 1.24 \times 10^5$ CFU/ml and $1.79 \times 10^5 \pm 1.19 \times 10^5$ respectively (Table 2).

Comparing the number of isolates from the two temperature treatment groups between V. parahaemolyticus and E.coli, more number of bacterial isolates were generally found from the V. parahaemolyticus group. The difference was significant at a P value of ≤ 0.05 . For both V. parahaemolyticus and E.coli, the 21.5°C treatment group showed higher number of bacterial isolates compared to the number of isolates from 37°C treatment group. Approximately 4.3 times more V. parahaemolyticus isolates and 1.5 times more E. coli isolates were found in the 21.5°C treatment group compared to the 37°C treatment group. However, no significant difference was found in the number of isolates when comparing each temperature group of the two strains (Figure 6).

Average number of bacterial isolates per gram samples of base 10 logarithm from 1%, 3% and 6% NaCl treatments were $1.90 \times 10^6 \pm 2.61 \times 10^6$ CFU/ml, $1.12 \times 10^6 \pm 8.57 \times 10^5$ CFU/ml, and $5.20 \times 10^5 \pm 3.88 \times 10^5$ CFU/ml respectively (Table 2). The result from the concentration of 6% NaCl treatment group was slightly lower than the other two treatment groups. However, the average number of bacterial isolates from the three different salinity treatments had no significant difference at a P value of ≥ 0.05 (Figure 7).

The 95% confidence level test was conducted to examine the null hypothesis, the slope of the numbers of inoculates and isolates of each treatment to be 1. The test comprised the numbers of bacterial isolates (*y*-units) and inoculates (*x*-units), and the linear relationship of the two variables was compared in Table 3. The result showed positively to the 95% confidence interval test were *V. parahaemolyticus* grown in 6% NaCl and *E. coli* grown at 37°C, and these groups were tested in different dates from February to June, 2008. The result from these two groups showed the 95% confident correlation between numbers of inoculates and bacterial isolates. There was no linear regression among the data results from other treatment groups since the result from the analysis showed no strong correlation.

DISCUSSION

Both temperature and salinity are important factors that control sea water densities (49). Singleton explained that fluctuations of environmental parameters, such as temperature, salinity, and pH, influences bacterial growths (53). Many researchers have studied the relationship between abundance of bacteria and changes in temperature and salinity. A number of studies explain broad temperature ranges of *V. parahaemolyticus* growth in *Crassostrea gigas* in both pre- and post-harvesting conditions. Permissible growth temperatures for *V. parahaemolyticus* ranges from a minimum of 8°C to a maximum of 44.5°C (1, 30). Horie indicated in 1966 that 37°C is an optimal temperature for growing *V. parahaemolyticus*, and it is also known as a human body temperature which is frequently used for microbiology laboratory practices for culturing *Vibrios* (30).

According to the U.S. Food and Drug Administration (FDA)'s Bacteriological Analytical Manual for *Vibrios*, 2% or 3% NaCl was suggested to grow *V. parahaemolyticus* under media conditions. The salinity in estuaries fluctuates majorly due to local heating and cooling cycles, run-off, coastal upwelling and advection (49). In Oregon, the dry summer and wet winter climates occur near coast lines, and Duan and Su reported that the occurrence of *V. parahaemolyticus* in the two oyster growing bays indicated 1.5 ppt to 33.3 ppt salinity levels throughout the year (17). The higher abundance of the bacteria is typically found during summer seasons when warmer temperatures and various ranges of salinity levels are found in the ocean water. The different effects on the bacterial adherence in *C. gigas* were initially hypothesized since these environmental conditions seem to affect the bacterial growth as well as for host animal interaction.

Data Analysis

A total of three or more repeated tests were done per each treatment, and the representative growth curves were measured (Figure 8 A and B). Under different environmental parameters, faster growth of both *E. coli* and *V. parahaemolyticus* generally occurred at 37°C. *V. parahaemolyticus* grew faster than *E.coli* under equal growth conditions of temperature and salinity level (Figure 3 and Table 1). As the U.S. FDA Bacteriological Analytical Manual suggested, *V. parahaemolyticus* were able grow in three selected NaCl concentration from 1% to 6% at the incubation temperature of 37°C in LB media. As a result, the rank orders of the bacterial growth are the NaCl concentration of 3%, 1%, and 6% with 3% being the fastest and 6% being the slowest.

Bacteria were grown under different environment parameters with different growth rates. The overall result showed lack of significant effects due to these growth conditions on the bacteria adherence in *C. gigas* since each *P* value test of the treatment group suggested no significant difference. The result from temperature treatments of 21.5°C and 37°C showed that *V. parahaemolyticus* isolates were generally ten times more than *E. coli* isolates. Comparing the number between inoculates before the oyster infection and bacterial isolates after the oyster infection, the number of *E. coli* and *V. parahaemolyticus* strains in temperature treatment were decreased approximately to a thousandth and a hundredth respectively. Therefore, more number of *E. coli* was washed out, and better adherence of *V. parahaemolyticus* to *C. gigas* was made from the temperature treatment group.

For testing linear regression, Pereira and her graduate students, Abourached and Taylor, suggested for running a 95% confidence interval test to evaluate whether the rate of

change in 'y-units' per 'x-units' was close to 1. With the analysis, we were able to interpret how different temperatures or salinity levels could affect and associate with bacterial adherences in *C. gigas*. The test is necessary to examine whether outliers or influential points distorted the data model. If the slope is in the 95% confidence interval of the slope of one, the correlation of the number of inoculates (*x-units*) and bacterial isolates (*y-units*) could be safely compared. However, if the slope is out of the 95% confidence ranges, neither the correlation nor the comparison of the two variables can be preceded to make strong results and data analysis. The complete result from the regression analysis is found in Table 3.

Majority of the results from treatment groups showed the slope was not close to one and did not validate the 95% confidence test (Figure 6 and Figure 7). This means that the data result had low precision, the research showed no special effect on the bacterial adherence due to temperature and salinity levels. According to *Stats Data and Models*, this approach was yet limited in the interpreting whether the bacterial adherence was due to the association of the two selected variables since having the association do not mean one of the variables causes the other variable (12).

Pereira and her graduate students states, randomizing the sample surveys in times or spaces and setting blocking variables necessary are important principles when designing an experiment. The two out of seven treatment groups had slopes ranging within the 95% confidence intervals of the slope of 1 (Table 3). Those groups were *V. parahaemolyticus* grown in 6% NaCl and *E. coli* grown at 37°C in different dates from February to June, 2008. They indicated 95% the confident correlations between inoculates and bacterial isolates. Considering the tests were conducted in different dates, data among inoculates and bacterial

isolates from those groups were sampled randomly and safely described that those variables are correlated.

Except the result analysis of the two treatment groups, the rest of the treatment groups had no validation through 95% confidence interval tests, and the slope of *x*-units and *y*-units were not close to one (Table 3). High variations in data distributions caused them not to pass the 95% confidence interval test. Lack of strong interpretation was made thereby limited in explaining the effect of bacterial adherence due to selected temperature and salinity parameters for bacterial growth. There was no significant effect on the bacterial adherence in *C. gigas* under the selected temperature and salinity levels.

Errors

According to Pereira and her graduate students, day effects were concerned due to lack of management to some unknown or uncontrollable source of variation, such as moisture levels and temperatures in the room where the experiment was performed in different time and space. Although appropriate sample size and temperature was carefully monitored throughout the study, some data were thrown out because of incomplete test results due to human errors. The final analysis report from Pereira and her students argued that the data collected from the same and different days were significantly different (Figure 4 and Figure 5). Within treatment groups, replicates of some treatments were done in the same day, and this might be responsible for a possible day effect and the overall result could be biased.

Another important factor, which might contribute the higher margin of errors, is the source of variation among animal samples. The average weight of 6.66g animal sample was used. The animal's defensive mechanisms (humoral and cellular) against microorganisms were

ignored since the experiment was designed conditions. Although equal weight of an animal sample was used per treatment group, general characteristics of the sample, such as origins, sizes, ages, or freshness were not recorded. The animal's defensive mechanisms (humoral and cellular) against microorganisms were ignored since the experiment was designed conditions. According to Otwell, the effective wash highly depends on how the animals were grown and might vary within samples based on the growth conditions in which how temperature, salinity, pH, turbidity, and water inflow rates were placed. No constant water inflow was made during the washing period, other than the six run rotations with sterile seawater. Although the washing was not done as similar as the conventional depurification practice, various environmental factors prior to harvests of the animals could still be considered. Variation within the animal sample were not clearly known or addressed during the investigation, and this could potentially cause for errors.

The research design initially implemented equal numbers of five replicates per treatment; however the average number of replicates was between three and five in this document since some data was discarded because of experimental errors. Those errors are associated with imbalanced mixing of homogenates and undesirable colony formations. Less number of replicates of tests and smaller number of sample sizes could potentially influence to have higher margin of errors to the result.

Human errors, such as mishandling of laboratory equipments and mismanaging time during experiments, might have caused potential errors. For instance, the time was delayed during the period between terminating the bacterial growth and inoculating into the animal sample solution. On a few occasions, shucking animals were taking too long prior to the infection, or the examiner had frequent mistakes when multiple tests were performed at once.

Due to these incidences, if unequal amounts of inoculates were possibly allocated for pour plating and infecting animal samples, the cell growth might not properly terminated, but rather be continued. Consequently, the number of inoculates that were suspended in the sample solution and other inoculates that were counted by the pour plate method might not be identical and be responsible for causing errors.

CONCLUSION

The genus *Vibrio* belongs to the family *Vibrionaceae* (59). *Vibrio parahaemolyticus* was initially discovered in the 1960s during the major food poisoning incidences in Japan (22). It is gram negative, facultative anaerobic with the size is between 0.5 nm and 0.8 nm wide and 1.4 nm and 2.6 nm long (52). It is not fecal-originated and naturally occurs in either temperate regions in deep sea or near shore marine waters including estuaries (3, 10, 23, 63). Unrevealing the new discoveries about the bacteria has continued, and experts have been saying that the bacteria might overcome its limited environmental conditions while evolving with their neighboring marine species over decades. The bacteria interact closely with marine organisms such as zooplanktons, crustaceans, mollusks and fish (16, 41). Studies have shown that the abundance of the bacteria could be found in the body of hosts including farmed animals, such as blue crabs from Chesapeake Bay (57), and roots of *Spartina alterniflora*, salt marsh grasses in Atlantic and northern Gulf Costs (10). These findings could be evidence to the mutual relationship between the bacteria and theses marine hosts (57).

Both pathogenic and non-pathogenic *V. parahaemolyticus* exist. The pathogenic form cause bacterial infections in shellfish, particularly oysters (16). The bacterial infections due to consumption of raw or improperly cooked oysters have been gradually increased seasonally in both the United States (7, 14, 16, 57, 58) and foreign countries such as those in Asia and Europe (35, 37, 60). Oysters are popular for the good sources of beneficial nutrients such as calcium, phosphorus, zinc, and relatively low cholesterol (16). Yet, the seafood safety management is the one of the key concerns of shellfish industries. To ensure public safety, the industries follow the governmental regulations and strict management policies as well as implement the newest technology to improve the quality of seafood products (16). Removing

Vibrio spp. has been particularly challenging to the modern technology, including depuration practices (43). Therefore, developing novel molecular technology to understand the mechanism of bacterial adherence to animal hosts, particularly oysters, is emphasized by researchers such as Dr. Claudia Häse in the department of Biomedical Sciences at Oregon State University (29). This research is a preliminary study to develop an assay that designed to be implemented for the basis of the Häse laboratory study.

The main objective was to develop an assay which could potentially examine optimal growth conditions for these bacteria of several different temperatures and salinity levels. The single factorial design of the two variables, temperature and salinity was implemented. A total of seven treatment groups include temperature treatment group of 21.5°C and 37°C of *V. parhaemolyticus* and *E.coli*, and salinity treatment group of 1%, 3%, and 6% NaCl of *V. parahaemolyticus*. The data results from *V. parahaemolyticus* treated at 37°C are also used as the results of the 3% NaCl treatment, since both treatments shared the identical bacterial growth conditions.

Four major steps include culturing bacteria under different environmental factors; inoculating the bacteria into animal stomach samples; washing the infected animals periodically; and homogenizing, followed by pour plate counts. The optimal growth conditions for *Vibrio parahaemolyticus* were at 37°C with 3% NaCl in Luria-Bertani (LB) media. From the result of this study, there was no significant difference in bacterial adherence among the two temperatures and three salinity treatment groups. Consequently, the study was not able to find optimal bacterial growth conditions for the oyster infection.

Effective time and temperature management were emphasized to control source of variation throughout the experiments. There was no strong precision of the ratio of the number of inoculates and bacterial isolates, since only two out of seven treatments were reached within the range of the 95% confidence intervals of the slope of one in the result of the regression test. In other words, the results of each treatment group were not consistent throughout, which caused the limited data interpretation of bacterial adherence relating to the two environmental factors. For future recommendation, preliminary studies should be carefully organized in order to minimize source of variability whether they are controllable or uncontrollable. Possible errors should be eliminated under realistic goal plans. Also, consistent numbers in each treatment and random blocking should be considered.

This research sets a foundation for the future designing of an assay of *V.* parahaemolyticus adherence in *C. gigas*. The study is based on the Claudia Häse Laboratory's investigation on the specific adherence mechanisms of *V. parhaemolyticus* to oysters. The thesis could be useful for student researchers whose study might primarily focus on molecular aspects of science relating to bacterial adherence in marine organisms. It anticipates learning about the pathogenic *V. parahaemolyticus* and host animals, such as *C. gigas* as well as improving seafood safety in regard to human consumption. Lastly, the study provides more insights to the current issues of bacterial contaminations in shellfish products.

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Exploratory Study on the Impacts in Economy and Culture due to
Vibrio parahaemolyticus Infections in Pacific Oyster (Crassostrea gigas) in Tong-young
South Korea

INTRODUCTION

Observation: Journal Log in Fall 2007 during Tong-yeong Visit

The sunset reflected on the ocean and white Styrofoam floats were arranged neatly along with long wooden bars in the day of my second trip to Tong-yeong. I figured somebody must be busy managing the fishery farms. Seeing more floats after floats, the ocean seemed to be the land for bountiful opportunities for old fishermen. As I had heard and read about the city before my departure, there were small islands surrounding the city of Tong-yeong. When I got close to the final destination in the intercity bus station, I was ready to explore the new town, located in south eastern end of the Korean Peninsula.

I stayed at a motel near the bus station for a night and noticed that the clerk carefully glanced at me. She asked how old I was and why I was visiting the town. After showing her respect and answering her question clearly, she seemed relieved and gave me the key to a room. I had several incidences in which Koreans mistook me for a teenager. I understood her intention, to figure out the young woman's trip agenda and responded her in a positive manner.

The day of my visit was to interview Mr. Jeong, who has owned an oyster business for forty years and lived in Yong-hori Kwangdo-myen, Tong-yeong. Next morning, I woke up early and walked around the streets. I stopped at a public information center. A staff spoke with heavy regional dialect and explained the city bus schedule. She also mentioned that the town was busy preparing for the upcoming annual General Lee's marathon race which was held in early December. According to her, many people were excited, and the city was expecting lots of tourists from other regions. As I indicated my plan of entering the race, she urged me to reserve a cheap place to stay prior to the race.

I decided to grab brunch and stopped by the restaurant close to the bus station. I remember reading a traveler's guide book mentioning about the *Chungmu Kimbab*, one of the most popular seafood dishes in the Tong-yeong region. It included rice roll covered with dried seaweed and served typically with squid salad and radish Kimchi. I often took photos of my food before I started tasting them, and I wasn't shy whether others in the same room would carefully look at my actions.

For strangers whom I came across, I might have been seen differently by my appearance and speaking the Korean language. Although I am bilingual in English and Korean, people in Gyeongnam province often speak the Korean language with the Gyeongnam regional dialect which is different from my own. As I commonly asked questions to strangers, I had hard a time understanding people's conversation when certain tones or fast speech which were associated with the regional dialect. I thought of how others might not understand my accent in Korean language as well.

Buses were running on schedules and only ran a few times a day, so I had to watch out in not missing the right one. I was a bit nervous waiting for the bus, seventy seven since I had to solely rely on the advice from the staff from the information center and the bus schedule was not documented anywhere. While waiting, I observed the public scenes and the general public.

At the station, there were many students commonly wearing school uniforms, and some elders were waiting for their buses with a few pieces of baggage. Some of them carried white Styrofoam containers tied with strings, which usually meant that they got fresh fish products inside. I had several experiences taking buses with some people carrying those containers, which ended up causing the smell to spread in the entire room of the bus. Although

I wanted to fully experience the public culture, I remember trying to hold my breath after avoid to breath the fish smell.

The rate for the city bus was inexpensive, less than a dollar per one-way ride to an unlimited distance within the bus route. Taking public transportation was beneficial since I got to see the town and a few times I was very lucky in that I interacted with riders or bus drivers and got free tour guides. While the bus was going through the downtown, General Lee Soon Sin's statues or cartoon logos frequently appeared and was the city's central mascot. As it was mentioned earlier, people in Tong-yeong seemed to be busy getting ready for the annual marathon race happening in a month.

General Lee Soon Sin (1545-1598) was a naval commander who lived during the *Joseon* era, the last kingdom of Korea before the Japanese's invasion in 1900s. Since then, Koreans have honored and celebrated his birthday annually. General Lee was a famous hero in Korean history, and he made numerous victories from the naval battles took in the southern coast of Tong-yeong. Where the bus entered the downtown, a large LCD screen was sitting on the top of a tall building and showed numerous advertisements of sight-seeing and regional art and craft, as well as seafood products including Pacific Oyster.

As the bus got into the residence area, modern style tall buildings and apartments appeared and seemed to explain about urban developments. According to City Hall of Tongyeong, the city has become more popular region for migrations of new residents, because geologically the city was surrounded by ocean and mountains, and abundant natural resources. I saw numerous restaurants around the residences.

When I tried dining out at a restaurant that mainly served oyster dishes, some restaurants owners had gotten awards for the best practice from City Hall or the chamber of

commerce which often attracts more customers. According to a Ministry of Marine Affairs and Fisheries (MOMAF) agent, it has been a popular trend for people to run a franchised oyster restaurant business in town and across the nation as the popularity of Pacific Oyster has risen. In addition, if the restaurants were advertised or appeared in radio or TV, more customers would visit and aid the success of the businesses.

On the way to the small oyster harvest, many small family farms were along the hillside of the mountain. The bus driver gave me a notice indicating the next station was my stop. When I got off, I saw well organized fishing equipment and a fisherman's boat standing near the deck. Where the water was closely attached to the inland, groups of anchovy swam and numerous empty oyster shells were laid in the water as if they were waiting for new larvae to take over. There were piles of oyster shells making a big mountain next to the harvesting facility. A man came out and invited me over to his shucking facility. When I asked whether he was Mr. Jeong, the owner of small scale shellfish business, he agreed and we started a conversation by mentioning my relation to his daughter since I have learned that position and relationship seemed to be important in business settings in Korea.

About a month later, I visited the town again for the ten kilometer race. When I was close to getting off from the bus, the bus driver cheered me to arrive there before the race starts. There were a total of fifty five hundred participants and I ran for ten kilometer in downtown next to Tong-yeong bays. I was surprised to see that there was a concert, and mostly entertainers were singing and dancing right next to the arrivals of the runners. Some people who appeared to be a group or members from the community played *Samulnori*, a band playing traditional percussion music while others seemed to enjoy their time entering the talent shows and singing contests.

For outsiders like me, it was bit of noise to fully relax my muscle after the long race. Further down from the arrival, there were booths serving free oyster dishes. Right next to the food serving tables, I saw a workshop on how to shuck oysters, and competitions were happening among the participants including kids and adults. A representative from the regional oyster cooperatives spoke loudly with a microphone announcing to the crowds about the oyster cooperative's campaign, "Eat oyster, love longer." Various other health aspects from consuming oysters were discussed in order to attract more participants for the event.

On the other side of the announcer, there was the regional representative of the Tongyeong Pacific Oyster served the numerous dishes with other volunteers. A total of eight oyster
dishes were presented. Little kids carried bags of dried anchovy and seemed to be having fun
eating oysters, therefore, I decided to give it a try. My favorite was the oyster pancake. The
participants emptied out their dishes after they were done eating and organized the recycled
ones, near the banner next to Tong-yeong oysters. The empty dishes were recycled and looked
very neat and clean.

With all the noises and food, the day was bright for all the participants from the race and was truly became the festival of Pacific Oyster. As I thought of my relatives in Inchon, S. Korea and friends and family in the U.S., I wished I could enjoy the moment that I had in Tong-yeong with them. And perhaps show them how they could be amazed with the creative cooking ideas for oyster dishes.



FIG. 9. Tong-yeong Bay in November 2007 Photo taken by Jee Lee



FIG. 10. Volunteers serving rice-cake soup served with Pacific Oyster during the 2007 annual General Lee Marathon Race held in Tong-yeong, South Korea Photo taken by Jee Lee

Introduction to Tong-yeong, South Korea



FIG. 11. The scenery of Tong-yeong cited on the City of Tong-yeong website in 2008

The population of Tong-yeong was approximately 140,397 in an area of 234.83km² in 1999. The population growth rate has been slowly approached to 1.3% in the last ten years. Male population was 50.3%; female was approximately 49.7%. Mostly ethnic Koreans are living in the city and foreigners were recorded approximately 0.8% of the city's total population. Primary industry (agriculture and fisheries) groups were 62.9%, next to secondary was 7.4%, and service industry represented as 29.7% of the total distribution of the city's industry.

