ABSTRACT

Title of Dissertation:	CLIMATE CHANGE AND *VIBRIO* SPECIES: INVESTIGATION OF ENVIRONMENTAL PARAMETERS ASSOCIATED WITH OCCURRENCE AND TRANSMISSION
	Kyle D. Brumfield, Doctor of Philosophy, 2023
Dissertation directed by:	Distinguished University Professor, Rita R. Colwell, Marine Estuarine Environmental Sciences

Climate change, associated with shifts in the geographical range of biological species, has become increasingly important in emergence and re-emergence of disease. *Vibrio spp.*, native to aquatic ecosystems, are commonly associated with aquatic invertebrates, notably crustaceans and zooplankton. Some species of the genus *Vibrio* cause infection in humans, of which *Vibrio cholerae*, the etiological agent of pandemic cholera, is the most documented. Pathogenic non-cholera *Vibrio spp.*, namely *Vibrio parahaemolyticus* and *Vibrio vulnificus*, cause gastroenteritis and also septicemia and extra-intestinal infections. They are responsible for a large number of public health emergencies in developed countries, including the United States. As sea temperatures rise and salinity profiles are altered, a pattern of poleward spreading of non-cholera *Vibrio spp*. has been observed globally, demonstrating significant geographic expansion of these bacterial populations, corroborated by an associated increase in the number of reported vibriosis cases. Since *Vibrio spp.*, including pathogenic vibrios, play an important role in the degradation of

polymeric substances, such as chitin, and in biogeochemical processes, they cannot be eradicated. Hence, routine monitoring and an early warning system are needed for public health preparedness. Since the 1960's, ongoing research has focused on environmental factors linked with occurrence and distribution of clinically relevant *Vibrio spp.* and their role in disease transmission. We have reported that lack of, or damage to, water, sanitation, and hygiene (WASH) infrastructure, coupled with elevated air temperatures, and followed by above average rainfall promotes exposure of a population to contaminated water, hence increases the risk of an outbreak of cholera. Global predictive intelligence models applicable to diseases caused by non-cholera *Vibrio spp.* are in development. The research reported here describes results of intensive sampling to detect and characterize *Vibrio spp.* in the Chesapeake Bay, Maryland, and the Florida Gulf Coast, the latter an area significantly impacted by Hurricane Ian, September 2022, with a spike in confirmed vibriosis cases and deaths during weeks following the storm. Results of this study provide confirmation of environmental predictors for *Vibrio spp.* and document long-term increase and extended seasonality of *Vibrio* populations in the Chesapeake Bay. Using satellite remote sensing data, we demonstrate the impact of extreme heat, precipitation, and other key environmental and geophysical factors (e.g., temperature, salinity, and chlorophyll) on prevalence of pathogenic *Vibrio spp*. in aquatic systems. This study lays the groundwork for a predictive intelligence system for *Vibrio spp.* and other pathogens under varying climatic scenarios.

CLIMATE CHANGE AND *VIBRIO* SPECIES: INVESTIGATION OF ENVIRONMENTAL PARAMETERS ASSOCIATED WITH OCCURRENCE AND TRANSMISSION

by

Kyle D. Brumfield

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2023

Advisory Committee:
Distinguished University Professor, Rita R. Colwell, Chair/Advisor
Research Professor, Anwar Huq, Co-Advisor
Professor, Amy R. Sapkota, Dean's Representative
Professor, Najib M. El-Sayed
Dr. Nur A. Hasan

Acknowledgements

Reflecting on the journey that has brought me to this point in my academic career, I am acutely aware of the remarkable individuals who have profoundly influenced both my personal and scholarly trajectory. Their guidance has been instrumental in enabling me to reach the culmination of this work. Without them, this achievement wound not have been possible.

First and foremost, I would like to express my deepest gratitude to my primary advisors, Dr. Rita R. Colwell and Dr. Anwar Huq. Since 2015, I have had the privilege of working closely with Dr. Colwell, who initially served as a member of my master's thesis committee at Plymouth State University (PSU). It was during my time at PSU that I had the opportunity to meet Dr. Huq, and they generously offered me the chance to pursue a Ph.D. in the Colwell/Huq Laboratory at the University of Maryland. Their tremendous support, mentorship, and encouragement have been invaluable to me as I grow as an independent researcher. Dr. Colwell and Dr. Huq's impact on my life extends beyond the realm of academia. Not only have they broadened my interests in microbial ecology, microbiome research, and infectious diseases, but they have also left an indelible mark on my personal life, ultimately shaping me into a better person.

Additionally, I am deeply indebted to Dr. Nur Hasan, who has played a pivotal role in my professional journey as a mentor, member of my advisory committee, and a guiding figure akin to an "academic big brother." Dr. Hasan's encouragement and willingness to lend an ear, whether I needed guidance in the laboratory or an honest perspective, have played a crucial role in my achievements. His influence has changed

my approach to scientific inquiry, challenging me to view my research from a broader perspective and uncover the underlying narrative behind it.

Furthermore, I would like to acknowledge the remaining members of my advisory committee, Dr. Najib El-Sayed and Dr. Amy Sapkota (Dean's Representative), for their extensive contributions. I am deeply grateful for their generous allocation of time, support, insightful advice, and overall goodwill extended during the preparation and review of this document. The kindness of Dr. El-Sayed and Dr. Sapkota, coupled with their encouragement and engaging discussions, has had a significant impact on shaping and enhancing the quality of my research.

I would also like to express my gratitude to Victoria Lord for her exceptional dedication in keeping the laboratory operational and ensuring everyone stays focused during the busiest of times. Vickie has consistently been there to offer her assistance, whether it's facilitating manuscript submissions or helping me reschedule my canceled flights, even during the late hours. Her caring nature shines through every interaction, and speaking with her always brightened my day. She has been, and continues to be, one of my strongest supporters, and I am particularly thankful for her unwavering advocacy.

On a more personal note, I must acknowledge the immense role my family has played in this journey. I consider myself incredibly fortunate to have parents and grandparents who continuously inspire me, providing endless motivation and affording me opportunities and experiences that have built my character. Through their exemplary actions, they have instilled in me the conviction that diligent perseverance can lead to great achievements. As I embark on the path of starting my own family, I

am especially grateful for my loving and supportive wife, Allison. Her love and encouragement accompany me in all my endeavors, and I am truly blessed to have her by my side.

Finally, I would like to recognize the extensive collaboration and contributions of my many colleagues, including lab partners, graduate students, visiting scientist, and co-authors, whose assistance and discussion has been essential to this endeavor throughout the years.

This work was supported in part by National Institutes of Health (R01ES030317A), National Science Foundation (EF-0813066, OCE1839171, CCF1918749, and CBET1751854), and National Aeronautics and Space Administration (80NSSC20K0814 and 80NSSC22K1044). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of this document.

Table of Contents

List of Tables

List of Figures

Chapter 1: Environmental parameters associated with incidence and transmission of pathogenic *Vibrio spp.*

Kyle D. Brumfield, Moiz Usmani, Kristine M. Chen, Mayank Gangwar, Antarpreet S. Jutla, Anwar Huq, and Rita R. Colwell. 2021. *Environmental Microbiology*, 23:7314-7340.

DOI: https://doi.org/10.1111/1462-2920.15716

Abstract

Vibrio spp. thrive in warm water and moderate salinity, and they are associated with aquatic invertebrates, notably crustaceans and zooplankton. At least twelve *Vibrio spp.* are known to cause infection in humans, and *Vibrio cholerae* is well documented as the etiological agent of pandemic cholera. Pathogenic non-cholera *Vibrio spp.*, e.g., *Vibrio parahaemolyticus* and *Vibrio vulnificus*, cause gastroenteritis, septicemia, and other extra-intestinal infections. Incidence of vibriosis is rising globally, with evidence that anthropogenic factors, primarily emissions of carbon dioxide associated with atmospheric warming and more frequent and intense heatwaves, significantly influence environmental parameters, e.g., temperature, salinity, and nutrients, all of which can enhance growth of *Vibrio spp.* in aquatic ecosystems. It is not possible to eliminate *Vibrio spp.*, as they are autochthonous to the aquatic environment and many play a critical role in carbon and nitrogen cycling. Risk prediction models provide an early warning that is essential for safeguarding public health. This is especially important for regions of the world vulnerable to infrastructure instability, including lack of 'water, sanitation, and hygiene' (WASH), and a less resilient infrastructure that is vulnerable to natural calamity, e.g., hurricanes, floods, and earthquakes, and/or social disruption and civil unrest, arising from war, coups, political crisis, and economic recession. Incorporating environmental, social, and behavioral parameters into such models allows improved prediction, particularly of cholera epidemics. We have reported that damage to WASH infrastructure, coupled with elevated air temperatures and followed by above average rainfall, promotes exposure of a population to contaminated water and increases the risk of an outbreak

of cholera. Interestingly, global predictive risk models successful for cholera have the potential, with modification, to predict diseases caused by other clinically relevant *Vibrio spp*. In the research reported here, the focus was on environmental parameters associated with incidence and distribution of clinically relevant *Vibrio spp.* and their role in disease transmission. In addition, molecular methods designed for detection and enumeration proved useful for predictive modeling and are described, namely in the context of prediction of environmental conditions favorable to *Vibrio spp.*, hence human health risk.

Introduction

Vibrio spp., ecologically significant aquatic bacteria comprising Gram-negative, motile rods with a facultative anaerobic metabolism, are identified taxonomically as members of the family *Vibrionaceae* in the *Proteobacteria*. *Vibrio spp.* are autochthonous to riverine, estuarine, and coastal environments, and also can be found in freshwater ecosystems globally, including *Vibrio cholerae* and *Vibrio mimicus*, depending on the ionic content of the water (Singleton et al., 1983). *Vibrio spp.* have evolved to occupy diverse niches in ecosystems and to develop complex lifestyles (Reen et al., 2006), an example being *V. cholerae* which attaches to the chitinous exoskeleton and the gut of crustaceans, from crabs to planktonic copepods—the latter serving as a vector for this human pathogen (Huq et al., 1983; Colwell, 1996; Rawlings et al., 2007). *Vibrio fischeri* colonizes the light emitting organ of the Hawaiian bobtail squid, *Euprymna scolopes*, establishing a bioluminescent symbiosis (Visick and McFall-Ngai, 2000), and barotolerant *Vibrio spp.* have been isolated from

sediment and fish collected from deep sea hydrothermal vents (Hasan et al., 2015). The ubiquity of *Vibrio spp.* in the aquatic environment has been confirmed by both culture dependent and culture independent methods (Eilers et al., 2000; Heidelberg et al., 2002). These bacteria have a high biomass (10^3 to 10^4 cells per ml of seawater), yet generally comprise a few percent of the total bacteria (Yooseph et al., 2010; Takemura et al., 2014). A recent molecular genetic analysis (culture independent) yielded data on archived plankton collected in the North Sea (Vezzulli et al., 2012). The results demonstrated a transition during the 1980s, namely *Vibrio spp.* become increasingly numerous members of the bacterial community—a transition that was found to correspond with sea surface warming (*ca.* 0.5°C) and increased incidence of marine-borne *Vibrio* diseases, particularly at higher latitudes (Vezzulli et al., 2020). That is, as water temperature increased, the increase was found to be correlated with human exposure to specific pathogenic *Vibrio spp.*, i.e., risk of infection, most notably for vulnerable groups, e.g., elderly, low-income households, or immunocompromised individuals.

Vibrio spp. occur in aquatic ecosystems as free-living single cells or in aggregates, e.g., within biofilms in high numbers, attached to various abiotic substrates, including sediment particles, marine snow, fecal material of marine animals, and silt particles in the water column (Barbieri et al., 1999; Heidelberg et al., 2002; Reidl and Klose, 2002; DeLoney-Marino et al., 2003). Biofilms, an assemblage of microbial cells that often consists of multiple microbial species/strains enclosed in a polysaccharide-like matrix, allow *Vibrio spp.* to persist under unfavorable conditions (McDougald and Kjelleberg, 2006; Lutz et al., 2013; Sultana et al., 2018). This phenomenon comprises

a component of the annual life cycle of *V. cholerae* in the natural environment (Sultana et al., 2018).

Vibrio spp. undergo horizontal gene transfer (HGT), by which genetic material is transferred among strains and even species. For example, toxigenic strains of *V. cholerae* acquired the ability to produce cholera toxin (CT, a primary virulence factor) by HGT via lysogenic bacteriophage (CTXφ) (Faruque and Mekalanos, 2012). Interestingly, the presence of CTXφ has been detected in other *Vibrio spp.*, e.g., *V. mimicus* (Shah M. Faruque et al., 1999; Boyd et al., 2000). Emergence of multidrug-resistant pathogens via HGT that has resulted in decreasing efficacy of antibiotics and is now a serious global problem. HGT can be considered a primary cause of the global rise in antibiotic resistance observed in many human pathogens (Redondo-Salvo et al., 2020; Che et al., 2021). *Vibrio spp.* have been shown to evolve continuously by gaining fitness traits through HGT, including genes encoding multidrug-resistance (Mohan Raj et al., 2019; Verma et al., 2019). HGT contributes to the ability of *Vibrio spp.* to adapt to specific niches, likely promoting speciation (Hunt et al., 2008). HGT and recombination events may influence the emergence of pandemic clones, such as *Vibrio parahaemolyticus* O3:K6 which appears to have acquired pathogenic characteristics from various bacterial species (Boyd et al., 2008). Broad distribution of virulence genes among environmental *Vibrio spp.* is well documented (Nishibuchi, 1996; Sechi et al., 2000; Klein et al., 2014; Hasan et al., 2015). Studies suggest several virulence factors can influence the pathogenicity of *Vibrio spp.* for humans, but the factors primarily are of environmental relevance (Vezzulli et al., 2008), allowing basic metabolic processes,

establishing symbiosis (McFall-Ngai, 2014), and/or modulating prey/predator relationships (Martínez, 2013) in a natural ecosystem.

Vibrio spp. are associated with a variety of marine organisms, e.g., corals (Kemp et al., 2018), fish (Halpern and Izhaki, 2017), mollusks (Silva et al., 2018), seagrass, sponges, shrimp (Vandenberghe et al., 1999; Polz et al., 2004), and zooplankton (Tamplin et al., 1990; Yang et al., 2017). *Vibrio spp.* interactions with microbial and multicellular hosts, in general, are commensal or mutualistic. However, at least twelve species of the genus *Vibrio* are pathogenic for humans (Baker-Austin et al., 2018), with *V. cholerae*, *V. parahaemolyticus*, and *Vibrio vulnificus* the most significant and well-studied of *Vibrio spp.* pathogenic for humans.

Vibrio infection (vibriosis) in humans historically has been recognized as either cholera or non-cholera infection. Cholera, an acute diarrheal disease caused primarily by consumption of water or food containing toxigenic *V. cholerae* (serogroups O1 and O139), has essentially been eliminated in developed countries by treatment and distribution of safe potable water. Non-cholera *Vibrio* infections typically result from consumption of contaminated shellfish, commonly oysters, or from direct contact with contaminated water through recreation and occupational activities. Non-cholera *Vibrio* infections are characteristically gastroenteritis, wound or ear infection, and/or septicemia.

Vibrio infections have a hallmark seasonal distribution with most cases occurring during the warmer months when water temperatures and salinity profiles are optimal for growth. When environmental conditions are unfavorable, some *Vibrio spp*. demonstrably enter a protective state, namely viable but nonculturable (VBNC),

whereby the bacterial cells become metabolically dormant. That is, VBNC cells cannot be cultured under standard laboratory conditions (Colwell, 2000). When environmental conditions become favorable, usually triggered by elevated temperature and changes in salinity, VBNC cells regain cultivability and virulence potential (Oliver, 2010). VBNC *V. parahaemolyticus* and *V. vulnificus* have been shown to have increased resistance to thermal, low salinity, and acidic inactivation, suggesting that this state comprises a component of a survival strategy in adverse environments (Wong and Wang, 2004; Nowakowska and Oliver, 2013). A key finding of Colwell and colleagues (1996) was that resuscitation of VBNC *V. cholerae* to a culturable state occurred following ingestion of VBNC *V. cholerae* by human volunteers, demonstrating non-culturable *V. cholerae* regain the capacity to multiply when exposed to the environment of the human intestine and retain virulence potential.

Little is known of the effect of climate change on marine prokaryotes, the largest living aquatic biomass, important because these prokaryotes have many essential roles in nutrient cycling and maintaining life on Earth. As the climate of the planet continues to change, sea surface temperature and precipitation patterns are altered, with mounting evidence of a rise in the incidence and distribution of *Vibrio spp*. in the aquatic environment and increased incidence of vibriosis in the human population (Jutla et al., 2011; Vezzulli et al., 2016; Froelich and Daines, 2020). In summary, *Vibrio spp.* are ubiquitous in the aquatic environment and important in carbon and nitrogen cycling (Li et al., 2017; Zhang et al., 2018). Hence, they cannot be eradicated. With the seventh pandemic of cholera in progress and increasing incidence of non-cholera *Vibrio spp.* infections, it is important to understand the ecology of *Vibrio*

spp. in context of a changing climate (Kaper et al., 1995; Colwell, 1996; Lobitz et al., 2000; Mutreja et al., 2011).

Models, such as the National Centers for Coastal Ocean Science (NCCOS) Probability Model for *V. vulnificus* Occurrence in Chesapeake Bay Water (Jacobs et al., 2014), the *Vibrio* Map Viewer (Semenza et al., 2017) developed by the European Centre for Disease Prevention and Control (ECDC), and the hydroclimatic risk models for cholera prediction (Khan et al., 2017, 2018), have been developed to predict increased incidence of *Vibrio spp.* in the environment. The design of future predictive models for *Vibrio spp.* requires inclusion of sociological and behavioral factors, especially those related to contact with the aquatic environment, e.g., bathing, swimming, fishing, and other water-related recreational and occupational activities, since these influence contact with pathogenic *Vibrio spp.* Providing advanced notice of heightened risk, by cell phone or social media notifications (Akanda et al., 2018), allows change in behavior patterns, notably those associated with water use, hygiene, food preparation, consumption of raw or undercooked seafood, and other behaviors associated with increased likelihood of contact with pathogenic *Vibrio spp*. It is important to note that low income countries are affected more by *Vibrio* infection than middle or high income countries, primarily because of lack of sanitation facilities and access to clean drinking water (Igbinosa and Okoh, 2008). Behavior and hygiene education, e.g., hand sanitation, adequate cooking procedures, and methods for preventing cross contamination between food/water sources, is considered an effective intervention strategy for preventing transmission of vibriosis. Targeting disadvantaged populations, e.g., those with low income, transient workers, and individuals with

medical risk factors, is important (Einarsdóttir et al., 2001; Liao et al., 2015). Studies by Huq, Colwell, and colleagues (Huq et al., 1996; Colwell et al., 2003) demonstrated that modification of water collection, by employing simple sari-cloth filtration, effectively removed zooplankton and particulate matter from drinking water and significantly reduced the number of cholera cases in Bangladeshi villages. Clearly, human ecology and behavior, with respect to incidence of *Vibrio* infections, are complex and important for risk assessment, yet effective at the individual level as well.

Warning systems for cholera are important because they provide decision-makers with time to deploy health personnel and resources in vulnerable areas. This was demonstrated in Haiti (Khan et al., 2017), Zimbabwe (Jutla et al., 2015b), Nepal (Khan et al., 2018), and Yemen (Camacho et al., 2018; Federspiel and Ali, 2018) where both individual and large scale intervention early in outbreaks, i.e., early warning systems, contributed to a reduction in the case fatality rate (Akanda et al., 2012). The objective of this study was to investigate environmental factors related to clinically relevant *Vibrio spp.*, their interaction with the environment, and disease transmission. Also discussed are recent advances in molecular methods and satellite sensor informed hydroclimatic processes allowing significantly improved epidemiological surveillance and prediction of conditions favorable for proliferation of *Vibrio spp*. and the diseases they cause.

Vibrio ecology and epidemiology

Approximately twelve *Vibrio spp*. are known to cause infections in humans, of which *V. cholerae, V. parahaemolyticus,* and *V. vulnificus* are considered most

significant (Newton et al., 2012; CDC, 2016, 2019a). The World Health Organization (WHO) estimates up to 4 million cases of cholera and more than 100,000 deaths each year globally (Ali et al., 2015). Furthermore, non-cholera *Vibrio spp.* are among the most common causes of foodborne infection in humans, primarily from consumption of raw or undercooked seafood. The number of *Vibrio* bacteria in fresh caught seafood is usually low, but improper storage during transportation to markets, restaurants, and domestic kitchens can cause numbers to rise above the infectious load. Climate change is increasingly associated with the rise in *Vibrio* infections as well (Vezzulli et al., 2016). The Centers for Disease Control and Prevention (CDC) has developed improved *Vibrio* surveillance programs for the U.S., e.g., Cholera and Other *Vibrio* Illness Surveillance (CDC, 2019a) and Foodborne Diseases Active Surveillance Network (FoodNet) (Tack et al., 2020). However, global *Vibrio* infection surveillance is limited, especially for developing countries. Because of under-reporting or failure to report infections, differences in reporting procedures, and lack of an international epidemiology system, the actual number of global *Vibrio* infections is uncertain (Baker-Austin et al., 2018). CDC estimates there are *ca.* 130 undiagnosed cases for each reported incidence of vibriosis globally (Newton et al., 2012).

In the U.S., *Vibrio spp.* are estimated to cause 80,000 illnesses, 500 hospitalizations, and 100 deaths annually, of which about 65% are foodborne (Newton et al., 2012), and foodborne-associated vibriosis essentially tripled in a short period of time, between 1996 and 2010 (Newton et al., 2012). According to FoodNet, with sites in 10 states and covering 15% of the U.S. population, there appears to have been a long-term increase in the reported number of *Vibrio* infections between 1996 and 2019

(**Figure 1 A**), with most *Vibrio* infections occurring in July and August (**Figure 1 B**). *V. parahaemolyticus* accounts for nearly half of the *Vibrio* infections, but *Vibrio alginolyticus* (*ca.* 9.8%), *V. vulnificus* (*ca.* 9.1%), *V. cholerae* (*ca.* 6.6%) are also important (**Figure 1 C**). Interestingly, most *Vibrio* infections (*ca.* 55%) occur in males, and roughly 75% in the elderly population over the age of 65 years (**Figure 1 D**). *V. parahaemolyticus* is responsible for an estimated 34,000 cases, with *ca*. $40 million in associated costs each year. Even though *V. vulnificus* infection is relatively rare (estimated 100-200 cases in the U.S. annually), the bacterium is associated with an increased mortality rate, greater than 50% for primary septicemia and *ca.* 15% for wound infections. It also carries an associated yearly economic burden of *ca.* $320 million (Bross et al., 2007; Ralston et al., 2011; Scallan et al., 2011; Hoffman et al., 2015; Muhling et al., 2017). Higher incidence of vibriosis in the U.S. occurs more frequently during warmer years, with the expectation that, as global warming continues, there will be a parallel increase in frequency and intensity of *Vibrio* infections.

A key issue related to risk of *Vibrio* infection is that the location of some of the largest population centers and geographic regions of economic activity in the U.S. is along the coast. Currently, *ca.* 40% of the total U.S. population lives in coastal counties, a number expected to rise in coming years (NOAA, 2021a). Furthermore, the nearly half of Americans who live in coastal counties also account for an elevated risk category, e.g., elderly and low-income households (NOAA, 2021a). According to the United Nations, an older population is projected for most regions of the world population in the next several decades. Globally, the share of the population aged 65

years or over increased from *ca.* 6% to *ca.* 9% between 1990 and 2019. That proportion is projected to rise further to *ca.* 16% by 2050 globally so that one in six individuals will be over the age of 65 (United Nations, 2020).

Seafood remains the single source of high-quality protein for many populations, and there has been more than a fivefold rise in global aquaculture production since 1990 (FAO, 2020). Hence, protein derived from seafood (especially aquaculture) is becoming increasingly important for both human health and the global economy. Concurrently, an increased number of environmental *Vibrio spp*. have been identified as causative agents of vibriosis in the aquaculture industry and are responsible for large-scale economic losses (Kim and Lee, 2017). A dramatic example is early mortality syndrome in shrimp, caused by *V. parahaemolyticus*, a disease responsible for annual losses estimated to be more than one billion USD (Zorriehzahra and Banaederakhshan, 2015).

Changes in climate, natural host, and/or habitat are linked to changes in size and composition of marine prokaryotic populations, including pathogenic *Vibrio spp.* (**Figure 2**). Many *Vibrio spp.* are known to be associated with crustaceans, shellfish, arthropods, chironomids, vertebrate fishes, waterfowl, protozoans, phytoplankton, and aquatic plants (Broza et al., 2008; Almagro-Moreno and Taylor, 2013). *Vibrio spp.* are exposed in antagonistic relationships with other procaryotes in the environment, such as phages that are capable of reducing *Vibrio* populations (Silva-Valenzuela and Camilli, 2019). It has been suggested that *Vibrio* phages can modulate outbreaks, influencing seasonality of cholera, and may play a role in emergence of new serogroups or clones (Faruque et al., 2005), but this hypothesis has been challenged (Alam et al.,

2006a). In addition to phages, *Vibrio spp.* interact with other organisms that can serve as a replication niche (Espinoza-Vergara et al., 2020), such as the heterotrophic protists that feed on *Vibrio spp.* (Berk et al., 1976). Protozoa engulf bacteria and package them into phagosomes, thereby exposing them to low pH, antimicrobial peptides, and metal ions which may promote virulence or resistance factors (Espinoza-Vergara et al., 2020). Environmental parameters influencing seasonal epidemics of vibriosis and specific factors shown in laboratory experiments to promote HGT, contribute to the emergence of pathogenic strains, as reported by Oberbeckmann and colleagues (2012) and Le Roux and Blokesh (2018). Clearly, the importance of the environment in the transmission and disease causation by *Vibrio spp.* is increasingly being documented. Because of the affinity of *Vibrio spp.*, in general, for warm water of low salinity, a capability to grow in and on appropriate hosts, and broad pathogenicity profile, they have been proposed as a useful environmental barometer for climate change (Vezzulli et al., 2016, 2020; Baker-Austin et al., 2017), a rational conclusion based on currently available evidence.

Vibrio cholerae

Detection and identification of *Vibrio cholerae*, the etiological agent of cholera, in environmental samples, especially water and food, were for many years successful only after the disease was diagnosed in patients during an outbreak (Pollitzer, 1954; Pollitzer et al., 1959; Barua, 1992; Azarian et al., 2016). Direct detection in water was made possible with the developments of epifluorescent microscopy (Xu et al., 1984; Huq et al., 1990; Hasan et al., 1994; Lowenhaupt et al., 1998) and molecular probes

(Hoshino et al., 1998; Bauer and Rørvik, 2007; Fykse et al., 2007; Nandi et al., 2012). Between outbreaks and during unfavorable environmental conditions, *V. cholerae* cells can enter the VNBC state and/or switch between motile and biofilm lifestyles, enhancing survival and allowing persistence in the environment, notably forming environmental reservoirs in and on copepods, bivalves, aquatic plants, protozoa, and abiotic substrates (Conner et al., 2016). It is now recognized that *V. cholerae* is naturally occurring in coastal and estuarine microbial ecosystems globally, including northern countries like Iceland (Haley et al., 2013). Copepods, a zooplankton comprising a significant component of the aquatic fauna of rivers, bays, estuaries, and the open ocean, are a major host, sufficiently significant to be concluded a vector. That is, *V. cholerae* attaches to the gut and carapace of copepods, comprising a significant component of the commensal flora and thereby serving as a vector of the causative agent of cholera. A single copepod can harbor up to 10^4 *V. cholerae* cells (Colwell and Spira, 1992), hence ingestion of untreated water containing a small number of copepods can promote disease (Cash et al., 1974; Huq et al., 1996; Colwell et al., 2003). Conditions favorable for multiplication of copepods and other chitinous zooplankton are associated with increase in number of *V. cholerae* cells, thereby promoting greater potential for disease (Huq et al., 1983; Colwell, 1996; Vezzulli et al., 2010; Islam et al., 2020).

Based on its surface lipopolysaccharide O-antigen structure, *V. cholerae* is identified according to serogroup (Chowdhury et al., 2017). Historically, only serogroup O1 was associated with epidemic or pandemic cholera, until strains of serogroup O139 were isolated from cholera patients in major outbreaks and

environmental samples. However, to date, *V. cholerae* O139 has been isolated only in South East Asia (Albert, 1994; Waldor et al., 1994; Alam et al., 2006a), whereas serogroup O1 occurs worldwide, including U.S. waters, e.g., Maryland and Louisiana estuaries (Colwell et al., 1981). *V. cholerae* O1 is responsible for the current seventh pandemic and for all previously reported cholera pandemics since 1817. *V. cholerae* non-O1/non-O139 (NOVC, i.e., non-agglutinating *V. cholerae*) is the causative agent of sporadic, yet significant infections that may range in severity from mild, e.g., acute gastroenteritis and otitis, to life-threatening, e.g., necrotizing fasciitis (Deshayes et al., 2015). However, compared to *V. cholerae* O1/O139 isolates, NOVC strains are a neglected group of human pathogens (Baker-Austin et al., 2018). It is worth noting that conversion of NOVC to O1 has been reported (Colwell et al., 1995), and NOVC is believed to contribute to emergence of new pathogenic strains with epidemic potential as a direct consequence of genetic recombination and HGT (Vezzulli et al., 2020). Hence, presence of NOVC should not be ignored in this era of global climate change.

The toxin-coregulated pilus (TCP, encoded by *tcpA*) and CT (encoded by *ctx*) are two main virulence factors of *V. cholerae* and allow the bacterium to colonize and establish infection. Isolates of *V. cholerae* serogroup O1 are categorized into two biotypes, classical and El Tor, each of which displays unique genotypic and phenotypic characteristics (Brumfield et al., 2018a). Purified *V. cholerae* isolates are available for modern scientific study only from the sixth (1899-1923) and seventh (1961-present) cholera pandemics but not from any of the earlier pandemics (Hu et al., 2016). The classical biotype is responsible for the sixth cholera pandemic. Selective sweeps, suggested to result from environmental pressures and natural selection, promoted

emergence of new *V. cholerae* variants (Stine and Morris Jr, 2014). For example, *V. cholerae* acquired mobile elements, namely *Vibrio* seventh pandemic islands I and II, and the El Tor pandemic, the seventh, began thereafter (Stine and Morris Jr, 2014). Seventh pandemic isolates were first described in Indonesia and have displaced classical biotype strains in the environment (Barua, 1992). Subsequently, emergence of O139 encoding genes prompted the rapid spread of cholera outbreaks throughout the Indian subcontinent (Faruque et al., 2003). The integrative conjugative element SXT, a mobile genetic element that is strongly correlated with antibiotic resistance, is reported to have led to a third major selective sweep. A fourth selective sweep prompted exchange of El Tor *ctx* allele for classical *ctx* in *V. cholerae* El Tor biotype strains (Mutreja et al., 2011) that produce higher levels of CT than previously reported (Koley et al., 2010; Carignan et al., 2016). Furthermore, NOVC strains inhabit estuarine and coastal waters globally and carry multiple virulence factors characteristic of *V. cholerae*, e.g., CT (Hasan et al., 2013), RTX toxins, hemolysins, and type III secretion system (Ceccarelli et al., 2015), as well as toxins exclusive to these strains, e.g., cholix toxin (Schwartz et al., 2019). Hence, it is necessary to enumerate the total number of *V. cholerae*, regardless of serotype, in the context of public health.

