Managing *Vibrio* Risk in Oysters

Angelo DePaola*
Angelo DePaola Consulting 12719 Dauphin Island Parkway, Coden, AL 36523, USA

SUMMARY

Vibriosis has increased more than any other illness caused by a pathogen in the United States (U.S.) food supply since the Centers for Disease Control and Prevention (CDC) FoodNet program began in 1996. Foodborne Vibriosis is almost exclusively associated with seafood, and most cases are linked to raw oyster consumption. *Vibrio parahaemolyticus* (Vp) and *V. vulnificus* (Vv), which are naturally occurring bacteria inhabiting coastal areas around the world, are the leading causes of seafood-associated illnesses and deaths, respectively, in the U.S. Forces of nature and man, including bacterial evolution, climate change, risky food-handling practices, shifting geographical and seasonal production and consumption patterns, expanding globalization, improved recognition/diagnosis and fractured regulatory oversight have spawned a perfect storm of ever-increasing rates of vibriosis reported in the U.S. Control authorities in states with the greatest morbidity and mortality in which rapid cooling mandates have been implemented have seen sustained Vv illness reduction burdens for five years and appear to be driving down Vp illnesses as well in recent years. To better inform risk management, national and international risk assessments have generated forecasting and cold chain confirmation tools calibrated to risk. Emerging post-harvest processing and pre-harvest controls could further reduce or practically eliminate the vibriosis risk associated with consumption of raw oysters.

OVERVIEW

Foodborne vibriosis is almost exclusively associated with seafood, and most illnesses are linked to raw oyster consumption *(30)*. *Vibrio parahaemolyticus* (Vp) and *V. vulnificus* (Vv), naturally occurring bacteria inhabiting coastal areas around the world *(30, 46)*, are the leading causes of seafood-associated illnesses and deaths, respectively, in the U.S. *(29)*. Vibriosis has increased more than illnesses caused by any other pathogen in the United States (U.S.) food supply since the Centers for Disease Control and Prevention (CDC) FoodNet program began in 1996 *(7)*.

Vp was first reported as a cause of disease in Japan during an outbreak in the 1950s *(24)*. Vp causes diarrhea in healthy individuals and, occasionally, bloody diarrhea. In rare cases, infections can become septic in those with serious underlying chronic illnesses *(50)*. Early on, it was noted that most clinical Vp strains produced a thermostable direct hemolysin (TDH) that was relatively rare (< 1%) in environmental or seafood isolates *(50)*. TDH production was universally considered a pathogenicity marker and some evidence indicated that it was a major virulence factor. TDH is coded by the gene tdh, which is the targeted by a variety of molecular assays to characterize pathogenicity of an isolate or enrichment for presence or most probable number (MPN) analysis *(1)*. Subsequently a TDH-related hemolysin (TRH) was identified and found to be coded by the *trh* gene. U.S. outbreaks in the 1970s were caused by cross-contamination of cooked shrimp and crabs by raw product *(12)*. Reports of sporadic cases linked to raw oyster consumption began in the 1990s, and the first oyster-associated outbreak occurred in the Pacific Northwest (NW) in 1997 and again in 1998 *(4)*. However, the wakeup call occurred in 1998, when consumption of raw Galveston Bay, TX oysters caused the largest Vp outbreak in U.S. history *(13)*. The culprit was the O3:K6 (Sequence type 3) strain that had emerged in 1996 in Asia; the outbreak spread to North America, signaling the first Vp Pandemic *(35)*. Within a few years, the pandemic had spread globally, with illnesses associated with food reported in every continent except Antarctica and Australia *(34)*. Chile was the most severely affected country, with thousands of cases reported in 2004, mostly associated with consumption of raw mussels *(25)*.

Vv was first reported in 1979 as a lactose-positive, halophilic pathogenic bacterium occurring along the U.S. Gulf Coast *(2)*. Two syndromes were described, one associated with wound exposure to seawater and the other a primary septicemia from consumption of raw oysters harvested from the Gulf Coast during warm months. The primary septicemia associated with Vv has the highest case fatality rate (35–50%) of any foodborne disease but is limited to individuals with underlying chronic illnesses, especially liver disorders *(23, 29)*. Vv ingestion does not cause serious illness in healthy individuals and is not associated with outbreaks. Other pathogenic *Vibrio* spp. such as *V. chlolerae* occur in oysters but are far less consequential for morbidity and mortality in the U.S. than Vp and Vv *(29)*. Although this article focuses on Vp and Vv, most of the information is applicable to these other pathogenic vibrios as well. Sanitary controls that form the backbone of shellfish safety globally do not protect the public against pathogenic vibrios. Instead, control of oyster-associated vibriosis in the U.S. is based on risk assessments conducted by the U.S. Food

*Author for correspondence: Phone: +1 251.455.3035; Email: andydepaola@gmail.com

and Drug Administration (FDA) on Vp (VPRA) and by the United Nations Food and Agricultural Organization (FAO) and the World Health Organization (WHO) for Vv (VVRA) that were released in 2005 *(23, 50)*. Vp and Vv levels in oysters at harvest were based on FDA data on their relationship to water temperature *(10, 16, 40)*. Levels at the time of consumption were largely determined by Vp and Vv growth rates *(8, 9, 27)* in oysters and time to cool the oysters below the minimum growth temperatures of 10° and 13°C, respectively *(23, 50)*, for these two pathogens.

This article examines the factors contributing to escalating *Vibrio* illness reporting and emerging practices and mitigations that are driving down illnesses in the most severely impacted regions of the U.S. The lessons learned in the U.S. have global implications for shellfish safety.

Perfect *Vibrio* Storm

Vast estuaries abound on U.S. coasts and create ideal habitats for growing oysters and vibrios. Forces of nature and man, including bacterial evolution, global warming, risky food-handling practices, shifting geographical and seasonal production and consumption patterns, expanding globalization, improved recognition/diagnosis and fractured regulatory oversight have spawned a perfect storm of ever-increasing rates of vibriosis reported in the U.S.

Bacterial evolution and emergence of virulent outbreak strains

Vp grows faster than any other pathogen, and its short generation time accelerates evolution and genetic diversity *(50)*. Vibrios also exchange genes with other marine bacteria and can readily acquire gene clusters from numerous phages (viruses that can lyse bacteria or be integrated into the bacterial genome). These processes are a source of genetic elements that can increase pathogenicity toward humans, most notably the gene system for the cholera toxin *(52)*. Although a direct link to phage-acquired pathogenicity in Vp or Vv has not been established, numerous and diverse lytic Vv phages occur in oysters *(20)*. A lysogenic phage has also been identified in the pandemic O3:K6 serotype *(42)*. Separate human-pathogenic variants have recently evolved from a benign Vp strain endemic to the NE Atlantic through acquisition of pathogenicity islands of unknown sources *(53)*. Many Vp and Vv strains and lineages occur simultaneously in the same area and even in the same oyster.

