THE WORLD IS YOUR OYSTER: A MULTIDISCIPLINARY APPROACH TO EVALUATE THE SPATIAL DISTRIBUTION, OCCURRENCE AND RISK OF *V. PARAHAEMOLYTICUS* IN OYSTERS AND WATER

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Dedication

To Jen and Maddy, this would not have been possible without the never-ending love and support.

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Chapter 1

Abstract

Vibrio parahaemolyticus (V. parahaemolyticus) and *Vibrio vulnificus* (*V. vulnificus)* are naturally occurring bacteria and are the leading causes of seafood-borne illness and death in the United States, respectively. While other foodborne illnesses in the United States are decreasing, *V. parahaemolyticus* and *V. vulnificus* cases continue to increase. Often illness from *V. parahaemolyticus* and *V. vulnificus* are associated with the consumption of raw oysters. The current understanding of *V. parahaemolyticus* and *V. vulnificus* are focused primarily on oysters harvested in the Gulf of Mexico and the East Coast, though there is a growing health risk globally and in Southern California. In this dissertation, a multidisciplinary approach was used to show the spatial distribution of *V. parahaemolyticus* at a global and local level and data were used to inform risk estimates from consuming Southern California oysters contaminated with *V. parahaemolyticus*. In this dissertation, a scoping literature review provided an up-to-date understanding of *V. parahaemolyticus* in water and oysters at a global scale. This highlighted the necessity for oyster-related research publication guidelines to compare studies globally. From the details generated in the literature review, a database was developed to aid in a collaborative effort to better understand *V. parahaemolyticus*. This dissertation was the first to assess *V. parahaemolyticus* and *V. vulnificus* in water and Pacific oysters in Southern California. There was a higher concentration of pathogenic *V. parahaemolyticus and V. vulnificus* in the water column compared to oysters. Additionally, *V. parahaemolyticus* concentration in water and oysters and *V. vulnificus* concentration in water were correlated with environmental covariates. Following this field study, a quantitative microbial risk assessment (QMRA) model was utilized to predict illness risk from *V. parahaemolyticus* in recreationally harvested oysters from Southern California along the "sea-to-fork" pathway. This study utilized regional-specific and national data to estimate *V.*

parahaemolyticus illness risk. The mean probability of illness per serving of oysters was 6.20 x 10^{-5} (95% confidence interval 5.653 x 10^{-7} – 1.92x 10^{-4}) from consumption of raw oysters harvested in Southern California following the "sea-to-fork" pathway. The mean *V. parahaemolyticus* illness risk per serving of oysters immediately following harvest was 5.61 x 10^{-5} (95% confidence interval 4.84 x 10^{-8} – 1.83 x 10^{-4}). The sensitivity analysis highlighted the relative importance of the initial concentration of *V. parahaemolyticus* in the oysters, number of oysters consumed, and concentration of pathogenic *V. parahaemolyticus* following transport in vibriosis risk.

Chapter 1: Introduction

Shellfish provided a high source of protein and nutrients as humans were on the brink of extinction over 164,000 years.[1–3] Today, oyster consumption remains a staple of diets around the world and while oysters have historically been consumed in large quantities, [4,5] consumption is actually increasing, particularly as aquaculture oyster demands surge.[6,7] The global production of oysters increased from 3 million to over 5.9 million tons between 2009 and 2019.[6–8] The Pacific oysters (*Magallan gigas,* formerly *Crassostrea gigas*) are the largest grossing aquaculture species In 2018, 643,549 tons of Pacific oysters were distributed globally, but only 23,797 tons were harvested in the United States.[8] The United States' oyster industry is worth $173 million but is recovering from unsustainable over-harvesting practices on the East Coast.[5,9] On the West Coast of the United States, the oyster industry continues to expand and Pacific oysters now account for 89% of oysters harvested on the West Coast.[10,11] On the West Coast, five species of oysters are commercially harvested.[12]

There have been human health concerns from pathogens, chemicals, and radionucleotides during the consumption of raw oysters.[13] Since as early as 1818, it was understood that oysters played an important role in pathogen transmission.[14] However, an oyster harvesting policy was not implemented in the United States until 1925.[15] The adverse health outcomes from most hazardous agents related to oyster consumption have decreased through effective policy and education.[16–19] However, *Vibrio* spp. related illness and death from consumption of raw oysters continue to increase and dominate the concern of scientists, industry, and public health.[20–22] The filter-feeding nature of oysters allows pathogens to be concentrated and harbored within the oyster meat.[14,23–25] Oysters can filter up to 190 L per day.[14,23–25]

Vibrio spp.

Vibrio spp., a naturally occurring gram-negative bacteria, are frequently isolated from fresh, estuarine, and seawater.[26] Of the 100 known *Vibrio* spp., at least 12 are capable of causing disease in humans. Of particular public health concern from the consumption of oysters is *V. parahaemolyticus* and *V. vulnificus*.[27,28] In the United States, approximately 80,000 illnesses, 500 hospitalizations, and 100 deaths occur from *Vibrio* infections (vibriosis) each year. Additionally, vibriosis associated with the consumption of seafood accounts for an estimated $250 million in health-related costs in the United States.[21,22,29] Consumption of raw oysters is responsible for approximately 90% of *V. parahaemolyticus* and *V. vulnificus* illnesses.[30,31] As other foodborne illness rates in the United States have decreased in recent decades, vibriosis rates have increased.[20,30]

V. parahaemolyticus is the leading cause of seafood-borne illness in the United States. There are an estimated 34,000 infections resulting in $168 million per year in medical costs each year in the United States.[21,29] *V. parahaemolyticus* often presents as mild gastroenteritis issues for 24 hours to 10 days, but recently, there has been an increase in *V. parahaemolyticus* infections causing wound infection and septicemia due to unidentified causes.[32,33] The inaugural *V. parahaemolyticus* outbreak occurred in Japan from the consumption of sardines in 1950.[34] The first outbreak in the United States occurred in 1971 in Maryland from the consumption of steamed crabs.[35] There have been over 49 reported outbreaks in the United States from *V. parahaemolyticus* between 1973 and 2019.[36–39] Between 2006 and 2019, there were five outbreaks associated with *V. parahaemolyticus* in the United States (Table 1) and there were 25 commercial oyster recalls and safety notices from *V. parahaemolyticus* in oysters from 2012 to 2020.[39,40] Additionally, there have been outbreaks associated with *V. parahaemolyticus* in oysters harvested from as far north as Alaska and in Scandinavian countries.[41,42] As both foodborne and environmentally acquired *V. parahaemolyticus* illnesses are increasing, it is imperative to have policies that are protective of human health to mitigate illness risk.

Table 1. *V. parahaemolyticus* outbreaks in the United States between 2006 and 2019.

Year	Food	Harvest Location	States involved	Cases	Hospitalizations	Deaths	Citation
2019	Oyster	Mexico	5	16	2	0	[43]
2018	Fresh crab meat	Venezuela	7	26	9	0	[44]
2013	Oyster and clam	Connecticut, Massachusetts, New York, Virginia	13	104	6	0	[45]
2012	Shellfish	No traceback	3	6	0	0	[46]
2006	Shellfish	Washington	3	177	3	0	[47]
Total	-	-	31	1265	20	0*	

*Although no deaths were attributed to *V. parahaemolyticus* in these outbreaks, there are an estimated 4 deaths per year from *V. parahaemolyticus*.[21]

V. parahaemolyticus is readily isolated from estuarine environments.[48] Temperature and salinity are most commonly associated with *V. parahaemolyticus* concentrations in the environment.[27,42,49] The bacterium grows most preferentially between 20 °C and 35 °C, however, it has been isolated in environments less than 10 °C and over 40 °C.[50] Additionally, *V. parahaemolyticus* has a wide salinity range conducive to growth, with optimal growth around 23 parts per thousand (ppt), allowing *V. parahaemolyticus* to be isolated in marine and estuarine environments.[50–52] While temperature and salinity are important co-variates in *V. parahaemolyticus* concentrations, several Pacific Northwest studies have found limited relationships between *V. parahaemolyticus* concentration with salinity and temperature.[53–56] As

V. parahaemolyticus cases continue to increase, it is critical to further elucidate environmental covariates impacting concentrations in water and oysters.

Pathogenic *V. parahaemolyticus* is not readily isolated from the environment. Pathogenic *V. parahaemolyticus* is identified by the detection of thermostable direct hemolysin (*tdh*) or tdh-related hemolysin (*trh*) genes.[57,58] Generally, the detection of *tdh* and *trh* is between 0 to 5% of isolates from environmental samples, but pathogenic *V. parahaemolyticus* has been isolated in up to 50% of water samples from South Carolina.[59–65] However, there have been outbreaks associated with limited concentrations of pathogenic *V. parahaemolyticus* and, approximately 10% of clinical *V. parahaemolyticus* cases lack either the *tdh* or *trh* gene, indicating an incomplete understanding of *V. parahaemolyticus* pathogenicity.[41,48,60,66] It is believed that type-three secretion systems play an integral role in pathogenesis, but this is not completely understood.[60,67] Additionally, several serotypes of *V. parahaemolyticus* are more frequently associated with outbreaks and pose a greater public health risk. The two most prominent outbreak-associated serotypes are O3:K6 and ST36.[48,68] As the O3:K6 and ST36 serotypes were identified, subsequent outbreaks in Peru, Alaska, Spain, Africa, and Maryland were recorded.[41,68–72] *V. parahaemolyticus* is a regional concern with global implications, however, the limited scientific understanding of the pathogenicity limits effective management to protect human health.

V. vulnificus, an opportunistic pathogen, is the leading cause of seafood-borne deaths in the United States with a case fatality rate of around 50% of the people infected with *V. vulnificus*.[73] *V. vulnificus* presents most frequently as primary septicemia from consumption of oysters and wound infection from exposure to environmental waters.[73,74] Consumption of oysters was associated with over 90% of *V. vulnificus* deaths from food between 2002 and 2007.[73] Of note, 80% of *V. vulnificus* illnesses and deaths were associated with pre-existing health conditions, such as diabetes or chronic liver illnesses, in men over the age of 60 as they have higher blood iron levels.[33,75–78] In the United States, there are approximately 100 cases of *V. vulnificus* annually, resulting in $260 million per year in healthcare-related costs.[29] *V. vulnificus* cases in the United States and globally are increasing at an alarming rate. Wound infections from *V. vulnificus* nearly doubled between 1988 and 2010 in the United States from both environmental and seafood exposures.[22,51]

The relationship of environmental co-variates and *V. vulnificus* concentrations are not fully elucidated, though water temperature and salinity are frequently significantly associated with *V. vulnificus* concentrations.[31] *V. vulnificus* grows preferentially in salinity between 10 ppt to 18 ppt but can grow between 2 ppt and 25 ppt.[76] Extreme weather events, such as hurricanes and drought, can alter salinity levels causing changes in *V. vulnificus* detection frequency. For example, large influxes of rainwater from hurricanes and storms increased *V. vulnificus* infections in New Orleans and the concentrations of *Vibrio* spp. detected in water in France.[79,80] Alternatively, a drought increased the salinity of a North Carolina estuary resulting in reduced *V. vulnificus* concentrations.[81] *V. vulnificus* can grow in water temperatures ranging from 13 °C to 30 °C.[76] At water temperatures below 13 °C, it can enter a viable but not culturable (VBNC) state which allows it to persist for up to 4.5 months.[76,82–84] As temperature and salinity profiles of coastal environments are impacted by natural disasters, climate change, and anthropogenic changes, it is imperative to monitor for the presence of *V. vulnificus* with molecular methods and culture based methods to help mitigate human health risks.

Pathogenic *V. vulnificus* is identified by the presence of virulence correlated gene for environmental genotypes (*vcgE*), virulence correlated gene for clinical genotypes (*vcgC*), or polymorphism in the pilus-type IV (*pilF*).[85–87] The *vcgE* gene is associated with 90% of the wound infection cases whereas the *vcgC* gene is associated with primary septicemia.[87] The *pilF* gene identifies pathogenic *V. vulnificus* as the pilus-type IV protein was shown to cause pathogenicity in mice with *Francisella tularensis*.[88] Pathogenic *V. vulnificus* is hypothesized to cause increased levels of disease in immunocompromised patients as *V. vulnificus* takes iron from the body in order to grow.[89,90] While there is a more complete understanding of the pathogenicity of *V. vulnificus* compared to *V. parahaemolyticus* the mechanism of action is complex and the relationship of pathogenic *V. vulnificus* with environmental covariates is not fully understood.

While *V. parahaemolyticus* and *V. vulnificus* have been public health concerns for over 70 years, there are numerous scientific knowledge gaps for these organisms. The current approaches to detect pathogenicity involve a culture-based enumeration step followed by molecular confirmation which limit our ability to properly protect human health. Additionally, there have been few published studies assessing *V. parahaemolyticus* and *V. vulnificus* concentrations and risk in Southern California. Taken together, there is a growing public health need to assess the prevalence and risk of *V. parahaemolyticus* and *V. vulnificus* in recreationally harvested oysters in Southern California to develop protective public health policies.

Pacific oyster

The Pacific oyster is currently commercially harvested from 17 countries, but has been introduced to 66 countries.[91] The Pacific oyster was introduced to the Pacific Northwest and California in the early 1900s as the native Olympia oyster was being over-harvested, but the Pacific oyster was not successfully cultured from the region until the 1920s.[92–94] Carlsbad

Aquafarm was the first to culture Pacific oysters in Southern California in the early 1990s.[95,96] Until the early 2000s, feral Pacific oysters were infrequently detected in Southern California, but are now endemic in Southern California.[11,97] Pacific oysters have proven to be an economically lucrative aquaculture species on the West Coast.[10,11]

Pacific oysters are capable of growing in water temperatures ranging from 3 °C to 35 °C and salinity from 10 ppt to 42 ppt.[92,98,99] However, Atlantic oysters are only successfully grown in water temperatures ranging from 20 °C to 30 °C and salinity from 10 ppt to 28 ppt.[92] Given temperature and salinity profiles of the harvesting water impacts the concentrations of *V. parahaemolyticus* and *V. vulnificus*, growing environments are important to microbial concentrations within oysters. Additionally, the broad environmental conditions a Pacific oyster can grow in may alter the phytoplankton community. This is important as a long-term assessment of phytoplankton speciation in Europe showed a relationship between diatom concentrations, *Vibrio* spp. populations, and vibriosis cases.[100] Other studies have demonstrated that *Vibrio* spp. more selectively attach with diatoms compared to other phytoplankton species.[101] The filtration rate of the Pacific oyster has been shown to be 1.5 times higher than Atlantic oysters at 20°C.[102,103] The physiological differences in filtration rate and size have the potential to impact pathogens concentrated within the oyster as there are differences in the ability to attach to the marine substrate and enter the oyster.[104] Taken together, there are physiological and environmental differences between Atlantic and Pacific oysters that may impact the ability of pathogens to concentrate in the oysters. Given wild Pacific oysters in Southern California is a recent phenomenon, it is imperative to understand the dynamics with pathogens of concern to best protect human health.[11]

Oyster policy

Health concerns from the consumption of oysters have prompted policy and regulation. Though rates of vibriosis are increasing globally, there is limited global collaboration and unity in forming or updating related concentration limits (Table 2).[20,21] In Japan and Canada, there is a stringent policy of 100 MPN (most probable number) per gram of *V. parahaemolyticus* permitted in oysters.[105,106] The 100 MPN per gram of food was based on the assumption that 100 MPN per gram of pathogenic *V. parahaemolyticus* was sufficient for infection.[105] The MPN is determined statically following a serial dilution of a homogenized oyster sample. Additionally, Japan and Canada have a robust reporting system for vibriosis. In Japan, cases of *V. parahaemolyticus* decreased by over 90 fold between 1998 and 2012 following the implementation of the 100 MPN per gram policy, however, there have been outbreaks associated with *V. parahaemolyticus* in oysters harvested in Canada.[105,107,108] The frequency of monitoring in Canada and Japan were not dictated in the guidelines. In the United Kingdom, ready-to-eat foods are required to have fewer than 1,000 CFU (colony forming units), lower than the median infection dose of 10,000 CFU/g, of *V. parahaemolyticus* per gram of *oysters*.[109,110] However, not all countries require evaluation of *Vibrio* spp. in or shellfish. European shellfish harvesting criteria evaluate the concentrations of *E*. coli in oyster meat.[111] Oyster meat with *E. coli* concentrations less than 230 MPN per gram require no additional depuration.[111] This microbial standard was shown to be more protective of human illness than current US guidelines, shown in table 2.[112] In Australia and New Zealand, raw mollusks, including oysters, are subject to a "maximum microbial concentration" standard which is 7.0 CFU of *E. coli* per gram and a mean acceptable microbial concentration of 2.3 CFU per gram of oysters.[113] While other countries have created policies to protect oyster consumers, the United States has not implemented a more protective policy in the face of increasing illnesses.

Table 2. Global microbial policy in oysters.

Country	Organism	Concentration Limits	Citation
Australia	*E. coli*	2.3 – 7 /g	[113]
Canada	*V. parahaemolyticus*	100 MPN/g	[86]
England	*V. parahaemolyticus*	1000 CFU/g	[109]
Europe	*E. coli*	230 MPN/g	[111]
Japan	*V. parahaemolyticus*	100 MPN/g	[85]
New Zealand	*E. coli*	2.3 – 7 /g	[113]
United States	*V. parahaemolyticus*	10,000 cells/oyster	[114]
	Fecal coliform	14 MPN/100 ml	[114]
	Total coliform	70 MPN/100 ml	[114]

Following a multi-state typhoid fever outbreak in 1924 linked to the consumption of oysters harvested from sewage-contaminated water, public health officials and shellfish industry representatives united to petition the US Surgeon General for safer shellfish.[13] From this meeting, the National Shellfish Sanitation Program (NSSP) Guide for Control of Molluscan Shellfish was created as a basis to regulate interstate commerce based on most current shellfish health issues.[15] In 1975, the US Food and Drug Administration (US FDA) wanted a larger role in regulating shellfish, however, this was rejected as it did not align with the goals of NSSP to keep policy collaborative.[13] Rather, the Interstate Shellfish Sanitation Conference (ISSC) was formed as a cooperative working group with the oyster industry, scientists, and national and state government to create a unified NSSP in 1982. The ISSC formed a memorandum of understanding with the US FDA in 1984 to develop of shellfish policy along the "sea-to-fork" pathway to ensure a safe product. Every two years, the ISSC formalizes an updated NSSP, a Guide for the Control of Molluscan Shellfish. Each state is then responsible for adopting standards based on these NSSP guidelines.

The NSSP has developed guidelines for oyster harvesting water. Shellfish harvesting waters impacted by point sources require five water quality samples a year, sites impacted by non-point sources require six samples a year, and remote shellfish harvesting water requires a minimum of two water quality samples a year. The NSSP requires shellfish water to have a median fecal coliform concentration of less than 14 per 100 ml using dilution tubes or membrane filtration (MF) methods. Additionally, the 90th percentile of samples may not exceed additional standards based on various methods. A five-tube decimal dilution test must not exceed 43 MPN per 100 ml, a three-tube decimal dilution test cannot exceed 49 MPN per 100 ml or 31 CFU per 100 ml for an MF (mTEC) test.[114,115] Oysters being imported into the United States must follow these harvesting water criteria.[116]

In addition to the fecal coliform concentration rule, the NSSP requires growing areas to be closed if there is greater than 10,000 MPN/g, determined by assessing the *tlh* gene, per gram of oyster meat.[114] The NSSP requires *Vibrio* Control Plans for states that have had either two or more *V. parahaemolyticus* infections within three years, two or more *V. vulnificus* infections within ten years, or if the monthly average water temperature is greater than 27.2 °C in the Gulf of Mexico or the Atlantic Ocean south of New Jersey, greater than 15.6 °C along the Pacific Ocean or the Atlantic Ocean north of New York.[114] *Vibrio* Control Plans are developed specifically for each state to reduce the risk of *V. parahaemolyticus* or *V. vulnificus* illness. Additionally, NSSP provide guidelines for post-harvest time-to-temperature control based on regional air temperatures. The NSSP time-to-temperature control ranges from 36 hours with air temperatures less than 10 °C to less than 12 hours in areas with air temperatures greater than 26.7 °C.[114] The NSSP provides recommendations for temperatures during oyster transport. It is recommended that internal oyster temperature remain below 10 °C or stored below 7.2 °C.[114] However, one study identified that the temperature of oyster shipments in the United States exceeded the recommended temperature on average for 2.5 hours.[117] Given the importance of cold chain in minimizing *Vibrio* spp. risks, additional studies developing strategies to better cool oysters during long transport times and conducting oyster samples *d*uring the supply chain rather than modeling to assess *Vibrio* would allow for increased understand and more protective policy during the post-harvest shipping process.

The California State *Vibrio* Control Plan is focused on *V. parahaemolyticus* concentrations in oysters.[118] In addition to the recommended *V. parahaemolyticus* levels in harvesting water from the NSSP, the California *Vibrio* Control Plan (VCP) suggests optional steps to reduce *V. parahaemolyticus* concentration if there is greater than 100 total *V. parahaemolyticus* MPN per gram or greater than 10 *tdh* or *trh* MPN per gram.[118] The California VCP requires stricter time-to-temperature control than the NSSP. From March through December, Southern California aquaculture farms are required to have oysters in temperature control within 5 hours though the average air temperature is less than 26.7 °C.[118]

Oysters from the Gulf of Mexico imported into California for commercial consumption during summer months must have undergone post-harvest processing to *V. vulnificus* levels less than 30 MPN per gram.[119,120] Additionally, bars and restaurants must have visible warnings about vibriosis from consuming raw oysters harvested in the Gulf of Mexico.[119,120] These policies have reduced *V. vulnificus* rates in California, however, the inclusion of Spanish and English warnings on menus and post-harvest processing of Gulf of Mexico oysters have not reduced *V. parahaemolyticus* cases.[30,121] Studies looking at sources of *V. parahaemolyticus* cases in California may allow for increased knowledge on exposure to *Vibrio*. Additionally, all commercial institutions serving oysters must keep oyster tags for traceability in case of an outbreak.[114,116]

While the ISSC develops policy for commercially harvested oysters, recreationally harvested oysters are omitted. The California Recreational Shellfish Harvesting numeric targets (SHEL Standard) are encompassed within the Basin Plans for several waterbodies in California..[122] The The SHEL water quality objective requires the monthly median fecal coliform level to be less than 14 MPN per 100 ml and no more than 10% of samples to exceed 43 MPN per 100 ml. However, these low standards are difficult to meet, are more protective than recreational water quality standards, and are not supported by human health data.[123–127] Numerous studies have shown a disconnect between fecal coliform concentrations in water and human pathogens in oysters or water.[128–133] Additionally, *V. parahaemolyticus* and *V. vulnificus* are not addressed by the current SHEL standards. However, *V. parahaemolyticus* concentrations in commercially harvested oysters in Southern California are addressed by the *Vibrio* control plan for California. Given there are feral Pacific oysters ubiquitously throughout Southern California, it is imperative to have a recreational shellfish policy that encompasses *V. parahaemolyticus* and *V. vulnificus* to best protect human health.

As Pacific oysters were not readily available for recreational harvesting in Southern California until about twenty years ago, there was a limited need for an appropriate recreational shellfish harvesting policy. However, as Pacific oysters are now endemic to Southern California, there is a need to understand potential health concerns from the consumption of recreationally harvested Pacific oysters.[11] The current policy is lacking regional studies that evaluate the concentration of *V. parahaemolyticus* and *V. vulnificus* in recreationally harvested oysters and their surrounding water throughout California.

Quantitative microbial risk assessment

Quantitative microbial risk assessment (QMRA) is a systematic approach to mathematically quantify human health risk. The QMRA framework consists of hazard identification, exposure assessment, dose-response, risk characterization, and risk communication. Hazard identification aims to understand the characteristics of the pathogen of interest. Following, the exposure assessment evaluates the fate and transport of the pathogen leading to human exposures, environmental conditions impacting the concentration of microorganisms, behavior patterns that impact exposure, such as consumption rates and serving size. The exposure assessment aims to understand human interactions with the pathogen. Dose-response models are pathogen-specific to quantify the relationship between the quantity of pathogen exposed to and the likelihood of developing an adverse health effect and can evaluate different health endpoints. Following, the risk characterization integrates the exposure assessment with dose-response to quantitatively estimate health outcomes. The risk analysis step allows for risk mitigation. Risk communication bridges the gap between science and the community as it is imperative to communicate the risks to stakeholders.[134] The QMRA framework has been used extensively to quantify risks from pathogens in natural and built environments. The QMRA framework has been utilized to inform safe drinking water standards, estimate illness risk from recreation water, evaluate consumption risk from leafy greens, and estimate health risk from seafood.[110,126,135–146]

The US FDA developed an extensive QMRA model for *V. parahaemolyticus* in oysters harvested in the United States. However, the QMRA model extensively utilized Atlantic oysters harvested in the Gulf of Mexico to develop harvesting and shipping policies.[110] The US FDA QMRA accepted risk standard was set at one case in 10,000 servings based on *V. parahaemolyticus* illness risk from oysters harvested in the Gulf of Mexico.[110] The estimated median health risk from oyster consumption in the US FDA model for the United States ranged from 2.1 x 10^{-6} to 4.4 x 10^{-4}.[110] The QMRA model fails to account for regional differences impacted by *V. parahaemolyticus* dynamics in local harvesting water, oyster health, and outbreak-associated serotypes of *V. parahaemolyticus*. QMRA models have been utilized to estimate the mean risk from *V. parahaemolyticus* in Brazil (3.6 x 10^{-3}, 95% CI 3.3 x 10^{-4} – 9.9 x 10^{-3}), Taiwan (8.56 x 10^{-5}, 95% CI 0.0 – 4.5 x 10^{-3}), Japan (1 x 10^{-10}), Australia (5.97 x 10^{-8}, 95% CI 3.04 x 10^{-8} – 2.04 x 10^{-7}), Canada (1.08 x 10^{-6}, 95% CI 3.04 x 10^{-8} – 4.00 x 10^{-6}), New Zealand (1.40 x 10^{-8}, 95% CI 1.10 x 10^{-9} – 4.57 x 10^{-8}), the Gulf of Mexico (3.75 x 10^{-4}, 95% CI 2.86 x 10^{-5} – 1.21 x 10^{-3}) and Chesapeake Bay (3.0 x 10^{-7} , 95% CI 1.0 x 10^{-7} – 7.0 x 10^{-7}).[147–151] The regional differences in health risks from *V. parahaemolyticus* in identified models highlight the importance of site-specific QMRA models to best understand and assess local health risks.

While QMRA is a useful tool, it has limitations. Each QMRA is only as accurate as the input data and often specific data needed for a scenario does not exist requiring the use of assumptions to fill gaps in the exposure assessment or dose-response relationship. The US FDA QMRA model has several key limitations. For example, the current dose-response model was estimated from feeding studies conducted with healthy, young men which fail to account for differences in oyster consumer vulnerabilities. Additionally, only one virulent strain of *V. parahaemolyticus* was assessed. Given there are outbreaks associated with different serotypes, an updated dose-response model is needed to better understand *Vibrio* spp. associated risks. Another example of a knowledge gap is the growth rate model utilized by the US FDA which assumed *V. parahaemolyticus* concentration would decline under temperature control.[110] However, a study conducted with Pacific oysters in the Pacific Northwest found no significant decay rate of *V. parahaemolyticus* in oysters held at 4 °C.[152] Taken together, there is a need to use regionally relevant information, or generate such data when not available, to make science-driven decisions about oyster harvesting and consumption.

There is a growing need to better understand *V. parahaemolyticus* risk at a global and local level as cases of vibriosis are increasing throughout the world as climate change is altering coastal communities and Pacific oyster production and consumption is increasing. While several risk models have evaluated the risk from consumption of raw oysters, there is no *V. parahaemolyticus* QMRA specific to Southern California.[110,147,148,151,153] Wild Pacific oysters are prevalent in Southern California waters and therefore it is critical to understand the human health risks posed by the consumption of oysters.

Addressing gaps in *Vibrio*-oyster-health

There is a pressing need to better understand *V. parahaemolyticus* and *V. vulnificus* from a global and local perspective to integrate public health interventions. However, the current oyster harvesting policy in the United States is antiquated and heavily based on studies conducted on the East Coast and the Gulf of Mexico with limited related human health data. Additionally, the current *V. parahaemolyticus* QMRA framework employed fails to truly consider regional differences in growing conditions, oyster species, and environmental covariates. Taken together, there are critical gaps in the current understanding of the *Vibrio*-oysters-health relationship, particularly in Southern California. Through the following chapters, I will explore the global distribution of *V. parahaemolyticus* in paired oyster and water samples; evaluate the prevalence of *V. parahaemolyticus* and *V. vulnificus* in oysters and water samples harvested from Newport Bay, California; and integrate environmental occurrence with “sea-to-fork” processes to understand human health risks from consumption of oysters harvested from Newport Bay, California.

Chapters 2, 3, and 4 cumulatively address some of the most pressing scientific gaps in the *Vibrio* spp. -oyster-health paradigm. Chapter 2 will provide up-to-date information on the global occurrence and spatial distribution of paired oysters and water samples. This work aims to summarize the current knowledge of *V. parahaemolyticus* in paired oyster and water samples and provide the basis for an open-access database using a systematic literature review. Chapter 3 will provide an understanding of the prevalence of *V. parahaemolyticus* and *V. vulnificus* in Pacific oysters to help inform health risks associated with the consumption of oysters in Southern California. This chapter aimed to generate background information on concentrations of *V. parahaemolyticus* and *V. vulnificus* in wild oysters and the overlying water column in a Southern California bay. Finally, Chapter 4 evaluated the health risk of *V. parahaemolyticus* from consumption of raw oysters. The objectives of this chapter are to quantify the risk of *V. parahaemolyticus* illness from recreationally harvested Pacific oysters harvested in Southern California and evaluate key places for public health interventions.

Taken together, this dissertation aims to evaluate and address critical knowledge gaps in the *Vibrio* spp.-oyster-health arena to advance shellfish safety. Findings from this dissertation may be utilized to understand *V. parahaemolyticus* distribution locally and globally to aid in decision making to reduce health risks from *V. parahaemolyticus* during oyster consumption. Additionally, this dissertation serves as the first study to address *V. parahaemolyticus* and *V. vulnificus* in recreationally harvested Pacific oyster from a Southern Californian estuary. A QMRA specific for *V. parahaemolyticus* in Pacific oysters harvested on the West Coast of the United States will be developed and will support regional guidance for *V. parahaemolyticus* and *V. vulnificus* oyster consumption.

Chapter 2: A global perspective to understanding the distribution of *V. parahaemolyticus* in oysters and water

Abstract

V. parahaemolyticus is frequently associated with human illness from consumption of raw oysters. The global distribution of *V. parahaemolyticus* in paired oyster and water samples was assessed by synthesizing 32 studies. The relationship of *V. parahaemolyticus* in water or oysters with environmental co-variates is not fully elucidated. The current knowledge of *V. parahaemolyticus* in water and oysters is limited by lack of accessible data, use of antiquated detection methodology, and limited environmental co-variates assessed. This literature review may serve as a foundation of knowledge of *V. parahaemolyticus* concentration and environmental parameters as future studies are conducted. Collaboration and continued synthesis of *V. parahaemolyticus* concentration in water and oysters may inform decision making abilities to mitigate *V. parahaemolyticus* illness.

Introduction

Between 1998 and 2018, global oyster exports increased from 244,713 to 708,688 tons as demand increased.[154] During the same time, oyster related foodborne illnesses increased while other foodborne illnesses decreased.[3,22,51] Human pathogens are commonly detected in oysters as their filter-feeding nature allows pathogens to be concentrated within the oyster at up to 100 times higher than in the water.[155] *Vibrio parahaemolyticus* (*V. parahaemolyticus*), a naturally occurring bacteria frequently isolated in coastal and estuarine environments, causes 34,000 infections per year in the United States resulting in $168 million per year in medical costs.[3,21,29,156] Typically, *V. parahaemolyticus* presents as self-limiting gastroenteritis but has increasingly been associated with wound infection and death from wound infection and ingestion.[30,32,33,110] There have been devastating outbreaks related to the movement of *V. parahaemolyticus* around the globe. Outbreaks in Peru, Alaska, Spain, Africa, and the United States highlight the global impact of strain variants of pathogenic *V. parahaemolyticus*.[41,68–71,157–160]

Pathogenic *V. parahaemolyticus* are identified by the occurrence of either the thermostable direct hemolysin (*tdh*) or tdh-related hemolysin (*trh*) genes.[57,58] Historically throughout the world, less than 5%, of environmental samples tend to have either the *trh* or *tdh* gene.[61,157] Detection frequency of virulent *V. parahaemolyticus* in the environment in South Carolina and Long Island has been increasing in recent years.[59,161] However, there may be a disconnect between environmental monitoring of *trh* and *tdh* genes and clinical cases as approximately 10% of clinical *V. parahaemolyticus* isolates lacked the presence of either the *tdh* or *trh* gene.[27,162] It is hypothesized that other factors, particularly type three secretion systems (T3SS), may be involved in inducing pathogenesis.[60,67] Though the presence of *V. parahaemolyticus* is often viewed as a regional concern, it is increasingly important to understand the fate and transport from a global perspective to better protect human health.

V. parahaemolyticus is frequently monitored and modeled in water and oysters. However, the relationship between the environment and concentrations of *V. parahaemolyticus* in water and oysters is complex and not fully elucidated.[31] Water temperature and salinity are often associated with the concentrations of *V. parahaemolyticus* in the water, but this association is not universal.[53,54,56,155,163,164] Other environmental parameters, such as chlorophyll A, colored dissolved organic matter, and zooplankton, may provide a key link between the concentration of *V. parahaemolyticus* in the water and oysters.[100,165] Environmental monitoring and modeling aids in understanding *V. parahaemolyticus* exposure potential but has not reliably related oyster variability and human health risks.

This work aims to summarize the current state of knowledge of *V. parahaemolyticus* in paired oyster and water samples and provide the basis for an open-access database using a systematic literature review. This review will provide up-to-date information on the global occurrence and spatial distribution of paired oysters and water samples. This synthesized data will highlight the need for a unified approach to reporting and assessing the prevalence of *V. parahaemolyticus* in oysters and water to better understand and mitigate human health risks.

Methods

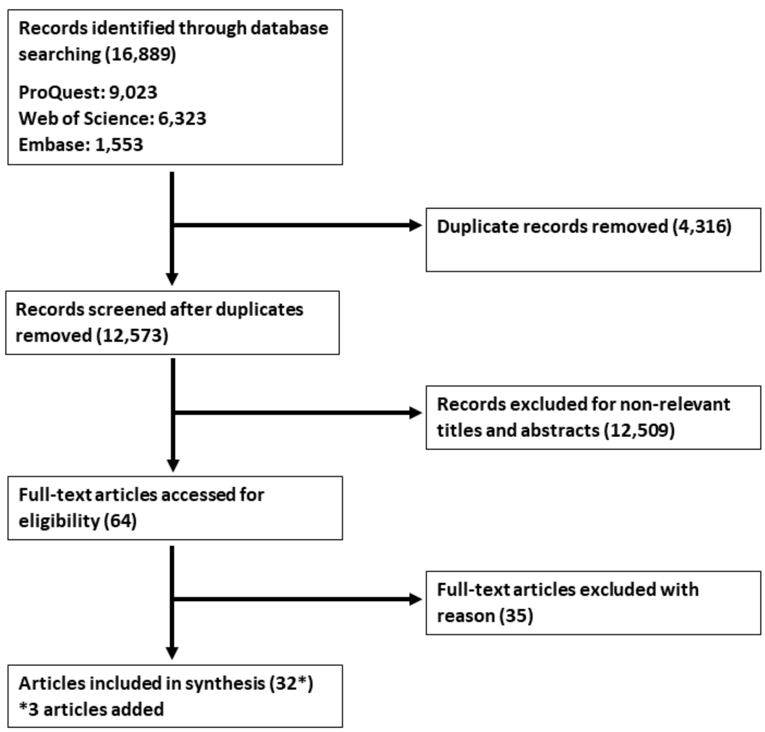
Literature search

Utilizing methodology for scoping reviews set forth by Arksey et al. (2005), a wide net was cast to capture the range of literature addressing concept areas of oysters, *V. parahaemolyticus*, and the environment (SI tables 1 – 5).[166] This methodology employed five steps: identify the research question, identify relevant studies, select the studies, chart the data, and finally, collate, summarize, and report the results. The search strategy was developed in collaboration with a University of Arizona public health librarian. Three databases were used to identify appropriate articles. The database Elsevier/Embase (1947 - current) was searched using keywords and controlled vocabulary (Emtree). Keyword searches in Clarivate/Web of Science (1898 - current) and the ProQuest Agricultural & Environmental Science Collection (1960 - current) were conducted. There were no limits placed on this search. On March 4, 2020, we identified 16,899 records and after removing duplicates in EndNote, imported the resulting 13,862 articles into Rayyan for screening.[167] Upon importing into Rayyan, duplicates were removed again resulting in 12,573 articles to be included for screening.

During the initial title screening process, articles that discussed *V. parahaemolyticus* in water and oysters from the same environment were included. Articles were excluded if: 1) studies were review articles, or a benchtop or laboratory-based study; 2) did not include *V. parahaemolyticus ;* 3) did not collect both oyster and water samples in the environment; or 4) sampled oysters from a market or post-harvest. Four authors participated as reviewers in the title screening process (Figure 1). Of the 12,573 titles reviewed, 12,509 were excluded.