Cholera

The disease, cholera, occurs predominantly in two forms: *epidemic*, characterized by sporadic or rampant occurrence of cases in an outbreak or *endemic*, associated with cases occurring annually at a continuous level, often with distinct

seasonal peaks in the number of cases. WHO defines a cholera endemic area as a particular geographic region where confirmed cholera cases have been detected consecutively during the past three years with evidence of local transmission—indicating the etiological agent of the disease had not been imported from a different geographic region (WHO, 2019). Cholera reemerges persistently in epidemic form, particularly along the coast in tropical areas (Colwell, 1996; Lipp et al., 2002; Camacho et al., 2018). Epidemiological surveillance has shown cholera outbreaks emerge, usually, in coastal regions before reaching inland (Nair et al., 1994; Kierek and Watnick, 2003; Alam et al., 2006a). However, cholera epidemics or outbreaks can occur in both endemic areas and in geographic locations where cholera does not occur regularly (CDC, 2012; WHO, 2019), i.e., epidemic areas (Jutla et al., 2013a, 2013b, 2015b; Khan et al., 2017, 2018), with the majority of the burden in Sub-Saharan Africa (Ali et al., 2015). However, there continue to be sporadic and erratic occurrences of apparently indigenous cholera occurring mostly around the coastal regions, namely the Gulf Coast of the U.S. and most recently in Hawaii (Maniam et al., 2020).

Vibrio parahaemolyticus

V. parahaemolyticus is a halophilic bacterium and like all *Vibrio spp.* is autochthonous to the aquatic environment (Joseph et al., 1982). *V. parahaemolyticus*, first named *Pasteurella parahaemolytica* until its taxonomy was updated to genus *Vibrio* (Fujino, 1953), was isolated by Professor Fujino during a 1950 shirasu food poisoning outbreak in Japan (Fujino, 1951). *V. parahaemolyticus* was first isolated in U.S. waters from diseased crabs by Krantz and colleagues (1969), who suggested that

the bacterium is part of the natural marine flora, with the ability to invade marine animals and a potential human problem. Subsequently, the bacterium was isolated from plankton (Kaneko and Colwell, 1973) and a variety of seafood including codfish, sardine, mackerel, flounder, clam, octopus, shrimp, crab, lobster, crawfish, scallop, and oyster (Liston, 1990). While most isolates from the environment or seafood are nonpathogenic (Nishibuchi and Kaper, 1995), *V. parahaemolyticus* is recognized as an opportunistic pathogen and one of the leading causes of seafood-derived food poisoning throughout the world (CDC, 2016, 2019a). Acute gastroenteritis is the primary symptom of an infection with virulent strains of the bacterium. However, contact with water containing *V. parahaemolyticus* can lead to wound infection and septicemia, with a life-threatening potential for individuals with preexisting medical conditions (Su and Liu, 2007). Early studies estimated *V. parahaemolyticus* was responsible for up to 30% of all food-poisoning cases in Japan (Jahangir Alam et al., 2002), and similar claims have been made in other parts of Asia (Koralage et al., 2012; Yu et al., 2013). Furthermore, *V. parahaemolyticus* has been identified as the leading cause of seafood-associated gastroenteritis in the U.S. (Mead et al., 1999) and China (Li et al., 2014) since the 1990s. CDC estimates over 45,000 illnesses each year in the U.S. alone result from consumption of raw or undercooked seafood, particularly shellfish (CDC, 2019b). The global distribution of *V. parahaemolyticus* underscores the importance of understanding its many virulence characteristics and their effect on the human host, as well as environmental factors, e.g., temperature and salinity, that influence abundance and distribution.

There is a strong correlation was observed between the ability to produce beta-hemolysis on Wagatsuma agar, known as the Kanagawa phenomenon (KP), and virulence of the bacterium. Most strains isolated from patients carry genes that encode an amyloid toxin that disrupts lipid microdomains and promotes cytotoxicity in the cell (Matsuda et al., 2010), i.e., thermostable direct hemolysin (TDH) or TDH-related genes (TRH) (Honda et al., 1987, 1988). TDH and TRH are rarely observed in environmental isolates, compared to clinical isolates (Joseph et al., 1982; Bej et al., 1999). However, clinical strains have been isolated that are negative for thermostable hemolysin genes suggesting other important virulence traits are carried by *V. parahaemolyticus* (Nishibuchi et al., 1992). Thus, while the virulence potential of the two genes has been accepted, not all clinical strains possess these genes. In fact, previous reports claim roughly 10% of clinical *V. parahaemolyticus* isolates are TDH/TRH negative (Miyamoto et al., 1969; Shirai et al., 1990; Okuda et al., 1997), suggesting full pathogenic mechanism of the bacterium is unclear. An additional hemolysin, the thermolabile hemolysin (TLH), which encodes phospholipase A2 (Zhang and Austin, 2005), has been characterized as not causing hemolysis on Wagatsuma agar, but has been observed in nearly all strains of *V. parahaemolyticus* (Bej et al., 1999). Accordingly, TLH has become the 'Gold Standard' for *V. parahaemolyticus* detection and identification (Bej et al., 1999; Johnson et al., 2012; Gutierrez West et al., 2013; De Silva et al., 2019).

The TLH/TDH/TRH gene profile typically determines the pathogenic load in seafood and is used to characterize pathogenic strains. However, surveillance of the bacterium by presence of hemolysin can be biased since these markers are present in

other *Vibrionaceae* species (Yáñez et al., 2015). Furthermore, studies indicate that strains lacking the toxins and genomic regions associated with pandemic isolates may still be pathogenic, evidence that other virulence factors are clinically relevant. The type III secretion system (T3SS) has been proposed as an indicator of virulence and two nonredundant T3SSs have been described in many *V. parahaemolyticus* strains (Park et al., 2004; Broberg et al., 2011). T3SS1 is involved in cytotoxicity and lethality in mice (Park et al., 2004; Hiyoshi et al., 2010; Broberg et al., 2011), while T3SS2 is suspected to play a role in environmental fitness (Park et al., 2004; Matz et al., 2011). However, Jones and colleagues (2012) described *V. parahaemolyticus* strains, collected from patients suffering gastroenteritis, that were negative for TDR, TRH, and T3SS genes, indicating these genes alone are not necessarily predictive of pathogenic potential.

Based on somatic (O) and capsular (K) surface antigens, there are currently over 80 described serotypes of *V. parahaemolyticus*. Serovar O3:K6 was first described as a pandemic isolate in 1996, during a major outbreak in India, and over 50% of strains isolated from the patients proved to be O3:K6 (Matsumoto et al., 2000; Nair and Hormazabal, 2005; Nair et al., 2007b). Soon after, *V. parahaemolyticus* O3:K6 was isolated in other countries in Asia, South America, North America, Africa, and Europe (Matsumoto et al., 2000; Nair and Hormazabal, 2005). Recently, other serovariants, most notably O1:K68, O1:K25, and O1:KUT (un-typable), have emerged and appear to have rapidly evolved from the original pandemic O3:K6 clone (Chowdhury et al., 2004; Chen et al., 2011). From 1996 to 2007, 22 pandemic serovariants were identified

from various regions of the world (Nair et al., 2007b), and at least five additional serovariants have since been described (Han et al., 2017).

Since 2012, *V. parahaemolyticus* serovar O4:K12 has also shown transcontinental epidemic expansion (Martinez-Urtaza et al., 2017), with O4:K12 isolates detected outside the region in which it was endemic, i.e., the U.S. Pacific Northwest, with infections reported along the U.S. northeastern coast and Spain (Martinez-Urtaza et al., 2013). Genome analysis of representative isolates from the Pacific Northwest, northeastern U.S., and Spain showed this *V. parahaemolyticus* population was highly dynamic and strains causing infection in the northeastern U.S. likely had diverged from the original lineage in the Pacific Northwest by cross-continent eastward expansion (Martinez-Urtaza et al., 2017). More recently, Abanto and colleagues (2020) reported transcontinental expansion of O4:K12 into Lima, Peru and showed long term persistence and presence of the isolates in the environment, suggesting successful establishment in environmental reservoirs. Precise sources and routes of pandemic clonal transmission of *V. parahaemolyticus* into Peru have yet to be determined. The available evidence suggests a link existing between the epidemic dynamics and spread of *Vibrio* infections in this region of South America and El Niño (Martinez-Urtaza et al., 2016), the latter providing ideal environmental conditions for growth of *Vibrio spp.*, namely heavy rains and heat waves. Subsequently, emergence of cases in Peru associated with new clones of *Vibrio* was concurrent with the El Niño and the causative agent of the outbreaks were *V. cholerae* in 1991 and *V. parahaemolyticus* in 1997 (Martinez-Urtaza et al., 2008a).

Multilocus sequence typing, an unambiguous procedure for characterizing bacterial isolates by genomic fingerprinting of essential house-keeping genes (Maiden et al., 1998), is employed routinely to compare genomic relatedness of *V. parahaemolyticus* clinical and environmental isolates (Turner et al., 2013; Baker-Austin et al., 2020a). *V. parahaemolyticus* sequence type (ST) 3, encoding *tdh*$^{(+)}$ and *trh*$^{(-)}$ virulence factors, typically is associated with the O3:K6 pandemic group of *V. parahaemolyticus* that emerged in Asia during the 1990's and spread rapidly to nearly all continents (Nair et al., 2007b; Han et al., 2019). *V. parahaemolyticus* isolates belonging to the second transcontinental O4:K12 group are classified as ST36 (Han et al., 2019). Nasu and colleagues (2000) identified a filamentous phage (f237) in a collection of O3:K6 isolates and suggested the eighth open reading frame (ORF8), located in the phage, encodes a putative adherence protein specific to post-1995 pandemic isolates. It has been suggested that, post-1995, O3:K6 pandemic isolates can be characterized using pandemic group-specific PCR (PGS-PCR) (Okura et al., 2003) and a unique/new sequence of ToxR, a virulence regulator of vibrios encoded by the *toxRS* operon, that shows variation across pandemic and non-pandemic strains potentially involved in overall virulence (Matsumoto et al., 2000). However, by themselves, these markers are not reliable for identification of the pandemic group (Bhuiyan et al., 2002; Chowdhury et al., 2004; Rahman et al., 2006; Jones et al., 2012). Genotyping of non-pandemic strains containing virulence factors remains an open question.

Vibrio vulnificus

V. vulnificus is a serious human pathogen common to estuarine waters globally. It is present in large numbers in sediment and molluscan shellfish, especially during warmer months of the year. *V. vulnificus* infections typically progress to septicemia from a wound infection and are contracted more commonly by individuals participating in recreational activities, such as swimming or fishing (Baker-Austin and Oliver, 2018). An infection usually progresses from diarrhea after consumption of raw or under cooked seafood, primarily oysters where it occurs in large numbers (10^5 g^{-1} or more). *V. vulnificus* is readily isolated from shellfish harvested from the Atlantic and Pacific coasts where *ca.* 75% of the retail oyster harvest has been reported to contain the bacterium, with highest rate of detection in the Gulf of Mexico (Oliver, 2006; Bowers et al., 2014). Furthermore, *V. vulnificus* can multiply rapidly in postharvest seafood improperly stored and/or transported. The fatality rate for *V. vulnificus* infections in humans is one of the highest of any water-borne pathogen, greater than 50% for primary septicemia, and it is responsible for *ca.* 95% of all waterborne and seafood related deaths in the United States (Bross et al., 2007; Jones and Oliver, 2009). *V. vulnificus* severe wound infections are characterized by necrotizing fasciitis or soft tissue and blood infection, with a case fatality rate of *ca.* 15% (Jones and Oliver, 2009). *V. vulnificus* infections notoriously become acute rapidly, and symptoms usually present within 24 h or less after exposure (Jones and Oliver, 2009). Unfortunately, even with aggressive therapy, the case-fatality rate is *ca*. 30-40% (Bross et al., 2007; Baker-Austin and Oliver, 2018).

The genomic, biochemical, and serological characteristics and the host range of *V. vulnificus* is categorized as comprising three biotypes. Biotypes 1 and 3 are predominantly associated with human disease and Biotype 2 with zoonotic infection of eels. Interestingly, wound infections in Israel caused by *V. vulnificus* Biotype 3 were reported to represent a *V. vulnificus* population emerging as a component of the impact of global warming on the eastern Mediterranean basin (Paz et al., 2007). More recently, *V. vulnificus* Biotype 3 has been identified as the causative agent of infections in Japan (Hori et al., 2017). Despite being characterized as an environmental pathogen, *V. vulnificus* Biotype 1 strains are typically subtyped as clinical (C), or environmental (E), based on genotypic differences correlated with the source of isolation (Rosche et al., 2005; Baker-Austin and Oliver, 2018). Biotype 1C strains have been reported to be responsible for nearly all cases of primary septicemia, whereas Biotype 1E strains traditionally are associated with wound infections. Whole-genome sequencing results reported by several investigators (Gulig et al., 2010; Morrison et al., 2012; Koton et al., 2015) revealed that *V. vulnificus* encodes a wide array of putative virulence factors, e.g., acid neutralization, capsular polysaccharide expression, iron acquisition, cytotoxicity systems, and expression proteins associated with adhesins and attachment (Jones and Oliver, 2009). *V. vulnificus* secreted cytolytic/hemolysin pore-forming toxin coded for by *vvhA* and auto processing RTX toxin encoded by *rtxA1* have been linked to increased virulence following intestinal colonization (Fan et al., 2001; Guo et al., 2018). Despite elegant research reported to date, specific biomarkers directly distinguishing pathogenic and non-pathogenic *V. vulnificus* strains have not yet been identified.

Other pathogenic *Vibrio spp.*

Vibrio spp. have been isolated from patients expressing mild to moderate diarrhea and from extraintestinal sites, such as the respiratory tract, the ear, a variety of wound infections, and from cerebrospinal fluid. Occasionally, CT and TCP associated genes have been reported in NOVC isolates (Hasan et al., 2013), and the presence of other virulence factors, such as Shiga-like cytotoxin, heat-stable enterotoxin, and hemolysins have also been detected in non-cholera *Vibrio spp.* (Nair et al., 1988; Ceccarelli et al., 2015). In the U.S., most cases of vibriosis have been identified as infections caused by *V. parahaemolyticus*, *V. alginolyticus*, or *V. vulnificus* (**Figure 1 C**), but up to 10% of the vibriosis reported yearly has been attributed to NOVC (Jones et al., 2013), suggesting a long term continual increase of such infections with time (Newton et al., 2012).

V. alginolyticus is a species representative of the marine microflora and has been identified as a causative agent of food poisoning and also bloodstream and soft tissue infections in humans. Ironically, *V. alginolyticus* is a significant pathogen of marine animals (Xie et al., 2020). The pathogenicity of *V. alginolyticus* is highly dynamic, with molecular surveillance showing virulence genes homologous to those of *V. cholerae* and *V. parahaemolyticus* widely distributed amongst *V. alginolyticus* strains isolated from the environment (Xie et al., 2005), including CT and pathogenicity islands (Sechi et al., 2000). The frequency of occurrence of *V. alginolyticus* infections has increased rather dramatically in recent years, very likely ascribable to a rising sea temperature globally (Baker-Austin et al., 2018). Between 1998 and 2012, more than 1,300 *V. alginolyticus* infections in humans were reported in the U.S., mainly

associated with exposure during participation in water related activities in the coastal states (Newton et al., 2012). During the same period, *V. alginolyticus* was reported to be a major cause of foodborne disease each year in America (**Figure 1**). In fact, the incidence of vibriosis caused by *V. alginolyticus* has increased more than 12-fold in recent years (Newton et al., 2012).

Vibrio fluvialis, an environmental bacterium, is considered an emerging human pathogen (Igbinosa and Okoh, 2010). Other *Vibrio spp*., including *Vibrio mimicus*, *Grimontia* (*Vibrio*) *hollisae*, *Vibrio furnissii*, *Vibrio metschnikovii*, *Vibrio cincinnatiensis*, and *Photobacterium* (*Vibrio*) *damsela*, are opportunistic pathogens for humans. No doubt as climate warming continues, those species will increasingly be recognized as human pathogens.

Molecular methods for detection and genomic analysis of *Vibrio spp.*

Advances in molecular methods for microbial detection, identification, and characterization, particularly high-throughput sequencing (HTS), have allowed researchers to investigate more completely the mode of emergence and transmission, and the dynamics of *Vibrio* diseases (Martinez-Urtaza et al., 2017; Weill et al., 2017, 2019). HTS provides useful insight into how *Vibrio spp.* spread in the environment and the mechanisms by which they are able to cause diseases, thereby permitting retrospective analysis of their evolution and the disease they cause (Mutreja et al., 2011; Hasan et al., 2012). The ability to predict and monitor disease outbreaks in real time is critical to managing risk associated with those caused by pathogenic *Vibrio spp.* (Baker-Austin et al., 2020b). By incorporating molecular techniques in environmental

studies of cholera, it has been found that climate change is playing a significant role in the ecology of *V. cholerae* (Colwell, 1996; Lobitz et al., 2000; Vezzulli et al., 2016, 2020). Historically, detection of *Vibrio spp.* in the environment had been challenging because these bacteria can enter the VBNC state, complicating detection if traditional culture is relied upon for detection. Enumeration by culture underestimates the total *Vibrio* population. This was shown for samples examined by fluorescent antibody, revealing the presence of *V. cholerae* when culture was not successful (Hasan et al., 1994; Nair et al., 2007a; Martinelli Filho et al., 2011; Kahler et al., 2015). Molecular methods, namely PCR and DNA sequencing, have proved to be more useful in circumventing lack of success when culture methods fail. In addition to detection, identification, and characterization of microbial communities, whole genome sequencing has been used to detect and identify virulence factors and antimicrobial resistance genes (Kimes et al., 2012). For environmental samples where the low abundance of *Vibrio* DNA, e.g., virulence genes and genetic markers of epidemic strains, is a challenge and not detectable by conventional methods, whole genome enrichment has proven valuable for direct genotyping and metagenomic analysis (Vezzulli et al., 2017). Perhaps more useful than the short read HTS provided by Illumina, e.g., NovaSeq, HiSeq, NexSeq, MiSeq, and Thermo Fisher (Ion Torrent), which produce reads of up to *ca.* 600 base pairs, long read sequencing, provided by PacBio and Oxford Nanopore instruments, have the potential to generate reads > 10 kb (Pollard et al., 2018). Long read sequencing routinely has been employed to produce high-quality reference genomes of *Vibrio spp.* isolates (Kanrar and Dhar, 2018; Dorman et al., 2019).

DNA metagenome sequencing (metagenomics) is a valuable technology for detection of all *Vibrio spp.* in the environment, including VBNC *Vibrio*, and is particularly useful because it simultaneously profiles virulence and antimicrobial resistance associated genes (Roy et al., 2018; Acharya et al., 2020; Brumfield et al., 2020c, 2021a). Most of the HTS studies reported to date have focused on clinical isolates obtained by culture previously from patient samples. Metagenomic sequencing allows generation of genome assemblies directly without culture, providing a more accurate composition of the microbiome and making it possible to detect, identify, characterize, and *Vibrio spp.* and their dynamics (Baker-Austin et al., 2020b). The shortcoming of DNA metagenomics is that viability or infectious potential of the microorganisms comprising the community cannot be determined without additional analyses to determine gene function and activity. Nevertheless, profile composition of all microorganisms present in a sample, e.g., bacteria, archaea, fungi, viruses, and protozoa, is highly informative (Brumfield et al., 2020c), and metagenomics can provided useful insight to disease outcome, as has been shown for the gut microbiome during *V. cholerae* infection (Levade et al., 2021) and for patients with skin and soft tissue infections caused by rare pathogens such as *V. vulnificus* (Wang et al., 2020). Most valuable is the ability of HTS to identify multiple pathogens comprising a poly-microbial infection (De et al., 2020), to estimate antimicrobial resistance (Zhelyazkova et al., 2021), and to establish the quantitative microbial risk of *Vibrio spp.* of human health significance in non-clinical environments (Singh et al., 2018; Noman et al., 2021). Genome based tools will play an important role in the advancement of predicative modeling because they provide an analysis of the composition and diversity

of *Vibrio spp.* populations in clinical and environmental samples, along with all other microorganisms present, including those in the VBNC state (Baker-Austin et al., 2020b).

Impact of climate and environment on diseases caused by *Vibrio spp.*

Studies have shown that climate change is associated with an increased frequency and intensity of extreme weather events, such as heatwaves and severe precipitation, which can also result in warmer and less saline waters in coastal regions (Ummenhofer and Meehl, 2017; Vezzulli et al., 2020). Marine heatwaves, analogous episodes in the oceans, are caused by anomalous ocean heating at the surface and are expected to exacerbate in a warming climate (Smale et al., 2019), with an irreversible impact on marine ecosystems (Holbrook et al., 2019). Similarly, marine oxygen concentrations have been declining globally during the past half century, and dead zones near the coast have multiplied 10-fold causing suffocation of marine life and severe ecological disruption (Breitburg et al., 2018). Hence, changes that occur in the aquatic environment can have a significant impact on abundance and distribution of marine microorganisms, and the impact of ocean warming on emergence and spread of environmental microbial pathogens is rightfully a developing concern (Harvell et al., 2002; Baker-Austin et al., 2013; Vezzulli et al., 2016).

Because of their high growth rate and rapid response to environmental signals, *Vibrio spp*. have been proposed as a microbial indicator of a changing global climate (Vezzulli et al., 2016; Baker-Austin et al., 2017). A number of studies have documented a pattern of poleward spreading of *Vibrio spp.*, demonstrating significant geographic

expansion of *Vibrio* populations (Baker-Austin et al., 2013, 2017; Vezzulli et al., 2016). This geographic expansion of *Vibrio* populations is corroborated by impact on human health (Watts et al., 2018), a prime example of which is the increase in NOVC related infections, a striking linkage of human disease and climate change (Vezzulli et al., 2020). Estuarine waters, typically warm and brackish, provide optimal environmental conditions for growth of *Vibrio spp.* (Johnson et al., 2012; Vezzulli et al., 2013) and have warmed faster than the open ocean, with climate change (Europen Environment Agency, 2012). Open oceans are generally associated with high salinities, low temperatures, and depleted nutrients that hinder growth of *Vibrio spp*. However, during summer months, there are periods of increased temperature and low salinity in coastal regions of the world, e.g., Chesapeake Bay in the eastern U.S., East China Sea surrounding Shanghai, and the Baltic Sea in northern Europe, considered 'Hot Spots' for risk of *Vibrio* infection (Semenza et al., 2017; Vezzulli et al., 2020). In addition to marine or coastal ecosystems, NOVC occurrence and infections have been reported in human populations near inland waters in regions of the world where cholera is not endemic (Vezzulli et al., 2020). As sea surface temperatures rise, the geographic range of pathogenic *Vibrio spp.* is expected to expand poleward and their abundance to increase in those regions where previously present in low or undetectable numbers. While it is worth noting that certain *Vibrio spp*., e.g., *Vibrio splendidus*, express virulence factors at low temperatures (Lattos et al., 2021), higher temperatures can serve as a selective pressure for strains with higher virulence potential or elevated expression of virulence factors that allow more effective host invasion (Vezzulli et al., 2020). Since *Vibrio spp.* exhibit seasonal patterns of growth, a changing climate likely

will induce extended seasonality of *Vibrio spp.*, with serious economic and public health implications (Baker-Austin et al., 2010).

Using a suite of Earth system model simulations and satellite remote sensing, Frölicher and colleagues (2018) recorded a doubling of the number of marine heat wave events globally between 1982 and 2016, with extreme warm sea surface temperatures persisting for days to months. These events were projected to increase with added global carbon emissions (Frölicher et al., 2018). Over the past 30 years, extreme heat events in Northern Europe have been linked to increased reports of *Vibrio* infections (Baker-Austin et al., 2013). In Finland and Sweden, a heatwave in 2014 was associated with an increased number of infections caused by *Vibrio spp.* (Baker-Austin et al., 2017). Similarly, a 60-year investigation of samples collected during a continuous plankton recorder survey showed prevalence of *Vibrio spp.* increased in the coastal North Sea, and the increase was correlated with warming sea temperatures and with increased number of infections caused by pathogenic *Vibrio spp.* in Northern Europe (Vezzulli et al., 2016). Extreme precipitation events have also been correlated with spikes in *Vibrio* infections as occurred following Hurricane Katrina in the Gulf Coast (CDC, 2005). Flash floods increase risk of infection and loss in aquaculture productivity since an abrupt decrease in salinity following a heavy rainfall and major flooding favors growth of pathogenic *Vibrio spp.* (Esteves et al., 2015). Intense rainfall will not only alter the salinity profile, but also nutrient availability in coastal rivers, with increasing inflow of freshwater. Stormwater runoff can also promote transmission of antimicrobial resistance and virulence associated genes among bacteria (Brumfield *et al.*, 2021). The evidence is clear that *Vibrio spp.* persist under unfavorable

environmental conditions by entering the VBNC state, but the effect with more extensive climate change is not yet known.

Anthropogenic impact on *Vibrio spp*.

Urban development, coastal engineering, sediment dredging, pollution, fishing, aquaculture, tourism, and mining are human activities that have a direct impact on marine ecosystems, including the microbial populations (Nogales et al., 2011). Global transport and discharge of ballast water by ocean going cargo vessels suggests a mechanism for dispersal of *Vibrio spp.*, evidenced by epidemiological surveys of waterborne diseases (Ruiz et al., 2000). Improper holding and transport of shellfish is another path by which *Vibrio spp.* can be dispersed (Love et al., 2020), providing a separate route for long-range dispersal (Nair et al., 2007b). Not only *Vibrio spp.* but also antibiotics released into the aquatic environment via wastewater discharge and runoff amplify the problem by antibiotic resistance gene transfer amongst *Vibrio spp.* in the aquatic environment (Vincent et al., 2019; Cuadrat et al., 2020). Phytoplankton blooms in vulnerable areas of the ocean resulting from anthropogenic activity (Michael Beman et al., 2005) are linked to increased zooplankton populations, notably the copepods that feed on phytoplankton, with a significant correlation of increased populations of copepods with phytoplankton blooms and *Vibrio spp.* (Huq et al., 1983). An abundance of phytoplankton is followed by zooplankton blooms and, subsequently, increase in *Vibrio* populations in nutrient-rich waters (Alam et al., 2006a; Vezzulli et al., 2015).

Environmental parameters and epidemiology

Remote sensing provides vast spatial coverage and near real-time observations that can significantly improve the capacity to predict occurrence of *Vibrio spp.* in the environment. By predicting when and where pathogenic *Vibrio spp.*, namely *V. cholerae*, proliferate, it is now possible to identify regions where increased interaction between human populations and these bacteria can occur, thereby determining risk of infection (**Figure 2**). These remote sensing capabilities have been leveraged extensively to investigate how environmental conditions affect incidence and distribution of *Vibrio spp.* and have only recently been incorporated into predictive models—portending the future of early warning systems for pathogenic *Vibrio spp.* and the diseases they cause, potentially for global public health in general. For example, by using remote sensing data, cholera (*V. cholerae*) risk can be predicted at the scale of available satellite observations (Khan et al., 2019a, 2019b). While there have been major advances in the spatial resolution of remote sensing data in recent decades (Nasr-Azadani et al., 2015, 2017; Khan et al., 2017, 2019a), data manipulation and system model simulations are required for accurate interpretation of the data, adding complexity to downstream analyses such as cholera risk prediction. Upon notice of impending risk, informed individuals can take precautions, including changing water collection and sanitation behavior, limiting consumption of raw seafood, or flushing open wounds with antiseptic following exposure to contaminated water. Remote sensing technology also enables prediction in regions disrupted by natural or anthropogenic disaster, where satellite observation may be the only accurate source of near real-time data (Voigt et al., 2007). While significant progress has been made in

understanding the effect of environmental factors on cholera, additional data collection will continue to improve remote sensing-based predictive models for pathogenic *Vibrio spp*. in addition to *V. cholerae*, the causative agent of Asiatic cholera.

A few studies have examined correlations of *V. cholerae* and other pathogenic *Vibrio spp.* with bulk environmental variables, e.g., temperature, salinity, nitrogen, and phosphate, as well as association with potential host organisms (Eiler et al., 2006; Turner et al., 2009; Vezzulli et al., 2009). However, the importance of environmental correlation remains undefined because the data can be fragmented across study locations and the sampling methods may vary. A meta-analysis conducted by Takemura and colleagues (2014) determined trends with respect to bulk water environmental variables and concluded that, while temperature and salinity are generally strongly predictive correlates, other parameters are inconsistent and overall patterns depend on taxonomic resolution. They provide evidence that *Vibrio spp.* can attach to many different organisms and the dynamics of *Vibrio spp.* may correlate better with narrowly defined niches. It is worth noting that while *Vibrio spp.* flourish when attached to the surfaces, these bacteria also have an alternative free-living lifestyle (Takemura et al., 2014).

Two leading models for *Vibrio* prediction are the NCCOS Probability Model for *V. vulnificus* Occurrence in Chesapeake Bay Water (Jacobs et al., 2014) and the *Vibrio* Map Viewer (Semenza et al., 2017) developed by the ECDC. Daily forecast models for *V. vulnificus* occurrence in Chesapeake Bay Water (**Figure 3 A**) are created from data gathered by large scale sampling in Chesapeake Bay between 2007-2010 and forced with the Chesapeake Bay Operational Forecast System (NOAA, 2021b), a

network of observed and model-generated hydrodynamic nowcast and forecast predictions of pertinent parameters, e.g., water level, current, salinity, and temperature, providing probability of incidence of *V. vulnificus* throughout the Chesapeake Bay. However, this model does not predict abundance of pathogens or risk of illness. The ECDC *Vibrio* Map Viewer (**Figure 3 B**) employs real-time remotely sensed data to calculate a Daily Suitability Index for *Vibrio* risk. The NCCOS Probability Model for *V. vulnificus* Occurrence can be visualized on the NOAA nowCoast (NOAA, 2021c) for a given day and the following day, while the ECDC *Vibrio* map viewer provides daily *Vibrio* risk indices for the current day and the forecast the next five days. Most pathogenic *Vibrio spp.* thrive in warm water with moderate salinity. Hence, temperature (thresholds of 15°C and 18°C) and salinity (thresholds of ~10-15 ppt and <26 practical salinity units) are key input for the NCCOS Probability Model for *V. vulnificus* occurrence and ECDC *Vibrio* map viewer, respectively. **Figure 3** depicts model output in the current version with maps of temperature (**Figure 3 C**) and salinity (**Figure 3 D**), for archived daily *Vibrio* predictions throughout the Chesapeake Bay during different seasons of the year. These models have been successful for short-term prediction of high *Vibrio spp.* abundance on a regional scale, and public health officials have been able to use the forecasts to target public safety messages and monitoring in the Chesapeake Bay and Europe, respectively. However, additional calibration is needed before they can be applied globally. As stated above, *Vibrio spp.* inhabit different ecological niches and encode unique virulence factors that allow different infection pathways that must be considered before treating all *Vibrio spp.* as a single cohesive unit (Takemura et al., 2014).

To reduce the complexity of risk prediction models, certain assumptions are made, e.g, when pathogens are present, risk is increased, and all individuals of a given region are at the same risk of infection. Age has also been shown to be important, as children under the age of five and the elderly have the highest disease burden of diarrheal disease (Faruque et al., 2004), but models typically assume that population age is constant. However, immunocompromised individuals or those with underlying conditions, e.g., diabetes, HIV, or liver disease, are more likely to face potentially fatal systemic *Vibrio* infections (Janda et al., 2015). More specifically, infections with *V. vulnificus* and NOVC septicemia show greatest risk for individuals with pre-existing conditions, often linked to age (Jones and Oliver, 2009). Previous studies have indicated that individuals with compromised immune systems or chronic liver disease, such as cirrhosis are up to 80 times more likely than healthy individuals to develop *V. vulnificus* primary septicemia (Jones and Oliver, 2009; Baker-Austin et al., 2017). Recent epidemiological data, such as liver cirrhosis trends in the United States (Scaglione et al., 2015), show similar correlation between gender and age as observed in *V. vulnificus* cases, with a disproportionate rate of cirrhosis in males and in older age groups (e.g. > 40 years old). Future research on *Vibrio* predictive modeling will need to incorporate human ecology and census data, with other bulk environmental variables, in addition to temperature and salinity, and association with potential host organisms. Clearly, for successful environmental predictive modeling, additional ground truth observations need to be included at fine genetic and environmental sampling scales to describe *Vibrio spp.* dynamics more completely (Takemura et al., 2014).