¹ Kim Yŏng

¹⁹⁹⁹ T'ong'yŏng Jiyŏk ui Kong'gan kujo wa Jiyŏk Kaebal Jŏl'yak (Development of spatial structures and region planning in T'ong'yŏng). *In* T'ong'yŏng Kŏjae Jiyŏk Yŏn'ku (The research study on T'ong'yŏng and Kŏjae regions). Pp.133-163. Kyungnam University Gyŏngnam Ji'yŏk Munjae Yŏn'gu'won (Kyungnam University the center of the research on the problems of the Gyŏngnam region), Masan: Kyungnam University Press.

Primary industries are agriculture and fisheries and secondary industries such as ship building as one of the major exporting businesses.² The city's economy in particular, recreation and tourism are heavily influenced by the recent global trend of tourism. According to a World Trade Organization (WTO) report, there was an 8.8% increase in the number of the travelers from overseas in South Korea from 1997 to 1998 and a total number of 42,500,000 foreign travelers visited in 1998.³

Both geographic features and climate patterns in southern coast of Gyeongnam province have allowed ideally for the large production of fisheries for both national and international consumption. The coast line is called the Rias Coast which means highly indented, and the length of the total coast line is 616,800km. The city of Tong-yeong includes fifty uninhabited and ninety inhabited islands.

The average winter water temperature is 2.1 °C. The average water temperature in August is 14.7 °C with an average rainfall of 1,358mm and this is known as relatively higher, compared to other regions in South Korea. The region has the most daylight compared to other regions in the nation.⁴ During the observational study in field sites, the open-ocean seemed to be one of the popular habitats for the growing of oysters in the town.

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² Kim Yŏng

¹⁹⁹⁹ T'ong'yŏng Jiyŏk ui Kong'gan kujo wa Jiyŏk Kaebal Jŏl'yak (Development of spatial structures and region planning in T'ong'yŏng). *In* T'ong'yŏng Kŏjae Jiyŏk Yŏn'ku (The research study on T'ong'yŏng and Kŏjae regions). Pp.133-163. Kyungnam University Gyŏngnam Ji'yŏk Munjae Yŏn'gu'won (Kyungnam University the center of the research on the problems of the Gyŏngnam region), Masan: Kyungnam University Press.

³ Son Haeshik

¹⁹⁹⁹ T'ong'yŏng Jiyŏk ui Kong'gan Hwalsŏng'haw Bang'an (Planning the spatial revitalization in the T'ong'yŏng region). *In* T'ong'yŏng Kŏjae Jiyŏk Yŏn'ku (The research study on T'ong'yŏng and Kŏjae regions). Pp.163-192. Kyungnam University Gyŏngnam Ji'yŏk Munjae (Kyungnam University the center of the research on the problems of the Gyŏngnam region), Masan: Kyungnam University Press.

⁴ Yŏm Malgu

¹⁹⁹⁹ T'ong'yŏng Jiyŏk ui Susanŏp Hwalsŏng'haw Bang'an (A plan for the revitalization of fisheries

According to Yeong Kim's research on the urban development and infrastructure plan of Tong-yeong, the bay region of the city is the nation's famous scenery, and has highest water qualities in the southeastern coast, which is called *Hanryeosudo*, a nationally recognized oceanic park.⁵ The coastal region is strictly protected by the Ministry of Maritime Affairs and Fisheries (MOMAF) of South Korea.⁶

For the economic needs of various human activities with the ocean, the community focuses on developing long term sustainable and ecological management and practices for natural resources. A total of seventy nine fishery cooperatives consist of approximately five thousand memberships. The cooperatives provide community outreach to educate members of fishing village while serving its primary role in the market distribution for sales of seafood products and in banking and insurance services. The Tong-yeong Gul Suhyeop (The Tong-yeong Oyster Hanging Culture Fisheries Cooperatives) celebrates the harvests of Pacific Oyster in spring and the annual event attracts hundreds of travelers from various places. 8

in the T'ong'yŏng region). *In* T'ong'yŏng Kŏjae Jiyŏk Yŏn'ku Yŏn'ku (The research study on T'ong'yŏng and Kŏjae regions). Pp.71 -83. Kyungnam University Gyŏngnam Ji'yŏk Munjae Yŏn'ku'won (Kyungnam University the center of the research on the problems of the Gyŏngnam region), Masan: Kyungnam University Press.

⁵ Kim Yŏng

¹⁹⁹⁹ T'ong'yŏng Jiyŏk ui Kong'gan kujo wa Jiyŏk Kaebal Jŏl'yak (Development of spatial structures and region planning in T'ong'yŏng). *In* T'ong'yŏng Kŏjae Jiyŏk Yŏn'ku (The research study on T'ong'yŏng and Kŏjae regions). Pp.133-163. Kyungnam University Gyŏngnam Ji'yŏk Munjae Yŏn'gu'won (Kyungnam University the center of the research on the problems of the Gyŏngnam region), Masan: Kyungnam University Press.

⁶ Ministry of Maritime Affairs and Fisheries (MOMAF)

²⁰⁰⁷ Mission statement. Electronic document. http://www.momaf.go.kr/.

⁷ Kim Yŏng

¹⁹⁹⁹ T'ong'yŏng Jiyŏk ui Kong'gan kujo wa Jiyŏk Kaebal Jŏl'yak (Development of spatial structures and region planning in T'ong'yŏng). *In* T'ong'yŏng Kŏjae Jiyŏk Yŏn'ku (The research study on T'ong'yŏng and Kŏjae regions). Pp.133-163. Kyungnam University Gyŏngnam Ji'yŏk Munjae Yŏn'gu'won (Kyungnam University the center of the research on the problems of the Gyŏngnam region), Masan: Kyungnam University Press.

⁸ Yi Jŏng'hun

^{2008 &#}x27;Kul ŭi Modŭn gŏt' 22nd T'ong'yŏng Kul Ch'ukjae ('All about oysters' the 22nd T'ong'yŏng oysters festival). Yŏnhap Nyusŭ, 3-wŏl 8-il (March, 8). Electronic document, http://news.naver.com/main/read.nhn? mode=LSD&mid=sec&sid1=101&oid=001&aid=0001991510, accessed May 29, 2008.

About eighty percent of the nation's total fish products are harvested, manufactured and marketed from the region's large fishing industry. The primary industry consisting of agriculture and fisheries fields, make up 62.9% of the total industries in Tong-yeong. The common harvested fish are anchovies, mackerel, saurel, and sea bream, and the popular commercial shellfish are oysters, hen cockle short neck clams, sea mussels, and abalones. In comparing the total industrial scales, fish farming is approximately 45%; 50% is done by catching, and the rest is composed of hatchery businesses.

The total area of oyster harvesting facilities in town is approximately 14,454.3 hectares. 10 In addition, a number of seafood markets and processing plants make up part of the city's fishing industry. Particularly oyster products from the region have fed the needs of consumers nationally and internationally. According to Lee from the National Fisheries Research and Development Institute (NFRDI), as of 2006, a total of seven registered shellfish harvesting facilities exports oyster products and they are approximately made of 34,385 hectares and located in the Southern coastal cities in South Korea. 11

According to Kim, his company should follow the U.S. Food and Drug Administration guideline, therefore keeping laboratory journals and follow the Hazard Analysis of Critical Control Point (HACCP) protocol in order to ensure food safety, particularly oyster products.

⁹ EnCyber

²⁰⁰⁸ T'ong'yong -si. Doosan Corporation Seoul: Electronic document, http://www.encyber.com/comm/board/bh list.php?tid=helpqna.

¹⁰ Kim Yŏng

¹⁹⁹⁹ T'ong'yŏng Jiyŏk ui Kong'gan kujo wa Jiyŏk Kaebal Jŏl'yak (Development of spatial structures and region planning in T'ong'yŏng). In T'ong'yŏng Kŏjae Jiyŏk Yŏn'ku (The research study on T'ong'yŏng and Kŏjae regions). Pp.133-163. Kyungnam University Gyŏngnam Ji'yŏk Munjae Yŏn'gu'won (Kyungnam University the center of the research on the problems of the Gyŏngnam region), Masan: Kyungnam University Press.

¹¹ Lee Taesik

^{2006,} Suchul-yong Paeryu SaengSan Haeyŏk Wisaeng Josa (Safety Examination of seas in which exporting shellfish productions are located) In Annual Report of National Fisheries Research and Development Institute (2006).

The manufacturers had to be aware of the particular needs and the regulations of other nations and his business's major international partners included industries in the Japan and the $U.S.^{12}$

¹² Kim, J. Personal Interview 26 Oct. 2007.

Crassostrea gigas

Oysters are Mollusks in the family *Ostreidae*. The two shells of oysters could be considered as skeletons, and other Molluscs spices include clams, scallops, and mussels. Two genera, *Ostrea* and *Crassostrea*, are popularly known as commercial oysters. Various nutrients per one hundred grams of the edible portion in oysters are composed of calcium, phosphorus, zinc, thiamin, riboflavin, niacin and relatively low cholesterol. Most Americans consume cooked oysters and common methods include stewing or cooking half shell. Raw oysters are frequently consumed in coastal regions. Shucked oysters are frequently used as a term describing when oysters are mentioned, and they are readily available across the country and frequently. ¹³

Cupped Oyster. ¹⁴ C. gigas is typically from 8 to 15cm in length, and the maximum length can go upto approximately 30 cm. ¹⁵ With a scale-like outer shell, the species is relatively large compared to other oyster species. The soft part is composed of nine major organs: anus, adductor muscle, gill, mouth, mantle, pericardium, rectum, and stomach. With a unique physical appearance, C. gigas has the darkest soft part among various other Crassostrea species. ¹⁶ In News Life, Cho, a shellfish producer from Wooyeong Susan Co., mentioned that C. gigas often

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¹³ Dore, Ian and Sandra Noel

¹⁹⁹¹ Shellfish: a guide to oysters, mussels, scallops, clams, and similar products for the commercial user. Pp. 8-9, 69-109. Van Nostrand Reinhold, New York: Springer.

¹⁴ Food and Agriculture Organization of the United Nations (FAO) © 2005-2009. Text by Helm, M.M. In: *FAO Fisheries and Aquaculture Department* [online]. Rome. Updated 8 June 2006. [Cited 7 January 2009]. http://www.fao.org/fishery/culturedspecies/Crassostrea_gigas, accessed June 29, 2008.

Food and Agriculture Organization of the United Nations (FAO) © 1988 Status of oyster culture in selected Asian countries by Lovatelli, A. *In*: FAO Fisheries and Aquaculture Department [online]. Bangkok http://www.fao.org/docrep/field/003/AB716E/AB716E00.HTM, accessed June 30, 2008

¹⁶ Food and Agriculture Organization of the United Nations (FAO) © 1988 Status of oyster culture in selected Asian countries by Lovatelli, A. *In*: FAO Fisheries and Aquaculture Department [online]. Bangkok http://www.fao.org/docrep/field/003/AB716E/AB716E00.HTM, accessed June 30, 2008.

attracts more customers, because it produces more meat compared to other oyster species in South Korea.¹⁷

C. gigas is currently widespread in coastal regions of Asia, Australia, Northern Africa and various parts of Europe. In North America, its range is from Alaska to Baja California. It is euryhaline, living in both fresh and saline waters. As temperature and salinity were mentioned to play critical roles in the growth of Vibrio parahaemolyticus from page 15 to 19, these physical factors have also significantly influenced oysters' growth. The level of the salinity in oyster growing bays is typically from 10‰ to 35‰. According to "Culture of the Pacific Oyster (Crassostrea gigas) in the Republic of Korea," the size of the shell is an indicator of the growth of C. gigas. The warmer temperature influences the positive growth of the shells. 19

The general stages of the oyster' life cycle studied by the Republic of Korea and reported was made by the United Nation Food and Agricultural Organization (FAO). Understanding the general life cycle of the oyster species was necessary in order to investigate research objectives on the economic and cultural impact due to *V. parahaemolyticus* infection in shellfish particularly oyster species. From spring to summer, the gonad is developed and produces gametes.²⁰ Salinity and temperature of water are the main environmental factors for the oyster'

30, 2008.

¹⁷ Yim Minhi

²⁰⁰⁷ Ka'mang-man Kul Yangshikjang ŏmindŭl ŭi Haru (Fishermen's day at oyster farms in *Ka'mak* bay. Nyusŭ Laip'ŭ (News Life). 3-wŏl 27-il (March, 27). Electronic document, http://blog.naver.com/newslifeblog?Redirect=Log&logNo=150017492329, accessed on August, 2007.

¹⁸ Food and Agriculture Organization of the United Nations (FAO) © 2005-2009 Text by Helm, M.M. *In*: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 8 June 2006. [Cited 7 January 2009]. http://www.fao.org/fishery/culturedspecies/Crassostrea_gigas, accessed June 29, 2008.

¹⁹ Food and Agriculture Organization of the United Nations (FAO) ©
1988 Status of oyster culture in selected Asian countries by Lovatelli, A. *In*: FAO Fisheries and Aquaculture Department [online]. Bangkok http://www.fao.org/docrep/field/003/AB716E/AB716E00.HTM, accessed June

Food and Agriculture Organization of the United Nations (FAO) © 1988 Status of oyster culture in selected Asian countries by Lovatelli, A. *In*: FAO Fisheries and Aquaculture Department [online]. Bangkok http://www.fao.org/docrep/field/003/AB716E/AB716E00.HTM, accessed June

spawning. *C. gigas* alternates its sex throughout life and generally beginning of its life as males and ending as females. Spawning generally occurs from July to August, and approximately fifty to a hundred million eggs or sperms are released in a single spawning. The high abundance of *V. parahaemolyticus* also has shown in these summer months.

The meat portion of the animal is also not as big, and the product is not as marketable as the one from winter months. The distribution of larvae occurs during a planktonic period, and this period is also highly affected by the water temperature. The range of the water temperature is typically between nineteen and twenty degree Celsius over three weeks and approximately twenty seven Celsius for approximately ten days. The larvae then settle out of the water column and move slowly through the bottom of the water column searching for suitable habitats in intertidal zones of estuarine environments. Settling is related to the amount of copper compounds and the velocity of water currents typically between five to seven centimeters per second.

In the following fall months, the soft part of *C. gigas* becomes leaner after the spawning and eventually fills up with glycogen during the winter season. Planktonic algae and organic matter are known for the main sources of *C. gigas*' diet. The preferable water depth in coastal regions is approximately between five and forty meters, and the larvae attach to rocks, debris, and other oyster shells. Larvae could also be found in mud or mud-sand bottoms.²²

^{30, 2008.}

²¹ Food and Agriculture Organization of the United Nations (FAO) ©

¹⁹⁸⁸ Status of oyster culture in selected Asian countries by Lovatelli, A. *In*: FAO Fisheries and Aquaculture Department [online]. Bangkok http://www.fao.org/docrep/field/003/AB716E/AB716E00.HTM, accessed June 30, 2008

Food and Agriculture Organization of the United Nations (FAO) © 1988 Status of oyster culture in selected Asian countries by Lovatelli, A. *In*: FAO Fisheries and Aquaculture Department [online]. Bangkok http://www.fao.org/docrep/field/003/AB716E/AB716E00.HTM, accessed June 30, 2008.

Commercial fisheries for *C. gigas* grew rapidly world-wide since the beginning of the 1900s, as the species imported into western countries, including the United States. In the U.S., *C. gigas* was originally imported from Japan in 1903. In 2006, the total catch reported for *C. gigas* to the FAO was over 30,000 tons. The FAO fisheries statistics showed that the global aquaculture production for *C. gigas* has continually grown since the 1950s, with the highest increase rate occurred since the early 1990s. The U.S. has become one of the largest consumers of *C. gigas* and the U.S. produces approximately 60% of the total global oysters. Approximately 60% to 70% of the total oysters produced are *C. gigas* from the Pacific Coast States.

The Republic of Korea is one of leading countries that produces *C. gigas* with approximately 11,609 tons in the FAO report in 1999, and the U.S. produced approximately 539 tons of *C. gigas* in that year.²³ *C. gigas* has been the most popularly harvested oyster species in South Korea since the industry began in the 1970. As a result from the introduction to the hanging method in the fisheries industry, the total capture of *C. gigas* were increased from 26,814 MT in 1969 to 268,775 MT in 1986.²⁴ In 2005, the production was approximately 77% (261,706 tons) of the total Korean shellfish industry according to the Ministry of Maritime Affairs and Fisheries (MOMAF) of South Korea.²⁵ According to the annual statistics on the cooperative sales of fishery product of South Korea, Korean sea food was exported to 104 countries and major partners includes Japan (62.1%), China (9.1%), the United States (7.4%),

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²³ Food and Agriculture Organization of the United Nations (FAO) © 2005-2009 Text by Helm, M.M. *In*: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 8

June 2006. [Cited 7 January 2009]. http://www.fao.org/fishery/culturedspecies/Crassostrea_gigas, accessed June 29, 2008.

Food and Agriculture Organization of the United Nations (FAO) © 1988 Status of oyster culture in selected Asian countries by Lovatelli, A. *In*: FAO Fisheries and Aquaculture Department [online]. Bangkok http://www.fao.org/docrep/field/003/AB716E/AB716E00.HTM, accessed June 30, 2008.

²⁵ Han'guk Susanhoe (Korea Fisheries Association) 2007 Korean Fisheries Yearbook. Pp. 85.

New Zealand (5.1%) and Thailand (4.8%) in 2005.²⁶ South Korea exported raw, canned, and frozen Pacific Oyster products to 25 countries and earned \$120,000,000 in 2005²⁷. The National Federation of Fisheries Cooperatives (NFFC) reported that the total number of production of *C. gigas* in 2005 was 27,320 M/T and \$61,204,000 (11,308 M/T) of these were exported.²⁸

The general background information to the International Degree research consists of my personal interactions with people and observations made throughout the research trip occurred in Tong-yeong, South Korea. Several features such as population, geographic features, the scale of oyster businesses in the major shellfish producing city of South Korea, Tong-yeong, were discussed extensively throughout the introduction section. The general background information sets an essential foundation for understanding economic and cultural aspects due to an impact of the *V. parhaemolyticus* infection in *C. gigas*. Getting to know the language, common dietary food, transportations, and other kinds of life-style features that are specifically unique to the region are recognized highly in order to demonstrate cross-cultural understanding and communication during one-on-one interactions between the Tong-yeong shellfisheries members and me. Next followed by the method section, the innovative approaches to the ID research method and lists of interview participant profiles are covered.

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²⁶ Han'guk Susanhoe (Korea Fisheries Association)

²⁰⁰⁷ Korean Fisheries Yearbook. Pp. 90.

²⁷ Yi Jong-hun

²⁰⁰⁵ Kul Sanŏp ŭi Hyŏn'hwang gwa Jŏn'mang (Present status and prospect of the oyster industry).

Pp. Tŭk'kang-1 (Crash course-1). Korea Institution of Science and Technology Information.

²⁸ Han'guk Susanhoe (Korea Fisheries Association) 2007 Korean Fisheries Yearbook. Pp. 272-274.

METHODS

Methods for the International Degree (ID) part of the thesis were used, including: library research, personal and phone interviews and participant observations. Various academic research papers were reviewed including Food and Agriculture Organization, the Gyeongnam Regional Research Summit from Kyungnam University, and the governmental agencies of the Republic of Korea, such as the Korea Food and Drug Administration (KFDA), the Ministry of Maritime Affairs and Fisheries (MOMAF), and the National Federation of Fisheries Cooperatives (NFFC). The interview was designed to be semi-structured which allowed a place for participants' preferences.

Prior to the interview process, the project was reviewed under the guidelines of Oregon State University Institutional Review Board (IRB), and the full approval was issued (IRB Application No. 3738) on September, 28 2007. The informed consent was translated into Korean by the student investigator following a review by Lim, a graduate student from Korean language program of Kookmin University Education Department. The consent forms were distributed to the participants prior to interview sessions in fall 2007.

Demographics of Interview Participants

A total of seven participants from Tong-yeong and Masan, South Korea participated in the interview. They were from small scale businesses which composed of less than or equal to twenty employees and a large scale business with more than sixty employees in the Tong-yeong region. In addition, officials of the Tong-yeong regional MOMAF and a research assistant from the shellfish division of NFFC in Tong-yeong office voluntarily participated and assisted the interview studies. The potential benefit of the exploratory study was carefully mentioned in order to encourage participants of the interview. By focusing on an example of one of the largest oyster producing communities of South Korea, an interaction between marine natural resources and human activities is to evaluate and the study provides an education to global communities.

Two sets of questions were presented to ask background information and economy or culture. For MOMAF and KFDA, the regulation section was added. The general background was initially inquired to get participants' profiles as well as to evaluate history, company mission, size of companies, and general views on microbial contamination issues in fisheries products. The purpose of the background information is to organize the participants' profiles and initially to engage active conversations in the course of investigation. Summaries to the general background information of each seven interview participants are listed as it follows.

Kim was a forty-five-year-old male, who owned a small scale oyster farm in Yongnam myen, Tong-yeong for thirty years. The size of the facility was about ten hectares and fifty thousand cultch (suhayen), equivalent to sixty oyster shells on a line.²⁹ A total of twenty workers were working at his shucking facility, and the company mainly sold oysters without shells.

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Jang was a fifty-five-year-old male who owned a small oyster farm for twenty years. His property included a total of eight hectares with forty thousand cultch (suhayen). He hired close to twenty five to thirty female workers for his shucking facility. He was using natural and artificial seedling to produce oysters.

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Sŏ was twenty-nine-year-old female working as a laboratory assistant for the oyster division of the National Federation of Fisheries Cooperatives (NFFC) in Tong-yeong. Starting from the end of October to the following year of March, her laboratory examined about four hundred oysters per five thousand oysters at the wholesale market. A total of forty nine employees were working at the Tong-yeong NFFC, in different branches which are including general, marketing, banking and mutual credits and cooperative insurance services.