Following the initial screening process, the inclusion and exclusion criteria became more stringent. In addition to the initial inclusion criteria, aquaculture studies were included during the abstract and full-text review if the farm harvested oysters from natural environments. In conjunction with the previous exclusion criteria, articles were excluded if: 1) studies that harvested oysters from manmade environments; 2) modeled, predicted, or forecasted *Vibrio*; 3) utilized sequence-based, 16S, or genomic methods to evaluate *V. parahaemolyticus as V. parahaemolyticus* was not quantified; or 4) non-English language articles. The abstracts of the remaining 150 articles were screened, and a further 86 were excluded. The full text of the remaining 64 studies were reviewed, resulting in the 29 studies included in this review. Ad-hoc screening of reference lists from included articles resulted in six additional articles to be screened following the described process. Of the articles screened post-hoc, three were added to be incorporated into the review. A total of 32 articles were included in the final full literature review step.

Figure 1. Scoping literature review flowchart.



Data extraction and visualization

Data were extracted from the 32 studies included in the full-text review in Microsoft Excel. Data from figures were extracted with WebPlotDigitalizer.[168] Extracted data included (when provided): location (e.g. country, city, latitude and longitude) and date of study; oyster species evaluated; environmental parameters assessed; occurrence or concentration of microbes; occurrence or concentration *of V. parahaemolyticus*; and described correlations from each article. Data tables were imported into R Studio.[169] Data sets of the environmental parameters, correlations, concentration of microbes, concentration of *V. parahaemolyticus* , and a reproducible R archive of data sets are available in the Supplemental Material and via GitHub (https://github.com/emcooksey/Global_Vp). An online interactive map was developed (https://emilycooksey.shinyapps.io/shiny/).

Results

The articles included in this review were published from 1970 to 2020. Of the final 32 articles, 24 (75%) were published after 2000. There were 22 studies in North America, five conducted in Asia, three in South America, one in Europe, and one in Australia. Interestingly, of the included studies conducted in North America, 21 (95%) were in the United States, seven studies were conducted on the East Coast, seven in the Gulf of Mexico, four in the Pacific Northwest, and three evaluated multiple coasts. On average, studies measured *V. parahaemolyticus* during 26 sampling events. The number of sampling events ranged from 4 to 156 events (Table 1). Seven species of oysters were assessed, with the Pacific Oyster (*Magellan gigas*, formerly *Crassostrea gigas*) being the most prevalent (28%). Eleven studies did not report oyster species.

Table 1. Number of sites, samples, and oyster species utilized.

Country	Year(s)	Number of Sites	Sampling Events	Oyster Species	Reference
Australia	2015 - 2016	1[a]	4	*Saccostrea cullullata*	65
Bahrain	1991 - 1992	2	8	*Pinctada radiata*	170
Brazil	2015 - 2016	1[b]	Does not specify	*Crassostrea rhizophorae*	171

Country	Year(s)	Number of Sites	Sampling Events	Oyster Species	Reference
	2008 - 2009	6	10	*Magallana gigas*	172
	1998 - 1999	1	10	*Crassostrea brasiliana*	19
Mexico	2001 - 2002	1[c]	266 total samples	Does not specify	173
Netherlands	1971	Does not specify	6	Does not specify	174
South Korea	2017	1[d]	12	*M. gigas*	175
	2017	9e	12	*M. gigas*	176
	2004 - 2016	9[f]	156	*M. gigas*	177
Taiwan	2008 - 2010	3[f]	120	*M. gigas*	178
USA	2009 - 2012	2	51	Does not specify	179
	2013 - 2015	4[b]	112 oyster sampling events, 125 water sampling events	*Crassostrea virginica*	180
	2013 - 2015	4[b]	112 oyster sampling events, 125 water sampling events	*C. virginica*	61
	2008 - 2010	3	Does not specify	Does not specify	53
	1989	16	10	*M. gigas*	181
	2006 - 2007	4	12	*C. virginica*	182
	2011	3	4	*C. virginica*	183
	1989	47	80 oyster samples, 91 water samples	Does not specify	184
	1984 - 1985	18	4	*C. virginica* and *M. gigas*	23

Country	Year(s)	Number of Sites	Sampling Events	Oyster Species	Reference
	1972	Does not specify	Does not specify	*C. virginica*	[185]
	2011	1	12	Does not specify	[63]
	2002 - 2003	2	24	Does not specify	[56]
	Does not specify	Does not specify	26	*M. gigas* and *Ostrea lurida*	[186]
	1972 - 1974	24	54 oyster samples, 49 water samples	Does not specify	[187]
	2006 - 2007	4	12	Does not specify	[163]
	2004 - 2008	3	12	*C. virginica*	[188]
	2001 - 2003	9	Site dependent	*C. virginica*	[189]
	2014	1[b]	9	*C. virginica*	[190]
	2004	2	102 surface water, 32 oyster sampling	Does not specify	[164]
	2009	4[b]	30	Does not specify	[191]
	2007 - 2008	26	39	*M. gigas*	[64]

[a]12 sites did not have oysters; [b]1 site did not have oysters; [c]11 sites did not have oysters; [d]7 sites did not have paired water and oyster samples; [e]9 sites did not have oysters; [f]2 sites did not have oysters

Environmental parameters

There were 12 different physio-chemical parameters assessed within at least one of the 32 studies. The most frequently reported physio-chemical parameters were water temperature (n = 26) and salinity (n = 21). Table 2 highlights the temperature and salinity descriptive parameters for all studies. The measured temperature ranged from 0 to 34°C with a median of 19°C. Salinity ranged from 0 to 48 ppt (parts per thousand), with a median of 24 ppt. All other measured physio-chemical parameters are summarized in SI Tables 6 - 10.

Table 2. Temperature and Salinity profiles for sampling sites

Country	Year	Site Name	Temperature (°C)				Salinity (ppt)				Reference
			Minimum	Mean	Maximum	Median	Minimum	Mean	Maximum	Median	
Australia	2015 - 2016	Site 7	-	-	-	-	-	-	-	-	65
Bahrain	1991 - 1992	Site 1	20.0	25.2	28.9	-	40.0	46.6	48.0	-	170
		Site 2	19.4	20.0	29.3	-	40.0	41.0	47.0	-	
Brazil	2015 - 2016	Cairu	-	27.0	-	-	-	20.0	-	-	171
	2008 - 2009	Site A	-	22.9	-	-	-	-	-	-	172
		Site B	-	23.3	-	-	-	-	-	-	
		Site C	-	24.6	-	-	-	-	-	-	
		Site D	-	24.9	-	-	-	-	-	-	
		Site E	-	24.9	-	-	-	-	-	-	
		Site F	-	25.2	-	-	-	-	-	-	
	1998 - 1999	Ariri Channel	19.2	22.7	28.0	21.8	16.0	18.5	21.0	18.0	19
Mexico	2001 - 2002	Isleta Tomato	-	-	-	-	-	-	-	-	173
Netherlands	1971	Oostemchelde estuary	-	-	-	-	-	-	-	-	174
South Korea	2017	Station 7	5.9	17.0	28.4	18.1	31.9	33.4	34.1	33.5	175
	2017	All Sites	9.0	18.0	27.0	18.5	29.0	32.0	34.0	32.5	176

	2004 - 2016	South Korea	9.0	-	27.7	-	-	36.2	-	-	177
Taiwan	2008 - 2010	Cigu	-	-	-	-	26.7	33.5	36.0	-	178
		Dongshih	-	-	-	-	28.0	33.6	36.3	-	
		Shengang	-	-	-	-	12.3	21.7	33.0	-	
USA	2009 - 2012	Tangier Sound	-	-	-	-	-	-	-	-	179
	2013 - 2015	Harlowe Creek	4.4	20.0	28.9	21.1	8.0	24.8	37.0	24.8	61,180
		Hoop Pole Creek	7.2	19.5	28.3	21.1	28.0	35.4	40.0	35.0	
		North River	7.8	17.5	26.9	17.5	21.0	29.8	37.0	29.5	
		South River	7.8	20.3	29.4	21.1	10.0	16.6	22.0	15.0	
	2008 - 2010	Gulf of Mexico	7.0	-	33.0	26.7	0.0	-	30.0	16.4	53
		Hood Canal	5.0	-	23.0	13.7	18.0	-	21.0	27.5	
		Tangier Sound	0.0	-	30.0	21.6	0.0	-	19.0	11.0	
	1989	Grays Habor	-	-	-	-	-	-	-	-	181
	2006 - 2007	Area 5	17.8	17.8	17.8	17.8	24.0	24.0	24.0	24.0	182
		Area 6	12.2	17.2	23.8	16.2	16.0	23.2	27.0	24.0	
		Area 8	14.0	21.2	30.3	19.6	20.0	26.0	32.0	25.0	
	2011	Chesapeake Bay	24.0	25.3	26.0	25.5	8.0	9.3	11.0	9.0	183
	1989	Growing Areas	15.5	-	22.5	-	23.6	-	30.5	-	184
	1984 - 1985	East Coast	-	17.0	-	-	-	-	-	-	23
		Gulf of Mexico	-	22.0	-	-	-	-	-	-	

		West Coast	-	15.0	-	-	-	-	-	-	
	1972	Galveston Bay	12.0	23.0	30.0	-	7.0	18.2	26.0	-	185
	2011	Dauphin Island Bay	20.0	21.8	25.0	21.0	3.7	10.9	25.0	12.0	63
	2002 - 2003	Tillamook Bay	8.0	13.0	18.0	13.0	-	-	-	-	56
		Yaquina Bay	7.0	12.0	18.0	11.0	-	-	-	-	
	Does not specify	Northwest Shellfish Sanitation Lab	6.0	14.8	23.0	15.0	-	-	-	-	186
	1972 - 1974	Galveston Bay	11.6	-	13.1	-	1.0	-	16.9	-	187
	2006 - 2007	USA	9.0	-	34.0	21.0					163
	2004 - 2008	Broad Creek	3.6	17.4	29.4	-	-	10.7	-	-	188
		Chester River	2.8	17.1	29.2	-	-	7.5	-	-	
		Eastern Bay	3.2	17.3	29.1	-	-	10.4	-	-	
	2001 - 2003	Breech Inlet	9.0	19.0	29.0	-	31.0	35.2	38.0	-	189
		Bullyard Sound	7.0	19.8	30.0	-	35.0	36.3	38.0	-	
		Copa Casino	14.0	20.7	28.0	-	8.0	22.0	29.0	-	
		Crabbank	7.0	19.4	29.0	-	23.0	30.3	37.0	-	
		Middle Bay	7.0	21.2	31.0	-	9.0	22.8	34.0	-	
		North Inlet	6.0	20.3	29.0	-	21.0	33.6	38.0	-	
		Pascagoula Harbor (Area 6)	26.0	28.4	31.0	-	3.0	16.2	25.0	-	

		Pass Christian Tonging Reef (Area 2 - 12)	8.0	21.9	34.0	-	6.0	15.0	26.0	-	
		Schooner Bay	9.0	18.9	29.0	-	29.0	31.3	35.0	-	
	2014	North Inlet	24.0	-	32.0	-	-	-	-	-	190
	2004	US FDA Gulf Coast Seafood Laboratory (GCSL)	22.4	-	33.8	-	10.0	-	28.0	-	164
		University of Southern Mississippi Gulf Coast Research Laboratory (GCRL)	22.4	-	33.8	-	4.0	-	13.0	-	
	2009	NB1	13.0	18.0	21.0	18.0	-	-	-	-	191
	2009 - 2010	NB2	12.0	20.1	23.0	21.0	-	-	-	-	
		CP1	13.0	23.1	28.0	25.0	-	-	-	-	
		CP2	17.0	22.7	28.0	23.1	-	-	-	-	
	2007 - 2008	Belfair park, Hood canal	12.6	18.2	23.8	18.5	3.0	18.9	30.0	20.0	64
		Case Inlet, North Bay	12.0	17.6	22.7	17.8	0.0	26.6	38.0	29.0	
		Cushman Park	13.3	17.7	22.9	17.3	10.0	24.2	30.0	26.5	
		Dabob Bay	10.5	16.8	27.3	16.9	6.0	17.2	28.0	16.5	

		DNR Beach 48	14.8	16.9	19.5	16.8	28.0	29.8	33.0	29.0	
		Duckabush	9.4	16.4	26.5	15.5	5.0	21.1	35.0	22.0	
		Eld Inlet	8.9	17.4	21.5	17.7	0.0	26.2	35.0	27.0	
		Grays Habor	12.4	16.9	21.9	17.0	15.0	27.3	35.0	28.0	
		Hama Hama, Hood canal	8.6	14.2	25.5	13.5	0.0	18.3	34.0	20.0	
		Hammersley Inlet	8.3	15.6	19.8	16.9	25.0	28.4	34.0	29.0	
		Hoodsport	5.9	13.6	19.8	13.9	10.0	24.1	33.0	25.0	
		Mike's beach, Hood canal	11.4	16.9	21.2	17.2	5.0	26.9	32.0	28.0	
		Misery point	12.0	16.9	21.2	17.2	0.0	27.6	36.0	29.0	
		Mystery Bay	12.1	16.5	20.9	16.7	15.0	30.9	36.0	31.0	
		Nahcotta	12.0	17.2	19.5	17.5	18.0	28.3	36.0	29.5	
		Oakland Bay	14.0	19.4	23.6	19.4	17.0	26.0	32.0	26.0	
		Potlatch, Hood canal	7.0	13.8	18.8	15.1	6.0	24.2	35.0	25.5	
		Quilcene Bay	10.9	16.9	23.4	17.6	6.0	26.9	35.0	28.5	
		Rocky Bay	12.7	17.7	22.3	18.5	0.0	24.2	35.0	28.0	
		Samish Bay	11.5	16.8	21.5	16.5	5.5	22.5	34.0	24.0	
		Sister's point, Hood canal	6.9	14.7	20.0	16.4	21.0	26.9	33.0	28.0	
		South Totten Inlet	7.3	15.4	20.9	15.9	25.0	30.8	37.0	30.0	
		Stackpole Harbor	18.7	18.7	18.7	18.7	28.0	28.0	28.0	28.0	
		Thorndyke Bay	12.5	14.4	15.5	14.8	26.0	29.4	32.0	30.0	
		Totten Inlet	8.1	16.7	26.0	17.6	20.0	27.1	36.0	26.5	

		Twanoh park, Hood canal	5.2	15.0	23.7	17.0	12.0	26.5	39.0	27.0	

Microorganisms

There was a total of 15 different microorganisms assessed in conjunction with *V. parahaemolyticus* in either water or oysters (Figure 2). Sixteen studies (50%) assessed for more than *V. parahaemolyticus* in oysters. There were 13 different microorganisms assessed in oysters. *V. vulnificus* was assessed in oysters in 10 studies (31%). Eleven different microorganisms were assessed in water by 15 studies (47%). *V. vulnificus*, the most frequently assessed microorganism in water, was evaluated in nine studies (28%).

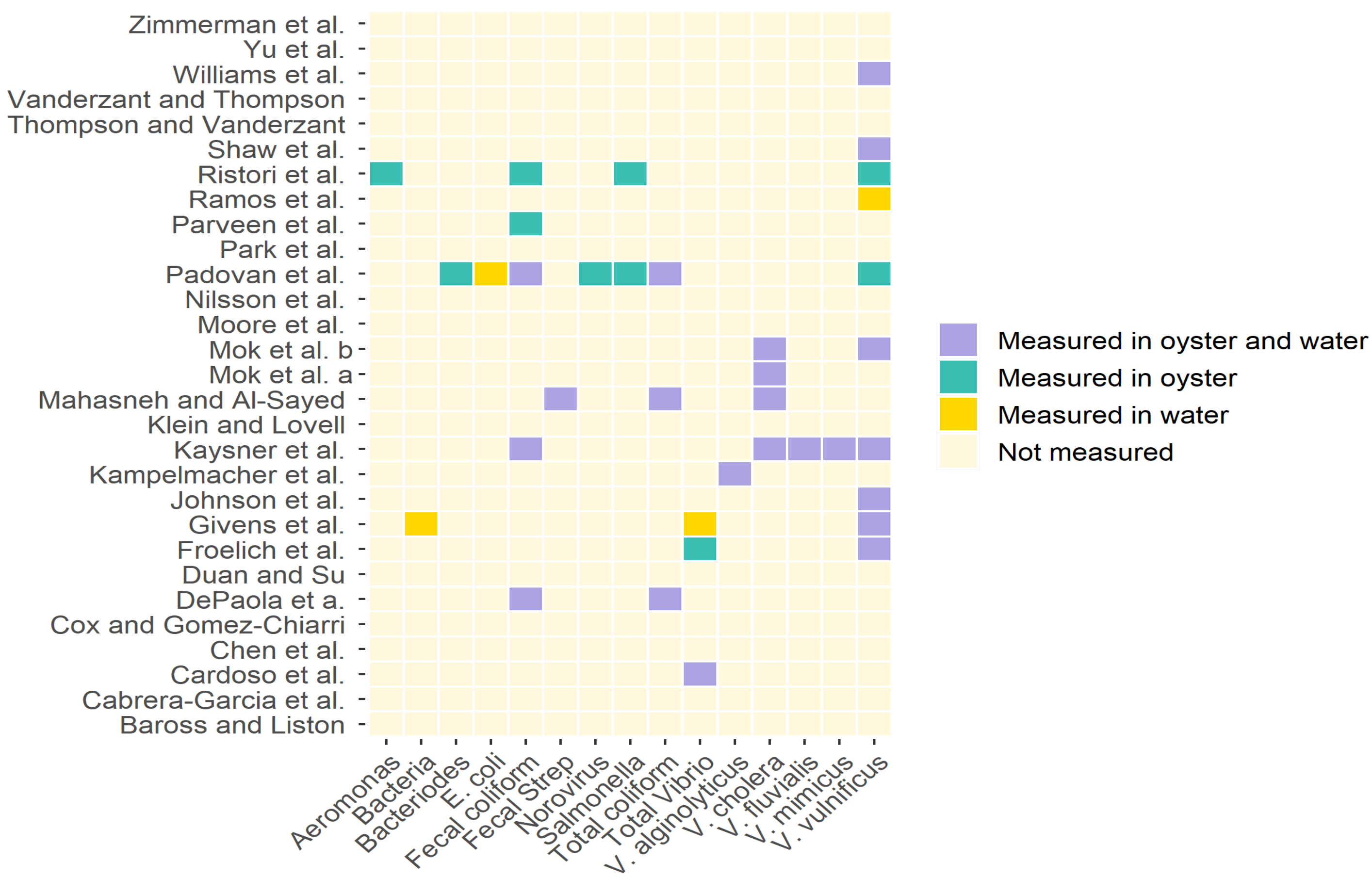


Figure 2. Heatmap of microorganisms other than *V. parahaemolyticus* assessed in articles. All studies included the measurement of *V. parahaemolyticus* in water and oysters.

V. parahaemolyticus concentrations and methods

Methods and frequency or concentration of *V. parahaemolyticus* in oyster and water samples are shown in tables 3 and 4, respectively. The concentration of *V. parahaemolyticus* in water ranged from 0 (below the limit of detection) to 1.85×10^6 CFU or MPN/ml and the frequency of detection ranged from 0 to 100%. The concentration of *V. parahaemolyticus* in oysters ranged from 0 to 1.71×10^5 CFU or MPN/g and the frequency of detection ranged from 4.7 to 100%. All studies assessed paired oyster and water samples, however, either frequency or concentration of *V. parahaemolyticus* was not reported for oysters or water in three (9.4%) and six (18.8%) studies, respectively. When reported, the limit of detection for MPN and CFU quantification methods ranged from 1 to 30, however, limit of detection is methodology specific.

Table 3. Concentration and frequency of *V. parahaemolyticus* in oyster samples as reported in original literature.

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
Australia	2015 - 2016	Site 7	-	- / -	10	-	PCR	65
Bahrain	1991 - 1992	Site 1	70	- / -	-	CFU/g	TCBS	170
		Site 2	1.4×10^4	- / -	-			
Brazil	2015 - 2016	Cairu	-	5.6×10^3/ 5.6×10^4	-	MPN/g	MPN, PCR	171
	2008 - 2009	Site A	2.5	- / -	-	MPN/g	MPN* PCR	172
		Site B	6.3	- / -	-			
		Site C	7.9	- / -	-			
		Site D	2.5	- / -	-			
		Site E	5.0	- / -	-			
		Site F	6.3	- / -	-			
	1998 - 1999	Ariri Channel	18	3.6 / 44	-	MPN/g	MPN, TCBS	19
Mexico	2001 - 2002	Isle Tomato	-	- / -	-	-	TCBS*	173
Netherlands	1971	Oostemcheld e estuary	-	- / -	4.7	-	BTB, TCBS, PTSS	174

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
South Korea	2017	All Sites	3.6×10^2	$30 / 2.1 \times 10^4$	39.5	MPN/100 g	MPN*	[175]
	2017	All Sites	1.5	0.3 / 46	41	MPM/g	MPN*	[176]
		Station 1	-	- / -	67			
		Station 10	-	- / -	42			
		Station 12	-	- / -	42			
		Station 2	-	- / -	25			
		Station 5	-	- / -	33			
		Station 6	-	- / -	18			
		Station 7	-	- / -	25			
		Station 9	-	- / -	58			
	2004 - 2016	NA	-	- / -	-	-	MPN-PCR*	[177]
Taiwan	2008 - 2010	Cigu	1.6	$0.04 / 2.5 \times 10^3$	-	CFU/ml	MPN-PCR*	[178]
		Dongshih	2.0	$0.04 / 2.0 \times 10^4$	-			
		Shengang	1.3×10^2	$3.2 / 3.2 \times 10^3$	-			
USA	2009 - 2012	Tangier Sound	-	- / -	9.1	-	CAV and TCBS PCR	[179]

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
	2013 - 2015	NA	-	- / -	-	-	CAV and TCBS; PCR	180 61
		Harlowe Creek	$3.4 X10^2$	$0.0 / 2.5 X10^3$	-	CFU/g		
		Hoop Pole Creek	$2.3 X10^2$	$0.0 / 2.5 X10^2$	-	CFU/g		
		North River	$1.5 X10^2$	$0.0 / 9.1 X10^2$	-	CFU/g		
		South River	$2.7 X10^2$	$0.0 / 1.8 X10^3$	-	CFU/g		
	2008 - 2010	All sites	16	$10 / 2.2 X10^4$	82	CFU /g	DP/CH* MPN-PCR*	53
		Gulf of Mexico	14	- / -	-			
		Hood Canal	0.6	- / -	-			
		Tangier Sound	4.2	- / -	-			
	1989	Grays Harbor	-	0.0 / 0.15	$1.0 X10^2$	MPN/g	MPN DP/CH	181
	2006 - 2007	NA	-	- / -	-	-	DP/CH*	182
	2011	Chesapeake Bay	$8.2 X10^4$	- / -	-	MPN/g	MPN-qPCR*	183
		Chesapeake Bay, Bottom	$7.9 X10^4$	- / -	-			

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
		Chesapeake Bay, Top	8.5 X10^4	- / -	-			
	1989	Willapa Bay, Growing areas	-	32 / 1.0 X10^4	71	MPN/g	MPN*	184
	1984 - 1985	Atlantic	30	- / -	-	cells/100g	HGFM*	23
	1984 - 1985	Gulf of Mexico	1.1 X10^2	- / -	-			
	1984 - 1985	Pacific	21	- / -	-			
	1972	Galveston Bay	25	- / -	-	MPN/g	MPN*	185
	2011	Dauphin Island	7.5 X10^4	1.1 X10^3 / 1.7 X10^5	-	Gene copies/g	DP/CH* PCR	63
	2002 - 2003	Tillamook Bay	-	3.0 / 27	5.6	MPN/g	MPN-PCR*	56
		Yaquina Bay	-	3.0 / 43	23			
	-	Northwest Shellfish Sanitation Lab	-	- / -	1.0 X10^2	-	Salt-starch agar, *Vibrio* maintenance medium,	186

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
							Human blood agar	
	1972 - 1974	Galveston Bay	-	0.0 / 1.1X10^3	59	CFU/g	MPN, TCBS Isolate confirmation	187
	2006 - 2007	All Sites	1.0 X10^2	10 / 2.8X10^3	87	CFU/g	MPN-qPCR* DP/CH*	163
	2004 - 2008	Broad Creek	1.4 X10^2	- / 6.0 X10^2	-	CFU/g	DP/CH* MPN-PCR*	188
		Chester River	1.1 X10^2	- / 4.3 X10^2	-	CFU/g		
		Eastern Bay	1.3 X10^2	- / 4.1 X10^2	-	CFU/g		
	2001 - 2003	Bullyard Sound	6.4 X10^2	5.0 / 6.3 X10^3	-	CFU/g	DP/CH* MPN-PCR*	189
		Pass Christian Tonging Reef	2.6 X10^2	5.0 / 1.7 X10^3	-			
		Breech Inlet	1.9 X10^2	10 / 8.4 X10^2	-			
		Copa Casino	2.2 X10^3	10 / 1.3 X10^4	-			
		Crabbank	1.2 X10^3	10 / 2.2 X10^4	-			

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
		Middle Bay	$3.0 X10^2$	$10 / 1.3 X10^3$	-			
		North Inlet	$2.9 X10^2$	$10 / 3.3 X10^3$	-			
		Pascagoula Harbor	$2.7 X10^2$	$30 / 6.0 X10^2$	-			
		Schooner Bay	82	$10 / 1.7 X10^2$	-			
	2014	North Inlet	-	- / -	72	-	TCBS, PCR	[190]
	2004	US FDA Gulf Coast Seafood Laboratory	$8.1 X10^2$	$3.8 X10^2 / 3.0 X10^3$	$1.0 X10^2$	Gene copies /g	DP/CH* MPN/PCR*	[164]
		University of Southern Mississippi Gulf Coast Research Laboratory	79	$0.2 / 1.6 X10^3$	$1.0 X10^2$			
	2009	CP1	$1.0 X10^2$	$1.0 / 4.3 X10^2$	-	MPN/g	MPN-qPCR*	[191]
	2009 - 2010	CP2	$2.1 X10^2$	$1.0 / 2.3 X10^3$	-			
		NB1	$9.4 X10^2$	$1.0 / 9.7 X10^3$	-			
		NB2	53	$2.0 / 2.5 X10^2$	-			
	2007 - 2008	Belfair park, Hood canal	$1.0 X10^3$	$0.3 / 4.6 X10^3$	-		MPN-qPCR*	[64]

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
		Case Inlet, North Bay	66	0.0 / 3.8 $X10^{2}$	-	Gene equivalent/ g		
		Cushman Park	24	0.0 / 75	-			
		Dabob Bay	27	0.0 / 93	-			
		DNR Beach 48	13	2.3 / 43	-			
		Duckabush	1.4 $X10^{2}$	0.0 / 93	-			
		Eld Inlet	3.3 $X10^{2}$	0.0 / 2.3 $X10^{3}$	-			
		Grays Harbor	52	0.0 / 9.3 $X10^{2}$	-			
		Hama Hama, Hood canal	1.1 $X10^{2}$	0.0 / 9.3 $X10^{2}$	-			
		Hammersley Inlet	4.2 $X10^{2}$	0.0 / 4.5 $X10^{3}$	-			
		Hoodsport	2.7 $X10^{3}$	0.0 / 4.6 $X10^{4}$	-			
		Mike's beach, Hood canal	3.8 $X10^{2}$	0.0 / 2.4 $X10^{3}$	-			
		Misery point	2.3 $X10^{2}$	0.0 / 1.5 $X10^{3}$	-			
		Mystery Bay	0.5	0.0 / 4.3	-			
		Nahcotta	65	0.0 / 1.5 $X10^{3}$	-			

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
		Oakland Bay	3.0 $X10^2$	0.0 / 4.3 $X10^3$	-			
		Potlatch, Hood canal	1.7 $X10^2$	0.0 / 2.3 $X10^3$	-			
		Quilcene Bay	2.0 $X10^3$	0.0 / 2.4 $X10^4$	-			
		Rocky Bay	15	0.0 / 93	-			
		Samish Bay	3.1	0.0 / 43	-			
		Sister's point, Hood canal	4.3 $X10^3$	0.0 / 4.6 $X10^4$	-			
		South Totten Inlet	2.5 $X10^2$	0.0 / 2.3 $X10^3$	-			
		Stackpole Harbor	1.5 $X10^2$	15 / 15	-			
		Thorndyke Bay	0.7	0.0 / 2.3	-			
		Totten Inlet	1.9 $X10^2$	0.0 / 1.2 $X10^3$	-			
		Twanoh park, Hood canal	5.6 $X10^3$	0.0 / 1.1 $X10^5$	-			

*US FDA Bacteriological Analytical Manual

DP/CH: Direct plating/colony hybridization

HGFM: Hydrophobic grid membrane filtration

Table 4. Concentration and frequency of *V. parahaemolyticus* in water.

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
Australia	2015 - 2016	Site 7	-	- / -	-	-	PCR	65
Bahrain	1991 - 1992	Site 1	6.8	- / -	-	CFU/ml	TCBS	170
		Site 2	9.4	- / -	-			
Brazil	2015 - 2016	Cairu	-	$5.6 X10^{2}$ / $1.8 X10^{4}$	-	MPN/ml	MPN, PCR	171
	2008 - 2009	Site A	$3.0 X10^{-2}$	- / -	-		MPN* PCR	172
		Site B	$3.0 X10^{-2}$	- / -	-			
		Site C	$4.0 X10^{-2}$	- / -	-			
		Site D	$3.0 X10^{-2}$	- / -	-			
		Site E	$6.0 X10^{-2}$	- / -	-			
		Site F	$3.0 X10^{-2}$	- / -	-			
	1998 - 1999	NA	-	- / -	-	-	MPN, TCBS	19
Mexico	2001 - 2002	Isle Tomato	-	- / -	-	-	TCBS*	173

<table>
<tr><th>Country</th><th>Year</th><th>Site Name</th><th>Mean</th><th>Minimum / Maximum</th><th>Frequency (%)</th><th>Units</th><th>Method</th><th>Reference</th></tr>
<tr><td>Netherlands</td><td>1971</td><td>Oostemchelde estuary</td><td>-</td><td>- / -</td><td>1.2</td><td>-</td><td>BTB, TCBS, PTSS</td><td>[174]</td></tr>
<tr><td rowspan="12">South Korea</td><td>2017</td><td>All Sites</td><td>3.2</td><td>2.9 / 9.1</td><td>18.8</td><td>MPN/ 100 ml</td><td>MPN*</td><td>[175]</td></tr>
<tr><td rowspan="10">2017</td><td>All Sites</td><td>5.0 X10^{-2}</td><td>3.0 X10^{-2} / 2.4</td><td>22.4</td><td>MPM/ml</td><td rowspan="10">MPN*</td><td rowspan="10">[176]</td></tr>
<tr><td>Station 1</td><td>-</td><td>- / -</td><td>41.7</td><td rowspan="9">-</td></tr>
<tr><td>Station 10</td><td>-</td><td>- / -</td><td>8.3</td></tr>
<tr><td>Station 12</td><td>-</td><td>- / -</td><td>25.0</td></tr>
<tr><td>Station 2</td><td>-</td><td>- / -</td><td>27.3</td></tr>
<tr><td>Station 5</td><td>-</td><td>- / -</td><td>0.0</td></tr>
<tr><td>Station 6</td><td>-</td><td>- / -</td><td>25.0</td></tr>
<tr><td>Station 7</td><td>-</td><td>- / -</td><td>25.0</td></tr>
<tr><td>Station 8</td><td>-</td><td>- / -</td><td>8.3</td></tr>
<tr><td>Station 9</td><td>-</td><td>- / -</td><td>16.7</td></tr>
<tr><td>2004 - 2016</td><td>NA</td><td>-</td><td>- / -</td><td>-</td><td>-</td><td>MPN-PCR*</td><td>[177]</td></tr>
</table>

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
Taiwan	2008 - 2010	All Sites	1.0	2.0×10^{-2} / 2.6×10^{2}	-	CFU/mL	MPN-PCR*	178
USA	2009 - 2012	Tangier Sound	-	- / -	83.2	-	CAV and TCBS PCR	179
	2013 - 2015	NA	-	- / -	-	NA	CAV and TCBS PRC	180
	2013 - 2015	Harlowe Creek	1.6×10^{1}	0.0 / 8.8×10^{1}	-	CFU/ml		61
		Hoop Pole Creek	4.9	0.0 / 2.0×10^{1}	-			
		North River	4.2	0.0 / 2.2×10^{1}	-			
		South River	3.9	0.0 / 4.0×10^{1}	-			
	2008 - 2010	All sites	0.4	1.0 / 2.0×10^{2}	69.5	CFU/ml	CP/CH* MPN-PCR	53
		Gulf of Mexico	0.8	- / -	-			
		Hood Canal	8.0×10^{-2}	- / -	-			
		Tangier Sound	0.3	- / -	-			

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
	1989	Grays Harbor	-	0.0 / 0.4	100.0	MPN/ml	MPN DP/CH	181
	2006 - 2007	NA	-	- / -	-	-	DP/CH*	182
	2011	Chesapeake Bay	1.8 X10^{1}	0.0 / 4.9 X10^{1}	-	CFU/ml	MPN-qPCR*	183
	1989	Willapa Bay, Growing areas	-	3.2 / 1.0 X10^{3}	53.8	MPN	MPN*	184
	1984 - 1985	Atlantic	1.9 X10^{-1}	- / -	-	Cells/ml	HGFM	23
		Gulf of Mexico	4.4 X10^{-1}	- / -	-			
		Pacific	2.0 X10^{-2}	- / -	-			
	1972	NA	-	- / -	-	-	MPN*	185
	2011	Dauphin Island	7.0 X10^{3}	1.1 X10^{1} / 2.8 X10^{4}	-	Gene copies/ml	DP/CH* PCR	63
	2002 - 2003	Tillamook Bay	-	3.0 / 1.5 X10^{1}	11.1	MPN/ml	MPN-PCR	56
		Yaquina Bay	-	3.0 / 1.5 X10^{1}	27.3			

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
	-	Northwest Shellfish Sanitation Lab	-	- / -	78.0	-	Salt-starch agar, *Vibrio* maintenance medium, Human blood agar	186
	1972 - 1974	Galveston Bay	-	0 / 9.3	49.0	CFU/ml	MPN, TCBS Isolate confirmation	187
	2006 - 2007	All Sites	1.4	1.0 / 4.0	49.0	CFU/ml	MPN-qPCR* DP/CH*	163
	2004 - 2008	NA	-	- / -	-	-	DP/CH* MPN-PCR*	188
	2001 - 2003	Bullyard Sound	9.3	5.0 / 3.1 X10^1	-	CFU/ml	DP/CH* MPN-PCR*	189
		Pass Christian Tonging Reef	5.5	5.0 / 1.0 X10^1	-			
		Breech Inlet	1.1 X10^1	1.0 / 3.0 X10^1	-			

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
		Copa Casino	$3.6 X10^1$	$1.0 X10^1$ / $1.6 X10^2$	-			
		Crabbank	$2.0 X10^1$	$1.0 X10^1$ / $1.5 x10^2$	-			
		Middle Bay	$1.3 X10^1$	$1.0 X10^1$ / $3.0 X10^1$	-			
		North Inlet	$2.3 X10^1$	$1.0 X10^1$ / $8.0 X10^1$	-			
		Pascagoula Harbor	6.0	$1.0 X10^1$ / $1.0 X10^1$	-			
		Schooner Bay	$1.3 X10^1$	$1.0 X10^1$ / $3.0 X10^1$	-			
	2014	North Inlet	-	- / -	58.0	-	TCBS, PCR	[190]
	2004	US FDA Gulf Coast Seafood Laboratory	$1.5 X10^1$	- / 0.5	98.0	Gene copies /ml	DP/CH* MPN/PCR*	[164]
		University of Southern Mississippi Gulf Coast Research Laboratory	4.5	0.1 / 0.8	100.0			
	2009	CP1	2.0	1.0 / $1.0 X10^1$	-	MPN/ml		[191]

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
	2009 - 2010	CP2	$4.2 X10^{1}$	$1.0 / 4.5 X10^{2}$	-		MPN-qPCR*	
		NB1	1.9	$1.0 / 1.0 X10^{1}$	-			
		NB2	2.5	$1.0 / 1.6 X10^{1}$	-			
	2007 - 2008	Belfair park, Hood canal	$2.4 X10^{3}$	$0.0 / 1.9 X10^{4}$	-	Gene equivalent/100g	MPN-qPCR*	64
		Case Inlet, North Bay	$6.8 X1\text{-}^{3}$	$0.0 / 7.8 X10^{4}$	-			
		Cushman Park	$1.8 X10^{4}$	$5.1 X10^{1} / 6.9 X10^{4}$	-			
		Dabob Bay	$9.4 X10^{3}$	$0.0 / 1.3 X10^{5}$	-			
		DNR Beach 48	$1.0 X10^{5}$	$1.3 X10^{2} / 4.1 X10^{5}$	-			
		Duckabush	$1.6 X10^{4}$	$0.0 / 4.3 X10^{5}$	-			
		Eld Inlet	$4.9 X10^{4}$	$0.0 / 7.6 X10^{5}$	-			
		Grays Habor	$1.6 X10^{4}$	$0.0 / 1.3 X10^{5}$	-			
		Hama Hama, Hood canal	$1.3 X10^{4}$	$0.0 / 1.4 X10^{5}$	-			
		Hammersley Inlet	$4.1 X10^{3}$	$0.0 / 5.0 X10^{4}$	-			
		Hoodsport	$8.6 X10^{3}$	$1.5 / 1.5 X10^{5}$	-			
		Mike's beach, Hood canal	$9.7 X10^{2}$	$0.0 /7.7 X10^{3}$	-			

Country	Year	Site Name	Mean	Minimum / Maximum	Frequency (%)	Units	Method	Reference
		Misery point	$9.6 X10^{3}$	$0.0 / 2.0 X10^{5}$	-			
		Mystery Bay	$3.8 X10^{3}$	$0.0 / 4.1 X10^{4}$	-			
		Nahcotta	$1.6 X10^{4}$	$0.0 / 2.9 X10^{5}$	-			
		Oakland Bay	$9.8 X10^{3}$	$0.0 / 2.3 X10^{5}$	-			
		Potlatch, Hood canal	$1.4 X10^{3}$	$0.0 / 1.5 X10^{4}$	-			
		Quilcene Bay	$1.1 X10^{4}$	$0.0 / 1.0 X10^{5}$	-			
		Rocky Bay	$1.4 X10^{4}$	$1.4 X10^{2} / 1.1 X10^{5}$	-			
		Samish Bay	$1.3 X10^{5}$	$0.0 / 1.9 X10^{6}$	-			
		Sister's point, Hood canal	$5.5 X10^{3}$	$0.0 / 6.7 X10^{4}$	-			
		South Totten Inlet	$3.0 X10^{4}$	$0.0 /6.6 X10^{5}$	-			
		Stackpole Harbor	$5.4 X10^{-1}$	$5.4 X10^{-1} / 5.4 X10^{-1}$	-			
		Thorndyke Bay	6.2 X10	$6.3 X10^{-1} / 2.3 X10^{3}$	-			
		Totten Inlet	$2.5 X10^{4}$	$0.0 / 6.7 X10^{5}$	-			
		Twanoh park, Hood canal	$4.4 X10^{3}$	$0.0 / 3.9 X10^{4}$	-			

*US FDA Bacteriological Analytical Manual

DP/CH: Direct plating/colony hybridization

HGFM: Hydrophobic grid membrane filtration

Correlations

Of the 32 studies, 12 (38%) did not assess for correlations or relationships between *V. parahaemolyticus* in oyster or water samples with environmental or biological covariates. The correlations and relationships between covariates and *V. parahaemolyticus* in water and oyster is shown in table 5. Temperature and salinity were most frequently assessed to determine a relationship of *V. parahaemolyticus* concentrations in water or oysters. Temperature was significantly associated or correlated with *V. parahaemolyticus* concentrations in water (n = 6) and oysters (n = 11). Temperature was evaluated for a relationship with *V. parahaemolyticus* in water and oyster samples in 17 and 19 studies, respectively. Salinity was associated with *V. parahaemolyticus* concentrations in oysters and water in six studies. However, some studies reported no significant associations with *V. parahaemolyticus* and temperature in water (n = 11) or in oysters (n = 5). There were 10 studies with no significant relationship between salinity and *V. parahaemolyticus* in either water or oysters. Of note, turbidity was associated with *V. parahaemolyticus* in oysters (n = 3) and water (n = 3), though it was infrequently assessed. Of these studies, 100% and 75% of these studies found turbidity to be correlated with *V. parahaemolyticus* in water and oysters, respectively. *V. parahaemolyticus* in water was associated with biological covariates in four studies, but there was infrequent association of *V. parahaemolyticus* in oysters and biological covariates (n = 8).