Cholera risk prediction

Climate has been established as a driver of cholera (Colwell, 1996; Pascual et al., 2000; Jutla et al., 2011), and seasonal outbreaks occur in regions of the world where the disease is endemic. Cholera seasonality is mostly observed as a single annual peak in human cases of the disease, but bi-annual peaks occur in the Bengal Delta region (Akanda et al., 2009; Jutla et al., 2015a). Furthermore, cholera surveillance is useful for aquatic ecosystems, with outbreaks occurring more often near river and coastal regions. Use of satellite remote sensing for cholera prediction was first proposed in 1996 (Colwell, 1996), and a number of studies have employed satellite remote sensing to identify relationships between *V. cholerae* epidemics and environmental parameters, such as sea surface temperature and height (Lobitz et al., 2000; Emch et al., 2008; Xu et al., 2016), chlorophyll (Constantin de Magny et al., 2008; Emch et al., 2008; Jutla et al., 2013a), precipitation (Eisenberg et al., 2013; Jutla et al., 2015b; Kirpich et al., 2015), and water storage (Jutla et al., 2015b). Insights from studies such as these provide a foundation for environment based cholera risk modeling and prediction.

Jutla, Whitcombe, and colleagues (2013b) incorporated these insights into the following hypothesis for prediction of epidemic cholera outbreaks: cholera epidemics occur where warm temperatures, followed by heavy precipitation and in combination with societal factors related to water insecurity, lead to an outbreak by increasing contact of the population with unsafe water (Jutla et al., 2015b). This hypothesis is based on conditions favorable for a cholera trigger and its transmission. Furthermore, it incorporates environmental factors, including temperature and precipitation, readily

observed with satellite remote sensing. These factors make the hypothesis widely applicable. The hypothesis has been supported by successful prediction of cholera outbreaks in South Sudan, the Central African Republic, Rwanda, Cameroon, Mozambique, and Zimbabwe (Jutla et al., 2015b). In fact, model simulations demonstrated that the Zimbabwe 2008 cholera epidemic would not have occurred without environmental forcing (Jutla et al., 2015b).

Models based on the epidemic cholera hypothesis demonstrate utility in evaluating post-disaster (natural or anthropogenic) cholera risk and provide an essential tool for public health officials. In January 2010, a catastrophic 7.0 M_W earthquake struck Haiti, destroying infrastructure and causing an estimated 250,000 deaths (Hasan et al., 2012). The earthquake was followed by an anomalously warm summer and heavy rainfall in November from Hurricane Tomas. While these natural disasters in Haiti pre-dated model development, the corresponding cholera epidemic was linked to above average air temperatures, anomalously high rainfall in the month preceding the outbreak, and damaged or lacking sanitation infrastructure (Jutla et al., 2013b). More recently, the model was used to provide near-real-time cholera risk prediction in Haiti after Hurricane Matthew (Khan et al., 2017). Predictions corresponded well with observed cholera cases, and comparison of model output pre- and post-hurricane demonstrated that damaged water and sanitation infrastructure, in conjunction with favorable environmental conditions, increased the risk of a cholera outbreak (Khan et al., 2017). In 2015, an earthquake struck Nepal, and application of the model demonstrated that swift, water and sanitation related humanitarian intervention prevented a cholera epidemic, despite favorable environmental conditions (Khan et al.,

2018). The absence of time series data for vector abundance and cholera case reports poses significant challenges for developing relationships with hydroclimatic processes using traditional modeling techniques for many parts of the world. However, the use of time series-independent algorithms (Jutla et al., 2011, 2013b; Khan et al., 2017, 2019b), that employ similarity principles to determine conditions of favorability for both hydroclimatic processes and sociological factors across geographic locations have proven useful in circumventing these issues. The underlying theory of the algorithms is: if a pathogen is present under certain hydroclimatic conditions in a given region, then under the same hydroclimatic scenarios, the risk of presence of the pathogen will be increased in other locations, given other societal conditions remain constant. Collectively, these studies suggest a cholera risk prediction model, especially one that utilizes near-real-time satellite observations of temperature and precipitation, could be used as a post-disaster rapid response tool to inform location and extent of interventions.

Non-cholera *Vibrio spp.* risk prediction

Several investigators have identified a positive correlation between temperature and abundance of pathogenic *Vibrio spp*. Environmental microbiology surveillance of the Chesapeake Bay found the incidence of *V. parahaemolyticus* correlated with water temperature. Water column temperatures between 14-19 °C were observed to be critical in the annual cycle of *Vibrio spp.* (Kaneko and Colwell, 1973). These observations have since been confirmed elsewhere in the U.S. (Duan and Su, 2005; Blackwell and Oliver, 2008; Johnson et al., 2012), Japan (Fukushima and Seki, 2004), India

(Rehnstam-Holm et al., 2014), and Europe (Deter et al., 2010; Böer et al., 2013; Vezzulli et al., 2016), among other locations. Climate anomalies, such as El Niño, can expand the geographic extent of *V. parahaemolyticus* outbreaks by transporting abnormally warm water to otherwise unfavorable regions (Martinez-Urtaza et al., 2010). Temperature has also been positively correlated with presence and abundance of *V. vulnificus*, as observed for multiple sample types, i.e., water, oyster, and sediments, and across multiple sampling regimes (Lipp et al., 2001; Fukushima and Seki, 2004; Johnson et al., 2012; Böer et al., 2013).

Temperature and salinity appear to be primary environmental drivers of non-cholera *Vibrio spp.* abundance. As mentioned previously, *V. vulnificus* displays distinct temperature and salinity tolerances, and the incidence of infection with *V. vulnificus* is related to environmental distribution of the bacterium (Baker-Austin et al., 2018). Seasonal geographic expansions of *V. vulnificus* have been observed in areas where temperatures have increased and saline water extends further into the coastal fresh water system (Blackwell and Oliver, 2008; Deeb et al., 2018). In addition, a field study carried out in Galicia, Spain demonstrated salinity to be the primary driver of *V. parahaemolyticus* distribution, with temperature serving as a secondary, modulating factor (Martinez-Urtaza et al., 2008b). Field studies have also examined the relationship between non-cholera *Vibrio spp.* and environmental variables, such as turbidity, chlorophyll, pH, dissolved oxygen, and other microorganisms (Blackwell and Oliver, 2008; Johnson et al., 2012; Wetz et al., 2014; Davis et al., 2017). The strength of these relationships differ among investigations (Johnson et al., 2012; Imamura et al.,

2017), making further study necessary to determine more accurately how the variables affect non-cholera *Vibrio spp.* occurrence and abundance.

While efforts to develop predictive models and to implement them are ongoing, several models are currently in operation. Models for several regions of the U.S. employ water temperature to predict *V. parahaemolyticus* concentrations and doubling times related to harvested oysters (FDA, 2005; NCCOS, 2018). The *V. vulnificus* model for Chesapeake Bay uses temperature and salinity to predict probability of occurrence (NCCOS, 2019). The European Centre for Disease Prevention and Control (ECDC) employs remotely sensed temperature and salinity to estimate risk of infection with non-cholera *Vibrio spp.* in the Baltic Sea (Semenza et al., 2017).

Satellite remote sensing provides additional useful information that can improve existing models. DeLuca and colleagues (2020) recently demonstrated models for *V. parahaemolyticus* that incorporate remotely sensed salinity, total suspended solids, and chlorophyll-a performed better than those that relied solely on sea surface temperature. Satellite observations likely will spur development of increasingly accurate *Vibrio* predictive models by providing data on additional environmental variables, notably those other than temperature and salinity that would otherwise be unavailable or of poor resolution. As the spatial and temporal resolution of satellite observations continue to improve, models may also be able to provide predictions that will be accurate, more frequently updated, and better able to resolve finer scale differences in risk of infection with *Vibrio spp*. The global coverage of satellite remote sensing clearly provides opportunities for modeling efforts to be expanded beyond current reach within the U.S. and Northern Europe.

It should be pointed out that predictive modeling relies on ground truth observations that can be used to train algorithms. Because of inconsistencies in reporting of infections caused by pathogenic *Vibrio spp.* globally and difficulties in detecting *Vibrio spp*. when traditional culturing methods are used, the predictive modeling for all *Vibrio spp*. is at present not uniform. Global interest and impact on health and the economy currently drive cholera prediction, hence it has been at the forefront of predictive risk assessment. More precisely stated, prediction of *V. cholerae* is driven by pathogenicity, thus modeling the virulence potential of other *Vibrio spp.* has been limited to predicting presence of the given *Vibrio spp*. Ground observations of non-cholera *Vibrio spp.* have been limited essentially to only a few locations, with most studies carried out in developed countries. Therefore, the existing models will need to be challenged by ground truth validation. Urquhart and colleagues (2014) showed that two models (Jacobs et al., 2014) have the potential to produce systematically different *V. vulnificus* probabilities when evaluated with the same environmental data input (Urquhart et al., 2014). Relationships that have been calculated between environmental variables and *Vibrio spp.* occurrence have also been found to be inconsistent among studies, demonstrating the necessity to develop a better understanding of those environmental factors driving infections caused by the various pathogenic *Vibrio spp*. As satellite remote sensing expands the domain within which models can be developed, additional ground observation studies to evaluate both presence and pathogenicity of *Vibrio spp*., including *V. cholerae*, will allow assessment of regional variation in environmental factors that influence *Vibrio spp.* incidence and distribution.

Conclusion

There is strong evidence that suggests the global ocean heat content of the world oceans is increasing and salinity profiles are changing in certain regions (**Figure 4**) and that warming temperatures are associated with both spread of pathogenic *Vibrio spp*. and emergence of human disease globally (Vezzulli et al., 2016; Baker-Austin et al., 2017). Because *Vibrio spp.* are predominantly autochthonous to the aquatic environment and have a role in cycling carbon and nitrogen, the diseases they cause cannot be eradicated from the environment. Therefore, early warning systems are essential to public health and for informing decision makers and those individuals whose risk of infection is high (**Figure 5**). Molecular methods for ground truth observation coupled with satellite remote sensing for prediction of the risk of infection with a pathogen is spurring progress towards such warning systems. Information gained using molecular methods increase the likelihood of detecting pathogenic *Vibrio spp*., including those in a viable but nonculturable state. Developments in satellite technology and the increasing number of advanced satellites orbiting the Earth provide modelers with access to near real-time environmental data. In turn, predictive models are being developed that improve the representation of pathogenic *Vibrio spp.* response to environmental conditions within the context of their autochthonous aquatic habitat and bounded by the framework of a changing climate driven increased potential of emerging infectious disease.

Figures

Figure 1: Pathogen surveillance of infections caused by pathogenic *Vibrio spp*., 1999-2019.

Graphs were created using the Foodborne Diseases Active Surveillance Network (FoodNet) Fast to display data for *Vibrio* infections (CDC, 2021). Where indicated, data are presented as number of infections per 100,000 population at FoodNet sites, which cover 10 states and *ca.* 15% of the United States population. **(A)** *Vibrio* infections by year. Shown is the incidence of infections caused by pathogenic *Vibrio spp*. Teal, all test methods; gold, culture confirmed, including those infections confirmed by culture only or by culture following a positive culture-independent diagnostic test (CIDT); purple, CIDT only. **(B)** Infections caused by pathogenic *Vibrio spp.* presented by month. Shown are monthly percentage of infections across all reported cases. **(C)** Distribution of infections caused by pathogenic *Vibrio spp*. Shown are percentage of infections caused by pathogenic *Vibrio spp.* across all reported cases. CIDT, culture-independent diagnostic test. **(D)** Demographics of infections caused by pathogenic *Vibrio spp*. The annual average incidence of infections is shown by age (left) and sex (right).

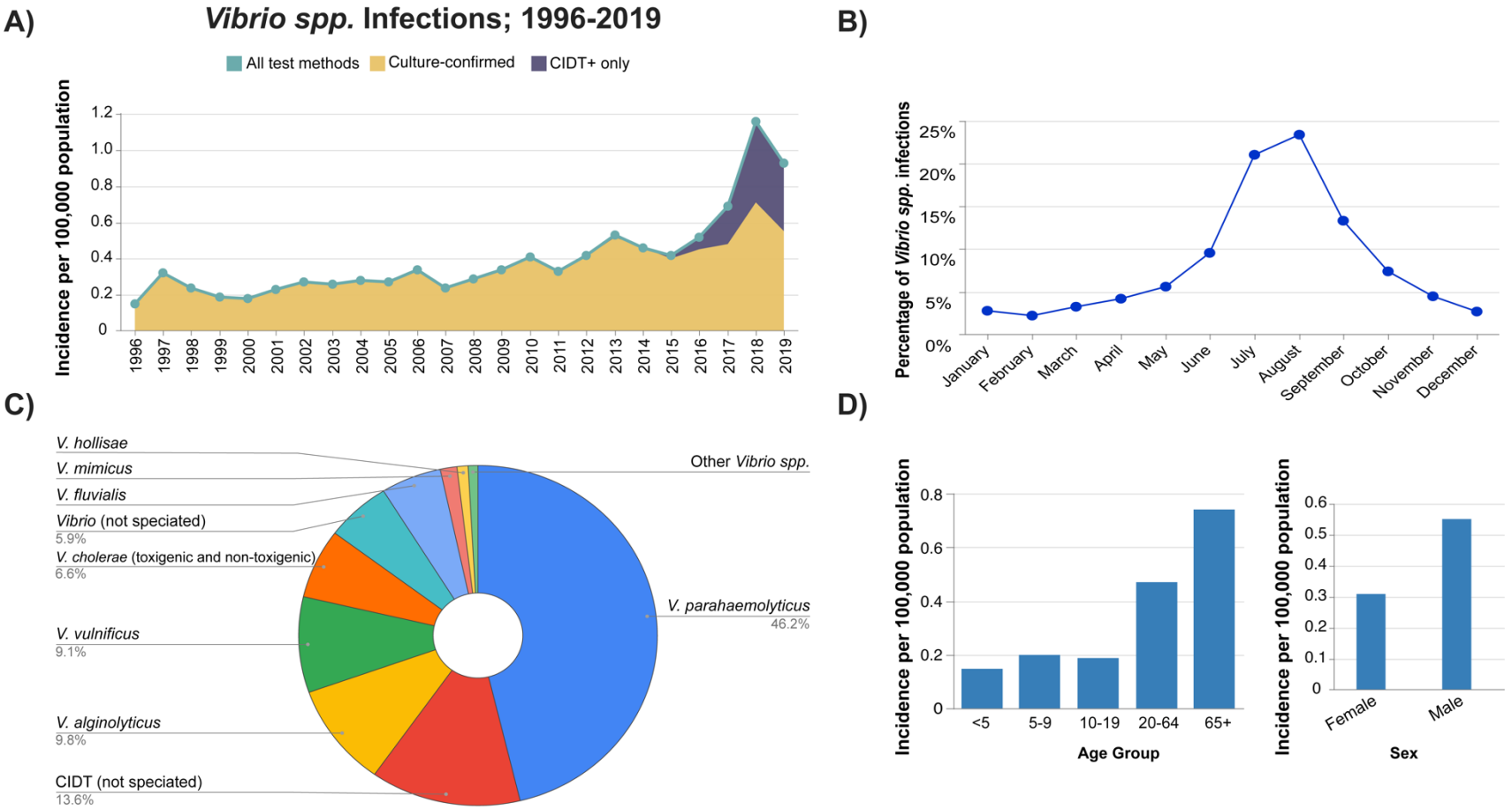

Figure 1: Pathogen surveillance of infections caused by pathogenic *Vibrio spp*., 1999-2019.

Figure 2: Interactions of *Vibrio spp*. and their natural environment.

Shown are biotic and abiotic factors influencing incidence and distribution of *Vibrio spp*. in the natural environment, adapted from (Sakib et al., 2018). Black arrows indicate potential reservoirs, e.g., zooplankton (primarily copepods), crustaceans, bivalves, fish, aquatic plants, phytoplankton, chironomids, waterfowl, and sediment; red indicates environmental *Vibrio spp.* antagonists, e.g., Vibriophages, protozoa, and predatory/competing bacteria. Abiotic factors, including temperature, sea surface height, precipitation, and extreme weather events, have the potential to alter the size and metabolic activity of populations of *Vibrio spp*. It is essential to determine the impact of abiotic factors on occurrence and distribution of pathogenic *Vibrio spp*. to safeguard public health. Image was created using the University of Maryland Center for Environmental Science Integration and Application Network Image Library (UMCES, 2021).

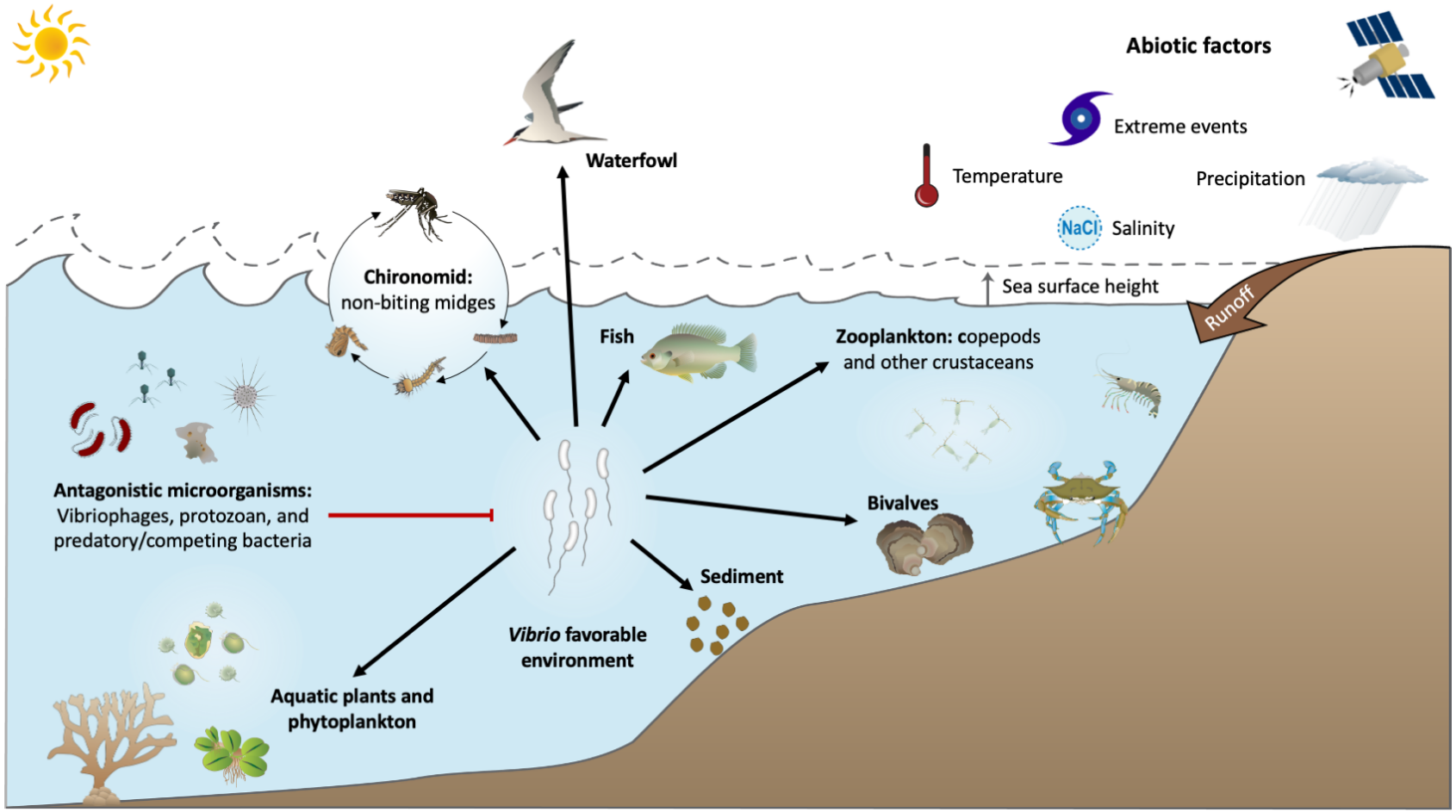

Figure 2: Interactions of Vibrio spp. and their natural environment.

Figure 3: Seasonal prediction of occurrence of Vibrio spp. in the environment for year 2020.

Shown are predicted daily occurrences of *Vibrio spp.* for the last day of each season (Winter, 29 Feb; Spring, 21 May; Summer, 31 Aug; and Fall, 30 Nov). **(A)** Occurrence of *V. vulnificus*. Incidence and distribution of *V. vulnificus* are based on the National Centers for Coastal Ocean Science (NCCOS) Probability Model for *V. vulnificus* Occurrence in Chesapeake Bay Water (Jacobs et al., 2014). **(B)** Daily risk of infection with pathogenic *Vibrio spp.* Shown are daily suitability indices using the European Centre for Disease Prevention and Control (ECDC) *Vibrio* Map Viewer (Semenza et al., 2017). **(C)** Temperature profiles. Time series of the area averaged sea surface temperature for each day as reported by Goddard Earth Services Data and Information Services Center Interactive Online Visualization and Analysis Infrastructure (Acker and Leptoukh, 2007). **(D)** Salinity profiles. Salinity averaged over top 1 m and corrected using Chesapeake Bay Interpretive Buoy System (CBIBS) was provided by NOAA Chesapeake Bay Operational Forecast System (CBOFS) (NOAA, 2021b) and visualized using NASA Panoply Data Viewer (NASA, 2021).

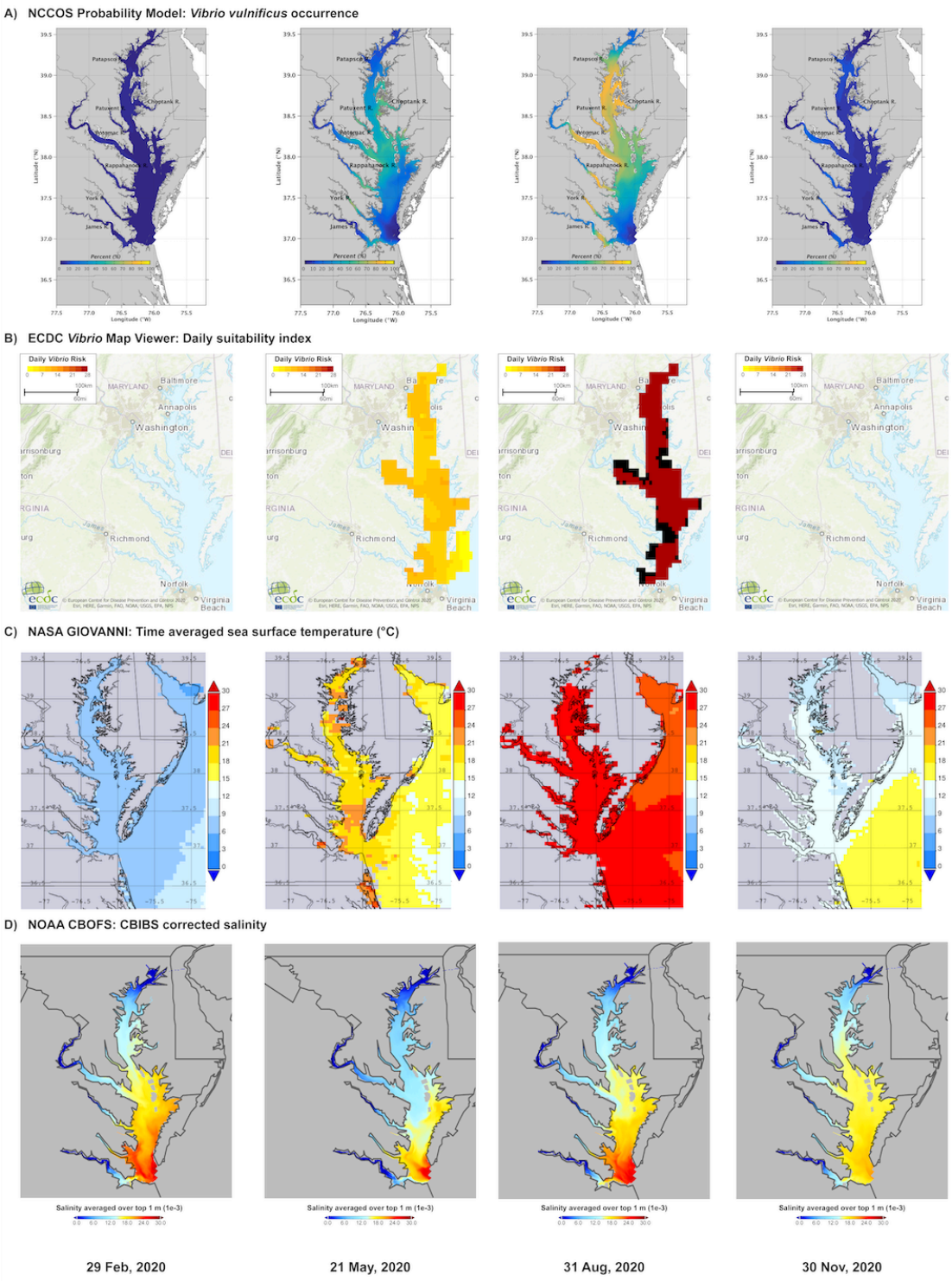

Figure 3: Seasonal prediction of occurrence of Vibrio spp. in the environment for year 2020.

Figure 4: Global Ocean Heat and Salt Content (1955-2020).

Data distribution for temperature and salinity observations are provided elsewhere (NOAA, 2021d). Pentadal averages are shown through 2016-2020; blue, 0-100 m; red 0-700 m; grey, 0-2000 m. **(A)** Mean temperature anomaly. Mean temperature anomalies have previously been detailed by Levitus and colleagues (2012). **(B)** Mean salt anomaly. Temperature calculations are extended to keep the fields current and include salinity anomalies.

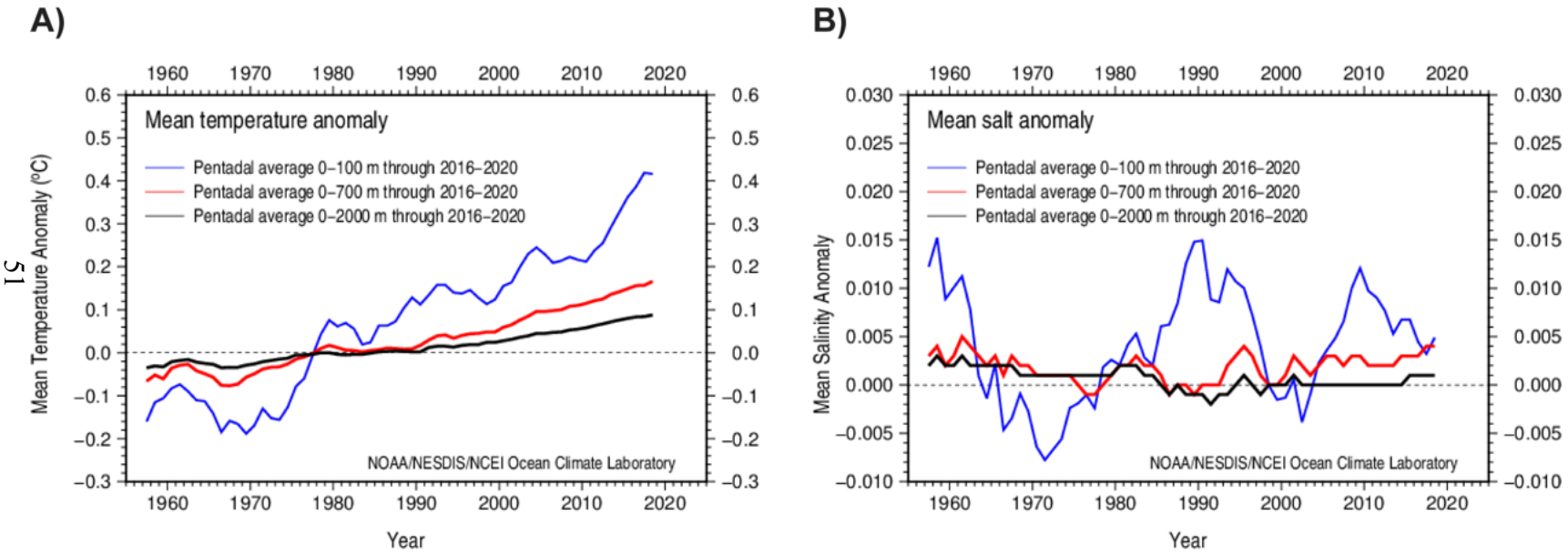

Figure 4: Global Ocean Heat and Salt Content (1955-2020).

Figure 5: Environmental and sociological aspects of *Vibrio spp*. transmission.

Pathways of transmission of pathogenic *Vibrio spp.* to humans with respect to environmental (blue) and sociological (red) factors. Disruption of these pathways—informed by early warning—present opportunities for public health intervention. Double slashes represent opportunities for public health intervention to disrupt pathways and reduce likelihood of infection.

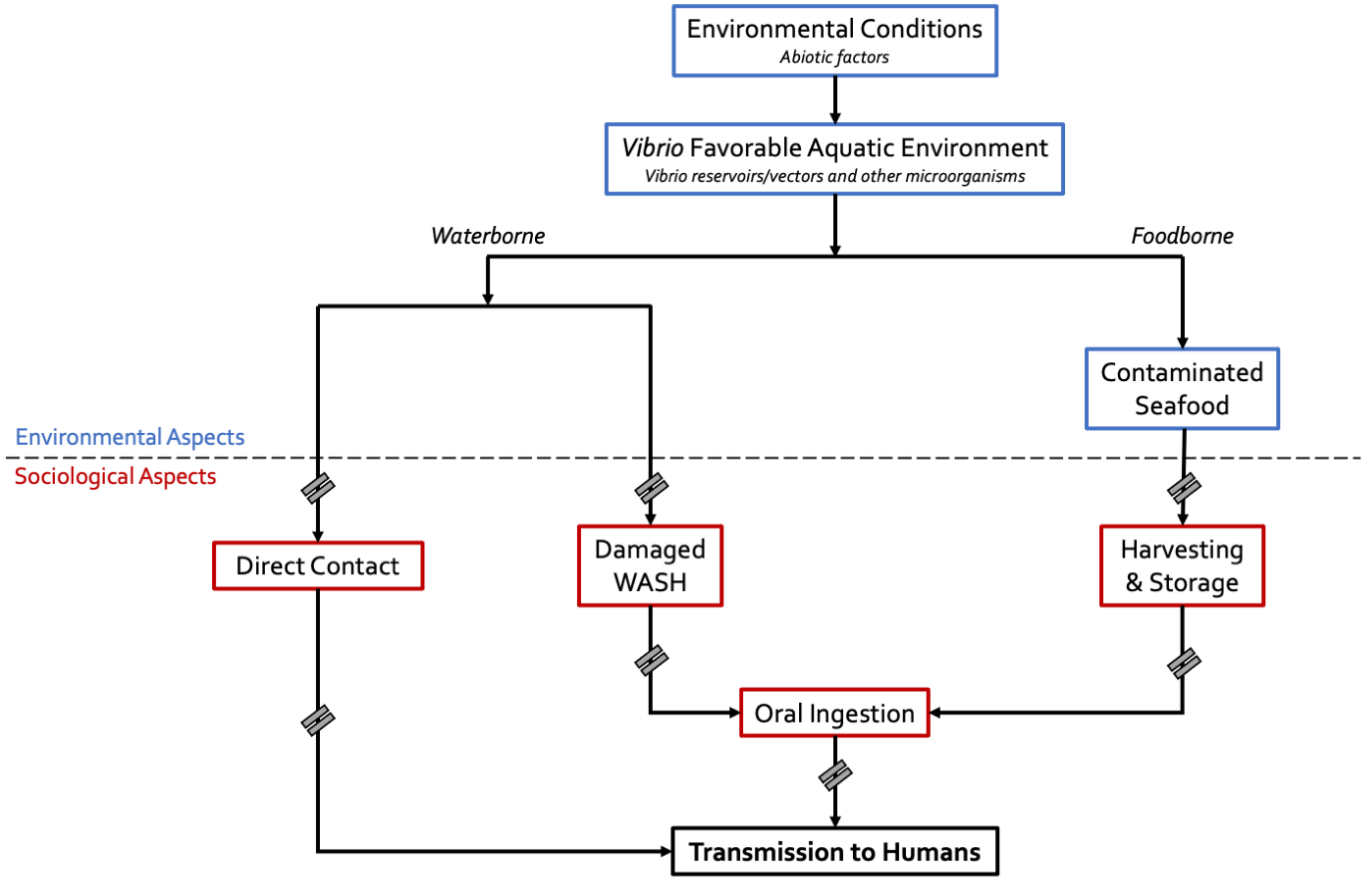

Figure 5: Environmental and sociological aspects of *Vibrio spp*. transmission.