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²⁹ Korea-US Aquaculture

²⁰⁰⁰ Pacific oyster Electronic document, http://www.lib.noaa.gov/korea/main_species /pacific.htm, accessed May 28, 2007.

Kim was a thirty-two-year-old male working directly with the chairman of DongwonMulSan. The company was considered to be largely scale oyster producing facility that was established in 1991. The property included eleven hectares in blue belt, Chung-jung-hae-yeuk or Chung-jung-su-yuck, a region in which federal government of South Korea protects the surface water quality for marine fisheries and natural resources. With mentioned the region got approval of the U.S. Food and Drug Administration HACCP regulation in the past every two years, and it was well known for the pristine water quality.

The company hired about sixty to seventy employees in the year when the interview was conducted. About 120 up to 160 employees were hired on average in 2006. The average workers' age was between thirty and forty, and all of them were female workers. The wages were depending on the amount of oysters that were shucked. Kim mentioned that a skillful full-time (forty hours per week) worker would earn about \$1,386.46 to \$1,848.74 per month.

The company produced about six hundred fifty tons of frozen oyster products and a thousand tons of raw oysters in 2007. In 2007, the export amounts were decreased compared to the record of 2006; approximately twenty percent of the frozen product was exported to the U.S., ten percent were exported to Japan and the rest were distributed to Korean market. In

³⁰ NHN Corp. ©

²⁰⁰⁸ Neibŏ Jishik-iN(Naver *Jishik* (knowledge)-iN). Electronic document, http://kin.naver.com/detail/detail.php?d1id=12&dir_id=12120101&eid=h3pglqAanMSyXnIbDhw9ITk+VdHwv4zy&qb=w7vBpMfYv6rAzCC/tb7ut84=&pid=fgLz%2Bsoi5UsssbgwEI8sss-169446&sid=STNvtG5UM0kAACb8f@8, accessed October 10, 2008.

2006, the company produced a total of two thousand tons of raw meat and five hundred tons of boiled oyster, and a total of eighty percent of them were exported to the U.S. and Japan.³¹

005

Jeong was a fifty-eight-year-old male who have owned his current shellfish business for the past forty years in Kwangdomyen of Tong-yeong. He has passion for his long-term career as the oyster producer and hired about ten to twenty employees throughout seasons. The average ages of his employees were from forty to sixty and most of them were about twenty female workers. He sold oysters without shells to the whole sellers or organized direct market sales to customers at the facility.

During the interview session, he expressed his passion for having a career in fisheries in Tong-yeong since 1960s, and his company worked hard to maintain the business. He also emphasized that providing safe food products to his customers is an important consideration for his company's sustainability. To do so, he thinks that the modern technology and scientific research for advancing the current method of shellfish productions as well as finding solution for the frequent agar bloom and bacterial contamination in shellfish such as Noro virus and Vibrio spp in the ocean environment.

³¹ Cho Sŏng-in

²⁰⁰⁶ Wŏnch'ik kwa Shinroe rŭl Batang ŭro Ch'oe'go ŭi Shikpum ŭl Saengsanhanŭn Dongwŏn Mulsan (Ju) (Dongwon Mulsan (Company): Producing the best quality seafood products based on fundamental rules and reliance). Electronic document, http://blog.empas.com/king4775/13142949, accessed March 4, 2007.

Ju was a thirty-five-year-old male who was a sales representative from the NFFC of Tong-yeong. His work deals with one year old Pacific Oyster, produced by the NFFC memberships in shellfish villages in Tong-yeong. He explained his daily duties at the whole sale market. Getting orders in the morning, his work was followed by two actions; one at noon and the other one at six o'clock in the evening. After he received oysters from the producers, he has washed all the meats and got ready for the sales. Approximately ten kilograms shucked oysters were sold per a day during the peak season, and between seventy and eighty percents of them are raw meat.

007

Lim was the director general from the Gyeongnam regional Ministry of Maritime affairs and Fisheries office in 2007. He oversaw all the seafood products produced in the Gyeongnam region including Pacific Oyster.

The General Structure of the Korean Shellfish Industry

The major three entities of the Korean shellfish industry in the study are the federal governmental institutions, the oyster cooperative group, and shellfish harvesters in Tongyeong. The main governmental institutions associated with the Korean shellfish industry are listed as follows. Ministry of Maritime affairs and Fisheries (MOMAF), also known as Haeyang Susan-Bu, is a cabinet-level division of the government of South Korea. It oversees a variety subdivisions and the nature of work involving with port safety, maritime shipping and develops policies for fisheries management in coastal environments. MOMAF also manages the regulation of shellfish product including oysters, through the National Fisheries Research and Development Institute (NFRDI) and the National Fisheries Products Quality Inspection Service (NFPQIS).³²

MOMAF includes regional office, the Masan Regional Maritime Affairs and Port
Office (MRMAPO) which is the jurisdiction of Gyeongnam province of South Korea. The
Tong-yeong Maritime Office (TMO) is one of the three major regional branches in the
MRMAPO and facilitates product exchanges and protect marine environment near the Tongyeong port as well as other local gates. Following the mission of the federal governmental
level in ensuring public safety, TMO's primary role is to enforce labeling and tags. For
instance, the container or bag that has final oyster products should have labels including the
product name, date and location of harvesting and the information about owners or companies
before sending to various markets. According to Lim, the director general of the Gyeongnam
regional MOMAF in 2007, the institution provides services to help investigate bacterial

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Ministry of Maritime affairs and Fisheries (MOMAF) 2007 Mission Statement. Electronic document. http://www.momaf.go.kr/, accessed April 20, 2007.

outbreak cases with respect to seafood safety. For example, during the *Noro* virus outbreak in Spring 2007, the MOMAF played important role in investigating and solving the situation with the virus contamination in oyster products in Tong-yeong shellfish harvesting sites. ³³ Both the Korea Food and Drug Administration (KFDA) examine and regulate the shellfish product for both national and international consumptions; the Gyeongnam regional governmental body of Ministry for Health, Welfare and Family Affairs (MIHWAF) is particularity responsible for the Tong-yeong region. ³⁴

The federal government such as MOMAF works closely with the fisheries cooperatives, such as the National Federation of Fisheries Cooperatives (NFFC). NFFC is a non-profit and non-governmental organization that provides benefits to both the government and members of Korean fisheries industries. The non-profit organization includes three major branches: administration, marketing, and financing. Each department plays critical roles in the Korean shellfish industry by managing market flows of fisheries products both nationally and internationally. ^{35, 36, 37}

In Tong-yeong, oyster cooperative groups host various educational outreach events in the community including workshops and lecture series about current markets, technology and research advancement in Mollusk productions. ^{38, 39, 40} The department of finance provides

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³³ Yim, G. Personal Interview 19 October 2007.

³⁴ Masan Regional Maritime Affairs and Port Office (MRMAPO) 2007General Information. Electronic document. http://masan.momaf.go.kr/english/index.html, accessed July 23, 2008.

National Federation of Fisheries Cooperatives (NFFC) 2008 Su'hyŏp Sogae (The introduction to the NFFC). Electronic document. http://www.suhyup.co. kr/intro/vision.jsp/, accessed July 23, 2008.

³⁶ Yim, G. Personal Interview 19 October 2007.

³⁷ Sŏ, J. Personal Interview 26 October 2007.

National Federation of Fisheries Cooperatives (NFFC) 2008General Services. Electronic document. http://www.suhyup.co.kr/eng/business/general.jsp, accessed July 23, 2008.

banking and loan services and manages the seasonal distribution of fisheries products. According to interviewees, their sale management assists fishermen to secure their income profits. ^{41, 42} When an oyster harvester from the Gamackman village, located in the southeastern coastal region of South Korea, has lost his profits due to the seasonal bacterial outbreak associated with their harvested oysters in 1996, the regional branch of the NFFC provided partial loan assistance. ⁴³

The third branch of the NFFC is the marketing department, and it handles the overall distributions of the fisheries products in nation-wide. To boost seasonal sales, the marketing department creates sales events with seasonally varies in cultural themes in different regions in national wide. A lot of their fisheries products are also supplied to Korean military bases and public schools.⁴⁴

One of the popular annual events sponsored by the Tong-yeong Oyster Hanging

Culture Fisheries Cooperatives (TOHCFC) is the General Lee Sunshine Marathon race in

December. Part of the 2007 winter event, the TOHCFC provides general public free oyster

dishes prepared in traditional or their own innovative recipes. Approximately seven thousand

people from different cities across the nation participated in the race held in Tong-yeong. They

Yim G. Personal Interview 19 October 2007.

³⁹ Yim G. Personal Interview 19 October 2007.

⁴⁰ Sŏ, J. Personal Interview 26 October 2007.

⁴¹ National Federation of Fisheries Cooperatives (NFFC) 2008 Banking Service. Electronic document. http://www.suhyup.co.kr/eng/busin

²⁰⁰⁸ Banking Service. Electronic document. http://www.suhyup.co.kr/eng/business/general.jsp, accessed July 23, 2008.

⁴² National Federation of Fisheries Cooperatives (NFFC)

²⁰⁰⁸ Mutual Credits and Cooperative Insurance Service. Electronic document, http://www.suhyup.co.kr/eng/business/mutual.jsp, accessed July 23, 2008.

⁴³ Yim Minhi

²⁰⁰⁷ Ka'mang-man Kul Yangshikjang ŏmindŭl ŭi Haru (Fishermen's day at oyster farms in *Ka'mak* bay. Nyusŭ Laip'ŭ (News Life). 3-wŏl 27-il (March, 27). Electronic document, http://blog.naver.com/newslifeblog?Redirect=Log&logNo=150017492329, accessed on August, 2007.

⁴⁴ National Federation of Fisheries Cooperatives (NFFC) 2008General Services. Electronic document. http://www.suhyup.co.kr/eng/business/general.jsp, accessed July 23, 2008.

were able to try raw and cook oysters as demo dishes and join shucking competitions after the races. ⁴⁵ Various age groups between elementary students to 60 years old participated in the event. This kind of event promotes the consumption of *C. gigas*, and increase the seasonal distribution. According to NFFC, their marketing events attract more customers and attribute in the positive economic growth of the shellfish industry in Tong-yeong. ⁴⁶

⁴⁵ Yi Jŏng-hun

²⁰⁰⁷ Naedal 2-il T'ong'yŏng-sŏ Yi Sun-shin Jangkun bae Ma'raton (The General *Yi Sun-shin* Marathon will be held on the 2nd of next month). Yŏnhap Nyusŭ (*Yŏnhap* News), 11-wŏl 28-il (November, 28). Electronic document, http://news.naver.com/main/read.nhn?mode=LSD&mid=sec &sid1=103&oid=001&aid=0001837869, accessed January, 2008.

⁴⁶ Sŏ J. Personal Interview 26 October, 2007.

The Economic Aspects of the Korean Shellfish Industry

Ocean water is used among Tong-yeong communities for various purposes. The region's leading industries are ship building and tourism which often directly utilize services from the Tong-yeong marine environment. A few interview participants have mentioned that fecal matters from live-stock businesses should be more strictly monitored by the government; otherwise it will possibly worsen the current ocean water quality and cause food borne infections in their oyster products. Due to these consequences, the overall economy in the sea food industry in southwestern coastal region has been affected negatively.

According to interviewees from the Tong-yeong shellfish industry, the coastal water quality is influenced heavily by the effluent from the city's water treatment plant as well as input from non-point surface water from the land. Especially growing shellfish products that are frequently consumed as raw or fresh, the water quality issues has been one of the key concerns of the effective management for the food safety of the seafood and shellfish. Therefore these harvesting plants along the coast lines are strongly sensitive to the coastal water quality issue.⁴⁷

Vibrio parahaemolyticus is a naturally occurring halophilic pathogen and known to be seasonally abundant in marine and estuarine environments world-wide. In South Korea, V. parhaemolyticus is also a leading cause of seasonal diarrhea infection among people, and the number of cases has gradually increased in recent years. So, a laboratory technician from the National Federation of Fisheries Cooperative in Tong-yeong, explained that a higher number of V. parahaemolyticus is typically found when the temperature is approximately 17°C. According to the National Fisheries Research and Development Institute (NFRDI) in South Korea, the

⁴⁷ Yun Hodong

^{2006,} Susan Sikpum Wisaeng Anjon Wihae Kwala'li Yon'gu (Research on the Sanitation, Safety, and Hazard Management of Marine Products) In Annual Report of National Fisheries Research and Development Institute (2006) Pp. 87 National Fisheries Research and Development Institute (NFRDI).

highest number of the most probably number (MPN) of *V. parahaemolyticus* occurred during summer months when oysters were not typically harvested from number of oyster harvesting areas in the Southern coast from 2004 to 2006. In the same report, there was the highest month was August, and pathogenic *V. parahemolyticus* was found approximately up to 1%.⁴⁸

Raw fish and shellfish have been popularly historically consumed among many Koreans for a long period. Today, research have found that the frequent consumption of raw oysters in Koreans' food diet could thereby be associated for a higher risk of getting infectious diseases due to *Vibrio spp.* popularly known species could be *V. Parahaemolyticus, V. vulnificus, V. cholera* as well as diarrhea-causing virus, such as *Noro* virus. ⁴⁹ According to the Korea Food and Drug Administration (KFDA), recent outbreak have occurred as more people consume oyster dishes in public settings, cafeterias of schools and companies, where food dishes are prepared at a large quantity. ⁵⁰

Improper sanitation during harvesting oysters with bacterial contaminates caused the major outbreak of *Vibrio parahaemolyticus* in the past reports. According to the Korean Food and Drug Administration (KFDA)'s Annual Food Borne Bacteria Report in 2003, several indirect factors for the nation's *V. parahaemolyticus* were correlated to consumers' behavior, consuming raw oysters.

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⁴⁸ Yun Hodong

^{2006,} Susan Sikpum Wisaeng Anjŏn Wihae Kwala'li Yŏn'gu (Research on the Sanitation, Safety, and Hazard Management of Marine Products) *In* Annual Report of National Fisheries Research and Development Institute (2006) Pp. 87 National Fisheries Research and Development Institute (NFRDI).

⁴⁹ Yun Hodong

^{2006,} Susan Sikpum Wisaeng Anjŏn Wihae Kwala'li Yŏn'gu (Research on the Sanitation, Safety, and Hazard Management of Marine Products) *In* Annual Report of National Fisheries Research and

Development Institute (2006) Pp. 87 National Fisheries Research and Development Institute (NFRDI). ⁵⁰ Korea Food and Drug Administration (KFDA)

²⁰⁰⁴ Shikjungdok Balsang Hyŏnhwang mit Yebang Daech'aek (The occurrence of food borne illnesses and prevention plan).

In 2003, the major typhoon *Maemi* caused for rapid changes in the oceanic climate in the southeastern regions. The fastest as 60 meter per second, the typhoon *Maemi* became the one of the largest catastrophic storms in the Korean history. The storm had lasted for seven hours from September 12 to September 13, 2003, with a total property loss amount \$3,537,027,446 and 10,975 victims.⁵¹ The nation's major oyster producing sites are located in Tong-yeong and Kuje, and the damage due to the storm event was severe.⁵² In the KFDA report, oysters were harvested earlier prior to the storm event in order to avoid further economic loss; however, ocean water temperature was reported warmer and perhaps provided optimal growth condition for *V. parahaemolyticus* during the outbreak incidence.

A total of 9 illness outbreaks with 76 patients were associated with the consumption of contaminated raw oysters. The economic loss of the Tong-yeong oyster industry was approximately \$76,999,999 throughout the catastrophic event. The significant loss or impact due to the bacterial outbreak is uncertain. The reported number of patients consumed raw oysters or related dish such as *Kimchi*. *Kimchi* is a popular Korean cabbage salad dish, which often include raw oysters as an additive. Jang from the NFFC in oyster division of Tong-yeong mentioned that the early distribution of oysters through marketing events added rapid the sporadic disease outbreak. ⁵⁴

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April 3, 2007.

⁵¹ National Institute for Disaster Prevention

²⁰⁰³ Field Survey Report of Damages Caused by Typhoon Maemi in 2003-Damaged by flood, storm surge, electric power system failure (9.12-9.13) The Ministry of Government Affairs and Home Affairs.

⁵² Jŏng Daesŏn

^{2003 &#}x27;Maemi' P'ihaeji Shikjungdok Yŏp'a Yijung-go ('*Maemi* (Typhoon)' damaged areas aftereffect of food borne diseases). Munhwa Ilbo (The *Munhwa* Newspaper) 10-wŏl 2-il (October, 2). Electronic document, http://news.naver.com/main/read.nhn?mode=LSD&mid=sec&sid1=102&oid=021&aid=0000046053, accessed April 3, 2007.

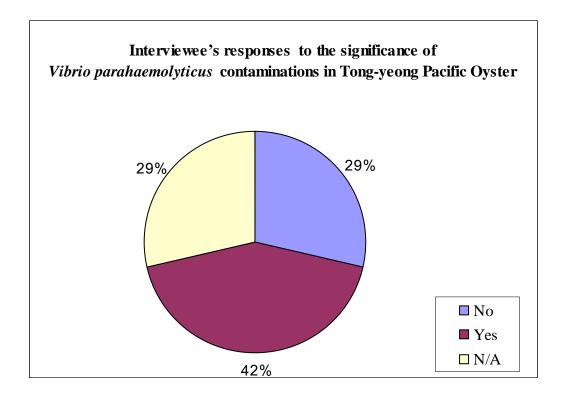
Sim Suhaw, Park Byŏng'gi and Lee Jongmin 2003 <'Maemi' Han-dal> Ssŏr'lŏng'han Jiyŏk Kyŏngjae (< One month with the 'Maemi (Typhoon)> the region's cool economy). Yŏnhap Nyusü (Yŏnhap News) 10-wŏl 9-il (October, 9). Electronic document, http://news.naver.com/main/read.nhn?mode=LSD&mid=sec&sid1=102&oid=001&aid=0000475860, accessed

⁵⁴ Jang Kyŏng-il

Questions were asked to interview participants to understand the impact of *V*.

parahaemolyticus contamination specifically in oyster products. Out of seven interviewees from the oyster industry in Tong-yeong, two mentioned no significant economic effects due to *V*.

parahaemolyticus infections in their businesses. They mentioned that fresh water species from different regions might consider dangerously with more severe economic loss. Yet, three interviewees responded strongly to the economic damage due to the *V. parhaemolyticus* infections in their business. The two other participants showed no response to the question.



All the interviewees argued that the recent *Noro* virus contaminations in shellfish have become the most serious economic concern in the Tong-yeong shellfish industry. This concerns significantly when dealing with exporting oyster products to countries, such as the U.S. According to NFRDI, diarrhea-causing virus was also detected from the waters of the

shellfish harvesters that are located near the cities in Gyeongnam Province. The problem with virus should have been occurred due to non-point water sources as well as discharged water from the inland in which human related waste water could have flew into the to the coast. ⁵⁵

According to the KFDA's annual food borne bacteria report of 2003, *V. parhaemolyticus* infection was mostly common at dining-outs in restaurants (12 cases with 272 patients) and in large social gatherings at school and public cafeterias (352 patients). In the same report a total of six cases with a total of 512 patients were reported due to food contaminated with *V. parahaemolyticus* in Gyeongnam province, and this was close to seventy percent of the total *V. parahaemolyticus* infection in the nation. ⁵⁶

Kim, an interviewee from a small scale shellfish-farm, who owned his business for thirty years, responded that almost every freshly harvested oyster would not be marketable if his oyster products had significant bacterial contamination. He also mentioned that sales rates for other shellfish products often dropped as the infection in oyster would be reported. As the reports were publicized, the economic losses became enormous. According to Kim, today's customers could be extremely cautious with their food choices as well as its safety of seafood. Customers would approach with a holistic view, such that if their shrimps were bad, this could mean that oysters and other neighboring species are bad.⁵⁷

Lim has owned his small seafood restaurant for eleven years in Ju'andong in Inchon, where the population of the total unit area within a county is recorded as approximately 420,591 in 2008.⁵⁸ The total sales would drop significantly in various times throughout

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⁵⁵ Yim D. Personal Interview 20 November 2007.

⁵⁶ Korea Food and Drug Administration (KFDA)

²⁰⁰⁴ Shikjungdok Balsang Hyŏnhwang mit Yebang Daech'aek (The occurrence of food borne illnesses and prevention plan).

⁵⁷ Kim J. Personal Interview 26 October 2007.

⁵⁸ Wŏn Gaech'ŏl

^{2008 2008-}nyŏn 1/4-bun'ki Gae'gan Inch'ŏn T'ong'kae Jipkae Kyŏlgwa Gongp'yo (The 2008

summer due to seasonal bacterial contaminations in his seafood products. The circumstances often lasted for at least ten days after the safety caution reports were notified which are frequently made by KFDA and many other new broadcasters. He has faced challenges in earning unstable incomes throughout his involvement with food industry due to unexpected bacterial outbreaks in seafood.⁵⁹

Jang, the director of the Tong-yeong Oyster Cooperative, mentioned the food safety management is a key concern for the NFFC when the industry continues to sustain their growth. To promote its long-term economic sustainability, the NFFC develop partnerships within the nation as well as through global networks. To avoid raw oyster contamination issues, the industries have developed creative oyster-dish recipes to manufacture canning and frozen shellfish products. Jang mentioned that the major concern has been emphasized about the food safety since strict regulations in food trades with countries, such as the U.S. and Japan in 2002. ⁶⁰ Also, Korean customers prefer to have safe and healthy diet for their well-being. ⁶¹

In a 2003 report, the NFFC constructed their plans to have frequent monitoring of water quality in shellfish harvesting sites, as well as inspections in processing plants and shucking facilities. It also increased educational and marketing programs to control market inflows in South Korea especially during summer seasons when frequent bacterial infections could potentially occur.

quarterly annual report on the *Inch* 'ŏn statistical total result). Bŏmu T'ong'kae Dangdamkwan (Judiciary statistics officer) *In* Incheon Metropolitan City Website Website ©2003-2008. Electronic document, http://www.incheon.go.kr/board/servlet/board?writeNo=5217&cmd=VIEW&page=42&bbsName=PRESS&category=&searchType=&searchString=&startDate=&endDate=&orderby=&requesturl=/board/servlet/board, accessed January 7, 2009.

