# Assessment of health risks in the elimination of food-borne pathogens

Assessment of Health Risks in the Elimination of Food-borne Pathogens *Vibrio parahaemolyticus* and *V. vulnificus* in Oysters

Shellfish are aquatic shelled mollusks (clams, oysters, and mussels) or crustaceans (shrimp and lobsters). Often sought by consumers for their unique flavors and health benefits, shellfish are an excellent source of omega-3 fatty acids, monounsaturated and polyunsaturated fats, and numerous minerals like zinc, copper, iron, and magnesium (Hosomi, 2012). However, consuming shellfish presents potential public health risks when not carefully prepared. Raw and improperly cooked mollusks have been shown to be vectors of infectious agents and marine biotoxins due to their high potential to host bacteria and viruses when filter-feeding from their environment (Budtz-Jørgensen, 2007). Infectious disease outbreaks have been reported in the United States since the late 1800s, many of which involve seafood-borne illnesses associated with human waste sewage effluent or bacterial pathogens indigenous to marine environments along coastal regions (Rippey, 1994).

Oysters, a common delicacy of seafood enthusiasts, are saltwater bivalves that consume aquatic microorganisms such as plankton and other organic particulates through filter-feeding (Jud, 2011). This process, however strategically efficient for these organisms, typically results in the accidental ingestion of any contaminants and pathogens present within the water column and thus evolves the bioamplification of contaminants within oysters (Jackson, 2009).

The *Vibrio* genus of bacteria, commonly associated with eating undercooked or raw seafood, are organisms widespread in marine environments and exist in greater numbers in warm, coastal waters such as the Gulf of Mexico (Thompson, 2005). There are 30 species in the genus *Vibrio* , 14 of which are pathogenic in humans (Faruque, 2008). *Vibrio* infections, or clinically referred to " vibriosis," are a result of consuming contaminated seafood, most commonly due to *Vibrio* parahaemolyticus and V. vulnificus (FDA, 2018).

The consumption of raw oysters, particularly those harvested in warmer months from the Gulf of Mexico, is strongly correlated with *Vibrio vulnificus* (Shapiro, 1998). Although foodborne illnesses attributed to *V. vulnificus* are rare, ~0. 04 illnesses per 100, 000 people, (Newton, 2012), this bacterium is the leading cause of seafood-related deaths in the United States (Oliver, 2013). *Vibrio parahaemolyticus* , the milder of the two *Vibrio* infections, causes watery diarrhea, abdominal cramps, nausea, vomiting, and septicemia, while *V. vulnificus* can become more severe, especially in hosts suffering from liver disease and iron storage disorders (Iwamoto, 2010). The seriousness of this illness is due to its high mortality rate (up to 50%) in patients with blood infections, or septicemia (Oliver, 2013). Nearly all cases of septicemia occur in patients with preexisting medical conditions, often liver disease (Shapiro, 1998).

Raw oyster consumption has been linked to *Vibrio* infections due to the high concentrations of these bacteria in the tissue of the bivalves (Budtz-Jørgensen, 2007). Improper cooking and cross-contamination to other food can also contribute to human infection. Proper control and monitoring of potential hazards within water sources have not been shown to eradicate

*Vibrio* contamination, but certain processing methods have been shown to make the consumption of oysters safer (FDA, 2018), such as freezing and cooking oysters prior to consumption and depuration of live oysters of contaminants.

Depuration is the process of placing aquatic organisms into a purified environment for a period of time to purge contaminants such as infections of bacteria and viruses, and intoxications of chemicals or biotoxins (Froelich, 2014). Practiced since the 1800s (Herdman, 1899), bivalve depuration is a necessary postharvest practice used even today and is effective in eliminating *Salmonella* and *Escherichia* coliform bacteria (Belding, 1909). However, depuration is not as effective in the removal of *Vibrio* species, due to their commonly prolific numbers and longevity (CDC, 2016). Complications with *Vibrio* removal prior to consumption is problematic as a large majority of seafood oyster species can carry V. vulnificus and/or V. parahaemolyticus especially during warm summer months when seafood consumption peaks and maintain their infections for long periods of time (Wright, 1996).

Depuration has demonstrated success in reducing bacteria, and effectiveness of many depuration techniques is dependent on a number of variables including the health of the harvested shellfish, parameters of the water within the depuration plant (salinity, temperature, and turbidity), type of pathogen, and severity of contamination (Jackson, 2009). However, current post-harvest processing methods for elimination of these pathogens are expensive and kill the oyster, changing their organoleptic properties, making them less appealing to some consumers (Larsen, 2015). Since the initial use of depuration in the 1800s, different techniques have been

modified over time to find the most successful and profitable means of harvesting safe seafood. Scientists are currently exploring depuration techniques involving salinity levels (Phuvasate, 2013), application of ultraviolet light and chlorination (Ramos, 2012), changes in temperature (Chae, 2009; Larsen, 2015), and the application of select bacteriophages (Rong, 2013).