Chapter 2: Predictive risk assessment for *Vibrio spp.* infections during climate change

Abstract

Vibrio spp. are autochthonous to the aquatic environment globally, and notably associated with zooplankton. Historically, some species/strains are known to cause disease in humans, with *Vibrio cholerae* O1/O139 and *Vibrio parahaemolyticus* O3:K6 having pandemic potential. Changing climatic conditions influence environmental factors associated with growth and reproduction of vibrios in the environment, hence are linked to increased incidence of *Vibrio* infections. Because *Vibrio spp*. play an ecologically significant role in biogeochemical cycles, their eradication is not possible, but prevention of *Vibrio* infections can be avoided by providing access to safe water and sanitary infrastructure. Methods to predict disease outbreaks caused by *Vibrio spp.* that employ remote sensing have proven useful in mitigating risk of cholera. Until recently, the virulence potential of non-cholera *Vibrio spp.*, except for *Vibrio parahaemolyticus*, has been considered to be minimal. In addition, factors associated with the emergence of disease is complex and have not included climate/weather processes but mainly microbiological and sociological determinants. During the past two decades, remote sensing has expanded the domain within which predictive models can be developed and ground observation studies carried out over the past fifty years have provided significantly greater amounts of data on both presence and pathogenicity of vibrios. Pathogenic *Vibrio spp.* and strains have been found to demonstrate niche preference, allowing assessment of regional variation in environmental factors influencing risk. Here, the objective was to analyze data from studies carried out since the 1960's and identify environmental determinants of occurrence and distribution of

pathogenic *Vibrio spp.*, allowing predictive modeling of the effect of climate change on incidence and distribution of *Vibrio spp.* infections.

Introduction

Despite advances in medical science and, for developed countries, distribution of safe water, infectious diseases continue to impact human populations in many parts of the world, especially where climate/weather processes intersect with population vulnerabilities, such as lack or loss of access to water, sanitation, and hygiene (WASH) infrastructure. In the United States, waterborne diseases cause *ca.* 7.2 million infections and *ca.* 7,000 deaths annually (CDC, 2023), with far greater numbers for low and middle income countries (LMIC) where more than two billion people lack access to safe water (WHO, 2022a). The numbers can be expected to be even higher in regions where extreme effects of climate change and population growth occur (WHO, 2022a). Emergence of disease in human populations is dynamic and influenced by social, economic, political factors, and environmental factors, in addition to the primary causative agent, e.g., microorganisms (viruses, bacteria, fungi, protozoa, and helminths). The microbial communities, including pathogens, often play a critical role in the biogeochemistry of both the planet and its biological inhabitants. The total number of microbial cells of the Earth biosphere is estimated to exceed 10^{30} (Turnbaugh and Gordon, 2008), with microbes themselves harboring up to 10^{31} phages (Dinsdale et al., 2008). In total, it has been estimated that the *ca.* 1,500 known bacteria pathogens capable of infecting humans, account for less than one percent of the total microbial population of the planet (Bartlett et al., 2022).

Thanks to advanced therapeutics and vaccines, the World Health Organization declared two diseases officially eradicated: smallpox and rinderpest, caused by *Variola* and *Rinderpest* viruses, respectively (Roser et al., 2014). Viruses are ideal candidates for eradication programs since they infect a single host primarily and rely on the host cell for energy and macromolecular synthesis machinery for genome replication and particle assembly (Rampersad and Tennant, 2018). Viruses infect all types of organisms and bacteriophages, i.e., viruses that infect bacteria, have the potential to drive evolution since they serve as vectors of horizontal gene transfer (HGT), a prime example of which is the toxigenic strain of *Vibrio cholerae*, etiological agent of cholera, acquiring the ability to produce cholera toxin (CT) by HGT carried on lysogenic bacteriophage CTX (Faruque and Mekalanos, 2012). In the aquatic environment, phages are critical to homeostasis of the aquatic ecosystem since they play a fundamental role in cycling nutrients that govern microbial diversity (Sime-Ngando, 2014). The genus *Vibrio* comprises ecologically significant species, some of which are pathogenic for humans and/or marine animals. Although phages have been shown to reduce populations of *Vibrio spp.* (Silva-Valenzuela and Camilli, 2019), debate continues whether phages modulate disease outbreaks, including cholera, by influencing seasonality of *Vibrio spp.* (Faruque et al., 2005; Alam et al., 2006a).

With respect to human disease, many pathogenic agents have environmental relevance and, therefore, will not be eradicated since they form commensal and/or mutualistic interactions with multicellular hosts and play a role in fundamental environmental processes. In fact, many *Vibrio spp*. have not been shown to be pathogenic and some are beneficial, contributing to degradation of polymeric

substances, such as chitin, and playing a role in carbon and nitrogen cycling (Thompson and Polz, 2006; Pruzzo et al., 2008; Le Roux and Blokesch, 2018; Zhang et al., 2018). Furthermore, *Vibrio spp.* with bioluminescent properties are important symbionts of marine organisms, notably *Vibrio fischeri* which provides squid with essential counterillumination (Visick and McFall-Ngai, 2000). In addition, luminescent *Vibrio cholerae* and *Vibrio vulnificus* strains have been reported to be associated with copepods and related crustacean species (Sochard et al., 1979; Oliver et al., 1986; Colwell, 2006; Grim et al., 2008; Zo et al., 2009). The symbiotic relationship between *Vibrio spp.* and copepods, zooplankton that comprise a significant component of aquatic fauna, has been well-documented (Kaneko and Colwell, 1973; Berk et al., 1976; Huq et al., 1983). Copepods are a major host of *Vibrio spp*., providing nutrient rich surfaces to which the bacteria attach, namely gut and carapace, comprising a significant component of their commensal flora and influencing gene expression of copepods (Almada and Tarrant, 2016). A single copepod can harbor up to 10^4 *V. cholerae* cells (Colwell and Spira, 1992), hence ingestion of untreated water containing a small number of copepods can promote disease (Cash et al., 1974; Huq et al., 1996; Colwell et al., 2003), a sufficiently significant activity for the copepod to be concluded a vector.

Many genetic traits expressed by microorganisms that are associated with virulence, e.g., hemolysins, capsular polysaccharides, flagella, proteases, attachment factors, type III secretion systems (T3SS), chitin binding proteins, iron acquisition systems, and colonization factors, can also be considered fitness factors, rendering the microbe more fit under specific environmental conditions (Johnson, 2013). However,

there is a link between mechanisms bacteria employ to acquire nutrients and those that confer virulence (Rohmer et al., 2011; Johnson, 2013). Hence, the hypothesis proposed here is that these pathogenic agents most likely evolved to persist in the environment and not to cause infection in a host.

Environmental persistence of bacteria is promoted by variable mutation and the acquisition and expression of genes, aided by genomic plasticity and HGT. The mechanisms by which *Vibrio spp.* cause disease are diverse and major human virulence factors have been detected in non-human pathogens (Klein et al., 2014; Khouadja et al., 2022). Furthermore, many mobile genetic elements and pathogenicity islands, characterized as primary virulence factors, encode genes which can improve fitness of *Vibrio spp.* in the environment, aside from causing disease in a host (Hacker and Kaper, 2000; Faruque and Mekalanos, 2003; Matz et al., 2011; Gennari et al., 2012). A prime example is the type 3 secretion system 2 (T3SS2), encoded within genomic island VPal-7 of *Vibrio parahaemolyticus* (O3:K6) pandemic clones. T3SS2 is the major virulence factor of pandemic *V. parahaemolyticus*, essential for enterotoxicity and development of clinical manifestations of the disease, but also increases environmental fitness of *V. parahaemolyticus*, namely in its interaction with bacterivorous protists (Matz et al., 2011; Jerez et al., 2022). Several reports have detected T3SS2-related genes in other *Vibrio* and non-*Vibrio* species, notably *Vibrio alginolyticus* (Zhou et al., 2013) and *V. cholerae* non-O1/non-O139 environmental isolates (Ceccarelli et al., 2015; Hounmanou et al., 2022).

An important mechanism employed by *Vibrio spp.* to survive unfavorable environmental conditions is by entering the viable but nonculturable (VBNC) state

(Colwell et al., 1985; Colwell and Huq, 1994; Colwell, 2000; Wong and Wang, 2004; Albertini et al., 2006; Nair et al., 2007a; Nowakowska and Oliver, 2013). The VBNC state allows bacteria to become metabolically dormant until environmental conditions again become favorable and VBNC cells regain cultivability, while having retained virulence potential (Colwell et al., 1996; Colwell, 2000; Oliver, 2010). Vibrios can also switch from motile to biofilm lifestyles and attach to zooplankton, primarily copepods, enhancing their long-term persistence as native inhabitants of the aquatic environment (Berk et al., 1976; Huq et al., 1984; Nair et al., 2007a; Rawlings et al., 2007). Historically, detection of vibrios in the environment had been challenging because of the ability of these bacteria to enter the VBNC state, complicating detection by traditional culture-based methods. However, fluorescent antibody assays and molecular techniques, e.g., polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), DNA sequencing, and metagenomics, have shown presence of *Vibrio spp*. when culture is not successful (Colwell and Huq, 1994; Hasan et al., 1994; Vezzulli et al., 2021; Chamanrokh et al., 2022).

Vibrio spp. have a hallmark seasonal distribution with most cases occurring during the warmer months when water temperatures and salinity profiles are optimal for growth. Changing climate has been linked to increased frequency of anomalous weather events, e.g., heatwaves, hurricanes, and severe precipitation, resulting in severe measurable impact in the marine environment and by changes in sea surface height, temperature, and salinity, with most severe impact observed among coastal communities (Ummenhofer and Meehl, 2017). What is beginning to be understood is that environmental factors also influence incidence and transmission of pathogenic

agents, i.e., proliferation, dissemination, and virulence, as well as transmission routes, e.g., via vectors, food, and water (Mora et al., 2022). Changes in human behavior can be associated with climate when people are brought into more frequent contact with pathogens in increased water-related activities during periods of extended warming (Baker-Austin et al., 2016; Mora et al., 2022). It is also worth noting that human activities and global travel contribute to dissemination and evolution of microorganisms, e.g., movement of infected individuals or by ballast water of ships (Colwell, 1996; Ruiz et al., 2000; Chinazzi et al., 2020). Climate change is associated with shifts in geographical range of species, increasingly important in emergence and re-emergence of disease (El-Sayed and Kamel, 2020). A prime example is climate-driven enhancement of incidence of pathogenic *Vibrio spp.*, which has been correlated with an increased number of infections caused by these bacteria (Colwell, 1996; Vezzulli et al., 2012, 2015; Baker-Austin et al., 2017; Froelich and Daines, 2020; Brumfield et al., 2021b; Vezzulli, 2022; Archer et al., 2023).

Given access to appropriate health care, treatment of vibriosis is typically uneventful, with oral or intravenous rehydration therapy. Antibiotics are sometimes used in severe or prolonged cases of disease, e.g., necrotizing fasciitis caused by *V. vulnificus* (Morris Jr. et al., 2023). However, bacterial resistance to antimicrobial agents is an emerging public health concern. Historically, Tetracycline has been shown to be an effective treatment for cholera, along with rehydration therapy, and is recommended as the first-line drug choice, followed by Azithromycin and Ciprofloxacin, according to the Centers for Disease Control and Prevention (CDC, 2022a). However, macrolides and fluoroquinolone drugs have also been used for treatment of cholera and other

Vibrio spp. infections, following emergence of antimicrobial resistant *Vibrio spp.* isolates (Colwell, 1996; Harris et al., 2012; Wong et al., 2015). While vaccines are used for cholera and also have been developed for *Vibrio spp.* infection in aquaculture, efficacy in humans is often not up to the desired standard, often requiring multiple doses which show protection for only a few years (CDC, 2022b). Vaccine hesitancy associated with effectiveness and preference for low-cost cholera treatment over prevention have limited their use in regions where the disease is endemic (Aziz et al., 2021). Hence, access to clean water, effective sanitation, and improved WASH infrastructure remain at the forefront of public health intervention. However, improvements in methods to predict infectious disease incidence, particularly community outbreaks and epidemics, employing remote sensing as a public health tool is appealing for mitigating risk of cholera and vibriosis (Jutla et al., 2010; Akanda et al., 2018). Data gathered using remote sensing show promise in estimating the risk of a cholera epidemic (Colwell, 1996; Lobitz et al., 2000), and such satellite derived prediction methods have been employed successfully (Jutla et al., 2010, 2013b, 2015b; Khan et al., 2017, 2019a; Usmani et al., 2022, 2023). A local population survey of slum areas in Bangladesh showed early warning systems can directly benefit vulnerable populations by increasing knowledge of the risk and reducing uncertainties associated with avoidance measures, namely willingness to pay for vaccines (Aziz et al., 2021).

Over the past five decades, extensive datasets have been generated on incidence and behavior of toxigenic *V. cholerae* and non-cholera *Vibrio spp*. in the ocean and estuarine environments through cross-sectional studies and also some long-term retrospective work, namely in the Bay of Bengal, Arabian Sea, Baltic Sea, Gulf of

Mexico, and Chesapeake Bay. Here are presented foundational observations providing evidence for environmental parameters directly and indirectly involved in distribution and occurrence of pathogenic *Vibrio spp.* in the environment and currently being incorporated into predictive modeling to allow observation regarding the effect of climate change on *Vibrio*-human interactions.

Vibrio spp. infections

At least 12 *Vibrio spp.* are known to cause infections in humans, of which *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* are currently considered most significant (Newton et al., 2012; CDC, 2016, 2019b; Waldor et al., 2018). *V. cholerae* is the causative agent of pandemic cholera, responsible for up to four million cases and more than 100,000 deaths each year globally (Ali et al., 2015). *V. parahaemolyticus* is a major cause of seafood-derived foodborne gastroenteritis (CDC, 2019b). *V. vulnificus*, also a common cause of foodborne illness, can cause severe necrotizing fasciitis and septicemia (Baker-Austin and Oliver, 2018), with a fatality rate among the highest of any waterborne pathogen, greater than 50% for primary septicemia, and responsible for *ca.* 95% of all waterborne and seafood-derived foodborne deaths in the U.S. (Bross et al., 2007; Baker-Austin and Oliver, 2018). However, with increasing reports of vibriosis in humans and marine animals globally, the infections caused by *V. alginolyticus* and *Vibrio fluvialis* are becoming increasingly relevant (Waldor et al., 2018; Brumfield et al., 2021b; CDC, 2021). In the U.S., *Vibrio spp*. are estimated to cause 80,000 illnesses and hundreds of deaths annually, of which *ca.* 65% are foodborne (Newton et al., 2012; CDC, 2019b). According to the Centers for Disease

Control and Prevention FoodNet, there are indications of a long-term increase in reported vibriosis between 1996 and 2019 (Brumfield et al., 2021b; CDC, 2021). In the Eastern U.S. between 1988 and 2018, *V. vulnificus* wound infections increased eightfold, i.e., from 10 to 80 cases per annum, and the northern case limit has shifted northwards 48 km per annum (Archer et al., 2023). Concerningly, this trend of increased vibriosis is expected to expand further in the coming years because of climatic change (Colwell, 1996; Lobitz et al., 2000).

Cholera

With descriptions of a cholera-like disease in India dating *ca.* 400-500 B.C (Bhishagratna, 1963), cholera is an ancient infectious disease transmitted notably via untreated drinking water carrying the causative agent, *V. cholerae*, and characterized by severe diarrhea and dehydration. It has been shown that cholera emerges in two forms: *epidemic*, characterized by sporadic or rampant occurrence of cases in an outbreak or *endemic*, associated with cases occurring continuously throughout the year, often observed with distinct seasonal peaks in the number of cases (Usmani et al., 2021). Coastal areas and riparian zones are most impacted by endemic cholera, where outbreaks usually emerge before reaching inland (Colwell, 1996; Lipp et al., 2002). Outbreaks are generally associated with natural (earthquake, hurricane, floods, etc.) and/or anthropogenic (wars, coups, political crisis, economic recession, etc.) disasters, notably when environmental conditions favor growth of *V. cholerae* in the environment (Usmani et al., 2021).

Historically, cholera outbreaks have occurred as a sporadic, often seasonal, disease prior to its emergence in epidemic form during the first recorded pandemic in 1817 (Colwell, 1996). The disease remains endemic in developing countries where access to safe water is problematic even though the causative agent is naturally occurring in estuaries and river systems globally (Colwell, 1996). *V. cholerae* is broadly classified according to serogroup by characterization of the surface lipopolysaccharide O-antigen structure. Serogroup O1 is associated with the ongoing seventh pandemic, which began in 1961, and all previously recorded cholera pandemics since 1817. In addition, based on a combination of biochemical traits and sensitivity to specific phages, *V. cholerae* O1 is further classified into biotypes, i.e., classical and El Tor (Kaper et al., 1995; Nair et al., 2002, 2006; Brumfield et al., 2017). The seventh pandemic is primarily associated with the El Tor biotype of *V. cholerae* O1 (7PET) and currently persists with large outbreaks in Africa and the Eastern Mediterranean and recurring outbreaks in the Americas, namely Haiti (WHO, 2022b). Debate whether recurrent outbreaks in Africa and the Americas are caused by intercontinental introduction or resurgence of endemic clonal populations of the bacterium is ongoing (Mutreja et al., 2011; Weill et al., 2017, 2019).

In the 1990's, a unique non-O1 *V. cholerae* (serogroup O139) emerged that closely resembled serogroup O1 isolates (Ramamurthy, 1993). Following its emergence, O139 isolates temporarily displaced the O1 serogroup in several parts of India and Bangladesh (Basu et al., 2000; Faruque et al., 2003). Serogroup O139 spread across Asia until the mid 2000's and continues to cause outbreaks, primarily in South East Asia (Albert, 1994; Waldor et al., 1994; Alam et al., 2006a; Luo et al., 2022;

Ramamurthy et al., 2022). Genomic investigation of O139 showed considerable genetic diversity within these isolates including variable copy numbers of CTX prophage (Waldor et al., 1994; Kimsey et al., 1998; Faruque et al., 2003) and novel integrative conjugative elements (ICE) encoding resistance to antibiotics, namely Sulfamethoxazole and Trimethoprim (SXT) and Streptomycin (Waldor et al., 1996; Spagnoletti et al., 2014). However, later studies showed deletion of a genomic region encoding resistance to SXT and Streptomycin occurring amongst O139 strains (Shah M Faruque et al., 1999). It is suspected that, due to a genetic switch from homogeneous toxin genotype to heterogeneous genotypes and loss of AMR, serogroup O139 failed to spread globally (Ramamurthy et al., 2022).

Following genomic sequencing and phylogenic analysis, it is concluded that *V. cholerae* O1 and O139 strains comprise a monophyletic clade, designated *V. cholerae* phylocore genome clade, which includes isolates from both the sixth and seventh pandemics and suspected to have evolved from a common ancestor within this clade (Chun et al., 2009). In contrast, O1/O139 non-agglutinating *V. cholerae* (NOVC, i.e., non-O1/non-O139) have showed significant genomic diversity and comprise a neglected group of human pathogens that cause sporadic, yet significant, infections ranging in severity from mild, e.g., acute gastroenteritis and otitis, to life threatening, e.g., necrotizing fasciitis. NOVC strains inhabit coastal waters globally and are known to carry a number of antimicrobial resistance and virulence coding genes characteristic of pandemic *V. cholerae* and non-cholera *Vibrio spp.* isolates, e.g., cholera toxin, repeats in toxin, hemolysins, T3SS, zona occludens toxin, and cholix toxin (Hasan et al., 2013; Ceccarelli et al., 2015; Schwartz et al., 2019; Zhao et al., 2022). A growing

number of studies have reported on the increased environmental fitness conferring enhanced virulence of NOVC isolates, e.g., acquisition of the T3SS (Dziejman et al., 2005; Zeb et al., 2019) and multidrug resistance (Luo et al., 2021), corroborated by an increased number of NOVC infections observed globally in recent years (Quilici et al., 2015; Vezzulli et al., 2020; Zhang et al., 2020; Brumfield et al., 2021b). Furthermore, NOVC is believed to contribute to global emergence of new pathogenic strains with epidemic potential as a result of genetic recombination and HGT (Vezzulli et al., 2020). A prime example being strains of the current pandemic, which belong to a single phyletic lineage, that have been diversified by HGT of naturally occurring NOVC strains, likely driven by environmental factors (Chun et al., 2009). Hence, monitoring pandemic and local isolates is a critical public health strategy for controlling current and future cholera outbreaks. The presence of NOVC should not be ignored in the current era of global climate change.

Cholera in the African Subcontinent

While human transmission is an important aspect of cholera progression in a population, the changing climate is increasingly linked to greater frequency of anomalous weather events, e.g., heatwaves, hurricanes, floods, and droughts, that have the potential to accelerate spread of pathogenic microorganisms. Various parts of Africa are under constant stress of climate change, notably intense flooding and extended drought, and cholera has been reported in several African countries (Khoury, 2023). Countries of the Horn of Africa, including Kenya, Ethiopia, and Somalia, have been severely impacted by drought, leading to increased number of climate refugees.

Similar refugee situations are occurring in other regions of Africa because of deadly flooding, e.g., Nigeria, and violent surges of civil unrest, e.g., Yemen. Due to the associated mass migration caused by either climate or anthropogenic conflicts, people in these regions are experiencing significant lack of access to safe water, proper sanitation, and WASH infrastructure, directly increasing risk of cholera and spread of disease caused by related pathogens.

Pandemic *V. parahaemolyticus*

V. parahaemolyticus was first described by Professor Fujino during a 1950 Shirasu food poisoning outbreak in Japan (Fujino, 1951). The bacterium was later isolated in diseased crabs from the Chesapeake Bay (Krantz et al., 1969) and found to be associated with plankton (Kaneko and Colwell, 1973) and a variety of seafood, including codfish, sardine, mackerel, flounder, clam, octopus, shrimp, crab, lobster, crawfish, scallop, and, notably, oyster (Liston, 1990). Having been isolated in coastal waters throughout the world (Kaneko and Colwell, 1973; Joseph et al., 1982; Nair et al., 2007b; Abanto et al., 2020; Gavilan et al., 2023), evidence suggests that *V. parahaemolyticus* is a member of the natural microbial flora, given specific environmental conditions such as temperature and salinity. While most *V. parahaemolyticus* strains are not considered pathogenic to humans, the bacterium, as do other vibrios, concentrates in filter-feeding shellfish, especially oysters, which are often consumed raw thereby exposing people to large doses of potentially pathogenic agents (Lovelace et al., 1968; Berk and Colwell, 1981; Froelich and Noble, 2016). In addition, certain strains have been shown to cause severe infections in marine fish

(Colwell and Grimes, 1984) and shrimp (Prithvisagar et al., 2021), with significant economic burden and aquaculture loss (Freitag et al., 2022).

V. parahaemolyticus is characterized by antigenic properties of the somatic (O) and capsular (K) antigens. The *V. parahaemolyticus* serovar O3:K6 was first identified during a major outbreak in India in 1996 and subsequently spread globally along with serovariants (Matsumoto et al., 2000; Nair and Hormazabal, 2005; Nair et al., 2007b; Ceccarelli et al., 2013), leading to a *V. parahaemolyticus* pandemic. Pandemic isolates generally share virulence profiles that are characterized by presence of specific genetic markers, e.g., thermostable direct hemolysin (*tdh*), absence of TDH-related hemolysin (*trh*), and a distinctive *toxRS* sequence (*toxRS/new*) amplifiable by group-specific PCR (Matsumoto et al., 2000). Between 1996 and 2007, 22 pandemic serovariants sharing these virulence characteristics were described in various regions of the world (Nair et al., 2007b). However, more recently, an increasing number of pandemic serotypes have been reported across Asia, Europe, the Americas, and Africa (Han et al., 2017). Since 2012, serovar O4:K12 has also been found to have transcontinental epidemic expansion, most notably having been detected in regions beyond where it was endemic, i.e., the U.S. Pacific Northwest. For example, *V. parahaemolyticus* O4:K12 strains were isolated along the U.S. northeastern coast, Spain (Martinez-Urtaza et al., 2013, 2017), and Peru (Abanto et al., 2020). This long-term persistence of isolates in the environment was suggested to be a result of successful establishment in environmental reservoirs, but evidence clearly indicates the aquatic environment to be its natural habitat. The precise source and route of pandemic transmission of *V. parahaemolyticus* continue to be debated. However, available evidence suggests a link between its

epidemic dynamics and the emergence of new *V. parahaemolyticus* clones in South America with El Niño, characterized by heavy rains and heat waves, contributing environmental conditions ideal for growth of *Vibrio spp.* (Martinez-Urtaza et al., 2008a, 2016). In the early 2000's, others have proposed that vibrios, autochthonous to the aquatic environment, were likely present in coastal waters, and Peruvian outbreaks resulted from environmental conditions occurring during El Niño phenomenon (Seas et al., 2000; Lipp et al., 2003; Gil et al., 2004; Colwell, 2006). Hence, the link between El Niño and vibriosis outbreak is a rational conclusion based on available evidence.

Vibrio-human interactions and climate change

In the early 1990's, the risk of increased *Vibrio spp.* infections was linked to conditions associated with a changing climate (Epstein et al., 1993; Colwell, 1996; Lipp et al., 2002; NIH, 2009). Among the first climate linked epidemiological observations on a global scale was of cholera epidemics related to climatic events, i.e., El Niño and the global distribution of copepods, the plankton host of *V. cholerae* (Colwell, 1996). Since *Vibrio spp.* and other pathogens are known to enter the VBNC state (Colwell et al., 1985; Colwell and Huq, 1994; Colwell, 2000; Wong and Wang, 2004; Albertini et al., 2006; Nair et al., 2007a; Nowakowska and Oliver, 2013), the failure of the early studies to detect vibrios in the natural environment can explain the lack of recognition of vibrios to comprise an autochthonous member of aquatic microbial communities. Advancements in laboratory methods provided data confirming *Vibrio spp.* to be autochthonous aquatic environmental inhabitants globally, playing an important role in biogeochemical cycles. With a deeper understanding of environmental parameters

influencing occurrence and distribution of vibrios, it is now clear that pathogenic vibrios, namely *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus*, are naturally present in the aquatic environment, notably in warm, low-salinity water.

Since it is estimated that over half of known human pathogenic diseases can be aggravated by climate change (Mora et al., 2022), changing climatic conditions and increasingly anomalous weather events may presage alterations in the geographical distribution of the causative agents. Changes in the intensity and frequency of hurricanes, associated with violent storm surge and severe precipitation, are among the most severe climatic catastrophes associated with disease outbreaks. For example, one of the largest cholera outbreaks occurred in 2016 during the months following Hurricane Mathew (Hasan et al., 2012), which poured rains over the southwestern coast of Haiti and crippled WASH infrastructure during periods of elevated air temperatures. More recently, *Vibrio spp.* infections were reported along coastal regions of the Florida Gulf Coast post Hurricane Ian, a destructive category 4 Atlantic hurricane that made landfall on Florida in September 2022. In the days following Ian, many Florida residents were impacted by sustained floodwaters, a problematic situation given that pathogenic *Vibrio spp.* thrive in warm and low-salinity waters (Singleton et al., 1982; Brumfield et al., 2021b). The Florida Department of health reported 38 cases and 11 vibriosis-associated deaths attributed to the storm (case fatality rate of *ca.* 28.9%), double the usual number for that time of the year (Florida Department of Health, 2023; Sodders et al., 2023).

In addition to the *V. cholerae* O1 pandemic and the ongoing pandemic caused by *V. parahaemolyticus* O3:K6, the rise in reported non-pandemic *Vibrio spp.*

infections is of particular concern. Numerous studies have linked climate change to increased risk of vibriosis caused by non-cholera *Vibrio spp.* However, it is worth noting that global cholera has further exacerbated, as of 2023, with an unprecedented number of larger and more deadly cholera outbreaks, most notably in regions of the world where the disease is not considered endemic (United Nations, 2022; WHO, 2022b). Furthermore, there has been a global increase in NOVC-related (Vezzulli et al., 2020) and non-cholera *Vibrio spp.* infections (Newton et al., 2012; Baker-Austin et al., 2017; Archer et al., 2023). In the U.S., recent reports suggest an increasing number of *V. vulnificus* wound infections along the U.S. eastern seaboard between 1988 and 2019 (Archer et al., 2023) and a long-term increase and extended seasonality of pathogenic *Vibrio spp*. in the Chesapeake Bay between 2009 and 2022 (Brumfield et al., 2023). It is estimated that *ca.* half a million non-cholera *Vibrio spp.* infections occurred worldwide in 2020 (Trinanes and Martinez-Urtaza, 2021). Furthermore, recent projections showed geographic areas suitable for proliferation of *Vibrio spp.* could cover 38,000 km of new coastal areas alone by 2100, and this was concluded for the most unfavorable scenario (Trinanes and Martinez-Urtaza, 2021). Hence, it can be concluded that, while climate is not the sole driver of this disease, it has the potential to accelerate outbreaks of *Vibrio spp.* infections, including cholera. Predictive risk assessment of environmental conditions favoring growth of pathogenic *Vibrio spp.* will be essential with respect to public health.

Developments in satellite technology and the increased number of advanced satellites orbiting the Earth can be used with near real-time environmental and sociological data to develop predictive models for risk assessment of *Vibrio spp.*

infection. Warning systems can provide decision-makers with time to deploy health personnel and resources to vulnerable areas. Cholera predictive modeling, demonstrated for Haiti (Khan et al., 2017), Zimbabwe (Jutla et al., 2015b), Nepal (Khan et al., 2018), Ukraine (Usmani et al., 2022), and Yemen (Usmani et al., 2023), has the potential, when coupled with both individual and large-scale intervention early in outbreaks, to contribute to reduction in case fatality rates (Akanda et al., 2012). Similar models have been proposed for *V. vulnificus* in the Chesapeake Bay (Jacobs et al., 2014) and non-cholera vibrios in the Baltic Sea (Semenza et al., 2017). However, predictive modelling relies on ground truth observations to train algorithms, and ground observations of non-cholera vibrios have been limited essentially to only a few locations, with most studies conducted in developed countries. Future modeling efforts will benefit from inclusion of sociological and behavioral factors related to contact with the aquatic environment, such as recreational and occupational activities, that influence contact with waterborne pathogens.