⁵⁹ Yim D. Personal Interview 20 November 2007.

⁶⁰ Jang Kyŏng-il

²⁰⁰³ Kul Sanŏp ui Baljŏn Bang'hyang (Developmental direction of the oyster industry). *In* Han'guk Yangsik (Korean Aquaculture). Pp.96-98 Han'guk Yangsik Hakhoe (Korean Aquaculture Society).

⁶¹ Ju J. Personal Interview 26 October, 2007.

Park from the Kyungnam University conducted a comprehensive research on the water quality in various shellfish harvesting sites in Tong-yeong and Kuje. According to the study, the water quality had gotten worse as the population had continuously grown in those urbanized cities in Gyeongnam province. Naturally occurring oyster larvae have become rarely in the harvesting sites, instead hatchery businesses for shellfish, particularly oysters, have been established for capturing healthy larvae and maintaining the suitable environment for spats in modern technology.

The NFFC spent approximately 620,000,000 won (\$600,193.60) in order to build sixteen new hatcheries facilities. Approximately 2,000,000,000 won (\$1,936,108.40) were used for building one hundred modern style shucking facility from 2003 to 2007. NFFC also established its own laboratory facility specifically for oysters, and the research fund was allocated approximately to 7,030,000 won (\$6,805) in 2003. ⁶³ During an interview with Sö, a laboratory assistant, from the Tong-yeong NFFC division, stated that the MOMAF provided research grant amount of \$3,000 for her laboratory facility in 2007. She mentioned that although it was the major reason that *V. parhaemolyticus* outbreak occurred in 2003, the laboratory was also established in the same year as restrict food safety issue had arisen. To safe practice of the hanging culture occurring in Tong-yeong and other neighboring bay areas, her laboratory team is primarily responsible for seasonal safety inspections at shellfish producing sites throughout year. She frequently samples water at the site from April to May for detecting the number of hazard microorganisms in the harvesting sites in Tong-yeong. In addition, Soe and her co-workers

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⁶² Kim Yŏng

¹⁹⁹⁹ T'ong'yŏng Jiyŏk ui Kong'gan kujo wa Jiyŏk Kaebal Jŏl'yak (Development of spatial structures and region planning in T'ong'yŏng). *In* T'ong'yŏng Kŏjae Jiyŏk Yŏn'ku (The research study on T'ong'yŏng and Kŏjae regions). Pp.133-163. Kyungnam University Gyŏngnam Ji'yŏk Munjae Yŏn'gu'won (Kyungnam University the center of the research on the problems of the Gyŏngnam region), Masan: Kyungnam University Press.

⁶³ Jang Kyŏng-il

²⁰⁰³ Kul Sanŏp ui Baljŏn Bang'hyang (Developmental direction of the oyster industry). *In* Han'guk Yangsik (Korean Aquaculture). Pp.96-98 Han'guk Yangsik Hakhoe (Korean Aquaculture Society).

inspect the harvested oysters at wholesale markets throughout year. The busiest sales season is through November until the following year in March. ⁶⁴ Typically, in fall seasons, about 5000 packages of 10 kg shucked oysters are sold per day during auctions, Sŏ and her laboratory team monitors about 10% of these prior to auction at the wholesale market in Tong-yeong.

The auction seasons are typically from October to June of the following year. During this period, monitoring especially for *Noro* virus and *V. parhaemolyticus* in the products is taking place. Shucking facilities are examined monthly for detecting bacterial toxin counts from April to May. According to Ju who is an oyster wholesaler broker working for the NFFC, he is responsible for washing sucked oysters before a two-hour auctions which start each day at noon and 6 pm.

Kim is a manager of a large scale oyster processing plant producing about six hundred fifty tons of frozen products and a thousand tons of raw products in 2007. A total of two thousand tons of raw meat and five hundred tons of boiled oyster, and eighty percent of them were exported to the U.S. and Japan. He described microbial concern and shellfish safe is the key in his factory. According to his approximation, the company spends about \$68899 (\forall 100,000,000) for the shellfish safety management in 2007. Number of hires is also important when considering the safety. The company hired about sixty to seventy employees in 2007 and about 120 up to 160 employees were hired on average in 2006.

Due to the reduction in oyster production in the following year, the number of employee dropped significantly to about 60 workers in the beginning of peak sale season when the interview was taken in October 2008. Kim mentioned that a skillful full-time (forty hours per week) worker who can shuck at a fast rate would earn about \$1,386.46 to \$1,848.74 per month.

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⁶⁴ Sŏ J. Personal Interview 26 October 2007.



FIG. 12. Pictures of a large-scale oyster production cited on an article: Producing the best quality seafood products based on fundamental rules and reliance in 2008

One of the challenges today facing in many rural industries has been with less number of younger workforces. The age of workers at Kim's company were consisting of between forty and sixty. More older age workers have tended to stay with primary industrial business such as many of oyster harvesting and it has been the long term demographic trend that more younger workforce are highly motivated in bigger cities and urban metro regions where high number of educational institutions and seek jobs in secondary and service industries.

Tong-yeong oysters are known as finest quality and highly valued for regionally developed food receipts for a long period. However, considering the seasonal fluctuation of oyster prices the bacterial outbreak could have potentially negatively had an impact on the economy. According to the 2006 sale record of Jeon's oyster company, the oyster price were lowest as \$2.00 (₩3000) highest as \$5.50 (₩8000) which the amounts were converted by the Yahoo Currency Converter program on Feb 17, 2009. The factors of which controls the overall market price depends upon the mount of produced versus the number of customers.

One of the biggest factor that affects in the price of the Tong-yeong oyster is due to illegally marketed foreign products often from China equally treated as the Tong-yeong regional oyster. Lim, Director General from MOMAF in Gyeongnam region describes that one of the key functions is to ensure by tagging and labeling the shellfish product prior to the market sale. Jeon, who owns a small scale harvesting facility, listed information will be tagged on all his products prior to sales and the information could be consisted of product name and amount, place of origin, dates and time of the harvest, and contact information.

Korean Dietary Cultures Related to Oyster Consumptions

Various aspects to the Koreans' traditional dietary seem to become a foundation of modern practices in the oyster consumption in South Korea. The record of oyster productions and human consumptions of the shellfish has been found during ancient periods in the mountainous peninsula which currently forms both North and South Koreas. Koryŏdokyŏ was published in the year 1123 A.D during *Koryŏ*, an ancient Korean State. It has a record of working classes frequently consumed seafood including mudfish, abalones, shrimps, pearl oysters, oysters, and crabs. According to Dong'kuk'yŏjisŭng'ram, a geography textbook written during the Chosŏn era (1392-1910 A.D.), oysters are known for native produce in seven provinces and seventy counties except in Kangwŏndo Province, located in the northeast side of South Korea. 65, 66, 67

A lot of cooking methods of Korean dishes are based on the use of popular holiday functions and various social gathering. Proper ways of preparing and presenting food dishes have been recognized highly in the Korean society. The level of formality could depend upon the usage of food in particular occasions. Four major ceremonies or formalities have evolved around lives' milestones of many Koreans, and those have been continued throughout modern days. The four formalities include coming-of-age, marriage, death and celebration of death anniversaries. Consuming the holiday dishes are recognized significantly recognized as a way

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⁶⁵ Yi Dusŏk

Jaemi'itgo Yu'ikhan Susanmul Yi'yagi (A story of fun and beneficial fisheries products). Pp. 111-113.

⁶⁶ NHN Corp. Dusanbaekkwasajŏn EnCyber & EnCyber.com

²⁰⁰⁸ Neibŏ Baekkwasajŏn (Naver Encyclopedia). Electronic document, http://100.naver.com/100.nhn? docid=13000, accessed January 17, 2009.

⁶⁷ NHN Corp.

²⁰⁰⁸ Neibŏ Kukŏsajŏn (Naver Korean dictionary). Electronic document, http://krdic.naver.com/detail.nhn? docid=10187800, accessed January 17, 2009.

to ward off illness and maintain health and well-being. Food ingredients especially for holiday cerebration are carefully selected for good quality and appearance since food dishes help people reflect on the significance of the holiday event and enlightened the festive mood. ⁶⁸, ⁶⁹, ⁷⁰

These four major features of the traditional dietary culture are based on the Confucianism. Those include courtesy toward elders, sense of family tradition, and sense of community. The first daughter-in-law's primary role has traditionally dedicated with preparing and serving dishes to elders of her household 71. The *p'um-ashi* tradition is an example of the fourth feature, the sense of community, and it is a form of household event involving wives from villages to make large quantities of *Kimchi*, pickled, fermented vegetables, before the first snow.

Oysters are popular for their beneficial nutrients such as calcium, phosphorus, and many other mineral elements. ⁷² A medical book written by Hŏ Jun, a medical officer from the Chosŏn era in the year 1596 A.D., mentions that oysters are the most precious marine

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⁶⁸Kang In-hee

¹⁹⁹⁷ Traditional Dietary Customs *In* Koreana Korean cultural heritage Volume IV Traditional Lifestyles Pp. 176-181 Korea Foundation.

⁶⁹ Park Tae-sun

¹⁹⁹⁷ Life's Milestones: Ceremonies and Food *In* Koreana Korean cultural heritage Volume IV Traditional Lifestyles Pp.182-187Korea Foundation.

⁷⁰ Yim Jae-hae

¹⁹⁹⁷ Holiday Customs and Food *In* Koreana Korean cultural heritage Volume IV Traditional Lifestyles Pp. 187-193 Korea Foundation.

⁷¹ Kang In-hee

¹⁹⁹⁷ Traditional Dietary Customs *In* Koreana Korean cultural heritage Volume IV Traditional Lifestyles Pp. 176-187 Korea Foundation

⁷² Dore, Ian and Sandra Noel

¹⁹⁹¹ Shellfish: a guide to oysters, mussels, scallops, clams, and similar products for the commercial user. Pp. 8-9, 69-109. Van Nostrand Reinhold, New York: Springer.

organism. It has smell and flavor (香味) and supports for fine skin and glowing complexion.⁷³

According to Choi, people often consume raw oyster dishes because of the unique texture of the animal. Raw seafood has been believed to be sacred since the animal should have grown healthy throughout its lifetime, and this is directly related to the freshness of the food item according to the interviewee.⁷⁴

Numerous proverbs in relation to oysters could be found in Korean language. ⁷⁵ Few examples are listed, and show certain experiences or advices that are shared among many Koreans.

글 같이 다친 여인[Kul gach'i dachin yŏin]

➤ Woman who keeps her chastity like oysters

글 같은 사나이[Kul gat'ŭn sanai]

Man like an oyster ➤ Man who holds his tongue well

남양원님 굴회 마시듯 [Namvang wŏnim kulhoe masidǔt]

As if the Namyang [region] county officer eats raw oysters. > Get something done swiftly.

배타는 어부의 딸은 얼굴이 까맣고 굴 따는 어부의 딸은 얼굴이 하얗다.

[Baetanŭn ŏbu ŭi ddarŭn ŏrlkul i kkamat ko kulddanŭn ŏbu ŭi ddarun hayata]

A daughter of a fisherman has tanned facial skin. But a daughter of an oyster harvester has white facial skin.

Oysters contain nutritious elements that are effectively making fine tones and destroy melanin, a dark brown pigment in facials.

2005 Kul Sanŏp ŭi Hyŏn'hwang gwa Jŏn'mang (Present status and prospect of the oyster industry).

Jaemi'itgo Yu'ikhan Susanmul Yi'yagi (A story of fun and beneficial fisheries products). Pp. 111-113.

⁷³ Yi Jong-hun

Pp. Túk'kang-1 (Crash course-1). Korea Institution of Science and Technology Information.

⁷⁴ Ju J. Personal Interview 10 December 2007

⁷⁵ Yi Dusŏk

Throughout the traditional dietary, Koreans enjoy having seasonal food, and some knew when to and not to eat oysters. Oysters are generally harvested from the end of October until the early April. ⁷⁶ During a market survey in the beginning of August, a shellfish vendor at an outdoor seafood market in Inchon mentioned less quality and taste of the oyster products were to be concerned with oyster products to their market customers during off-harvesting season, which are generally from the early summer in June to the beginning of fall in September.

An observation participant from the fish market mentioned that the appearance of the animal's meat is important when distinguishing good quality product during the season. The meat portion should be large and contain milk white color without any odor. The fresh one should have tender textures. According to Yi, oysters are generally shucked and contained in salt water, customers should be careful to distinguish for healthy oysters since they are not usually able to check them by tasting, touching or smelling the products.⁷⁷

Some features that are similar to the old customs are related to significant gender role in food consumption, particular way of preparing shellfish items, and old tradition of celebrating holidays. According to Sŏ and other interviewees from the Tong-yeong shellfish industry, the industry boosts the city of Tong-yeong's economy during high peak seasons of shellfish production typically from early November to March in the following year.

The Oyster Hanging Culture Fisheries Cooperatives (OHCFC) owned a total of 5,016hactares with 20.000 licensed personnel whose business is related to Pacific Oyster. Yi from the Tong-yeong branch of the OHCFC reported that the approximately 4,000,000 labors

Jŏng J. Personal Interview 20 November 2007
 Yi Dusŏk

Jaemi'itgo Yu'ikhan Susanmul Yi'yagi (A story of fun and beneficial fisheries products). Pp. 111-113.

are annually hired and earned 100,000,000,000 won (\$ 77.4 million) in 2005. During the high peak season, more job openings at shucking facilities and processing plants in Tongyeong attract labors, particularly female workforce. During the participant interviews, the female workforce was observed to be dominant across the Tong-yeong shellfish industry as well as local seafood markets.

In responses to how companies prevent bacterial contaminations in their products, Kim, a facility manager of a large-scaled shellfish producer, mentioned that his workers are frequently trained with sanitary education, and the company provides necessary equipment with regard to the safety requirement of industrial partners from the U.S. and Japan. The workers had been trained with the Hazard Analysis and Critical Control Point (HACCP) protocol and safety education has been implemented. Kim's company's oyster products exports to the U.S. and Japan as well as sold nationally. The HACCP protocol generally follows the agreement with countries such as the U.S. and Japan and partnered with the European Union. The U.S. FDA inspects Kim's shellfish producing facility every other year as part of trade agreements, and helps enforcing the selfish safety issue.

According to Kim, the HACCP protocols includes criteria for monitoring water quality and temperature, packaging, examining the potential hazard due to insecticides as well as microbial contaminant such as pathogenic bacteria and viruses. Temperature should be maintained under 7 °C at all time. Managing the time has also been critical for the safety and sanitation, and employees are trained to conduct the processing work from harvesting for less than about four hours. They started to teach their employees about proper sanitary gears, such

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⁷⁸ Yi Jong-hun

²⁰⁰⁵ Kul Sanŏp ui Hyŏn'hwang gwa Jŏn'mang (the present condition and prospect of oyster industries). Pp. Tŭk'kang-1 (Crash course-1), Korea Institution of Science and Technology Information.

as company uniforms, laboratory gowns, wearing hats at work. As I observed the facility through a guided tour, the supervisor of the shucking facility periodically called for the washing hands or maintaining the facility's cleanliness at all times.⁷⁹

When asked how the workers would get compensated, Kim mentioned that workers would receive pay stubs without special benefits such as health insurance, vacation, and sick leaves. A full-time skillful employee would get paid at approximately 2,000,0000 won (\$1,800) per month. The range of the average ages of workers was composed between forties and sixties and most of them were hired as full time. Throughout the participant observation, the nature of work seemed to be heavily involved in physical activities from the workers such as long periodic standing, squatting, lifting water tubs, and carefully shucking oysters. ⁸⁰

Sŏ emphasized that a lot of house wives would work in oyster productions in Tongyeong and seemed to wait for the peak season. According to her, the gender role in oyster industry is significant; traditionally, female labor force could be seen thought to be more patient and careful, compared to male. Sŏ described that especially Korean housewives are often took the lead on household chores, thereby female involvements in shellfish productions seemed to be much correlated with the traditional women's role in Korea.⁸¹

Modern technology brings innovative ideas to Koreans dietary culture. Some customs seem to be remained different, whereas others might be similar to the old customs. It provides great convenience to their kitchens as diversification of service industries that produces holiday dishes and especially *Kimchi* products in large quantities. 82 I observed that certain

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⁷⁹ Kim J. Personal Interview 26 October 2007.

⁸⁰ Kim J. Personal Interview 26 October 2007.

⁸¹ Sŏ J. Personal Interview 26 October 2007.

⁸² Park Wan Su

holiday dishes such as rice cake, rice drink, Korean pan-cakes and many others were sold all throughout seasons in stores. These changes seemed to make house wives' job more convenient.

Kimchi, a pickled, fermented vegetables are frequently consumed throughout Koreans meals. Depending on the kind of *Kimchi*, raw oysters could be added and the major types of *Kimchi* are made out of shredded Chinese cabbage and white radish that contain seasoning ingredients such as chile pepers, garlic, onions, and fish sauce. ⁸³ My Korean grandmother who often prepares dishes for her household mentioned that she no longer needed to make *Kimchi* at home, because it was cheaper to purchase from the local groceries. When a question was asked to industry members, oysters are typically known for an additive for *Kimchi*, and the Koreans' tradition of preparing the winter seasonal *Kimchi* has been significantly correlated to the annual peak sales of oysters. ⁸⁴

Many Koreans celebrate major holidays, such as *Chusuk*, Korean people's thanksgiving holiday and *New Year* holiday with family and friends. On the other hand, some also take vacations during those major holidays. These factors are highly related to number of food consumptions, particularly raw oysters. The meat of oysters were often served with soy sauce, seasoned hot soybean paste, green mustard paste or a mixture of oil and salt.⁸⁵

The recent survey on the oyster consumption explained that the raw oyster consumption had been the highest among other cooking methods in South Korea. The result from the study was shown; 61.6% consumptions were preferably made at home and 52% were

^{2000.} Kimchi Dae-ryang Sangsangwa San-uphaw Kisul (Large-Scale Production of Kimchi and Industrial Movement and Technology) Kimchi Ui-gwahack Hackgwa Kisul (*Kimchi* Medical Department Journal Volume 6, pp. 170-173

⁸³ Kittler, Amela Goyan and Sucher, Kathryn

^{2000.} Cultural Foods Traditions and Trends Pp. 275-287

⁸⁴ Ju J. Personal Interview 10 December 2007.

⁸⁵ Koreana Korean cultural heritage Volume IV Traditional Lifestyles, Korea Foundation 1997.

from dining-out at hotels and restaurant businesses. ⁸⁶ According to KFDA, more disease outbreaks often occur when people consume raw oyster under deficient sanitization during these holiday trips and often in summer months when the ocean water temperature rises. Improper ways to store foods during the trip and cultural tradition of eating fresh raw oysters had easily became the most problematic concerns. ^{87, 88, 89, 90}

Depending on climate condition as well as environmental factors in ocean water of harvesting facility, oysters are generally harvested from the late fall to early spring of the following year. ⁹¹ When I asked a question to a local fresh seafood market in Inchon, South Korea during a market survey, no raw oysters were sold from the early summer to the beginning of fall roughly from June to September, and vendors did not carry oysters, since the less quality and taste were concerned with oyster products.

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⁸⁶ Kim Hyŏng

^{2008 &}quot;Kul Hoero Mŏngnŭn gae Choi'go" (" Oyster best if consumed as raw"). Busan Ilbo (The *Busan* Newspaper), 2-wŏl 27-il (February 27). Electronic document, http://www.busanilbo.com/news2000/html/2008/0227/0C0020080227.1018111148.html, accessed June, 2008.

⁸⁷ Korea Food and Drug Administration

²⁰⁰⁷ Yŏrŭm chŏl Jang'yŏm bibŭrio Shikjungdok Jui (Notification for the summer food borne disease due to *Vibrio parahaemolyticus*). Technical Report Shikjungdok Yebang Kwanri T/F Tim (Food borne illnesses surveillance T/F team), Korea Food and Drug Administration. Electronic document,

http://search.kfda.go.kr/kfda/kfda_search.jsp?SortField=NOTICE_DATE, accessed March 20, 2008.

⁸⁸ Korea Food and Drug Administration

^{2007.} Kŏn'kang hago Jŭl'gŏun Han'gawi Shikjungdok Joshim hasaeyo (Wish for your health and have a great holiday Please watch out for the food borne disease). Technical Report Shikjungdok Yebang Kwanri T/F Tim Tim (Food borne illnesses surveillance T/F team), Korea Food and Drug Administration. Electronic document, http://www.kfda.go.kr/open_content/kfda/news/press_view.php?seq=1278, accessed February 10, 2008.

⁸⁹ Korea Food and Drug Administration

^{2003.} Saengkul ro'innan Shikjungdok Yebang Hongbo (A public information on the prevention of food borne illnesses due to raw oyster consumption). Technical Report Shikpum Kwalrikkwa (The department of food management), Korea Food and Drug Administration. Electronic document, http://www.kfda.go.kr/open_content/kfda/news/press_view.php?seq=257#, accessed February 10, 2008.