Climate change

Remarkable seasonal and regional expansion of Vv and Vp illnesses and outbreaks have followed the warming of seawater, especially since 1998. Most striking is the geographical expansion of Vp outbreaks to Alaska *(37)* and Chile *(25)*. An unexpected consequence of geographical expansion is the increasing risk with increasing latitude. The attack rate on three consecutive cruise ship outbreaks in the Prince William Sound, AK was about 30% with consumption of 1–6 oysters *(37)*, an attack rate >1000-fold higher than the VPRA predictions *(33)*. The AK outbreak occurred >1000 Km further north than previously reported Vp outbreaks *(37)*. In southern Chile, Vp infections reached epidemic levels for the first time in history *(25)*. A recent draft Food and Agricultural Organization (FAO)/World Health Organization (WHO) report on updating *Vibrio* risk assessment has identified the introduction or emergence of Vp outbreak strains to high latitude areas as the greatest foodborne vibriosis threat.

The Vv risk season historically spanned from May to October in the U.S. Gulf Coast *(46)*. However, since 1998, the number of days with water temperatures >20°C have increased in April and November, and oyster-associated illnesses in these shoulder months are similarly frequent to those of summer months *(33)*. During a La Niña anomaly in 1998, water temperatures were 3–4°C higher than historical averages, and there were 13 reported Vv septicemia illnesses, the most of any month on record *(33)*. Less stringent time/temperature controls outside the historical Vv risk season and higher consumption likely contributed to this surge in illnesses.

Globalization

Growth of international trade has provided avenues for introduction of emerging pathogenic vibrios on a global scale. Cargo ships take on millions of liters of harbor water as ballast for stabilization on the high seas and discharge it to facilitate cargo transfer at the destination port. Many ports are highly polluted and have water temperatures and salinities that favor *Vibrio* abundance and survival during the voyage. This phenomenon was first documented in 1991, when the Latin American epidemic *V. cholerae* strain was discovered in ballast water of a number of ships in Mobile Bay, Alabama that had taken on water from various Latin American ports where this strain had become endemic *(36)*. The same epidemic strain had previously been found in oysters from several growing areas in coastal Alabama, but these areas had fortunately been closed to harvest for purposes of conservation *(15, 41)*. The pandemic O3:K6 Vp strain appears to have been introduced into major ports in North and South America, Asia, Europe and Africa in the late 1990s. Many of the resulting outbreaks were identified retrospectively by analysis of archived isolates *(34)*. Systematic surveys of ballast water were not conducted to confirm its role in the spread of Vp. Many countries also permit wet storage/depuration practices related to bivalve mollusks from other regions or countries, often with little oversight. Many consider these risky practices responsible for the introduction of the O4:K12 (Sequence Type 36) strain from the U.S. Pacific NW to Oyster Bay, New York and to Galicia, Spain in 2012 *(32)*. This strain, endemic to the NE Atlantic coast, remains the dominant cause of illnesses in that region *(6, 53)*. The outbreak in Galicia was on a cruise ship and was associated with using ice from a shellfish depuration plant to cool cooked seafood; the attack rate approached 100% *(32)*. This outbreak was identified retrospectively months later, as Europe does not have a systematic vibriosis surveillance system. Vp attack rates associated with consumption of raw shellfish tend to be much lower, and sporadic illnesses such as occur in the U.S. are much less likely to be detected without systematic surveillance *(32)*.

Harvest practices

Vibrios are normal microflora of oysters and proliferate in their tissues at temperature-dependent rates before and after harvest *(8, 9, 23, 27, 50)*. When oysters are taken out of the water or emerge intertidally, their shells close and they cannot purge vibrios. As the vibrios proliferate in the oyster tissues, the risk of illness in those who consume the oysters also increases. Historic harvest practices worldwide expose oysters to warm ambient air, allowing vibrios to proliferate exponentially.

Unlike many other animal food sources, oysters are not slaughtered during harvest and can live for days out of water. Thus, spoilage and shelf life were of little economic concern in the case of live oysters than for less risky but perishable seafood products that have long been subjected to rapid cooling. Market surveys of live U.S. oysters in 1998–1999 and 2007 frequently found Vp and Vv levels greater than 100,000 MPN/g in samples from the Gulf and Mid-Atlantic during summer months *(11, 18)*, levels 100-fold greater than at harvest and approaching the maximum levels than can be caused by temperature abuse of live oysters. The U.S. industry and regulators were long reluctant to mandate the stringent time/temperature controls needed to prevent growth of Vp and Vv. Many doubted the relationship between exposure and risk, and most harvesters operated out of small boats that were considered impractical for on-board cooling. Typically, industry had up to ten or even more hours to place oysters under refrigeration, even for the warmest areas and seasons. Stacking burlap sacks of oysters onto pallets and loading them into refrigerators at that time was standard practice. The VPRA and VVRA assumed that ten hours was needed for the internal temperature of the sacks to be below 10°C, the minimum Vp growth temperature *(23, 50)*.

Shifting seasonal and geographical production and consumption patterns

Late fall and winter, especially around holidays such as Thanksgiving, are historically the times of highest oyster demand as well as the periods when vibriosis risk is lowest. Glycogen levels in oyster tissues peak in the winter after the spawning season, increasing yield and organoleptic "fatter" appeal. With the recent advent of triploid oysters that do not spawn, aquaculture production of these oysters has increased to meet the growing demand for half-shell oyster consumption during the peak summer vacation season in coastal areas, when vibriosis risk is greatest. The VPRA has estimated that half of all oysters were consumed raw, based on data from the 1990s *(50)*. Industry and state regulators assume that most farmed oysters are now consumed raw. Perhaps more consequential to increasing vibriosis numbers is the geographical shift of production to the Pacific NW in the late 1990s and the Northeast (NE) Atlantic over the past decade. The increased production in these regions primarily reflect farmed oysters destined for raw consumption. While *Vibrio* abundance in oysters is an order of magnitude lower in these cooler regions than in the warmer Gulf waters, epidemiological data indicates that the Vp risk per serving of oysters from higher latitude areas may be an order of magnitude higher than for Gulf oysters during the Vp risk season *(3, 4, 5, 6, 11, 18, 19, 50)*. Fortunately, the *Vibrio* season in the Pacific NW and NE Atlantic is short, beginning in mid-June and typically being over sometime in September. The Vv risk remains negligible in these cooler regions, as cooler waters suppress its growth far more than growth of Vp *(11, 18, 31, 46)*.

Human host susceptibility

The VVRA used CDC data that indicated that ~7% of the U.S. population had underlying health conditions that predisposed them to primary Vv septicemia, based on data from the 1990s *(23)*. As people age they are more likely to have chronic illnesses and to use medications that increase susceptibility to infectious diseases, including vibriosis. Changes in rates of alcoholism, cancer, diabetes and infectious diseases such as HIV, and hepatitis B and C could influence the pool of high-risk individuals.