*Salinity*

High salinity treatment of oysters is one new method under scientific investigation as previous research has shown a significant decrease in *Vibrio vulnificus* concentrations in live oysters held at high salinities (Larsen, 2013; Motes, 1996). *V. vulnificus* numbers are also known to be positively correlated with water temperature (Drake, 2007; Kelly, 1985) and it is possible that reducing the tank temperature during depuration will increase the efficacy of this method.

In addition to *Vibrio vulnificus* , raw oyster consumption is also a risk factor for another foodborne pathogen, *Vibrio parahaemolyticus* , the leading cause of seafood-borne gastroenteritis in the United States (DePaola, 1990). *Vibrio parahaemolyticus* numbers are also influenced by water temperature and salinity (DePaola, 1990, 2003; Johnson, 2010; Zimmerman, 2007) and as a result abundance of this pathogen may also be impacted by depuration procedures.

A 2013 study conducted by Sureerat Phuvasate and Yi-Cheng Su focused on various levels of salinity to determine the most effective method to reduce *V. parahaemolyticus* . Five clinical strains were obtained from the FDA and

each strain was grown in a broth at 37°C for 18-24 hours. Each culture was then streaked on to individual plates and incubated at 37°C for an additional 18-24 hours. A single colony was obtained from each plate and transferred to a tube and incubated for 37°C for 4 hours. Cultures were harvested to be used for inoculation. Oysters were initially placed into a tank with a salinity of 30 ppt to simulate artificial seawater and were maintained at room temperature for 2-4 hours. Oysters were placed into groups verifying from 6-8 per group and they were placed into six different containers of verifying salinity: 5, 10, 15, 20, 25, and 30 ppt.

Oysters were inoculated with the *V. parahaemolyticus* and depuration was conducted at 12. 5°C with salinity levels of 10, 20, 25, and 30 ppt for 5 days. Oyster movement was monitored every 5 minutes for a 24 hours period and a measurement of > 0. 05 cm indicated movement. Results indicated that oysters exposed to higher levels of salinity exhibited greater movement and those exposed to levels of 5 ppt showed no movement at all. This is significant due to the belief that oyster movement is associated with water-pumping activity; therefore, oysters are more likely to remove contaminants in higher levels (≥20 ppt) of salinity when compared to lower levels.

Phuvasate's results showed a greater than 3. 0-log reduction of *V. parahaemolyticus* in oysters depurated in 30 ppt salinity. Phuvasate concludes, " salinity levels of 20 ppt or higher appear to be favorable for oysters to maintain biological activity, therefore, a greater reduction of *V. parahaemolyticus* was observed in oysters depurated in salinity levels between 20 and 30 ppt." Phuvasate's study coincides with previous research that indicated that *V. vulnificus* shows greater survivability when exposed to

low levels of salinity, demonstrating the success of salinity as a useful method of oyster depuration.

*Ultraviolet Light and Chlorination*

Depuration using a combination of ultraviolet (UV) light with chlorinated seawater has also been shown effective in eliminating *Vibrio* species in oysters. UV light is a potential alternative to typical depuration techniques in that this process does not harm nor kill the oysters.

Roberta Juliano Ramos et al set out to measure the effectiveness of UV treatment on contaminated oysters in a 2012 study that ultimately demonstrated that UV light will reduce both *V. parahaemolyticus* and *V. vulnificus* with or without the treatment of chlorinated water.

Five strains of *Vibrio parahaemolyticus* and *V. vulnificus* were used as the inoculate. 120 oysters were prepared by immersing them in a tank of UV-sterilized seawater for a period of six hours at room temperature. The seawater was then inoculated first with the *V. parahaemolyticus* cocktailthen inoculated with *V. vulnificus* cocktail and continuously circulated with air for an 18-hour period. 90 of the 120 oysters were contaminated in this tank leaving 30 oysters as the control group.

All 120 oysters were placed into a single depuration tank containing 350 liters of UV sterilized seawater that was maintained at room temperature. Three methods of depuration were analyzed: untreated, UV light only, and UV light combined with a chlorine solution.