Table 5. Number of studies that reported *V. parahaemolyticus* correlations with environmental and biological covariates.

Covariate	Water, not significant	Water, significant	Oyster, not significant	Oyster, significant
Chlorophyll A	2	-	3	-

Dissolved oxygen	1	1	4	1
Month	1	-	-	1
pH	-	-	3	-
Precipitation	-	1	1	-
Salinity	12	6	15	6
Sediment	1	-	-	-
Suspended Particulate Matter	-	1	-	1
Temperature	11	6	8	11
Tidal height	-	1	1	-
Total Suspended Solids	2	-	1	-
Turbidity	-	3	1	3
Water Depth	-	1	2	-
Wet or Dry season	-	-	-	1
Wind	-	-	1	-
E. coli	-	1	-	-
Fecal coliform	1	1	4	1
Surface water, *V. vulnificus*	-	1	-	-
Total aerobic bacteria	1	1	2	1
Total coliform	-	1	2	1
Total *Vibrio* spp.	-	2	1	-

Database

An interactive database and map were developed to show the concentrations of *V. parahaemolyticus* in water and oysters (Figure 2). In this database, users will be able to access and search the synthesized data from this literature review. Additionally, users will be able to submit their data to be added to the map.

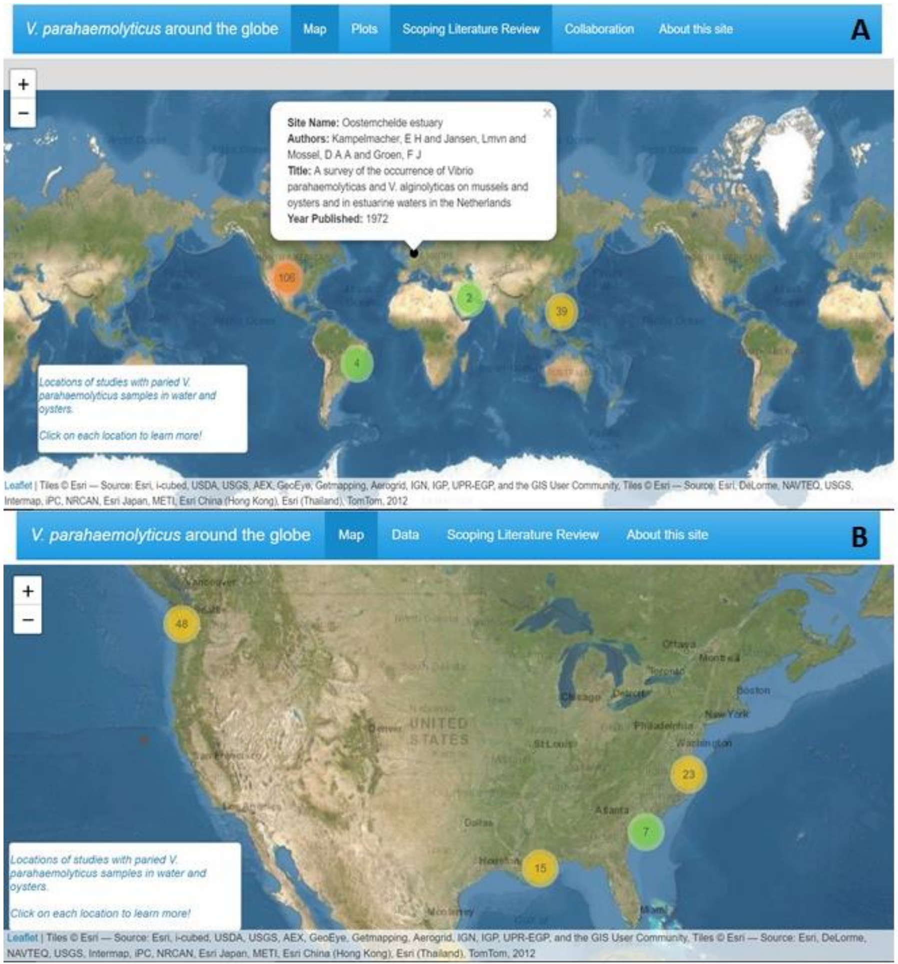


Figure 2. A screenshot of the interactive database providing searchable spatial distribution of *V. parahaemolyticus* concentrations in water and oyster samples from the synthesized data identified in the current review at a global (A) and United States (B) perspective

Discussion

This study assessed the spatial distribution of *V. parahaemolyticus* in oyster and water samples throughout the world utilizing a scoping literature review. It synthesized critical environmental co-variate and statistical relationships of *V. parahaemolyticus* in oysters and water from 32 articles. Additionally, it developed a framework for an open access database for *V. parahaemolyticus* related data. There is a disproportionate number of studies conducted in the USA. Additionally, environmental co-variates were not consistently associated or correlated with concentration of *V. parahaemolyticus* in the water.

There are numerous detection methods for *V. parahaemolyticus* in oysters and water. However, the most common detection methods utilized for *V. parahaemolyticus* in oysters and water are antiquated as it detects *V. parahaemolyticus* from culture-based samples with molecular confirmation. The Bacteriological Analytical Manual enumeration method approved by the US FDA for detection of *V. parahaemolyticus* involves a multi-day culture-based method followed by a confirmation step.[192] While this method must be followed for commercially distributed oysters in the United States, advanced detection methods may be employed for research purposes.[114] Innovative approaches, such as digital droplet PCR or matrix-assisted laser desorption ionization time-of-flight (MALDI TOF), may allow for a better understanding of *V. parahaemolyticus* concentration in the environment due to lower sensitivity and absolute quantification purposes.

A key limitation of this review was exclusion of previous literature reviews, post-harvest processing, method development, or genetic sequencing. Additionally, this literature review focused only on studies assessing *V. parahaemolyticus* in paired environmental oysters and water samples. Moving forward, these studies should be assessed and integrated as *V. parahaemolyticus* prevalence and concentrations is location dependent. Additionally, the inclusion of sediment or other environmental reservoirs may play a critical role in understanding the movement of *V. parahaemolyticus* in an ecosystem.

Additionally, an outbreak in Alaska and studies conducted in the Gulf of Mexico and United States Atlantic coast highlight the importance of a single oyster in *V. parahaemolyticus* exposure risk.[41,61,62,190] The US FDA requires pooled oyster homogenate for *V. parahaemolyticus* assessment. However, there is a need to understand the role of oyster-to-oyster variability by individual samples to understand *V. parahaemolyticus* health risks.[192] The "hot oyster" phenomenon, a single oyster with up to 100 times higher concentration of *V. parahaemolyticus* compared to oysters harvested in the same location, shows the potential extreme variability of *V. parahaemolyticus.*[190] "Hot oysters" have been detected in the Gulf of Mexico, South Carolina, and North Carolina.[61,62,190] Variability in the oysters may play a role in differences in *V. parahaemolyticus* concentrations, and therefore, human exposure. Particularly as *V. parahaemolyticus* exposure risk in oysters and estuarine water will only continue to increase with climate change, it is imperative to identify and embrace alternative detection methods to best protect public health.[42,49,193–195]

There is a need for a unified approach to holistically understanding environmental co-variates associated with *V. parahaemolyticus* in water and oysters to mitigate human health risks, particularly as cases of vibriosis continue to increase. Previous literature reviews have assessed *V. parahaemolyticus* in water and oysters.[196,197] Of note, there were inconsistent environmental covariates with *V. parahaemolyticus* in the environment in each review. Of note, the relationship of *V. parahaemolyticus* and turbidity has been supported by other studies and should continue to be explored as *V. parahaemolyticus* can attach to sediment and suspended particulates in the environment.[196,198] Additionally, V. *parahaemolyticus* has been associated or correlated with copepods, dinoflagellate blooms, and plankton.[100,199,200] The inconsistent associations or correlations with *V. parahaemolyticus* and environmental co-variates highlight a disconnect in current monitoring approach. Moving forward, environmental and ecology studies of *V. parahaemolyticus* should include turbidity and other understudied environmental co-variates. Further work is needed to elucidate the relationship of *V. parahaemolyticus* and the environment to allow for better monitoring and predictive models.

The lack of unity in reported *V. parahaemolyticus* results coupled with limited access to data limits efficient synthesis of data. Guidelines should be developed to provide a basis for the minimum information necessary for reporting *V. parahaemolyticus* concentrations and minimum environmental parameters measured. It is recommended to report geographical locations, environmental parameters for each site, and *V. parahaemolyticus* concentration in water and oysters for each site. Ideally, it is recommended to provide concentration and environmental data in supplemental information. It is imperative to have a collaborative network of academic, government, and oyster harvesters monitor coastal communities to protect human health from *V. parahaemolyticus* illness.

The open access database developed provides equitable access to *V. parahaemolyticus* and environmental data allowing for a greater synthesis of information to tease apart complex environmental relationships.[201] Printed summaries of findings and links to publications from the findings will be provided to shellfish farmers, government agencies, and academics to aid in the use of this tool. Equitable access to environmental parameters will allow for increased capacity to utilize hindcasting models following an outbreak to assess critical events. It is imperative to collaboratively utilize to mitigate emerging global health issues. For example, the *Vibrio* Sustainability tool developed by the European Centre for Disease Prevent and Control (https://www.ecdc.europa.eu/en/publications-data/vibrio-suitability-tool) allows *Vibrio* species to be forecasted with remotely sensed sea surface temperature and salinity following increased vibriosis cases.[193] The database developed will act as a central location for researchers to utilize data to develop predictive models and to serve as a living document as more research is identified and conducted.

Conclusion

This literature review summarized the global distribution of *V. parahaemolyticus* in paired oysters and water samples. Continued efforts to identify critical environmental covariates in addition to temperature and salinity will help further explain the prevalence of *V. parahaemolyticus* in oysters and water. Additionally, a unified reporting system or database may allow for additional collaborations develop models to better under *V. parahaemolyticus* concentrations in water and oysters. Findings from this literature review will improve our understanding of how *V. parahaemolyticus* is distributed around the world and will inform decision on how to reduce health risks form *V. parahaemolyticus*.

Chapter 3: Prevalence of total and pathogenic *V. parahaemolyticus* and *V. vulnificus* in water and wild pacific oysters from a southern Californian estuary

Abstract

V. parahaemolyticus and *V. vulnificus* are naturally occurring bacteria associated with illness from the consumption of raw oysters. The objectives of this study were to investigate the distribution of total and pathogenic *V. parahaemolyticus* and *V. vulnificus* in water and recreationally harvested oysters from a Southern California estuary. Concentrations of *V. parahaemolyticus* and *V. vulnificus* were assessed from 12 sites in Newport Bay, California in August and September 2019 in water and transplanted wild Pacific oysters. Culture-based enumeration followed by molecular confirmation was utilized to detect total and pathogenic *V. parahaemolyticus* and *V. vulnificus* in water and oyster samples. Pathogenic *V. parahaemolyticus (trh+)* was detected in 12.8% of water and 0% of oyster samples. Pathogenic *V. vulnificus* (*pilF+*) was detected in 57.3% of water and 17.6% of oysters. Total *V. parahaemolyticus* and *V. vulnificus* concentrations in water were correlated with chlorophyll A concentration, water temperature, salinity, and fecal coliforms levels, however co-variants were co-correlated which potentially masked true relationships. Total *V. parahaemolyticus* concentrations in oysters were associated with chlorophyll A concentration and salinity. *V. vulnificus* concentration in oysters was not correlated with any co-variates. There was a greater concentration of *V. parahaemolyticus* and *V. vulnificus* in water compared to oyster samples. There have been limited studies to date evaluating *V. parahaemolyticus* and *V. vulnificus* prevalence in California. These findings support the need for increased studies of *V. parahaemolyticus* and *V. vulnificus* to

assess trends and environmental covariates of *V. parahaemolyticus* and *V. vulnificus* in California bays.

Introduction

Vibrio spp., a naturally occurring bacteria, is frequently isolated in estuarine water and shellfish.[51] While there are over 100 identified species of *Vibrio*, 12 are primarily responsible for human illness.[27,28] Of these 12, *V. parahaemolyticus* and *V. vulnificus* are most often identified as causing illness from seafood consumption. Annually in the U.S., vibriosis associated with the consumption of seafood accounts for an estimated $250M in health-related costs, with consumption of raw oysters responsible for approximately 90% of *V. parahaemolyticus* and *V. vulnificus* illnesses.[21,22,29–31] As other foodborne illness rates in the U.S. have decreased in recent decades, vibriosis rates have been increasing.[20,30] *V. parahaemolyticus* risk from oyster consumption is also expected to increase further with increasing sea surface temperatures under various climate change scenarios.[202,203]

V. parahaemolyticus has become an increasingly significant emerging infectious organism over the last two decades.[3,22,49] It has been the primary agent in multiple outbreaks in shellfish and it is responsible for the majority of seafood-borne illness.[38,41,47,157,204] Often *V. parahaemolyticus* is the culprit of self-limiting gastroenteritis, but can result in wound infections and death.[30,33] Pathogenic *V. parahaemolyticus* is traditionally identified by the presence of thermostable direct hemolysin (*tdh*) or tdh related hemolysin (*trh*) gene.[57,58] However, approximately 10% of clinical cases have been reported without detection of either the *tdh* or *trh* gene, suggesting the mechanism of pathogenicity is not completely understood for *V. parahaemolyticus*, and that measurement should not be focused only on detection of the pathogenic genes.[48,60,66] Studies have highlighted the potential role of type III secretion systems in pathogenicity for *V. parahaemolyticus* illness but the exact mechanism of pathogenicity has yet to be elucidated.[60,67]

V. vulnificus, an opportunistic pathogen, causes 95% of seafood-related deaths as it has an approximately 50% mortality rate in those infected with *V. vulnificus*. .[73] Often the *V. vulnificus* deaths are men over the age of 60 with at least one chronic illness, such as diabetes or liver cirrhosis.[33,75–78] Often *V. vulnificus* causes septicemia and wound infection, commonly resulting in death or amputation of the infected area, but it can also cause gastroenteritis.[73,74] Pathogenic *V. vulnificus* is identified by the presence of pilus-type IV assembly protein gene (*pilF*), virulence correlated clinical gene (*vcgC*), and the virulence correlated clinical gene (*vcgE*).[85–87,205]

Expanding shellfish aquaculture coupled with rising seawater temperatures creates a need to understand *Vibrio* spp. concentrations in the environment and shellfish in previously understudied areas. While there have been studies assessing *V. parahaemolyticus* and *V. vulnificus* concentrations in Washington and Oregon oysters, few studies have assessed the prevalence along the California Coast, and even fewer studies have evaluated *Vibrio* spp. levels in wild oysters associated with recreational harvesting. In one study at southern California beaches, low concentrations of pathogenic *V. parahaemolyticus* and no *V. vulnificus* were detected.[206]

Generally, higher concentrations of *V. parahaemolyticus* are reported in the Gulf of Mexico, however, several studies have found high concentrations of pathogenic *V. parahaemolyticus* in water from the Pacific Northwest.[53,54] In contrast, limited detection of *V. vulnificus* in shellfish and water in the Pacific Northwest has been reported.[53,207] The relationship of environmental covariates and *V. parahaemolyticus* is not fully elucidated on the West Coast. Several Pacific Northwest studies have found no relationships between *V. parahaemolyticus* and environmental parameters such as harmful algal blooms (HABS), salinity, and temperature.[53–56] Other studies in the Pacific

Northwest found temperature to be a strong predictor of *V. parahaemolyticus* presence.[55,56] The relationship between pathogenic *V. parahaemolyticus* and *V. vulnificus* with environmental factors is not always similar to total *V. parahaemolyticus* or *V. vulnificus*. For example, pathogenic *V. parahaemolyticus* was correlated with turbidity but not temperature though total *V. parahaemolyticus* was associated with temperature and not turbidity in the Gulf of Mexico.[163,164] Additionally, concentration of pathogenic *V. parahaemolyticus* was not related to total *V. parahaemolyticus* in France and the Gulf of Mexico.[163,164,208]

The dynamics of *V. parahaemolyticus* and *V. vulnificus* in water and oysters have shown to be regionally specific, with limited information regarding prevalence in Southern California. This study aimed to generate background information on levels of total *V. parahaemolyticus* and *V. vulnificus* and pathogenic *V. parahaemolyticus* and *V. vulnificus* in wild oysters and the overlaying water column in a Southern California bay during the summer, when water temperatures are the warmest, however oysters can be harvested year round. This study aimed to provide a better understanding of the prevalence of *V. parahaemolyticus* and *V. vulnificus* in wild Pacific oysters, assess how those levels may vary across a single bay, and inform potential environmental co-variates of *V. parahaemolyticus* and *V. vulnificus* levels.

Methods

Study location and approach

Newport Bay, an urbanized estuarine water body located in Southern California, was selected for this study.[130] Lower Newport Bay is highly urbanized, has several working harbors, and is tidally influenced. Upper Newport Bay has a 3.9 km^2 wildlife refuge characterized by open water, mudflats, and saltwater and freshwater marshes.[209] Upper Newport Bay is heavily influenced by stormwater and freshwater inputs from two creeks: San Diego Creek and Santa Ana Delhi Channel.[209,210]

Wild oysters (*Magallana gigas*, formerly *Crassostrea gigas*) were collected, held in disinfected seawater, and then transplanted to 12 sites throughout Newport Bay, California (Figure 1) for a 6-week period.[211] Sites were selected that were geographically representative of the Bay: Newport Bay was divided into three regions based on location and salinity. The Front bay encompasses sites 1 – 6, Middle bay sites 7 – 9, and the Back bay sites 11 - 13. Pacific oysters are naturally occurring at sites 1 – 8, while at sites 9, 11, 12, and 13 oysters are not naturally found but were transplanted to these locations for this study.

Figure 1. Locations of shellfish harvest sites.

Oyster harvesting

In July 2019, oysters (n=1000) were harvested over a three-day period (July 31 – August 2) throughout the Newport Bay, California. Oysters were transported to tanks containing disinfected, flow-through seawater located at Kerckhoff Marine Laboratory, Newport Bay, California (33.60, -117.88) within four hours of harvesting.

Oysters were arranged on perforated stacked trays in four 0.283 m^3 tanks for 14 days. Flow-through seawater from Newport Bay, California was filtered through a sand filter at 15 – 20 gallons per minute and further disinfected with Classic UV 80-Watt Series light (Aqua Ultraviolet, Temecula, California, USA) before entering the tanks. 200 µL of Kent Marine Aqueon PhytoMax was added to each tank every 48 hours.

Oyster deployment

Following the two-week hold period, a total of 1000 oysters were deployed across the 12 sites in 23 mm mesh bags (Ketcham Supply) one week before the first sample collection day. At the time of oyster deployment, a 1 L grab sample of water was collected at each site. In addition, oysters were taken and processed on selective and nonselective media as described below to enumerate background levels of *V. parahaemolyticus* and *V. vulnificus* in the deployed oysters.

Oyster sample collection and processing

Paired oyster and water samples were collected at 4-time points between August 8th, 2019, and September 25th, 2019 at each site. During each sampling event, oysters were collected and transported on ice and processed within four hours of collection from each site at the Southern California Coastal Water Research Project (SCCWRP) laboratory.

Shellfish were scrubbed with water and a brush then sprayed with ethanol prior to being shucked, and the meats of 10 shellfish were pooled and blended in a sterile stand blender (Warring, Torrington, CT) for 60 seconds. 25 g of shellfish homogenate was then weighed out and diluted with sterile phosphate-buffered saline (PBS) at a 1:1 w:v ratio and blended for another 60 seconds.

Oyster homogenate was used for *Vibrio* spp. enumeration (described below). Fecal coliforms in oyster homogenate was assessed utilizing a five-tube most probable number (MPN) method, following standard methods.[212]

Water sample collection and processing

Water grab samples were collected in sterile 2L Nalgene containers and transported on ice from each site to the SCCWRP laboratory for enumeration of *Vibrio* spp. An additional water grab sample was collected and transported to Orange County Public Health Laboratory for fecal coliform enumeration. All water samples were processed within six hours of collection.

Water samples were vacuum-filtered through a 47 mm diameter, 0.45 µm pore sized mixed cellulose ester filter which was then used for *Vibrio* enumeration (described below). Filters intended for fecal coliform enumeration were placed on mFC, and incubated for 22 – 24 hours at 44.5 °C, following standard methods (APHA Method 9221).[213]

Measurement of environmental parameters

Water temperature and salinity were measured with a YSI Model Pro30 at the time of collection. To evaluate total chlorophyll A in water, 100 mL was filtered through a glass fiber filter (Whatman It Maidstone UK) for each site and sampling event. The filter was stored in aluminum foil at -80°C until further analysis. The filter was extracted in 100% acetone in the dark for 24 hours. Total chlorophyll A was measured in all water samples using a non-acidification method and a Trilogy Turner Design Fluorometer, as previously described.[214]

Vibrio enumeration

TCBS and CHROMagar *Vibrio* were used to enumerate total *Vibrio* spp. and potentially pathogenic *Vibrio* spp., respectively. Oyster homogenate was plated on Thiosulfate Citrate Bile Salts Sucrose (TCBS) (BD Difco) and CHROMagar *Vibrio* (CHROMagar, Paris, France) and incubated for 18 - 24 hours at 37 °C. *V. parahaemolyticus* and *V. vulnificus c*oncentrations were determined by counting visible pink and blue colonies, respectfully, and adjusting for dilution.[215,216] The total number of colonies on TCBS and the number of presumptive *V. parahaemolyticus* and *V. vulnificus* colonies on CHROMagar *Vibrio* were recorded. Data were reported as CFU/mL and CFU/g for water and oysters, respectively. The concentration of confirmed and pathogenic *V. parahaemolyticus* and *V. vulnificus* were multiplied by the percentage of molecularly confirmed (by PCR) isolates.

Molecular analysis

Up to 10 presumptive *V. parahaemolyticus* and *V. vulnificus* isolates per site per sampling event were stored in ultrapure water. To extract DNA, colonies were boiled for 10 minutes at 100 °C then centrifuged for 10 minutes at 10,000 g.[61] DNA was stored at -20 °C until molecular analysis was performed. Presumptive *V. parahaemolyticus* and *V. vulnificus* isolates were first confirmed for speciation then assessed for the presence of virulence-associated genes. Presumptive *V. parahaemolyticus* was assessed for the *toxR* gene to confirm *V. parahaemolyticus*.[217] Following, all *toxR* positive isolates were assessed for pathogenicity by presence of the *tdh* and *trh* genes.[218,219] All presumptive *V. vulnificus* isolates were assessed for the *vvhA* gene to confirm species, then assessed for *pilF* to confirm pathogenicity.[85,220] Following the identification of *vvhA*, the presence of the *vcgE* or *vcgC* gene was assessed.[220] *V. parahaemolyticus* DNA (F113A), *V. vulnificus* DNA (JY 1301), and synthetic DNA (IDT, Coralville, Iowa, USA) were used as positive controls for *V.*

parahaemolyticus , *V. vulnificus,* and the toxin genes, respectively. Molecular grade water was used as a negative control.

The qPCR reaction mixtures for all genes contained 6 µl template DNA or control, 10 µL of PerfeCTa® SYBR® Green FastMix® Reaction Mix (Quanta bio, USA), 0.024 µL (0.1 µM) of forward primer, 0.024 µL (0.1 µM) of reverse primer, and 7.95 µL of nuclease-free water for a 24 µL qPCR reaction. Details for thermal cycling conditions for each gene are provided in table 1. All qPCR analysis was completed using a CFX96 Real-Time PCR Detection System (Bio-Rad).

Table 1. Primer and reaction conditions used for the PCR amplification of *V. parahaemolyticus* and *V. vulnificus.*

Gene	Forward / Reverse	Sequence (5' - 3')	Annealing temperature (°C)	Annealing time (s)	Citation
toxR	Forward	gtc ttc tga cgc aat cgt tg	60.2	30	[217]
	Reverse	ata cga gtg gtt gct gtc atg			
trh	Forward	ttg gct tcg ata ttt tca gta tct	59.7	90	[221]
	Reverse	cat aac aaa cat atg ccc att tcc g			
tdh	Forward	gta aag gtc tct gac ttt tgg ac	60.0	30	
	Reverse	tgg atat aga acc ttc atc ttc acc			
vvhA	Forward	agc ggt gat ttc aac g	56.5	30	[220]
	Reverse	ggc cgt ctt tgt tca ct			
vcgE	Forward	ctc aga aag gct caa ttg ac	56.5	30	
	Reverse	gat taa cgc tgt aag gcc g			
vcgC	Forward	agc tgc cga tag cga tct	61.8	30	
	Reverse	tga gct aac gcg agt agt gag			
pilF	Forward	cga ttg gta ggc aat aga c	60.0	30	[85]
	Reverse	gca act caa cct caa gac g			

Statistical analysis

All statistics and data visualization were performed in R (R Core Team, 2020). Microbial data were assessed for normality then log-transformed. Following, Spearman rank correlations were performed to evaluate relationships between *V. parahaemolyticus* and *V. vulnificus* levels in oysters versus water and when compared to other parameters (water temperature, salinity, chlorophyll A, fecal coliform concentrations).

Results

Environmental parameters

Average temperature, salinity, and chlorophyll A concentration at each site during the study are shown in figure 2 and SI table 1. The Back Bay had significantly lower salinity ($p < 0.01$) and significantly higher chlorophyll A concentration ($p < 0.01$) than the Front- and Middle- Bay. Though there were no significant differences in water temperatures in Newport Bay ($p = 0.9$), although water temperatures were overall higher in the Back bay.

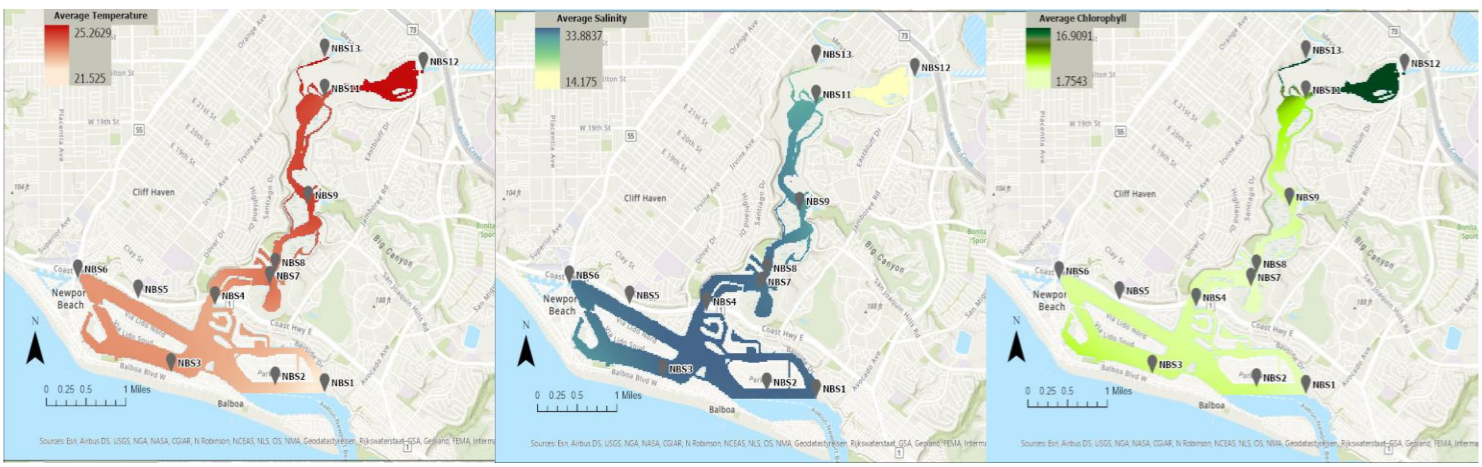


Figure 2. Spatial representation of the average temperature (° C), salinity (ppt), and chlorophyll A (µg/L) levels measured in the Newport Bay during this study.

<u>Vibrio</u> spp. in Newport Bay oysters

Confirmed *V. parahaemolyticus* was detected in 72.5% of the oyster samples (n = 58), with concentrations that ranged from 99 to 3400 CFU/g (Figure 3 and 4). Overall, *trh* was only detected in 1 sample, at site 6, but was not positive for toxR. No *tdh* was detected in any oyster samples for the duration of the study (SI table 2). The concentration of *V. parahaemolyticus* was significantly higher in oysters deployed in the Back Bay compared to the Front Bay ($p = 0.02$). There was no significant difference in the concentration of *V. parahaemolyticus* in the Front and Middle bays ($p > 0.05$) or Back and Middle Bays ($p > 0.05$). *V. parahaemolyticus* levels in oysters were also consistent over the duration of the study; concentrations did not vary significantly by sampling week ($p > 0.05$).

V. vulnificus was detected in 19.6% of the oyster samples (n = 16), with concentrations of *V. vulnificus* in oysters that ranged from 13.2 to 1775 CFU/g (Figure 3 and 4). Of the *V. vulnificus* confirmed isolates in oysters (n = 16), 12.5% (n = 2) were positive for the pathogenic *pilF* gene (SI table 3). Pathogenic *V. vulnificus* was detected in the Front bay (n = 1), Back bay (n = 1). The concentration of *V. vulnificus* in oysters across all sites in week 1 was significantly different than concentrations in week 2 ($p = 0.029$) and week 6 ($p = 0.02$) (SI Figure 1). There was no significant difference in *V. vulnificus* concentrations by Newport Bay region ($p > 0.05$) or site ($p > 0.05$).

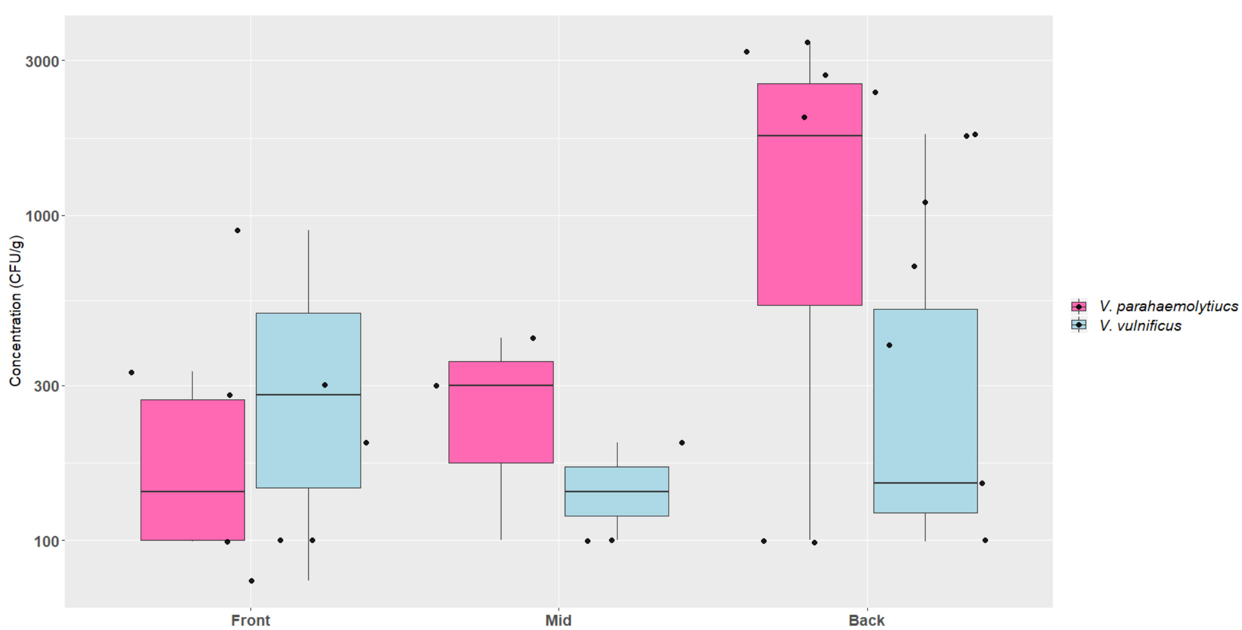


Figure 3. Concentrations of *V. parahaemolyticus* and *V. vulnificus* in oysters by region.

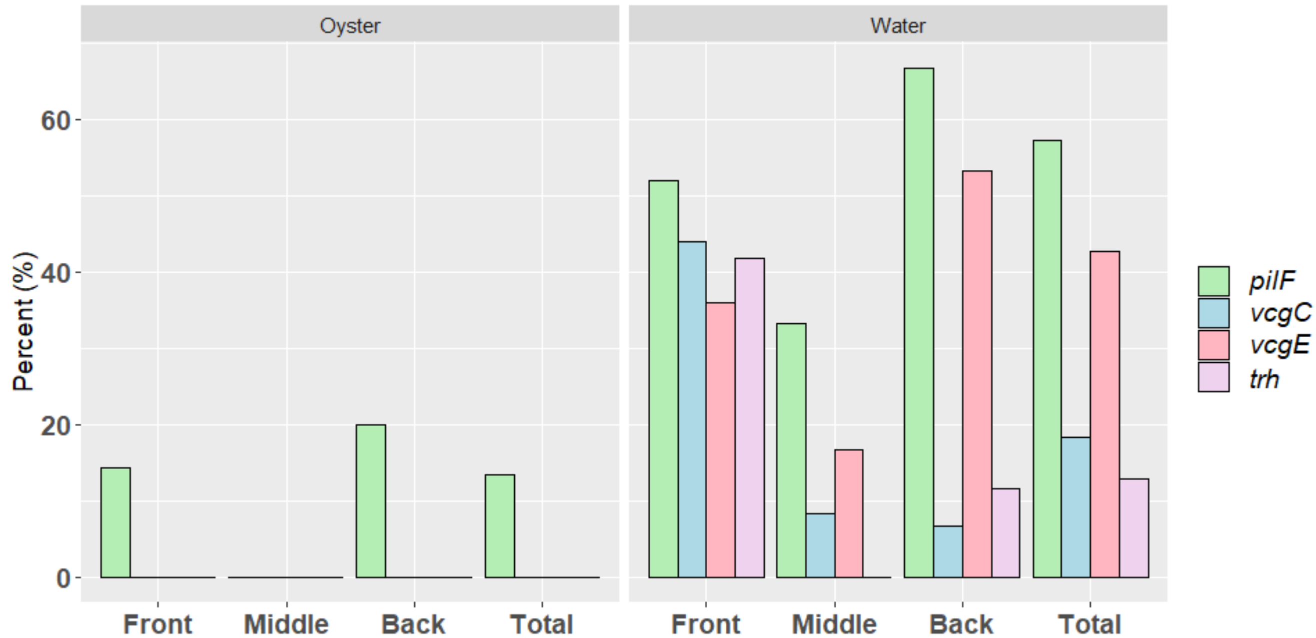


Figure 4. Percent detection of virulence-associated *V. parahaemolyticus* and *V. vulnificus* genes in oysters and water by region.