⁹⁰ Sim Yŏng'gu

²⁰⁰⁷ Shikyakch'ŏng 8,9-wŏl Jang'yŏm bibŭrio Shikjungdok 'Juibo' (Korea Food and Drug Administration (KFDA) August and September *Vibrio parahaemolyticus* associated food borne illnesses 'caution') SBS Nyŏsŭ (*SBS* News), 7-wŏl 25-il (July, 25). Electronic document, http://news.sbs.co.kr/section_news/news_read.jsp? news id=N1000289160, accessed August, 2007.

⁹¹ Jŏng J. Personal Interview 14 November, 2007.

As raw oysters could be consumed seasonally, during the off-season typically staring from the late spring, the industry produces more canned products than raw oysters, because the water quality is suitable for pathogens to grow actively in ocean water environment in which the shellfish harvesting occurs. ⁹² New recipes for cooked oyster dishes have been created by the oyster division of National Federation of Fisheries Cooperatives (NFFC) and are popular for all ages. Those include oysters and rice dumpling soup, oyster porridge, oyster pancake, raw oyster salad, fried oysters with sweet and sour sauce, steamed and grilled oysters or/and with shrimp, broiled oysters, kettle rice pot with oyster, sweet and spice oysters, raw oysters with noodle, oyster soup, grilled oysters with hollandaise sauce, fresh shucked oysters with mornay sauce, and oyster o'gratin. ⁹³

The NFFC organizes a lot of outreach events to attract customers, and education people about cooking methods and nutritional facts on oysters. ⁹⁴ On March 22nd, 2008, the 13th annual event invited hundreds of participants for the oyster shucking contests and cultural performances by local artists, such as traditional paper mask preservation group and folk play groups in Gyeongnam province. Five thousand canned oyster products were donated from the NFFC and distributed to the customers with free of cost. The cooking demonstrations were sponsored by shellfish wholesalers and involved the wives from the oyster cooperatives and volunteers from Geyongnam regional shellfish industry. ⁹⁵ It seemed like that oysters have

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⁹² Sŏ J. Personal Interview 26 October, 2007.

⁹³ Oyster Hanging Culture Fisheries Cooperatives

²⁰⁰⁰ Kul Yori T'ŭksŏn (Special selection of oyster dishes). Electronic document, http://www.oyster.or.kr/,accessed June, 2008

⁹⁴ Sŏ J. Personal Interview 26 October, 2007.

⁹⁵ Yi Jŏng'hun

^{2008 &#}x27;Kul ŭi Modŭn gŏt' 22nd T'ong'yŏng Kul Ch'ukjae ('All about oysters' the 22nd T'ong'yŏng oysters festival). Yŏnhap Nyusŭ, 3-wŏl 8-il (March, 8). Electronic document,

http://news.naver.com/main/read.nhn?mode=LSD&mid=sec&sid1=101&oid=001&aid=0001991510, accessed May 29, 2008.

become a huge part of the regional culture by hosting holidays and market events by the NFFC.

Continuing the old dietary traditions the shellfish industry have utilized the sale exchange through e-commerce businesses as a successful strategy of distributing large quantity oyster products to a number of shoppers with less food safety hazard. Ju, a wholesales representative, discussed that a lot of Koreans are now exposed to information technology (IT). The on-line or TV shopping is popular. Some described that using IT through internet and mass media is especially convenient for busy working classes in Korea. Once the oyster products are purchased, customers could receive the ordered products within twenty-four hours from the wholesales with affordable shipment cost. Also, those shopping venues provide information that people often rely on and trust and those are price ranges, origins, pictures, discounts, coupons, sellers' contact information and so on. 96

Involving both government and citizens, Korea is one of the leading countries promoting the fast growth in the field of IT. The internet seemed to open a wide venue for ecommerce. Approximately a quarter of the total internet users exposed to internet activities such as businesses, shopping, getting higher education. The general public popularly utilizes on-line based college entrance exam tutor/ programs. The presidential election was held on line in 2003 followed by No Moohyen regime. ⁹⁷ In the same year, more than 100,000 subscribers were recorded for the Skylife, a popular TV cable broadcast, which included various shopping channels and news.

⁹⁶ Website of Korean Oyster

⁹⁷ Yŏnap Nyusŭ (Yonhapnews) Agency

²⁰⁰³ A comprehensive Handbook on Korea, Korea Annual. Pp.170-172 Yŏnap Nyusŭ (Yonhapnews) Agency.

The seafood industry seemed to frequently use the today's technology of mass media. ⁹⁸ Jeong, an interview participants mentioned that the convenience of their current tele-communication technology had became important for the industry since more people carry cell phones today. According to *A Comprehensive Handbook on Korea in* 2003, approximately 11.97 million users were accessed to the wireless internet using their cell phones, and 36 million out of 48 million, close to 75% of the nation's population carried cell phones that year. For an example, Jeong mentioned that when the outbreak was announced or significant numbers of bacteria were detected from shellfish or harvesting waters, producers were able to receive text messages from the government reporting the bacterial incidences on their cell phones.

During the President No Moo-Hyen regime from 2003 to 2008, the government enforced to increase the access of internet in rural regions, and approximately thirty million people were able to access the internet at home. Eight out of ten households had a personal computer. A study from the same report also showed that the ages of forties who surf internet were recorded more than one million compared to the previous year. ⁹⁹ More increases in the use of e-commerce were expected in recent years, and this had influenced in both culture and economy of the shellfish business in Tong-yeong. ¹⁰⁰

Some interviewees argued for various controversial views while utilizing the modern information technology According to Lim, the director general from the MRMAFO in Gyeongnam province in 2007, when bacterial outbreaks associated with shellfish consumptions occur, news media post the announcements and Koreans customers are now

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¹⁰⁰ Ju J. Personal Interview 14 November 2007.

⁹⁸ Yim G. Personal Interview 19 October 2007.

⁹⁹ Yŏnap Nyusŭ (Yonhapnews) Agency 2003 A comprehensive Handbook on Korea, Korea Annual Pp170-172.

responding very quickly and swiftly to those incidences. Today's customers could be extremely cautious on their food safety issues. Consequently, their holistic views might allow ideas such as their shrimps were bad, this could mean that oysters and other related marine species are bad.

Mr. Lim has owned a small seafood restaurant for the last eleven years in the urbanized intercity of the Inchon metro, located in the Northwest of South Korea. Lim mentioned that his total sales decreases significantly each Summer due to certain kinds of bacterial outbreaks in seafood products. The circumstances often lasted for at least ten days after the reports were publicized. Therefore he had to face the challenge of earning the unstable income throughout his restaurant-business career. The damage from the economy loss in the industry could be detrimental according to Jang's report of the Tong-yeong NFFC. ¹⁰¹

When a number of news broadcasters announced, the bacterial infections in shellfish particularly oysters, the message could be generated repeatedly or often the most harmful effect from the infection to human health. According to A Comprehensive Handbook on Korea in 2003, women spent most their time surfing internet than men. When I stayed with my relatives in Inchon for a couple of weeks, my grandmother, who were frequently took care of preparing meals for her family, watched TV in average about four to six hours. A study also showed women spent more of their time surfing internet than men. 103

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¹⁰¹ Jang Kyŏng-il

²⁰⁰³ Kul Sanŏp ui Baljŏn Bang'hyang (Developmental direction of the oyster industry). *In* Han'guk Yangsik (Korean Aquaculture). Pp.96-98. Han'guk Yangsik Hakhoe (Korean Aquaculture Society).

¹⁰² Lee Yeong-ho

^{2005.} *Vibrio vulnificus* bupjeong Jeonyeom Byeong Jijeong-e Daehan MoonjeJŏm-mit Daecheck (Problems and countermeasures on *Vibrio vulnificus* as nationally notifiable diseases) Electronic document, http://www.basemi.net/basemi/mir.php?mode=sub03_04&mode2=&tbmode=tb_view&tbcode=mir_sub03_04&uid=118&fid=118&thread=A&cur_page=1

¹⁰³ Yŏnap Nyusŭ (Yonhapnews) Agency 2003 A comprehensive Handbook on Korea, Korea Annual Pp170-172.

As more people were exposed to the media and depend on their livelihood in certain TV or internet programs, considering how accurate or precise the information seems to be highly controversial. I was not able to find credible resources that talked about how mass media or informational technology frequently had dealt with the microbial issues specifically realted to *V. parahaemolyticus* infection in oysters and its impact on economy or culture in South Korea. However, majority of my interviewees from the Tong-yeong oyster industry argued that misuse or misinterpretation in mass media such as TV and internet sources seemed to exist. In addition to the economy down fall due to seasonal *V. parhaemolyticus* outbreak incidences in oyster products, the kind of information might have negatively affected the economy of the shellfish industry in Tong-yeong, South Korea, and have influenced in the change of Korean dietary culture



FIG. 13. Variety Oyster Dishes The oyster dishes are listed from left to right and from top to bottom: Oysters with vegetable cooked in a casserole, raw oysters, fried oysters with sweet and sour sauce, kettle rice pot with oyster, raw oysters with spicy sauce over rice noodle, oyster porridge. The photographs are cited on the Oyster Hanging Culture Fisheries Cooperatives http://www.oyster.or.kr/

DISCUSSION & CONCLUSION

Infectious and parasitic diseases are the second most common leading cause of death according to the World Health Organization (WHO). ¹⁰⁴ The naturally occurring pathogenic *Vibrio parahaemolyticus* has been the leading cause of gastroenteritis in humans world-wide since Japanese medical researchers discovered the bacteria during the major outbreak that occurred in the 1950s. According to the Food Borne Diseases Active Surveillance Network, the rising concern over the *V. parahaemolyticus* infection has gotten significant public attention in the U.S. due to the highest level infection rate, compared to *Salmonella*, *Campylobacter*, *Listeria*, *E. coli* and other pathogenic microorganisms over the last decade.

Common sources of gastroenteritis outbreaks associated with pathogenic *V*.

parahaemolyticus include crabs, shrimps, lobsters, and oysters. The International Degree (ID)

research has chiefly explored a popular commercial oyster species, *Crassostrea gigas*.

According to the United Nation Food and Agriculture Organization, the U.S. has been one of the largest consumers of the global oysters in 2006. South Korea has exported *C.gigias* products to numerous other countries including the major partners, the U.S and Japan.

The exploratory study aims to understand how bacterial outbreaks, particularly pathogenic *V. parahaemolyticus*, have influenced the culture and the economy in the Tong-yeong shellfish industry in South Korea. It includes perspectives of the Tong-yeong shellfish industry members in both pre- and post- harvesting settings. The conclusion section evaluates one of the major *V. parhaemolyticus* outbreaks that happened in Gyeongam region in 2003. It evaluates the

¹⁰⁴ Colwell, R. R.

^{2006.} A Global and Historical Perspective of the Genus *Vibrio*, p. 3-11. *In* F. L. Tompson, B. Austin, J. Swings, and A. S. f. Microbiology. (ed.), The *BIOLOGY of VIBRIOS*. Blackwell Publishing, Washington, D.C. ¹⁰⁵ Colwell. R. R.

^{2006.} A Global and Historical Perspective of the Genus *Vibrio*, p. 3-11. *In* F. L. Tompson, B. Austin, J. Swings, and A. S. f. Microbiology. (ed.), The *BIOLOGY of VIBRIOS*. Blackwell Publishing, Washington, D.C.

selected method for the research study and further summarizes recent challenges that the Tongyeong oyster industry has faced in regard to the food safety and microbial contamination in shellfish products.

There have been great potential for exchange of ideas and resources as people seek to create more international fishery businesses opportunities in modern years. The environmental health issues with *V. parhaemolyticus* in shellfish products are significantly considered by policy makers, researchers, and business partners. The ID research is aimed to provide insights on the current environmental health issues affecting global citizens. Its purpose is to mobilize crosscultural understanding and communication with a holistic and interdisciplinary approach in research academia.

Personal and phone interviews and participant observations were conducted in fall 2007 in South Korea. Foreign textbooks and literature were collected mostly through various on-line sources as well as at educational institutions such as libraries of Oregon State University, Kyunamnam University, Korean National Assembly Library and Korean National Fisheries Research and Development Institution. The major groups of the shellfish industry in this study are governmental institutions, a leading national non-profit banking organization, and independent private businesses producing and marketing Pacific Oyster.

Korean National Federation of Fisheries Cooperatives (NFFC) is owned primarily by 1,969 fishermen cooperatives groups and 560,000 fishery stakeholders and has a long history of becoming an ally between federal governments and fishermen. The Korean Ministry of Maritime Affairs and Fisheries (MOMAF) regulates fishery products or investigates outbreaks incidences and shellfish handlers in all stages of handling shellfish products. The three major branches are

administration, financing, and marketing which collaboratively work to manage the market flow of fisheries products including *C. gigas* both domestically as well as internationally.

A lot of small scale oyster harvesters in Tong-yeong sell their products at the wholesale fish market run by NFFC. During the wholesale event, NFFC's laboratory technicians monitor for *V. parahaemolyticus* and other pathogens such as *Noro* virus and *Vibrio vulnificus*. One of the NFFC's major efforts is to manage the food safety in fishery products, and they emphasize public outreach and host numerous educational events that teach the general public about seafood quality and safety management with healthy food recipes and provide research-based information.

NFFC oversees the Tong-yeong Oyster Cooperative (TOC) division. The TOC has developed strategic plans to effectively manage food safety in fishery products and to further engage various research development activities that are partially supported by the major fisheries government such as Korean Ministry of Maritime Affairs and Fisheries (MOMAF).

Tong-yeong is located in the Southwestern Coast of the Republic of Korea, and the city is surrounded by *Hanryeosudo*, a nationally recognized oceanic park with 140 islands. The region's geographic features of Rias coastline and the oceanic climate allow for success in industries such as agriculture, fisheries, and ship building.

The average water temperatures in Tong-yeong were approximately 15.5 °C at the near shore and 16.3 °C at the outer shore with the salinity levels ranging from the lowest at 31.06‰ to the highest at 32.97‰. The city is considered to be slowly urbanizing, yet is till considered a rural community in the nation. The tourism industry has emphasized more attractions to diverse group of visitors each year. About 140,397 residents, mostly composed of ethnic Koreans, live in an area of 234.83km². Most of them frequently use Gyeongnam regional dialect. Approximately

14,454.3 hectares are taken by the oyster harvesting facilities in Tong-yeong mostly practicing hanging cultures, which has been one of the most common harvesting method used in South Korea for the large quantity shellfish production since 1969.

The locally-based, small- and large-scale oyster producers have contributed strongly to the economic variability in Tong-yeong. As the global demand for the *C. gigas* consumption has gradually increased, the Korean shellfish industry has been the leading producer for this marine species and approximately 77%, 261,705 tons, of the total national oyster capture occurred in Tong-yeong, South Korea in 2006.

With the growing industry, more jobs have been created, and competitions among different oyster businesses have occurred. Higher advancement in modern technology and research development brought quality products to a larger number of customers in modern days. Yet, the industry has to inevitably face the challenge of dealing with the region's environmental degradations and the worsening effects in water quality in coastal environment caused by the growth of human population and urbanization.

During the 60 meter per second, the largest catastrophic storming event in the recent Korean history, the industry learned the toughest lesson from the significant economic loss, approximately up to \$76,999,999 on the total captured oysters in 2003. *V. parhaemolyticus* contamination in oyster products was partially associated with the incident, yet the significant amount lost due to the bacterial outbreak is not clearly understood. The director of NFFC emphasized in his 2003 annual Tong-yeong shellfish report that customers today actively responded to the safety of their food products, and the Tong-yeong oyster industry's long-term

sustainability had primarily depended upon how well their shellfish products were managed in a sanitized environment.

According to the Korean National Fisheries Research and Development Institute (NFRDI), the Most Probable Number (MPN) of *V. parahaemolyticus* occurs highly during summer months in which oysters, such as Agust are not typically harvested when approximately less than 1% was pathogenic *V. parahaemolyticus*. Public health concerns due to the pathogenic *V. parhaemolyticus* contamination in food have increased significantly in recent years as the FDA's annual report showed the continually-increasing number of the bacterial infection in South Korea.

According to the Korea Food and Drug Administration (KFDA), the major outbreak of *V. parahaemolyticus* in 2003 could have been related to the consumer's behavior of favoring raw or undercooked oyster consumptions without proper sanitization in large public settings such as cafeteria or restaurant as well as in private settings. During the 2003 storm event, the major shellfish-harvesting cities in the Southern coast faced worsening effects from the *V. parahaemolyticus* outbreak in their shellfish products including oysters and other ethnic foods that used oyster additives such as *Kimchi*.

The public interview data has shown that approximately 42% responded that their shellfish businesses were concerned about *V. parhaemolyticus* in their shellfish products and that *V. parahaemolyticus* significantly impacted their economy; approximately 29% responded that there was no economic significance in their businesses due to *V. parahaemolyticus* infection, and another 29% did not respond. The *Noro* virus infection in Pacific Oyster and sudden algae bloom occurring in shellfish farming waters were emphasized repetitively and discussed by all

interviewees and seemed to be the biggest concern in regard to the Tong-yeong shellfish industry's economy.

Although there are no relevant research studies done on the economic impact from *V*.

parahaemolyticus infection in Pacific Oyster, the long-term consumers' behavior of consuming raw oysters seems to correlate with the KFDA's report, and the evidence from the same report that has shown that *V. parhaemolyticus* infection occurs highest from Gyeonam province compared to other provinces in the nation. This finding has generated the research focuses on the amount of money spent on shellfish safety management and sanitization by members of the Tong-yeong oyster industry.

The NFCC seemed to hold the central role in Tong-yeong since it oversees a number of shellfish productions owned by private owners and is heavily involved in distributing and banking services. The NFCC spent approximately \$2,543,106 to build new modern style shucking facilities and hatcheries and to establish the company's research facility. The company conducted frequent monitoring of water quality at different harvesters and planned to increase the number of inspections in shellfish handling facilities in town. Some of the company's marketing events invited a large number of people and introduced healthy and safe ways of preparing oyster dishes.

To understand *V. parhaemolyticus* infection in pre- and post-harvesting conditions in Tong-yeong, it was necessary to compare modern and old implications in dietary culture of South Korea. The historical records of human consumptions and productions of oysters find evidence that seafood has been the central part of Korean dietary sources for hundreds of years. This tradition has been maintained throughout the modern time, and approximately 40% of

animal protein comes from the seafood in the Korean diet and there are numerous food recipes for oysters according to the director general from the MOMAF.

Numerous cooking methods traditionally have been carefully developed for as their usage in particular social functions. The food ingredients used to be prepared by females members in the household. The food dishes are believed to become rich sources for well-being of humans as well as for the festive mood in holidays. Major features of the Korean traditional dietary culture are based on Confucianism which emphasizes primary features based on gender role, age and social groups.

A public survey was conducted to identify consumers' behaviors in oyster consumption in 2008 in South Korea. The result showed that the raw oyster consumption is the highest among various cooking methods which are frequently consumed at home and diners such as hotels and restaurant businesses. Questions were raised to understand what possible cultural implications or thoughts are existing when considering the common behavior of consuming raw or undercooked oyster products.

In the medical book written by Ho Jun in 1596 A.D., oysters are described to be the most precious marine organisms which carry out smell and flavor (香味) and contain nutritious elements that support fine skin and glowing complexion. A seafood expert from South Korea mentions that people often seek the unique texture of raw oyster dishes. For example, raw oysters are frequently tasted with sauces or dressings such as soy sauce, seasoned hot soybean paste, green mustard paste or a mixture of oil and salt. Raw dishes also have been traditionally fermented for the winter storage, yet, they are frequently found all throughout year since the

industrialization made it possible for producing larger quantities of the fermented products in modern days.

Interestingly, the raw oysters are known to be the popular dish which goes well with *Kimchi*, a pickled fermented vegetable that appears in almost every Korean meal. The high rates of oyster sales are occurring typically during the late fall when there is the major production of winter *Kimchi* or, during holidays such as *Chusuck*, the three-day national holiday for celebrating the thanksgiving and *Sul*, the NewYear celebration similar to ones followed by western and Chinese calendars.

After hearing from the wholesaler's perspectives on the frequent raw oyster consumption in the Tong-yeong fish market, it seems to be that freshly shucked oysters have long been considered to present the animal's health throughout their lifetimes. This would bring beneficial effects for the well being of humans as well as for carrying out festive moods to customers in various social settings. Today, the significant female role in food preparation at homes and in the current workforce of the Tong-yeong shellfish industry seems to be continually remained as similar as the old tradition.

It is my personal observation that the shellfish industry has faced innovative changes which often bring challenges and new assets in dealing with old and new implications in the current practicing. The shellfish industry leaders in Tong-yeong have argued for keeping the shellfish product safe in all stages of handling of animals when considering the industrial economic survival in national and international markets. As one of the interviewees mentioned, today's customers want to purchase their oyster items in more safe and convenient ways.

One of the efficient ways for the marketing strategy is to utilize sales through internet or other sources of mass media such as televisions or mail. The informational technology through the mass-media has perhaps created new venues for some shoppers and buyers who seem to rely on businesses' trust by obtaining information about their products. The advantage from this method is that the information about the products are publicized; and the information containing in the publication are price ranges, origins, pictures, discounts, coupons, and sellers' contact information.