All methods showed a reduction in *V. parahaemolyticus* after 48 hours of depuration with the smallest change in the control group. UV light treated oysters showed a reduction of *V. parahaemolyticus* by 2. 4 log while UV light plus chlorine showed a reduction by 3. 1 log after 48 hours of depuration. This study also noted there were no significant difference in *V. parahaemolyticus* population between 24 and 48 hours of depuration.

The reduction of *V. vulnificus* was not as significant. Little reduction occurred in the control group while chlorine had no significant effect. UV light treated oysters showed a reduction of *V. vulnificus* of 2. 5 log while UV light plus chlorine reduced V. vulnificus by 2. 4 log. There were no observed differences in *V. vulnificus* between 18 and 48 hours of depuration.

Ramos' study was not only one of the first to report efficacy of this depuration technique on oysters grown along the Brazilian coast, but also suggested that the process could rely on ultraviolet light alone.

*Temperature Effects*

*Vibrio vulnificus* has been shown to fluctuate concentrations coinciding with seasonal changes in estuarine waters (Tamplin, 1982). Water temperature has been considered the major factor that controls the *V. vulnificus* concentration. Warmer water (> 20°C) with moderate salinity (5-25 ppt) shows the highest abundances of this organism (Bryan, 1999). Lower seasonal temperature has also shown to result in a lower abundance of *V. vulnificus* related instances decreases which has led to an undetectable level during the colder months during winter.

The abundances of both pathogens in oysters are positively correlated with temperature, thus ingestion of raw oysters during the warm summer months is a risk factor for contracting illness from these bacteria.

In a 2004 study by Mark A. Randa and Martin F. Polz, the reduction of *Vibrio parahaemolyticus* and *V. vulnificus* in American oysters based on temperature effects on depuration was investigated. A cocktail consisting of five strains of either *V. parahaemolyticus* or *V. vulnificus* was used to inoculate raw oysters. Oysters were depurated in artificial seawater at different temperatures: 5, 10, 15, and 22°C. Oysters that were depurated at 22°C had little effect on reducing total counts of either *V. parahaemolyticus* or *V. vulnificus* in the contaminated oysters. *V. parahaemolyticus* counts were reduced by 1. 2 MPN/g while *V. vulnificus* counts were reduced by 2. 0 log MPN/g after 48 hours of depuration. When water temperature was reduced to 15°C, the depuration efficiency increased in reducing *V. parahaemolyticus* up to 2. 1 log MPN/g and *V. vulnificus* to total reduction of 2. 9 log MPN/g. Reductions of *V. parahaemolyticus* and *V. vulnificus* in oysters increased to 2. 1 and 2. 9 log MPN/g. Depuration time was conducted for 48 hours at 15°C. An extended depuration of 96 hours at 15°C produced greater results in reduction of *V. parahaemolyticus* and *V. vulnificus* , 2. 6 log MPN/g and 3. 3 log MPN/g, respectively. Randa’s results had shown that temperature had a great impact on the pathogenic nature and success of *Vibrio* bacteria in shellfish.

*Bacteriophage Application*

Bacteriophages, or phages, are viruses that infect host bacteria and utilize host mechanisms to replicate. Since their discovery by French scientist Felix d'Herelle in 1917, scientists have been investigating their potential uses within the public health realm and beyond. One of many applications of bacteriophages is the control of contamination in seafood and has recently been under the lens by scientists (Wommack, 2000; Rong 2015; Jung 2016).

Bacteriophages are being used in other research domains as a safe bio-controlling agent such as their use in eliminating pathogens within food products. Rong Rong, Hong Lin, and Jingxue Wang set out to investigate the potential to reduce *V. parahaemolyticus* by using the bacteriophage *VPp1* during depuration in a 2014 study.

Oysters were monitored at different levels of infection that ranged from 105, 106, and 107 CFU/ml of *V. parahaemolyticus* with three MOI values of 0. 1, 1, and 10. The oysters were depurated in temperatures of 12, 16, 20, and 22°C for 36 hours. The most effective reduction occurred at 16°C with MOI of 0. 1 and decreased *V. parahaemolyticus* by 2. 35-2. 76 log CFU/g.

Through this process, Rong's study successfully demonstrated the potential use of bacteriophages during the depuration process to maximize the removal of *Vibrio parahaemolyticus* in oysters.

*Summary*

In conclusion, commercial depuration procedures are widely used to treat shellfish, such as oysters, harvested from contaminated sites. While depuration techniques are complex processes involving a number of

variables effecting success, ongoing research efforts to minimize public health risks when eating shellfish are producing safer and safer seafood.

However, it should be noted that the most effective public health strategy when producing seafood products is to focus on good water quality in production areas rather than removal of contamination after events

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