Vibrio spp. in Newport Bay water

Confirmed *V. parahaemolyticus* was detected in 50% of water samples collected, with average concentrations that ranged from 8.35 x 10^4 to 3.65 x 10^8 CFU/mL (Figure 4 and 5) by site. Of the confirmed *V. parahaemolyticus* isolates, 12.8% (n = 10) contained the pathogenic *trh* gene, *tdh* was not detected in any water sample (Figure 4 and SI table 2). Pathogenic *V. parahaemolyticus* (*trh* +) concentrations ranged from 1.21 x 10^5 to 9.16 x 10^7 CFU/mL.

Confirmed *V. vulnificus* was detected in 54.2% of the water samples collected, with average concentrations ranging from 1 x 10^5 to 1.17 x 10^9 CFU/mL by site (Figure 4 and 5). Significantly higher concentrations of *V. vulnificus* were observed in the Back bay compared to the Front (p = 0.009) and Middle bays (p = 0.02), but no significant difference in samples from Front and Middle bays ($p > 0.05$). The *pilF, vcgE, and vcgC* genes were detected in 57.3% (n = 47), 35.4% (n = 35), and 15.2% (n = 15) of confirmed *V. vulnificus* isolates, respectively (Figure 4).

The concentration of pathogenic *V. vulnificus* (*pilf*+) ranged from 2.75×10^5 to 1.02×10^9 CFU/mL (Figure 5). There was a significantly higher concentration of pathogenic *V. vulnificus* detected in the Back bay compared to the Front Bay ($p = 0.04$), but no significant difference in the Front and Middle bays ($p > 0.05$) or Middle and Back bays ($p > 0.05$). Of note, the *pilF* gene was more frequently detected in isolates from Back bay water (63.8%) than the Middle (8.5%) and Front bays (26.7%) (SI table 3).

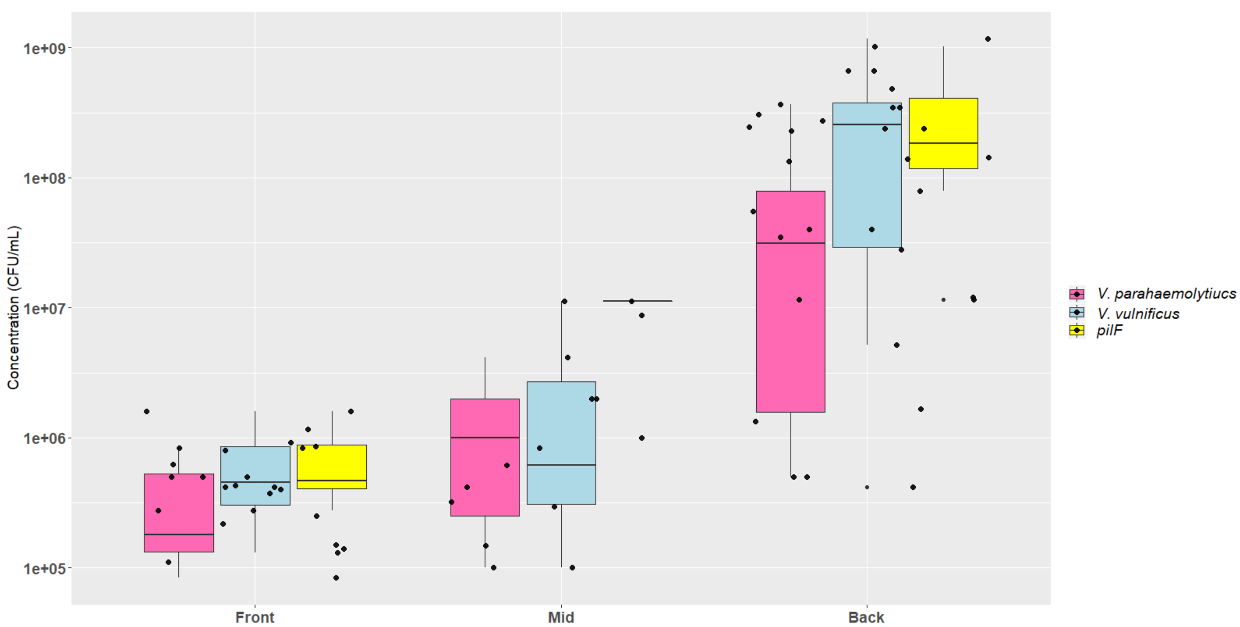


Figure 5. Concentration of *V. parahaemolyticus* and *V. vulnificus* in water by region.

Environmental and biological co-variates with <u>*V. parahaemolyticus*</u> *and* <u>*V. vulnificus*</u>

Pathogenic and non-pathogenic *V. parahaemolyticus* and *V. vulnificus* were isolated more frequently in the water column versus oyster tissues. *V. parahaemolyticus* concentrations in the oyster tissues and water column were significantly correlated ($r = 0.57$, $p < 0.05$), while concentrations of *V. vulnificus* were not correlated between oyster tissue and water column ($p > 0.05$) (Figure 6). *V. vulnificus* and *V. parahaemolyticus* concentrations in the water columns were significantly correlated with salinity, chlorophyll A, and water column fecal coliform concentrations (Figure 6). *V. parahaemolyticus* in oyster tissues were significantly correlated with salinity, chlorophyll A, and water column fecal coliform concentrations, while *V. vulnificus* in oysters was not associated with any of the environmental covariates measured (Figure 6). Concentration of the *vcgC* and *vcgE* genes were significantly correlated with *pilF* gene in water ($r = 0.85$ and 0.98, respectively, $p < 0.05$).

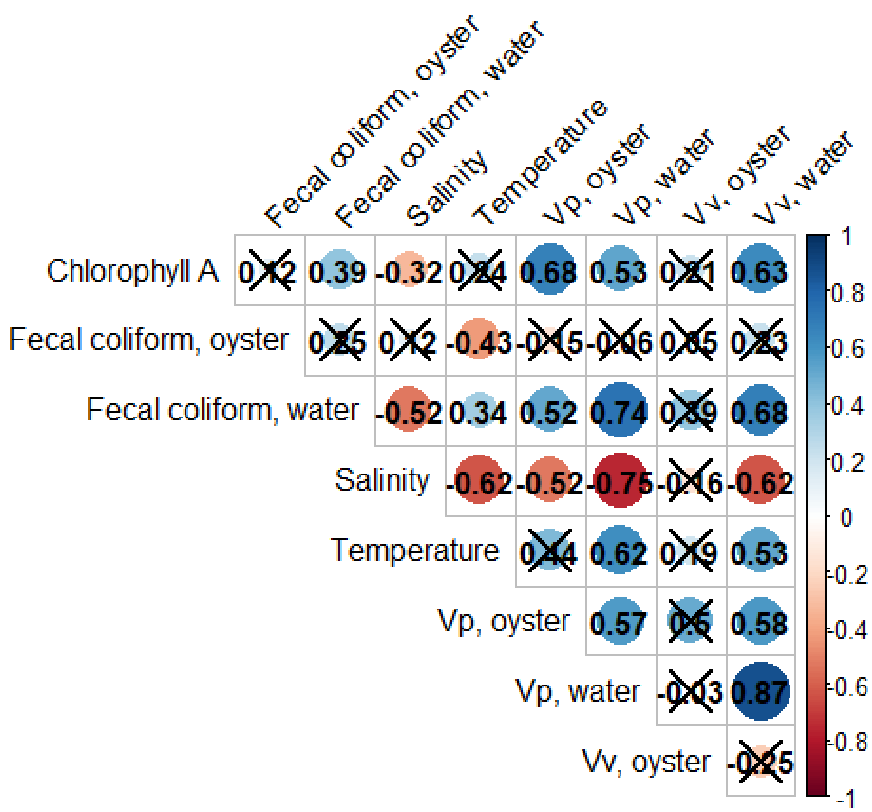


Figure 6. Spearman rank correlation coefficient of *V. parahaemolyticus* (Vp) and *V. vulnificus* (Vv) in water and oysters. Insignificant p-values ($p > 0.05$) are crossed out.

Discussion

This study aimed to further elucidate *Vibrio* spp. dynamics by quantifying total and potentially pathogenic *V. parahaemolyticus* and *V. vulnificus* and assessing environmental co-variates in Pacific oysters and water from high spatial resolution sampling of a Southern California bay. Overall, levels varied depending on site and differences were observed in the different environmental co-variates associated with *V. parahaemolyticus* and *V. vulnificus* levels in the water column and oyster tissues. Results from this study support the notion that regionalized studies are needed to understand *Vibrio* dynamics, with significant differences observed in concentration even within one bay.

Higher concentrations of both *V. parahaemolyticus* and *V. vulnificus* were detected in the water column, when compared to the oyster tissues. Higher concentrations of *V. parahaemolyticus* in the water column when compared to shellfish tissues is supported by a recent study in the Pacific Northwest but contrasts with studies conducted in the Gulf of Mexico and on the East Coast that show oysters can harbor *Vibrio* concentrations up to 100 x more than the water.[61,62,64,190] Given this was a six-week study, further studies should be conducted to evaluate concentrations of *Vibrio* spp. in parallel in the water column and shellfish tissues, to better understand accumulation potential and if sampling one matrix can be predictive of the other.

V. parahaemolyticus isolates were recovered from water and oysters at all sites within Newport Bay. The concentrations of *V. parahaemolyticus* isolated from oysters was less than the US FDA recommended risk threshold of 10,000 CFU/g.[110] Pathogenic *V. parahaemolyticus* was not detected in oysters, and met the State of California *Vibrio* control plan recommended level of 10 CFU/g for pathogenic *V. parahaemolyticus* in oyster meat, with no *tdh* detected in any of the oyster or water samples collected.[118] The infrequent detection of *tdh* during the current study is surprising as prior studies along the Washington Coast have detected *tdh* in up to 85% of *V. parahaemolyticus* in water samples, with *tdh* detected in oyster meats at concentrations up to 430 MPN/g and 15 ge/g.[53–55,64] The detection of *trh+* but *toxR-* may be indicative of *Vibrio alginolyticus* as it can harbor the *trh* gene.[222] The percentage of pathogenic *V. parahaemolyticus* isolated from Newport Bay water (12.8%) falls within levels observed in other regions (e.g. 2.4% in North Carolina, 78% in Mississippi, and 30% in Alabama) and the lack of detection of pathogenic *V. parahaemolyticus* in oysters is unsurprising as it can vary significantly by region (e.g. 0.7% in North Carolina, 3.7% in Australia, 3% in Chesapeake Bay, 50% in South Carolina, 57% in Long Island Sound, and 31.9% in Rhode Island).[59,61,65,161,164,188,191] The presence of *V. parahaemolyticus* in shellfish on the West Coast is of particular public health concern, as the attack rate in an outbreak in Alaska with *V. parahaemolyticus* was 29%, higher than predicted by the US FDA.[41,110] The higher attack rate could be indicative of increased pathogenesis of *V. parahaemolyticus* or related to health of consumers. Together, these results indicate the need for local assessments of oyster harvesting environments.

V. vulnificus was detected in both oyster and water samples throughout Newport Bay. This species has not previously been reported in Southern California and has been reported infrequently in the Pacific Northwest.[54,64,206] The sporadic detection of *V. vulnificus* in oysters was not explained directly by the environmental parameters assessed, however, the low frequency of detection may have limited the ability to assess correlation with environmental and microbial parameters in this study. Even though there are no naturally occurring Pacific oysters in the Back bay portion of Newport Bay (where *V. vulnificus* was detected most frequently and at the highest concentrations in water), the high detection frequency of pathogenic *V. vulnificus* (57.3%) in water is a public health concern as this bacteria is commonly associated with wound infections from water exposures.[73] Additionally, detection of *V. vulnificus* in this study was in close proximity to sites where no *V. vulnificus* was previously detected.[206] Infections associated with *V. vulnificus* are rare but increasing, with detection in the current study highlighting the need to better understand exposure potentials and more thorough evaluations for *V. vulnificus* in water and oysters.[22,77]

Current California recreational shellfish harvesting policy utilizes the presence of fecal coliform in the water column to determine shellfish safety, while commercial oyster harvesters assess both *V. parahaemolyticus* levels in oyster tissues and fecal coliform levels in the water column.[118] In this study, there was a disconnect in concentrations of fecal coliforms in the water and concentrations of *Vibrio* spp. in oysters. Although the commercial shellfish industry is widely aware of the risks associated with *V. parahaemolyticus* and follows strict collection and handling guidelines to reduce the likelihood of illness associated with *V. parahaemolyticus* exposure, these same practices aren't required, with guidance varying regionally, for recreationally harvested shellfish. Fecal coliforms have historically been used as a proxy for fecal contamination but have shown a limited relationship with *Vibrio* spp. concentration.[178,223,224] This is unsurprising as *V. parahaemolyticus* and *V. vulnificus* are naturally occurring rather than associated with human or animal waste and points to the importance of an integrated approach for evaluating recreationally harvested shellfish safety that includes guidance surrounding *Vibrio* spp.

Conclusion

The present study was one of the first to evaluate *V. parahaemolyticus* and *V. vulnificus* levels in Southern California in paired water and oyster samples, with detection of both species in water and oysters in varying concentrations throughout Newport Bay, CA. This study highlights the importance of regional studies as *Vibrio* spp. dynamics in the water column and in shellfish tissues vary dramatically by region, and in this study varied significantly even within the single Bay targeted. In addition, this study focused on *Vibrio* spp. levels in wild, transplanted oysters (as opposed to commercially grown oysters) and the surrounding water column, highlighting the need for guidance surrounding *Vibrio* spp. in recreational settings.

Chapter 4: A quantitative microbial risk assessment model estimating the *V. parahaemolyticus* illness risk from wild oysters harvested in southern California along the "sea-to-fork" pathway

Abstract

Consumption of raw oysters poses a health risk to consumers. This study estimated the risk of *V. parahaemolyticus* illness from the consumption of recreationally harvested oysters in Southern California with a quantitative microbial risk assessment (QMRA) framework pre- and post- "sea-to-fork" pathway. A sensitivity analysis was utilized to understand influential input parameters on illness risk from consumption of raw oysters following the "sea-to-fork" pathway. The mean probability of illness per serving of oysters was 6.20×10^{-5} (95% confidence interval 5.65×10^{-7} – 1.92×10^{-4}) from consumption of raw oysters harvested in Southern California following the "sea-to- fork". The mean *V. parahaemolyticus* illness risk per serving of oysters immediately following harvest was 5.61×10^{-5} (95% confidence interval 4.84×10^{-8} – 1.83×10^{-4}). The sensitivity analysis highlighted the importance of the initial concentration of *V. parahaemolyticus* in the oysters, number of oysters consumed, and concentration of pathogenic *V. parahaemolyticus* following transport in vibriosis risk. This study highlights mitigation strategies for *V. parahaemolyticus* illness risk from consumption of raw oysters along the "sea-to-fork" pathway.

Introduction

The United States produced 36 million pounds of oysters worth approximately $186 million in 2017.[225] Given the filter feeding nature of oysters, human pathogens are frequently detected.[155] Consumption of raw oysters has historically been connected with illness and death.[226] One oyster-related pathogen of particular concern is *Vibrio parahaemolyticus* (*V. parahaemolyticus*) which causes approximately 34,000 infections a year, with $168 million per year in medical associated costs, lost wages, and premature death in the USA.[21,29] Cases have continued to increase since 1996.[22] *V. parahaemolyticus* primarily leads to self-limiting gastroenteritis but infrequently causes severe wound infections and sepsis.[30,33] It has been implicated in five seafood outbreaks since 2006 and causes the majority of seafood-borne illness in the United States.[31,39,51,227] There is a growing concern about the increasing rates of V. *parahaemolyticus* associated illnesses from the United States oysters and aquaculture industry.[3,22]

The US Food and Drug Administration (US FDA) and the Interstate Shellfish Sanitation Conference (ISSC) collaborate to deliver safe shellfish to the consumer.[114] The ISSC offers guidance for shellfish harvesting, processing, and shipping in the National Shellfish Sanitation Plan (NSSP) Guide for Control of Molluscan Shellfish.[114] The NSSP has set minimum requirements to ensure safe and sanitary shellfish during the "sea to fork" pathway. The NSSP has extensive guidelines for harvesting, handling, shipping, and distribution of shellfish such as water quality assessments in shellfish harvesting areas, shellfish tag requirements for commercial retail, and temperature controls to mitigate illness from shellfish.[114] In addition, *Vibrio* Control Plans are implemented in 20 states with: (1) two or more *V. parahaemolyticus* cases in the past three years; (2) an outbreak within five years; (3) average water temperature greater than 27.2°C in the Gulf of

Mexico and the Atlantic Ocean south of New Jersey, or greater than 15.6 °C along the Pacific Ocean or the Atlantic Ocean north of New York as required by the NSSP.[114] The *Vibrio* control plans establish time to temperature controls, the duration from harvest to placement of oysters into temperature control or wet storage/depuration tanks, for each harvesting area within each state to reduce the potential re-growth of *V. parahaemolyticus* in harvested oysters. Post-harvest time to consumption and storage temperatures are critical in controlling *Vibrio parahaemolyticus* in post-harvested oysters. Temperature control with cold chain implementation has been shown to reduce *V. parahaemolyticus* growth in post-harvest oysters [117,149] and the risk of illness from oyster consumption.[110,148] However, *Vibrio* related illness rates have increased from consumption of oysters in recent decades despite the implementation of *Vibrio* control plans.

Quantitative microbial risk assessment (QMRA) uses a mathematical approach to allow for an understanding of health risk and can be used to inform best management practices. The QMRA framework utilizes a four-step process involving hazard identification, exposure assessment, dose-response, and risk assessment to quantify the likeliness of a health outcome.[134] QMRA models have been used to evaluate the human health risk from *V. parahaemolyticus* in Atlantic oysters in the USA[110,149] and Brazil[147]; Pacific oysters harvested in Taiwan[148,202] and Australia[228]; shrimp in Malaysia[135]; blood clams in Malaysia[229] and Taiwan[230]; and short Mackerels in Malaysia[231]. However, there are limited existing QMRA models informed by regional pathogen occurrence in oysters in the United States.

The occurrence of human pathogens in oysters, oyster safety policies, and oyster risk models applied throughout the US are largely built on data collected outside of Southern California. Wild Pacific oyster are accessible throughout Southern California estuaries for recreational harvesting.[11] Recreationally harvesting of oysters include oysters harvested from public water. The current Total

Maximum Daily Load (TMDL) for Newport Bay, California requires Newport Bay water with recreational shellfish to have fecal coliform levels less than 14 most probable number (MPN)/ml . However, fecal coliform and other fecal indicator bacteria concentrations in water do not account for *V. parahaemolyticus* illness risk.[178,223,224] Additional information specific to Southern California *V. parahaemolyticus* and associated risks is necessary. Therefore, this study aimed to fill critical Pacific oyster data gaps necessary to advance shellfish safety. The objectives of this study were to quantify the risk of illness from recreationally harvested Pacific oysters harvested in Southern California and to evaluate key public health interventions designed to reduce risks.

Methods

Overview and model structure

The risk of illness from *V. parahaemolyticus* with oyster consumption in Southern California was estimated. The risk assessment model was constructed with harvest, post-harvest, and consumption parameters from experimental studies in Southern California on Pacific oysters and the literature (Figure 1 and Table 1).[147,148,202] The *V. parahaemolyticus* illness risk was estimated for oyster consumption pre- and post- "sea-to-fork" pathway. The pre- "sea-to-fork" pathway scenario assessed risk from oyster consumption with the initial concentrations of *V. parahaemolyticus* at harvest. The post- "sea-to-fork" pathway was assessed with the harvest, post-harvest, and consumption modules. The harvesting module assessed concentration of total and pathogenic *V. parahaemolyticus* at the time of harvest, air temperature of Newport Bay, and growth rate of *V. parahaemolyticus* when exposed to air temperatures greater than 7.2°C. Following, the distribution module included distributions for the duration and temperature of shipping, amount of time oyster shipments was exposed to temperature abuse scenarios with air temperature greater than 7.2°C, and the growth rate of *V. parahaemolyticus.* The consumption module accounts for time and temperature oysters are stored before consumption, the weight of wild Pacific oysters, and oyster consumption. The concentration of pathogenic *V. parahaemolyticus* was calculated to estimate the probability of *V. parahaemolyticus* illness.

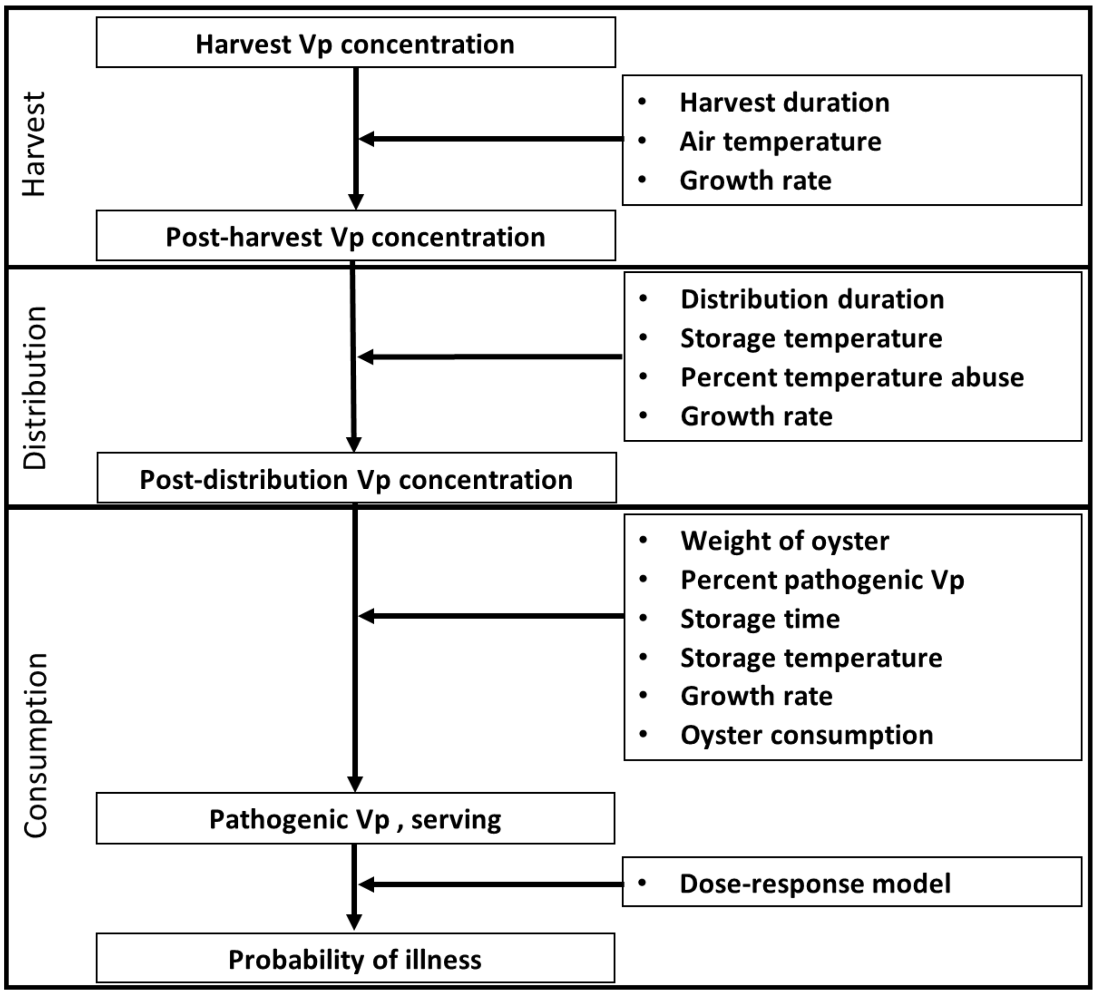


Figure 1. Model of the QMRA of *V. parahaemolyticus* in Southern California Pacific oysters.

Table 1. Parameters and calculations for estimated *V. parahaemolyticus* (Vp) illness risk from oyster consumption.

Parameter	Units	Distribution	Values / Equations	Citation
Vp concentration	CFU/ g	Lognormal	Log mean: 1.29 Standard deviation: 2.25	Cooksey et al., in prep
Harvest duration	Hr	Uniform	Minimum: 0 Maximum: 5	118
Southern California air temperature	°C	Normal, truncated	Mean: 17.0 Standard deviation: 2.41 Minimum: 12.6 Maximum: 20.5	232
Harvest Vp concentration	CFU/ g		$Vp\ Concentration + 0.00372\ (Air\ temperature\ during\ harvest - 5.4)$	149
Distribution duration	Hr	Normal, truncated	Mean: 81.3 Standard Deviation: 51.1 Minimum: 0	117
Distribution temperature	°C	Lognormal, truncated	Log mean: 3.65 Standard deviation: 0.143 Minimum: 1.439	117
Percent temperature abuse	%	Gamma, truncated	Shape: 0.327 Scale: 0.0408 Minimum: 0.001Maximum: 50.83	117
Temperature abuse duration		Calculated	Percent temperature abuse * Distribution time	
Vp distribution concentration	CFU/ g	Calculated	$\text{Harvest growth} + 0.00372(\text{Temperature during distribution} - 5.4)$	149
Consumption storage temperature	°C	Normal, truncated	Mean: 11.3 Standard deviation: 5.10 Minimum: 5 Maximum: 17	233
Consumption storage time	Hr	Pert	Minimum: 0 Maximum: 106.8	

Parameter	Units	Distribution	Values / Equations	Citation
			Mode: 168	
Vp concentration consumption	CFU/g	Calculated	$\text{Distribution growth} + 0.00372(\text{Temp}, \text{consumption} - 5.4)$	
Oyster mass	g	Weibull, truncated	Shape: 2.305 Scale: 21.785 Minimum: 3.88 Maximum: 45.31	This study
Pathogenic Vp	%	Uniform	Minimum: 0.00 Maximum: 0.055	
Oyster per serving	g	Pert	Minimum: 1 Maximum: 12 Mode: 6	[41,147,148]
Dose	CFU	Calculated	Percent pathogenic Vp * Vp concentration in oyster * Oyster per serving	
Illness risk		Point	1-(1+(dose/β)) ^ (-α) α: 0.6 β: 1310000	[110]
California oyster production	Oyster	Uniform	Minimum: 12,476,412 Maximum: 13,455,628	[118]
Annual oyster serving		Calculated	$\frac{\text{California oyster production}}{\text{Serving}/\text{Oyster mass}}$	
California attributable risk		Calculated	$\text{Annual oyster servings} * \text{Risk} * 10^{-5}$	

α and β are likelihood factors used to define the dose-response curve

Harvest

The concentration of *V. parahaemolyticus* and pathogenic *V. parahaemolyticus* in oysters and water from Southern California were enumerated as previously described in Cooksey et al. (In prep). Briefly, oysters obtained from Newport Bay, California during August and September of 2019 were assessed using culture and molecular methods to determine the concentration of total and pathogenic *V. parahaemolyticus*. *V. parahaemolyticus* concentration was only assessed at sites where Pacific oysters are naturally occurring. The probability distribution for the concentration of *V. parahaemolyticus* in oysters was estimated with a truncated lognormal distribution. The best fit model was developed using prior knowledge and comparing goodness of fit criteria. The Akaike information criteria (AIC) for *V. parahaemolyticus* concentration was 229.27. A uniform distribution assumed to estimate a low percent of pathogenic *V. parahaemolyticus* (*trh*+) detected in oysters in Southern California oysters. Though no pathogenic *V. parahaemolyticus* was detected in oysters from Newport Bay, pathogenic *V. parahaemolyticus* is detected in low prevalence, between 0 – 5%, from Southern California and in Newport Bay water.(Personal communication, California Department of Public Health) To be protective of public health, the minimum percent of pathogenic *V. parahaemolyticus* was 0% and the maximum was 5.5% as this is similar to what is seen in commercial harvesting areas in Southern California. (Personal communication, California Department of Public Health)

The time-to-temperature control in Southern California, established by the California *Vibrio* Control Plan, allows a maximum of 5 hours from harvest to temperature control between March and December and 18 hours between January and February.[118] A uniform distribution was assumed estimate the time until placed in temperature control. Daily average air temperatures in Newport Bay from 1981 to 2020 were used to estimate the air temperature at time of harvest in the current study as this was the location of recreationally harvested oysters (SI table 1).[232] A normal distribution for air temperature was assumed. Temperature ranged from 12.6 to 20.5 °C, with an average temperature of 17.0 °C.

The impact of duration until oysters were stored at or below 7.2 °C in temperature control, as assessed to estimate potential regrowth of *V. parahaemolyticus* in oysters. The growth rate of *V. parahaemolyticus* in Atlantic oysters was obtained from previous studies (Eq 1).[149] An inactivation rate was not included in the model as studies conducted in the Pacific Northwest and Southern California (SI Table 2) found no significant inactivation in culturable *V. parahaemolyticus* concentration when holding oysters at 4 °C.[152] It was assumed that *V. parahaemolyticus* growth rates will be similar in Pacific and Atlantic oysters in storage environments with a temperature greater than 7.2 °C to be protective of public health.

$Growth\,rate = VpConc + 0.00372(Temp - 5.4)$ (1)

Post-harvest: Processing and distribution

The commercial distribution of oysters to consumers allows for more widely consumed products and is important economically to oyster harvesters. It is recognized that *V. parahaemolyticus* concentrations can increase during processing and distribution with improper storage temperatures. The NSSP necessitates ambient storage temperatures to be below 7.22 °C and oyster meat below 10 °C; higher storage temperatures is considered temperature abuse.[114] Though precautions and guidelines are in place to mitigate temperature abuse, a study conducted in the Pacific Northwest and Mid-Atlantic showed 18% of shipments experienced temperature abuse.[117] This study utilized the time and temperature for the Pacific Northwest Pacific oyster from Love et al. (2020) to estimate temperature and time during distribution.[117] Distribution time ranged from

6.833 to 217.3 hours, with an average of 84.33 hours. The distribution for the time was characterized by a truncated normal distribution. The normal distribution model used had an AIC value of 539.23. The distribution temperatures ranged from -2.5 °C to 23.0 °C. The distribution temperature was estimated with a truncate log normal distribution. The log normal model for distribution temperature had an AIC goodness of fit value of 154195.6

When oyster shipments were exposed to temperatures over 7.22 °C, the average duration was for 4.84 hours.[117] The amount of time oyster shipments spent in temperature abuse scenarios ranged from 0 hours to 79.8 hours.[117] The percent of time oyster shipments spent in temperature abuse ranged from 0 to 50.8%. A truncated gamma distribution with a shape of 0.327 and scale of 0.041 was used to estimate percent of shipment time the oyster shipment was in temperature abuse. The gamma distribution had an AIC value of 316.97. The amount of time an oyster shipment would spend at temperatures greater than 7.22 °C was estimated to calculate the increase in *V. parahaemolyticus* growth using the previously described growth rate (eq 1).

Time and temperature at retail

Retail distributers, such as restaurants, are a vital link in the supply chain. A 2007 study in the United States evaluated oyster storage temperatures and time held at 258 retail distribution points in nine states (n = 382 sampling events). Of the 258 establishments, 39% were restaurants or raw bars, 59% were seafood markers, and 2% were wholesale distributers. The mean storage time was 8.9 days, though 10.7% (41/382) were stored for over 14 days.[233] Consumption storage duration was estimated with a pert distribution. In the retail study, the average consumption storage temperature was less than 5°C, but 15% of the consumption storage temperatures were above 10 °C. The maximum temperature reported was 17°C.[233] From this study, it was estimated the mean storage temperature was 11.3°C with a standard deviation of 5.1°C.[228] A truncated normal distribution was used to estimate the consumption storage temperature at retail. Growth rate of *V. parahaemolyticus* in oysters following consumption storage time and temperature at retail was calculated (eq 1).

Oyster size and consumption

The weight of Pacific oysters was determined by individually weighing 144 recreationally harvested Pacific oysters. The Pacific oysters were harvested from Newport Bay, California (33.61, -117.91) on September 21, 2020. Oysters were transported on ice to the University of Arizona for processing. The mean weight of wild oysters was 19.3 +/- 8.97 g, which is larger than aquaculture Pacific oysters and Atlantic oysters (SI table 3).[110] The distribution for oyster weight was characterized by a Weibull distribution with a shape of 2.31 and a scale of 21.8. The gamma distribution model was the best fit with an AIC of 1030.35. Oyster consumption was estimated with a pert distribution, with a maximum of 12, most frequent as 6, and minimum 1.[41,147,148] The total weight of oysters consumed was determined by multiplying the oyster weight by consumption. It was assumed all oysters were consumed raw to be most protective of human health.

Dose-response

The risk of illness from consumption was calculated using the Beta-Poisson dose-response formula for *V. parahaemolyticus.* The α and β are parameters of the Beta-Poisson dose-response model developed by FDA (2005) with pooled exposure data from human feeding studies using a *tdh* hemolysin positive strain of *V. parahaemolyticus*.[110] The health endpoint was illness as defined as diarrhea event.[110] It was assumed the dose-response for *trh* positive strains was similar to *tdh*.

Risk characterization

Monte Carlo methods in R Studio were used to calculate the risk of *V. parahaemolyticus* illness from consumption of Pacific oysters harvested in Southern California.[169] The probability of illness from consumption of an oyster infected with pathogenic *V. parahaemolyticus* was estimated with a Monte Carlo simulation. There were 10,000 iterations performed sampling from the input and distribution parameters. The seed was set to 15. The probability of illness was estimated with eq 2.

$$\Pr(ill) = 1 - \left(1 + \left(\frac{Dose}{\beta}\right)^{-\alpha} \right) \quad (2)$$

Sensitivity analysis

A sensitivity analysis was used to show the influential parameters in the model variability. The Spearman rank correlation coefficient was utilized to identify influential input parameters on *V. parahaemolyticus* illness risk. The input distributions were initial *V. parahaemolyticus* concentration, harvest temperature, harvest duration, distribution temperature, distribution duration, distribution temperature abuse, retail temperature, retail duration, serving size, and percent of pathogenic *V. parahaemolyticus*. The input parameters were ranked based on the strength of the correlation coefficient with *V. parahaemolyticus* illness risk, where -1 is a perfect inverse relationship and 1 is a perfect positive correlation. All calculations were performed in R.

Dose response

For example, dose-response curve and QMRA models were estimated using data from the Alaska oyster outbreak and information from the US FDA QMRA to highlight differences in estimated health risks from consumption of raw oysters. To do this, an exponential dose-response model was used to estimate the probability of *V. parahaemolyticus* illness under each scenario (SI table 4)

Three scenarios were used to calculate the concentration of *V. parahaemolyticus* consumed in raw oysters. Scenario 1 and scenario 2 utilized the concentrations of *V. parahaemolyticus* in oysters harvested in Alaska following several outbreaks. Scenario 3 is the concentration of *V. parahaemolyticus* in oysters from the Gulf of Mexico in the summer from the US FDA QMRA risk model. It was assumed Pacific and Atlantic oysters were similar in weight.

The number of oysters consumed in Alaska and the Gulf of Mexico (SI Table 5) was then utilized to calculate the dose of *V. parahaemolyticus* a person would receive (Table 2). It was assumed 12 oysters were consumed in the Gulf of Mexico for the FDA scenarios and only one oyster consumed for Alaska scenarios given the reported consumption frequency in each scenario

Results

The probability of illness from consumption of raw oysters harvested from Southern California was estimated for oysters at before and after the “sea-to-fork” pathway (Figure 2). There was a statistically significant difference between *V. parahaemolyticus* illness risk from consumption of raw oysters before and after the “sea-to-fork” pathway (Wilcoxon rank sum test, $p < 0.001$). Consuming an oysters at the time of harvest has a lower median risk compared to the median risk following the “Sea-to-Fork” pathway. The mean *V. parahaemolyticus* illness risk per serving of raw oysters harvested in Southern California immediately following harvest was 5.61×10^{-5} (95% confidence interval (CI) $4.84 \times 10^{-8} – 1.83 \times 10^{-4}$). The mean probability of illness per serving of raw oysters following the “sea-to-fork” pathway was 6.20×10^{-5} (95% CI $5.653 \times 10^{-7} – 1.92 \times 10^{-4}$).The mean *V. parahaemolyticus* illness risk per serving of raw oysters harvested in Southern California immediately following harvest was 5.61×10^{-5} (95% confidence interval (CI) $4.84 \times 10^{-8} – 1.83 \times 10^{-4}$). Consumption rates of recreational harvested oysters is unknown in Southern

California. The mean probability of illness per serving of raw oysters following the “sea-to-fork” pathway was 6.20×10^{-5} (95% CI 5.653×10^{-7} – 1.92×10^{-4}).