Improved recognition, reporting and diagnostics

Greater recognition of vibriosis in the health care community due to high profile outbreaks and improved diagnostics, especially real time PCR, has likely reduced underreporting *(1, 45)*. However, CDC revised its underreporting rate for Vp in 2011 from 20:1, which was used in the VPRA, to >150:1 *(38, 48)*. The VPRA had assumed 2800 Vp oyster-associated cases annually in 2005, but annual U.S. foodborne Vp illnesses are now estimated to be around 50,000 *(48, 50)*. The estimated underreporting rate for vibriosis, with the exception of that caused by Vv, is much higher than for other major foodborne pathogens *(48)*. CDC has recently implemented culture-independent diagnostic testing protocols in many states, which will likely detect vibriosis evidence that may have been undetected by historical culture-dependent testing protocols *(3)*. Authorities agree that this will complicate comparisons with historical culture-dependent data even if the new approach more accurately reflects actual risk. Reported illness could increase while risk is declining, as the result of improved management practices.

Fractured regulatory oversight

The Interstate Shellfish Sanitation Conference (ISSC), which makes shellfish safety policy for the National Shellfish

Sanitation Program (NSSP), meets biennially to update mandates and guidance for the Model Ordinance. Unlike agencies involved with most other foods under FDA's purview, in which the agency is authorized to develop food safety regulations, the NSSP is a cooperative program between Federal Agencies including FDA, National Oceanic and Atmospheric Administration (NOAA), Environmental Protection Administration and CDC, state control authorities and industry. *Vibrio* policy has dominated the ISSC debate since the late 1980s. The debate has been contentious, often pitting region against region, especially with regard to Vv when illnesses were traced exclusively to the Gulf region. An FDA proposal to ban harvest of Gulf oysters from May through October was narrowly defeated in the 1990s. This was followed by a plan in 2000 to reduce the Vv illness rate by 60% over a 7-year period. Educating high-risk consumers and increasing post-harvest processing (PHP) capacity were the key elements of this plan. Soon after the Vv illness reduction plan was adopted, California passed a bill to prohibit importation of Gulf oysters from May through October unless they had undergone a PHP to reduce Vv most probable number (MPN) to < 3 per gram. The ISSC censored California for this action, and CA lost its voting privilege even though the policy was effective in preventing Vv illnesses in CA *(51)*. However, the ISSC Vv illness reduction plan failed to meet the 60% illness reduction goal. The industry and states requested that FDA develop a Vv risk calculator for scenario analysis of proposed refrigeration controls for Vv risk reduction. FDA produced a Vv risk calculator based on the VVRA *(23)*. Calculator inputs were monthly average water and air temperatures, time between harvest and refrigeration, and time to cool to 13°C (55°F), the minimum Vv growth temperature, after the start of refrigeration. The output was risk per 100,000 servings, and the goal was to reduce risk from five illnesses to two per 100,000 meals. A new regime that came to FDA after the 2008 presidential election attempted to mandate PHP of Gulf oysters during the Vv season, based on Hazard Analysis Critical Control Point (HACCP) rules, an action that united nearly all parties at ISSC to defeat this unilateral FDA action. Gulf harvesters complained that they had invested heavily in refrigerated harvest vessels in anticipation of a rapid cooling mandate. The conference adopted a Vv control plan based on the FDA Vv risk calculator that went into effect in 2010. FDA was heavily criticized in the press and admonished by the Gulf congressional delegation for overreaching its authority. Although FDA officially maintained its position for mandatory PHP, a provision was included in the Food Safety Modernization Act (FSMA) to prevent unilateral action by FDA to mandate PHP of oysters for Vv control.

Just as the Vv risk season began in 2010, the largest manmade environmental disaster in history, the Deep-Water Horizon Oil Spill, occurred in the Gulf. Virtually all state and regulatory oversight was appropriately focused on the oil spill, which threatened coastal areas in all Gulf states. Florida lifted conservation bag limits in Apalachicola Bay, fearing that the oil would wipe out the crop. Compliance to the new cold chain mandates were largely ignored by state authorities at a time when FDA was also occupied by the oil spill and still clinging to mandatory PHP as their Vv policy. The result was a near-record number of Vv illnesses in 2010, which remained well above the VVRA baseline annual average of 32 through 2012. FDA influence at ISSC continued to diminish, reaching a low point at the time of the San Antonio, TX biennial meeting in January 2014. FDA had introduced a number of *Vibrio* control proposals in 2013 after the worst *Vibrio* season on record; none were adopted. Most notable was a proposal for immediate cooling (50°F within an hour of harvest) of oysters during the *Vibrio* risk season. FDA substituted a proposal to study effects of immediate cooling, as no states were supporting the immediate cooling mandate.

MANAGING AND MITIGATING VIBRIOSIS RISK: CHALLENGERS AND OPPORTUNITIES

Vibrios will come after you

Vibrios present unique food safety challenges. They are ubiquitous to aquatic foods and proliferate faster than any other pathogen. Vibrios are constantly evolving, creating more resilient and virulent strains. They cross oceans on ships in ballast waters and likely in biofouling communities, on ship hulls and cause pandemics unlike any other major foodborne pathogen. Global warming is expanding their geographical and seasonal range and creating the greatest Vp threat at the invasion frontier. These changes have been stimulated by misuse of antibiotics, fossil fuel dependence and globalization, and will likely continue to increase the vibriosis threat for the foreseeable future. Shellfish growing areas in higher latitudes such as in Europe that have rarely been associated with vibriosis may be a climate anomaly away from a major outbreak and could become the vibriosis epicenter in a warmer world. The oyster industry has no control over the forces of nature and globalization and should focus on adapting and preparing for an increasingly *Vibrio*-friendly environment.