Environmental parameters for predictive assessment of *Vibrio*

Research of environmental parameters associated with occurrence and transmission of pathogenic *Vibrio spp.* has been ongoing since the 1960's. *Vibrio spp.* are bacteria that, under favorable conditions will replicate rapidly, and, hence, are highly responsive to environmental stimuli and adaptable to a changing environment. In general, growth of vibrios in the environment is directly proportional to ambient environmental conditions, whereby increased temperatures enhance their proliferation and spread in the environment. Cholera and noncholera disease have varying routes of

environmental transmission, primarily attributed to the spread of *V. cholerae* via fecal-oral routes in an epidemic setting with loss of WASH infrastructure and appropriate environmental conditions (Usmani et al., 2021). Furthermore, it has been found that pathogenic strains show niche preference, which will vary across species and clonal populations (Zo et al., 2009; Brumfield et al., 2023). Hence, it is reasonable to conclude that all *Vibrio spp.* cannot be treated as a single cohesive unit and multiple predictive systems are needed to capture the risk of infection caused by these bacteria.

A hypothesis was proposed (**Figure 6**) for environmental parameters influencing trigger of epidemic cholera by Jutla and colleagues (2013b) and validated globally (Jutla et al., 2010, 2013b, 2015b; Khan et al., 2017, 2019a; Usmani et al., 2022, 2023), whereby anomalously high (defined as more than one standard positive deviation above the long-term average) temperatures followed by anomalously high precipitation, over a period of four weeks, in a region of damaged or compromised WASH infrastructure, facilitated interaction between contaminated water and the human population and comprised an environment favorable for triggering an epidemic. Under the hypothesis, if one or more of the conditions are not satisfied, the region would be at lower risk to experience an epidemic. In addition, several investigators proposed association of *V. cholerae* with environmental parameters, including sea surface temperature and height (Lobitz et al., 2000; Emch et al., 2008), chlorophyll (Constantin de Magny et al., 2008; Emch et al., 2008; Jutla et al., 2013a), precipitation (Jutla et al., 2015b; Kirpich et al., 2015), water storage (Jutla et al., 2015a), and salinity (Singleton et al., 1982; Khan et al., 2018), and suggested for use in cholera risk prediction.

For NOVC and non-cholera vibrios, modeling efforts have focused on temperature and salinity as primary input (Jacobs et al., 2014; Levy, 2018). Research carried out in the Chesapeake Bay since the 1970's (Kaneko and Colwell, 1973, 1974, 1977; Colwell et al., 1977, 1981; Zo et al., 2009; Brumfield et al., 2023) has shown that *Vibrio spp.* have a distinct seasonality, since they are found in greater numbers during warmer months of the year. Others have also reported temperature to be a strong predictor of the incidence and distribution of *Vibrio spp*. in the environment (Colwell, 1996; Martinez-Urtaza et al., 2010; Johnson, 2013; Jacobs et al., 2014; Vezzulli et al., 2016; Parveen et al., 2020; Brumfield et al., 2023; Doni et al., 2023). Studies of culturable vibrios in the Chesapeake Bay (Kaneko and Colwell, 1977; Brumfield et al., 2023) have identified two critical sea temperature thresholds, whereby when an initial increase in *Vibrio* density was observed between 14°C and 15°C and when water temperatures rose above 25°C, maximum counts were observed.

Independent of temperature, salinity is understood to influence growth and activity of *Vibrio spp.*, with Na^+ required for growth (Singleton et al., 1982). Hence, salinity is known to be a strong predictor of *Vibrio* abundance (Singleton et al., 1982, 1983; Jacobs et al., 2014; Levy, 2018; Parveen et al., 2020). In general, vibrios have been shown to persist in a wide range of salinities, generally between 5 and 20 ppt. Increased to maximum abundance of *Vibrio* has been observed when salinities are between 10 and 15 ppt (Kaper et al., 1979; Joseph et al., 1982; Huq et al., 1984; Jacobs et al., 2014; Levy, 2018; Brumfield et al., 2023). However, it should be noted that when salinity profiles are below optimal thresholds, *Vibrio spp*. have been shown to thrive in freshwater as well as coastal ecosystems (Singleton et al., 1983) in association with a

variety of living organisms, notably chitinous exoskeletons of copepods (Huq et al., 1984; Colwell, 1996; Vezzulli et al., 2010), or other factors such as the ionic sparing effect of Ca^{++} in freshwater and Mg^{++} in seawater (Joseph et al., 1982; Singleton et al., 1982; Colwell and Spira, 1992).

Clearly, copepods are an important reservoir for *Vibrio spp.*, but spatiotemporal distributions of zooplankton are difficult to quantify. A few studies have shown the ability to detect zooplankton swarms via satellite ocean color (Strömberg et al., 2009; Basedow et al., 2019). However, this is an emerging field of research and remote sensing to measure zooplankton populations has been limited to a few species, without ability to detect low abundance populations. As a result, investigators have used chlorophyll pigments as a proxy for zooplankton abundance (Colwell, 1996; Lobitz et al., 2000). Since zooplankton feed on phytoplankton, correlation of the zooplankton population surges following phytoplankton blooms can be calculated. In addition, phytoplankton blooms are an indicator of sunlight, temperature, and nutrients, which in addition to affecting growth of phytoplankton, promote proliferation of vibrios (Constantin de Magny and Colwell, 2009; Shaw et al., 2014b; Thickman and Gobler, 2017). Hence, chlorophyll is an important predictor of *Vibrio* abundance (Constantin de Magny et al., 2008; Deter et al., 2010; Julie et al., 2010; Jutla et al., 2013b; Namadi and Deng, 2023). A caveat for chlorophyll as a proxy for zooplankton abundance is that modeling efforts would benefit by including specific lag times, e.g., 1 month, to account for a subsequent increase in zooplankton numbers and associated microbial flora (Colwell, 1996; Lobitz et al., 2000; Namadi and Deng, 2021).

Conclusions

In summary, climate change is reported to have an environmental influence in most regions of the world and more so in coastal communities, notably in overall warming trends, altered precipitation patterns, and changes in jet streams and ocean currents. *Vibrio spp.*, because they are naturally occurring in coastal waters globally, possess a high growth rate, and rapid response to environmental stimuli, significant geographic expansion of pathogenic strains with correlated by impact on public health, i.e., increased number of infections caused by these pathogens, is in progress (Lovelace et al., 1968; Krantz et al., 1969; Kaneko and Colwell, 1973, 1974; Colwell et al., 1977; Huq et al., 1984, 2005; Colwell, 1996; Baker-Austin et al., 2013, 2017; Froelich and Daines, 2020; Vezzulli et al., 2020; Brumfield et al., 2021b, 2023; Olson, 2022; Vezzulli, 2022; Archer et al., 2023). Factors favoring growth of vibrios in the environment influenced by climate variability are shown in **Figure 7**. Trend of increased incidence of vibriosis infections is expected in coming years (Colwell, 1996; Lobitz et al., 2000). Early warning systems will be essential to safeguard public health and inform both decision makers and individuals for whom risk of vibriosis infection is high. Cholera research has laid the groundwork for predative intelligence systems in varying climatic scenarios. New models are in preparation to improve representation of ecological niche preferences for pathogenic subspecies.

Figures

Figure 6: Cholera trigger mechanism.

Adapted from “Environmental Factors Influencing Epidemic Cholera,” by Jutla et al., 2013, The American Journal of Tropical Medicine and Hygiene 89 (3), 597-607.

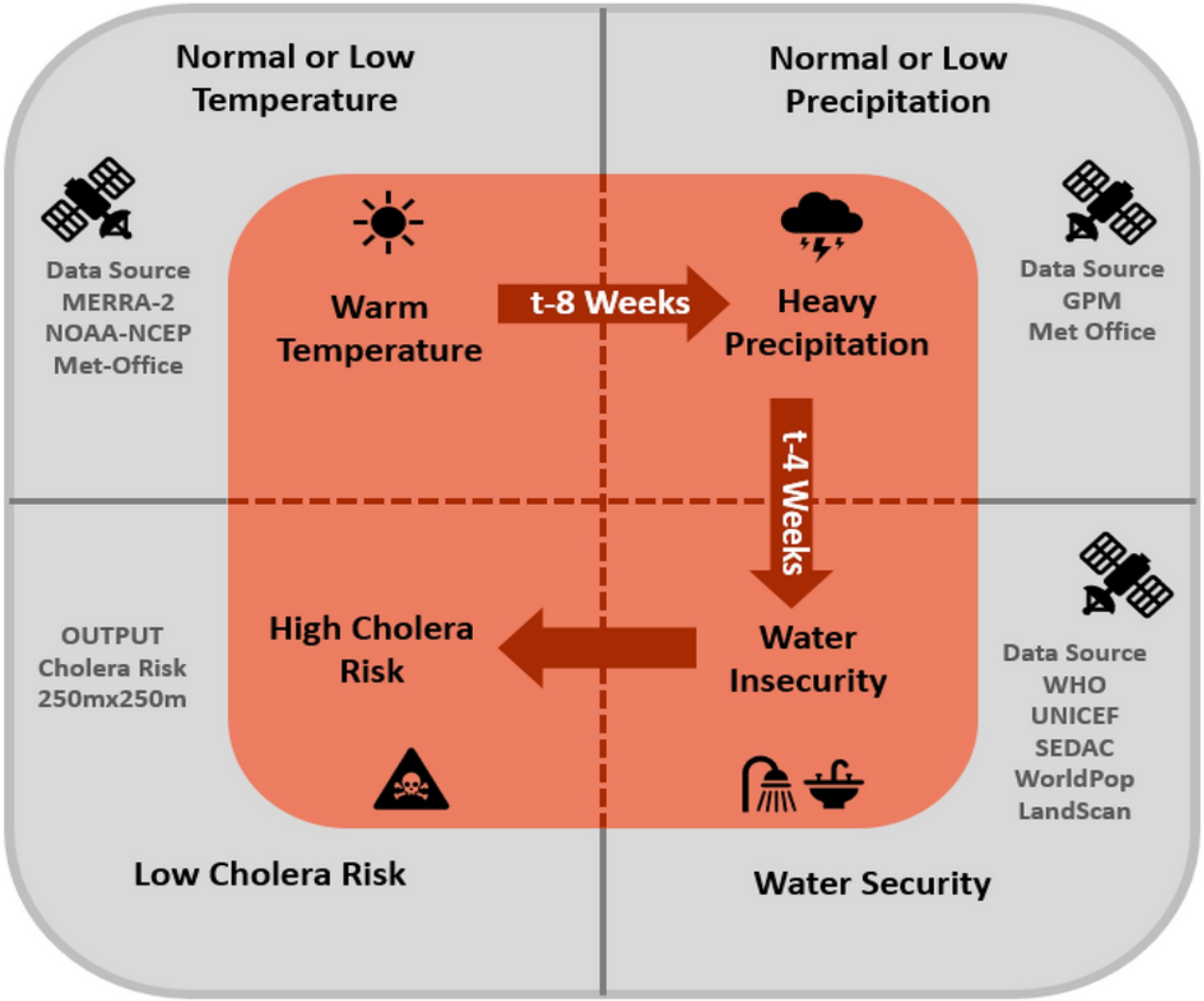

Figure 6: Cholera trigger mechanism.

Figure 7: Hierarchical model for environmental transmission of *Vibrio* associated with climate change.

Adapted from "Effects of Global Climate on Infectious Disease: The Cholera Model," by Lipp et al., 2002. Clinical Microbiology Reviews 15(4), 757-770. SST, sea surface temperature; DO, dissolved oxygen; UV, ultra-violate wavelength.

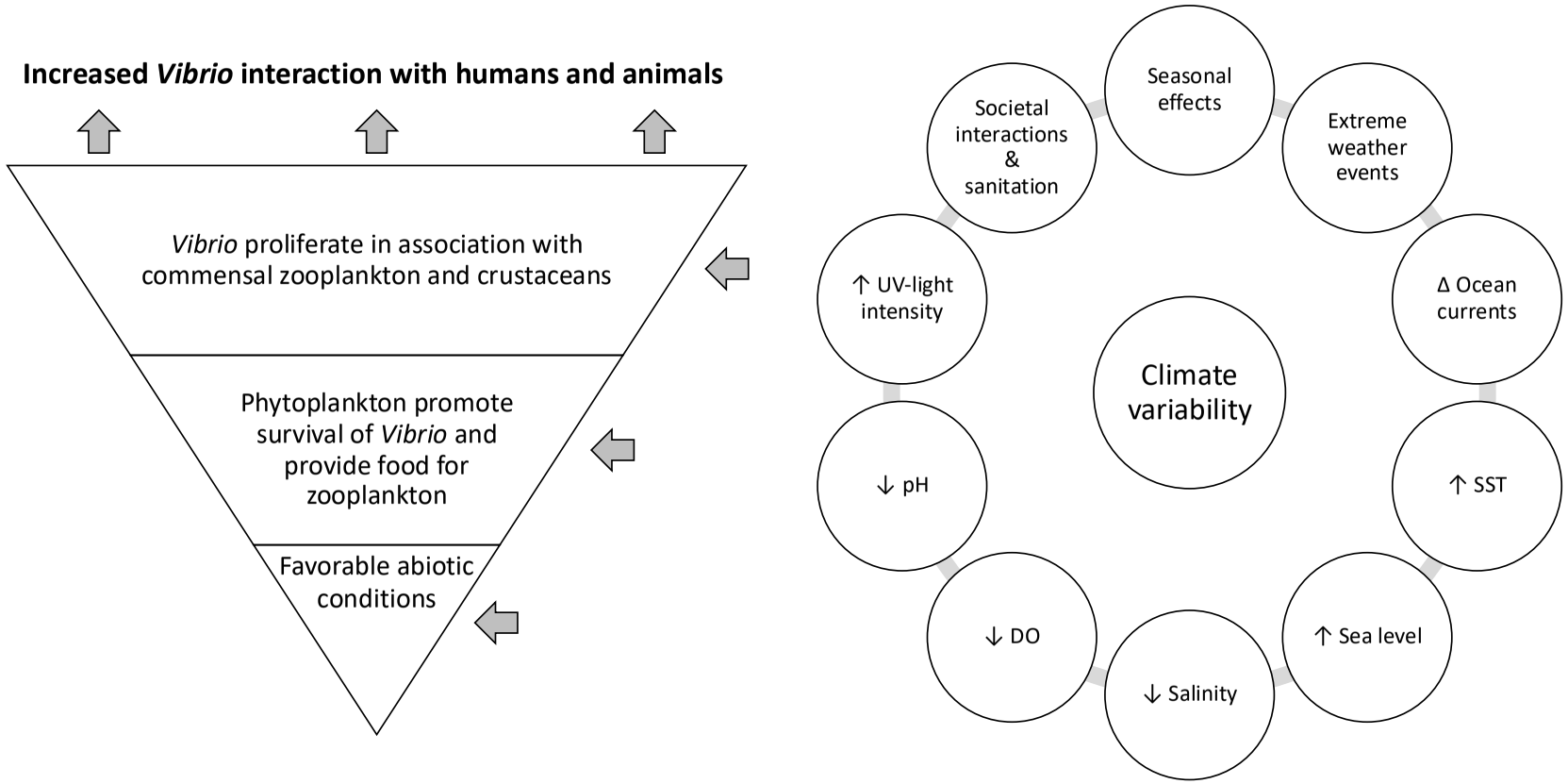

Figure 7: Hierarchical model for environmental transmission of *Vibrio* associated with climate change.

Chapter 3: Environmental factors influencing occurrence of *Vibrio parahaemolyticus* and *Vibrio vulnificus*

Kyle D. Brumfield, Arlene J. Chen, Mayank Gangwar, Moiz Usmani, Nur A. Hasan, Antarpreet S. Jutla, Anwar Huq, and Rita R. Colwell. 2023. *Applied Environmental Microbiology*, e00307-23.

DOI: https://doi.org/10.1128/aem.00307-23

Abstract

Incidence of vibriosis is rising globally, with evidence that changing climatic conditions are influencing environmental factors that enhance growth of pathogenic *Vibrio spp.* in aquatic ecosystems. To determine the impact of environmental factors on occurrence of pathogenic *Vibrio spp*., samples were collected in the Chesapeake Bay, Maryland, during 2009-2012 and 2019-2022. Genetic markers for *Vibrio vulnificus* (*vvhA*) and *Vibrio parahaemolyticus* (*tlh*, *tdh*, *trh*) were enumerated by direct plating and DNA colony hybridization. Results confirmed seasonality and environmental parameters as predictors. Water temperature showed a linear correlation with *vvhA* and *tlh* and two critical thresholds were observed, an initial increase in detectable numbers (> 15 °C) and a second increase when maximum counts were recorded (> 25 °C). Temperature and pathogenic *V. parahaemolyticus* (*tdh* and *trh*) were not strongly correlated, however, the evidence showed these organisms to persist in oyster and sediment at colder temperatures. Salinity (10-15 PPT), total chlorophyll *a* (5-25 µg/L), dissolved oxygen (5-10 mg/L), and pH (8) were associated with increased number of *vvhA* and *tlh*. Importantly, a long-term increase in *Vibrio spp.* numbers was observed in water samples between the two collection periods, specifically at Tangier Sound (lower bay), with the evidence suggesting an extended seasonality for these bacteria in the area. Notably, *tlh* showed a mean positive increase that was *ca.* threefold overall, with the most significant increases observed during the fall. In conclusion, vibriosis continues to be a risk in the Chesapeake Bay region. A predictive intelligence system to assist decision makers, with respect to climate and human health, is warranted.

Importance

The genus *Vibrio* includes pathogenic species that are naturally occurring in marine and estuarine environments globally. Routine monitoring for *Vibrio* species and environmental parameters influencing their incidence is critical to provide a warning system for the public when risk of infection is high. In this study, occurrence of *Vibrio parahaemolyticus* and *Vibrio vulnificus*, both potential human pathogens, in Chesapeake Bay water, oysters, and sediment samples collected over a 13-year period were analyzed. The results provide a confirmation of environmental predictors for these bacteria, notably temperature, salinity, and total chlorophyll *a*, and their seasonality of occurrence. New findings refine environmental parameter thresholds of culturable *Vibrio* species and document a long-term increase in *Vibrio* populations in the Chesapeake Bay. This study provides a valuable foundation for development of predicative risk intelligence models for *Vibrio* incidence during climate change.

Introduction

Changing climatic conditions have been linked to an increased frequency of anomalous weather events, e.g., heatwaves, hurricanes, and severe precipitation, that result in severe impact on the marine environment, notably changes in sea surface height, temperature, and salinity, and for coastal communities (Ummenhofer and Meehl, 2017). What is beginning to be understood is that environmental factors also influence incidence and transmission of pathogenic agents, i.e., proliferation,

dissemination, and virulence, as well as transmission routes, notably via vector, food, and water (Mora et al., 2022). Changes in human behavior are also associated with climate by bringing people into more frequent contact with pathogens from increased water-related activities during periods of extended warming (Baker-Austin et al., 2016; Mora et al., 2022). In the U.S., it is estimated that *ca*. 7.15 million waterborne illnesses occur annually (Collier et al., 2021). Climate change associated with shifts in the geographical range of species, is increasingly important in the emergence and re-emergence of disease (El-Sayed and Kamel, 2020). A prime example is climate-driven enhancement of the incidence of pathogenic *Vibrio* (Colwell, 1996; Vezzulli et al., 2012, 2015; Baker-Austin et al., 2017; Froelich and Daines, 2020; Brumfield et al., 2021b; Vezzulli, 2022; Archer et al., 2023).

The genus *Vibrio* comprises ecologically significant Gram-negative bacteria native to the aquatic environment globally and their incidence is strongly influenced by environmental parameters (Singleton et al., 1982; Colwell, 1996; Brumfield et al., 2021b). *Vibrio spp*. thrive in warm water with moderate salinity (Joseph et al., 1982) and are associated with aquatic invertebrates, notably crustaceans, zooplankton, and bivalves, all of which are known to impact occurrence of these bacteria in the environment (Lovelace et al., 1968; Krantz et al., 1969; Huq et al., 1983; Brumfield et al., 2021b). Copepods, zooplankton comprising a significant component of aquatic fauna, are a major host of *Vibrio spp*., including *Vibrio parahaemolyticus* and *Vibrio vulnificus*, and considered a vector of *Vibrio cholerae* (Colwell et al., 1977; Huq et al., 1983; Colwell, 1996; Rawlings et al., 2007; Martinelli Filho et al., 2020). Similarly, *Vibrio spp*. have been shown to concentrate in filter-feeding shellfish, especially

oysters, which are often consumed raw, thereby exposing people to large doses of potentially pathogenic agents (Lovelace et al., 1968; Berk and Colwell, 1981; Froelich and Noble, 2016).

Vibrio spp. are known to cause infection in humans, with *V. cholerae* being the etiological agent of cholera, an acute diarrheal disease caused by consumption of food or water containing pathogenic strains of the bacterium. The seventh cholera pandemic is in progress and the disease continues to plague the modern world, notably when climate/weather processes, microbiological parameters, and sociological determinants intersect with population vulnerabilities and loss of water, sanitation, and hygiene (WASH) infrastructure (Usmani et al., 2021, 2022). However, in addition to *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* have been proven historically significant (Waldor et al., 2018; Brumfield et al., 2021b). *V. parahaemolyticus*, first described by Professor Fujino during a 1950 shirasu food poisoning outbreak in Japan (Fujino, 1951), is a major cause of seafood-derived foodborne gastroenteritis in the United States (CDC, 2019b). *V. vulnificus*, first reported in the U.S. in 1976 by Hollis et al. (Hollis et al., 1976), is also a common cause of foodborne illness and can cause severe extraintestinal infections, including necrotizing fasciitis and septicemia (Baker-Austin and Oliver, 2018). The bacterium has a fatality rate among the highest of any waterborne pathogen, i.e., greater than 50% for primary septicemia. It also is responsible for *ca*. 95% of all waterborne and seafood-derived foodborne deaths in the U.S. (Bross et al., 2007; Baker-Austin and Oliver, 2018). In the U.S., *Vibrio spp*. are estimated to cause 80,000 illnesses and hundreds of deaths annually, of which *ca*. 65% are foodborne (Newton et al., 2012; CDC, 2019b). According to the Centers for Disease

Control and Prevention FoodNet, which has sites in 10 states covering 15% of the U.S. population, there is indication of a long-term increase in reported vibriosis between 1996 and 2019 (Brumfield et al., 2021b; CDC, 2021). In the Eastern U.S. between 1988 and 2018, *V. vulnificus* wound infections increased eightfold, i.e., from 10 to 80 cases per annum, and the northern case limit has shifted northwards 48 km per annum (Archer et al., 2023). Disturbingly, this trend of increased vibriosis is expected to continue significantly into the future because of climate change.

Compared to clinical isolates, traditionally most *V. parahaemolyticus* strains collected from the environment are not considered pathogenic to humans, i.e., when primary virulence factors are absent, namely thermostable direct hemolysin (*tdh*) and thermostable direct-related hemolysin (*trh*) which encode potential lysis of human erythrocytes (DePaola et al., 2000; Drake et al., 2007; Letchumanan et al., 2014; Raghunath, 2015). However, it has been estimated that up to *ca*. 27% of *V. parahaemolyticus* clinical isolates do not encode *tdh* and/or *trh* (Jones et al., 2012), suggesting other virulence factors exist. In fact, many environmental isolates lacking *tdh* and/or *trh* can be highly cytotoxic to human gastrointestinal cells (Raghunath, 2015) with potential to cause acute gastroenteritis (Ottaviani et al., 2012). Furthermore, *tdh*/*trh* negative *V. parahaemolyticus* strains can cause severe infections in marine fish (Colwell and Grimes, 1984) and shrimp (Prithvisagar et al., 2021), resulting in significant economic burden and aquaculture loss (Freitag et al., 2022). Nonetheless, both *tdh* and *trh* are commonly used to identify pathogenic *V. parahaemolyticus* strains (Waldor et al., 2018). An additional hemolysin, thermolabile hemolysin (*tlh*) which encodes phospholipase A2 (Zhang and Austin, 2005), has been observed in nearly all

strains of *V. parahaemolyticus* and is commonly employed as a marker for species detection and identification (Drake et al., 2007). In contrast, the mechanisms by which *V. vulnificus* causes infection in humans are more complex (Drake et al., 2007). While a few putative virulence markers have been suggested to be associated with clinical strains, e.g., pilus-type IV assembly genes (Paranjpye and Strom, 2005; Roig et al., 2010) and virulence-correlated gene (*vcg*) (Rosche et al., 2005), the extent to which they are responsible is unclear, and no single virulence gene has yet been identified that distinguishes pathogenic and non-pathogenic isolates (Baker-Austin and Oliver, 2018). However, the *V. vulnificus* hemolysin A gene (*vvhA*) has been established as reliable species-specific biomarker (Wright et al., 1985). Since *Vibrio spp*. play a critical role in the degradation of polymeric substances, such as chitin, nutrient cycling, and in other biogeochemical processes (Thompson and Polz, 2006; Pruzzo et al., 2008; Le Roux and Blokesch, 2018; Zhang et al., 2018), they cannot be eradicated from the environment.

While *V. cholerae* and non-cholera vibrios have varying routes of environmental transmission, primarily attributed to the ability of *V. cholerae* to thrive in freshwater as well as coastal ecosystems (Singleton et al., 1982) through association with a variety of living organisms, e.g., exoskeleton of chitin (Colwell, 1996; Vezzulli et al., 2010), treatment of vibriosis and cholera is similar and generally includes oral or intravenous rehydration therapy (CDC, 2019b). However, antibiotics are used in prolonged and severe cases, notably wound infections caused by *V. vulnificus* (Morris Jr. et al., 2023). Whereas cholera has essentially been eliminated in developed countries by treatment and distribution of safe potable water, pathogenic non-cholera vibrios

continue to be a significant public health burden, especially following anomalous climatic events. Significant variability has been reported in their abundance and behavior among regions of a country and the world. Studies of environmental parameters influencing the ecological niche of pathogenic *Vibrio* strains can be helpful in developing an early warning system and risk reduction strategies.

Here, we describe analysis of environmental parameters influencing occurrence and abundance of pathogenic *Vibrio spp*. in samples collected in the Chesapeake Bay over a 13-year span, with samples collected between 2009-2012 and 2019-2022. Specifically, we determined association of environmental parameters with abundance of *V*. *parahaemolyticus* and *V*. *vulnificus*, with the intent to improve existing models for assessing risk of infection with pathogenic *Vibrio spp*. and vibriosis in a changing climate.

Materials and Methods

Site description

Methods employed for sample collection and processing have been described previously in detail (Chen et al., 2017). A summary of methods relative to this study is provided here, whereby samples were collected from the Chesapeake Bay, Maryland, USA, during two separate three-year sampling events spanning 13 years. Between June 2009 and August 2012, water, oyster (Eastern Oyster, *Crassostrea virginica*), and sediment samples were collected at two stations, i.e., Chester River (CR) and Tangier Sound (TS), totaling 111 sampling events. The Chesapeake Bay, an inlet of water

enclosed on three sides by land, is the largest estuary in the U.S. CR is a major tributary in the northern portion of the Chesapeake Bay comprising brackish water, while TS is an inlet of the larger Atlantic Ocean with a more saline water content and is located further south. Between April 2019 and August 2022, water and oyster samples were collected from 12 stations throughout the Chesapeake Bay, totaling 85 sampling events (**Figure 8**). Details of each sampling station location, number of sampling events and samples collected, and station abbreviations are detailed in **Table 2**.

Sample collection

Between 2009 and 2012, sampling (water, oyster, and sediment) was done twice per month during warmer months (June through August) and once per month the rest of the year. Between 2019 and 2022, sampling (water and oyster) was done weekly during the warmer months and twice per month during the rest of the year. However, between March 2020 and July 2020, no samples were collected due to laboratory restrictions associated with COVID-19. Water (12 L) was collected 0.3 m below the surface using a Van Dorn water sampler (WildCo, Buffalo, NY) and stored in a sterile Nalgene carboy (Thermo Fisher Scientific, Waltham, MA, USA). The carboy was rinsed three times with sample water from the site prior to collection. Up to 30 oysters were collected by dredging and stored in clean double zipper freezer bags. Sediment (*ca*. 100 g) was collected using a stainless-steel scoop and stored in sterile Nalgene bottles (Thermo Fisher Scientific, Waltham, MA, USA). Samples were transported to the laboratory in a cooler with ice packs, ensuring that samples did not come in direct contact with the ice packs. Temperature of the coolers was monitored to ensure that it

did not reach above 8 °C during transport (< 4 hrs) using a LogTag® single trip temperature alert indicator (LogTag Recorders, Auckland, New Zealand). Temperature of the water samples was recorded upon arrival to the laboratory, and samples were stored at 4 °C until processing (< 12 hrs). It is worth noting that internal analysis of split samples processed immediately, i.e., no refrigeration, and following refrigeration for 12 hrs showed no significant change in the number of culturable bacteria detected in homogenized oyster tissue or water samples at a volume of > 10 L, using methods detailed below.

Environmental parameters

During each sampling event, water temperature, pH, optical dissolved oxygen (DO), salinity, conductivity, and total dissolved solids (TDS) were measured 0.3 m below the surface and 0.3 m above the bottom using a handheld water probe (Eureka, Austin, TX). Air temperature was recorded at the time of collection. Total chlorophyll *a* (chl-a) was measured by high-performance liquid chromatography. Briefly, volumes up to 250 ml of water were filtered using 25 mm glass microfiber filters (Whatman GF/F; Cytiva, Marlborough, MA) and stored at -80 °C. For samples collected between 2009 and 2012, concentrations of total chl-a were measured in methanol extracts on a Cary model 50 UV-visible light spectrophotometer, as described previously (Letelier et al., 1993; Johnson et al., 2012). For samples collected between 2019 and 2022, concentrations of chl-a (total and active) and pheophytin were measured in acetone extracts on a Shimadzu UV 2401PC spectrophotometer following University of Maryland Center for Environmental Science (UMCES) standard operating procedures

for Fluorometric Determination of chl-a in waters and sediments of Fresh/Estuarine/Coastal Areas (UMCES, 2022), modified from United States Environmental Protection Agency (EPA) Methods No. 445.0 (EPA, 1997) and American Public Health Association (APHA) Standard Methods No. 10200H.3 (APHA, 2012). Chl-a analysis for 2009-2012 and 2019-2022 was performed in experimental triplicate and duplicate, respectively, and results are presented as the average.

Sample processing

Details of sample processing have been reported in other publications (Johnson et al., 2012; Chen et al., 2017). Briefly, samples were treated following methods outlined in the Bacteriological Analytical Manual for food sampling/preparation of sample homogenate (Andrews and Hammack, 2019) and *Vibrio* (Kaysner et al., 2019). Notably, water samples were shaken vigorously 25 times in 30 cm arc in 7 s. Oysters were rinsed and scrubbed under running deionized water to remove debris from the shell and opened using a sterile shucking knife. Oyster tissue in equal part phosphate buffered saline (PBS; pH 7.4) was homogenized in a sterile blender for 90 s. Sediment samples were weighed in a sterile conical tube and vortexed in equal part PBS for 90 s. Following homogenization, all dilutions were done in appropriate media within 15 min.