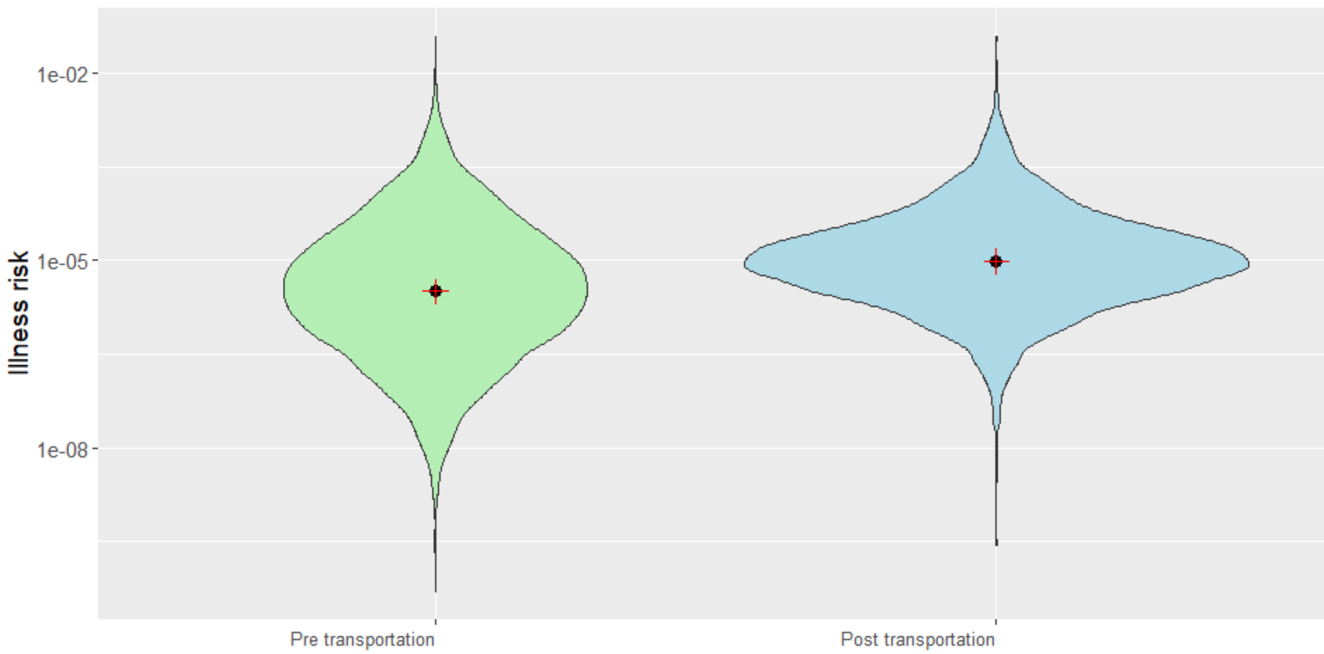


Figure 2. *V. parahaemolyticus* illness risk from consumption of raw recreationally harvested oysters in Southern California pre- and post- “sea-to-fork” pathway the black dot represents the median and the red cross represents the mean.

A sensitivity analysis of the “sea-to-fork” pathway for oysters highlighted the importance of several parameters on the outcome variable (risk of illness) including the initial concentration *of V. parahaemolyticus* in the oysters, number of oysters consumed, and concentration of pathogenic *V. parahaemolyticus* following transport (Figure 3). The most influential input parameter was initial concentration of *V. parahaemolyticus*, followed by the percent pathogenic *V. parahaemolyticus* and serving size. Time and temperature at the retail point were also found to heavily influence final illness risk.

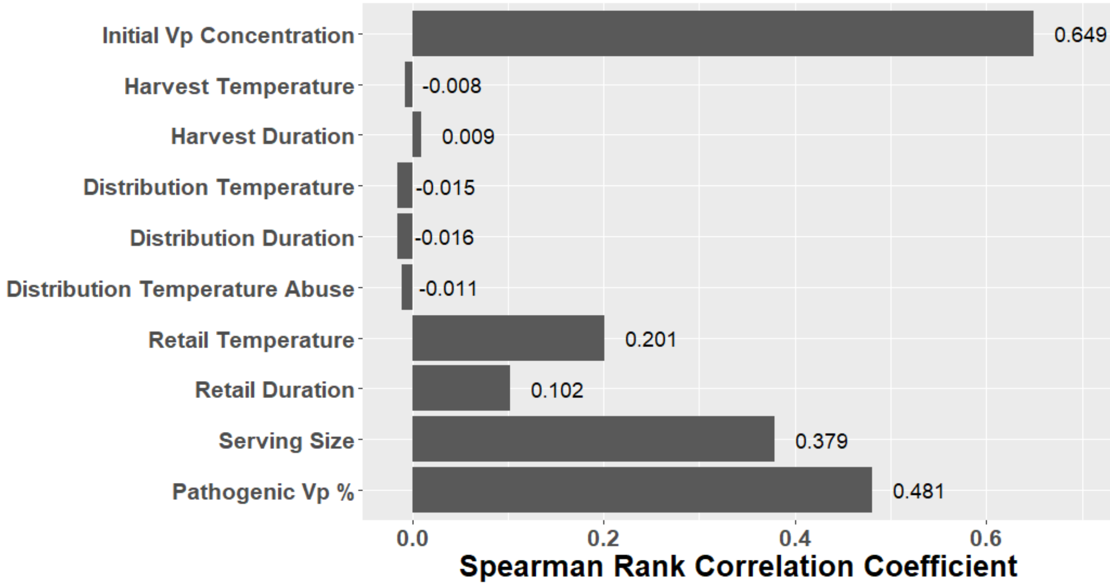


Figure 3. Sensitivity analysis highlighting influential variables on *V. parahaemolyticus* (Vp) illness risk following the "sea-to-fork" pathway.

The mean illness risk from *V. parahaemolyticus* of 6.20×10^{-5} (95% CI 5.65×10^{-7} – 1.92×10^{-4}) following the "sea to fork" pathway was compared to the mean estimated illness risks during the summer from Brazil (3.6 x 10^{-3}, 95% CI 3.3 x 10^{-4} – 9.9 x 10^{-3}), Taiwan (8.56 x 10^{-5} 95% 0.0 – 4.5 x 10^{-3}), Japan (1 x 10^{-10}), Australia (5.97 x 10^{-8}, 95% CI 3.04 x 10^{-8} – 2.04 x 10^{-7}), Canada (1.08 x 10^{-6}, 95% CI 3.04 x 10^{-8} – 4.00 x 10^{-6}), New Zealand (1.40 x 10^{-8}, 95% CI 1.10 x 10^{-9} – 4.57 x 10^{-8}), the Gulf of Mexico (3.75 x 10^{-4}, 95% CI 2.86 x 10^{-5} – 1.21 x 10^{-3}) and Chesapeake Bay (3.0 x 10^{-7} , 95% CI 1.0 x 10^{-7} – 7.0 x 10^{-7}) (Figure 4).[147–151] The estimated mean risk per exposure from this study was lower than Brazil and the Gulf of Mexico, similar to Taiwan, and higher than Canada, Chesapeake Bay, Australia, New Zealand, and Japan.

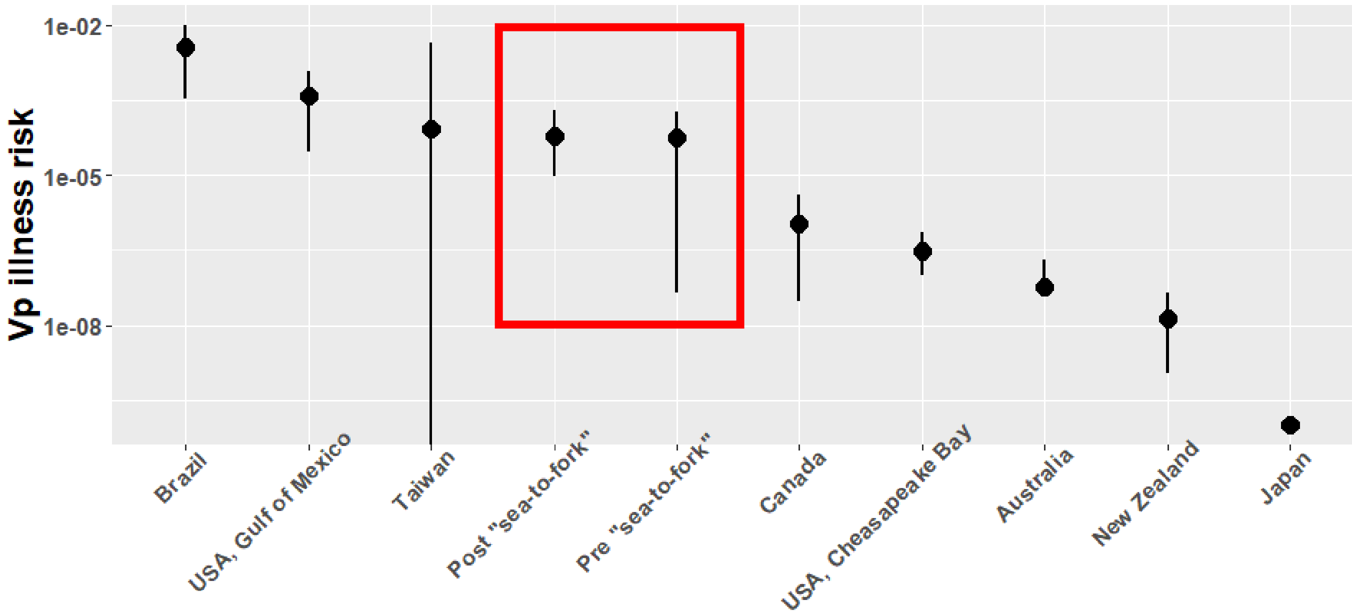


Figure 4. Mean *V. parahaemolyticus* (Vp) illness risk per exposure and 95% confidence intervals from previous QMRA. The red box is highlighting the estimated mean risk from the current study.

. The dose for total *V. parahaemolyticus* and pathogenic *V. parahaemolyticus* was calculated. The dose from Scenario 1 was used to calculate k for the Alaska Cruises (Table 3). From this, the dose-response curve was plotted to see the implication of different k values from the Alaska cruise outbreaks compared to a scenario from the US FDA QMRA model (Figure 5 and 6).

Table 2. Dose of *V. parahaemolyticus* consumed per serving of oysters. Cells highlighted in yellow were used to calculate the dose.

Scenario	Total *V. parahaemolyticus*	Pathogenic *V. parahaemolyticus*
	Median/mode	Median/mode
Scenario 1 (Alaska consumption)	31.5	23.31
Scenario 2 (Alaska consumption)	52.5	38.85
US FDA	378000	648

Table3. Calculated k values for each scenario.

	Attack rate	Total *V. parahaemolyticus*	Pathogenic *V. parahaemolyticus*
		Scenario 1.1 k	Scenario 1.2 k
Cruise 1	0.21	0.00748	0.0101
Cruise 2	0.42	0.0173	0.0234
Cruise 3	0.27	0.00999	0.0135
FDA	0.5	1.834E-06	0.00107

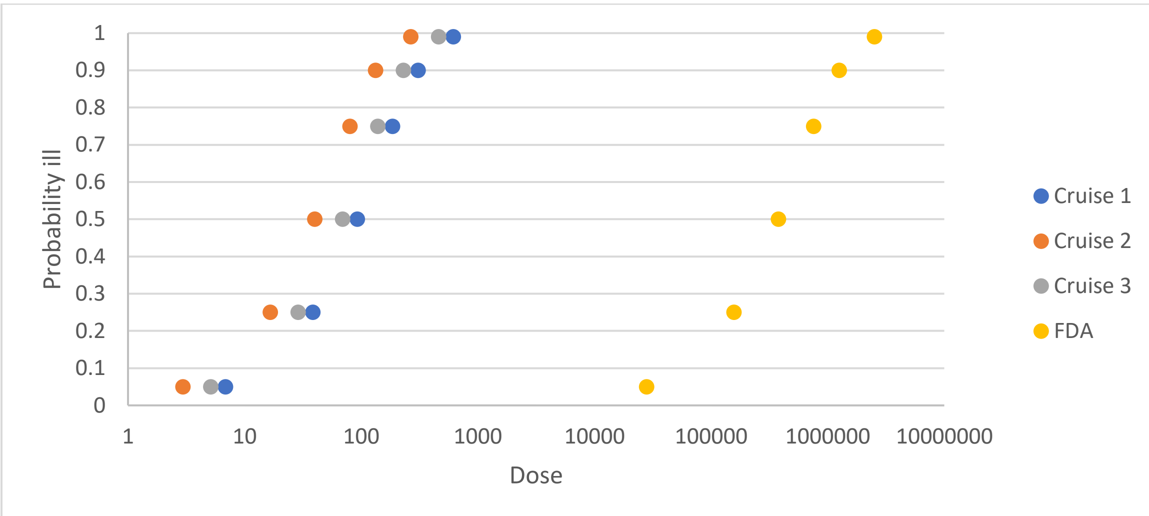


Figure 5. Calculated dose-response curves with total *V. parahaemolyticus*.

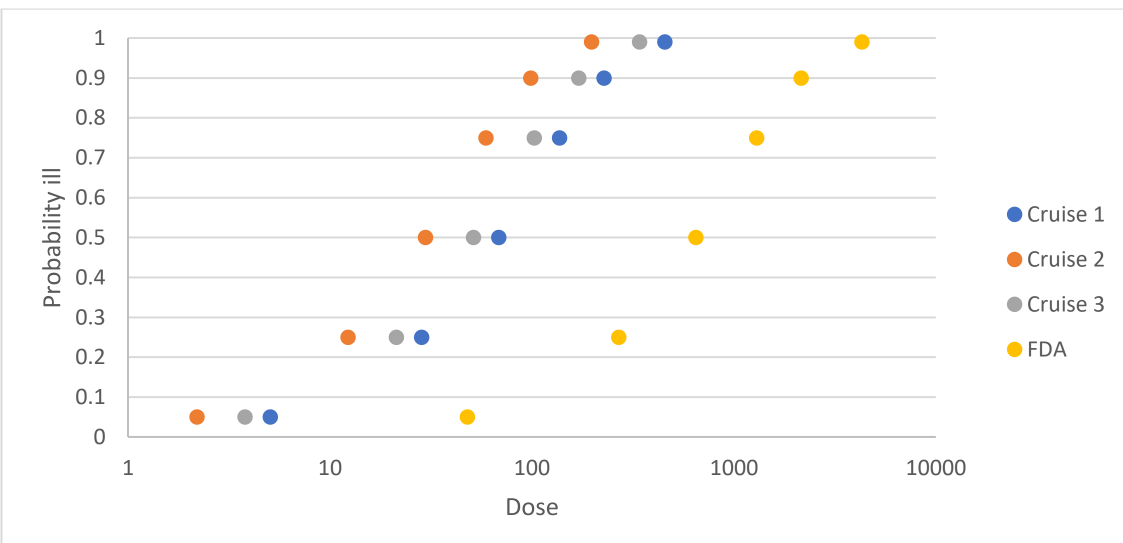


Figure 6. Calculated dose-response curves with pathogenic *V. parahaemolyticus*

Given the Alaska cruise scenarios had a smaller k, therefore increased illness risk, the QMRA model developed in Chapter 3 was used to estimate the risk in each scenario. From this, the estimated illness risk was evaluated (Figure 7). The differences in median illness risk from consumption with the US FDA dose-response model compared to the dose-response model modified from the Alaska cruise outbreak is important as it highlights that the current QMRA and dose-response model may not be the most protective of public health from *V. parahaemolyticus* risk. Moving forward, incorporating a ratio of the attack rates to develop a modified dose-response relationship would allow a better understanding of illness risk from *V. parahaemolyticus* strains. Additionally, it is recommended to assess other virulence factors, such as *trh+* and type three secretion systems and dose-response relationships.

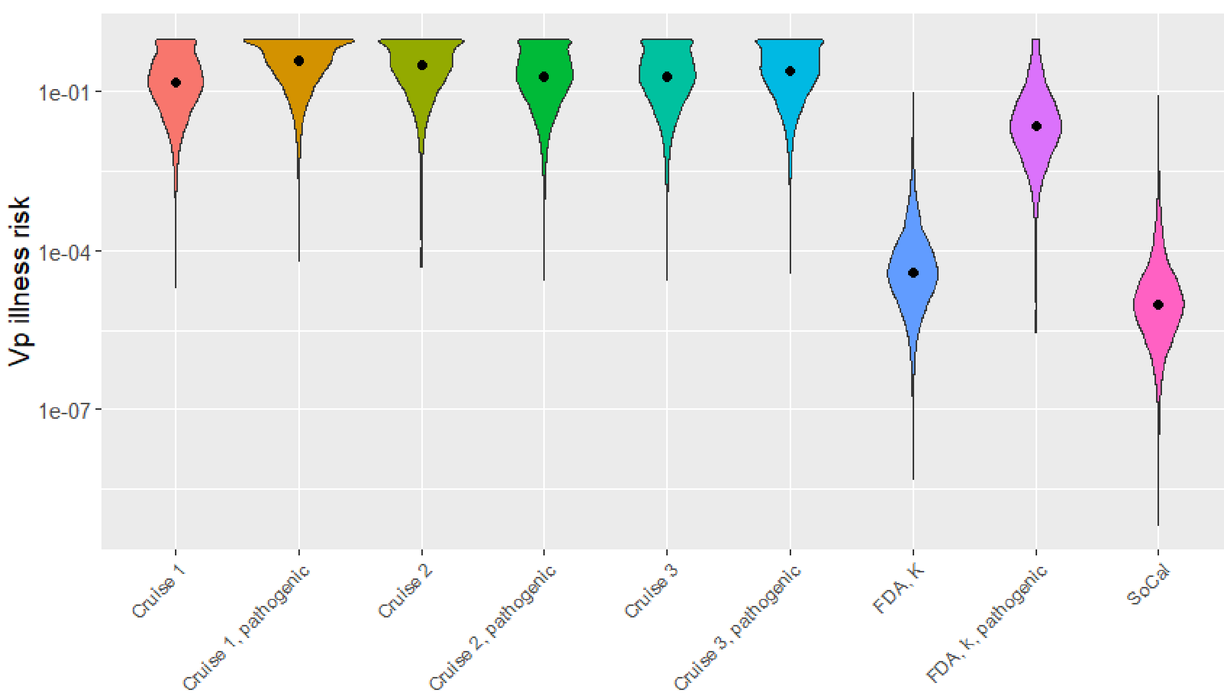


Figure 7. Estimated *V. parahaemolyticus* illness risk from each scenario. The black dot represents the median illness risk for each scenario.

Discussion

This is the first QMRA model specific *to V. parahaemolyticus* illness from consumption of Pacific oysters harvested in Southern California. A QMRA was performed to assess the risk of illness from *V. parahaemolyticus* in oysters harvested from Southern California along the "sea-to-fork" pathway. The mean *V. parahaemolyticus* illness risk from Pacific oyster consumption pre- (5.61 x 10^{-5}, 95% CI 4.84 x 10^{-8} – 1.83 x 10^{-4}) and post- (6.20 x 10^{-5} , 95% CI 5.653 x 10^{-7} – 1.92x 10^{-4}) "sea-to-fork" pathway were lower than the estimated mean risk in the FDA model for the Gulf of Mexico (3.75 x 10^{-4} , 95% CI 2.86 x 10^{-5} – 1.21 x 10^{-3}). There was a significant difference in mean *V. parahaemolyticus* illness risk from oysters before and after the "sea-to-fork" pathway. The differences in mean *V. parahaemolyticus* risk in different locations highlights regional differences and supports more regional guidelines to reduce the risk of *V. parahaemolyticus* illness from raw oyster consumption.

This QMRA model incorporates recreationally harvested and commercially harvested oysters. The risk from recreationally harvested oysters in Newport Bay was lower compared to the "Sea-to-Fork" pathway, however, there is limited information regarding temperature and time of storing recreationally harvested oysters. Recreational oysters may be exposed to higher temperature during transportation and consumption (i.e. no put on ice following harvest) that may increase the health risks from *V. parahaemolyticus* exposure. Increased education of the importance of proper storage conditions will be critical for recreationally harvested oysters. Additionally, given the aquaculture oysters are smaller than recreational oysters, the exposure of *V. parahaemolyticus* may be over estimated. A QMRA model should be developed for West Coast oysters with *V. parahaemolyticus* concentrations from aquaculture farms to better understand the *V. parahaemolyticus* exposure risk. Though the QMRA model developed was not specific for either commercial or recreational oysters, it provides a foundation of knowledge that can be incorporated into best practice methods to reduce *V. parahaemolyticus* illness risk as the concentration of *V. parahaemolyticus* at the time of harvest was most influential on *V. parahaemolyticus* health risk.

V. parahaemolyticus concentrations and illness rates are higher in the months of June-September with warmer water temperatures, Pacific oysters harvested at other times of year may have a lower concentration of *V. parahaemolyticus* , peak commercial harvesting is in the winter, but recreationally harvested oysters may be consumed at any time of the year.[3] Further studies should evaluate the quantities of *V. parahaemolyticus* present in Pacific oysters harvested in Southern California in October through June to understand seasonal distribution to better estimate risk. Additionally, it was assumed all Pacific oysters harvested in California were consumed raw. As cooking oysters is effective at reducing *V. parahaemolyticus* quantities, consumption of cooked oysters would also lower risk of *V. parahaemolyticus* illness.[234] However, cooking oysters may not inactivate preformed toxins and further work should look at the inactivation of pathogenic *V. parahaemolyticus* in cooked oysters.[235]

The sensitivity analysis highlighted the importance of the initial concentration of *V. parahaemolyticus*, serving size, and concentration of pathogenic *V. parahaemolyticus*. Similarly, sensitivity analysis for *V. parahaemolyticus* in Taiwan and Brazil with spearman rank correlation test highlight the importance of initial concentration of *V. parahaemolyticus* illness risk.[147,148] Transportation temperature had a strong spearman rank correlation (0.42) with risk in Brazil and Australia, however, it was less influential in Taiwan illness risk (0.21) and in the current study.

The serving size in Taiwan was less influential on illness risk (spearman rank correlation co-efficient: 0.07) compared to the current study's QMRA and Brazil (spearman rank correlation co-efficient: 0.18). Additionally, a QMRA conducted in Taiwan and Brazil highlighted the importance of climate change in driving *V. parahaemolyticus* illness risk concentrations from increase of sea surface temperatures.[147,202] Differences in sensitivity analysis taken with differences in risk estimates across the globe supports the need for more region-specific QMRA to protect human health.

As *V. parahaemolyticus*-related illness and oyster harvesting increase in Southern California oysters during a six-week study were used to assess *V. parahaemolyticus* concentrations. Additional studies should be conducted to better understand the relationship of *V. parahaemolyticus* concentrations in oysters and environmental co-variates to allow for a predictive risk model. Utilizing farmed aquaculture oysters and harvesting water would allow this model to be more specific to aquaculture oysters. As Newport Bay represents only one water body, it will be imperative to evaluate the prevalence of *Vibrio* spp. in other water bodies along the West coast to better understand the impact of health risk in each location as *V. parahaemolyticus* prevalence varies by location.

The *V. parahaemolyticus* growth rate used in this model has not been validated for Pacific oysters. The Pacific oyster growth rate may be influenced by the oyster microbiome, growing environment, and concentrations of *V. parahaemolyticus* in the oyster, therefore a growth rate specific to Pacific oysters is necessary to better mitigate *V. parahaemolyticus* persistence during transportation[117,228] and aid in the development of needed regional models to address increasing *V. parahaemolyticus* risk.[31,236] The growth rate of *Vibrio* spp. in Pacific oysters has been estimated by oyster homogenate, inoculated irradiated oysters, predictive modeling, and in alkaline peptone water.[148,228,237,238] However, these studies may fail to accurately estimate *V. parahaemolyticus* growth in live post-harvest Pacific oysters. Developing a growth rate specific to *V. parahaemolyticus* in Pacific oysters will allow for a better understanding of potential re-growth during the "sea-to-fork" pathway. Differences in growth rate between oyster species support the need for regional guidelines for oyster safety.

This risk model assumes the concentrations of total and pathogenic *V. parahaemolyticus* in oysters consumed does not vary oyster-to-oyster. However, "the hot oyster" phenomenon, an individual oyster with an exponentially higher concentration of *V. parahaemolyticus* compared to surrounding oysters, has highlighted that individual oysters can drive exposure risks with an exponentially higher concentration of *V. parahaemolyticus*.[61,62,190] *V. parahaemolyticus* concentration in oysters is often as a composite sample of oysters, therefore a high concentration of *V. parahaemolyticus* in a single oyster may not be taken into account.[239] Future studies should assess *V. parahaemolyticus* concentrations in both composite and single oysters to better understand environmental or oyster physiological factors that may increase the likelihood of "hot" oysters and increased exposure risk. Additionally, the concentration of pathogenic *V. parahaemolyticus* in oysters was assumed to be similar to commercial harvesting grounds. Given the concentration of total *V. parahaemolyticus* may not be reflective of pathogenic *V. parahaemolyticus*, it is imperative to continue to evaluate and monitor the concentration of pathogenic *V. parahaemolyticus* in recreational harvested oysters.[208]

Another limitation is the lack of specific supply chain time and temperature data from California oyster harvesters. The shipping time and temperature were from farmed oysters from Washington State.[117] The shipping time and temperature were from farmed oysters from Washington State.[117] However, there may be differences in California and Washington cold chains. For example, there are 145 entities certified to partake in the shipping of shellfish in California, but only 91 in Washington.[240] There may be differences in the number of participants in each cold chain and duration of the cold chain which may increase frequency of temperature abuse. However, there may be differences in the California and Washington cold chain. For example, there are 145 entities certified to ship shellfish in California, but only 91 in Washington.[240] The complexity of the supply chain has the ability to increase temperature abuse or time of distribution but there is limited information on exactly how the supply chains in the different West coast states compare to each other and the rest of the US.[117,241,242] While the time and temperature are more specific to the West Coast in this QMRA, there are likely to be regional differences in air and water temperatures between Washington State and Southern California which may influence post-harvest *V. parahaemolyticus* growth. Incorporation of a more specific time and temperature for California oyster transport would allow for a better understanding of specific scenarios that drive health risks.

The current dose-response, developed by the FDA, is limited for numerous reasons. The strain of *V. parahaemolyticus* used in the current dose-response model is positive for the *tdh* gene, not *trh*, as the pathogenic genes may have different dose response models. Additionally, the current QMRA dose-response assessment is known to be derived from a less virulent bacterium than serotypes associated with outbreaks and reported illnesses on the West Coast.[41,68,243] The *tdh+ V. parahaemolyticus* strain from the West Coast had a mean infection dose as low as $10^3 – 10^4$ cells rather than the median infectious dose of 10^7 cells estimated in the dose-response model estimated by the US FDA.[110,194] This highlights the potential need to re-evaluate the current standards to protect human health. The under reporting of *V. parahaemolyticus* illness as it generally causes mild gastrointestinal illness limits the ability to quantity the prevalence of *V. parahaemolyticus* illness, however, as the population ages there may be an increase vulnerability to *V.*

parahaemolyticus illness.[21]One of the limitations to address health risks is the incomplete understanding of the mechanism of pathogenicity.[194] As the mechanism of pathogenicity within humans is not fully understood, it limits the ability to directly measure pathogenicity from the consumption of naturally grown oysters. Further elucidation of the mechanism of pathogenicity may allow for better environmental detection, in turn reducing vibriosis risk.

Conclusion

This study showed the median *V. parahaemolyticus* illness risk from consuming recreationally harvested oysters in Southern California was less than the median illness risk estimated by the US FDA. The risk from recreational harvested oysters in Southern California was higher during the "sea-to-fork" pathway. Mitigation strategies during harvest and temperature storage before consumption may help reduce *V. parahaemolyticus* illness risk from consumption of raw aquaculture oysters. Finding from these studies may be applied to aquaculture oyster farms in Southern California to help reduce *V. parahaemolyticus* illness

Chapter 5: Conclusion

Main findings and implications for public health

There is a pressing need to better understand *V. parahaemolyticus* and *V. vulnificus* from a global and local perspective as cases of vibriosis continue to increase.[22] The overall goal of this dissertation was to understand *V. parahaemolyticus* from a global and local perspective to inform public health interventions. To achieve this goal, this dissertation evaluated the global distribution of *V. parahaemolyticus* in paired oyster and water samples, quantified the prevalence of *V. parahaemolyticus* and *V. vulnificus* in recreational oysters and water samples harvested from Southern California, and integrated environmental occurrences in a "Sea-to-Fork" process to understand *V. parahaemolyticus* health risks from consumption of recreationally harvested oysters from Southern California. In a collaborative effort, key gaps in the current understanding of the *Vibrio*-oyster-health relationship were assessed.

Through a scoping literature review, 32 scientific manuscripts were synthesized to evaluate the global distribution of *V. parahaemolyticus* in paired oyster and water samples. While other literature reviews have assessed *Vibrio* spp. and *V. parahaemolyticus* concentrations in water and oysters, this was the first to evaluate *V. parahaemolyticus* in paired oysters and water samples. Most of the studies were conducted in the United States and focused on the Gulf of Mexico or the Atlantic Coast of the United States. There was generally a higher concentration of *V. parahaemolyticus* in oysters than water. Limited correlations or associations with environmental covariates or microorganisms and *V. parahaemolyticus* were identified. Critical gaps in the current understanding of the environmental covariates and concentration of *V. parahaemolyticus* in oyster and water were identified because of the inconsistent availability of data. From this, an open-access database was developed to promote equitable access and collaborative research. The information provided from the current literature review in conjunction with future work will allow for meta-analysis, forecast, or hindcast models to predict *V. parahaemolyticus* illness prevalence in absence of water or oyster samples. As *V. parahaemolyticus* continues to be a pathogen of concern, the literature review and database will aid in the ability to evaluate the spatial distribution of *V. parahaemolyticus.*

As *V. parahaemolyticus* is an emerging pathogen of concern for many coastal communities, it will be important to understand the dynamics between *V. parahaemolyticus* and the environment. This dissertation was the first known study to assess and detect total and pathogenic *V. parahaemolyticus* and *V. vulnificus* in wild Pacific oysters in Southern California. The utilization of deployed oysters allowed for the assessment of microbial accumulation. There was a significantly higher concentration of *V. parahaemolyticus* and *V. vulnificus* in water and oyster samples in the Back bay compared to the Front bay and Middle bay oysters and water. Additionally, there was higher detection of pathogenic *V. parahaemolyticus* and *V. vulnificus* in the water column compared to oyster tissue. There were mixed associations with temperature, salinity, and chlorophyll A with concentrations of *V. parahaemolyticus* in water and oysters and *V. vulnificus* in water. Differences in *Vibrio* spp. dynamics in Southern California compared to the East Coast and the Gulf of Mexico highlight the need for regional policies and guidance.

This study showed that there were differences in environmental covariates and concentrations of *V. parahaemolyticus* and *V. vulnificus* in oysters and water, supporting the need for increased regional monitoring of *V. parahaemolyticus* and *V. vulnificus* in recreational oysters to protect human health. It is recommended, particularly in months with water temperature greater than 15.5 °C, as recommended by the ISSC *Vibrio* Control Plans on the West Coast, to integrate the evaluation of *V. parahaemolyticus* and *V. vulnificus* in oyster tissue and water into the state monitoring plan currently used for assessing paralytic shellfish poisoning or recreational water quality in areas with recreational oyster harvesting. It is important to assess for *V. parahaemolyticus* and *V. vulnificus* in both the oyster tissue and water column as the concentrations may vary, representing different exposure potentials for different exposure routes. Additionally, understanding the presence of *V. vulnificus* in recreational water may help provide medical treatment for wound infections. Given the limited case numbers of *V. vulnificus* and *V. parahaemolyticus* wound infections, it is not recommended to close recreational areas in estuary environments due to the presence of either bacteria. Rather, increased education to at-risk individuals (i.e. immunocompromised men who are more at risk than other oyster consumers due to physiological differences) is recommended to be aware and observant of *Vibrio* spp. symptoms.

While the US FDA developed a QMRA model for *V. parahaemolyticus* in oysters, the model was primarily focused on oysters harvested from the Gulf of Mexico. The QMRA developed for oysters harvested in Southern California is the first specific to Pacific oysters harvested on the West Coast. This QMRA model further evaluated the impact of storage time and temperature during transportation for Pacific oysters harvested in the Pacific Northwest and at consumption locations. The mean probability of illness per serving of oysters was 6.20×10^{-5} (95% confidence interval $5.653 \times 10^{-7} – 1.92\times 10^{-4}$) from consumption of raw oysters harvested in Southern California following the "sea-to-fork". The mean *V. parahaemolyticus* illness risk per serving of oysters immediately following harvest was 5.61×10^{-5} (95% confidence interval $4.84 \times 10^{-8} – 1.83 \times 10^{-4}$). The sensitivity analysis highlighted the importance of including the initial concentration of *V. parahaemolyticus* in the oysters, number of oysters consumed, and concentration of pathogenic *V. parahaemolyticus* following transport in vibriosis risk.

Beyond this work, the QMRA model sheds light on important drivers of *V. parahaemolyticus* illness risks with the sensitivity analysis that can be utilized by recreational and commercial oyster harvesters, scientists, and policymakers. Given pre-harvest conditions, such as concentrations of total and pathogenic *V. parahaemolyticus* were influential in illness risk from raw oysters, integration of post-harvest processing and more frequent *V. parahaemolyticus* monitoring in oyster meat is warranted. Post-harvest processing, such as gamma-radiation and high-pressure processing, may change the flavor profile and texture of the oyster, but offer effective mitigation strategies to reduce *V. parahaemolyticus* concentrations in raw oysters between 2 and 7 logs.[244–247] Incorporating either post-harvest processing method is a viable mitigation strategy to reduce *V. parahaemolyticus* concentrations, and therefore illness risk. Alternatively, a more frequent evaluation of *V. parahaemolyticus* in oysters during harvest would allow for a better understanding of the concentration of *V. parahaemolyticus.* For recreationally harvested oysters, *V. parahaemolyticus* and *V. vulnificus* should be evaluated from intertidal oysters harvested at low tide to represent the worst case scenario of *V. vulnificus* and *V. parahaemolyticus* increase due to air temperature above 7.2 °C for an extended period.[110,114] It is recommended to assess the concentrations of *V. parahaemolyticus* and *V. vulnificus* in oysters grown closest to the surface of the water and on the bottom as harvest location can impact the concentrations of pathogenic *V. vulnificus* concentrations.[248].[248] *V. parahaemolyticus* and *V. vulnificus* should be evaluated with a MPN method followed by speciation and pathogenic gene PCR confirmation step. Additionally, it is recommended to utilize the heat shock protein 60 (HSP60) amplicon to identify pathogenic genes (*tdh* and *trh*) for *V. parahaemolyticus* specific for the sampling location to allow for the optimization of PCR primers.[249] It is recommended to evaluate *V. parahaemolyticus* and *V.*

vulnificus concentrations in six to 12 individual oysters as the "hot oyster", significantly higher concentration of *V. parahaemolyticus*, is not accounted for with a pooled homogenate sample.[61,62,190] While post-harvest processing and increased monitoring reduce vibriosis risk, they would also increase the cost of growing and harvesting oysters. Therefore, state and national government would need to provide aid or subsidies to oyster harvesters to reduce the burden of reducing health risks.

While the QMRA model developed is a useful decision tool, it is important to understand potential differences in illness risks related to regional strain variations of *V. parahaemolyticus*. A *V. parahaemolyticus* outbreak in Alaska from raw oyster consumption highlighted a difference in illness risk from strain variations on the West Coast with an average attack rate of 29%, significantly higher compared to the expected illness rate modeled by the current dose-response model utilized by US FDA and this QMRA model.[41,110] There is a need for an updated dose-response model for *V. parahaemolyticus* to account for differences in strain pathogenicity and other virulence factors.

Limitations and future direction

The literature review provided an up-to-date synthesis of the global distribution in *V. parahaemolyticus* in paired water and oyster samples. Numerous studies have evaluated *V. parahaemolyticus* concentrations in water, oysters, and other environmental samples (i.e. sediment, aquatic plants, and other bivalves) that should be integrated into the database to more broadly understand the global distribution and environmental covariates associated with *V. parahaemolyticus*. While this dissertation was focused on recreationally harvested oysters, it will be important to integrate knowledge about recreationally harvested oysters with aquaculture oysters to best understand health risks from both recreational and aquaculture oysters. It is recommended to include studies that assess *V. parahaemolyticus* concentrations post-harvest or at markets into the database to allow for a greater understanding of the "sea-to-fork" pathway. Integrating the "sea-to-fork" pathway with environmental monitoring at a global scale will allow for the development of a QMRA model to estimate illness risk from *V. parahaemolyticus* in regional environments. An important next step is to conduct literature reviews assessing the occurrence of *V. parahaemolyticus* in non-paired oyster and water samples, concentration post-harvest, and in market studies to expand the impact of the database and global understanding of the spatial distribution and health risks from *V. parahaemolyticus* in both recreational and commercial oysters.

An interesting next step in understanding *V. parahaemolyticus* in oysters and water would be incorporating the spatial distribution of oyster pathogens, such as the Oyster Herpes Virus (OsHV-1), *Perkinsus marinus* (Dermo), and *Haplosporidium nelsoni* (MSX) into the *V. parahaemolyticus* database. Given climate change is a critical factor in each disease, a further understanding of pathogen movement will be critical for aquaculture and human health. This would allow for an increase in knowledge of oyster health, environmental parameters, distribution of oyster and human pathogens. Additionally, the collaboration of numerous experts may shed light on the interconnection of human and oyster pathogens of concern. Moving forward, it will be imperative to synthesize our understanding of human health-related and oyster health-related pathogens to holistically understand microbial ecology interactions driving human health risks in the oyster harvesting environment.