Game changer

Sometimes things have to get worse before they get better, as happened in 2012, when the Pacific outbreak Vp strain (O4:K12 serotype/Sequence type 36) invaded Oyster Bay, NY *(32)*. Reported illnesses increased by more than an order of magnitude, causing extended closure of areas in Long Island Sound, including major areas in Connecticut (CT). In 2013, this outbreak strain spread from Virginia (VA) to Massachusetts (MA) and caused over 100 reported illnesses *(6)* leading to unprecedented closures and recalls. The industry perceived this as an existential threat and became more receptive to controls that could restore summer operation, which is critical to their half-shell business model. CT control authorities responded by proposing a mandate for immediate cooling to 50°F within an hour, similar to the FDA proposal that was getting no traction at ISSC. However, because they lacked the *Vibrio* expertise and credentials to sell this plan to a skeptical industry, they requested FDA assistance to help them make the case for immediate cooling. This marked the return of FDA relevance and influence on the control of shellfish-associated vibriosis. FDA brought a team of scientists, regulators and policy makers to CT for a highly contentious public hearing in December 2013. FDA recommended the use of ice slurries on harvest vessels, arguing that this was the fastest and most reliable method to cool oysters. Industry generally considered ice slurries to be too expensive and impractical to cool oysters on harvest vessels and argued that immersion of oysters in ice slurries would be lethal to oysters. Prominent industry members said they would terminate summer harvest if immediate cooling was mandated. Ultimately, CT moved forward with the immediate cooling mandate for the 2014 *Vibrio* season spanning June through September. The plan allowed mechanical refrigeration or other means that reduced the oyster tissue temperature to 50°F within an hour of removal from the water. The boldness of this immediate cooling mandate and courage of the CT authorities cannot be overstated, as leadership was betting their jobs on the uncertain success of this plan. The 2014 Vp season would be an experiment to test the skill of VPRA risk models and the credibility of FDA science. Twenty-one oyster-associated Vp illnesses were linked solely to CT harvest areas in 2013; therefore, more than one case in 2014 would result in failure to achieve the predicted 95% illness reduction. CT harvest was closed for the majority of the risk season in 2013, increasing the challenge. The dominant CT harvester initially terminated harvest operations but soon began the use of ice slurries, after other harvesters had implemented this practice without any issues of oyster mortality or quality. The plan succeeded, as only one Vp illnesses was solely attributed to CT harvest areas in 2014, and no closures or recalls were triggered. NY and MA adopted more stringent refrigeration controls in 2014 than in 2013 for outbreak areas but did not mandate immediate cooling, as had been mandated in CT, and experienced closures in 2014. The immediate cooling mandate in CT remains in effect to date, with success similar to that in 2014. The CT immediate cooling mandate is now triggered by 20°C water temperature instead of by the June 1 predetermined date, as Vp illnesses have not been associated with lower water temperatures in the state. NY and MA have also tightened refrigeration controls, and cases have declined sharply since the 2013 peak. Use of ice slurries is more challenging for the intertidal harvest that is prominent in MA, than for vessels used in subtidal harvest areas in CT.

WA state, which pioneered oyster aquaculture in the U.S., has become a leader in oyster production and numbers of Vp illnesses. Much of the half-shell production is intertidal, which allows Vp proliferation in oysters during emergence at low tide *(44)*. After a multistate outbreak in 2006 *(6)*, WA mandated increasingly more stringent refrigeration controls but were only able to maintain baseline levels of cases. Increasing production, warmer summers and a high-performing Vp reporting system may have been factors in the inability to drive down illness numbers. Reported illnesses may have been much higher without these control efforts. In 2015, Washington state (WA) passed a new Vp rule after a couple of years of negotiations with growers. Areas were designated as low, medium and high risk based on historical illness reports. Proactive triggers were established for either reduction of time to refrigeration or closures based on historical risk, water and air temperatures. Proactive controls based on actual conditions are a paradigm shift from past reactive controls based on reported illnesses. Reactive controls of closures and recalls were considered ineffective and punitive, as the lag between harvest, consumption, illness reporting and trace back typically took weeks and often occurred after the peak risk period and sometimes after the Vp season. WA cases have deceased substantially, but the state's oyster production is still linked to more Vp cases than are linked to any other state.

FDA *Vibrio* initiatives

By 2013, FDA senior leadership that had historically presented FDA's position at the ISSC Biennial meetings had disengaged and were focused on FSMA. Less senior officials were not authorized to negotiate policy and their positions were not taken seriously by the Conference. As the 2013 *Vibrio* risk season began, FDA management detailed me to a new role to coordinate *Vibrio* policy for the Agency and to develop "fabric" solutions to control vibriosis risk. *Vibrio* management has historically been reactionary, in response to illnesses, unlike management of other shellfish hazards under the NSSP. Shellfish toxins and pollution have long-established monitoring programs and proactive triggers to close areas when risk was considered unacceptable based on a body of scientific evidence. Attack rates from toxins and enteric bacteria and viruses associated with shellfish consumption tend to be much higher than rates of sporadic cases from naturally occurring vibrios, providing greater support for mandated closures. Additionally, patrol of closed areas for compliance is much simpler than monitoring compliance with cooling mandates aboard harvest vessels.

It was critical for FDA to take leadership in establishing an effective program for cold chain verification. Cooperation from all parties would be required to tackle this challenging problem. Systematic communication between the research, policy and regulatory arms of FDA on *Vibrio* control was lacking, and misperceptions were rampant. Monthly conference calls were conducted between research, policy and management to establish priorities and explore approaches. Monthly calls to CDC epidemiologists were also implemented to improve communication between federal agencies. A Course Advisory Group (CAG) was formed to develop *Vibrio* educational materials and included FDA regional shellfish specialists who oversee and evaluate State shellfish programs. The Vibrio Assistance Review Board (VARB) was created to encourage and prioritize collaboration among state and FDA researchers and risk assessment experts. FDA also became more active in ISSC committees such as that working on methods validation, and new/improved methods for *Vibrio* and other hazards have been adopted at an unprecedented rate. The research products from the VARB collaborations expands ownership of the science to state collaborators and in some cases to industry.

The 2010 Vv Control Plan mandated time/temperature controls that greatly reduced time to refrigeration to as little as an hour from harvest and also limited time to the no-growth temperature of 55°F for the first time. However, the number of illnesses over the next several years were well above the baseline number before the plan was adopted, and there was growing evidence of noncompliance. Communication with the *Vibrio* CAG played an important role in increasing oversight of the regional shellfish specialist on compliance, with greater support from subject matter experts and management. Since 2013, reports of noncompliance to time/temperature mandates have been infrequent, and Vv illnesses numbers have sustained historic decreases of around 40% from the historic VVRA baseline. The fabric of a cooperative *Vibrio* program continues to be strengthened with these ongoing efforts and is essential to meeting future *Vibrio* challenges.

Cold chain confirmation

Minutes matter during times of typical summer temperatures, because Vp and Vv levels increase more than 1% every minute after harvest until the oysters are cooled *(23, 50)*. The effectiveness of rapid chilling of oysters on board harvest vessels in CT confirmed that the risk could be reduced proportionally to suppression of Vp growth *(50)*. However, land-based refrigeration remains the dominant practice, and the NSSP Vp Control Plan only mandates minimum times to first refrigeration. States under the NSSP Vv Control Plan and a few other states, including CT and WA, also require processors to reduce oyster tissue temperatures to below the no-growth temperature of 55°F and 50°F *(23, 50)*, respectively, within established time limits. Harvesters and processors are largely doing the best they can to prevent *Vibrio* growth during the periods of greatest risk, when most illnesses are reported. While the low-hanging fruit of rapid cooling has been picked, any break in the cold chain after processing provides a window of opportunity for *Vibrio* proliferation. Processors have long been slaves to a distribution chain plagued by unscrupulous operators who would turn off refrigeration during transport or unload live oysters on docks without refrigeration for hours. Many retailers and consumers are unaware of the importance of the cold chain in managing *Vibrio* risks and unwittingly handle oysters under conditions that allow vibrios to proliferate. The need to confirm the cold chain of an item destined for raw consumption is arguably more critical for food safety in the case of oysters than in the case of any other food.