The mass-media marketing phenomenon has created competitions in the market. As a result, fair quality products are conveniently available for a large number of customers in different time and places. Some argue that this kind of marketing strategy is preventing people from getting sick due to the pathogenic contaminants in oyster product such as *V*.

parhaemolyticus. Others seem concerned about the marketing strategy which has been heavily depended upon the usage of mass-media since publicized information could potentially treat seasonal outbreak incidences as the truth and could end up threatening a large number of sales to customers who highly consider of their food choices in terms of health and well-being of humans. It seems to be controversial in utilizing the marketing phenomenon when considering the overall economy of the Tong-yeong shellfish industry since the abundant information can be useful as well as become a harmful effect since it could deliver misleading or overanalyzing stories of microbial concerns in shellfish products and creating more health threats to the general public.

Most of the interview participants mentioned that harvesting environments had been altered, and maintaining good water quality had been the major concern when dealing with the infectious diseases through seafood consumption. Their concerns seemed to make sense that

competition generated based on informational technology opens for a larger business marketing opportunity in both the nation and worldwide. This requires high technology and research advancement. Although the total capture of oysters has increased since the beginning of the industry in the 1950s, one of the interview participants mentioned that the price for the oyster product had remained similar as it appeared in the past. Instead of the oysters being valued as regionally-specific, today's oyster products are often available everywhere in groceries or from invisible shelves on-line or, from TV shows with company brands, thereby lacking the sense of being regionally specific.

The research method was composed of participant observations, interviews, and literature reviews. Prior to the visit to South Korea, an informal interview was undertaken with an oyster hatchery farm in Tillamook, Oregon by support from the OSU Extension Services. The participant observation and interviews were conducted anonymously to ensure the ethical consideration for the participants, and the informed consent handed prior to the interview was previously approved by the Oregon State University Institutional Review Board in fall 2007. One of the benefits from these two approaches was to get credible information from various perspectives of participants whose answers reflected the knowledge and the experiences of their long-term involvements in the industry.

Launching new directions to each step of discoveries, my interactions with the participants provided opportunities for me to enhance my interpersonal communication skills in the consideration of cross-cultural understanding. These allowed me to build extensive networking opportunities with industry professionals throughout the research. I noticed that people seemed to make certain assumptions about me based on nationality, class, status, gender,

etc. These kinds of assumptions could have resulted in providing incorrect answers or misleading information when responding to the questionnaire during the interviews.

The majority of literature used in the study was obtained from academic sources such as governmental documents, research journals or magazines, publicized reports and newspapers, and books written by experts from South Korea. However, finding of foreign textbooks was challenging, and search opportunities were limited, especially after my return from South Korea to the U.S. A lack of credibility in some source types could have prevented me from getting strongly valid arguments in the study. Overall, the research method was able to provide valid answers and has achieved the core objectives by addressing for the pathogenic *V. parahaemolyticus* contamination issues in Pacific Oyster in Tong-yeong, South Korea.

A small scale oyster harvester stated an important finding of my research which is that food products seem to generally become more available through largely-packaged items rather than freshly-produced items in South Korea. They are less common for being specific to the region. Rather, the current competitive mode in the shellfish market has projected toward food products that are easily available to national and global customers. Considering the rising concern over the infectious disease rates across the world and the U.S.'s view on dealing with *V. parhaemolyticus* and other infectious diseases in shellfish products, the competitive nature of the current global oyster market seems to require hurdles and much expenses to manage safety and sanitization issues when considering sustainable directions for the future of the Tong-yeong shellfish industry's global marketing strategies of *C. gigas*, the Korean government's marine policies, and the Tong-yeong community.

While the price of products has remained relatively the same as in the past, inevitable factors shared by the members of the industry have been that of collaboratively dealing with environmental degradation and public health issues. The Tong-yeong shellfish community has faced the challenges with their worsening the marine water quality. This seemed to be the major issue while cultivating and managing their culturally-and economically-viable shellfish animals, particularly *C. gigias*.

Throughout the course of my investigation, it seemed like that conservation of the ocean environment is important when considering sustainability of the old fishery towns like Tongyeong where the business has historically influenced the dietary culture and practices among people in South Korea. Members of the industry have and will try subtly opting into the fast changing regional and global market patterns. They are seeking solutions by cooperating to teach newest technology and research-based information to their own members. They distribute oyster products in available strategies: in-person at the fishery markets, on-line or in mass media-based distributions. Some discussed how purchase on-line or mass-media marketing seemed to be far better choices for customers since the sellers can sell large quantities of shellfish products, and the customers can obtain products with relatively low prices safely without microbial concerns. A few have mentioned that consuming freshly shucked oysters directly at the harvesting sites can better prevent microbial contamination from happening since animals would be freshly-harvested and bacterial growth conditions would be better managed as temperatures and time lengths between harvest and consumption of the oyster products could be effectively controlled.

Understanding the 21st century's human health issues seems to be highly related to shared knowledge and experiences among different people and communities. In *The BIOLOGY* of *VIBRIOS*, Colwell emphasizes that globalization allows more frequent exchanges of people

and goods which encourages "dynamic international scientific enterprise" among different countries. More collaborative approaches such as "interdisciplinary research in the biological, physical, and social science," seem to be the key solutions when dealing with infectious disease issues worldwide.

This thesis provides helpful insights on how one of the world's leading shellfish industries in South Korea deals with human health when considering pathogenic *V. parahaemolyticus* infection in their oyster products. Both economy and culture in the Tongyeong shellfish industry have been fully responsive to on-going microbial concerns in Korean oyster products. The industry has sought its long-term sustainability by adopting new modern technology and research advancement through informational technology while educating fishermen and the general public about shellfish sanitization and safety. As an example, there was a bilateral agreement between the U.S. National Oceanic and Atmospheric Administration and the Ministry of Maritime Affairs and Fisheries of the Republic of Korea on the Arrangement for Scientific and Technical Cooperation in Integrated Coastal and Ocean Resources

Management in 2000. This agreement has allowed for both countries to collaboratively improve and exchange aquaculture data.

Overall, the ID research study potentially has exemplified how prospective researchers can narrow down a global issue of pathogenic *V. parahaemolyticus* infections in *C. gigas*. As I would like to invite a broad audience profile including colleagues, peers, and family members, each one of us seems to contribute in some way to the global health issue, perhaps directly by learning about the environmental health issue of this study or perhaps indirectly by casting our votes to elect future leaders who would have significant influence on diplomatic considerations in the world. As a consumer, I had often neglected to understand the hard work and the

dedication from many helping hands in my food products whether they are producers, sellers, regulators, and researchers. My research has thoroughly presented me with various perspectives that on oyster products for human consumption. My career aspiration is becoming an educator or researcher in the field of environmental science and natural resources, and the outcome from this study will be greatly shared with youth and adult participants in various academic settings in the future.

APPENDICICES

Appendix 1. The final report from the OSU Statistics Department

Final Report

Date: 05/29/08

Project title: An in vitro assay development for the Adherence of Vibrio parahaemolyticus to

Pacific Oysters Project #: c0708-86 Client: Jee Lee

Degree: undergraduate senior Major Professor: Dr. Claudia Hase Department: Bioresource Research

Consultants: Carole Abourached, Aimee taylor

Background

Vibrio parahaemolyticus is a bacterium that exists in both pathogenic and non-pathogenic forms. It is halophilic (requires salt) and can be isolated from marine and estuarine environments. Pathogenic Vibrio parahaemolyticus produces several toxins and can invade host cells like fish and shellfish, such as squid, mackerel, tuna, crab, shrimp, oysters, and clams. It causes gastroenteritis in humans, including acute bacterial diarrheas and food poisonings for many individuals who consume raw shellfish, especially oysters. It has been a significant cause of serious food born illness. In the United States, about 2789 cases were annually associated with V. parahaemolyticus infection through the consumption of raw oysters from 1998 to 2003.

A study showed a correlation between the number of *V. parahaemolyticus* infection among patients and the occurrence of the organism in the environment. It was reported that the high bacterial density was shown in warmer seasons of the year (higher than 17 °C) and at low salinity (lower than 6.5 %).

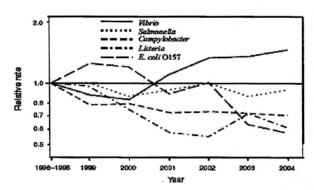


FIGURE 1. Relative rates compared with 1996–1998 baseline period of laboratory-diagnosed cases of infection with Campylobacter, Escherichia coli O157, Listeria, Salmonelia, and Vibrio, by year — Foodborne Diseases Active Surveillance Network, United States, 1996–2004

(http://www.cdc.gov/mmwR/preview/mmwrhtml/mm5414a2.htm#fig1)

Objectives

The objectives of this research are to:

- 1- Determine the optimum growth conditions of *V. p.* (temperature, salinity) in order to understand the specific adherence mechanism of *V. p.* to oysters.
- 2- Compare V. p. response to E. coli

Description of the experiment

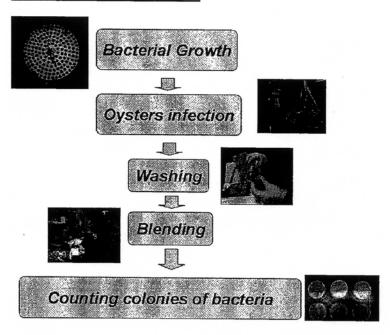


FIGURE 2. Steps of the experiment

V. p. were grown under 2 different temperatures: 20°C or room temperature and 37°C which is the body temperature. The salinity level was 3% in both cases. Other V. p. bacteria were grown under 3 salinity levels: 1, 3 or 6% and the temperature in these cases was 37°C. Note that the bacteria grown at 37°C and 3% salinity were common to both experiments. So, this treatment was done only once. V. p. has 3 growth phases: lag phase, exponential phase, and stationary phase. The bacteria used in the experiment were at the exponential phase. E. coli was used as a baseline control. It was grown under 20°C and 37°C but with no salt added. After the bacteria were grown in the various conditions mentioned above, tissue slices from fresh oysters were infected with V. p or E.coli. The number of bacteria used to infect the oysters was determined (Input). Also, the weight of infected oysters was taken. After finishing the infection, the oyster slices were mixed in the rotator for one hour then, washed, homogenized, then diluted with phosphate buffered saline (PBS) and plated on LB agar plate. The number of bacterial colonies and total bacteria were calculated (output).

Assistant Required

- The client wants to know which conditions are the best to allow adherence of V.
 p. to oysters.
- 2- She wants to compare V. p. to E. coli.
- 3- She would like to fit a curve to the growth of *V. p.* The bacteria that were used in the experiment were at the exponential growth stage as mentioned above but experiments on the growth of the bacteria were done at the beginning in order to decide at which stage to take the bacteria and use them in the infection.

Sources of error

source of ellor
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formed

There are variations in the conditions from one day to another for example the electricity went off in one of the days, in addition to the variation of room temperature from one day to another and from one month to another. In fact, the experimental work was done from February until June. So the atmospheric conditions were variable.

The results of the experiment would have much less error if the client did the different treatments on the same day, then blocking by day can be done.

- Note that some of the values are missing (3 observations out of 31 missing).
- Experimental error was unavoidable because timing is critical in such experiments since the bacteria growth can not be stopped.

Clarifications

what is the scope of inference

- Was a single batch of oyster used? If yes, the scope of inference is limited. The results could be similar or quite different with a different batch of oysters.
- Why are there multiple rows in the data set with the same weight? Are they measurements on the same experimental unit?
- Were there multiple plates for each experimental unit (EU = 2 oysters)? If yes, then Oyster must be in the model and a separate column in the spreadsheet is needed for Oysters so it is clear which lines correspond to the same oyster.
- Are missing data large values? If yes, then ignoring them in the analyses will cause a bias.

trentners Pary

Recommendations

What kind 1. of graph done?

- 1- DATA PRESENTATION: Create a table that has the treatments across the top and the dates for the rows. Sort by date. Each cell will contain the number of experimental units that were done on that treatment x day combination. This will help determine which treatments were done at a certain day.
- 2- DATA TRANSFORMATION: The data ranges over 1000 fold and the variation will not be the same over the full range of data. A log transformation would be the remediation to the heterogeneity of variance in the data.

y= log (x)

RESPONSE: The relationship between the numerator and denominator of the response Y = output/input = Z/X should be studied to make sure that it is appropriate to use the ratio in the analysis. A search for outliers in the input and output must be done. It is often best to take logs of both (Z and X) and then analyze log(Z) as a function of log(X). If the slope is close to 1, then it is probably OK to use the ratio in the analysis.

a less interested

Y= log(2) What is this mean?

A POWER OUTAGE

Who output taken out of the date

4- POWER OUTAGE: The days of power outage should be <u>flagged</u> so they can be taken out of the data if needed since they probably involve a large source of error due to the lack of control of the temperature at those days.

5- MULTIPLE PLATES PER EXPERIMENTAL UNIT (EU = 2 oysters):

Simplified analysis: If there are N plates per EU with N constant through the experiment, then averaging makes sense over the N plates to give one number per EU. Then the data is analyzed with the sample size being the number of EU's (number of groups of 2 oysters) rather than the number of measurements.

NOTE: For log transformation, the log for each plate needs to be taken and the average of the log is obtained to get a single number per EU. The backtransformation of the mean log of the response will give the GEOMETRIC (rather than arithmetic) MEAN back on the original scale. Reference: Statistical Sleuth, log transformation in index.

Varying number of plates per EU: If there are N_i plates per EU with N_i varying then it is not possible to just average them, except as an approximate analysis. If the N_i vary only slightly, then the result will be a good approximation to the full analysis. If the N_i vary a lot, then the simplified analysis may not be a very good approximation.

what dos

TEMPERATURE EFFECT: since the room temperature was varying, then temperature could be used as a continuous covariate. This might help to see a treatment effect.

Instead of comparing the #E temper atures

7- DAY EFFECT: There are variations of the conditions from one day to another which makes day a confounding factor in the experiment. All the treatments were not done on each day to make it possible to block by days and take out the source of variation due to days from the variation due to experimental error. So, it is

as categories, use t°C as a continuous pariable it so the response of its related to the t°C by regression in other winds response depends on the #t values of t°C in other winds

10- MULTIPLE COMPARISONS WITH THE BEST: In the context of less variable data and a more careful experimental design with appropriate blocking and randomization, after fitting an appropriate model the "multiple comparisons with the best" procedure could be used as a way of selecting "the set of treatments or single treatment (if possible) that provides the most desirable result." Reference: Kuehl pages 98ff. For the current data set, there is probably too much variation, so that the client will not be able to detect any differences between the treatments. Therefore, this may be a useful tool in future experiments.

References on using SAS to fit mixed models:

1) SAS for Mixed Models, 2nd edition (2006) by Littell, et al

2) Contemporary Statistical Models for the Plant and Soil Sciences (2000) by Schabenberger and Pierce

OSU Ware CD

STAT Graphics.

Copy > parte data into the STATCT.

From

Appendix 2. Institutional Review Board (IRB) Report



Institutional Review Board • Office of Sponsored Programs and Research Compliance
Oregon State University, 312 Kerr Administration Building, Corvallis, Oregon 97331-2140
Tel 541-737-3437 | Fax 541-737-3093 | http://oregonstate.edu/research/RegulatoryCompliance/HumanSubjects.html | IRB@oregonstate.edu

CERTIFICATION OF EDUCATION

FOR THE ETHICAL USE OF HUMAN PARTICIPANTS IN RESEARCH PROJECTS

The Oregon State University Institutional Review Board (IRB) requires that all research staff and students engaged in thesis/dissertation/projects or research involving human participants must certify that they have completed an educational component in the ethical use of human participants in research. The full version of this policy is available at: http://oregonstate.edu/research/RegulatoryCompliance/HumanSubjects.html.

To satisfy the training requirement, the IRB believes that the CITI web-based resource indicated below provides coverage of the ethical principles and procedures to be used when conducting human subjects research. However, education can be in a variety of forms such as the NIH web-based tutorial listed on the next page, graduate seminars, professional or university-sponsored workshops, monographs, or other comparable means of study.

To document that the required training has occurred, this form must be submitted, *only once*, to the Human Protections Administrator. IRB staff maintains a database of submitted Certification of Education forms. A form must be on file for all research staff working on a given project before the review and consideration of any IRB request will occur.

This certification also responds to the NIH policy requiring proof of education for NIH sponsored research and provides documentation of the required education. Investigators and key personnel (please see the next page for a description of key personnel and a link to the full NIH policy) should print this form, indicate the resource(s) reviewed, sign, and forward this form for signature by the Human Protections Administrator and the OSU Authorizing Official.

Resource in which training in the ethical use of human participants was received (a brief description of recommended resources are on the next page): Collaborative IRB Training Initiative CITI: https://www.citiprogram.org/default.asp Other (describe specifically on a separate page)	
I, (insert Researcher's name)	, attest that I have successfully completed the
	9/28/07
Signature	Date
OREGON STATE UNIVERSITY INSTITUTIONAL REVI	EW BOARD APPROVAL:
Human Protections Administrator, Office of Sponsored Programs and Research Compliance	Date
OSU approves the use of the indicated resource. The individua resources indicated to receive education in the ethical use of hu	
☐ Check here if the Authorizing Official Certification is required (for NIH sponsored applications only) OREGON STATE UNIVERSITY AUTHORIZING OFFICIAL CERTIFICATION:	
Signature Pagent S. Louve, Institutional Authorizing Official and Director, Office of Sport	Date

Collaborative IRB Training Initiative (CITI)

https://www.citiprogram.org/default.asp

The CITI educational program consists of several modules, developed by experts in the IRB community, focused on the different aspects of ethical considerations involved in Biomedical and Social Behavioral research involving human participants. Registration is required for this tutorial. Each module and associated quiz should take approximately 30 minutes to complete. If the CITI program is selected, the IRB requires the following modules be reviewed to satisfy the mandatory educational component. Reviewing these modules will help to establish an understanding of the historical perspectives, ethical principles and federal regulations associated with the conduct of research with human subjects.

- 1 "History and Ethical Principles SBR"
- 2 "Defining Research with Human Subjects SBR"
- 3 "The Regulations and the Social Behavioral Sciences SBR"
- 4 "Assessing Risk in Social and Behavioral Sciences SBR"
- 5 "Informed Consent SBR"
- 6 "Privacy and Confidentiality SBR"
- 7 "Research with Protected Populations, Vulnerable Subjects – An Overview"

The IRB strongly recommends that other modules having relevance to the proposed research should be completed as appropriate. For example, the under the "Optional Modules" menu the "International Research – SBR" module should be completed for projects involving international research.

Once the appropriate modules have been completed, submit the completed OSU IRB Certification of Education form (which is the page immediately preceding this one).

Other

If the individual listed on this form utilized another source of education, describe the training received in detail (attach a separate sheet). Be sure to indicate the type of education received (e.g., workshop, seminar) and the specific topics covered. The OSU IRB expects that each individual certifying "Other" training has occurred has read "The Belmont Report" (available at: http://www.hhs.gov/ohrp/humansubjects/guidance/belmont.htm). It describes and explains the ethical principles that should be followed by investigators: respect for persons, beneficence and justice.

National Institutes of Health Tutorial

http://cme.cancer.gov/clinicaltrials/learning/humanparticipant-protections.asp

This program has been developed by the U. S. Department of Health and Human Services, the National Cancer Institute, and the National Institutes of Health specifically for NIH grantees. This medically oriented tutorial provides a brief introduction into common concepts, principles, and issues related to protection of human participants. The tutorial will help research staff identify research activities that involve human participants, as well as help researchers understand how to protect the rights and welfare of all human participants involved in research.

The tutorial should take approximately one hour to complete. A Certificate of Completion is available once the tutorial has been completed; however, that form does not replace the requirement to submit a completed Certification of Education form (the page immediately preceding this one).

Key Personnel:

According to the NIH policy, documentation of education completed in the protection of human subjects is required for each individual identified as "key personnel" in the proposed research. Key personnel include all individuals responsible for the design and conduct of the study. However, OSU's IRB has decided to include any personnel who will be responsible for the design or conduct of the study, have access to the human subjects, or have access to identifying and confidential information (including but not limited to name, email address, and social security number). The full NIH policy is available at: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-00-039.html

Revised: 03-05



Institutional Review Board • Office of Sponsored Programs and Research Compliance Oregon State University, 312 Kerr Administration Building, Corvallis, Oregon 97331-2140 Tel 541-737-4933 | Fax 541-737-3093 | http://oregonstate.edu/research/osprc/rc/humansubjects.htm

TO: Bryan Tilt Anthroplogy

IRB #: 3738 - Exploratory Study on the Impacts of Vibrio Parahaemolyticus Outbreak in Economy and Culture in Tongyoung, South Korea (Student Researcher: Jeehye Lee)

Level of Review: Exempt Expiration Date: 10-22-08 Approved Number of Participants: 12 The referenced project was reviewed under the guidelines of Oregon State University's Institutional Review Board (IRB). The IRB has approved the: (X) Initial Application () Continuing Review () Project Revision

with a (if applicable): () Waiver of documentation of Informed Consent A copy of this information will be provided to the full IRB committee.

- CONSENT FORM: All participants must receive the IRB-stamped informed consent document. If the consent is in a format that could not have stamp placement (i.e. web site language, email language, etc), then the language must be exactly as the IRB approved it.
- PROJECT REVISION REQUEST: Any changes to the approved protocol (e.g. protocol, informed consent form(s), testing instrument(s), research staff, recruitment material, or increase in the number of participants) must be submitted for approval before implementation.
- **ADVERSE EVENTS:** Must be reported within three days of occurrence. This includes any outcome that is not expected, routine and that result in bodily injury and/or psychological, emotional, or physical harm or stress.
- CONTINUING REVIEW: A courtesy notice will be sent to remind researchers to complete the continuing review form to renew this project, however - it is the researcher's responsibility to ensure that continuing review occurs prior to the expiration date. Material must be submitted with adequate time for the office to process paperwork. If there is a lapse in approval, suspension of all activity including data analysis, will occur.
- **DEVIATION/EXCEPTIONS:** Any departure from the approved protocol must be reported within 10 business days of occurrence or when discovered.