The oyster microbiome may play a key role in the relationship between *Vibrio* spp. concentrations in the water and the environment as it impacts the immune response of the oyster.[250] While not specific to recreationally harvested oysters, an important next step is understanding the role of oyster genetics in the concentration of *V. parahaemolyticus* and *V. vulnificus* in aquaculture oysters as genetically modified triploid oysters account for over 30% of aquaculture Pacific oysters on the West Coast of the USA and up to 80% of Atlantic oysters in Virginia.[251,252] Triploid oysters are advantageous as they grow faster preventing mass oyster mortality from oyster parasites such as Dermo and MSX, and are harvestable during the summer months.[253–256] However, the development of triploid oysters alter the genetics may allow the oyster to be more readily colonized by *V. parahaemolyticus* and *V. vulnificus*. Additionally, the triploid oysters allow for summer harvesting as *V. parahaemolyticus* and *V. vulnificus* concentrations are highest in the water column. Taken together, there is a need to elucidate the role of modified genetics in *V. parahaemolyticus* and *V. vulnificus* concentrations in oysters to understand health risks, particularly in the summer months.

The six-week study used in the current study to assess *V. parahaemolyticus* and *V. vulnificus* in oysters and water accounted for the highest concentrations of *V. parahaemolyticus* and *V. vulnificus* in oysters and water but failed to account for the seasonality. It is recommended a longer-term study be undertaken to assess for seasonality in Southern California. Understanding the seasonality of *V. parahaemolyticus* and *V. vulnificus* in the water column, oysters, and sediment may allow for a better understanding of important reservoirs when water temperatures are lower or critical environmental parameters are driving *V. parahaemolyticus* or *V. vulnificus* concentrations. Additionally, it is recommended to use naturally occurring oysters rather than deployed oysters to allow for a better understanding of *Vibrio* spp. concentrations within harvestable oysters in Newport Bay. With additional sampling, a more robust regression analysis may be developed to better assess the relationship of *V. vulnificus* and *V. parahaemolyticus* with environmental covariates. Taken together, a long-term study will allow for a greater understanding of *Vibrio* spp. dynamics with respect to environmental parameters and seasonality and harvesting to aid in the ability to inform decisions to be protect human health from *V. parahaemolyticus* and *V. vulnificus* exposure in raw oysters harvested in Southern California.

The QMRA for *V. parahaemolyticus* developed in this dissertation was a combination of input parameters from recreationally harvested oysters and commercial oysters. To improve the QMRA for either recreationally harvested oysters or aquaculture oysters, more regionally specific information is necessary. Increased regional monitoring of *V. parahaemolyticus* in recreational oysters is necessary to understand the relationship with environmental co-variates. Additionally, it is important to understand human consumption behavior with recreational harvested oysters. This QMRA was the first to incorporate both the supply chain and restaurant storage time and temperature. However, it is necessary to better understand the cold chain process, particularly at end point consumption locations (i.e. restaurants or bars) as the sensitivity analysis from the *V. parahaemolyticus* QMRA highlighted influence of the time and temperature of oyster storage at the end consumption location. The supply chain in the USA is primarily understood by recent studies conducted in Washington and Virginia, however, there was no information regarding endpoint consumption time and temperature in these studies.[117,149] Additionally, the endpoint time and temperatures in the QMRA model were assumed comparable to a study conducted in 2007 from states other than California. There is a need for further understanding of the time and temperature of oyster storage at the endpoint distributer. Additional information regarding handling and storage of oysters would allow for mitigation efforts to aid in *V. parahaemolyticus* risk reduction strategies.

A critical next step to further the understanding of *Vibrio*-oyster-health will be to identify the mechanism of pathogenicity for *V. parahaemolyticus* as the mechanism of pathogenicity for *V. parahaemolyticus* is not fully understood. This is crucial to understand the genes involved to better assess for pathogenic *V. parahaemolyticus* in the environment. One way to assess for the virulence factors driving pathogenicity in *V. parahaemolyticus* is to utilize enteroid methods. Recent studies have used humans and mouse enteroid models in combination with fluorescence microscopy to understand the virulence factors of *Salmonella enterica* serovar Typhimurium.[257] It is proposed that scientists use enteroid models to assess the role of known virulence factors, such as *trh, tdh*, and type 3 secretion systems, in human infection. It is understood there are limitations with enteroid models, however, their use allows for the assessment of virulence factors in human models without exposing humans. A greater understanding of the mechanism of pathogenicity would allow for better detection of virulent *V. parahaemolyticus* in the environment. Currently, there are about 10% of cases that would not be detected by current environmental monitoring approaches as these cases do not have the traditionally monitored *trh* or *tdh* gene. Connecting the bench top understanding of pathogenicity with environmental monitoring will provide a better understanding of health risk from exposure to *V. parahaemolyticus* and will guide decision making to best protect human health.

The study conducted in Southern California was the first to detect pathogenic *V. vulnificus* in the water column in Newport Bay, California. Given *V. vulnificus* illnesses are rising as climate change is altering the spatial distribution, particularly with recreational exposure, it is of public health concern to better understand driver of health risk.[42] It will be critical to evaluate a wound infection dose response model for *V. vulnificus* to better understand exposure risks. As a majority of *V. vulnificus* illness and death are associated with older or immunocompromised males with elevated iron level in blood and liver disease, it will be important to utilize older or immunocompromised mice to understand the dose-response.

No *V. vulnificus* QMRA was developed from these studies for several reasons. For one, there are only approximately 50 cases of *V. vulnificus* illness or death reported each year from seafood consumption. Additionally, the dose-response model does not account for wound infection. Particularly as climate change changes coastal community estuarine environments, it will be critical to developing a dose-response for wound infection from *V. vulnificus*. A wound infection QMRA for *V. vulnificus* may be critical in evaluating health risks from water exposure.

The limitations and next steps highlighted all come together to develop regionally specific QMRA models. Key limitations in the QMRA developed for *V. parahaemolyticus* are assuming wild oysters are transported and consumed similarly to aquaculture oysters, there is no difference in pathogenicity in *trh* and *tdh*, and oyster consumption is similar on the East and West Coast. Though our QMRA model provided a framework for West Coast specific *V. parahaemolyticus* risks, it integrates regional and national data. Moving forward, studies should assess the national data utilized in this model to make a more specific model for the West Coast. Given Carlsbad Aquafarm has historical *V. parahaemolyticus* data, a logical next step is to develop a QMRA model specific for aquaculture harvested oysters. This model could identify critical spots for public health interventions to prevent additional *V. parahaemolyticus* illness associated with the aquaculture farm. Moving forward, as QMRA models become more regional, a site specific application could be developed for harvest locations to aid in decision making about oyster safety for the oyster harvesters by integrating harvest concentration of *V. parahaemolyticus* with the "sea-to-fork" pathway to understand if the oyster may cause illness in the consumer.

While the work presented in this dissertation has been disseminated to scientists, it is vital to continue to disseminate the information to the government, oyster harvesters, the public, and other stakeholder groups. One way to inform recreational oyster harvesters would be to provide a clear summary of the current state of science and knowledge gaps during the permitting process. The knowledge gained from these studies supports the need for regional guidelines to account for differences in *V. parahaemolyticus* and *V. vulnificus* concentrations, transport conditions, and environmental covariates. Regional guidelines will allow for safer oyster consumption. Oysters provide a sustainable protein source and have the potential to save humanity, again, but science and policy need a collaborative plan from the sea up to protect oyster consumers from *V. parahaemolyticus* and *V. vulnificus* illness.

Appendix A. Chapter 2

SI Table 1. Key word search terms for oysters utilized in the scoping literature review and number of results for each data base.

Keywords \ Database	Web of Science	Embase	ProQuest Ag&Env
keywords	TS= (oysters OR shellfish OR clams OR seafood OR bivalve OR aquaculture OR mariculture OR *oestridae* OR *ostreidae* OR *crassostrea* OR *magallana* OR *ostrea* OR *saccostrea*)	oysters OR shellfish OR clams OR seafood OR bivalve OR aquaculture OR mariculture OR *oestridae* OR *ostreidae* OR *crassostrea* OR *magallana* OR *ostrea* OR *saccostrea*	oysters OR shellfish OR clams OR seafood OR bivalve OR aquaculture OR mariculture OR *oestridae* OR *ostreidae* OR *crassostrea* OR *magallana* OR *ostrea* OR *saccostrea*
Results	346,162	64,405	325,888

SI table 2. Key word search terms for Vibrio utilized in the scoping literature review and number of results for each data base.

Keywords \ Database	Web of Science	Embase	ProQuest Ag&Env
keywords	TS= (*Vibrio* OR *V. parahaemolyticus* OR *Vibrio parahaemolyticus* OR vibrionaceae)	*vibrio* OR *'v parahaemolyticus'* OR *'vibrio parahaemolyticus'* OR vibrionaceae	*Vibrio* OR *V. parahaemolyticus* OR *Vibrio parahaemolyticus* OR vibrionaceae
Results	76,336	36,522	63,377

SI table 3. Key word search terms for environment utilized in the scoping literature review and number of results for each data base.

Keywords \ Database	Web of Science	Embase	ProQuest Ag&Env
keywords	TS= (Ecology OR Environmental OR Microbial Ecology OR Aquatic Microbiology OR Marine Water OR	ecology OR environmental OR 'microbial ecology' OR 'aquatic microbiology' OR 'marine water' OR	Ecology OR Environmental OR Microbial Ecology OR Aquatic Microbiology OR Marine Water OR Estuary OR Ocean OR Fresh Water OR

	Estuary OR Ocean OR Fresh Water OR freshwater OR Environment OR Estuarine OR population density OR surface water OR Bay OR Water OR Embayment)	estuary OR ocean OR 'fresh water' OR freshwater OR environment OR estuarine OR 'population density' OR 'surface water' OR bay OR water OR embayment	freshwater OR Environment OR Estuarine OR population density OR surface water OR Bay OR Water OR Embayment
Results	17,910,028	3,189,663	8,963,178

SI table 4. Key word search terms for occurrence utilized in the scoping literature review and number of results for each data base.

Keywords \ Database	Web of Science	Embase	ProQuest Ag&Env
keywords	TS= (Characterization OR Pattern OR Distribution OR Prevalence OR Identification OR Classification OR Incidence OR Density OR Concentration OR Season)	characterization OR pattern OR distribution OR prevalence OR identification OR classification OR incidence OR density OR concentration OR season	Characterization OR Pattern OR Distribution OR Prevalence OR Identification OR Classification OR Incidence OR Density OR Concentration OR Season
Results	24,101,931	8,576,545	8,782,552

SI Table 5. Combined key word search terms utilized in the scoping literature review and number of results for each data base.

Keywords \ Database	Web of Science	Embase	ProQuest Ag&Env
	TS= (oysters OR shellfish OR clams OR seafood OR bivalve OR aquaculture OR mariculture OR *oestridae* OR *ostreidae* OR *crassostrea* OR *magallana* OR *ostrea* OR *saccostrea*) AND TS=(*Vibrio* OR *V. parahaemolyticus* OR *Vibrio parahaemolyticus* OR vibrionaceae) AND TS=(Ecology OR Environmental OR Microbial Ecology OR Aquatic Microbiology OR Marine Water OR Estuary OR Ocean OR Fresh Water OR freshwater OR Environment OR Estuarine OR population density OR surface water OR Bay OR Water OR Embayment) AND TS=(Characterization OR Pattern OR Distribution OR Prevalence OR Identification OR Classification OR Incidence OR Density OR Concentration OR Season)	(oysters OR shellfish OR clams OR seafood OR bivalve OR aquaculture OR mariculture OR *oestridae* OR *ostreidae* OR *crassostrea* OR *magallana* OR *ostrea* OR *saccostrea*) AND (*vibrio* OR *'v parahaemolyticus'* OR *'vibrio parahaemolyticus'* OR vibrionaceae) AND (ecology OR environmental OR 'microbial ecology' OR 'aquatic microbiology' OR 'marine water' OR estuary OR ocean OR 'fresh water' OR freshwater OR environment OR estuarine OR 'population density' OR 'surface water' OR bay OR water OR embayment) AND (characterization OR pattern OR distribution OR prevalence OR identification OR classification OR incidence OR density OR concentration OR season)	(oysters OR shellfish OR clams OR seafood OR bivalve OR aquaculture OR mariculture OR *oestridae* OR *ostreidae* OR *crassostrea* OR *magallana* OR *ostrea* OR *saccostrea*) AND (*Vibrio* OR *V. parahaemolyticus* OR *Vibrio parahaemolyticus* OR vibrionaceae) AND (Ecology OR Environmental OR Microbial Ecology OR Aquatic Microbiology OR Marine Water OR Estuary OR Ocean OR Fresh Water OR freshwater OR Environment OR Estuarine OR population density OR surface water OR Bay OR Water OR Embayment) AND (Characterization OR Pattern OR Distribution OR Prevalence OR Identification OR Classification OR Incidence OR Density OR Concentration OR Season)
Results	6,323	1,553	9,023

SI table 7. Chlorophyll A, dissolved organic carbon, and suspended particulate matter report in studies. Empty cells represent the absence of reported parameter and study data.

Country	Site Name	Chlorophyll A (μg/L)				Dissolved Organic Carbon (mg/L)			Suspended Particulate Matter (mg/L)			Reference
		Minimum	Average	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	
Australia	All Sites											65
Bahrain	Site 1											170
	Site 2											
Brazil	All Sites											171
	All Sites											172
	Ariri Channel											19
Mexico	Isleta Tomate											173
Netherlands	Oostemchelde estuary											174
South Korea	Station 7											175
	All Sites											176
	All Sites											177
Taiwan	Cigu											178
	Dongshih											
	Shengang											
USA	Tangier Sound											179
	All Sites											180
	All Sites											61
	All sites											53
	Gulf of Mexico	2.0		316.2	13.8	2.8	11.2	5.1	3.2	316.2	26.3	

Country	Site Name	Chlorophyll A (μg/L)				Dissolved Organic Carbon (mg/L)			Suspended Particulate Matter (mg/L)			Reference
		Minimum	Average	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	
	Hood Canal	3.5		39.8	2.8	1.0	5.6	1.7	0.8	199.5	4.0	
	Tangier Sound	2.8		125.9	12.9	2.5	25.1	3.7	0.5	25.1	7.4	
	Grays Harbor											181
	All sites											182
	Chesapeake Bay											183
	All Sites											184
	All Sites											23
	Galveston Bay											185
	Dauphin Island Bay											63
	Tillamook Bay											56
	Yaquina Bay											
	Northwest Shellfish Sanitation Lab											186
	Galveston Bay											187
	Oyster											163
	Water											
	All sites	2.5	8.3	17.4								188
	Broad Creek		6.7									
	Chester River		9.3									

Country	Site Name	Chlorophyll A (µg/L)				Dissolved Organic Carbon (mg/L)			Suspended Particulate Matter (mg/L)			Reference
		Minimum	Average	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	
	Eastern Bay		8.9									
	All Sites											189
	All Sites											190
	US FDA Gulf Coast Seafood Laboratory (GCSL)		7.9									164
	University of Southern Mississippi Gulf Coast Research Laboratory (GCRL)		13.5									
	All Sites											191
	Belfair park, Hood canal	0.4	2.2	8.3	1.4							64
	Case Inlet, North Bay	1.0	6.8	24.3	4.3							
	Cushman Park	0.6	5.0	14.6	3.1							
	Dabob Bay	0.3	2.6	24	1.1							
	DNR Beach 48	1.9	3.6	5.3	3.5							
	Duckabush	0.3	3.2	20.4	2.2							
	Eld Inlet	0.8	7.6	31.2	5.7							

Country	Site Name	Chlorophyll A (μg/L)				Dissolved Organic Carbon (mg/L)			Suspended Particulate Matter (mg/L)			Reference
		Minimum	Average	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	
	Grays Harbor	0.1	3.5	9.8	3.2							
	Hama Hama, Hood canal	0.2	1.7	7.2	0.9							
	Hammersley Inlet	0.5	3.0	8.4	3.0							
	Hoodsport	0.4	3.8	27.4	2.1							
	Mike's beach, Hood canal	0.5	5.1	24.6	3.2							
	Misery point	0.8	6.8	58.3	3.1							
	Mystery Bay	0.9	13.9	62.9	8.1							
	Nahcotta	0.3	10.0	84.2	4.4							
	Oakland Bay	0.4	8.5	118.4	3.6							
	Potlatch, Hood canal	0.5	3.9	17.9	2.4							
	Quilcene Bay	0.2	4.7	34.2	1.6							
	Rocky Bay	1.9	5.4	15.3	3.7							
	Samish Bay	1.1	3.1	16.8	2.1							
	Sister's point, Hood canal	0.5	3.3	24.6	2.4							
	South Totten Inlet	0.5	8.3	70.4	3.2							
	Stackpole Harbor	20.7	20.7	20.7	20.7							
	Thorndyke Bay	1.9	3.9	5.1	4.3							
	Totten Inlet	0.9	3.4	15.9	2.5							

Country	Site Name	Chlorophyll A (µg/L)				Dissolved Organic Carbon (mg/L)			Suspended Particulate Matter (mg/L)			Reference
		Minimum	Average	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	
	Twanoh park, Hood canal	0.1	2.7	16.8	1.5							

SI table 8. Dissolved oxygen reported for each study. Empty cells represent the absence of parameter and study data.

Country	Site Name	Surface Dissolved Oxygen (ppm)			Bottom Dissolved Oxygen (ppm)			Dissolved Oxygen (ppm)			Reference
		Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	
Australia	All Sites										65
Bahrain	Site 1							4.9	5.9	6.7	170
	Site 2							5.9	5.8	6.2	
Brazil	All Sites										171
	All Sites										172
	Ariri Channel										19
Mexico	Isleta Tomate										173
Netherlands	Oostemchelde estuary										174
South Korea	Station 7										175
	All Sites										176
	All Sites										177
Taiwan	Cigu	4.1	7.3	10.1	4.1	7.9	12.9				178
	Dongshih	4.2	7.6	9.3	4.1	9.9	25.8				
	Shengang	2.9	5.9	8.2	3.8	5.8	8.2				
USA	Tangier Sound										179
	All Sites										180
	All Sites										61
	All sites										53
	Gulf of Mexico										
	Hood Canal										
	Tangier Sound										
	Grays Harbor										181

Country	Site Name	Surface Dissolved Oxygen (ppm)			Bottom Dissolved Oxygen (ppm)			Dissolved Oxygen (ppm)			Reference
		Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	
	All sites										182
	Chesapeake Bay								5.0		183
	All Sites										184
	All Sites										23
	Galveston Bay										185
	Dauphin Island Bay										63
	Tillamook Bay										56
	Yaquina Bay										
	Northwest Shellfish Sanitation Lab										186
	Galveston Bay							7.0		11.5	187
	Oyster										163
	Water										
	All sites							5.3		13.8	188
	Broad Creek								8.3		
	Chester River								9.5		
	Eastern Bay								9.5		
	All Sites										189
	All Sites										190
	US FDA Gulf Coast Seafood Laboratory (GCSL)										164

Country	Site Name	Surface Dissolved Oxygen (ppm)			Bottom Dissolved Oxygen (ppm)			Dissolved Oxygen (ppm)			Reference
		Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	
	University of Southern Mississippi Gulf Coast Research Laboratory (GCRL)										
	All Sites										191
	Belfair park, Hood canal										64
	Case Inlet, North Bay										
	Cushman Park										
	Dabob Bay										
	DNR Beach 48										
	Duckabush										
	Eld Inlet										
	Grays Harbor										
	Hama Hama, Hood canal										
	Hammersley Inlet										
	Hoodsport										
	Mike's beach, Hood canal										
	Misery point										
	Mystery Bay										

Country	Site Name	Surface Dissolved Oxygen (ppm)			Bottom Dissolved Oxygen (ppm)			Dissolved Oxygen (ppm)			Reference
		Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	
	Nahcotta										
	Oakland Bay										
	Potlatch, Hood canal										
	Quilcene Bay										
	Rocky Bay										
	Samish Bay										
	Sister's point, Hood canal										
	South Totten Inlet										
	Stackpole Harbor										
	Thorndyke Bay										
	Totten Inlet										
	Twanoh park, Hood canal										

SI table 9. pH, wind velocity, and turbidity reported by each study. Empty cells represent the absence of parameter and study data.

Country	Site Name	pH			Wind Velocity (MPH)		Turbidity (NTU)				Reference
		Minimum	Mean	Maximum	Minimum	Maximum	Minimum	Mean	Maximum	Median	
Australia	All Sites										65
Bahrain	Site 1	7.6	7.8	8.0							170
	Site 2	7.7	7.8	8.0							
Brazil	All Sites		7.5								171
	All Sites										172
	Ariri Channel										19
Mexico	Isleta Tomate										173
Netherlands	Oostemchelde estuary										174
South Korea	Station 7										175
	All Sites										176
	All Sites										177
Taiwan	Cigu	6.9	7.6	8.0							178
	Dongshih	7.6	7.8	8.1							
	Shengang	7.3	7.7	8.5							
USA	Tangier Sound										179
	All Sites										180
	All Sites										61
	All sites										53
	Gulf of Mexico										
	Hood Canal										
	Tangier Sound										
	Grays Harbor										181

Country	Site Name	pH			Wind Velocity (MPH)		Turbidity (NTU)				Reference
		Minimum	Mean	Maximum	Minimum	Maximum	Minimum	Mean	Maximum	Median	
	All sites										182
	Chesapeake Bay										183
	All Sites										184
	All Sites										23
	Galveston Bay										185
	Dauphin Island Bay										63
	Tillamook Bay										56
	Yaquina Bay										
	Northwest Shellfish Sanitation Lab										186
	Galveston Bay	8.0		8.8	3.0	25.0					187
	Oyster						1.0		47.0	8.0	163
	Water						0.0		47.0	7.0	
	All sites	7.3		8.5			0.5		2.5		188
	Broad Creek		7.8					1.2			
	Chester River		7.7					0.9			
	Eastern Bay		7.9					1.1			
	All Sites										189
	All Sites										190
	US FDA Gulf Coast Seafood Laboratory (GCSL)							6.1			164
	University of Southern							10.9			

Country	Site Name	pH			Wind Velocity (MPH)		Turbidity (NTU)				Reference
		Minimum	Mean	Maximum	Minimum	Maximum	Minimum	Mean	Maximum	Median	
	Mississippi Gulf Coast Research Laboratory (GCRL)										
	All Sites										191
	Belfair park, Hood canal										64
	Case Inlet, North Bay										
	Cushman Park										
	Dabob Bay										
	DNR Beach 48										
	Duckabush										
	Eld Inlet										
	Grays Harbor										
	Hama Hama, Hood canal										
	Hammersley Inlet										
	Hoodsport										
	Mike's beach, Hood canal										
	Misery point										
	Mystery Bay										
	Nahcotta										
	Oakland Bay										

Country	Site Name	pH			Wind Velocity (MPH)		Turbidity (NTU)				Reference
		Minimum	Mean	Maximum	Minimum	Maximum	Minimum	Mean	Maximum	Median	
	Potlatch, Hood canal										
	Quilcene Bay										
	Rocky Bay										
	Samish Bay										
	Sister's point, Hood canal										
	South Totten Inlet										
	Stackpole Harbor										
	Thorndyke Bay										
	Totten Inlet										
	Twanoh park, Hood canal										

SI table 10. Total suspended solid, turbidity, and water depth reported by each study. Empty cells represent the absence of parameter and study data.

Country	Site Name	Total Suspended Solid (mg/L)	Turbidity (m)	Water Depth (m)	Reference
Australia	All Sites				65
Bahrain	Site 1			9.5	170
	Site 2			20.0	
Brazil	All Sites				171
	All Sites				172
	Ariri Channel				19
Mexico	Isleta Tomate				173
Netherlands	Oostemchelde estuary				174
South Korea	Station 7				175
	All Sites				176
	All Sites				177
Taiwan	Cigu				178
	Dongshih				
	Shengang				
USA	Tangier Sound				179
	All Sites				180
	All Sites				61
	All sites				53
	Gulf of Mexico				
	Hood Canal				
	Tangier Sound				
	Grays Harbor				181
	All sites				182
	Chesapeake Bay	25.0	20.0		183
	All Sites				184
	All Sites				23
	Galveston Bay				185
	Dauphin Island Bay				63
	Tillamook Bay				56
	Yaquina Bay				
	Northwest Shellfish Sanitation Lab				186
	Galveston Bay				187
	Oyster				163
	Water				

Country	Site Name	Total Suspended Solid (mg/L)	Turbidity (m)	Water Depth (m)	Reference
	All sites				188
	Broad Creek				
	Chester River				
	Eastern Bay				
	All Sites				189
	All Sites				190
	US FDA Gulf Coast Seafood Laboratory (GCSL)				164
	University of Southern Mississippi Gulf Coast Research Laboratory (GCRL)				
	All Sites				191
	Belfair park, Hood canal				64
	Case Inlet, North Bay				
	Cushman Park				
	Dabob Bay				
	DNR Beach 48				
	Duckabush				
	Eld Inlet				
	Grays Harbor				
	Hama Hama, Hood canal				
	Hammersley Inlet				
	Hoodsport				
	Mike's beach, Hood canal				
	Misery point				
	Mystery Bay				
	Nahcotta				
	Oakland Bay				
	Potlatch, Hood canal				
	Quilcene Bay				
	Rocky Bay				
	Samish Bay				
	Sister's point, Hood canal				
	South Totten Inlet				
	Stackpole Harbor				
	Thorndyke Bay				
	Totten Inlet				
	Twanoh park, Hood canal				

Appendix B. Chapter 3

SI Table 1. Summary data for environmental parameters measured by site.

Site	Temperature (° C)		Salinity (ppt)		Chlorophyll A (µg/L)	
	Mean (SD)	Minimum / Maximum	Mean (SD)	Minimum / Maximum	Mean (SD)	Minimum / Maximum
NBS1	21.5 (0.90)	20.4 / 22.5	33.8 (0.33)	33.3 / 34.0	1.87 (0.61)	1.08 / 2.37
NBS2	22.4 (0.50)	21.7 / 22.9	33.9 (0.10)	33.8 / 34.0	2.70 (0.15)	2.51 / 2.82
NBS3	23.1 (0.22)	22.8 / 23.3	33.6 (0.60)	32.8 / 34.2	2.80 (0.64)	2.23 / 3.66
NBS4	22.8 (0.13)	22.6 / 22.9	33.8 (0.19)	33.6 / 34.0	2.23 (0.83)	1.14 / 3.12
NBS5	23.0 (0.36)	22.5 / 23.3	33.5 (0.26)	33.1 / 33.7	2.38 (0.67)	1.62 / 2.99
NBS6	23.0 (0.50)	22.4 / 23.6	33.3 (0.56)	32.5 / 33.7	2.28 (0.32)	2.06 / 2.76
NBS7	23.6 (0.27)	23.2 / 23.8	33.3 (0.15)	33.2 / 33.5	1.76 (0.46)	1.18 / 2.21
NBS8	23.2 (0.29)	22.9 / 23.6	33.6 (0.17)	33.4 / 33.8	1.76 (0.74)	0.72 / 2.44
NBS9	24.2 (1.07)	23.0 / 25.2	32.4 (1.72)	30.1 / 33.7	2.14 (0.84)	1.28 / 3.30
NBS11	23.8 (0.74)	22.9 / 24.7	31.7 (2.59)	27.9 / 33.6	4.07 (1.32)	2.77 / 5.27
NBS12	25.2 (1.91)	22.5 / 26.8	14.2 (7.76)	7.00 / 23.1	16.15 (10.61)	3.76 / 25.18
NBS13	25.3 (0.55)	24.7 / 25.9	24.7 (5.72)	17.8 / 29.8	17.3 (16.21)	2.60 / 38.34

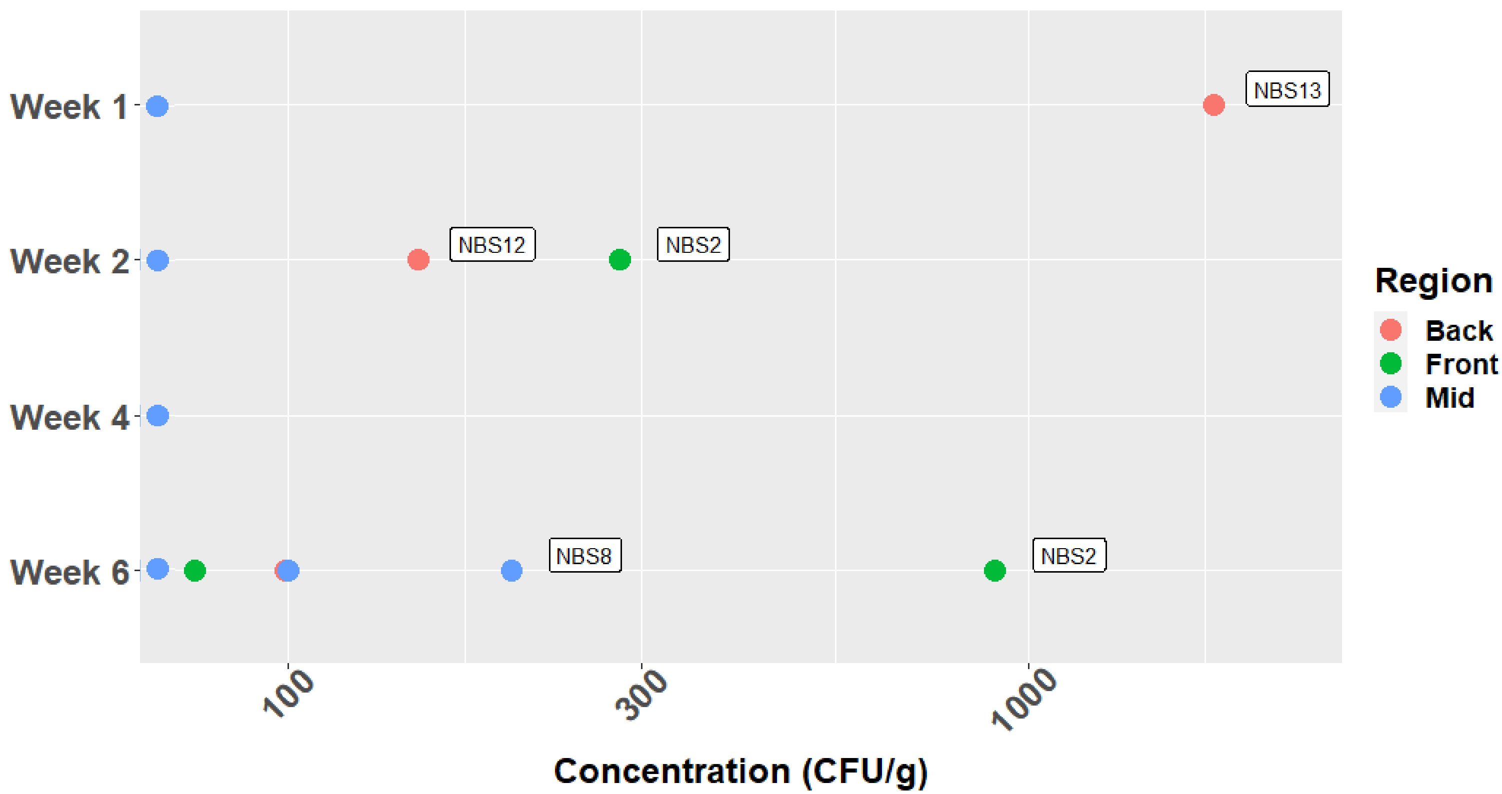
SI table 2. Summary data of *V. parahaemolyticus* isolates by site

Site	Presumptive *V. parahaemolyticus* isolates			Confirmed *V. parahaemolyticus* isolates (*toxR*)			*tdh*		trh	
	Oyster	Water	Total	Oyster	Water	Total	Oyster	Water	Oyster	Water
1	6	20	26	3	1	4	0	0	0	0
2	0	23	23	0	4	4	0	0	0	0
3	5	14	19	3	2	4	0	0	0	1
4	3	16	19	3	0	3	0	0	0	1
5	3	16	19	1	2	3	0	0	0	1
6	10	17	27	1	3	4	0	0	1	2
7	3	15	18	3	2	5	0	0	0	0
8	2	17	19	0	4	4	0	0	0	0
9	3	19	22	2	17	19	0	0	0	0
11	11	14	25	10	10	20	0	0	0	2
12	11	20	31	11	14	25	0	0	0	2
13	22	21	43	21	19	40	0	0	0	1
Day 1	3	NA	3	0	NA	0	0	NA	0	NA
Day 2	5	NA	5	0	NA	0	0	NA	0	NA
Total	87	212	299	58	78	135	0	0	1	10

Limit of detection was 1 CFU per g or mL; NA was not sampled

SI Table 3. Summary data of *V. vulnificus* isolates by site

Site	Presumptive *V. vulnificus* isolates			Confirmed *V. vulnificus* isolates (*vvhA*)			*pilF*		*vcgE*		*vcgC*	
	Oyster	Water	Total	Oyster	Water	Total	Oyster	Water	Oyster	Water	Oyster	Water
1	4	13	17	1	0	1	0	0	0	0	0	0
2	25	24	49	6	3	9	1	1	0	0	0	1
3	1	21	22	0	10	10	0	1	0	2	0	6
4	23	3	26	0	3	3	0	3	0	0	0	3
5	5	19	24	0	2	2	0	2	0	1	0	1
6	3	23	26	0	7	7	0	6	0	6	0	0
7	2	23	25	0	4	4	0	2	0	0	0	1
8	16	26	42	2	3	5	0	0	0	0	0	0
9	9	19	28	1	5	6	0	2	0	2	0	0
11	19	27	46	2	16	18	0	8	0	7	0	1
12	10	37	47	1	18	19	0	14	0	12	0	2
13	28	24	52	2	11	13	1	8	0	5	0	0
Day 1	3	0	3	1	NA	1	0	NA	0	NA	1	NA
Day 2	8	0	8	1	NA	1	1	NA	0	NA	0	NA
Total	156	259	415	17	82	99	3	47	0	35	1	15



SI Figure 1. Concentrations of *V. vulnificus* in oyster by sampling week and region. Site number is displayed when concentrations of *V. vulnificus* was greater than 100 CFU/g.

Appendix C. Chapter 4

Table S2. Newport Bay air temperatures used to estimate average air temperature distribution.

Day	January	February	March	April	May	June	July	August	September	October	November	December
1	54.3	54.8	56.6	57.4	60.4	62.5	66	68.7	68.7	66.4	61.9	57.8
2	54.7	55.7	56.2	57.6	60.9	62.8	66.2	68.6	68.2	66.2	62.3	57.2
3	54.5	55.6	56	57.6	61.1	63	66	68.5	68.5	65.7	62	57.5
4	54.6	56	56	58	61.2	63.2	66	68.3	68.3	65.9	61.4	57.3
5	54.9	55.8	56.3	58.6	61	63.2	66.1	68.2	68.4	65.9	61.2	57.2
6	55.1	55.9	56.6	58.4	61.2	63.3	66.1	68.5	68.4	65.8	61.5	56.8
7	55.5	55.9	56.6	58.5	60.9	63.3	66.5	68.6	68.3	65.6	61.4	56.9
8	55.1	56	56.7	58.7	61.1	63.3	66.4	68.4	68	65.2	61.1	56.8
9	55.4	55.9	56.7	58	61	63.4	66.8	68.7	68	65.4	61.1	56.8
10	55.1	55.9	56.3	58.3	61	63.7	67	68.9	67.6	64.8	60.6	56.8
11	55.3	56.1	56.5	58.7	61.1	63.9	67	68.8	67.7	64.5	60.1	56.6
12	55.6	56.3	57	58.7	61.3	64	67.1	68.5	67.4	64.4	60.1	56.7
13	55.5	56.3	56.9	59.1	61.5	64.1	67.5	68.8	67	64.6	60.3	56
14	55.6	55.9	56.7	58.9	61.8	64.1	67.4	68.5	67	65	60	55.9
15	55.3	56.1	56.9	58.8	61.8	64	67.6	68.5	67.1	64.8	59.6	55.9
16	55.9	56.2	57.3	59	62	64	67.5	68.4	67.3	64.5	59.6	55.9
17	55.7	56.2	57.5	58.9	61.9	64.2	67.7	68.6	67.5	64.4	59.5	56.1
18	55.5	55.9	57.1	58.9	61.9	64.4	67.7	68.5	67.1	64.2	58.9	55.8
19	55.2	55.8	57.4	59.3	61.8	64.5	67.9	68.6	66.7	64.1	59.1	55.7
20	55.1	55.8	57.8	59.3	61.8	65	67.9	68.2	66.9	64.3	59.2	55.7
21	54.7	56.1	57.4	59.3	61.8	65	67.9	68.1	66.6	63.9	58.9	55.6
22	54.8	56.1	57	59.3	61.8	64.9	67.7	68.3	66.7	63.6	58.9	55.3
23	55.1	56.4	57.1	59.9	62	65.1	68	68.4	66.8	64	58.7	55.3

Day	January	February	March	April	May	June	July	August	September	October	November	December
24	55.7	56.1	57.2	59.6	61.8	65	68	68.4	66.9	63.7	58.6	55.2
25	55.7	55.7	57.8	59.5	61.7	65.1	68.5	68.5	67	63.4	58.8	55.6
26	55.6	56.3	57.6	59.6	62.1	65.5	68.3	68.5	66.5	62.8	58.3	55
27	55.9	55.9	57.7	59.9	62.1	65.7	68.5	68.6	66.5	63.1	58.2	54.6
28	55.5	55.7	58.2	60.1	62.5	65.5	68.5	68.4	66.8	62.9	57.9	55.2
29	55.4	56.5	57.8	60	62.5	65.3	68.5	68.4	66.6	62.6	57.9	55.6
30	55.5		57.8	60	62.7	65.6	68.6	68.4	66.4	62.5	57.7	54.7
31	54.9		57.8		62.6		68.6	68.3		62.1		54.9

SI Table 2. Concentration and frequency of *V. parahaemolyticus* from Pacific oysters held at 4 and 10 °C for 7 days.