Tools for cold chain confirmation include thermometers, time/temperature recorders (TTR) and indicators (TTI). These tools complement each other and should be used in conjunction. Inserting probe thermometers between the oyster valves provides the most accurate snapshot of internal temperature in real time but is laborious and lacks cold chain history. Infrared thermometers are more commonly used because they are more convenient, providing reasonably accurate measurements quickly without the need to open containers or destroy product. However, they measure surface temperatures, which may differ from internal temperatures, especially when bags are stacked on pallets. TTRs provide complete and detailed cold chain history but may not be representative of the range of conditions in heterogeneous loads or lots unless they are widely distributed. Internal positioning is best for detecting slow cooling, and external placement is best for detecting cold chain breaks. Miniaturized TTRs can be placed inside oysters, and this approach has been employed to validate cooling processes. However, TTR data typically must be downloaded to a computer to be observed, and these data are not integrated with risk. TTIs calibrated to *Vibrio* doubling times based on FDA and FAO/WHO *Vibrio* risk assessment growth models have become available recently *(8, 9, 23, 27, 50)*. TTI's are inexpensive and easily interpreted and can be applied to every container, providing exquisite granularity in a heterogeneous environment such as a refrigerated truck. Unlike TTIs that are mandated by FDA for botulism toxin control in modified atmospheric packaged fresh seafood, *Vibrio* TTIs are optional for cold chain confirmation for *Vibrio* control in shellfish. Ultimately, retailers and consumers, who are most impacted by vibriosis, may drive TTI usage in the industry.

***Vibrio* risk forecasting tools**

The VPRA and VVRA risk models have also been integrated with satellite imagery and other observations of sea surface temperature and salinity to estimate *Vibrio* levels in oysters and the corresponding risk. The efficacy of this approach was demonstrated using archived data from a survey of Vp levels Alabama oysters *(21)* and sea surface temperature from derived satellite imagery from that period *(47)*. Strong agreement was observed between observed and predicted water temperatures and Vp levels based on satellite imagery. NOAA in collaboration with FDA, State control authorities and academia has since developed publicly available forecasting products for *Vibrio* levels in water and oysters. Recently, forecasts of Vp doubling times in oysters based on VPRA models and air temperature forecasts from the Puget and Long Island Sounds have become available on the internet *(43)*. Doubling times during Vp risk season in the Puget Sound can range to more than a day to less than an hour between the early morning and mid-afternoon in the same day. There are also efforts to improve the accuracy of *Vibrio* models with region specific data on the relationship of *Vibrio* levels with other water quality data beyond temperature and salinity *(14)*.

Post-harvest processing (PHP) and cooking

Vibrios are much more susceptible than other foodborne pathogens to most disinfection measures. Cooking virtually eliminates the risk and is the most effective measure, especially for high-risk consumers with chronic underlying illnesses. PHP can be as effective as cooking in preventing illness caused by vibrios while preserving most of the organoleptic qualities of raw oysters *(17, 51)*. Mild heat treatment was commercialized in the 1990s to eliminate the Vv risk of Gulf oysters, creating a new category of oyster usage, "PHP" that could be used on the label to indicate added safety. While PHP was capable of reducing *Vibrio* populations by orders of magnitude, it was incapable of achieving total *Vibrio* inactivation and zero risk. ISSC adopted the label "processed to reduce Vp and/or Vv to nondetectable levels." "Nondetectable" was originally defined as < 3 MPN/g but was changed to < 30 MPN/g based on *Vibrio* risk assessments *(23)*. This policy opened the door to an extraordinary period of oyster PHP innovations that are unparalleled for any other food commodity. Freezing coupled with extended frozen storage soon emerged as the most popular PHP, as this is a simple and inexpensive method that also extends shelf life. More innovative processing using high hydrostatic pressure to inactivate vibrios also makes shucking oysters easier by separating the adductor muscle from shell attachment. Irradiation kills vibrios but unlike other PHPs, is not lethal to oysters. Validation is required for PHPs with an added safety label, but many processors are freezing oysters without making any labeling claims, thus avoiding the expense of validation and verification. Industry experts estimate that about 15% of the half-shell oyster market represent PHP products. Perhaps no other product in the food supply has been subjected to the range and extent of PHP to successfully reduce pathogen risk as oysters have been. A U.S. survey of PHP oysters demonstrated that Vp and Vv levels were typically orders of magnitude lower than 30 MPN/g, and I am not aware of any definitive evidence that vibriosis has been linked to PHP oysters *(17)*.

Pre-harvest controls

Pre-harvest controls were not modeled in early *Vibrio* risk assessments, as they were considered ineffective or impractical for controlling naturally occurring pathogens in oysters. The potential effectiveness of this approach to reducing Vv levels was first demonstrated in the 1990s by relaying oysters to high salinity (>32 ppt) Gulf of Mexico waters *(39)*. Vv levels were consistently reduced to < 3 MPN/g within 17 days, but this approach has not been commercialized. Pre-harvest controls were first implemented in response to the 2004 Alaska outbreak linked to a warming anomaly. In 2005, AK control authorities mandated lowering gear containing oysters below the thermocline for a month before entry into commerce when water temperatures reached 15°C. Vp levels decreased by approximately one log unit in oysters held below the thermocline (< 10°C), compared with those in gear at the surface, and fewer illnesses were reported than in 2004 under similar climate conditions. Some harvesters in British Columbia, Canada have adopted this approach to reduce Vp levels to < 100/g, the Canadian Vp standard. Another approach, relocation of oysters out of site, was implemented in Katama Bay, MA in 2015 after several years of reactionary closures for exceeding NSSP illness allowances. Oysters moved into cooler Atlantic Ocean waters outside of the bay prior to being sold have not been linked to any illnesses, and Katama was not closed in the three years afterwards. There is also growing evidence that on-shore depuration of oysters in refrigerated seawater (12.5°C) can reduce Vp levels by several log units within a week. These findings have sparked industry interest, but this approach has not yet been commercialized *(49)*. On-shore depuration expands treatment options far beyond refrigeration. Innovative approaches abound; physical forces (UV, electrical, magnetic), chemical (antibiotics, polyphenol) and biological agents (phage, bacteria) and many more have been proposed to aid *Vibrio* depuration.