Direct plating and DNA colony hybridization

Methods to enumerate *V. parahaemolyticus* and *V. vulnificus* populations via direct plating and DNA colony hybridization (DPCH) have been described previously (Parveen et al., 2008; Johnson et al., 2010, 2012; Kaysner et al., 2019). For samples collected between 2009 and 2012, water (1 ml), oyster (0.1 g, 0.01 g) and various sediment concentrations (up to 0.05 g [wet weight]) were used for DPCH. For 2019-2022 samples, water (1 ml) and oyster (100 µl of oyster homogenate) were used. Respective sample volumes were spread plated in duplicate on T1N3 agar (1% tryptone, 3% NaCl [pH 7.2]) and *V. vulnificus* agar (VVA; 2% peptone, 3% NaCl, 1% cellobiose, 0.06% bromothymol blue [pH 8.2]) to enumerate *V. parahaemolyticus* and *V. vulnificus*, respectively. Following incubation at 37 °C for 16-18 hrs, Whatman 541 ashless filters (Whatman, Kent, ME) were used to lift colonies from each plate. Filters were probed using alkaline phosphatase conjugated oligonucleotide probes (DNA Technology A/S, Risskov, Denmark) specific for *V. parahaemolyticus* (*tlh*) or *V. vulnificus* (*vvhA*); samples collected between 2009 and 2012 were also screened for *V. parahaemolyticus* virulence markers (*trh* and *tdh*). Probes, listed in **Table 3**, were visualized using Nitro blue tetrazolium chloride/5-Bromo-4chloro-3-indolyl (NBT/BCIP) Ready-to-Use Tables (Roche, Basel, Switzerland), per manufacturer instructions. Probe-positive colonies, i.e., those purple-brown in color, were counted, and are presented as the average of duplicate hybridizations. Sterile PBS (1 mL) spread onto T1N3 agar or VVA was used as blank control. To ensure the deionized water used to scrub oysters did not contain traces of culturable *Vibrio* bacteria, 1 mL was spread onto T1N3 or VVA during each hybridization. Cultures of *V. parahaemolyticus* (ATCC

17803 [*tlh*+/*tdh*-/*trh*+], TX2103, [*tlh*+/*tdh*+/*trh*-], and AQ4037 [*tlh*+/*tdh*-/*trh*+]) and *V. vulnificus* (ATCC 27562 [*vvhA*+]) were prepared under standard growth conditions in Luria-Bertani broth at 37 °C overnight with aeration, and 1 µl was spotted onto T1N3 agar or VVA three times for each hybridization to serve as positive and negative controls, respectively.

Statistical analysis

Statistical analysis was done using R v.4.2 (R Core Team, 2022), and figures were generated using ggplot2 (Wickham, 2016) and plotly (Sievert, 2020). Environmental parameters and DPCH results were compared among locations, seasons, and collection periods. For comparisons of DPCH results, the average number of colony forming units (CFU) detected across duplicate hybridizations was used, following normalization to sample volume for water (CFU/ml), oyster (CFU/0.1 g), and sediment (CFU/0.1 g). Where appropriate, data were log-transformed using log1p method, i.e., [log(x+1)]. To evaluate the relationships of environmental parameters and DPCH results, surface recordings were used for water samples and bottom recordings were used for oyster and sediment samples. Both individual stations and pooled data were evaluated for both collection periods. However, since samples collected between 2009 and 2012 were representative of >50 sampling events from each of two stations, 15 sampling events were randomly selected from both CR and TS using the "sample" command from the base R software package. To ensure consistency of subsampling across data analysis, the seed was set to seven, which was chosen by random number generation. To test for significance of the observed differences of means between two

groups, Wilcoxon test was used, while one-way analysis of variance (ANOVA) was used to compare differences of means among more than two groups. Linear regression and Kendall's tau correlation methods were used to quantify the relationship of DPCH results with temperature. A nonparametric regression model, i.e., locally weighted smoothing (LOESS), was used to visualize the relationship of DPCH results with environmental parameters lacking linear correlation. Boxplots were used to summarize distributions by indication of interquartile range. Scatterplots were used to visualize DPCH results relative to environmental parameter values. Normalized kernel density estimates, i.e., a smoothed version of the histogram, were used to visualize the count of samples demonstrating > 10 CFU relative to each environmental parameter value.

Results

Environmental parameters

Between June 2009 and August 2012, a total of 111 total field observations were recorded from CR and TS, and between April 2019 and August 2022, 86 total field observations were recorded from 12 stations (**Figure 8**). A summary of environmental parameters for each station is detailed in **Table 2**. During all of the sampling, ranges of surface water temperature and DO were similar across the stations. However, between 2009 and 2012, differences were observed for salinity, conductivity, and TDS, namely at TS which showed higher values compared to CR (Wilcoxon, $p < 0.05$) (**Figure 9A**), with greatest differences observed during the summer and fall. Similarly, between 2019 and 2022, higher salinity, conductivity, and TDS values were

recorded at TS compared to other stations (ANOVA, $p < 0.05$) (**Figure 9B**). For total chl-a, pheophytin, and active chl-a, the highest values were recorded at the Upper Patuxent River (UPR) station, while lowest values were recorded at TS (ANOVA, $p < 0.05$). For other environmental parameters little variation was observed among the other stations, but values varied by season at each station, respectively.

Distribution of *V. parahaemolyticus* and *V. vulnificus* employing DPCH

DPCH was used to determine incidence and abundance of *V. parahaemolyticus* and *V. vulnificus* in various sample types (**Figure 10**). Overall, DPCH detection rates varied significantly by season and were highest in sediment samples, followed by oyster and water. Between 2009 and 2012, the abundance of *vvhA* was similar between TS and CR for all sample types, but *tlh* was detected at higher abundance at the TS station for water and oyster samples (Wilcoxon, $p < 0.05$) (**Figure 10A**). Overall, *trh* and *tdh* were detected at similar abundance at both stations. No differences between stations were observed for sediment samples, among all genetic markers tested. The *vvhA* marker was detected at higher abundance than *tlh* for all sample types (Wilcoxon, $p < 0.05$). Both *vvhA* and *tlh* were detected at higher abundance than *trh* and *tdh* (Wilcoxon, $p < 0.05$). Between 2019 and 2022, concentrations of *tlh* and *vvhA* showed no overall difference, with respect to sampling station (ANOVA, $p > 0.05$) (**Figure 10B**), whereby *tlh* and *vvhA* were detected at similar abundance to each other for water and oyster samples (Wilcoxon, $p > 0.05$).

Temporal evaluation of *V. parahaemolyticus* and *V. vulnificus*

To evaluate how concentrations of *V. parahaemolyticus* and *V. vulnificus* may have changed over time, DPCH results for water samples were compared between the two collection periods. Across all stations, no overall difference was observed between the two collection periods for *vvhA*, but *tlh* concentrations increased significantly during the 2019-2022 collection period compared to 2009-2012, whereby median log1p values increased from 0.4 to 2.4 (Wilcoxon, $p < 0.05$) and mean positive values increased from *ca.* 5 CFU/ml to *ca.* 18 CFU/ml (**Figure 10C**). With respect to season, an increase for *vvhA* was observed during the fall, whereby median log1p values increased from 1.5 to 3 (Wilcoxon, $p < 0.05$) and mean positive values increased from *ca.* 18 CFU/ml to *ca.* 37 CFU/ml (**Figure 10D**). Similarly, *tlh* showed significant increases during all seasons (Wilcoxon, $p < 0.05$), with the greatest increase observed during the fall whereby median log1p values increased from 0.55 to 2.67 and mean positive values increased from *ca.* 3 CFU/ml to *ca.* 22 CFU/ml.

Further evaluation of water samples at TS allowed for temporal evaluation of *vvhA* and *tlh* from the same station between collection periods. Water samples from the TS station showed no overall difference for environmental parameters, including temperature, salinity, conductivity, TDS, DO, and total chl-a, between the two periods (Wilcoxon, $p > 0.05$). Like the comparison of all stations, no statistical difference was observed for the concentration of *vvhA*, but an increase was observed for *tlh*, whereby median log1p values increased from 0.41 to 2.43 (Wilcoxon, $p < 0.05$) and mean positive values increased from *ca.* 5 CFU/ml to *ca.* 18 CFU/ml (**Figure 10E**). With respect to seasonality, *vvhA* did not show a significant difference, but *tlh* showed

significant differences during the summer, fall, and winter (Wilcoxon, $p < 0.05$), with the greatest increase observed during the fall whereby median log1p values increased from 0.55 to 2.67 and mean positive values increased from *ca.* 2 CFU/ml to *ca.* 22 CFU/ml (**Figure 10F**).

Seasonal impact on incidence of pathogenic *Vibrio*

To assess the impact of seasonality, the DPCH results were evaluated with respect to water temperature at the time of sample collection (**Figure 11**). For each sample type and location, the range of DPCH detection and percentage of positive samples relative to the total number of samples is shown in **Table 4**. The number of *tlh* and *vvhA* determinations showed strong linear association with temperature, where highest abundance occurred in the warmer months of the year. For samples collected between 2009 and 2012 (**Figure 11A**), the greatest association with temperature was observed in oyster samples for both *vvhA* and *tlh* (Tau > 0.5) (**Figure 11B**). However, it is worth noting that *vvhA*, and occasionally *tlh*, were detected in higher numbers during the winter months in sediment samples collected from both CR and TS stations. Hence, the correlation between temperature and *vvhA* was low for sediment samples (Tau < 0.1). Compared to *tlh*, negative results were more frequent using DPCH to quantitate *trh* and *tdh* populations in water, oyster, and sediment samples from both stations between 2009 and 2012. Both *tdh* and *trh* were detected during warmer months, with higher detection in oyster and sediment samples. Like *tlh* and *vvhA*, the *trh* and *tdh* detection rates were highest for sediment, followed by oyster and water. Pathogenic *V. parahaemolyticus* (*trh* and *tdh*) were detected more frequently during the winter

months in sediment compared to water and oysters. With exception of *trh* in oysters (Tau = 0.4), the relationship with temperature was not strongly correlated. A similar pattern of seasonality was observed for samples collected between 2019 and 2022 (**Figure 11C**), whereby *tlh* and *vvhA* showed a stronger association with temperature for oyster samples (*tlh*, Tau = 0.64; *vvhA*, Tau = 0.66) compared to water samples (*tlh*, Tau =0.37; *vvhA*, Tau = 0.56) (**Figure 11D**).

Environmental parameters associated with incidence

The relationship among environmental parameters and DPCH results was further investigated to determine optimal ranges of each environmental parameter associated with genetic marker abundance. **Figure 12** shows analysis of pooled data for both collection periods. Temperature, salinity, and total chl-a were determined to be strong predictors of increased abundance of both *vvhA* (**Figure 12A**) and *tlh* (**Figure 12B**), forming distinct clusters compared to sampling events with low abundance or those for which genetic markers were not detected. Analysis of individual stations, shown as scatterplots and overlayed kernel density of each genetic marker detected above the threshold of 10 CFU per hybridization can be found in the supporting information (**Figure 13**).

<u>Temperature</u>

With respect to seasonal temperature, occurrence of both *tlh* and *vvhA* increased proportionally with temperature during both collection periods (Tau > 0.4), with the

greatest association observed for *vvhA* occurrence in oyster samples (**Figure 12C**). As temperature increased, the percent of positive samples also increased, and the temperature threshold for occurrence of *tlh* and *vvhA* in >50% of the samples was determined to be > 15 °C (**Figure 12D**). A second temperature range, indicative of increased to maximum abundance marker abundance, was observed when temperatures were > 25 °C. However, it should be noted that in a few cases increased *tlh* and *vvhA* numbers were observed in oyster and sediment samples at lower temperatures during the 2009-2012 collection period (**Figure 13**). Incidence of both *trh* and *tdh* was associated with temperatures > 20 °C in oyster and water samples, but moderate abundance of these markers was detected occasionally at lower temperatures in sediment samples compared to water and oyster samples.

Salinity

The overall relationship between salinity and abundance of both *tlh* and *vvhA* was nonlinear, demonstrative of a downward curve (**Figure 12E**). Evaluation of pooled data showed salinity ranges associated with positive samples above the threshold of 30 CFU/hybridization to be between 6 PPT and 18 PPT for *vvhA* and 8 PPT and 16 PPT for *tlh* (**Figure 12F**). However, a salinity range between 10 PPT and 15 PPT was associated with increased to maximum abundance marker abundance. For samples collected between 2009 and 2012, the optimal salinity ranges for *tlh* and *vvhA* differed between CR and TS stations, whereby the salinity of CR was lower than TS (**Figure 13**). Moderate abundance of *vvhA* was associated with salinity ranges between 3 PPT

and 12.5 PPT at CR and ranges between 10 PPT and 17 PPT at TS. Differences in salinity between stations were more prominent for *tlh* in water (CR, 3-8 PPT; TS, 12-16 PPT) compared to oyster and sediment (CR, 4-15 PPT; TS, 10-17 PPT). For samples collected between 2019 and 2022, salinity ranges associated with moderate abundance were more similar among sampling stations, with exception of *tlh* detection in water at Choptank River (CHR) and UPR stations (6-12 PPT) compared to TS (12-17 PPT). For *vvhA* in water, salinity ranges associated with moderate abundance were observed between 7 and 17 PPT, and a similar range was observed for both *tlh* and *vvhA* in oyster samples.

Chlorophyll and pheophytin

Like salinity, the relationship of total chl-a and detection of *tlh* and *vvhA* was observed as a nonlinear downward curve (**Figure 12G**). Across both collection periods, total chl-a values associated with an increased number of positive samples > 30 CFU/hybridization for both *vvhA* and *tlh* were determined to be between 5 µg/L and 30 µg/L (**Figure 12H**). However, higher values were observed, ranging from 20 µg/L to 40 µg/L at the UPR station (**Figure 13**). For *trh* and *tdh*, a peak density was observed around 10 µg/L, but the overall range of detection was like that of *tlh* and *vvhA*. For samples collected between 2019 and 2022, optimal ranges for pheophytin (4-8 µg/L) and active chl-a (10-20 µg/L) were observed to be associated with *tlh* and *vvhA* detection at increased abundance.

Other environmental parameters

The relationship of other environmental parameters and *Vibrio* abundance was consistent across collection periods and location, observed as a nonlinear relationship with an acceptable range for occurrence of genetic markers. For all markers, pH ranges between 7 and 9 were associated with moderate detection, and pH values closer to 8 were generally associated increased abundance (**Figure 13**). DO ranges between 4 mg/L and 15 mg/L were associated with moderate detection of *vvhA* and *tlh*. The DO range for increased abundance of *tlh* and *vvhA* was observed between 5 mg/L and 8 mg/L, which was also within the range for presence of pathogenic *V. parahaemolyticus* markers. Ranges for conductivity (15-22 mg/L) were associated with increased abundance of *vvhA* and *tlh* during both periods. Between 2009-2012, TDS ranges (10-15 g/L) were associated with increased abundance, while slightly higher values were observed (15-25 g/L) during the 2019-2022 collection period.

Discussion

Climate change is recognized to be associated with increased frequency of anomalous weather events, which can result in warmer and less saline waters along the coast (Ummenhofer and Meehl, 2017). In general, *Vibrio spp*. thrive in warm water and moderate salinity (Joseph et al., 1982; Brumfield et al., 2021b). An increasing number of studies document a pattern of poleward spreading of *Vibrio spp*., demonstrating significant geographic expansion of *Vibrio spp*. populations (Colwell, 1996; Vezzulli et al., 2012, 2016; Baker-Austin et al., 2013, 2017; Froelich and Daines, 2020; Vezzulli,

2022), corroborated by associated impacts on human health—especially in coastal communities, including the eastern seaboard of the U.S. (Archer et al., 2023) and Chesapeake Bay (Watts et al., 2018; Teodoro and Nairn, 2020). Unfortunately, the trend of cases of vibriosis is expected to increase further in coming years as a result of climate variabilities, notably changes in frequency and magnitude of anomalous weather events, e.g., heatwaves, hurricanes, floods, and droughts (Huq et al., 1984, 2005; Colwell, 1996; Baker-Austin et al., 2013, 2017; Vezzulli et al., 2020; Brumfield et al., 2021b). A prime example is the number of *V. parahaemolyticus* and *V. vulnificus* cases reported along coastal regions of the Florida Gulf following Hurricane Ian, a destructive Category 4 Atlantic hurricane that made landfall on FL in September 2022. There were 74 confirmed cases of *V. vulnificus* infection and 17 deaths reported that year—nearly double the normal number for the area (Florida Department of Health, 2023). It is reasonable to suspect that changes in frequency, intensity, and duration of extreme weather events will affect the ecology of pathogenic *Vibrio spp*., notably in the Chesapeake Bay, which is already experiencing major climate-driven changes, namely sea level rise and flooding associated with storms and hurricanes (Teodoro and Nairn, 2020). Hence, it is important to understand the ecology of *Vibrio spp*. and environmental parameters influencing their occurrence and transmission in a changing climate.

A primary objective of the research reported here was to determine microbial ecological niche profiles that can be incorporated in predictive risk assessment models and real-time remote sensing data for geographic regions of the world. Risk prediction models provide an early warning, essential to safeguarding public health, especially in

regions vulnerable to infrastructure instability (e.g., lack of WASH infrastructure), natural calamity (e.g., hurricanes, floods, and earthquakes), and/or social disruption and civil unrest (e.g., wars, coups, political crisis, and economic recession). For example, the risk of cholera, can now be predicted at the scale of available satellite observation and favorable environmental conditions (Khan et al., 2019a, 2019b). The model was employed to assess the risk of cholera in Ukraine (Usmani et al., 2022) and Yemen (Usmani et al., 2023), countries suffering damaged or severely crippled civil infrastructure, following which the human population is at risk of health disasters. It is important to note that both cholera and vibriosis have varying routes of environmental transmission, primarily attributed to the ability of *V. cholerae* to thrive in freshwater through its salinity requirements (Singleton et al., 1982) and association with a variety of hosts with chitinous exoskeletons (Colwell, 1996; Vezzulli et al., 2010). Other models, such as the National Centers for Coastal Ocean Science (NCCOS) Probability Model for *V. vulnificus* Occurrence in Chesapeake Bay Water (Jacobs et al., 2014) and the *Vibrio* Map Viewer (Semenza et al., 2017) developed by the European Center for Disease Prevention and Control (ECDC), have been developed to predict increased incidence of *Vibrio spp.* in the environment. Data from this study will be used to refine the established models by providing additional environmental parameter thresholds, including temperature, salinity, and chlorophyll, for *V. parahaemolyticus* and *V. vulnificus*. Design of future predictive risk assessment models for *Vibrio spp.* will benefit from inclusion of sociological and behavioral factors related to contact with the aquatic environment, such as swimming, fishing, and other recreational/occupational activities, that influence contact with waterborne pathogenic agents.

Over the past five decades, extensive datasets have been generated with respect to incidence and behavior of *V. cholerae* and non-cholera *Vibrio spp*. in the ocean and estuarine environments through cross-sectional studies, including long-term retrospective work (Berk et al., 1976; Huq et al., 1983, 1996; Colwell and Huq, 1994; Colwell, 1996; Mutreja et al., 2011; Hasan et al., 2012; Johnson et al., 2012; Vezzulli et al., 2016; Baker-Austin et al., 2017; Chen et al., 2017; Martinez-Urtaza et al., 2017; Parveen et al., 2020). These studies, reviewed by Brumfield et al. (Brumfield et al., 2021b), provide evidence for environmental parameters as drivers, both directly and indirectly, of the incidence of pathogenic *Vibrio spp*. in the environment.

The results presented here represent one of the most intensive sampling campaigns for *Vibrio spp*. occurrence and distribution, allowing determination of seasonal variation of *Vibrio* abundance. However, a caveat should be noted that *Vibrio spp*. enter the viable but nonculturable (VBNC) state (Colwell et al., 1985; Roszak and Colwell, 1987; Colwell and Huq, 1994), whereby the cells become metabolically dormant. VBNC cells cannot be cultured employing standard laboratory media, yet detectable using molecular genetic methods (Colwell, 2000). *V. parahaemolyticus* and *V. vulnificus* have been shown to have increased resistance to thermal stress, low salinity, and acidic inactivation when in the VBNC state, suggestive of a survival strategy under adverse conditions (Wong and Wang, 2004; Nowakowska and Oliver, 2013). Interestingly, studies have shown alternative methods, such as PCR, qPCR, LAMP, and DNA sequencing, to be more sensitive for detection of genetic markers than DPCH (Parveen et al., 2008; Johnson et al., 2012; Jacobs et al., 2014; Vezzulli et al., 2017; Brumfield et al., 2020c). Additionally, improved recovery of pathogenic *V.*

parahaemolyticus by DPCH has been observed following enrichment (Nordstrom and DePaola, 2003). The results reported here were for culturable *Vibrio* detected using DPCH without enrichment, hence likely underestimating total *Vibrio* populations. Alternative methods will be helpful in the future for more precise quantification of *Vibrio spp*. Complementary research is underway employing whole genome shotgun sequencing of archived *Vibrio* isolates to determine the influence of environmental factors on *Vibrio spp*. genomic diversity, including virulence and antimicrobial resistance.

Genetic markers of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in various sample matrices were quantified using DPCH (**Figure 8**), and the results support findings of previous studies using similar culture-based methods in estuarine waters of Louisiana, Maryland, Mississippi, Georgia, South Carolina, and Washington, USA, (Kaneko and Colwell, 1973, 1974; Colwell and Spira, 1992; Colwell, 2006; Parveen et al., 2008; Johnson et al., 2012). These studies and related work in the northern Gulf of Mexico (Johnson et al., 2010), conducted approximately at the same time, showed relatively few *tdh*/*trh* positive samples. Results provided here detected pathogenic *V. parahaemolyticus* in *ca*. one third of oyster and sediment samples and *ca.* 14% of water samples collected between 2009 and 2012 (**Table 4**). The higher rates of detection of pathogenic *V. parahaemolyticus* in oyster and sediment samples suggest a niche of pathogenic vibrios, i.e., increased abundance of *trh*/*tdh* bacteria at temperatures < 10 °C but lack of linear correlation with temperature. These observations support previous work showing the preferable niche of pathogenic *Vibrio spp.* in matrices other than water, especially at lower temperatures (DePaola et al., 2003; Givens et al., 2014). An

in situ study conducted in the Mediterranean Sea showed culturable *Vibrio spp*. to be present year-round in sediment (Vezzulli et al., 2009). It can be concluded that oysters and sediment function as reservoirs of pathogenic *Vibrio spp*. and represent different communities than those in water, contributing to seasonality of *Vibrio spp*. during unfavorable conditions.

Results of this study reaffirm temperature as a strong predictor of the incidence and distribution of *Vibrio spp*. (measured using *tlh* and *vvhA*) in the environment (Colwell, 1996; Martinez-Urtaza et al., 2010; Johnson et al., 2012; Jacobs et al., 2014; Vezzulli et al., 2016; Parveen et al., 2020; Vezzulli, 2022). A similar study of culturable *V. parahaemolyticus* populations in the Chesapeake Bay between 1970 and 1971 (Kaneko and Colwell, 1977) found *V. parahaemolyticus* numbers below detectable levels in the water column from January to early April until water temperatures rose above 14 °C. However, a rapid increase in counts from 0 to 10^3 CFU/100 ml were found in the period from April to early June, and maximum counts (6.2×10^3 CFU/100 ml) were observed in the middle of July. Subsequently, *V. parahaemolyticus* numbers stayed around 10^3 CFU/100 ml until the middle of October when the water temperature dropped below 15 °C (Kaneko and Colwell, 1977). A similar pattern of seasonality was observed here (**Figure 9**), whereby two critical temperature thresholds were observed at 15 °C and 25 °C. That is, between 14°C and 15°C, an initial increase in *Vibrio* density was observed, and when temperatures rose above 25 °C, maximum counts were observed (**Figure 10**).

While critical temperature thresholds have been fairly consistent over the years, the presumptive *V. parahaemolyticus* populations demonstrate a long-term seasonal

increase between 2019 and 2022, compared to similar locations in the 1970's (Kaneko and Colwell, 1973, 1974, 1977; Kaper et al., 1981). Maximum counts of *V. parahaemolyticus* in water samples during summer months were within the same order of magnitude, i.e., ≈ 10^3 CFU/100 ml, but detectable values were more frequent during winter and early spring, with lowest numbers recorded as late as March. During 2019-2022, the numbers remained high to the end of fall. Samples collected between 2019 and 2022, showed mean *tlh* numbers significantly increased across all seasons (*ca.* threefold) for 2019-2022 compared to 2009-2012, with greatest increase observed during the fall for both *tlh* (*ca.* sevenfold) and *vvhA* (*ca.* twofold). Similarly, water samples collected at TS, the station consistently sampled during both 2009-2012 and 2019-2022 collection periods, showed increase in *tlh* during summer, fall, and winter with greatest increase in the fall. However, no significant difference was observed for *vvhA* at TS between collection periods, which might be due to the fact that *V. vulnificus* has been found to be concentrated in the mid-bay, upper bay, and western estuaries compared to stations in the lower bay (Banakar et al., 2011). Hence, additional sampling from more diverse locations is required to elucidate changes in *V. vulnificus* populations over time. Nonetheless, *Vibrio spp.* exhibit seasonal patterns of growth, and these results suggest an extended seasonality, notably during the fall.

The methods for processing oyster samples differed between collection periods, i.e., weight (2009-2012) compared to volume (2019-2022). Thus, direct quantitative comparison could not be made. However, the rate of detection using genetic markers was similar, e.g., (Parveen et al., 2008) employed DPCH during 2004-2005, and detected *tlh* in *ca.* 80% of oyster samples. In this study *ca.* 70% (2009-2012) and *ca.*

85% (2019-2022) of the oyster samples were positive for *tlh*. Oyster samples evaluated by weight during 2009-2012 values were low, according to U.S. Food and Drug Administration (FDA) guidance for *V. parahaemolyticus* (< 10^4 CFU/g) in ready-to-eat foods (FDA, 2022). Overall, *tlh* detection rates were about 10% lower for water compared to oysters, and for all genetic markers, DPCH detection rates were highest in sediment, followed by oyster and water, in agreement with previous reports (Johnson et al., 2012).

Salinity proved to be a strong predictor of *Vibrio* abundance, in agreement with findings of previous studies (Randa et al., 2004; Jacobs et al., 2014; Deeb et al., 2018; Levy, 2018; DeLuca et al., 2020). Overall, the relationship between *Vibrio* abundance and salinity indicated a nonlinear downward curve (**Figure 10**), with moderate abundance of *vvhA* and *tlh* between 7 and 17 PPT. Maximum numbers were observed when the salinity was between 10 and 15 PPT, in agreement with (Jacobs et al., 2014) for *V. vulnificus* and optimal salinity gradient of 11.5 PPT.

Chlorophyll serves as an important predictor of *Vibrio* abundance (Constantin de Magny et al., 2008; Julie et al., 2010; Jutla et al., 2013b; Scro et al., 2022; Namadi and Deng, 2023), especially since chlorophyll indicates density of phytoplankton populations and, thereby, serves as an indicator of zooplankton which feed on phytoplankton (Colwell, 1996; Lobitz et al., 2000). Since zooplankton feed on phytoplankton, correlation of zooplankton population surges following phytoplankton blooms can be calculated. *Vibrio spp.* are commonly associated with zooplankton, primarily copepods, whereby they provide nutrient-rich surfaces to which the bacteria attach (Berk et al., 1976; Huq et al., 1983). Abundance of phytoplankton, followed by

a zooplankton bloom, can be used to predict increase in the *Vibrio* populations in nutrient rich waters (Constantin de Magny et al., 2008). Phytoplankton blooms are also an indicator of nutrient runoff introducing organic matter, leading to proliferation of *Vibrio spp*. (Shaw et al., 2014a; Thickman and Gobler, 2017). Here, the relationship between (*tlh* and *vvhA*) and chlorophyll was found to be nonlinear (**Figure 10**), with an acceptable range of increased occurrence of genetic markers according to chlorophyll pigments, including total chl-a (5-25 µg/L), namely pheophytin (4-8 µg/L), and active chl-a (10-20 µg/L). With respect to pathogenic *V. parahaemolyticus* (*trh* and *tdh*), a peak density was observed around 10 µg/L. However, chlorophyll as a proxy for zooplankton abundance would benefit by including specific lag times, e.g., one month post sample collection, to account for a subsequent increase in zooplankton (Colwell, 1996; Lobitz et al., 2000; Jutla et al., 2012).

Conclusions

With a high growth rate and rapid response to environmental signals, *Vibrio spp*. have been proposed as a microbial indicator of a changing global climate, corroborated by impact on public health, namely increased number of infections caused by these pathogens (Lovelace et al., 1968; Krantz et al., 1969; Kaneko and Colwell, 1973, 1974; Colwell et al., 1977; Huq et al., 1984, 2005; Colwell, 1996; Baker-Austin et al., 2013, 2017; Froelich and Daines, 2020; Vezzulli et al., 2020; Brumfield et al., 2021b; Olson, 2022; Vezzulli, 2022; Archer et al., 2023). Hence, it is important to understand the ecology of *Vibrio spp*. and those environmental parameters influencing their occurrence and transmission. This study, employing field sampling in the

Chesapeake Bay confirmed results of earlier studies, with respect to numbers of pathogenic *Vibrio spp*. and their correlation with environmental factors, including seasonality, temperature, salinity, and chlorophyll. In addition, correlative differences between response of total and pathogenic *V*. *parahaemolyticus* and *V*. *vulnificus* to environmental parameters were determined. It is concluded that pathogenic *Vibrio spp*. cannot be treated as a single cohesive unit, since subspecies/strains may inhabit differential niches. Further, different population composition was observed for oyster and sediment compared to water and linked to *Vibrio* seasonality. A long-term increase in *Vibrio* populations in the Chesapeake Bay was determined, particularly during fall months of the year suggesting an extended seasonality of these bacteria has occurred. These results provide a baseline useful for future investigations, both in the Chesapeake Bay and globally, to evaluate changes in pathogenic *Vibrio* populations relative to climate change over time. The results obtained to date will be used in developing environmental and climate niche models for non-cholera *Vibrio spp*., namely *V*. *parahaemolyticus* and *V*. *vulnificus*.

Acknowledgements

The authors are grateful to the Department of Natural Resources and Maryland Department of the Environment for their exceptionally generous support during the study, namely in providing logistical assistance for sample collection. Their support made this work possible.

Tables

Table 1: Sampling location information in Chesapeake Bay.

[a] Wa = water; Oys = oyster; Sed = sediment; n = number of sampling events.

[b] Stations with more than three sampling events are presented as: minimum; median; maximum. If fewer than three sampling events were conducted at a given location, all values are shown. (n.d.) = not determined.

[c] For samples collected between 2009 and 2019, concentrations of total chl-a were measured in methanol extracts using methods described in (Letelier et al., 1993; Johnson et al., 2012), performed in experimental triplicate and presented as the average. For samples collected between 2019 and 2022, concentrations of chl-a (total and active) and pheophytin were measured in acetone extracts following methods described in (UMCES, 2022), performed in experimental duplicate and presented as the average.

Table 1: Sampling location information in Chesapeake Bay.