Temperature (°C)	Time (Hr)	Presumptive colonies	Confirmed colonies	Confirmed frequency	Presumptive Concentration (CFU/g)	Confirmed Concentration (CFU/g)
4	0	2	1	0.33	20	6.67
4	2	1	1	1.00	10	10.00
4	6	2	0	0.00	20	0.00
4	12	8	0	0.00	100	0.00
4	24	9	0	0.00	120	0.00
4	36	5	2	0.29	60	17.14
4	48	2	2	0.50	20	10.00
4	72	20	1	0.05	320	15.24
4	96	6	5	0.45	100	45.45
4	120	10	2	0.17	110	18.33
4	144	13	0	0.00	180	0.00
4	168	20	0	0.00	90	0.00
20	0	4	2	0.50	40	20.00
20	2	10	1	0.10	80	8.00
20	6	1	0	0.00	10	0.00
20	12	6	0	0.00	400	0.00
20	24	13	0	0.00	1000	0.00
20	36	2	2	1.00	20	20.00
20	48	12	8	0.67	200	133.33
20	72	0	0	0.00	0.1	0.00
20	96	13	5	0.38	750	288.46
20	120	2	1	0.50	50	25.00
20	144	20	0	0.00	600	0.00
20	168	20	1	0.05	800	40.00

SI Table 3. Weight of Pacific oysters harvested from Newport Bay.

Unique ID	Oyster mass (g)
1	9.97
2	24.54
3	21.5
4	18.35
5	25.18
6	11.5
7	17.14
8	22.78
9	15.36
10	16.4
11	22.86
12	26.9
13	29.26
14	15.45
15	8.28
16	7.88
17	7.76
18	18.44
19	13.82
20	25.26
21	26.5
22	5.9
23	23.3
24	17.06
25	37.46
26	24.33
27	25.03
28	6.85
29	10.53
30	4.29
31	21.06
32	25.54
33	13.52
34	8.56
35	12.53
36	12.54
37	18.49

Unique ID	Oyster mass (g)
38	9.46
39	8.49
40	24.83
41	5.93
42	38.81
43	10.83
44	21.52
45	13.19
46	11.19
47	9.7
48	13.73
49	6.5
50	18.65
51	16.33
52	13.42
53	15.75
54	15.16
55	16.24
56	16.77
57	11.37
58	18.81
59	16.71
60	14.33
61	25.29
62	28.86
63	14.51
64	23.85
65	20.29
66	23.588
67	5.78
68	24
69	19.79
70	13.17
71	13.31
72	19.7
73	11.01
74	6.23
75	22.84
76	21.56

Unique ID	Oyster mass (g)
77	8.13
78	19.44
79	31.17
80	7.07
81	8.69
82	11.63
83	27.37
84	13.81
85	8.33
86	17.73
87	35.46
88	17.64
89	16.86
90	33.77
91	29.17
92	3.88
93	24.78
94	11.28
95	24.42
96	22.45
97	28.02
98	6.57
99	11.36
100	31.81
101	16.52
102	45.31
103	23.77
104	9.22
105	21.86
106	18.75
107	16.73
108	32.25
109	17.48
110	19.53
111	16.48
112	24.24
113	24.24
114	15.4
115	32.24

Unique ID	Oyster mass (g)
116	39.27
117	8.88
118	8.37
119	19.43
120	39.27
121	37.66
122	23.96
123	29
124	39.55
125	21.74
126	18.02
127	11.21
128	17.83
129	39.51
130	23.07
131	31.5
132	19.6
133	15.3
134	25.3
135	13.15
136	20.56
137	38.22
138	29.48
139	31.99
140	29.37
141	17.52
142	26.72
143	9.69
144	8.48

SI Table 4. Scenarios used to calculate concentration of *V. parahaemolyticus* in oysters

	Scenario 1: Alaska		Scenario 2: Alaska		Scenario 3: US FDA	
V. parahaemolyticus concentration, oyster	2.1	MPN/g	3.5	MPN/g	2100.0	/g
Pathogenic *V. parahaemolyticus*	74	%	74	%		
	1.554	MPN/g	3	MPN/g	3.6	/g
Approximate oyster weight	15	g	15	g	15	g

SI Table 5. Oyster consumption frequency in the USA and Alaska

	Minimum	Median/mode	Maximum
US FDA	6	12	24
Alaska cruises	1	1	6

References

1. Cawthra HC, Anderson RJ, De Vynck JC, Jacobs Z, Jerardino A, Kyriacou K, Marean CW. Migration of Pleistocene shorelines across the Palaeo-Agulhas Plain: Evidence from dated sub-bottom profiles and archaeological shellfish assemblages. *Quat Sci Rev*. 2020;235:106107. doi:10.1016/j.quascirev.2019.106107

2. NPR. Who Ate the First Oyster? Cave May Hold an Answer. Accessed February 4, 2021. https://www.npr.org/templates/story/story.php?storyId=15391834

3. CDC. *National Enteric Disease Surveillance: COVIS Annual Summary, 2014*.; 2016. Accessed June 24, 2020. http://www.cdc.gov/

4. Thurston-Enriquez JA, Haas CN, Jacangelo J, Gerba CP. Chlorine inactivation of adenovirus type 40 and feline calicivirus. *Appl Environ Microbiol*. 2003;69(7):3979-3985. doi:10.1128/AEM.69.7.3979

5. Mackenzie CL. Causes underlying the historical decline in eastern oyster (*Crassostrea virginica* Gmelin, 1791) landings. *J Shellfish Res*. 2007;26(4):927-938. doi:10.2983/0730-8000(2007)26[927:CUTHDI]2.0.CO;2

6. Bostock J, McAndrew B, Richards R, Jauncey K, Telfer T, Lorenzen K, Little D, Ross L, Handisyde N, Gatward I, Corner R. Aquaculture: global status and trends. *Philos Trans R Soc B Biol Sci*. 2010;365(1554):2897-2912. doi:10.1098/rstb.2010.0170

7. FAO. 2. Aquaculture production of fish and. Accessed October 29, 2020. http://www.fao.org/3/T8211E/t8211e03.htm

8. FAO. *Oysters Aquaculture Production by Species and Country or Area*.; 2020.

9. NOAA. Fisheries of the United States. In: ; 2013:41-50. doi:10.1029/ln010p0041

10. Dumbauld BR, Ruesink JL, Rumrill SS. The ecological role of bivalve shellfish aquaculture in the estuarine environment: A review with application to oyster and clam culture in West Coast (USA) estuaries. doi:10.1016/j.aquaculture.2009.02.033

11. Crooks JA, Crooks KR, Crooks AJ. Observations of the non-native Pacific oyster (*Crassostrea gigas*) in San Diego County, California. *Calif Fish Game*. 2015;101(2):101-107.

12. NOAA. Commercial Shellfish Aquaculture on the West Coast.

13. ISSC. *ISSC Mission: Comprehensive Safety*.; 2011. Accessed September 15, 2020. http://www.issc.org

14. Pasquier J. *Essai Médical Sur Les Huîtres, Etc*.; 1818. Accessed February 4, 2021. https://books.google.com/books?hl=en&lr=&id=fxCy18oVyDEC&oi=fnd&pg=PR3&ots=8c_sMTLHO4&sig=dp9Hyw7CfFPzM4KA19hCbrfmMBo

15. ISSC. *Interstate Shellfish Sanitation Conference Consitution, Bylaws, Adn Prodcedures*.; 2009. www.issc.org

16. Potasman I, Paz A, Odeh M. Infectious outbreaks associated with bivalve shellfish consumption: A worldwide perspective. *Clin Infect Dis*. 2002;35(8):921-928. doi:10.1086/342330

17. Lees D. Viruses and bivalve shellfish. *Int J Food Microbiol*. 2000;59(1-2):81-116. doi:10.1016/S0168-1605(00)00248-8

18. Konno T, Chimoto T, Taneichi K, Deno M, Yoshizaya T, Kimura O, Sibaki H, Konno M,

Kojima H. Oyster-associated hepatitis A. *Hokkaido Igaku Zasshi*. 1983;58(5):553-555. doi:10.1001/jama.1975.03260100035016

19. Ristori CA, Iaria ST, Gelli DS, Rivera ING. Pathogenic bacteria associated with oysters (*Crassostrea brasiliana*) and estuarine water along the south coast of Brazil. *Int J Environ Health Res*. 2007;17(4):259-269. doi:10.1080/09603120701372169

20. Crim SM, Griffin PM, Tauxe R, Marder EP, Gilliss D, Cronquist AB, Cartter M, Tobin-D'Angelo M, Blythe D, Smith K, Lathrop S, Zansky S, Cieslak PR, Dunn J, Holt KG, Wolpert B, Henao OL, Centers for Disease Control and Prevention (CDC). Preliminary incidence and trends of infection with pathogens transmitted commonly through food - Foodborne Diseases Active Surveillance Network, 10 U.S. sites, 2006-2014. *MMWR Morb Mortal Wkly Rep*. 2015;64(18):495-499. Accessed August 6, 2020. http://www.ncbi.nlm.nih.gov/pubmed/25974634

21. Scallan E, Hoekstra RM, Angulo FJ, Tauxe R V., Widdowson MA, Roy SL, Jones JL, Griffin PM. Foodborne illness acquired in the United States-Major pathogens. *Emerg Infect Dis*. 2011;17(1):7-15. doi:10.3201/eid1701.P11101

22. Newton A, Kendall M, Vugia DJ, Henao OL, Mahon BE. Increasing rates of vibriosis in the United States, 1996-2010: Review of surveillance data from 2 systems. *Clin Infect Dis*. 2012;54(SUPPL.5). doi:10.1093/cid/cis243

23. Depaola A, Hopkins LH, Peeler JT, Wentz B, Mcphearson ' RM. Incidence of *Vibrio parahaemolyticus* in U.S. coastal waters and oysters from shellfish- growing areas in. *Appl Environ Microbiol*. 1990;56(8):2299-2302. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC184726/pdf/aem00089-0031.pdf

24. Allen K. The Transmission of Typhoid by Sewage Polluted Oysters. *Public Health Pap Rep*. 1899;25:154-15465.

25. Rippey SR. Infectious diseases associated with molluscan shellfish consumption. *Clin Microbiol Rev*. 1994;7(4):419-425. doi:10.1128/CMR.7.4.419

26. Thompson FL, Iida T, Swings J. Biodiversity of Vibrios. *Microbiol Mol Biol Rev*. Published online 2004. doi:10.1128/mmbr.68.3.403-431.2004

27. Lovell CR. Ecological fitness and virulence features of *Vibrio parahaemolyticus* in estuarine environments. *Appl Microbiol Biotechnol*. Published online 2017. doi:10.1007/s00253-017-8096-9

28. CDC. *Vibrio* species causing vibriosis. Published 2019. Accessed June 24, 2020. https://www-cdc-gov.ezproxy4.library.arizona.edu/vibrio/index.html

29. Ralston EP, Kite-Powell H, Beet A. An estimate of the cost of acute health effects from food- and water-borne marine pathogens and toxins in the USA. *J Water Health*. 2011;9(4):680-694. doi:10.2166/wh.2011.157

30. National Center for Emerging C, Infectious Diseases Z. *National Enteric Disease Surveillance: COVIS Annual Summary, 2014.*; 2014. Accessed June 24, 2020. http://www.cdc.gov/

31. Froelich BA, Noble RT. *Vibrio* bacteria in raw oysters: Managing risks to human health. *Philos Trans R Soc B Biol Sci*. 2016;371(1689). doi:10.1098/rstb.2015.0209

32. Yeung PSM, Boor KJ. Epidemiology, pathogenesis, and prevention of foodborne *Vibrio parahaemolyticus* infections. *Foodborne Pathog Dis*. Published online 2004.

doi:10.1089/153531404323143594

33. Weis KE, Hammond RM, Hutchinson R, Blackmore CGM. *Vibrio* illness in Florida, 1998-2007. *Epidemiol Infect*. 2011;139(4):591-598. doi:10.1017/S0950268810001354

34. Fujino T, Okuno Y, Nakada D, Aoyama A, Mukai T, Ueho T. On the bacteriological examination of shirasu food poisoning. *Med J Osaka Univ*. Published online 1953.

35. Dadisman TA, Nelson R, Molenda JR, Garber HJ. *Vibrio parahaemolyticus* gastroenteritis in Maryland I. Clinical and epidemiologic aspects. *Am J Epidemiol*. 1972;96(6):414-426. doi:10.1093/oxfordjournals.aje.a121474

36. CDC. *National Enteric Vibrio Cases Reported to CDC, 1997 - 1998*.; 1999.

37. CDC. *National Enteric Vibrio Cases Reported to CDC, 2004*.; 2006.

38. Daniels NA, Mackinnon L, Bishop R, Altekruse S, Ray B, Hammond RM, Thompson S, Wilson S, Bean NH, Griffin PM, Slutsker L. *Vibrio parahaemolyticus* infections in the United States, 1973-1998. *J Infect Dis*. 2000;181(5):1661-1666. doi:10.1086/315459

39. CDC. Selected Outbreaks. Accessed October 30, 2020. https://www-cdc-gov.ezproxy4.library.arizona.edu/vibrio/outbreaks.html

40. ISSC. Notices. Accessed October 30, 2020. http://www.issc.org/notices

41. McLaughlin JB, DePaola A, Bopp CA, Martinek KA, Napolilli NP, Allison CG, Murray SL, Thompson EC, Bird MM, Middaugh JP. Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. *N Engl J Med*. 2005;353(14):1463-1470. doi:10.1056/NEJMoa051594

42. Baker-Austin C, Trinanes JA, Taylor NGH, Hartnell R, Siitonen A, Martinez-Urtaza J.

Emerging *Vibrio* risk at high latitudes in response to ocean warming. *Nat Clim Chang*. 2013;3(1):73-77. doi:10.1038/nclimate1628

43. CDC. Multistate outbreak of gastrointestinal illnesses linked to oysters imported from Mexico. Published 2019. Accessed March 10, 2021. https://www-cdc-gov.ezproxy4.library.arizona.edu/vibrio/investigations/rawoysters-05-19/index.html

44. CDC. *Vibrio parahaemolyticus* infections linked to fresh crab meat imported from Venezuela. Published 2018. Accessed March 10, 2021. https://www-cdc-gov.ezproxy4.library.arizona.edu/vibrio/investigations/vibriop-07-18/index.html

45. CDC. *Vibrio parahaemolyticus* illnesses associated with consumption of shellfish, United States, 2013. Published 2013. Accessed March 10, 2021. https://www-cdc-gov.ezproxy4.library.arizona.edu/vibrio/investigations/vibriop-09-13/index.html

46. Haendiges J, Rock M, Myers RA, Brown EW, Evans P, Gonzalez-Escalona N. Pandemic *Vibrio parahaemolyticus*, Maryland, USA, 2012. *Emerg Infect Dis*. 2014;20(4):718-720. doi:10.3201/eid2004.130818

47. CDC. Vibrio parahaemolyticus infections associated with consumption of raw shellfish-Three states, 2006. Morbidity and mortality weekly report. Published August 2006. Accessed March 10, 2021. https://www-cdc-gov.ezproxy4.library.arizona.edu/mmwr/preview/mmwrhtml/mm55d807a1.htm

48. Oliver JDJD, Baker-Austin C, Oliver JDJD, Alam M, Ali A, Waldor MK, Qadri F, Martinez-Urtaza J. *Vibrio* spp. infections. *Nat Rev Dis Prim*. 2018;4(1):7. doi:10.1038/s41572-018-0005-8

49. Froelich BA, Daines DA. In hot water: effects of climate change on *Vibrio* –human interactions. *Environ Microbiol*. Published online March 10, 2020:1462-2920.14967. doi:10.1111/1462-2920.14967

50. US FDA. Bad bug book: Handbook of foodborne pathogenic microorganisms and natural toxins. *Bad bug B Handb Foodborne Pathog Microorg Nat Toxins*. Published online 2012:292.

51. Baker-Austin C, Trinanes J, Gonzalez-Escalona N, Martinez-Urtaza J. Non-Cholera *Vibrios*: The microbial barometer of climate change. *Trends Microbiol*. 2017;25(1):76-84. doi:10.1016/j.tim.2016.09.008

52. Baker-Austin C, Stockley L, Rangdale R, Martinez-Urtaza J. Environmental occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: A European perspective. *Environ Microbiol Rep*. Published online 2010. doi:10.1111/j.1758-2229.2009.00096.x

53. Johnson CN, Bowers JC, Griffitt KJ, Molina V, Clostio RW, Pei S, Laws E, Paranjpye RN, Strom MS, Chen A, Hasan NA, Huq A, Noriea NF, Grimes DJ, Colwell RR, Noriea Iii NF, Grimes DJ, Colwell RR. Ecology of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in the coastal and estuarine waters of Louisiana, Maryland, Mississippi, and Washington (United States). *Appl Environ Microbiol*. 2012;78(20):7249-7257. doi:10.1128/AEM.01296-12

54. Paranjpye RN, Nilsson WB, Liermann M, Hilborn ED, George BJ, Li Q, Bill BD, Trainer VL, Strom MS, Sandifer PA. Environmental influences on the seasonal distribution of *Vibrio parahaemolyticus* in the Pacific Northwest of the USA. *FEMS Microbiol Ecol*. 2015;91(12). doi:10.1093/femsec/fiv121

55. Flynn A, Davis BJK, Atherly E, Olson G, Bowers JC, DePaola A, Curriero FC. Associations

of environmental conditions and *Vibrio parahaemolyticus* genetic markers in Washington state Pacific oysters. *Front Microbiol*. 2019;10:2797. doi:10.3389/fmicb.2019.02797

56. Duan J, Su Y-C, An U. Occurrence of *Vibrio parahaemolyticus* in Two Oregon Oyster-growing Bays. *J Food Sci*. 2005;70(1):M58-M63. doi:10.1111/j.1365-2621.2005.tb09047.x

57. Nishibuchi M, Fasano A, Russell RG, Kaper JB. Enterotoxigenicity of *Vibrio parahaemolyticus* with and without genes encoding thermostable direct hemolysin. *Infect Immun*. 1992;60(9):3539-3545. doi:10.1128/iai.60.9.3539-3545.1992

58. Honda T, Ni Y, Miwatani T. Purification and characterization of a hemolysin produced by a clinical isolate of Kanagawa phenomenon-negative *Vibrio parahaemolyticus* and related to the thermostable direct hemolysin. *Infect Immun*. 1988;56(4):961-965. doi:10.1128/iai.56.4.961-965.1988

59. Jones JL, Lüdeke CHM, Bowers JC, DeRosia-Banick K, Carey DH, Hastback W. Abundance of *Vibrio cholerae*, *V. vulnificus*, and *V. parahaemolyticus* in oysters (*Crassostrea virginica*) and clams (*Mercenaria mercenaria*) from Long Island Sound. *Appl Environ Microbiol*. 2014;80(24):7667-7672. doi:10.1128/AEM.02820-14

60. Klein SL, Gutierrez West CK, Mejia DM, Lovell CR. Genes similar to the *Vibrio parahaemolyticus* virulence-related genes *tdh*, *tlh*, and *vscC2* occur in other Vibrionaceae species isolated from a pristine estuary. Published online 2014. doi:10.1128/AEM.02895-13

61. Williams TC, Froelich BA, Phippen B, Fowler P, Noble RT, Oliver JD. Different abundance and correlational patterns exist between total and presumed pathogenic *Vibrio vulnificus* and *V. parahaemolyticus* in shellfish and waters along the North Carolina coast. *FEMS*

Microbiol Ecol. 2017;93(6). doi:10.1093/femsec/fix071

62. Kaufman GE, Bej AK, Bowers J, DePaola A. Oyster-to-oyster variability in levels of *Vibrio parahaemolyticus*. *J Food Prot*. 2003;66(1):125-129. doi:10.4315/0362-028X-66.1.125

63. Givens CE, Bowers JC, Depaola A, Hollibaugh JT, Jones JL. Occurrence and distribution of *Vibrio vulnificus* and *Vibrio parahaemolyticus* - potential roles for fish, oyster, sediment and water. *Lett Appl Microbiol*. 2014;58(6):503-510. doi:10.1111/lam.12226

64. Nilsson WB, Paranjpye RN, Hamel OS, Hard C, Strom MS. *Vibrio parahaemolyticus* risk assessment in the Pacific Northwest: it’s not what’s in the water. *FEMS Microbiol Ecol*. 2019;95:27. doi:10.1093/femsec/fiz027

65. Padovan A, Kennedy K, Rose D, Gibb K. Microbial quality of wild shellfish in a tropical estuary subject to treated effluent discharge. *Environ Res*. 2020;181(November 2019):108921. doi:10.1016/j.envres.2019.108921

66. Jones JL, Lüdeke CHM, Bowers JC, Garrett N, Fischer M, Parsons MB, Bopp CA, DePaola A. Biochemical, serological, and virulence characterization of clinical and oyster *Vibrio parahaemolyticus* isolates. *J Clin Microbiol*. 2012;50(7):2343-2352. doi:10.1128/JCM.00196-12

67. Park K-S, Ono T, Rokuda M, Jang M-H, Iida T, Honda T. Cytotoxicity and enterotoxicity of the Thermostable Direct Hemolysin-Deletion Mutants of *Vibrio parahaemolyticus*. *Microbiol Immunol*. 2004;48(4):313-318. doi:10.1111/j.1348-0421.2004.tb03512.x

68. Martinez-Urtaza J, Baker-Austin C, Jones JL, Newton AE, Gonzalez-Aviles GD, DePaola A. Spread of Pacific Northwest *Vibrio parahaemolyticus* Strain. *N Engl J Med*.

2013;369(16):1573-1574. doi:10.1056/nejmc1305535

69. Gonzalez-Escalona N, Gavilan RG, Toro M, Zamudio ML, Martinez-Urtaza J. Outbreak of *Vibrio parahaemolyticus* sequence type 120, Peru, 2009. *Emerg Infect Dis*. Published online 2016. doi:10.3201/eid2207.151896

70. Ansaruzzaman M, Lucas M, Deen JL, Bhuiyan NA, Wang XY, Safa A, Sultana M, Chowdhury A, Balakrish Nair G, Sack DA, Von Seidlein L, Puri MK, Ali M, Chaignat CL, Clemens JD, Barreto A. Pandemic serovars (O3:K6 and O4:K68) of *Vibrio parahaemolyticus* associated with diarrhea in Mozambique: Spread of the pandemic into the African continent. *J Clin Microbiol*. Published online 2005. doi:10.1128/JCM.43.6.2559-2562.2005

71. Haendiges J, Jones J, Myers RA, Mitchell CS, Butler E, Toro M, Gonzalez-Escalona N. A nonautochthonous U.S. strain of *Vibrio parahaemolyticus* isolated from Chesapeake Bay oysters caused the outbreak in Maryland in 2010. *Appl Environ Microbiol*. Published online 2016. doi:10.1128/AEM.00096-16

72. Martinez-Urtaza J, Powell A, Jansa J, Rey JLC, Montero OP, Campello MG, López MJZ, Pousa A, Valles MJF, Trinanes J, Hervio-Heath D, Keay W, Bayley A, Hartnell R, Baker-Austin C. Epidemiological investigation of a foodborne outbreak in Spain associated with U.S. West Coast genotypes of *Vibrio parahaemolyticus*. *Springerplus*. Published online 2016. doi:10.1186/s40064-016-1728-1

73. Jones MK, Oliver JD. *Vibrio vulnificus*: Disease and pathogenesis. *Infect Immun*. 2009;77(5):1723-1733. doi:10.1128/IAI.01046-08

74. Oliver JD. Wound infections caused by *Vibrio vulnificus* and other marine bacteria.

Epidemiol Infect. Published online 2005. doi:10.1017/S0950268805003894

75. Ito H, Shibayama A, Abe M, Antoku S, Nawata H, Isonishi M, Fujita M, Kato S. *Vibrio vulnificus* septicemia and necrotizing fasciitis in the patients with liver cirrhosis and diabetes mellitus. *J Diabetes Mellit*. 2012;02(01):122-125. doi:10.4236/jdm.2012.21020

76. Oliver JD. The Biology of *Vibrio vulnificus*. *Microbiol Spectr*. Published online 2015. doi:10.1128/microbiolspec.ve-0001-2014

77. Baker-Austin C, Oliver JD. *Vibrio Vulnificus: New Insights into a Deadly Opportunistic Pathogen*. Vol 20. Blackwell Publishing Ltd; 2018:423-430. doi:10.1111/1462-2920.13955

78. Bross MH, Soch K, Morales R, Mitchell RB. *Vibrio vulnificus* infection: Diagnosis and treatment. *Am Fam Physician*. 2007;76(4):539-544. Accessed October 12, 2020. www.aafp.org/afp.

79. Nigro OD, Hou A, Vithanage G, Fujioka RS, Steward GF. Temporal and spatial variability in culturable pathogenic *Vibrio* spp. in Lake Pontchartrain, Louisiana, following hurricanes katrina and rita. *Appl Environ Microbiol*. 2011;77(15):5384-5393. doi:10.1128/AEM.02509-10

80. Esteves K, Hervio-Heath D, Mosser T, Rodier C, Tournoud M-G, Jumas-Bilak E, Colwell RR, Monfort P. Rapid proliferation of *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio cholerae* during freshwater flash floods in French Mediterranean coastal lagoons. Published online 2015. doi:10.1128/AEM.01848-15

81. Froelich BA, Williams TC, Noble RT, Oliver JD. Apparent loss of *Vibrio vulnificus* from North Carolina oysters coincides with a drought-induced increase in salinity. *Appl Environ*

Microbiol. 2012;78(11):3885-3889. doi:10.1128/AEM.07855-11

82. Oliver JD, Hite F, McDougald D, Andon NL, Simpson LM. Entry into, and resuscitation from, the viable but nonculturable state by *Vibrio vulnificus* in an estuarine environment. *Appl Environ Microbiol*. Published online 1995. doi:10.1128/aem.61.7.2624-2630.1995

83. Oliver JD. The viable but nonculturable state in bacteria. *J Microbiol*. Published online 2005.

84. Saux MF Le, Hervio-Heath D, Loaec S, Colwell RR, Pommepuy M. Detection of cytotoxin-hemolysin mRNA in nonculturable populations of environmental and clinical Vibrio vulnificus strains in artificial seawater. *Appl Environ Microbiol*. 2002;68(11). doi:10.1128/aem.68.11.5641-5646.2002

85. Baker-Austin C, Lemm E, Hartnell R, Lowther J, Onley R, Amaro C, Oliver JD, Lees D. PilF polymorphism-based real-time PCR to distinguish *Vibrio vulnificus* strains of human health relevance. *Food Microbiol*. 2012;30(1):17-23. doi:10.1016/j.fm.2011.09.002

86. Roig FJ, Sanjuán E, Llorens A, Amaro C, Sanjuán E, Llorens A, Amaro C. pilF polymorphism-based PCR To distinguish *Vibrio vulnificus* strains potentially dangerous to public health. *Appl Environ Microbiol*. 2010;76(5):1328-1333. doi:10.1128/AEM.01042-09

87. Rosche TM, Yano Y, Oliver JD. A rapid and simple PCR analysis indicates there are two subgroups of *Vibrio vulnificus* which correlate with clinical or environmental isolation. *Microbiol Immunol*. Published online 2005. doi:10.1111/j.1348-0421.2005.tb03731.x

88. Chakraborty S, Monfett M, Maier TM, Benach JL, Frank DW, Thanassi DG. Type IV pili

in *Francisella tularensis*: Roles of *pilF* and *pilT* in fiber assembly, host cell adherence, and virulence. *Infect Immun*. 2008;76(7):2852-2861. doi:10.1128/IAI.01726-07

89. Bogard RW, Oliver JD. Role of iron in human serum resistance of the clinical and environmental *Vibrio vulnificus* genotypes. *Appl Environ Microbiol*. Published online 2007. doi:10.1128/AEM.01551-07

90. Kim HY, Ayrapetyan M, Oliver JD. Survival of *Vibrio vulnificus* genotypes in male and female serum, and production of siderophores in human serum and seawater. *Foodborne Pathog Dis*. Published online 2014. doi:10.1089/fpd.2013.1581

91. Ruesink JL, Lenihan HS, Trimble AC, Heiman KW, Micheli F, Byers JE, Kay MC. Introduction of non-native oysters: Ecosystem effects and restoration implications. *Annu Rev Ecol Evol Syst*. 2005;36(1):643-689. doi:10.1146/annurev.ecolsys.36.102003.152638

92. Shatkin G, Shumway SE, Hawes R. Considerations regarding the possible introduction of the pacific oyster (*Crassostrea gigas*) to the Gulf of Maine: A review of global experience. *J Shellfish Res*. 1997;16(2):463-477.

93. Harris J. *Aquatic Invasive Species Profile: Pacific Oyster,* Crassostrea Gigas *(Thunberg, 1793)*.; 2008.

94. Barrett EM. *The California Oyster Industry*.; 1963.

95. Moore, Thomas, Moore J. *Status of the Fisheries Report 2008: 21 Culture of Oysters*.; 2008.

96. Carlsbad Aquafarm. History. Accessed February 9, 2021. https://carlsbadaquafarm.com/history/

97. Cohen AN, Harris LH, Bingham BL, Carlton JT, Chapman JW, Lambert CC, Lambert G,

Ljubenkov JC, Murray SN, Rao LC, Reardon K, Schwindt E. Rapid assessment survey for exotic organisms in southern California bays and harbors, and abundance in port and non-port areas. *Biol Invasions*. 2005;7(6):995-1002. doi:10.1007/s10530-004-3121-1

98. Walne PR. Culture of bivalve molluscs: 50 years' experience at Conwy. *Cult bivalve molluscs 50 years' Exp Conwy*. 1979;(Ed. 2).

99. Mann R, Burreson EM, Baker PK. The decline of the Virginia oyster fishery in Chesapeake Bay: considerations for introduction of a non-endemic species, *C. gigas* (Thunberg, 1793). *J Shellfish Res*. 1991;(10):379-388. Accessed February 8, 2021. https://scholarworks.wm.edu/vimsarticles/1276

100. Vezzulli L, Grande C, Reid PC, Hélaouët P, Edwards M, Höfle MG, Brettar I, Colwell RR, Pruzzo C. Climate influence on *Vibrio* and associated human diseases during the past half-century in the coastal North Atlantic. *Proc Natl Acad Sci U S A*. 2016;113(34):E5062-E5071. doi:10.1073/pnas.1609157113

101. Main CR, Salvitti LR, Whereat EB, Coyne KJ. Community-Level and species-specific associations between phytoplankton and particle-associated *Vibrio* species in delaware's inland bays. *Appl Environ Microbiol*. Published online 2015. doi:10.1128/AEM.00580-15

102. Powell EN, Hofmann EE, Klinck JM, Ray SM. Modeling oyster populations: I. A commentary on filtration rate. Is faster always better? Published online 1992.

103. Kobayashi M, Hofmann EE, Powell EN, Klinck JM, Kusaka K. A population dynamics model for the Japanese oyster, *Crassostrea gigas*. *Aquaculture*. 1997;149(3-4):285-321. doi:10.1016/S0044-8486(96)01456-1

104. Froelich B, Oliver JD. The interactions of *Vibrio vulnificus* and the oyster *Crassostrea virginica*. *Microb Ecol*. Published online 2013. doi:10.1007/s00248-012-0162-3

105. Hara-Kudo Y, Kumagai S. Impact of seafood regulations for Vibrio parahaemolyticus infection and verification by analyses of seafood contamination and infection. *Epidemiol Infect*. 2014;142(11):2237-2247. doi:10.1017/S0950268814001897

106. Canadian Food Inspection Agency. Bacteriological guidelines for fish and fish products (end product). Published 2019. Accessed February 3, 2021. https://www.inspection.gc.ca/food-safety-for-industry/food-safety-standards-guidelines/bacteriological-guidelines/eng/1558757049068/1558757132060

107. Hara-Kudo Y, Saito S, Ohtsuka K, Yamasaki S, Yahiro S, Nishio T, Iwade Y, Otomo Y, Konuma H, Tanaka H, Nakagawa H, Sugiyama K, Sugita-Konishi Y, Kumagai S. Characteristics of a sharp decrease in *Vibrio parahaemolyticus* infections and seafood contamination in Japan. *Int J Food Microbiol*. 2012;157(1):95-101. doi:10.1016/j.ijfoodmicro.2012.04.019

108. Health Canada’s Food Directorate. Information document with request for input and scientific data: *Vibrio parahaemolyticus* in oyster shellstock intended for raw consumption. Published 2019. Accessed February 3, 2021. https://www.canada.ca/en/health-canada/services/food-nutrition/public-involvement-partnerships/request-input-scientific-data-vibrio-parahaemolyticus-oyster-shellstock-intended-consumption/consultation.html

109. HPA. Guidelines for assessing the microbiological safety of ready-to-eat foods placed on the market. *Heal Prot Agency, London*. 2009;(November):33.

110. US FDA. Quantitative risk assessment on the public health impact of pathogenic Vibrio

parahaemolyticus in raw oysters. *Food Drug Adm US Dep Heal Hum Serv*. Published online 2005. Accessed June 24, 2020. https://www.fda.gov/food/cfsan-risk-safety-assessments/quantitative-risk-assessment-public-health-impact-pathogenic-vibrio-parahaemolyticus-raw-oysters

111. EC. Regulation (EC) No 853/2004 of the European parliment and of the council of 29 April 2004 laying down specific hygiene rules for food of animal origin. *Off J Eur*. 2004;L226(853):69-74.

112. Lee RJ, Reese RA. Relating the bivalve shellfish harvesting area classification criteria in the United States and European Union programmes. Published online 2014. doi:10.2166/wh.2013.128

113. Food Standards Australia New Zealand. Food Standards Code. Australian Government Federal Legislation.

114. NSSP. Guide for the control of molluscan shellfish 2019 revision. *Risk Manag*. Published online 2019:549. Accessed July 1, 2020. http://www.fda.gov/Food/GuidanceRegulation/FederalStateFoodPrograms/ucm2006754.htm

115. Meschke JS, Boyle D. Shellfish and Microbial Source Tracking. In: *Microbial Source Tracking*. ASM Press; 2014:137-179. doi:10.1128/9781555815769.ch5

116. US FDA. Food Safety Modernization Act (FSMA). Published online 2011. Accessed October 13, 2020. https://www.fda.gov/food/food-safety-modernization-act-fsma/full-text-food-safety-modernization-act-fsma

117. Love DC, Kuehl LM, Lane RM, Fry JP, Harding J, Davis BJK, Clancy K, Hudson B. Performance of cold chains and modeled growth of *Vibrio parahaemolyticus* for farmed oysters distributed in the United States and internationally. *Int J Food Microbiol*. 2020;313:108378. doi:10.1016/j.ijfoodmicro.2019.108378

118. CDPH. *2020* Vibrio Parahaemolyticus *Control Plan For Cysters in Commercial Shellfish Growing Areas of California*.; 2020.

119. CDPH. *Restrictions on Raw Gulf Oysters Limitations on the Sale of Raw Gulf Oysters in California*.; 2015.

120. CDPH. *Raw Gulf Oysters: Labeling, Written Warnings and Additional Requirements*.

121. Vugia DJ, Tabnak F, Newton AE, Hernandez M, Griffin PM. Impact of 2003 state regulation on raw oyster-associated *Vibrio vulnificus* illnesses and deaths, California, USA. *Emerg Infect Dis*. 2013;19(8):1276-1280. doi:10.3201/eid1908.121861

122. US EPA. Clean Water Act: National Pollutant Discharge Elimination System (NPDES). *Clean Water Act*. Published online 1972. Accessed February 2, 2021. https://www.epa.gov/cwa-404/clean-water-act-section-402-national-pollutant-discharge-elimination-system

123. Board CSWRC. *Ocean Plan Triennial Review Workplan 2011-2013 Issue 5 | Beneficial Uses and Adoption of Fecal Coliform Standard for Shellfish Harvesting Area*.