Global tracking of emergence and movement of Vp outbreak strain

The key assumption of the VPRA that all pathogenic strains with *tdh* are equally infectious has long since been proven erroneous by orders of magnitude *(33, 50)*. Nearly 95% of predicted illnesses were attributed to the Gulf, where *tdh*+ strains are more than ten-fold more abundant than in other U.S. regions. While few reported illnesses are associated annually with the Gulf harvest *(3)*, the introduction of the pandemic O3:K6 Vp strain into Galveston Bay, TX in 1998 caused attack rates comparable to that of norovirus, with hundreds of reported illnesses and likely thousands of unreported illnesses *(13)*. Improving systems for recognizing and tracking the emergence and global spread of highly infectious outbreak strains is critical to mitigating the Vp risk. FDA initiated this effort in 2008 with the creation of a publicly accessible Multi-locus Sequence Typing (MLST) site at Oxford (UK) University *(26)*. The site is transitioning toward Whole Genome Sequencing (WGS), which provides greater resolution for distinguishing strains and tracking evolution. State health departments are sharing illness isolates with investigators doing cutting-edge research expanding knowledge on routes of spread and genetics of virulence *(53)*. However, authorities must become more proactive in implementing appropriate and more timely controls when these super pathogens invade. Reactive closures typically lag weeks behind peak risk, and the magnitude of the 2013 outbreak in the NE Atlantic could have been largely mitigated with more aggressive and timely cooling requirements. Certainly, control authorities also need to prevent invasions of outbreak strains by ending the depuration/wet storage of shellfish from other regions in flow-through systems that can release Vp or other hazards into the local environment. This practice appears to have resulted in a large outbreak in Galicia, Spain caused by the Pacific Vp outbreak strain. Ballast water controls have improved in response to mandates from selected governments, but the maritime community needs to address this more aggressively.

Microbial performance standards and trade

Japan and Canada have mandated performance standards set at 100 Vp/g. The Japanese standard is for all seafood and was implemented along with a suite of controls, including improved cold chain actions, and sanitary measures such as prohibiting use of harbor water for seafood processing and reducing contamination of fish flesh with their *Vibrio*-laden intestinal contents. Vp illnesses have plummeted, but attribution to specific controls remains uncertain *(28)*. Canadian controls are limited to oysters and were implemented after a Vp outbreak in 2015 linked to oysters produced in British Columbia (BC). BC producers have adopted measures to sink culture gear into cooler waters, and compliance to this standard has been good. While there have been no Vp outbreaks since the 100/g standard was implemented, sporadic cases occur annually. Reducing Vp levels below 100/g would be much more challenging in most U.S. shellfish growing waters, which are much warmer than those at the higher Canadian latitudes. Such a standard would likely provide little public health benefit, as Vp illnesses are much less frequently associated with Gulf and Mid-Atlantic oysters, which have Vp levels much higher than those of oysters from the Pacific NW and the NE Atlantic areas. Additionally, Codex guidance on controlling pathogenic vibrios in bivalve shellfish does not recommend the use of microbial performance standards *(22)*. Legal food importation has not been implicated in *Vibrio* outbreaks and is only occasionally linked to sporadic illnesses.

Industry is the most important *Vibrio* risk manager

In contrast to control of other shellfish hazards, such as pollution-associated pathogens, chemicals or toxins from harmful algal blooms, which are managed by the control authority with closures, control of vibrios is largely done by industry, especially the harvesters. The aquaculture industry is ripe for development of best management practices programs for shellfish that are calibrated to risk for pre- and post-harvest controls. Many growers have branded oysters for the boutique market, and the high value of farmed oysters merits additional safety expenditures beyond minimum NSSP mandates. Implication in illnesses tarnishes the brand and exposes the grower to litigation well beyond that for wild harvesters, who tend not to carry insurance. Farmed oysters have not yet implicated in Vv illnesses, which often trigger lawsuits. Incoming oyster farmers are not only more invested; they tend to be extraordinarily well educated. The number and ratio of oyster farmers with doctorate degrees in Alabama where I live is greater than at FDA's Gulf Coast Seafood Laboratory on Dauphin Island, AL, where I worked for 37 years! A more enlightened industry may signal a paradigm shift in the role of science, which historically has not been welcomed by the oyster industry. As science identifies new or expanding hazards, mandates typically follow, and these are often perceived by industry as over-precautionary. Authorities are risk averse, and it is in their interest to reduce risk as much as possible to insulate them from blame in the event of illnesses or outbreaks. Mandates, along with permitting burdens, are shrinking the operating bandwidth of oyster production and are crushing the industry.

Industry ownership of science could expand the operating bandwidth through better characterization of *Vibrio* risk and other hazards to inform more measured controls that track with risk. For example, mandated re-submergence times, a key element of emerging pre-harvest controls for aquaculture practices, vary from state to state and by gear type. States require re-submergence periods ranging from a week to a month after a 24–30 h desiccation step used to control biofouling in off-bottom containers. FDA studies have been used by Alabama authorities to reduce re-submergence times from 2 weeks to a single week but are limited to one of several gear types and have not been transferable to other states. These studies require years of research using expensive and complex methods approved by NSSP and conducted by a certified laboratory, both of which are in short supply. The many permutations of cultural practices, regions, oyster species and purposes overwhelm the very slight *Vibrio* diagnostic capacity. These limitations delay sanction or recognition of pre-harvest controls, such as relocation to colder waters, which could virtually eliminate risk. More accessible and affordable diagnostics could empower industry to implement best practices and to evaluate emerging technology that could drive down risk. Dockside testing for paralytic shellfish poison has been used by industry to manage that hazard in shellfish in the NE Atlantic for years. If the industry continues to recruit people who embrace science and if technology provides user friendly/affordable diagnostics, an era of enlightenment could improve production efficiency and reduce risk from all hazards. Improving safety and reducing uncertainty could stimulate production and grow the market share of oysters and other bivalve mollusks, arguably the greenest and most sustainable animal source of protein in the food supply.

ACKNOWLEDGMENTS

I thank the many colleagues and collaborators who have contributed, and continue to contribute to this article. I appreciate the assistance of Dorothy Jean McCoubrey in reviewing this manuscript.