Date: 10-23-07

Forms are available at: http://oregonstate.edu/research/osprc/rc/humansubjects.htm.

If you have any questions, please contact the IRB Human Protections Administrator at IRB@oregonstate.edu or by phone at (541) 737-8008.

Espiraz 4daus

Elisa Espinoza Fallows

Elisa

IRB Human Protections Administrator

() Waiver of Consent

Initial Application

Please read through the entire application before beginning. Requested information must be typed and submitted to the Human Protections Administrator, Office of Sponsored Programs and Research Compliance, 312 Kerr Administration Bldg. Be sure to allow adequate time for review and comments. Incomplete requests will delay the review process. Applications will be returned without review if the application involves technical language without common explanations or if the application is poorly constructed grammatically. Send an email to IRB@oregonstate.edu or call (541) 737-8008 with any questions.

Project Title:			IRB Application #: Assigned by IRB Office	
Exploratory Study on the im				
Economy and Culture in Oys Principal Investigator:	sters industry in Tong	gyoung, South Korea Department:		
Bryan Tilt		Anthropology		
PI Email:		, and a pology	PI Telephone:	
Bryan.Tilt@oregonstate.edu	1		(541) 737-3896	
Student Researcher:	•	Class or Degree Program (if		
Jeehye Lee		International Degree		
Primary Contact Person:	Email:	International Degree	Telephone:	
Jeehye Lee	leejee@onid.o	rst.edu	(503) 706-8329	
Campus or US Mail Address (to sen	, ,		Date:	
6205 SW 183 rd Terrace Bea	•		09/15/2007	
http://oregonstate.edu/r		oard meetings and submissionsubjects.htm	on deadlines can be found	d at:
2. Method of Submission:				
	ment) sent in mail. Fo	tion and appropriate material or Exempt from Full Board nit 3 copies.		
		ate materials as email attache e the application.	ments. The signature pa	ige
3. External Funding (present	t or proposed):			
☐ Yes Contract or grant Funding source: If funded by NII		ding subcontracts), submit (a copy of the grant.	
⊠ No				
Kerr Administration Building, Corvallis, Or	egon 97331-2140			Rev. 03

4. Certification of Education:

All research staff involved in this project must receive training in the ethical use of human participants in research. To document this training, the Certification of Education form must be submitted (available at: http://oregonstate.edu/research/osprc/rc/humansubjects.htm). The Certification of Education form is NOT the confirmation issued by the educational tutorial. The Certification of Education form needs to be submitted only once for each researcher. *Submission of all necessary certificates is a prerequisite to review.

Research Staff Name	Role in Project	Certification of Education Submitted
Bryan Tilt	Principal Investigator	⊠ Yes □ No*
Jeehye Lee	Student Researcher	☐ Yes ☐ No*
		☐ Yes ☐ No*
pathological/diagnostic specimens, if the	eeks fied data or samples?*	information is recorded by
Risk/Benefit Assessment:		, and the second
Minimal risk		
Greater than minimal risk, but holds	prospect of direct benefit to subjects	
Greater than minimal risk, no prosp knowledge about the subject's disor	ect of direct benefit to subjects but likely der or condition	to yield generalizable
Research not otherwise approvable problem affecting the health or welf	but presents an opportunity to understand, are of the subjects.	prevent, or alleviate a serious

Initial Application Institutional Review Board

5.

6.

7.

8.

Rev. 03/2006 Page 2 of 4

9.	Su	bject Population:		
		mber of subjects that will be enrolled over the life of the stud order to enroll more than the number specified, a Project R		
		rticipant age range (check all that apply): pulations designated with an asterisk (*) are vulnerable pop	ulati	ions and ineligible for exempt review.
		*0-7: Youth (include parental consent form)	\boxtimes	18-65
		*8-17: Youth (include assent and parental consent)		65 and older
		pulations targeted in this research (check all that apply): pulations designated with an asterisk (*) are vulnerable pop	ulati	ions and ineligible for exempt review.
		*Persons with mental/emotional/developmental disabilities		*Pregnant women/fetuses/IVF
		Gender imbalances – all or more of one gender		*Prisoners
		*Minority group(s) and non-English speakers		Elderly subjects
10.	If t	he research involves any of the following, check the appro	pria	ate box:
		Audio or videotaping Ineligible for Exempt review	\boxtimes	Survey/questionnaire
		Deception Requires review at Full Board level		Behavioral observation
		Radiation Complete and submit Attachment A	\boxtimes	Study of existing data
		Human materials (i.e., blood or other bodily secretions) Complete and submit Attachment B		Microorganisms or recombinant DNA
		Waiver of documentation (signature) of informed consent <i>Include justification in the protocol</i>		
		Waiver of informed consent Include justification in the protocol		
	\boxtimes	Consent material in another language Include consent material in other language and an English qualifications of translator and of research staff obtaining		
		Other research site (i.e. school, tribal reservation, etc) Provide documentation of the approval of the relevant IRB Name of other research site(s):	, scl	nool principal, tribal office, etc.
	\boxtimes	International research site Provide documentation of the approval of the relevant IRB Name of international research site(s):	, coi	mmunity leader, FWA, etc.
		Submitted to another institution's IRB for review Name of institution:		,

Initial Application Institutional Review Board Rev. 03/2006 Page 3 of 4

11. Attachments (check all that apply):	
Protocol (required)	Grant (required for NIH, DHHS, PHS funded projects)
	☐ Recruiting tools (scripts for recruitment/screening)
Assent Document	☐ Test instruments (e.g., questionnaires, surveys)
Attachment A: Radiation	☐ Material in other languages
Attachment B: Human Materials	Additional information (e.g., debriefing materials)
Approvals from other research site	es (other IRB, school principal, tribal office, etc)
12. Will the study need to be registered	with ClinicalTrials.gov?
☐ Yes For more information: http☒ No	o://www.oregonstate.edu/research/osprc/rc/humansubjects.htm
13. Conflict of Interest:	
the welfare of human subjects. If this	that there are no conflicts of interest in research projects that could affect study presents a potential conflict of interest, additional information will ples of potential conflicts of interest may include, but are not limited to:
 business in which the faculty mem A researcher participating in resea A researcher or family member as research activities related to the re 	rch on a technology, process or product developed by that researcher suming an executive position in a business engaged in commercial or searchers University responsibilities rving on the Board of Directors of a business from which that member
For more information: http://oregonst.	ate.edu/research/osprc/rc/conflictofinterest.htm
Conflict of Interest Statement:	
	a potential financial gain to you, a member of your family, or any of the earance of a potential conflict of interest?
Yes Please describe any potent consent document.	tial conflicts of interest in a cover letter and disclose in the informed
Has this potential conflict	been disclosed and managed? Yes* No
⊠ No	
Final IRB approval cannot be granted	until all potential conflicts of interest have been managed. until all potential conflict matters are settled. The full IRB committee closure of conflict statement in the consent form.
By signing below, I certify that the above involving human participants, including r the IRB.	information is accurate and complete. I understand that research ecruitment, may not begin until full approval has been granted by
Signature	Date
Principal Investige	•
	ill or fax this page with the PI's signature to the Human Protections Administrator. Rev. 03/2
Application tional Review Board	Page 4

Exploratory Study on the Impacts of *Vibrio parahaemolyticus* Outbreak in Economy and Culture in Oysters Industry in Tongyoung, South Korea

Principal Investigator: Bryan Tilt, Department of Anthropology Student Researcher: Jeehye Lee, Undergraduate Student

Study Protocol

- 1. Brief Description. The exploratory study examines how the bacterial outbreaks due to *Vibrio parahaemolyticus* have impacts on economy and culture in Tongyoung, located in Southeast in South Korea. Circumstances of both pre-harvest and post-harvest of oysters will be investigated in order to understand the influence of bacterial outbreak, such as the major one in 2003, and its consequences to economy and culture in Tongyoung. During the study, it will be explored how the contamination of *Vibrio parhaemolyticus* producing toxin would be happened during pre- and post-harvests processes. The research will also focus on how the culture has evolved with bacterial outbreak in Tongyoung. The previous research conducted was based on laboratory science; therefore the overall research aims for learning the diverse issues of bacterial outbreak in shellfish industry. This interdisciplinary approach embraces both social and biological science societies internationally. Upon completing the research, I will write a combined thesis for International Degree and Bioresource Research. Findings from this project will be used for presentations in various educational settings.
- **2. Background and Significance.** In the United States, *Crassostrea gigas* was originally imported from Japan in 1900's. It is also the most popular harvested oysters' species in South Korea since 1970s. Its Korean name is called Cham Gul. Approximately 70% of Korean shellfish industry was composed by oysters industry in 2006. The number of production has been gradually increased in every year, and these products are consumed nationally and internationally. Tongyoung is the major city of oysters industry, located in Kyungnam province in South Korea. It has an advantage of the oysters production since its unique geographic feature closes to the Southern coast, precidine environmental condition, and abundant natural resources. In summer 2003, however, there was a major outbreak of *Vibrio parahaemolyticus* in oysters in South Korea and 71 people were reported associating with gastroenteritis after consuming of oysters dishes from home, restaurants and various food handling places. About 70% of the total cases of the national *Vibrio* outbreak were related with consumption of oysters. The economic loss of the major shellfish distributor was approximately \$76,999,999. Considering the patterns of annual bacterial outbreak is necessary to evaluate how its influence to people's life within the industry. The possible outcome from the study is to make recommendation to the decision makers and other educators about economic and cultural value of oysters.
- **3. Methods and Procedures.** The exploratory study will be undertaken during November 2007. Specific methods and procedures for the study are as follows.
 - a. Recruitment: A total of twelve (12) subjects will be recruited to participate in the study. Subject identification and recruitment will take place according to the procedures outlined below, under "participant population" and "subject identification and recruitment,"
 - b. Semi-structured interview: Study subjects will participate in a semi-structured interview, to be conducted in a place convenient for the subject. Potential interview locations include offices, and places of employment for study subjects. It also will be an alternative choice to conduct phone interviews for any particular limitation or concern. The semi-structured interview protocol is attached to this IRB application. Study subjects will be composed of small and large scale oysters farmers and businesses, Ministry of

- Maritime Affairs and Fisheries, Korea Food and Drug Administration, National Federation of Fisheries Cooperatives in Tongyoung, and traditional and modern fish markets. Interviews will last approximately 1 hour or 1.5 hours.
- c. Analysis: The researcher, who is a bilingual in Korean and English, will take written notes of the subjects' responses to interview questions. No tape recording will be used. Content analysis and open coding of interview notes will be undertaken at a later time. Participants' identities will be kept confidential. A unique identification code, rather than the participants' names, will be used during analysis of the data.

4. Risks/Benefit Assessment.

- a. Risks: Possible scenario of the risks are some participants may consider some questions sensitive and regarded as invasion of privacy. As my investigation includes the economic loss from the major outbreak, the victims of the event may take questions responsive. Some participants may feel that recruitment via other government officials constitutes a form of coercion. Some participants may feel pressured by their responsibility of being official and providing accurate information. In order to minimize these risks, potential subjects will be given the consent form at the time of first contact (see attached consent form). They will be informed fully as to the goals of the study and the expectations placed upon them. To the extent possible, subjects will be interviewed individually and in private in order to avoid coercion from peers. Participants will be informed as part of the consent form that their participation is voluntary, that they may choose not to answer any question, and that they may end their participation in the study at any time.
- b. Benefits: This study is for academic purposes; participants will likely not benefit directly from this study. The study has scholarly and practical importance. However it will benefit to Tongyoung village by providing a general overview on how bacterial outbreaks generates. In addition, this will also provide important feedback to local governments on the economic and cultural aspects of shellfish industry, particularly oysters in Kyungnam province, South Korea. This will be helpful to the educational settings of various age groups. It also opens to doors communities in Oregon State University and Kyungnam University. The exploratory study will be used a preliminary study for the further study that food safety and management.
- d. Conclusion: In summary, the risks of this study to individual participants, as described above, are minimal. The benefits of the study, including building institutional relationships and recognizing of the issues of economy and culture outweigh the risks.
- **5. Participation Population.** Twelve groups will be interviewed from local regions in Kyungnam province. There will be four groups of two; these four groups will be consisted of small and large scaled oysters farmers and traditional and modern seafood market places sales representatives in Tongyoung. The other four groups of one will be recruited from National Federation of Fisheries Cooperatives in Tongyoung, government agent from Ministry of Maritime Affairs, Korea Food and Drug Administration, National Fisheries Research and Development Institute in Kyungnam province in South Korea.
- **6. Subject Identification and Recruitment.** Interview participants will be selected using a sample of convenience (snowball sample), with Korean collaborators from the Ministry of Maritime Affairs

and Kyungnam University helping to select appropriate interview participants. We will not exclude participants based on characteristics such as gender or ethnicity. Minors will not participate in this study.

- **7. Compensation.** Participants in this study will not be compensated.
- **8. Informed Consent Process.** Every study participant will be given an informed consent form that describes the purpose, methods, risks and benefits of this study (see attached consent form; Korean translation to be completed before commencing research). The consent form will follow the structure and content recommended in the OSU IRB template. Each informed consent form, after explaining the purposes, procedures, risks and benefits of the study, will ask the participant for a signature indicating his/her voluntary participation in the study under the terms described in the consent form. In case of phone interview, the informed consent process will be undertaken at least a week prior to the interviewing date. The informed consent form will be sent to participants electronically, fax or via mail with an envelope and a prepaid stamp. During the first phone contact, Jeehye Lee, the principal investigator will explain a brief overview of the informed consent form. The participants may contact Jeehye Lee for any discussion or clarification of the form.
- **9. Anonymity or Confidentiality.** In every case, participants' identities will be kept confidential. Upon agreeing to participate in this study, subjects will be given a unique identification code (marked "subject ID code" on the semi-structured interview protocol). This code, and not the direct identifier (e.g. name) will be used for all data analysis pertaining to this study. The link to the direct identifier will be kept separate from the information in the data files, and will be reactivated only if and when a larger study is undertaken in the future. Only the researcher (Jeehye Lee) will have access to identifiers and data files.

Exploratory Study on the Impacts of *Vibrio parahaemolyticus* Outbreak in Economy and Culture in Oysters Industry in Tongyoung, South Korea

Principal Investigator: Bryan Tilt, Department of Anthropology Student Researcher: Jeehye Lee, Undergraduate Student

The research question is 'How big a problem is bacterial contamination in oyster industry in Tongyoung? What problems does it cause and occur? How the economic loss due to *Vibrio* outbreaks in your area? Also, how the incidences have an effect on various practices in your field?' Total six groups were included as small and large scaled oyster harvesters, Ministry of Maritime Affairs (MMA) & Korea Food and Drug Administration (KFDA), managers from traditional and modern seafood markets and a researcher in Tongyoung. My research question will be asked to all these seven groups.

All the groups except MMA and KFDA and a researcher contain two sets of questions dividing into sections: background information and economy or culture. In addition to the sections, MMA and KFDA, is composed of a regulation section. Background information section inquires about the subject and its organization: history, company mission, scales, and so on. The purpose of the background information is to clearly understand the subject and its organization and not to mislead any information during interviews. Also some questions would support exploring possible transitional routes to bacterial contaminations to oysters. In each group, slightly dissimilar questions were addressed in the section of economy or culture. Lastly I include a research since the subject would have an input to the research question and their research work would eventually respond to solve the bacterial contamination issue in Tongyoung in a scientific way.

[1] Small scaled oyster harvester & Large scaled oyster harvester

Background Information[BI]

- 1. What are your name and age?
- 2. What is your company's name?
- 3. How many employees working in your business?
- 4. What are genders of your workers?
- 5. How long have your employees worked for the business or its related areas?
- 6. Provide me general information about the background of your business; how long have you operated your business? What is your company's mission statement?
- 7. What are the common ages of oysters do you sell in the market?
- 8. What are ways to bacterial contamination in pre-harvest condition and in post-harvest condition?
- 9. How to prevent from getting bacterial contamination in those two conditions (mentioned in the question 8)?

Economy/Culture [EC]

- 1. What is the average yield of the annual oyster harvest in your company in kilograms and in dollar value? Do you export your oyster product? If you do, tell me about the annual production, scales and so on.
- 2. What is the impact of bacterial outbreak on your income?
- 3. How many people in average work for your company?
- 4. What do you do in order to protect the health of oysters against bacterial contamination?

- What is your budget to protect the health of oysters, specifically against outbreak of *Vibrio parahaemolyticus*?
- 5. What kinds of agencies help you financially and institutionally? If Korean government support you from dealing with bacterial outbreaks, what kind of supports have you had and from them? How did the assistance benefit you and your business?
- 6. Explain the life cycle of Pacific oysters (Cham Gul) grown in Tongyoung
- 7. When is the popular harvest season? How is Chu Seok related to the season?
- 8. What is the geographical feature of your site and what kind of changes in climate and geographical features in the last 10 years? How did these changes have influences in your business?
- 9. How would you prevent from getting loss from the annual *Vibrio parahaemolyticus* outbreak?
- 11. Do you do any special ceremonial events or cultural practices before/after the harvest?
- 12. What kinds of common methods do you use for harvesting oysters? How are these methods adopted and related to the geographic features of Tongyoung?
- 13. After the harvest, explain the kinds of final products you make. What are common methods of selling your final products? For example, you may sell your products through traditional markets, on-line, or direct transaction between the general public.
- 14. Who are general consumers of your products? How are final products situated? For example, do you sell oysters containing their shells or not?
- 15. What do other common bacterial contaminations become problems in the oyster industry?

[2]Ministry of Maritime Affairs (MMA) & Korea Food and Drug Administration (KFDA)

Background Information[BI]

- 1. What are your name, and age?
- 2. What are the major roles and general body structure of MMA in the relation to the Korean shellfish industry, specific to oyster business in Tongyoung?
- 3. What other governmental agencies that inspect oysters in South Korea?
- 4. What other common bacterial contaminations have become problems in the oyster industry?
- 5. If you release news to the general public and members in the shellfish community in Tongyoung, what resources do you use?

Regulation[R]

- 1. How do MMA and KFDA monitor oysters? What kind of steps and frequencies of examinations? How do they regulate oysters in order to avoid bacterial contamination, especially *Vibrio parahaemolyticus*?
- 2. Once, there are reports of outbreaks announced from media sources, how those incidences could be taken care of. Are there any kinds of compensations to the victims or responsibility given to the producers/ sellers?
- 3. According to the major outbreak of *Vibrio parahaemolyticus* in 2003 due to *Vibrio parahaemolyticus*, the climate change, caused to increase ocean temperature and eventually resulted the incidences of bacterial outbreak. How would you prevent the future incidence that would be similar to the incidence in 2003?
- 4. What kind of beneficial service or program you provide to Korean shellfish industry and how do you communicate with members of the industry?
- 5. How would you protect general public against exposure to bacterial outbreaks due to the

consumption of raw oysters? What are ways for consumers to be aware of the safety of seafood while maintaining the food practices with oysters in South Korea?

Economy/Culture[EC]

- 1. In Tongyoung, what is the annual production of oysters? Explain the beneficial and geographic feature in the region. When was the current outbreak in Tongyoung and how much was the economic loss in Tongyoung due to that incidence?
- 2. What are common Korean oyster dishes and is there any correlation of their preparation for/ against the contamination of bacteria?
- 3. How the current outbreaks have effects in the current shellfish market in Tongyoung, specific to oysters?
- 4. What are the major causes of the annual Vibrio parahaemolyticus outbreak in oyster industry in South Korea? Specifically identify the situations in situations of pre-harvest and post-harvest.

[3]National Federation of Fisheries Cooperative

Background Information

- 1. What are your name, age and position?
- 2. What are the major roles of National Federation of Fisheries Cooperative in the relation to the Korean shellfish industry, specifically oysters?
- 3. How is the organization unique, compared to other bankers from South Korea?
- 4. How many are the organizations in Tongyoung?
- 5. Explain the summary of the history of your organization in Tongyoung and how your organization has coexisted with oysters industry in Tongyoung?

Economy/Culture

- 1. Do you provide any financial supports or assistantships if members from oysters industry in Tongyoung when they face challenges or losses due to bacterial outbreak and other disasters?
- 2. What are ways to adjust the price of oysters? What is related to the highest or the lowest prices of oysters in the recent years? How does a bacterial outbreak influence in its market value? The latest bacterial outbreak happened in 2003 can be an example.
- 3. What happen with the market value of oysters before and after the bacterial outbreak in oysters?
- 4. What is the average annual yield of harvested oysters in Tongyoung?
- 5. How does oyster production influence the total Tongyoung's economy?

Traditional seafood market

Background Information

- 1. What are your name, age and position?
- 2. How long have you worked for the business?
- 3. Explain the migration of oysters after its harvests? Where do you initially get your oyster products?
- 4. When is the most popular season to get and sell oysters in Tongyoung?
- 5. What are the common ages of oysters do you sell in the market?

- 6. What becomes a problems for other common bacterial contamination in the past?
- 7. Approximately how many days do you preserve oysters products after you purchased from the primary market or the harvesting site? How do you deal with the left over products or what do you do in order to avoid having the left over ones?
- 8. What are the classification of the average customers?
- 9. What is your organization called? When was your organization established? How long have you worked for the business or related businesses?

How much seafood, especially oyster products, do you handle? What other products do you deal with?