Collection Period	Station (abbr.)	latitude	longitude	Sample media[a]; No. sampling events	Temperature (°C)[b]	Salinity (PPT)[b]	pH[b]	Conductivity (mS/cm)[b]	Optical Dissolved Oxygen (mg/L)[b]	Total Chl-a (mg/L)[b,c]	Pheophytin (mg/L)[b,c]	Active Chl-a (mg/L)[b,c]
2009-2012	Chester River (CR)	39.0859796	-76.164233	Wa, Oys, Sed; n = 57	0.50; 19.80; 30.20	3.2; 8.43; 12.5	5.77; 7.55; 8.8	5.79; 14.61; 21.00	5.20; 7.23; 15.39	0.06; 5.19; 41.39	n.d	n.d
	Tangier Sound (TS)	38.1086036	-75.870907	Wa, Oys, Sed; n = 54	0.1; 21.48; 30.14	7.3; 13.77; 19.10	6.12; 7.70; 8.42	11.27; 15.12 18.88	5.36; 7.44; 19.5	0.03; 3.05; 10.19	n.d	n.d
2019-2022	Broad Creek (BC)	38.74596	-76.248124	Wa, Oys; n = 2	25.90; 29.20	8.80; 14.10	7.60; 8.20	16.93	6.40; 6.50	15.56; 16.48	5.27; 6.79	12.62;12.66
	Chester River (CR)	39.0859796	-76.164233	Wa, Oys; n = 1	25.2	3.72	7.32	6.82	8	21.76	9.28	16.59
	Choptank River (CHR)	38.6006577	-76.07417	Wa, Oys; n = 16	2.90; 22.05; 28.90	8.70; 10.50; 13.00	7.40; 7.80; 8.50	15.03; 12.27; 21.73	5.90; 7.31; 13.40	9.04; 15.29; 25.54	3.32; 4.87; 7.79	6.78; 12.78; 22.55
	Cox Creek (COX)	38.9283561	-76.304835	Wa, Oys; n = 6	3.71; 16.93; 26.43	7.62; 12.05; 14.47	7.70; 7.97; 8.98	13.18; 20.39; 23.80	7.20; 9.41; 12.86	6.72; 13.88; 61.95	1.98; 4.63; 22.96	5.61; 11.29; 49.135
	Lower Patuxent River (LPR)	38.325942	-76.466414	Wa, Oys; n = 7	9.30; 26.50; 28.70	10.40; 12.75; 13.80	7.40; 8.05; 8.20	17.71; 20.39; 22.73	3.85; 5.94; 11.40	7.70; 12.36; 25.07	2.66; 3.35; 6.27	6.06; 10.62; 21.55
	Upper Patuxent River (UPR)	38.4606929	-76.646907	Wa, Oys; n = 17	3.40; 22.14; 28.50	5.96; 10.10; 11.90	6.60; 7.60; 8.60	10.58; 16.22; 20.22	5.00; 7.35; 14.10	5.81; 27.64; 137.82	2.09; 7.72; 29.00	4.64; 22.52; 127.52
	Miles River (MR)	38.842556	-76.232417	Wa, Oys; n = 1	16.8	14.2	8.1	23.44	9.7	12.05	2.76	10.51
	Sandy Point (SP)	39.001192	-76.392426	Wa, Oys; n = 1	26.08	6.54	7.51	11.51	6.11	21.78	7.72	17.48
	South River (SR)	38.937111	-76.518583	Wa, Oys; n = 1	30.9	6.7	8.1	11.81	7.1	74.98	8.91	69.91
	St. Mary's River (SMR)	38.1565346	-76.438875	Wa, Oys; n = 4	3.40; 21.50; 29.80	8.00; 8.77; 9.20	8.60; 8.70; 8.90	13.70; 15.88; 20.57	7.90; 9.30; 10.71	5.66; 18.93; 36.38	2.06; 4.14; 6.02	4.51; 16.61; 32.98
	Tangier Sound (TS)	38.1086036	-75.870907	Wa, Oys; n = 15	1.75; 24.36; 29.66	11.44; 14.96; 17.00	7.70; 8.00; 8.170	19.27; 23.05; 27.62	6.02; 7.60; 16.70	4.78; 11.60; 21.41	2.00; 2.85; 6.03	3.67; 10.21; 18.23
	Wicomico River (WR)	38.2757472	-76.824132	Wa, Oys; n = 15	5.60; 15.00; 29.96	5.96; 10.10; 11.90	7.90; 8.35; 8.70	5.05; 15.70; 20.58	5.00; 7.35; 14.10	9.46; 16.88; 40.59	3.00; 4.88; 10.05	6.48; 14.57; 37.64

Table 2: Probes used in this study for DPCH.

[a] All probes were modified by (5') conjugation with alkaline phosphatase.

[b] Isolate characterized previously (Chen et al., 2017)

Table 2: Probes used in this study for direct plating and DNA colony hybridization.

Description (reference)	Primer/Probe	Sequence (5'-3')[a]	Control strain
thermolabile hemolysin	*tlh*-AP	AAAGCGGATTATGCAGAAGCACTG	*V. parahaemolyticus* ATCC 17803
thermostable direct hemolysin	*tdh*-AP	ACTTTGCTTTCAGTTTGCTATTGGCT	*V. parahaemolyticus* TX2103 (*tdh*+/*trh*-)[b]
thermostable direct-related hemolysin	*trh*-AP	GGTTCTATTCCAAGTAAAATGTATTTG	*V. parahaemolyticus* AQ4037 (*tdh*-/*trh*+)[b]
V. vulnificus hemolysin *vvhA*	*vvhA*-AP	GAGCTGTCACGGCAGTTGGAACCA	*V. vulnificus* ATCC 27562

Table 3: Summary for *Vibrio parahaemolyticus* and *Vibrio vulnificus* detection employing direct plating and DNA colony hybridization.

[a] Stations with more than three sampling events are presented as: maximum (median of positive samples). If fewer than three sampling events were conducted at a given location, all values are shown. Brackets represent proportion of positive samples relative to total number of samples. All assays were performed in duplicate and are presented as the average, prior to statistical evaluation.

[b] For samples collected between 2009-2012, values shown as CFU/g; For samples collected between 2019-2022, values shown as CFU/100 µl.

Table 3: Summary for *Vibrio parahaemolyticus* and *Vibrio vulnificus* detection employing direct plating and DNA colony hybridization.

Collection period	Site	Water (CFU/ml)[a]				Oyster[a,b]				Sediment (CFU/g)[a]			
		vvha	*tlh*	*trh*	*tdh*	*vvha*	*tlh*	*trh*	*tdh*	*vvha*	*tlh*	*trh*	*tdh*
2009-2012[a]	Chester River (CR)	80 (3.5) [0.69]	31.5 (1.5) [0.46]	1.5 (<1) [0.07]	26 (1.5) [0.12]	1.73×10^{4} (515) [0.71]	7.4×10^{3} (72.5) [0.57]	705 (25) [0.27]	80 (12.5) [0.18]	1.12×10^{7} (1.87×10^{3}) [0.74]	1.03×10^{7} (465) [0.65]	550 (110) [0.30]	270 (75) [0.29]
	Tangier Sound (TS)	145 (6) [0.70]	52 (1.5) [0.69]	39 (2) [0.19]	33.5 (1) [0.17]	2.43×10^{4} (440) [0.81]	1.56×10^{4} (535) [0.74]	280 (50) [0.35]	650 (40) [0.22]	1.24×10^{4} (105) [0.70]	2.00×10^{5} (45) [0.62]	870 (5) [0.26]	600 (60) [0.24]
2019-2022[a]	Broad Creek (BC)	119; 15.5 [1]	58.5; 36.25 [1]			310; 225 [1]	770; 55 [1]						
	Chester River (CR)	34.5 [1]	12 [1]			175 [1]	70 [1]						
	Choptank River (CHR)	82 (7) [0.5]	63.5 (8.5) [0.94]			610 (185) [0.69]	925 (70) [0.94]						
	Cox Creek (COX)	111 (27) [1]	27.5 (14.5) [1]			810 (402.5) [0.83]	1.79×10^{3} (525) [1]						
	Lower Patuxent River (LPA)	50.5 (2.5) [0.71]	26 (17.5) [1]			1.03×10^{3} (265) [0.71]	770 (225) [0.86]						
	Upper Patuxent River (UPA)	53.5 (5) [0.75]	195 (13.75) [1]			3.28×10^{4} (95) [0.75]	1.28×10^{4} (172.5) [0.94]						
	Miles River (MR)	3 [1]	<1 [1]			105 [1]	55 [1]						
	Sandy Point (SP)	7 [1]	4 [1]			45 [1]	90 [1]						
	South River (SR)	31 [1]	9 [1]			245 [1]	280 [1]						
	St. Mary's River (SMR)	63.5 (10.75) [1]	17 (14) [0.75]			3145 (80) [1]	640 (45) [0.75]						
	Tangier Sound (TS)	164.5 (12.75) [0.67]	47.5 (11.75) [0.93]			960 (430) [0.75]	2.78×10^{3} (425) [0.75]						
	Wicomico River (WR)	115.5 (6.75) [0.67]	32 (7.25) [0.93]			965 (165) [0.71]	76 (37.5) [1]						

Figures

Figure 8: Map of sampling locations in Chesapeake Bay.

Map shows eastern seaboard of the United States. Inset map shows sampling locations, indicated by red diamonds. Scale bar corresponds to distance shown using World Map Data from Natural Earth (Massicotte and South, 2023).

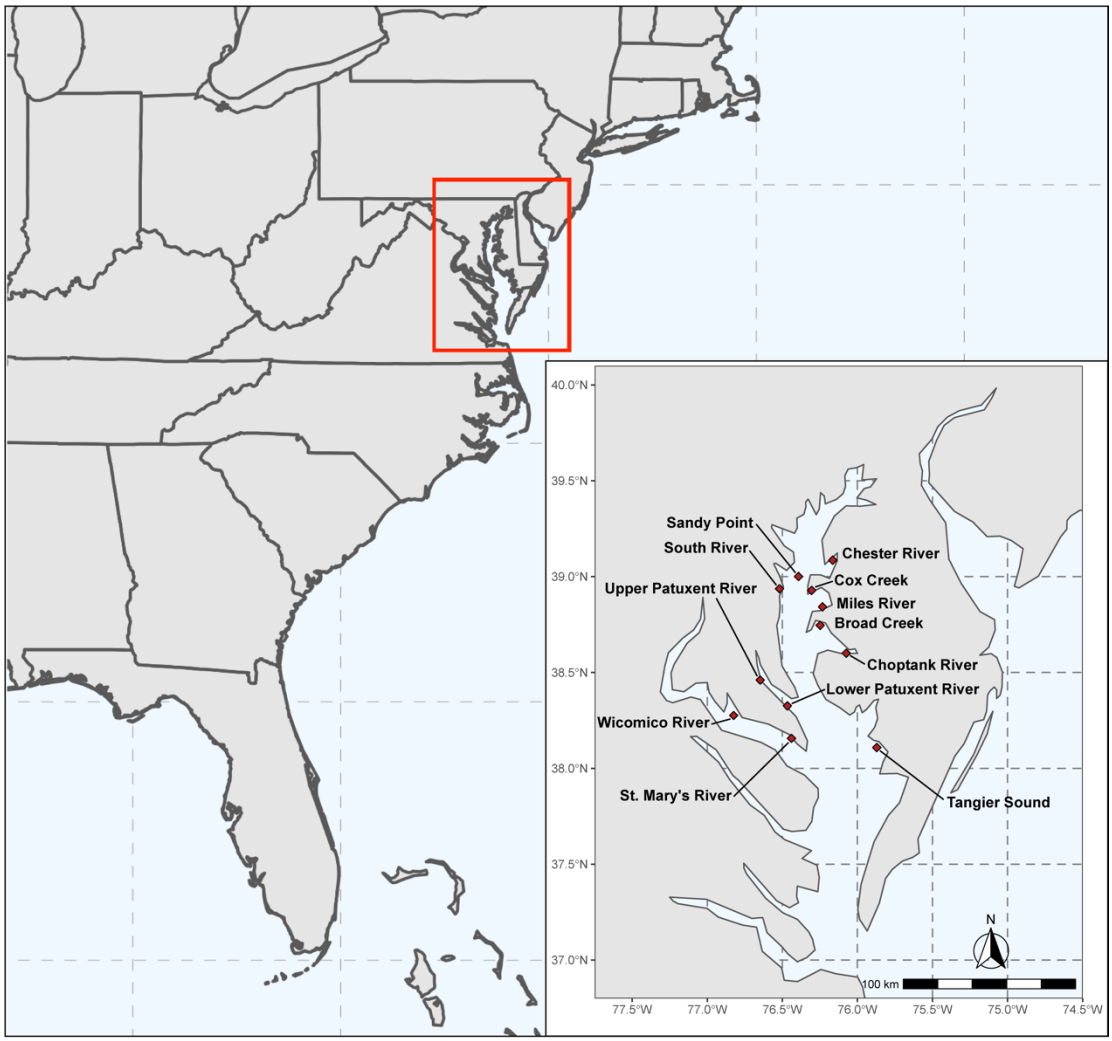

Figure 8: Map of sampling locations in Chesapeake Bay.

Figure 9: Box plots of environmental parameters.

Boxes summarize distribution by indication of interquartile range (IQR), with median shown as the center bar of each group. Whiskers represent 1.5 times the IQR. Additional circles indicate outlier values. Stations abbreviated as Chester River (CR), Tangier Sound (TS), Choptank River (CHR), Upper Patuxent River (UPR), and Wicomico River (WR). Shown are environmental parameters for samples collected between (A) 2009-2012 and (B) 2019-2022.

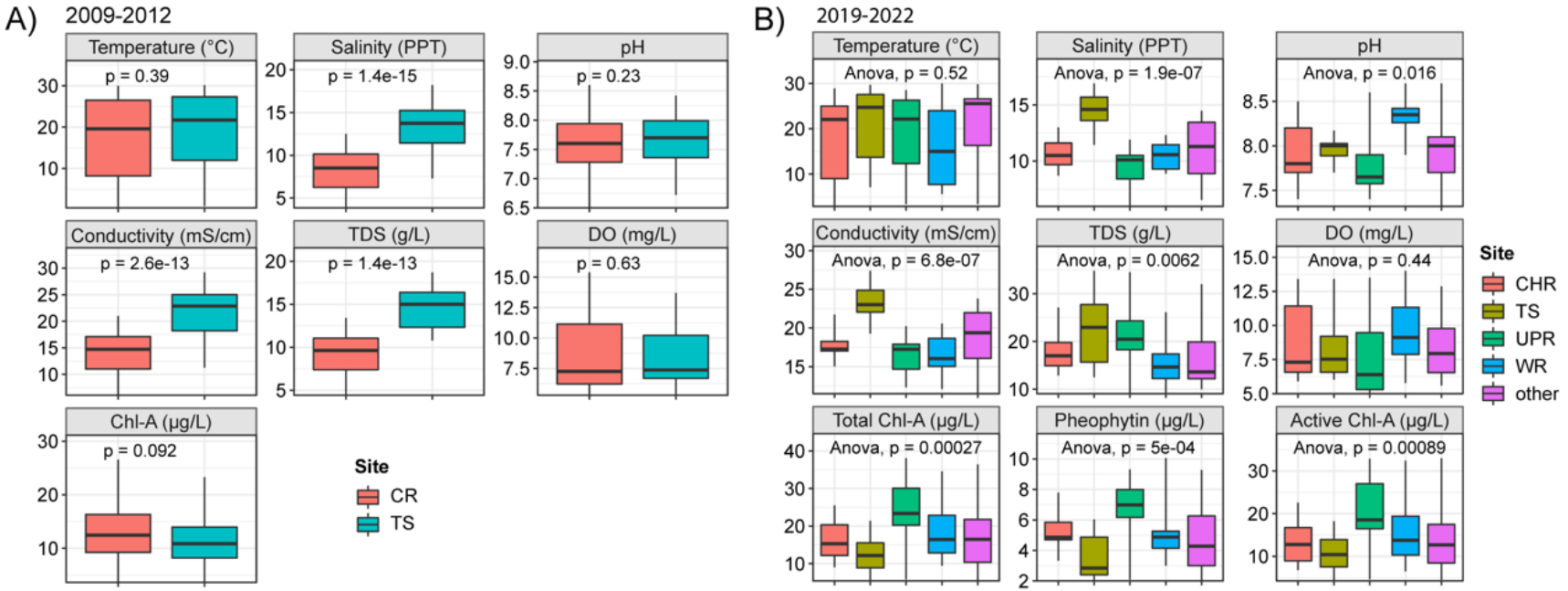

Figure 9: Box plots of environmental parameters.

Figure 10: Box plots of direct plating and DNA colony hybridization (DPCH) results.

Boxes summarize distribution by indication of interquartile range (IQR), with median shown as the center bar of each group. Whiskers represent 1.5 times the IQR. Additional circles indicate outlier values. "log1p" represents log(x+1). Stations abbreviated as Chester River (CR), Tangier Sound (TS), Choptank River (CHR), Upper Patuxent River (UPR), and Wicomico River (WR). Shown are DPCH results for samples collected between (A) 2009-2012 and (B) 2009-2012, along with comparison of collection periods at (C) all stations, (D) all stations by season, (E) Tangier Sound, and (F) Tangier Sound by season.

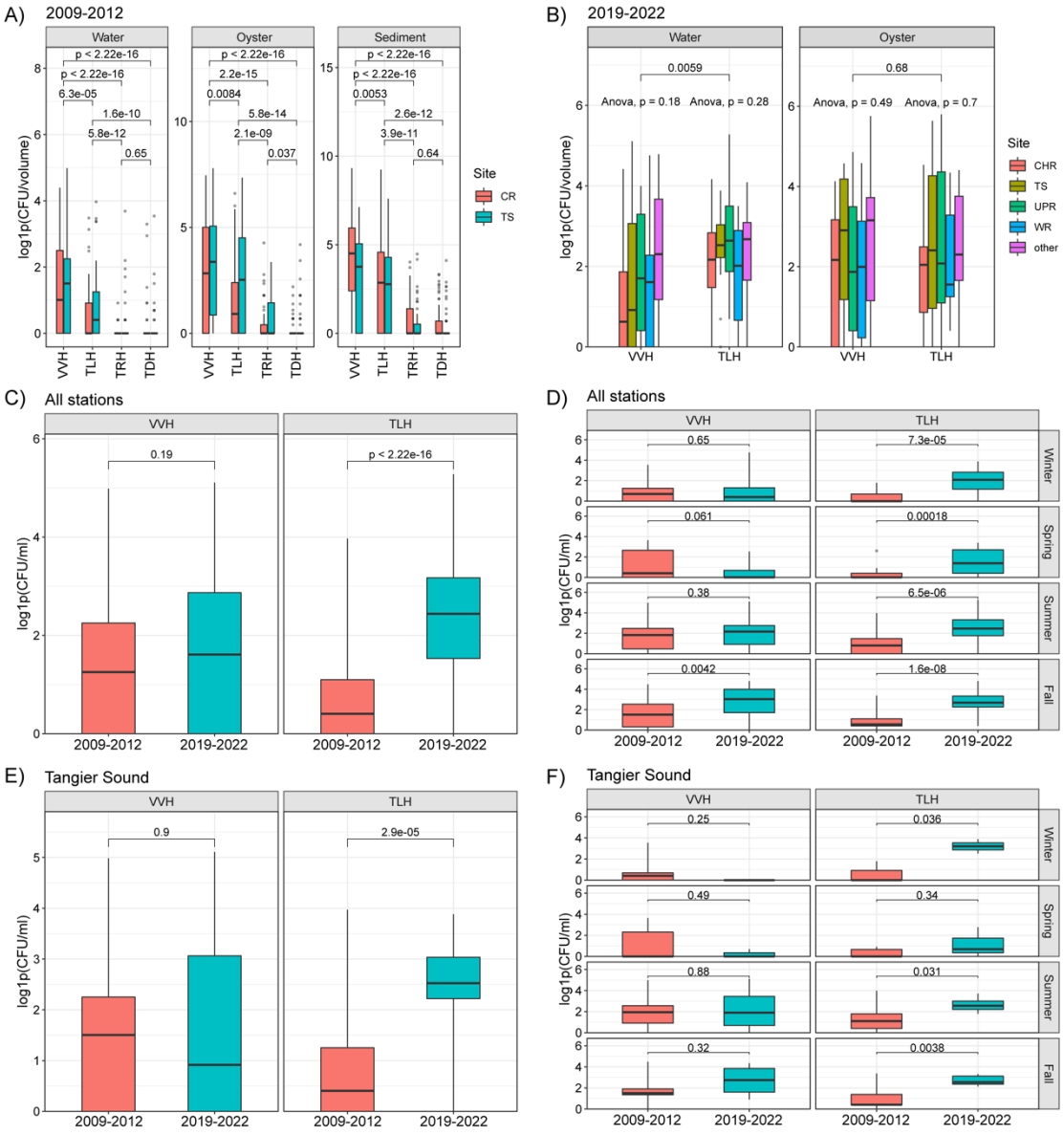

Figure 10: Box plots of direct plating and DNA colony hybridization (DPCH) results

Figure 11: Seasonal association of direct plating and DNA colony hybridization (DPCH) results.

Bar plots show DPCH results on the right axis and temperature values (shown by black lines) on the left. Scatterplots include linear regression, with 95% confidence intervals represented by shaded regions. Correlations among axis variables were respectively generated using Kendall's tau method. "log1p" represents log(x+1). Stations abbreviated as Chester River (CR), Tangier Sound (TS), Choptank River (CHR), Upper Patuxent River (UPR), and Wicomico River (WR). Shown are DPCH results and temperature for samples collected between (A-B) 2009-2012 and (C-D) 2019-2022.

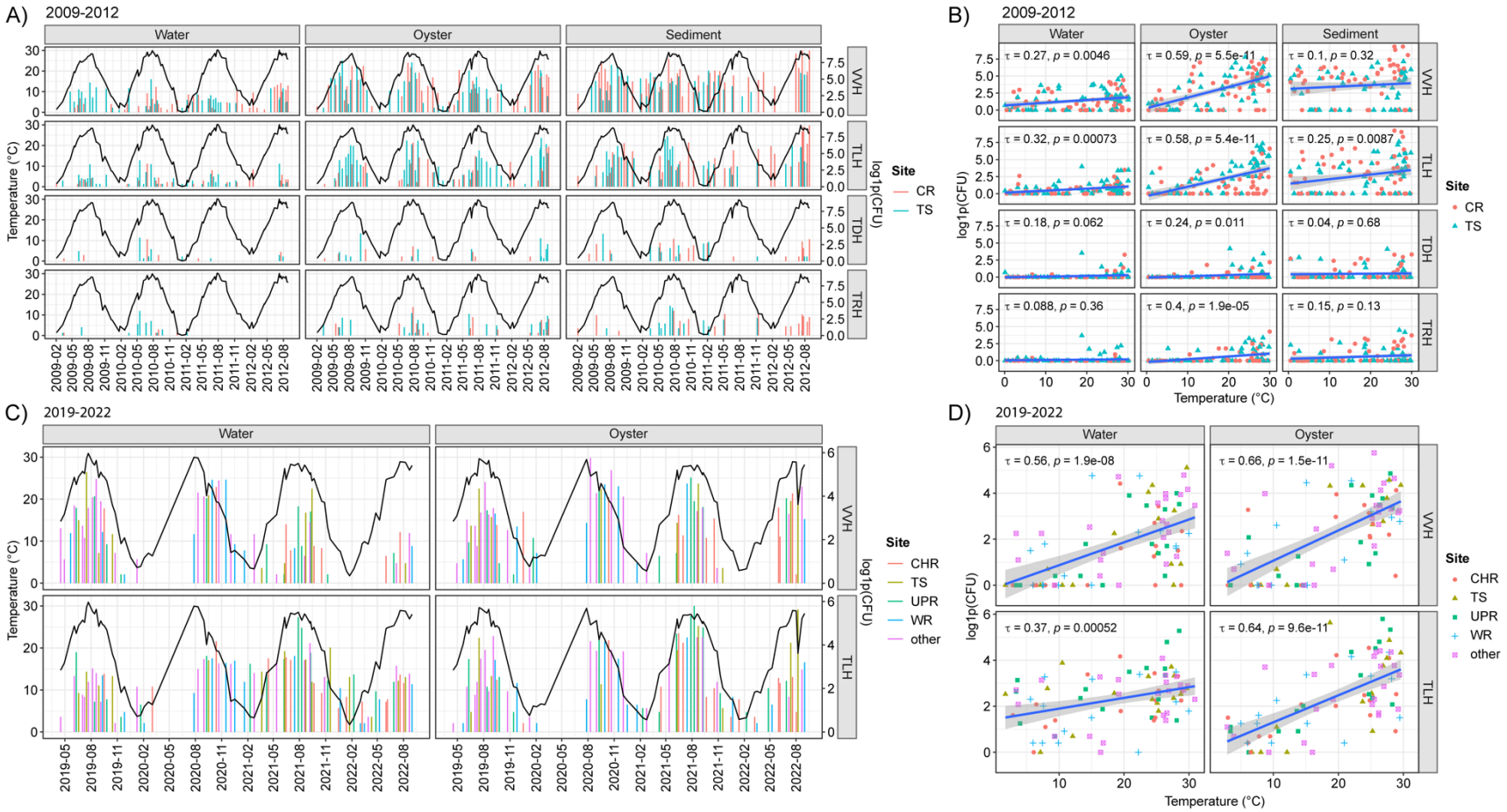

Figure 11: Seasonal association of direct plating and DNA colony hybridization (DPCH) results.

Figure 12: Association of temperature, salinity, and total chlorophyll-a with occurrence of Vibrio vulnificus and Vibrio parahaemolyticus.

Three-dimensional scatterplots are shown for temperature, salinity, and total chlorophyll-a, whereby marker size and color are indicative of detection and abundance of **(A)** *vvhA* and **(B)** *tlh*. Two-dimensional scatterplots show the association of environmental parameters with direct plating and DNA colony hybridization results, and include linear regression, with 95% confidence intervals represented by shaded regions. Correlations among axis variables for temperature and DPCH results were respectively generated using Kendall's tau method. Nonparametric regression model, i.e., locally weighted smoothing (LOESS), was used to visualize the relationship of DPCH results with environmental parameters lacking linear correlation, i.e., salinity and total chlorophyll-a. "log1p" represents log(x+1). Bar plots represent the percent of positive samples above defined hybridization thresholds, following the binning of environmental parameter values. Two-dimensional scatter plots and bar plots are shown for **(C-D)** temperature, **(E-F)** salinity, and **(G-H)** total chlorophyll-a.

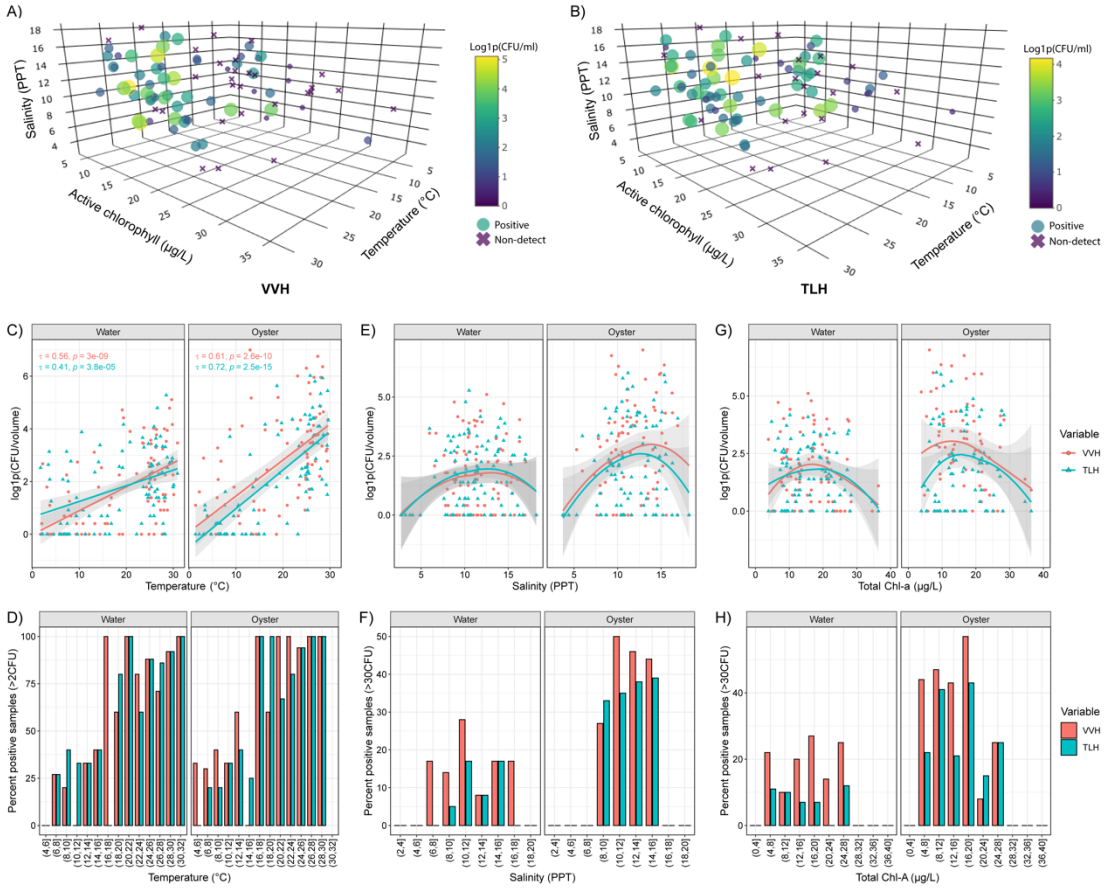

Figure 12: Association of temperature, salinity, and total chlorophyll-a with occurrence of *Vibrio vulnificus* and *Vibrio parahaemolyticus*.

Figure 13: Environmental parameters associated with moderate genetic marker density.

Scatterplots show normalized kernel density of each genetic marker detected above the threshold of 10 CFU per hybridization, with respect to environmental parameter values. "log1p" represents log(x+1). Stations abbreviated as Chester River (CR), Tangier Sound (TS), Choptank River (CHR), Upper Patuxent River (UPR), and Wicomico River (WR). Panels represent samples collected between (left) 2009-2012 and (center) 2019-2022 and (right) pooled data from both collection periods. Environmental parameters are shown as **(A)** temperature, **(B)** salinity, **(C)** total chlorophyll-a, **(D)** pH, and **(E)** DO.

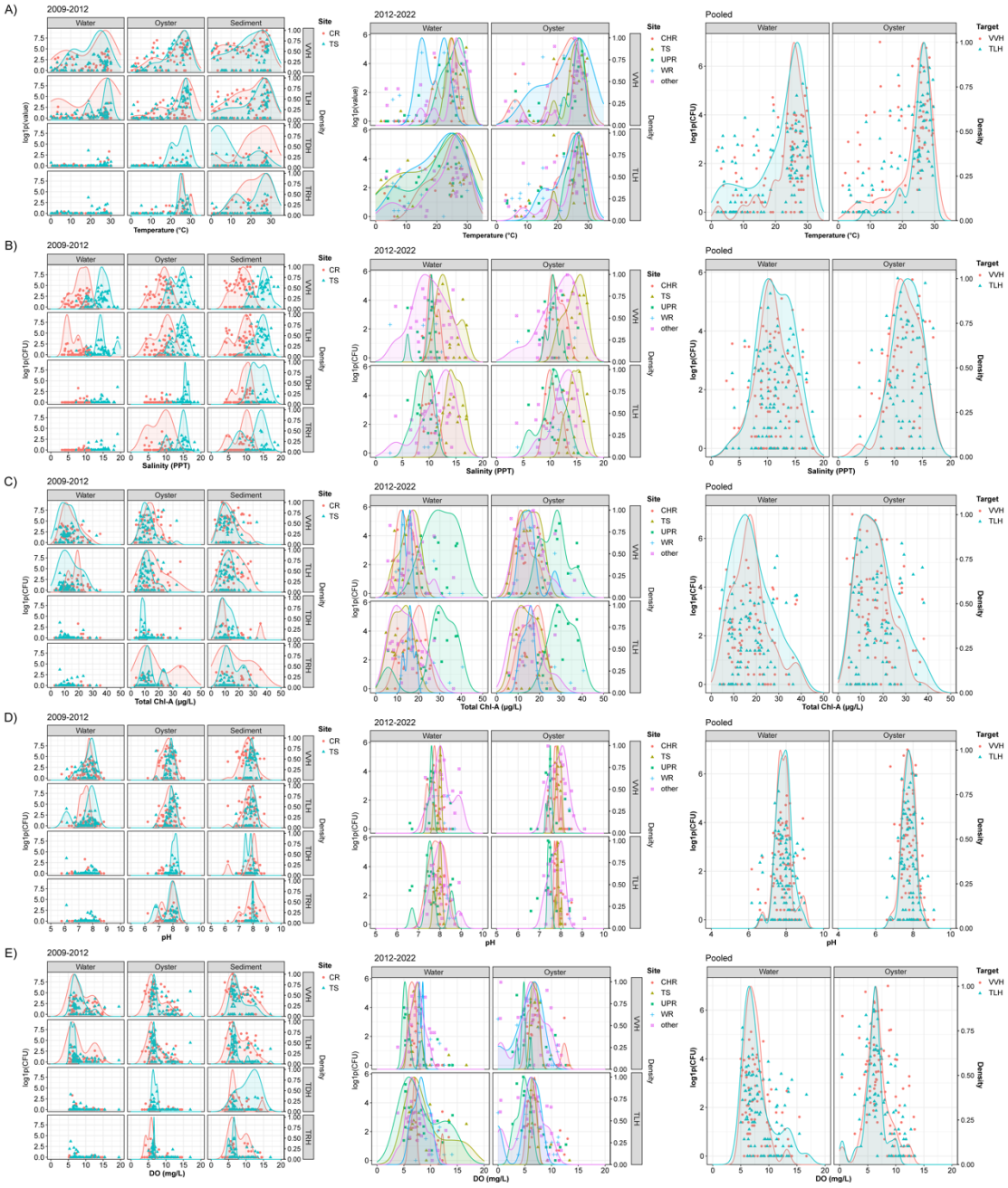

Figure 13: Environmental parameters associated with moderate genetic marker density.