REFERENCES

1. Blackstone, G. M., J. L. Nordstrom, M. C. L. Vickery, M. D. Bowen, R. F. Meyer, and A. DePaola. 2003. Detection of pathogenic *Vibrio parahaemolyticus* in oyster enrichments by real time PCR. *J. Microbiol. Meth.* 53:149–155.
2. Blake, P. A., M. H. Merson, R. E. Weaver, D. G. Hollis, and P. C. Heublein. 1979. Disease caused by a marine *Vibrio*: clinical characteristics and epidemiology. *New Engl. J. Med.* 300:1–5.
3. Burdette. E. 2017. *Vibrio parahaemolyticus* trends, 2007-2015. Interstate shellfish sanitation conference *Vibrio parahaemolyticus* workshop. September 6, 2017. http://www.issc.org/Data/Sites/1/media/presentations/vpworkshopbaltimore/erin-burdette-national-vp-pdf.
4. Centers for Disease Control and Prevention. 1998. Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters — Pacific Northwest, 1997. *Morb. Mortal. Wkly. Rep.* 47:457–462.
5. Centers for Disease Control and Prevention. 2006. V*ibrio parahaemolyticus* infections associated with consumption of raw shellfish — three states, 2006. *Morb. Mortal. Wkly. Rep.* 55:854–856.
6. Centers for Disease Control and Prevention. 2014. Notes from the field: Increase in *Vibrio parahaemolyticus* infections associated with consumption of Atlantic Coast shellfish – 2013. *Morb. Mortal. Wkly. Rep.* 63:335–336.
7. Centers for Disease Control and Prevention. 2014. National Surveillance of Bacterial Foodborne Illnesses (Enteric Diseases). http://www.cdc.gov/nationalsurveillance/botulism-surveillance.html.
8. Cook, D. W. 1994. Effect of time and temperature on multiplication of *Vibrio vulnificus* in postharvest Gulf coast shellstock oysters. *Appl. Environ. Microbiol.* 60:3483–3484.
9. Cook, D. W. 1997. Refrigeration of oyster shellstock: conditions which minimize the outgrowth of *Vibrio vulnificus. J. Food Prot.* 60:349–352.
10. Cook, D. W., J. C. Bowers, and A. DePaola. 2002. Density of total and pathogenic *(tdh+) V. parahaemolyticus* in Atlantic and Gulf Coast molluscan shellfish at harvest. *J. Food. Prot.* 65:1873–1880.
11. Cook, D. W., P. O'Leary, J. C. Hunsucker, E. M. Sloan, J. C. Bowers, R. J. Blodgett, and A. DePaola. 2002. *Vibrio vulnificus* and *Vibrio parahaemolyticus* in U.S. retail shell oysters: A national survey from June 1998 to July 1999. *J. Food Prot.* 65:151–159.
12. Daniels, N., L. MacKinnon, R. Bishop, S. Altekruse, B. Ray, R. Hammond, S. Thompson, S. Wilson, N. Bean, P. Griffin, and L. Slutsker. 2000. *Vibrio parahaemolyticus* infections in the United States, 1973–1998. *J. Infect. Dis.* 181:1661–1666.
13. Daniels, N. A., B. Ray, A. Easton, N. Marano, E. Kahn, A. L. McShan, L. Del Rosario, T. Baldwin, M. A. Kingsley, N. D. Puhr, J. G. Wells, and F. J. Angulo. 2000. Emergence of a new *Vibrio parahaemolyticus* serotype in raw oysters. *JAMA.* 284:1541–1545.
14. Davis, B. J. K., J. M. Jacobs, M. F. Davis, K. J. Schwab, A. DePaola, and F. C. Curriero. 2017. Environmental determinants of *Vibrio parahaemolyticus* in the Chesapeake Bay. *Appl. Environ. Microbiol.* 83:e1147–17.
15. DePaola, A., G. M. Capers, M. L. Motes, O. Olsvik, P. I. Fields, J. Wells, I. K. Wachsmuth, T. A. Cebula, W. H. Koch, F. Khambaty, M. H. Kothary, W. L. Payne, and B. A. Wentz. 1992. Isolation of Latin American epidemic strain of *Vibrio cholerae* 01 from U.S. Gulf Coast. *Lancet* 339:624.
16. DePaola, A., L. H. Hopkins, J. T. Peeler, B. Wentz, and R. M. McPhearson. 1990. Incidence of *Vibrio parahaemolyticus* in U.S. coastal waters and oysters. *Appl. Environ. Microbiol.* 56:2299–2302.
17. DePaola, A., J. L. Jones, K. E. Noe, R. H. Byars, and J .C. Bowers. 2009. Survey of postharvest-processed oysters in the United States for levels of *Vibrio vulnificus* and *Vibrio parahaemolyticus. J. Food Prot.* 72:2110–2113.
18. DePaola, A., J. L. Jones, J. Woods, W. Burkhardt III, K. R. Calci, J. A. Krantz, J. C. Bowers, K. Thammasouk, D. Williams-Hill, K. Kasturi, R. H. Byars, and E. Jacobs. 2010. Bacterial and viral pathogens in live oysters: U.S. market survey 2007. *Appl. Environ. Microbiol.* 76:2754–2768.
19. DePaola, A., C. A. Kaysner, J. Bowers, and D. W. Cook. 2000. Environmental investigations of *Vibrio parahaemolyticus* in oysters following outbreaks in Washington, Texas and New York (1997–1998). *Appl. Environ. Microbiol.* 66:4649–4654.
20. DePaola, A., M. L. Motes, A. M. Chan, and C.A. Suttle. 1998. Phages are abundant and diverse in oysters *(Crassostrea virginica)* collected from the Gulf of Mexico. *Appl. Environ. Microbiol.* 64:346–351.
21. DePaola, A., J. L. Nordstrom, J. C. Bowers, J. G. Wells, and D. W. Cook. 2003. Seasonal abundance of total and pathogenic *Vibrio parahaemolyticus* in Alabama oysters. *Appl. Environ. Microbiol.* 69:1521–1526.
22. Food and Agricultural Organization. Guidelines on the application of general principles of food hygiene to the control of pathogenic *Vibrio* species in seafood. GAC/GL 73–2010. 2010. Rome, Italy.
23. Food and Agricultural Organization and World Health Organization. Risk assessment of *Vibrio vulnificus* in raw oysters: Interpretative summary and technical report. 2005. Rome, Italy, FAO. Microbiological Risk Assessment Series No. 8.
24. Fujino, T., Y. Okuno, D. Nakada, A. Aoyoma, K. Fukai, T. Mukai, and T. Ueho. 1953. On the bacteriological examination of shirasu food poisoning. *Med. J. Osaka Univ.* 4:299–304.
25. González-Escalona, N., V. Cachicas, C. Acevedo, M. L. Rioseco, J. A. Vergaro, J. Romero, and R. T. Espejo. 2005. *Vibrio parahaemolyticus* diarrhea, Chile, 1998 and 2004. *Emerg. Infect. Dis.* 11:129–131.
26. González-Escalona, N., J. Martinez-Urtaza, J. Romero, R. T. Espejo, L. A. Jaykus, and A. DePaola. 2008. Molecular phylogenetics of *Vibrio parahaemolyticus* strains by multilocus sequence typing. *J. Bacteriol.* 190:2831–2840.
27. Gooch, J. A., A. DePaola, J. C. Bowers, and D. L. Marshall. 2002. Growth and survival of *Vibrio parahaemolyticus* in postharvest American oysters. *J. Food Prot.* 65:970–974.
28. Hara Kudo, Y., and S. Kumargai. 2012. Characteristics of a sharp decline in *Vibrio parahaemolyticus* infections and seafood contamination in Japan. *Intl. J. Food Microbiol.* 157:95–101.
29. Iwamoto, M., T. Ayers, B. E. Mahon, and D. L. Swerdlow. 2010. Epidemiology of seafood-associated infections in the United States. Clin. *Microbiol. Rev.* 23:399–411.
30. Kaneko, T., and R. R. Colwell. 1976. Incidence of *Vibrio parahaemolyticus* in Chesapeake Bay. *Appl. Microbiol.* 30:251–257.
31. Kaysner, C. A., C. Abeyta, M. M. Wekell, A. DePaola, R. F. Stott, and J. M. Leitch. 1987. Virulent strains of *Vibrio vulnificus* from estuaries of the United States West Coast. *Appl. Environ. Microbiol.* 53:1349–1351.
32. Martinez-Urtaza, J., C. Baker-Austin, J. L. Jones, A. E. Newton, G. D. Gonzalez-Aviles, and A. DePaola. 2013. Spread of Pacific Northwest *Vibrio parahaemolyticus* strain. *New Engl. J. Med.* 369:1573–1574.
33. Martinez-Urtaza, J., J. C. Bowers, J. Trinanes, and A. DePaola. 2010. Climate anomalies and the increasing risk of *Vibrio parahaemolyticus* and *Vibrio vulnificus* illnesses. *Food Res. Int.* 43:1780–1790.