Economy/Culture

- 1. How is the price of your oysters products set?
- 2. How long do you usually work in a day?
- 3. Explain your preparation of the oyster products. How many shells do you open in a day? What are most popular ones to your customers- oysters with shells or without shells?
- 4. What kinds of agencies help you financially and institutionally? If Korean government support you from dealing with bacterial outbreak, such as *Vibrio parahaemolyticus*, what kind of support have you gotten?
- 5. How did the assistantship benefit you and your business?
- 6. After hearing bacterial outbreak, did the news have any impact on your business? Overall all, any impact on your business?
- 7. What are ways to the contamination of bacteria in the post-harvest condition?
- 8. How can you prevent your products from bacterial contamination?

Modern seafood market

Background Information

- 1. What is your name, age and position? What is your organization called? When was your organization established?
- 2. How long have you worked for the business?
- 3. How much seafood, especially oysters products, do you handle? What other products do you deal with? What kind of oysters products do you sell in the market?
- 4. If you could classify the average consumers in your store-ages, genders, regions, and so on?
- 5. Explain the migration of oysters' products after their harvest? Where do you get your initial oysters products?
- 6. What are the common ages of oysters do you sell in the market?
- 7. What other common bacterial contamination become problems in the oyster industry?
- 8. Approximately how many days do you preserve the oyster product after your purchased from the primary markets or the harvesting sites?
- 9. Did you get any support from governmental agencies or others for your economic loss due to bacterial outbreak in the past? If you did, what kind of support did you get? If you did not, how did you deal with the consequences? If there were any reason, why couldn't you get any support?

Economy/Culture

- 1. How is the price of the oysters products adjusted? How many workers works for the oyster products?
- 2. Explain your preparation to the oyster products?

- 3. When is the most popular season to get and sell oysters?
- 4. What are ways to expose to bacterial contamination during the post-harvest period?
- 5. How could you prevent from getting bacterial contamination in steps after oysters have released from the harvesting sites?
- 6. How do you hear about bacterial outbreak? Any influence in your business?

Researcher

- 1. What are your name, position, and age?
- 2. Briefly describe your research. How is it related to issues with *Vibrio parahaemolyticus* in pacific oysters? How does the mechanism of separation between bacteria and oysters' tissue cell work? Why does it important to get healthy larvae?
- 3. How long has your study been and your reasons to get involve with the study? How does your study would benefit to the economy and the culture of Tongyoung's shellfish industry?
- 4. What is the trend of Vibrio outbreaks in pacific oysters in South Korea for the last five years?
- 6. In the common situation, what ways to the bacterial contamination? Associate with any incidences happened in the past.



DEPARTMENT of ANTHROPOLOGY

Oregon State University, 238 Waldo Hall, Corvallis, Oregon 97331

Tel 541-737-4515 | Fax 541-737-3650 | www.oregonstate.edu/dept/anthropology

INFORMED CONSENT DOCUMENT

Project Title: Exploratory Study on the Impacts of Vibrio parahaemolyticus Outbreak in Economy

and Culture in Oysters Industry in Tongyoung, South Korea

Principal Investigator: Bryan Tilt, Department of Anthropology **Student Researcher:** Jeehye Lee, Undergraduate Student

WHAT IS THE PURPOSE OF THIS STUDY?

You are being invited to take part in a research study on how the bacterial outbreak, specifically *Vibrio parahaemolyticus*, have impacts on economy and culture in Tongyoung, South Korea. The goals of the study are to learn more about your community, how these impacts exist in both situations of pre-and post-harvests of oysters. The results of this study will be used for technical reports, scientific publications, and public presentations. The study will help government official to understand how shellfish industry and communities in Tongyoung have evolved the culture and the economic development. The study that you will be participated in will be also set perspectives to the global communities.

WHAT IS THE PURPOSE OF THIS FORM?

This consent form gives you the information you will need to help you decide whether to be in the study or not. Please read the form carefully. You may ask any questions about the research, the possible risks and benefits, your rights as a volunteer, and anything else that is not clear. When all of your questions have been answered, you can decide if you want to be in this study or not.

WHY AM I BEING INVITED TO TAKE PART IN THIS STUDY?

You are being invited to take part in this study because you are a government official, shellfish farmers, or sales representative in Tongyoung, South Korea. I am interested in your perspective on your community and on rural industry.

WHAT WILL HAPPEN DURING THIS STUDY AND HOW LONG WILL IT TAKE?

If you agree to participate in this study, I would like to interview you, in your office or in another convenient place. I will ask you questions about general overview of oyster production, the past and present economic conditions of your community as well as kinds of support that you are getting or giving for maintaining the food safety. You can tell us your answers, and we will write them down. If you agree to take part in this study, your involvement will last for about one hour or one and a half of an hour.

WHAT ARE THE RISKS OF THIS STUDY?

You may consider some of the interview questions to be sensitive. Some people do not want to respond to questions for research. If for any reason you do not want to answer a question, please let me know and I will skip it. You may end your participation in this study at any time.

WHAT ARE THE BENEFITS OF THIS STUDY?

Oregon State University • IRB Study #:3738 Approval Date: 10/23/07 Expiration Date:

This study is for academic purposes; you may not benefit directly from taking part in this study. However, I hope that the results of this study will support to the educational settings of various age groups. The research will possibly open diverse issues of Korean shellfish to broad communities. The study can be used as preliminary study for any further research opportunity to promote seafood safety and management.

WILL I BE PAID FOR PARTICIPATING?

You will not be paid for being in this research study.

WHO WILL SEE THE INFORMATION I GIVE?

The information you provide during this research study will be kept confidential to the extent permitted by law. To help protect your confidentiality, you will be given an identification code so that your answers will not be directly linked to your name. The identification code will be stored in a computer, and only the researchers will have access to it. If the results of this project are published your identity will not be made public.

DO I HAVE A CHOICE TO BE IN THE STUDY?

If you decide to take part in the study, it should be because you really want to volunteer. You will not lose any benefits or rights you would normally have if you choose not to volunteer. You can stop at any time during the study and still keep the benefits and rights you had before volunteering. You will not be treated differently if you decide to stop taking part in the study.

If for any reason you do not want to answer a question, please let us know and we will skip it. You may end your participation in this study at any time. If you choose to withdraw from this project before it ends, the researchers may keep information collected about you and this information may be included in study reports.

WHAT IF I HAVE QUESTIONS?

If you have any questions about this research project, please contact: Bryan Tilt, the principal investigator at Oregon State University (1-541-737-3896, Bryan.Tilt@oregonstate.edu) or Jeehye Lee, a student researcher at Kyungnam University (010-3980-8329, leejee@onid.orst.edu).

If you have questions about your rights as a participant, please contact the Oregon State University Institutional Review Board (IRB) Human Protections Administrator, at 1-541-737-4933 or by email at IRB@oregonstate.edu.

•	ant's Name (printed):		
Sionati	ure of Participant)	 (Dat	e)
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o guar	are of randopandy	`	,



DEPARTMENT of ANTHROPOLOGY

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Let 541-727-4515 | Fax 541-727-2650 | www.onegonatate.edu/dept/anthropology

실현연구에 관련한 동의 서

렌구 계목: 장염 비보리오 관련 식용목이 한국 동영의 생물관련 과유 산업에 미치는 경계적 문화적 영향 **렌구 최종 책임자:** 보막이런 널트(Bryzn Tilt), 한유학부 교수 **대학생 조교:** 미지해(Jeelsyo Lee)

친구 과제의 목적은 구성한가?

여러분은 장염 비보리의 식공목과 관련하여 한국 동맹의 생물을 포함한 권반적인 피유산업에 미치는 경제적, 문화적 맹함에 관한 한 실험연구에 초대되셨습니다. 이 연구의 목적은 한국의 제1 의 글 생산지인 동맹에 관해서 지식을 넓히고 글 제조에서 관매까지 건반적인 문제로 발생하는 식공목과 그 맹함을 분석하는데 있습니다. 연구 결과는 건은 보고지, 과학자, 공공회의에서의 발표 동에서 이용이 가능한 것으로 여겨집니다. 그러므로 당신의 참여는 보다 넓게 지구순의 한 구성원으로서 그리고 여러분의 이야기는 사회 과계 과흥의 대공에게 전해된 수 있다는 공요한 의미를 무고 있습니다. 최공적으로 이러한 장염 비보리의학 관련한 식공목 한상이 생물관련 과유 산업과 대상이 동맹 지역 내에서 어떻게 상호 관련을 맺는지 문화적, 경계적인 측면을 연구하여 그 상관성에 대한 결과는 경부 및 공공기관 등의 다양한 성기를 면 여본에게 미래사업에 있어서 다시를 검토해를 수 있는 좋은 기회가 되기를 검실히 바라는 바입니다.

심문 조사 / 한터뷰 등록시가 지나는 목적은 구성한가?

이 동의서는 여러분이 실근조사 참여의 유무에 따르는 권리가 보장님을 알려드립니다. 현구와 한터뷰의 전반적인 내용, 발생 가능한 참여자의 위험 또는 부담강 그리고 참여의 이정과 참여한의 권리에 대해서는 거희가 여러분께 당면히 실명을 해드려야 하는 부분이며 의무임을 알려드립니다. 위의 거론된 사항에서 의존정의 해답을 찾은 후 동의의 유무가 여러분의 관단에 의해 결정됩니다.

왜 이 실문 조사 / 인터뷰에 등해야 합니까?

역하분은 경우, 공공기관의 관계자에게나 군양식업자 또는 공명 과무 산업과 관련이 되었기에 이번, 설문조사 임원으로 초대되셨습니다. 본 한터뷰가 공명지역과, 어촌에 관련하여 역하분의 전해와 의견을 함께 나눌수 있는 기회가 되고 바랍니다.

실문조사/인터뷰는 구세적으로 어떤 것 이며 얼마 정도의 시간이 소요됩니까?

한터뷰에 참여를 용하신다면, 여러분의 사무실이나 그밖의 다른 공공시설에서 한터뷰를 실행할수 있습니다. 한터뷰에서 게시되는 민준들은 금계조업과 관련한 권한적인 사항과 이러한 계조업이 동맹내의 관합기억에서의 경계성과 식품위생과 관련하여 여러분의 입장과 처기에 비우어 보아 어떠한 도움을 받거나 또는 어떠한 것을 계공하는기의 여분에 관련합니다. 여러분에서 대답하실 때 전구권은 메모를 적을 것이며 취약하신다면 이 한터뷰는 1 시간내기 1 시간 30 분가장의 시간 동안 진행 될 것입니다.

실근조사/인터뷰 참석에 대한 위험 부담에는 어떠한 점문이 있나요?

인강한 구계를 다꾸는 권론이 있을 시에, 대답에 용하지 않을 권리가 있으며 권론자는 그러한 경우 문항을 생략하고 넘어할 수 있습니다. 그리고 여러분은 한터뷰를 거부한 권리가 있으므로 먼게론지 보통에 한터뷰를 동안 한 수 있습니다.

Oregon State University - IRB Study # 3788 Approval Date: 10/23/07 Expiration Date:



Requested information must be submitted to the Human Protections Administrator, Office of Research Integrity, 308 Kerr Administration Bldg. Incomplete reports will delay the review process and may result in a suspension of the project.

Project Title:			IRB Application #:
Exploratory Study on the Impa Economy and Culture in Tongy		emolyticus Outbreak in	3738
Principal Investigator:		Department:	
Bryan Tilt		Anthropology	
PI Email:			PI Telephone:
Bryan.Tilt@oregonstate.edu			(541) 737-3896
Student Researcher:		Class or Degree Program (if	requirement for student):
Jeehye Lee		International Degree F	Program
Primary Contact Person:	Email:		Telephone:
Jeehye Lee	jeehye33@gmail	.com	(503) 706-8329
Campus or US Mail Address (to send co	orrespondence):		Date:
121 NW 21 st St. APT #22			10/06/2008
Level of Review:	Funding Source:		Approval Valid Through:
Exempt from Full Board	No External Fund	ding Source	OSU IRB
 Date project closed: 10/05/2 Reason for closing of project (e.g., unable to enroll participation) 	: project completed	d, project completed)	
3. Have all research activities b	een concluded, inclu	ding data analysis? 🛛 🖂	Yes No
If "No", then study cannot be available at http://www.oreg			
4. Total number of participants	s approved for the st	udy: <u>7</u>	
5. Provide a final subject repor	t:	During the Past Year	Cumulative Not Accrual Applicable

Provide a final subject report:	During the Past Year	Cumulative Accrual	Not Applicable
Number of subjects who consented (or enrolled):	7		
Number of consented subjects who voluntarily withdrew: (If any subjects have withdrawn, on separate sheet describe how many and the reasons for their withdrawal. Include whether decision to withdraw was directly related to study.)	0		
Number of consented subjects lost to contact:	1		
Number of consented subjects withdrawn by the PI:			\boxtimes
Number of consented subjects who completed the study:	7	,	

6. Were yo	u able to recruit participants in the ethnic	c and/or gender categories indicated in your proposal
☐ Yes ☐ No ☐ Not a	On a separate sheet, explain why not.	
7. Have the	ere been any participant complaints since	the last review?
☐ Yes ☑ No	On a separate sheet, describe the complain	nt and how it was handled.
8. Have the	ere been any breaches of participant confi	identiality since the last review?
☐ Yes ⊠ No	On a separate sheet, describe the breach an	nd how it was handled.
9. Have an	y unexpected risks, problems, or adverse	events been encountered since the last review?
⊠ No 10. Include	Yes No Has an ADVERSE EVENT Yes No Has a PROJECT REVISIO the following documents:	T form been completed for this project? ON form been completed for this project?
☐ Citat	f summary of the study progress to date. tion list of any related papers, abstracts, pres at progress report, if federally funded. If this	sentations, etc. from this study. s is a competitive renewal, submit the application.
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Summary of the International Degree Research Progress

The final report summarizes the student investigator's (Jeehye Lee) currrent progress of her undergraduate research and the finding of the International Degree (ID) project (IRB #3738): Exploratory Study on the impacts of Vibrio parahaemolyticus Outbreak in Economy and Culture in Tong-yeong, South Korea, conducted from Oct. 2007 to Oct. 2008 and assisted primarily by Dr. Bryan Tilt from the OSU Anthropology Department. The student investigator's personal outcomes from the study are also included in this final report.

Summary of the Research in Progress

From October to December in 2007, the student investigator conducted interviews including a total of seven participants as well as two participant observations in Gyeongnam province of South Korea. All the consent forms were distributed and collected prior to the interview sessions, and the copies of each consent forms was sent via mail in South Korea. The data collected from the interviews provided general information in the demographics of interview participants and answered questions addressing the impacts on economic and culture due to *Vibrio parahaemolyticus* contamination in Pacific oysters in Tong-yeong oyster industry. The personal interaction with the members from the shellfish industry greatly assisted to the student investigator, by setting the background information and knowledge of the Korean shellfish industry in the beginning of the investigation.

The following month in January 2008, with the help of the principal investigator, Dr. Bryan Tilt, the student analyzed data and focused on the secondary research mainly collected at the library. The secondary research focused on the frequent topics that were discussed during the interviews in 2007. Two public presentations were conducted in Spring 2008. First presentation became a practice run of the thesis presentation and was purposely given to faculty members and students from the BioResource Research (BRR) Department. The second public presentation invited a broad attendance-profile and was designed to meet the requirement for the Benjamin A. Gilman International Scholarship Program. The focus of the second presentation was related to the student investigator's personal growth from the study-broad experience in South Korea. A documentary movie that she produced was shared among audiences and deals with issues such as self-identities and lessons from the oversea experience. Please see the enclosed for the details of these presentations for your future reference.

The majority of the investigation is completed as of October 2008. A rough draft of the ID thesis document includes overview, introduction, method, data and result, discussion, and conclusion. In the future, a final public presentation on the student's combined joint thesis of the BRR Interdisciplinary Program and the ID Program as her undergraduate research project in Fall 2008.

Discussion of the Research Findings

The overall findings of bacterial outbreaks and its impact to culture and economy of the oyster industry in Tong-yeong, South Korea were comprehensively discussed throughout the ID thesis document. *Vibrio* infection is the one problematic concern and ensuring food safety regards in the shellfish production have helped each other by three major bodies in Korean shellfish industry. The major bodies are Ministry of Maritime Affairs and Fisheries (MOMAF), National Federation of Fisheries Cooperatives (NFFC), and individual shellfish handlers from private businesses. One of many functions that the governmental bodies involve with the issue with the safety of shellfish products are focusing on environmental conservation, which

primarily focuses on the water quality issue in regions where shellfish productions occurred. In addition, examining the shellfish production, labeling origins of the shellfish products, and informing the general public on the current disease outbreak issues are taken actively by governing efforts. The NFFC contributes the various services including administration, marketing, and financing. It is the leading organization owned by fishermen, fisheries stakeholders, and the general public.

South Korea has been one of the leading global oyster distributors since the early 1990s. Crassostrea gigas, commonly called Pacific oysters or Japanese oysters, have been the most popularly harvested oyster species in South Korea since the industry began in the 1970s, and its production was approximately 77% (261,706 tons) of the total Korean shellfish industry in 2006. Approximately 11,909 tons of C. gigas was produced from South Korea according to Food and Agriculture Organization and the total Pacific Oysters production was 150,443,773 tons with 45,285,328,267 Korean won values (\$44,531,077.78) in South Korea in 2006. The locally based small and large scale shellfish producers have provided the strong economic viability to the city of Tong-yeong and an interview participant from the NFFC mentioned that it had been a great economic opportunity for female workers, particularly their average ages, between 40 to 60, since they could find jobs working for shucking facilities during the high peak seasons. In the annual report of 2003, the representative from Tong-yeong division of the NFFC emphasized that today's primary concern in shellfish production was related to food safety and sanitization and those were the most important consideration in order to sustain the economy of the industry for both national and international markets. One of the interviewee of large scale shellfish productions argued that his company enforced strict guidelines to train their workers to persist the HACCP plan followed by the United State Food and Drug Administration (US FDA). The distributing good quality shellfish products to his global customers which were majorly from in Japan and the U.S., were important for his company's economic sustainability. The largest oyster distributor, the NFFC allocated a set-budget amount, close to \$2,500,000.40 for their science research, building modernized shucking facilities and hatcheries to manage food safety regards in oyster production in 2003.

The long-term traditions of the cooking methods for oysters have been consuming raw oysters as an additive to Kimchi (pickled vegetable salad) as well as the most popular dish, particularly the best served during the holidays, such as Chusuk (Korean Thanksgiving) and Seol (Chinese New Year) holidays. According to the secondary research, this trend is still similar to modern days. More of the general public have become aware of the importance of recognizing the current science-based research and news. Numerous responses from the interviewees and other resources expressed that these could potentially have a negative impact on the shellfish business, since they could overwhelmingly document the facts about pathogens in seafood and present dangerous aspect in public health. It seemed to be that modern technology such as electronic commerce (e-commerce), information technology, food-processing plants, modernized markets facilities and newly designed shellfish harvesting plants are important. They have critically become a positive vehicle for continuing the survival of the Korean's long-term cultural practice of consuming fresh raw oysters. For instance, an interview participant from the wholesale mentioned that the majority of his customers who were working class wives sought on-line shopping opportunities, because they consumed the fresh shucked oysters miles away from their home. The choices provided by the e-commerce businesses were highly viable in the current market situation, because less time and cost were associated for his customers and the customers prefer to have facts and information about the oyster products by visiting websites.

Student Investigator's Learning Outcomes

The rationale of the ID study was to explore the significant problematic concerns with pathogenic *Vibrio parahaemolyticus* in shellfish industry, particularly oysters in Tong-yeong, South Korea. Initially, I was thrilled to visit a new city that I had never been to but also nervous since I wasn't sure whether I planned enough to conduct an overseas research. With helpful advice from Dr. Bryan Tilt and the assistance provided by the Institutional Review Board, I was able to safely plan on my participant interviews. I recruited a total of seven interviewees including fishermen and governmental agents in the fishing village in Tong-yeong, South Korea. I was able to build networks and practice my communication skills with an understanding of cross-cultural barriers. The experience also enabled me to enhance technical skills that provided me with better knowledge of human interactions with their natural resources.

In addition to my prior experience with the BRR research, given the similar topics working closely with Dr. Claudia Hase from the OSU microbiology department, the exploratory study of the ID research with Dr. Bryan Tilt has added valuable aspects to my investigation. In addition to my academic development, I gained communication skills which allowed me to practice both English and Korean throughout the project. I have become more confident with my linguistic skills within those languages.

Overall, my learning outcomes from the study abroad experience helped me to evaluate myself and taught me to greater appreciate my college education and have respect for my surrounding neighbors, perhaps with a greater worldview. My personal growth and academic achievement were recognized publically. I was honored at the All-University Student Recognition Awards in April, and received the Clara L. Simerville Award for International Education, recognizing an OSU undergraduate with "innovative or creative contributions to international understanding on campus and/or in the community," and appeared in the *Asian Reporter Newspaper*. My academic goal is to achieve a Bachelor's of Science in BioResource Research (BRR) with focuses in water resources and toxicology and a Bachelor's of Art in International Degree program (ID) in the Fall of 2008. My long-term career goal is to become an educator or a researcher who cares about deeper knowledge in science to provide assistance in my community, as well as demonstrate innovative ideas for better sustainable living conditions. I hope to keep promoting awareness of international education in the society that I belong to and how others could learn about themselves and neighbors from other countries. Thank you very much for your support and willingness to assist in my undergraduate research study.

Citation of the International Degree Research

The citation includes books, journal and news articles, on-line sources, and personal interviews. All these categories were collected both in the United States and the Republic of Korea during the period of the student investigator's International Degree (ID) project, which was conducted from Oct. 2007 to Oct. 2008. Majority of sources were written in Korean language and translated with English alphabets. The foreign texts were carefully interpreted by the student investigator as well as her college from the Education department from Kookmin University whose native language is Korean.

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