124. Newport Bay study to examine scientific basis for shellfish water-quality standard - Southern California Coastal Water Research Project. Accessed February 3, 2021. https://www.sccwrp.org/news/newport-bay-study-examine-scientific-basis-shellfish-

water-quality-standard/

125. Sinigalliano CD, Fleisher JM, Gidley ML et al. Traditional and molecular analyses for fecal indicator bacteria in non-point source subtropical recreational marine waters. *Water Res*. 2010;44(13):3763-3772. doi:10.1016/j.watres.2010.04.026

126. Soller JA, Schoen ME, Varghese A, Ichida AM, Boehm AB, Eftim S, Ashbolt NJ, Ravenscroft JE. Human health risk implications of multiple sources of faecal indicator bacteria in a recreational waterbody. *Water Res*. 2014;66:254-264. doi:10.1016/j.watres.2014.08.026

127. Stewart JR, Gast RJ, Fujioka RS, Solo-Gabriele HM, Meschke JS, Amaral-Zettler LA, Del Castillo E, Polz MF, Collier TK, Strom MS, Sinigalliano CD, Moeller PDR, Holland AF. The coastal environment and human health: Microbial indicators, pathogens, sentinels and reservoirs. In: *Environmental Health: A Global Access Science Source*. Vol 7. BioMed Central; 2008:S3. doi:10.1186/1476-069X-7-S2-S3

128. Hood MA, Ness GE, Blake NJ. Relationship among fecal coliforms, *Escherichia coli*, and *Salmonella* spp. in shellfish. *Appl Environ Microbiol*. 1983;45(1):122-126. doi:10.1128/aem.45.1.122-126.1983

129. Wright AC, Harwood VJ. *Vibrios* . In: *Foodborne Infections and Intoxications*. Elsevier Inc.; 2013:113-128. doi:10.1016/B978-0-12-416041-5.00007-X

130. Jiang SC, Chu W, He J-WW. Seasonal detection of human viruses and coliphage in Newport Bay, California. *Appl Environ Microbiol*. 2007;73(20). doi:10.1128/AEM.01370-07

131. Noble RT, Fuhrman JA. Enteroviruses detected by reverse transcriptase polymerase chain

reaction from the coastal waters of Santa Monica Bay, California: low correlation to bacterial indicator levels. In: *The Ecology and Etiology of Newly Emerging Marine Diseases*. Vol 460. Springer Netherlands; 2001:175-184. doi:10.1007/978-94-017-3284-0_16

132. McAnulty JM, Rubin GL, Carvan CT, Huntley EJ, Grohmann G, Hunter R. An outbreak of Norwalk-like gastroenteritis associated with contaminated drinking water at a caravan park. *Aust J Public Health*. 2010;17(1):36-41. doi:10.1111/j.1753-6405.1993.tb00102.x

133. Geldenhuys JC, Pretorius PD. The occurrence of enteric viruses in polluted water, correlation to indicator organisms and factors influencing their numbers. *Water Sci Technol*. 1989;21(3):105-109. doi:10.2166/wst.1989.0086

134. Haas CN, Rose JB, Gerba CP. *Quantitative Microbial Risk Assessment: Second Edition*.; 2014. doi:10.1002/9781118910030

135. Abdullah Sani N, Ariyawansa S, Babji AS, Hashim JK. The risk assessment of *Vibrio parahaemolyticus* in cooked black tiger shrimps (*Penaeus monodon*) in Malaysia. *Food Control*. 2013;31(2):546-552. doi:10.1016/j.foodcont.2012.10.018

136. Hamilton KA, Chen A, de-Graft Johnson E, Gitter A, Kozak S, Niquice C, Zimmer-Faust AG, Weir MH, Mitchell J, Gurian PL. *Salmonella* risks due to consumption of aquaculture-produced shrimp. *Microb Risk Anal*. 2018;9:22-32. doi:10.1016/j.mran.2018.04.001

137. Teunis PFM, Medema GJ, Kruidenier L, Havelaar AH. Assessment of the risk of infection by *Cryptosporidium* or *Giardia* in drinking water from a surface water source. *Water Res*. 1997;31(6):1333-1346. doi:10.1016/S0043-1354(96)00387-9

138. WHO. Guidelines for drinking water quality. *Guidel Drink Qual*. 2008;1.

139. US EPA. Quantitative microbial risk assessment to estimate illness in freshwater impacted by agricultural animal sources of fecal contamination. *USEPA 822-R-10-005*. 2010;(December).

140. Rodrigues VFV, Rivera ING, Lim KY, Jiang SC. Detection and risk assessment of diarrheagenic *E. coli* in recreational beaches of Brazil. *Mar Pollut Bull*. 2016;109(1):163-170. doi:10.1016/j.marpolbul.2016.06.007

141. Schoen ME, Ashbolt NJ. Assessing pathogen risk to swimmers at non-sewage impacted recreational beaches. *Environ Sci Technol*. 2010;44(7):2286-2291. doi:10.1021/es903523q

142. Tseng LY, Jiang SC. Comparison of recreational health risks associated with surfing and swimming in dry weather and post-storm conditions at Southern California beaches using quantitative microbial risk assessment (QMRA). *Mar Pollut Bull*. 2012;64(5):912-918. doi:10.1016/j.marpolbul.2012.03.009

143. Pearce-Walker J, Bright KR, Canales RA, Wilson AM, Verhougstraete MP. Managing leafy green safety from adenoviruses and enteroviruses in irrigation water. *Agric Water Manag*. 2020;240. doi:10.1016/j.agwat.2020.106272

144. Shuval H, Lampert Y, Fattal B. Development of a risk assessment approach for evaluating wastewater reuse standards for agriculture. In: *Water Science and Technology*. Vol 35. Elsevier Science Ltd; 1997:15-20. doi:10.1016/S0273-1223(97)00228-X

145. Hamilton AJ, Stagnitti F, Premier R, Boland AM, Hale G. Quantitative microbial risk assessment models for consumption of raw vegetables irrigated with reclaimed water. *Appl*

Environ Microbiol. 2006;72(5):3284-3290. doi:10.1128/AEM.72.5.3284-3290.2006

146. Bouwknegt M, Verhaelen K, Rzezutka A, Kozyra I, Maunula L, von Bonsdorff CH, Vantarakis A, Kokkinos P, Petrovic T, Lazic S, Pavlik I, Vasickova P, Willems KA, Havelaar AH, Rutjes SA, de Roda Husman AM. Quantitative farm-to-fork risk assessment model for norovirus and hepatitis A virus in European leafy green vegetable and berry fruit supply chains. *Int J Food Microbiol*. 2015;198:50-58. doi:10.1016/j.ijfoodmicro.2014.12.013

147. Costa Sobrinho P de S, Destro MT, Franco BDGM, Landgraf M. A quantitative risk assessment model for *Vibrio parahaemolyticus* in raw oysters in Sao Paulo State, Brazil. *Int J Food Microbiol*. 2014;180:69-77. doi:10.1016/j.ijfoodmicro.2014.04.008

148. Huang Y-SS, Hwang C-AA, Huang L, Wu VC-HH, Hsiao H-II. The risk of *Vibrio parahaemolyticus* infections associated with consumption of raw oysters as affected by processing and distribution conditions in Taiwan. *Food Control*. 2018;86:101-109. doi:10.1016/j.foodcont.2017.10.022

149. Love DC, Lane RM, Davis BJK, Clancy K, Fry JP, Harding J, Hudson B. Performance of cold chains for Chesapeake bay farmed oysters and modeled growth of *Vibrio parahaemolyticus*. *J Food Prot*. 2019;82(1):168-178. doi:10.4315/0362-028X.JFP-18-044

150. WHO. *Risk Assessment of* Vibrio Parahaemolyticus *in Seafood*.; 2011.

151. Ndraha N, Wong H chung, Hsiao HI. Managing the risk of *Vibrio parahaemolyticus* infections associated with oyster consumption: A review. *Compr Rev Food Sci Food Saf*. 2020;19(3):1187-1217. doi:10.1111/1541-4337.12557

152. Cowan M, Finston T, Marshall R, Paul |, De La Bastide Y, Bastide PY. Post-harvest temperature regimes for Pacific oysters (*Crassostrea gigas*) and growth response of *Vibrio parahaemolyticu* s. *Aquac Res*. 2019;50(11):3471-3476. doi:10.1111/are.14289

153. WHO. *Risk Assessment of Vibrio Vulnificus in Raw Oysters*.; 2005. doi:10.1016/j.ijfoodmicro.2007.07.013

154. FAO. FAO statistics. *Ecosyst Serv*. 2013;2:38-44. Accessed January 5, 2021. www.fao.org/fishery/static/Yearbook/YB2018_USBcard/index.htm

155. DePaola A, Nordstrom JL, Bowers JC, Wells JG, Cook DW. Seasonal abundance of total and pathogenic *Vibrio parahaemolyticus* in Alabama oysters. *Appl Environ Microbiol*. 2003;69(3):1521-1526. doi:10.1128/AEM.69.3.1521-1526.2003

156. Daniels NA, Shafaie A. A review of pathogenic *Vibrio* infections for clinicians. *Infect Med*. Published online 2000.

157. DePaola A, Kaysner CA, Bowers J, Cook DW. Environmental investigations of *Vibrio parahaemolyticus* in oysters after outbreaks in Washington, Texas, and New York (1997 and 1998). *Appl Environ Microbiol*. 2000;66(11):4649-4654. doi:10.1128/AEM.66.11.4649-4654.2000

158. CDC. Outbreak of Vibrio parahaemolyticus Infection Associated With Eating Raw Oysters and Clams Harvested From Long Island Sound—Connecticut, New Jersey, and New York, 1998. *JAMA*. 1999;281(7):603. doi:10.1001/jama.281.7.603-jwr0217-2-1

159. Martinez-Urtaza J, Baker-Austin C, Jones JL, Newton AE, Gonzalez-Aviles GD, DePaola A. Spread of Pacific Northwest *Vibrio parahaemolyticus* Strain. *N Engl J Med*. Published

online 2013. doi:10.1056/nejmc1305535

160. Paranjpye R, Hamel OS, Stojanovski A, Liermann M. Genetic diversity of clinical and environmental vibrio parahaemolyticus strains from the Pacific northwest. *Appl Environ Microbiol*. 2012;78(24). doi:10.1128/AEM.01531-12

161. Gutierrez West CK, Klein SL, Lovell harles R. High frequency of virulence factor genes *tdh*, *trh*, and *tlh* in *Vibrio parahaemolyticus* strains isolated from a pristine estuary. *Appl Environ Microbiol*. Published online 2013. doi:10.1128/AEM.03792-12

162. Letchumanan V, Chan KG, Lee LH. *Vibrio parahaemolyticus*: A review on the pathogenesis, prevalence, and advance molecular identification techniques. *Front Microbiol*. Published online 2014. doi:10.3389/fmicb.2014.00705

163. Johnson CN, Flowers AR, Noriea NF, Zimmerman AM, Bowers JC, DePaola A, Grimes DJ. Relationships between environmental factors and pathogenic *Vibrios* in the northern gulf of Mexico. *Appl Environ Microbiol*. 2010;76(21):7076-7084. doi:10.1128/AEM.00697-10

164. Zimmerman AM, DePaola A, Bowers JC, Krantz JA, Nordstrom JL, Johnson CN, Grimes DJ. Variability of total and pathogenic *Vibrio parahaemolyticus* densities in Northern Gulf of Mexico water and oysters. *Appl Environ Microbiol*. 2007;73(23):7589-7596. doi:10.1128/AEM.01700-07

165. Froelich B, Gonzalez R, Blackwood D, Lauer K, Noble R. Decadal monitoring reveals an increase in *Vibrio* spp. concentrations in the Neuse River Estuary, North Carolina, USA. Karunasagar I, ed. *PLoS One*. 2019;14(4):e0215254. doi:10.1371/journal.pone.0215254

166. Arksey H, O'Malley L. Scoping studies: Towards a methodological framework. *Int J Soc Res Methodol Theory Pract*. 2005;8(1):19-32. doi:10.1080/1364557032000119616

167. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev*. 2016;5(1):210. doi:10.1186/s13643-016-0384-4

168. Rohatgi A. WebPlotDigitizer . Published 2020. Accessed February 17, 2021. https://automeris.io/WebPlotDigitizer

169. R Studio Team. R Studio. *RS ed http//www.rstudio.com/*. Published online 2016.

170. Mahasneh AM, Al-Sayed HA. Seasonal incidence of some heterotrophic aerobic bacteria in Bahrain pelagic and nearshore waters and oysters. *Int J Environ Stud*. 1997;51(4):301-312. doi:10.1080/00207239708711088

171. Cardoso MD, Lemos LS, Roges EM, de Moura JF, Tavares DC, Matias CAR, Rodrigues DP, Siciliano S. A comprehensive survey of *Aeromonas* spp. and *Vibrio* spp. in seabirds from southeastern Brazil: outcomes for public health. *J Appl Microbiol*. 2018;124(5):1283-1293. doi:10.1111/jam.13705

172. Ramos RJ, Miotto LA, Miotto M, Silveira Junior N, Cirolini A, da Silva HS, Rodrigues D dos P, Vieira CRW. Occurrence of potentially pathogenic *Vibrio* in oysters (*Crassostrea gigas*) and waters from bivalve mollusk cultivations in the South Bay of Santa Catarina. *Rev Soc Bras Med Trop*. 2014;47(3):327-333. doi:10.1590/0037-8682-0069-2014

173. Cabrera-García ME, Vázquez-Salinas C, Quiñones-Ramírez EI. Serologic and molecular characterization of *Vibrio parahaemolyticus* strains isolated from seawater and fish products of the Gulf of Mexico. *Appl Environ Microbiol*. 2004;70(11):6401-6406.

doi:10.1128/AEM.70.11.6401-6406.2004

174. Kampelmacher EH, van Noorle Jansen LM, Mossel DAA, Groen FJ. A survey of the occurrence of *Vibrio parahaemolyticus* and *V. alginolyticus* on mussels and oysters and in estuarine waters in the Netherlands. *J Appl Bacteriol*. 1972;35(3):431-438. doi:10.1111/j.1365-2672.1972.tb03719.x

175. Mok JS, Ryu A, Kwon JY, Park K, Shim KB. Abundance, antimicrobial resistance, and virulence of pathogenic *Vibrio* strains from molluscan shellfish farms along the Korean coast. *Mar Pollut Bull*. 2019;149(April):110559. doi:10.1016/j.marpolbul.2019.110559

176. Mok JS, Ryu A, Kwon JY, Kim B, Park K. Distribution of *Vibrio* species isolated from bivalves and bivalve culture environments along the Gyeongnam coast in Korea: Virulence and antimicrobial resistance of *Vibrio parahaemolyticus* isolates. *Food Control*. 2019;106:106697. doi:10.1016/j.foodcont.2019.06.023

177. Park K, Mok JS, Ryu AR, Kwon JY, Ham IT, Shim KB. Occurrence and virulence of *Vibrio parahaemolyticus* isolated from seawater and bivalve shellfish of the Gyeongnam coast, Korea, in 2004–2016. *Mar Pollut Bull*. 2018;137(July):382-387. doi:10.1016/j.marpolbul.2018.10.033

178. Yu WT, Jong KJ, Lin YR, Tsai S en, Tey YH, Wong H chung. Prevalence of *Vibrio parahaemolyticus* in oyster and clam culturing environments in Taiwan. *Int J Food Microbiol*. 2013;160(3):185-192. doi:10.1016/j.ijfoodmicro.2012.11.002

179. Chen AJ, Hasan NA, Haley BJ, Taviani E, Tarnowski M, Brohawn K, Johnson CN, Colwell RR, Huq A. Characterization of pathogenic *Vibrio parahaemolyticus* from the Chesapeake Bay, Maryland. *Front Microbiol*. 2017;8(DEC):1-10. doi:10.3389/fmicb.2017.02460

180. Froelich BA, Phippen B, Fowler P, Noble RT, Oliver JD. Differences in Abundances of Total *Vibrio* spp., *V. vulnificus*, and *V. parahaemolyticus* in Clams and Oysters in North Carolina. Published online 2016. doi:10.1128/AEM.02265-16

181. Kaysner CA, Abeyta C, Stott RF, Lilja JL, Wekell MM. Incidence of urea-hydrolyzing *Vibrio parahaemolyticus* in Willapa Bay, Washington. *Appl Environ Microbiol*. 1990;56(4):904-907. doi:10.1128/aem.56.4.904-907.1990

182. Johnson CN, Flowers AR, Young VC, Gonzalez-Escalona N, Depaola A, Noriea NF, Grimes DJ. Genetic relatedness among *tdh* + and *trh* + *Vibrio parahaemolyticus* cultured from Gulf of Mexico oysters (*Crassostrea virginica*) and surrounding water and sediment. *Microb Ecol*. 2009;57(3):437-443. doi:10.1007/s00248-008-9418-3

183. Shaw KS, Jacobs JM, Crump BC. Impact of Hurricane Irene on *Vibrio vulnificus* and *Vibrio parahaemolyticus* concentrations in surface water, sediment, and cultured oysters in the Chesapeake Bay, MD, USA. *Front Microbiol*. 2014;5(MAY):204. doi:10.3389/fmicb.2014.00204

184. Kaysner CA, Abeyta C, Stott RF, Krane MH, Wekell MM. Enumeration of *Vibrio* species, including *V. Cholerae*, from samples of an oyster growing area, Grays Harbor, Washington. *J Food Prot*. 1990;53(4):300-301. doi:10.4315/0362-028x-53.4.300

185. Vanderzant C, Thompson CA, Ray SM. Microbial flora and level of *Vibrio parahaemolyticus* of oysters (*Crassostrea virginica*), water and sediment from Galveston Bay. *JMilk Food Technol*. 1973;36(9):447-452. doi:10.4315/0022-2747-36.9.447

186. Baross J, Liston J. Occurrence of *Vibrio parahaemolyticus* and related hemolytic *Vibrios* in marine environments of Washington State. *Appl Microbiol*. 1970;20(2):179-186.

doi:10.1128/aem.20.2.179-186.1970

187. Thompson CA, Vanderzant C. Relationship of *Vibrio parahaemolytiucs* in oysters, water and sediment, and bacteriological and environmental indices. *J Food Sci*. 1976;41(1):117-122. doi:10.1111/j.1365-2621.1976.tb01115.x

188. Parveen S, Hettiarachchi KA, Bowers JC, Jones JL, Tamplin ML, Mckay R, Beatty W, Brohawn K, DaSilva L V., Depaola A. Seasonal distribution of total and pathogenic *Vibrio parahaemolyticus* in Chesapeake Bay oysters and waters. *Int J Food Microbiol*. 2008;128(2):354-361. doi:10.1016/j.ijfoodmicro.2008.09.019

189. Moore JG, Ruple A, Ballenger-Bass K, Bell S, Pennington PL, Scott GI. Snapshot of *Vibrio parahaemolyticus* densities in open and closed shellfish beds in Coastal South Carolina and Mississippi. *Environ Monit Assess*. 2014;186(11):7949-7960. doi:10.1007/s10661-014-3979-z

190. Klein SL, Lovell CR. The hot oyster: levels of virulent *Vibrio parahaemolyticus* strains in individual oysters. *FEMS Microbiol Ecol*. 2017;93(2):1-8. doi:10.1093/femsec/fiw232

191. Cox AM, Gomez-Chiarri M. *Vibrio parahaemolyticus* in Rhode Island coastal ponds and the estuarine environment of narragansett bay. *Appl Environ Microbiol*. 2012;78(8):2996-2999. doi:10.1128/AEM.07519-11

192. Kaysner, Charle Depaolo, Angelo, Jones J, FDA, Kaysner, Charles Depaolo, Angelo, Jones J, DePaola AJ, Kaysner, Charle Depaolo, Angelo, Jones J, Kaysner, Charle Depaolo, Angelo, Jones J, FDA. BAM Chapter 9: Vibrio | FDA. In: *Bacterial Analytical Manual*. ; 2004:1-45. Accessed October 14, 2020. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-9-vibrio

193. Semenza JC, Trinanes J, Lohr W, Sudre B, Löfdahl M, Martinez-Urtaza J, Nichols GL, Rocklöv J. Environmental suitability of *Vibrio* infections in a warming climate: An early warning system. *Environ Health Perspect*. 2017;125(10). doi:10.1289/EHP2198

194. Martinez-Urtaza J, Bowers JC, Trinanes J, DePaola A. Climate anomalies and the increasing risk of *Vibrio parahaemolyticus* and *Vibrio vulnificus* illnesses. *Food Res Int*. Published online 2010. doi:10.1016/j.foodres.2010.04.001

195. Cavicchioli R, Ripple WJ, Timmis KN et al. Scientists' warning to humanity: microorganisms and climate change. *Nat Rev Microbiol*. 2019;17(9):569-586. doi:10.1038/s41579-019-0222-5

196. Johnson CN. Influence of Environmental Factors on *Vibrio* spp. in Coastal Ecosystems. *Microbiol Spectr*. 2015;3(3). doi:10.1128/microbiolspec.VE-0008-2014

197. Takemura AF, Chien DM, Polz MF, Ceccarelli D, Huq A, Grimes DJ. Associations and dynamics of Vibrionaceae in the environment, from the genus to the population level. Published online 2014. doi:10.3389/fmicb.2014.00038

198. Johnson CN, Bowers JC, Griffitt KJ, Molina V, Clostio RW, Pei S, Laws E, Paranjpye RN, Strom MS, Chen A, Hasan NA, Huq A, Noriea NF, Grimes DJ, Colwell RR. Ecology of vibrio parahaemolyticus and vibrio vulnificus in the coastal and estuarine waters of Louisiana, Maryland, Mississippi, and Washington (United States). *Appl Environ Microbiol*. 2012;78(20):7249-7257. doi:10.1128/AEM.01296-12

199. Turner JW, Malayil L, Guadagnoli D, Cole D, Lipp EK. Detection of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* with respect to seasonal fluctuations in temperature and plankton abundance. *Environ Microbiol*. 2014;16(4):1019-

1028. doi:10.1111/1462-2920.12246

200. Greenfield DI, Gooch Moore J, Stewart JR, Hilborn ED, George BJ, Li Q, Dickerson J, Keppler CK, Sandifer PA. Temporal and environmental factors driving *Vibrio vulnificus* and *V. parahaemolyticus* populations and their associations with harmful algal blooms in South Carolina detention ponds and receiving tidal creeks. *GeoHealth*. 2017;1(9):306-317. doi:10.1002/2017gh000094

201. Huston P, Edge V, Bernier E. Reaping the benefits of Open Data in public health. *Canada Commun Dis Rep*. 2019;45(10):252-256. doi:10.14745/ccdr.v45i10a01

202. Ndraha N, Hsiao HI. The risk assessment of *Vibrio parahaemolyticus* in raw oysters in Taiwan under the seasonal variations, time horizons, and climate scenarios. *Food Control*. 2019;102:188-196. doi:10.1016/j.foodcont.2019.03.020

203. Ortiz-Jiménez MA. Quantitative evaluation of the risk of *Vibrio parahaemolyticus* through consumption of raw oysters (*Crassostrea corteziensis*) in Tepic, Mexico, under the RCP2.6 and RCP8.5 climate scenarios at different time horizons. *Food Res Int*. 2018;111:111-119. doi:10.1016/j.foodres.2018.05.012

204. CDC. Outbreak of Vibrio parahaemolyticus infections associated with eating raw oysters--Pacific Northwest, 1997. *MMWR Morb Mortal Wkly Rep*. 1998;47(22):457-462.

205. Warner E, Oliver JD. Population structures of two genotypes of *Vibrio vulnificus* in oysters (*Crassostrea virginica*) and seawater. *Appl Environ Microbiol*. Published online 2008. doi:10.1128/AEM.01434-07

206. Dickinson G, Lim K-Y ying, Jiang SC. Quantitative microbial risk assessment of pathogenic

Vibrios in marine recreational waters of Southern California. *Appl Environ Microbiol*. 2013;79(1):294-302. doi:10.1128/AEM.02674-12

207. Kaysner CA, Abeyta C, Wekell MM, Depaola A, Stott, And RF, Leitch JM. *Virulent Strains of* Vibrio Vulnificus *Isolated from Estuaries of the United States West Coast*. Vol 53.; 1987.

208. Julie D, Solen L, Antoine V, Jaufrey C, Annick D, Dominique HH. Ecology of pathogenic and non-pathogenic *Vibrio parahaemolyticus* on the French Atlantic coast. Effects of temperature, salinity, turbidity and chlorophyll a. *Environ Microbiol*. 2010;12(4):929-937. doi:10.1111/j.1462-2920.2009.02136.x

209. Newport Bay Conservancy. Bay History. Accessed February 16, 2021. https://newportbay.org/the-bay/bay-history/

210. Nezlin NP, Kamer K, Stein ED. Application of color infrared aerial photography to assess macroalgal distribution in an eutrophic estuary, upper Newport Bay, California. *Estuaries and Coasts*. 2007;30(5):855-868. doi:10.1007/BF02841339

211. Salvi D, Macali A, Mariottini P. Molecular phylogenetics and systematics of the bivalve family Ostreidae based on rRNA sequence-structure models and multilocus species tree. *PLoS One*. 2014;9(9). doi:10.1371/journal.pone.0108696

212. US FDA. BAM Chapter 4: Enumeration of *Escherichia coli* and the coliform bacteria. 2019;(September 2002):1-18. Accessed February 15, 2021. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria

213. APHA. *Standard Methods for Examination of Water and Wastewater*.; 2017.

214. Smith J, Gellene AG, Hubbard KA, Bowers HA, Kudela RM, Hayashi K, Caron DA. *Pseudo-nitzschia* species composition varies concurrently with domoic acid concentrations during two different bloom events in the Southern California Bight. *J Plankton Res*. 2018;40(1):1-17. doi:10.1093/plankt/fbx069

215. Froelich BA, Phippen B, Fowler P, Noble RT, Oliver JD. Differences in Abundances of Total *Vibrio* spp.,*V. vulnificus*, and *V. parahaemolyticus* in Clams and Oysters in North Carolina. Björkroth J, ed. *Appl Environ Microbiol*. 2017;83(2). doi:10.1128/AEM.02265-16

216. Di Pinto A, Terio V, Novello L, Tantillo G. Comparison between thiosulphate-citrate-bile salt sucrose (TCBS) agar and CHROMagar *Vibrio* for isolating *Vibrio parahaemolyticus*. *Food Control*. 2011;22(1):124-127. doi:10.1016/j.foodcont.2010.06.013

217. Kim YB, Okuda J, Matsumoto C, Takahashi N, Hashimoto S, Nishibuchi M. Identification of *Vibrio parahaemolyticus* strains at the species level by PCR targeted to the *toxR* gene. *J Clin Microbiol*. 1999;37(4). doi:10.1128/jcm.37.4.1173-1177.1999

218. Honda T, Abad-Lapuebla MA, Ni Y, Yamamoto K, Miwatani T. Characterization of a new thermostable direct haemolysin produced by a Kanagawa-phenomenon-negative clinical isolate of *Vibrio parahaemolyticus*. *J Gen Microbiol*. 1991;137(2):253-259. doi:10.1099/00221287-137-2-253

219. Nishibuchi M, Kaper JB. Nucleotide sequence of the thermostable direct hemolysin gene of *Vibrio parahaemolyticus*. *J Bacteriol*. 1985;162(2):558-564. doi:10.1128/jb.162.2.558-564.1985

220. Warner EB, Oliver JD. Multiplex PCR assay for detection and simultaneous differentiation

of genotypes of *Vibrio vulnificus* biotype 1. *Foodborne Pathog Dis*. 2008;5(5):691-693. doi:10.1089/fpd.2008.0120

221. Bej AK, Patterson DP, Brasher CW, Vickery MCL, Jones DD, Kaysner CA. Detection of total and hemolysin-producing *Vibrio parahaemolyticus* in shellfish using multiplex PCR amplification of tl, tdh and trh. *J Microbiol Methods*. 1999;36(3):215-225. doi:10.1016/S0167-7012(99)00037-8

222. González-Escalona N, Blackstone GM, DePaola A. Characterization of a Vibrio alginolyticus strain, isolated from Alaskan oysters, carrying a hemolysin gene similar to the thermostable direct hemolysin-related hemolysin gene (trh) of Vibrio parahaemolyticus. *Appl Environ Microbiol*. 2006;72(12):7925-7929. doi:10.1128/AEM.01548-06

223. Blackwell KD, Oliver JD. The ecology of *Vibrio vulnificus, Vibrio cholerae*, and *Vibrio parahaemolyticus* in North Carolina Estuaries. *J Microbiol*. Published online 2008. doi:10.1007/s12275-007-0216-2

224. Leight AK, Crump BC, Hood RR. Assessment of fecal indicator bacteria and potential pathogen co-occurrence at a shellfish growing area. *Front Microbiol*. 2018;9(MAR):384. doi:10.3389/fmicb.2018.00384

225. NOAA Fisheries. U.S. Aquaculture. Accessed June 22, 2020. https://www.fisheries.noaa.gov/national/aquaculture/us-aquaculture

226. Wittman RJ, Flick GJ. *Human Health, and Control Strategies*. Vol 16.; 1995. Accessed March 19, 2021. www.annualreviews.org

227. Baker-Austin C, Oliver JD, Alam M, Ali A, Waldor MK, Qadri F, Martinez-Urtaza J. Vibrio

spp. infections. *Nat Rev Dis Prim*. 2018;4(1). doi:10.1038/s41572-018-0005-8

228. Fernandez-Piquer J, Bowman JP, Ross T, Estrada-Flores S, Tamplin ML. Preliminary stochastic model for managing *Vibrio parahaemolyticus* and total viable bacterial counts in a pacific oyster (*Crassostrea gigas*) supply chain. *J Food Prot*. 2013;76(7):1168-1178. doi:10.4315/0362-028X.JFP-12-401

229. Malcolm TTH, Cheah YK, Radzi CWJWM, Kantilal HK, Martinez-Urtaza J, Nishibuchi M, Son R. Microbial risk assessment of *Vibrio parahaemolyticus* in bloody clams in Malaysia: A preliminary model from retail to consumption. *Microb Risk Anal*. 2016;4:43-51. doi:10.1016/j.mran.2016.07.002

230. Yamamoto A, Iwahori J, Vuddhakul V, Charernjiratragul W, Vose D, Osaka K, Shigematsu M, Toyofuku H, Yamamoto S, Nishibuchi M, Kasuga F. Quantitative modeling for risk assessment of *Vibrio parahaemolyticus* in bloody clams in southern Thailand. *Int J Food Microbiol*. 2008;124(1):70-78. doi:10.1016/j.ijfoodmicro.2008.02.021

231. Tan CW, Malcolm TTH, Premarathne JMKJK, New CY, Kuan CH, Thung TY, Chang WS, Loo YY, Rukayadi Y, Nakaguchi Y, Nishibuchi M, Radu S. Preliminary quantitative microbial risk assessment of pathogenic *Vibrio parahaemolyticus* in short mackerel in Malaysia. *Microb Risk Anal*. 2019;12:11-19. doi:10.1016/j.mran.2019.02.001

232. National Weather Service. NWS San Diego.

233. DePaola A, Jones JL, Woods J, Burkhardt W, Calci KR, Krantz JA, Bowers JC, Kasturi K, Byars RH, Jacobs E, Williams-Hill D, Nabe K. Bacterial and viral pathogens in live oysters: 2007 United States market survey. *Appl Environ Microbiol*. 2010;76(9):2754-2768. doi:10.1128/AEM.02590-09

234. CDC. *Vibrio* and Oysters.

235. Raimondi F, Kao JPY, Fiorentini C, Fabbri A, Donelli G, Gasparini N, Rubino A, Fasano A. Enterotoxicity and cytotoxicity of Vibrio parahaemolyticus thermostable direct hemolysin in in vitro systems. *Infect Immun*. 2000;68(6). doi:10.1128/IAI.68.6.3180-3185.2000

236. Spaur M, Davis BJK, Kivitz S, Depaola A, Bowers JC, Curriero FC, Nachman KE. A systematic review of post-harvest interventions for *Vibrio parahaemolyticus* in raw oysters. *Sci Total Environ*. 2020;745:140795. doi:10.1016/j.scitotenv.2020.140795

237. Yoon KS, Min KJ, Jung YJ, Kwon KY, Lee JK, Oh SW. A model of the effect of temperature on the growth of pathogenic and nonpathogenic *Vibrio parahaemolyticus* isolated from oysters in Korea. *Food Microbiol*. 2008;25(5):635-641. doi:10.1016/j.fm.2008.04.007

238. Cowan M, Finston T, Marshall R, Paul |, De La Bastide Y. Post-harvest temperature regimes for Pacific oysters (*Crassostrea gigas*) and growth response of *Vibrio parahaemolyticus*. *Aquac Res*. Published online 2019:50. doi:10.1111/are.14289

239. Kaysner, Charles Depaolo, Angelo, Jones J, DePaola AJ, Kaysner, Charle Depaolo, Angelo, Jones J, Kaysner, Charle Depaolo, Angelo, Jones J, FDA. BAM Chapter 9: *Vibrio*. In: *Bacterial Analytical Manual*. ; 2004:1-45. Accessed October 14, 2020. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-9-vibrio

240. US FDA. *October 2020 Interstate Certified Shellfish * Shippers List * Fresh and Frozen Oysters, Clams, Mussels, Whole or Roe-on Scallops*.; 2020. Accessed October 26, 2020. http://www.issc.org

241. Fernandez-Piquer J, Bowman JP, Ross T, Tamplin ML. Predictive models for the effect of storage temperature on *Vibrio parahaemolyticus* viability and counts of total viable bacteria in Pacific oysters (*Crassostrea gigas*). *Appl Environ Microbiol*. 2011;77(24):8687-8695. doi:10.1128/AEM.05568-11

242. Love DC, Lane RM, Kuehl LM, Hudson B, Harding J, Clancy K, Fry JP. Performance and conduct of supply chains for United States farmed oysters. *Aquaculture*. 2020;515:734569. doi:10.1016/j.aquaculture.2019.734569

243. Turner JW, Paranjpye RN, Landis ED, Biryukov S V., González-Escalona N, Nilsson WB, Strom MS. Population structure of clinical and environmental *Vibrio parahaemolyticus* from the Pacific Northwest coast of the United States. Schuch R, ed. *PLoS One*. 2013;8(2):e55726. doi:10.1371/journal.pone.0055726

244. Ma L, Su YC. Validation of high pressure processing for inactivating *Vibrio parahaemolyticus* in Pacific oysters (*Crassostrea gigas*). *Int J Food Microbiol*. Published online 2011. doi:10.1016/j.ijfoodmicro.2010.10.037

245. Ye M, Huang Y, Chen H. Inactivation of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in oysters by high-hydrostatic pressure and mild heat. *Food Microbiol*. Published online 2012. doi:10.1016/j.fm.2012.05.009

246. Mahmoud BSM. Reduction of *Vibrio vulnificus* in pure culture, half shell and whole shell oysters (*Crassostrea virginica*) by X-ray. *Int J Food Microbiol*. Published online 2009. doi:10.1016/j.ijfoodmicro.2009.01.023

247. Bruner DM, Huth WL, McEvoy DM, Morgan OA. Consumer valuation of food safety: The case of postharvest processed oysters. *Agric Resour Econ Rev*. Published online 2014.

doi:10.1017/S1068280500004330

248. Canty R, Blackwood D, Noble R, Froelich B. A comparison between farmed oysters using floating cages and oysters grown on-bottom reveals more potentially human pathogenic *Vibrio* in the on-bottom oysters. *Environ Microbiol*. 2020;22(10):4257-4263. doi:10.1111/1462-2920.14948

249. Jesser KJ, Noble RT. *Vibrio* ecology in the Neuse River Estuary, North Carolina, characterized by next-generation amplicon sequencing of the gene encoding heat shock protein 60 (hsp60). *Appl Environ Microbiol*. 2018;84(13):e00333-18. doi:10.1128/AEM.00333-18

250. King WL, Jenkins C, Seymour JR, Labbate M. Oyster disease in a changing environment: Decrypting the link between pathogen, microbiome and environment. *Mar Environ Res*. 2019;143:124-140. doi:10.1016/j.marenvres.2018.11.007

251. Nell JA. *Farming Triploid Oysters*. Vol 210. Elsevier; 2002:69-88. doi:10.1016/S0044-8486(01)00861-4

252. Hudson K. *Virginia Shellfish Aquaculture Situation and Outlook Report. Results of the 2018 Virginia Shellfish Aquaculture Crop Reporting Survey.*; 2019. doi:10.21220/V5BD8N

253. Dgremont L, Garcia C, Frank-Lawale A, Allen SK. Triploid oysters in the Chesapeake Bay: Comparison of diploid and triploid Crassostrea virginica. *J Shellfish Res*. 2012;31(1):21-31. doi:10.2983/035.031.0103

254. Ragone Calvo LM, Calvo GW, Burreson EM. Dual disease resistance in a selectively bred eastern oyster, *Crassostrea virginica*, strain tested in Chesapeake Bay. *Aquaculture*.

2003;220(1-4):69-87. doi:10.1016/S0044-8486(02)00399-X

255. Evans S, Langdon C. Effects of genotype × environment interactions on the selection of broadly adapted Pacific oysters (*Crassostrea gigas*). *Aquaculture*. 2006;261(2):522-534. doi:10.1016/j.aquaculture.2006.07.022

256. Dégremont L, Bédier E, Boudry P. Summer mortality of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). II. Response to selection for survival and its influence on growth and yield. *Aquaculture*. 2010;299(1-4):21-29. doi:10.1016/j.aquaculture.2009.11.017

257. Geiser P, Di Martino ML, Ventayol PS, Eriksson J, Sima E, Al-Saffar AK, Ahl D, Phillipson M, Webb DL, Sundbom M, Hellström PM, Sellin ME. *Salmonella enterica* serovar typhimurium exploits cycling through epithelial cells to colonize human and murine enteroids. *MBio*. 2021;12(1):1-18. doi:10.1128/mBio.02684-20