34. Martinez-Urtaza, J., L. Simental, D. Velasco, A. DePaola, M. Ishibashi, Y. Nakaguchi, M. Nishibuchi, D. Carrera-Flores, C. Rey-Alvarez, and A. Pousa. 2005. Pandemic *Vibrio parahaemolyticus* O3:K6, Europe. *Emerg. Infect. Dis.* 11:1319–1320.

35. Matsumoto, C., J. Okuda, M. Ishibashi, M. Iwanaga, P. Garg, T. V. Rammamurthy, H. Wong, A. DePaola, Y. B. Kim, M. J. Albert, and M. Nishibuchi. 2000. Pandemic spread of an O3:K6 clone of *Vibrio parahaemolyticus* and emergence of related strains evidenced by arbitrarily primed PCR and toxRS sequence analysis. *J. Clin. Microbiol.* 38:578–585.

36. McCarthy, S. A., and F. M. Khambaty. 1994. International dissemination of epidemic *Vibrio cholerae* by cargo ship ballast and other nonpotable waters. *Appl. Environ. Microbiol.* 60:2597–2601.

37. McLaughlin, J. B., A. DePaola, C. A. Bopp, K. A. Martinek, N. P. Napolilli, C. G. Allison, S. L. Murray, E. C. Thompson, M. M. Bird, and J. P. Middaugh. 2005. Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. *New Engl. J. Med.* 353:1463–1470.

38. Mead, P. S., L. Slutsker, V. Dietz, L. F. McGaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607–625.

39. Motes. M. L., and A. DePaola. 1996. Offshore suspension relaying to reduce levels of *Vibrio vulnificus* in oysters *(Crassotrea virginica). Appl. Environ. Microbiol.* 62:3875–3877.

40. Motes, M. L., A. DePaola, D. W. Cook, J. E. Veazey, J. C. Hunsucker, W. E. Garthright, R. J. Blodgett, and S. J. Chirtel. 1998. Influ

41. ence of water temperature and salinity on *Vibrio vulnificus* in northern Gulf and Atlantic Coast oysters *(Crassostrea virginica). Appl. Environ. Microbiol.* 64:1459–1465.

42. Motes, M. L., A. DePaola, S. Zywno-Van Ginkel, and M. McPhearson. 1994. Occurrence of toxigenic *Vibrio cholerae* O1 in oysters in Mobile Bay, Alabama: an ecological investigation. *J. Food Prot.* 57:975–980.

43. Nasu, H., T. Iida, T. Sugahara, Y. Yamaichi, K. Park, K. Yokoyama, K. Makino, H. Shinagawa, and H. Honda. 2000. A filamentous phage associated with recent pandemic *Vibrio parahaemolyticus* O3:K6 strains. *J. Clin. Microbiol.* 38:2156–2161.

44. National Oceanic and Atmospheric Administration. 2019. *Vibrio* predictive models. Available at: https://products.coastalscience.noaa.gov/Vibrioforecast/.

45. Nordstrom, J. L., C. A. Kaysner, G. M. Blackstone, M. C. L. Vickery, J. C. Bowers, and A. DePaola. 2004. Ecological factors affecting *Vibrio parahaemolyticus* levels in Pacific Northwest oysters. *J. Food Prot.* 67:2178–2182.

46. Nordstrom, J. L., M. C. L. Vickery, G. M. Blackstone, S. L. Murray, and A. DePaola. 2007. Development of a multiplex real-time PCR assay with an internal amplification control for the detection of total and pathogenic *Vibrio parahaemolyticus* in oysters. *Appl. Environ. Microbiol.* 73:5840–5847.

47. Oliver, J. D. 1989. *Vibrio vulnificus*, p. 569–600. *In* M.P. Doyle (ed.), Foodborne bacterial pathogens. Marcel Dekker, Inc. New York.

48. Phillips, A. M. B., A. DePaola, J. Bowers, S. Ladner, and D. J. Grimes. 2007. An evaluation of the use of remotely sensed parameters for prediction of incidence and risk associated with *Vibrio parahaemolyticus* in Gulf Coast oysters *(Crassostrea virginica). J. Food Prot.* 70:879–884.

49. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* 17:7–15.

50. Shen, X., Y-C Su, C. Liu, T. Oscar, and A. DePaola. 2019. Efficacy of depuration of *Vibrio parahaemolyticus* in oysters *(Crassostrea gigas). Food Microbiol.* 79:35-40.

51. U.S. Food and Drug Administration 2005. Quantitative risk assessment of the public health impact of pathogenic *Vibrio parahaemolyticus* in raw oysters. U.S. Food and Drug Administration, Washington, D.C. Assessed on 2/12/19 at: https://www.fda.gov/ downloads/food/scienceresearch/.../ucm196915.pdf.

52. Vugia, D. J., T. Farzaneh, A. E. Newton, M. Hernandez, and P. M Griffin. 2013. Impact of 2003 state regulation on raw-oyster-associated *Vibrio vulnificus* illnesses and deaths, California, USA. *Emerg. Infect. Dis.* 19:1276–1280.

53. Waldor, M. K., and J. J. Mekalanos. 2007. Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* 272:1910–1914.

54. Xu, F., N. Gonzalez-Escalona, K. P. Drees, R. P. Sebra, V. S. Cooper, S. H. Jones, and C. A. Whistler. 2017. Parallel evolution of two clades of an Atlantic-epidemic pathogenic lineage of *Vibrio parahaemolyticus* by independent acquisition of related pathogenicity islands. *Appl. Environ. Microbiol.* 83:e01168–17.