Chapter 4: Genomic diversity of *Vibrio spp.* and metagenomic analysis of pathogens in Florida Gulf Coastal waters following Hurricane Ian

Kyle D. Brumfield, Moiz Usmani, Sanneri Santiago, Komalpreet Singh, Mayank Gangwar, Nur A. Hasan, Michael Netherland Jr., Katherine Deliz Quiñones, Christine Angelini, Norman L. Beatty, Anwar Huq, Antarpreet S. Jutla, and Rita R. Colwell. 2023. *mBio*, under review.

Abstract

Changing climatic conditions influence parameters associated with growth of pathogenic *Vibrio spp.* in the aquatic environment. Post Hurricane Ian in September 2022, a category four storm that made landfall in Florida, the State reported a sharp increase in the number of confirmed *Vibrio spp.* infections. During October 2022, water and oyster samples were collected from three stations in Lee County in an area significantly impacted by Hurricane Ian and where the largest number of vibriosis infections and deaths associated with Ian were reported. *Vibrio spp.* were isolated and whole genome sequencing and phylogenetic analysis were done, with the focus on *Vibrio parahaemolyticus* and *Vibrio vulnificus* to provide genetic insight into pathogenic strains circulating in the environment. Metagenomic analysis of water samples provided insight with respect to human health-related factors, notably detection of approximately 12 pathogenic *Vibrio spp.*, virulence and antibiotic resistance genes, and mobile genetic elements, including the SXT/R391 family of integrative conjugative elements. Environmental parameters were monitored as part of a long-term time series analysis done using satellite remote sensing. In addition to anomalous rainfall and storm surge, changes in sea surface temperature and chlorophyll concentration during and after Ian favored growth of *Vibrio spp.* In conclusion, genetic analysis coupled with environmental data and remote sensing provides useful public health information, hence, constitute a valuable tool to proactively detect and characterize environmental pathogens, notably vibrios. These data can aid development of early warning systems by yielding a larger source of information for public health in an era of climate change.

Importance

Evidence suggests warming temperatures are associated with spread of potentially pathogenic *Vibrio spp.* and emergence of human disease globally. Following Hurricane Ian, the State of Florida reported an increase in the number of reported *Vibrio spp.* infections and deaths linked to the storm. Hence, monitoring of pathogens, including vibrios, and environmental parameters influencing their occurrence is critical to public health. Here, DNA sequencing was used to investigate the genomic diversity of *Vibrio parahaemolyticus* and *Vibrio vulnificus*, both potential human pathogens, in FL coastal waters post Hurricane Ian. Additionally, the microbial community of water samples was profiled to detect presence of *Vibrio spp.* and other microorganisms (bacteria, fungi, protists, and viruses) present in the samples. Long-term environmental data analysis showed changes in environmental parameters during and after Ian were optimal for growth of *Vibrio spp.* and related pathogens. Collectively, results will be used to develop predictive risk models during climate change.

Introduction

Climate change is reported to be associated with increased frequency of anomalous weather events, notably heatwaves and severe precipitation which can significantly impact the marine environment, especially along the coast (Ummenhofer and Meehl, 2017). Environmental factors, such as temperature, salinity, chlorophyll, and sea surface height, can influence incidence and transmission of pathogenic agents by impacting proliferation, dissemination, and virulence of microorganisms in the

environment. Climatic conditions also have the potential to influence human behavior by a more frequent contact with pathogens through water-related activities during periods of warming (Baker-Austin et al., 2016; Mora et al., 2022). Hence, climate change associated with shifts in the geographical range of microbial species, has the potential to influence emergence and re-emergence of disease (El-Sayed and Kamel, 2020). A dramatic example is the significant geographic expansion of pathogenic *Vibrio spp.*, a finding corroborated by their impact on public health, namely increased numbers of reported vibriosis infections in humans, as well as aquaculture loss (Colwell, 1996; Vezzulli et al., 2012, 2015; Baker-Austin et al., 2017; Froelich and Daines, 2020; Brumfield et al., 2021b, 2023; Vezzulli, 2022; Archer et al., 2023).

Vibrio spp. are autochthonous to the aquatic environment, notably in high concentrations along the coast and their incidence is strongly influenced by environmental conditions (Brumfield et al., 2021b). *Vibrio spp.* play an important role in the degradation of polymeric substances, such as chitin, and in other significant biogeochemical processes (Joseph et al., 1982; Le Roux and Blokesch, 2018; Brumfield et al., 2021b). *Vibrio spp*. occur in aquatic ecosystems as free-living single cells or in aggregates, e.g., within biofilms in high numbers and attached to various abiotic substrates. They have been shown to be commensals and symbionts of aquatic invertebrates, such as crustaceans, zooplankton, and bivalves, all of which are known to host these bacteria (Lovelace et al., 1968; Krantz et al., 1969; Sochard et al., 1979; Huq et al., 1983; Colwell, 2006; Grim et al., 2008; Zo et al., 2009; Brumfield et al., 2021b). Copepods, zooplankton comprising a significant component of aquatic fauna, are a major host of *Vibrio spp*. and considered a vector of *V*. *cholerae* (Colwell et al.,

1977; Sochard et al., 1979; Huq et al., 1983; Colwell, 1996; Rawlings et al., 2007). *Vibrio spp*. concentrate in filter-feeding shellfish, especially oysters, which are often consumed raw thereby exposing people to large doses of potentially pathogenic agents (Lovelace et al., 1968; Berk and Colwell, 1981; Froelich and Noble, 2016). Furthermore, *Vibrio spp.* with bioluminescent properties are important symbionts of marine organisms, including *Vibrio fischeri* which colonizes the light-emitting organ of the Hawaiian bobtail squid, *Euprymna scolopes* (Ruby et al., 2005), and luminescent *Vibrio cholerae* and *Vibrio vulnificus* strains reported to be associated with copepods and related crustacean species (Sochard et al., 1979; Oliver et al., 1986; Colwell, 2006; Grim et al., 2008; Zo et al., 2009).

Several species of the genus *Vibrio* cause severe infection in humans, primarily related to consumption of contaminated seafood or exposure to water containing the pathogens (Waldor et al., 2018). *V. cholerae* is well-documented as the etiological agent of cholera, the seventh cholera pandemic of which is in progress and continues to plague the modern world, notably when climate/weather processes, microbiological parameters, and sociological determinants intersect with population vulnerabilities and loss of water, sanitation, and hygiene (WASH) infrastructure (Usmani et al., 2021, 2022). In addition to *V. cholerae*, *Vibrio parahaemolyticus* and *V. vulnificus* have proven historically significant (Waldor et al., 2018; Brumfield et al., 2021b). *V. parahaemolyticus*, first described by Professor Fujino during a 1950 shirasu food poisoning outbreak in Japan (Fujino, 1951), is a major cause of seafood-derived foodborne gastroenteritis in the United States (CDC, 2019b). *V. vulnificus*, first reported in the U.S. in 1976 (Hollis et al., 1976), is also a common cause of foodborne

illness and can cause severe extraintestinal infections, including necrotizing fasciitis and septicemia (Baker-Austin and Oliver, 2018). The bacterium has a fatality rate that is one of the highest of any waterborne pathogen, i.e., greater than 50% for primary septicemia. It also is responsible for *ca*. 95% of all waterborne and seafood-derived foodborne deaths in the U.S. (Bross et al., 2007; Baker-Austin and Oliver, 2018). In the U.S., *Vibrio spp*. are estimated to cause *ca.* 80,000 illnesses and hundreds of deaths annually, of which *ca*. 65% are foodborne (Newton et al., 2012; CDC, 2019b). According to the Centers for Disease Control and Prevention FoodNet, which has sites in 10 states covering 15% of the U.S. population, there is indication of a long-term increase in reported vibriosis between 1996 and 2019 (Brumfield et al., 2021b; CDC, 2021). In the Eastern U.S. between 1988 and 2018, *V. vulnificus* wound infections increased eightfold, i.e., from 10 to 80 cases per annum, and the northern case limit has shifted northwards geographically at *ca.* 48 km per annum (Archer et al., 2023). Environmental factors linked to climate change have the potential to enhance incidence and genomic diversity of these pathogens in the environment, especially in coastal communities, and this trend of increased vibriosis is expected to continue (Colwell, 1996; Lobitz et al., 2000).

A key issue related to risk of vibriosis outbreaks is the high population density and economic activity along coastal areas and the U.S. eastern seaboard is a prime example. In fact, *ca.* 40% of the total U.S. population lives in coastal communities, with nearly half in an elevated health risk category, e.g., elderly and/or low-income households (NOAA, 2021e). The State of Florida (FL) has the longest coastline of any of the 48 contiguous states in the U.S. and *ca.* 22% of the population of FL is over the

age of 65 (United States Census Bureau, 2020). Furthermore, many FL residents rely on protein from seafood, especially aquaculture. While FL does have distinct seasons, i.e., warmest waters in the summer and early fall, the climate is generally warm year-round which allows for increased duration and frequency of recreational and occupational activities, along with an extended seasonality of *Vibrio spp*. abundance.

Pathogenic *Vibrio spp*. that cause cholera and vibriosis have been reportable diseases in FL since 1981 (CDC, 2019b). Between 2004 and 2021, over 3,000 vibriosis cases and 205 deaths were reported in FL (Florida Department of Health, 2023). *V. parahaemolyticus* and *V. vulnificus* accounted for over *ca.* 40% of the vibriosis cases and have been of particular interest to the State because of their potential association with locally harvested shellfish (Florida Department of Health, 2023). Between 2000 and 2021, there has been an average of *ca.* 34 (ranging from 13 to 55) *V. parahaemolyticus* and *ca.* 35 (ranging from 15 to 51) *V. vulnificus* cases reported each year, with most infections occurring between May and October (Florida Department of Health, 2023). However, it is worth noting that *V. vulnificus* infections accounted for *ca*. 78% of the confirmed deaths associated with vibriosis during this period.

On September 28, 2022, Hurricane Ian, a destructive category 4 storm made landfall in southwestern FL, bringing anomalously heavy rainfall, high winds (*ca.* 240 km/hr), and dangerous surf to the area, resulting in a rise in seawater levels, flash flooding, and mass destruction of infrastructure around coastal areas. In the days following Hurricane Ian, many FL residents were impacted by sustained floodwaters, a problematic situation given that pathogenic *Vibrio spp.*, namely *V. vulnificus* and *V. parahaemolyticus*, thrive in warm and low-salinity waters (Singleton et al., 1982;

Brumfield et al., 2021b). According to the FL Department of Health (FDH), there have been 74 reported cases of *V. vulnificus* infections, with 17 confirmed deaths in 2022, nearly double the usual number for that time of the year (Florida Department of Health, 2023). Hence, routine monitoring and predictive intelligence models warning decision makers and individuals when risk of infection of *Vibrio spp.* (and other pathogens) is high are essential to safeguarding public health.

Here, we describe whole genome sequencing (WGS) analysis to characterize pathogenic *Vibrio spp.* isolates (*V. vulnificus* and *V. parahaemolyticus*) recovered from water and oyster samples along the FL Gulf Coast (FGC) following Hurricane Ian. We also profiled water samples, employing shotgun metagenomic sequencing (SMS), for detection of *Vibrio spp.* and related pathogens, along with their virulence factors (VFs), antimicrobial resistance genes (ARGs), and mobile genetic elements (MGEs). These results provide useful information for future investigations, both for inhabitants of the FGC and globally, to evaluate incidence of pathogenic *Vibrio* populations relative to climate change over time.

Materials and methods

Environmental surveillance

The track, timeline, and windspeed data for Hurricane Ian were retrieved from the International Best Track Archive for Stewardship (IBTrACS) project (Knapp et al., 2010). Chlorophyll and sea surface temperature (SST) data were recovered for three locations, Cutthroat Clams (CC; 26.569889, -82.135616), Clam Key (CK; 26.546667,

-82.079722), and White Booth Seafood (WBS; 26.36675, -82.03819), from the Moderate Resolution Imaging Spectroradiometer (MODIS) carried on the Terra satellite (NASA, 2023). MODIS data products are available at 4 km × 4 km spatial resolution. A time series analysis was performed to determine the potential impact of anomalous weather events before (July 24, 2022 – September 23, 2022), during (September 24, 2022 – September 30, 2022), and after (October 1, 2022 – November 30, 2022) Hurricane Ian. Anomalous percentages were calculated for sea surface temperature (SST) and chlorophyll using the following equation: $Percent\ change = \frac{x - LT\ avg}{LT\ avg} \times 100$ whereby "x" represents a given time period, i.e., before, during, or after, and "$LT\ avg$" represents mean of long-term data over 10 years for the same spatial resolution. Hence, anomaly percentage results in a positive or negative value, suggesting deviation for a given variable from the long-term mean. That is, negative anomalous percentages imply that a given variable decreased during 2022 with respect to the previous 10 years and positive values imply an increase.

Vibrio spp. infections

Number of confirmed *Vibrio spp.* infections and associated deaths reported by the State of FL was retrieved from FDH (Florida Department of Health, 2023) and are presented as total number of *V. vulnificus* infections and deaths at the county level for 2022.

Site description and sample collection

Methods employed for sample collection and processing have previously been described in detail (Brumfield et al., 2023). A summary of methods relative to this study is provided here, whereby samples were collected from three stations (CC, CK, and WBS) on October 26, 2022. At each station, water (7 L) was collected and stored in clean 3.7 L bottles. The bottles were rinsed three times with sample water from each site prior to collection. Roughly 12 oysters were collected from CK and WBS and stored in clean double zipper freezer bags. Oysters were not able to be collected from CC. All samples were transported to the laboratory in a cooler with ice, ensuring samples did not come in direct contact with the ice packs and stored at 4 °C until further processing. During each sampling event, water temperature, pH, optical dissolved oxygen (DO), and salinity were measured 0.3 m below the surface and 0.3 m above the bottom using a handheld water probe (Eureka, Austin, TX).

Sample processing

Samples were treated following methods outlined in the Bacteriological Analytical Manual for food sampling/preparation of sample homogenate (Andrews and Hammack, 2019) and *Vibrio* (Kaysner et al., 2019), as described previously (Brumfield et al., 2023). Notably, water samples were shaken vigorously 25 times in 30 cm arc in 7 s. From each station, 250 ml of water was concentrated four times (totaling 1 L) using syringe filtration with four 0.22 μm pore size Sterivex Filter Units (Millipore Sigma, MO). Oysters were rinsed and scrubbed under deionized water to remove debris from the shell and opened using a sterile shucking knife. Oyster tissue in equal part

phosphate buffered saline (PBS; pH 7.4) was homogenized in a sterile blender for 90 s. Filter units and homogenized oyster tissue (500 µl) were stored at -80 °C in DNA/RNA Shield Stabilization Solution (ZymoResearch, CA). Following homogenization, subsequent enrichment steps were done within 15 min.

Alkaline peptone water enrichment

Samples were inoculated at various concentrations using alkaline peptone water (APW; 10% peptone, 1% NaCl [pH 8.5]). Briefly, three volumes of unfiltered water (1L, 100 ml, and 10 ml) and homogenized oyster tissue (10 ml, 1 ml, and 100 µl) were resuspended in 10 × APW, each in triplicate, and incubated overnight at 37 °C with moderate aeration (orbit diameter 2.5 cm × 30 rpm). The following morning, an aliquot (500 µl) of each APW enriched sample was stored at -80 °C in DNA/RNA Shield Stabilization Solution (ZymoResarch, CA).

Isolation of *Vibrio spp*.

A loopful of pellicle from each APW enriched sample was subcultured on selective media, including *Vibrio* specific chromogenic agar (CHROMagar, Paris, France), thiosulfate citrate bile-salts sucrose (TCBS) agar (Oxoid, Ontario, Canada), and M190 *Vibrio vulnificus* agar (FDA, 2017), and incubated overnight at 37 °C. Presumptive *Vibrio spp.* colonies were purified on Luria-Bertani agar (Difco, NY) and maintained under standard bacteriological conditions for *Vibrio spp*. (Brumfield et al.,

2017, 2018b). Confirmation and identification of *Vibrio spp*. was done using established molecular assays, as outlined (*vide infra*).

Preparation of genomic DNA

Genomic DNA was prepared from pure cultures grown under standard conditions in Luria-Bertani broth with aeration at 37 °C overnight (16 hr), using the ZymoBIOMICS DNA Miniprep Kit (ZymoResearch, CA). DNA extracts were further purified using DNA Clean and Concentrator Kit (ZymoResearch, CA), with a final elution volume of 80 µl.

Polymerase Chain Reaction

PCR methods have previously been established for detection of members of the genus *Vibrio* and species-specific markers. Amplified products were fractionated by electrophoresis through 1.5 % (w/v) agarose gel along with a 100 bp molecular weight marker (HyperLadder™, BioLine, Swedesboro, NJ), and visualized using SafeGLO Pre-Stain (BioLInk, San Francisco, CA). For quality control, a no template control (NTC) consisting of nuclease free water and positive/negative controls were included with each reaction. Primers and respective controls used in this study are detailed in **Table 4**.

Next generation sequencing

Samples analyzed by next generation sequencing included WGS of purified culture isolates identified by PCR as *V. vulnificus* (Vv-*toxR*$^+$ and *vvhA*$^+$; n = 12; **Table 5**) or *V. parahaemolyticus* (Vp-*toxR*$^+$ and *tlh*$^+$; n = 9; **Table 6**) and SMS of water samples (**Table 7**) from each location, both filter-concentrated (n = 3) and APW enriched (analyzed in duplicate; n = 6). Double stranded DNA concentration was measured using the Qubit 3.0 fluorometer (ThermoFisher, MA). Sequencing libraries were prepared using NEBNext Ultra II FS Library Prep Kit for Illumina (New England Biolabs, MA) sequencing, using the HiSeq 4000 System (Illumina, CA) with 150 bp paired end reads. WGS was performed targeting >200 × genome coverage (>10 M paired reads) and shotgun metagenomic sequencing was done targeting >30 M paired reads. For quality control, a NTC and a sequencing standard, i.e., ZymoBIOMICS Community Standard (ZymoResearch, CA), were included in the sequencing run. Read quality was confirmed using FastQC (Andrews, 2019). Adapter sequences were removed and low-quality bases were trimmed using Trimmomatic (Bolger et al., 2014). Processed reads were further analyzed for comparative genomics and metagenomic community profiling, as described below.

Comparative genomics

Processed sequencing read libraries of purified culture isolates were assembled into contigs using the St. Petersburg genome assembler (SPAdes) (Kulikov et al., 2012), with options "--careful" and "--cov-cutoff auto" to reduce the number of mis-

assemblies and remove low-coverage contigs. Small contigs (< 500 bp) were discarded. Assembly statistics, completeness, and genome quality were assessed using the Quality Assessment Tool for Genome Assemblies (QUAST) (Gurevich et al., 2013) and CheckM (Parks et al., 2015). Draft genome assemblies were annotated using the Rapid Annotation Using Subsystem Technology (RAST) tool kit (Brettin et al., 2015), and characterized for carriage of antimicrobial drug-, biocide-, and metal-resistance determinants employing the Microbial Ecology Group Antimicrobial Resistance (MEGARes) database (Doster et al., 2020), virulence factors via the Virulence Factor Database (VFDB) (Liu et al., 2018), and MGE via the Mobile Genetic Element database (MGEdb) (Johansson, 2023), using ABRicate (Seemann, 2020) with identity and coverage thresholds set to 60%. Multilocus sequence types (MLST) were predicted using the MLST software program (Seemann, 2022) with the PubMLST database (Jolley et al., 2018). Identified MLST profiles were submitted to the Public Databases for Molecular Typing and Microbial Gene Diversity (pubMLST) (Jolley et al., 2018). Isolates identified as *V. parahaemolyticus* were subjected to serotyping by profiling serogroup-specific genes based on WGS data, using VPsero (Bian et al., 2021).

Vibrio spp. phylogenetics

To evaluate genomic relatedness of 21 purified culture isolates of this study with known *Vibrionaceae* strains, draft genome assemblies were compared with 77 representative *Vibrio spp.* genomes with established taxonomic lineages, as defined previously (Jiang et al., 2022). *Shewanella oneidensis* MR-1 (BV-BRC genome ID 211586.12) was used as an outgroup to root the tree. Phylogenetic trees were built using

tools from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) (Brettin et al., 2015). Briefly, Codon Tree method was used to select up to 1,000 genes from cross-genus protein families (PGfams), allowing for two gene duplications within a single genome and two genomes missing a member of a particular homology group. Coding DNA (amino acid sequences) from selected genes was analyzed using the Randomized Axelerated Maximum Likelihood (RAxML) algorithm with automatic model selection (Stamatakis, 2014) to identify the best model for protein alignment. Resulting codon trees were viewed through the Phylogenetic Tree Viewer in the BV-BRC software suite (Brettin et al., 2015).

Similar methods were used to further classify isolates from FL within existing *V. vulnificus* and *V. parahaemolyticus* clonal populations. Briefly, 12 isolates identified as *V. vulnificus* were analyzed along with 88 representative strains (López-Pérez et al., 2019) using the BV-BRC Codon Tree method, as mentioned previously, but up to five gene duplications and genome deletions were allowed. Similarly, the nine isolates identified as *V. parahaemolyticus* were analyzed along with 91 representative strains (Miller et al., 2021), allowing for 10 gene duplications and genome deletions. Resulting codon trees were visualized using the Interactive Tree of Life (iTOL) (Letunic and Bork, 2021).

Metagenomic community profiling

Genomic sequences in public repositories can have a diverse range of genome statistics, e.g., reference size, number of contigs, assembly status (i.e., complete, chromosome, scaffold, contig), N50 values, etc., which may introduce bias towards

higher quality and complete genomes, thereby making abundance quantification unreliable. To circumvent this issue, metagenomic community profiling was done using the up-to-date bacterial core gene (UBCG) algorithm (Chalita et al., 2020) with the EzBioCloud database (Yoon et al., 2017). When extracting UBCG sequences, all references end up being represented by the same number of genes and their sequence sizes are nearly identical, making detection and abundance estimation more reliable. The 92 core genes (bacteria and archaea) currently hosted by the UBCG algorithm were extracted from the EzBioCloud database to create a core gene database for metagenomic profiling. First, the potential presence of bacterial and archaeal species was surveyed for each metagenomic sample read using Kraken2 (Wood et al., 2019) and a pre-built core gene database (Chalita et al., 2020) containing k-mers (k = 35) of reference genomes obtained from the EzBioCloud database (Yoon et al., 2017). Fungi, protists, and viral strains (full genomes) were extracted from the NCBI RefSeq database (O'Leary et al., 2016) and added to the core gene database. After acquiring a list of candidate species, a custom bowtie2 (Langmead and Salzberg, 2012) database was built from species detected during the Kraken2 (Wood et al., 2019) analysis. Sample reads were mapped against the bowtie2 database using the "--very-sensitive" option and a Phred quality score threshold of 33. SAMtools (Li et al., 2009) was used to convert and sort the resulting bam file. Coverage of the mapped reads against the bam file was obtained using BEDtools (Quinlan and Hall, 2010). To avoid false positive calls, reads mapping to a given species were quantified only if the total coverage of their core genes (bacteria and archaea) or genome (fungi, protists, and virus) was at least 25%. Species abundance was calculated using the total number of reads counted and normalized

species abundance was calculated using the total length of the respective reference sequence. Measures of alpha diversity (species richness, Shannon, and Simpson) were calculated using the EzBioCloud software platform (Yoon et al., 2017), as described previously (Brumfield et al., 2022b). Briefly, Species richness was defined using the abundance-based coverage estimator (ACE) algorithm (Chao and Lee, 1992). Shannon entropy of counts was calculated based on the description given in the Species Diversity and Richness manual (Seaby and Henderson, 2006). However, log base 2 (log_2) was used as default instead of the natural logarithm (log_e). Simpson's index was defined as "1 – *Dominance*" whereby dominance represents the probability of selecting two individuals from the same species, with replacement (Hammer, 2003).

ARG, VF, and MGE profiles were produced using separate pre-built bowtie2 (Langmead and Salzberg, 2012) reference gene databases composed of the NCBI National Database of Antibiotic Resistant Organisms (NDARO) database (NCBI, 2023), VFDB (Liu et al., 2018), or MGEdb (Johansson, 2023). Metagenomic reads were mapped against respective databases using bowtie2, as previously mentioned. For each gene detected, depth and coverage were calculated using mpileup script from the SAMtools software package (Li et al., 2009).

Data availability

Statistical analysis was done using R v.4.2 (R Core Team, 2022), EzBioCloud (Yoon et al., 2017), and BV-BRC (Brettin et al., 2015). Figures were generated using ggplot2 (Wickham, 2016), iTOL (Letunic and Bork, 2021), and Phylosmith (Smith, 2019). Sequencing data generated for all samples included in this study are deposited

in the National Center for Biotechnology Information Sequence Read Archive database under BioProject ID PRJNA978582. Accession numbers and MLST profiles for draft genome assemblies of *V. vulnificus* (**Table 5**) and *V. parahaemolyticus* (**Table 6**) and microbiome sample sequencing read libraries (**Table 7**) are provided in the supplementary information.

Results

Anomalous weather events associated with Hurricane Ian

Hurricane Ian made landfall on September 28, 2022, just south of Charlotte County near Pirate Harbor, with maximum sustained winds of 145 mph (**Figure 14A**). On October 26, 2022, samples were collected from three stations adjacent to this area (**Figure 14B**). Environmental parameters recorded at the time of sample collection are detailed in **Table 8**. Overall, water temperature, pH, and DO were similar across the three stations. However, salinity was higher at CC (30 PPT), compared to the CK and WBS stations (*ca.* 20 PPT). Following long-term time series analysis (**Figure 14C**), it was observed that all stations experienced above average SST before Hurricane Ian, varying between 0.6 and 2.26 percent, within half standard deviation compared to the previous 10 years. During Hurricane Ian (September 25-30, 2022), CC and CK experienced a decrease in SST. Following Ian (October 1 – November 30, 2022), all stations showed greater than average SST, varying between 0.7 and 2.75 percent. With respect to chlorophyll, all sampling sites indicated above average chlorophyll concentration before the hurricane, varying between 1.3 and 45 percent. Notably, the

CK station indicated significant chlorophyll variability, greater than one standard deviation compared to the previous 10 years. However, during and after Ian, these locations experience positive anomalous chlorophyll with values as high as five times the standard deviation.

V. vulnificus clinical reports

In 2022, the total number of confirmed *V. vulnificus* cases (74) and deaths (17) for the State of FL was roughly double that of 2021 (cases = 34; deaths =10) and 2020 (cases = 36; deaths = 7). The incidence of *V. vulnificus* infections increased from 2008 to 2022 (Tau = 0.71, p = 0.0028) (**Figure 14D**). The long-term increase in *V. vulnificus* infections, excluding 2022, was significant (Tau = 0.64, p = 0.013). However, the number of reported deaths did not increase significantly from 2008 to 2022 (Tau < 0.3, p >0.05). In 2022, the highest number of reported cases (28) and deaths (8) was observed in Lee County, an area significantly impacted by Hurricane Ian (**Figure 14A**). Other areas reporting *V. vulnificus* deaths were located along the coast, except for Polk County which had been in the path of Ian, reporting one death in 2022. In 2022, Escambia, Santa Rosa, and Brevard counties also reported the largest number of cases (≤5 cases).

Polymerase chain reaction detection of *Vibrio spp.*

Detection of genetic markers for the genus *Vibrio* (16S rRNA), *V. parahaemolyticus* (*tlh*, *tdh*, and *trh*), *V. vulnificus* (*vvhA*), and *V. cholerae* (*rfb*-O1, *rfb*-

O139, and *ctxA*), was done for water and oyster samples (**Table 4**). *Vibrio spp.* 16S rRNA marker was detected in all APW enriched samples (water and oyster) from each location. Successful detection of *tlh* and/or *vvhA* was possible only with APW enrichment, with PCR analysis of DNA done directly on oyster homogenate and Sterivex filters negative in all samples. The *tlh* marker was detected in enriched water samples at all locations and APW enriched oyster samples collected at CK. *V. parahaemolyticus* primary virulence factors (*tdh* and *trh*) were not detected. The *vvhA* marker was detected in CK and WBS enriched water samples but not in oyster samples. Markers coding for toxigenic *V. cholerae* (*ctxA*, *rfb*-O1, and *rfb*-O139) were not detected.

PCR characterization of *Vibrio spp.*

Following APW enrichment and subculture plating on selective media (ChromAgar, TCBS, and VVA), a total of 21 presumptive *Vibrio spp.* were isolated from the water (n = 14) and oyster samples (n = 7). PCR for the genus *Vibrio* (16S rRNA) was positive for all isolates. *V. parahaemolyticus toxR*$^{+}$ isolates (n = 9) were *tlh*$^{+}$ but negative for *tdh* and *trh*. Similarly, *V. vulnificus toxR*$^{+}$ isolates (n = 12) were *vvhA*$^{+}$. All isolates were negative for *V. cholerae toxR* and toxigenic markers (*rfb*-O1, *rfb*-O139, and *ctxA*).

Comparative genomics of *Vibrio spp.* isolates employing whole genome sequencing

The 21 presumptive *Vibrio spp.* isolates were subjected to WGS and comparative genomics. Following annotation, phylogenetic trees were built from the core genome, determined by alignment of coding sequences from selected isolates included in the analysis to evaluate genetic relatedness of the *Vibrio spp.* isolated in this study to reference genomes previously reported (**Figure 15**). A search for homologous genes returned 344 coding sequences (*ca.* 7.13×10^4 amino acids in length) shared by all genomes. The best fit protein model found by RAxML was LG. Within the *Vibrionaceae* phylogeny, each species formed coherent clusters in taxonomic subclades. Notably, the *V. cholerae* clade comprised *V. cholerae*, *Vibrio mimicus*, *Vibrio cincinnatiensis*, *Vibrio metschnikovii*, *Vibrio furnissii*, *Vibrio fluvialsis*, and *Vibrio diazotrophicus*. The 12 suspected *V. vulnificus* isolates obtained in this study formed a distinct cluster with reference *V. vulnificus* strains. Similarly, nine presumptive *V. parahaemolyticus* isolates clustered with reference *V. parahaemolyticus* strains. All *V. parahaemolyticus* strains were placed in a subclade within the *Harveyi* clade that also included *Vibrio alginolyticus*, *Vibrio diabolicus*, *Vibrio campbellii*, *Vibrio owensii*, and *Vibrio harveyi*.

Nearest neighbors of isolates from this study were identified using core-genome phylogeny in the context of major clonal linages for *V. vulnificus* (**Figure 16A**) and *V. parahaemolyticus* (**Figure 16B**). Homologous gene search returned 1,000 coding sequences, the maximum number allowed by the software program for *V. parahaemolyticus* (*ca.* 3.88×10^5 amino acids; best fit model, HIVB) and 982 for *V. vulnificus* (*ca.* 3.02×10^5 amino acids; best fit model, JTT).

Phylogenetic relatedness of *Vibrio vulnificus* isolates

The 12 *V. vulnificus* isolates (four from oyster and eight from water) were assigned to eight MLST profiles (using alleles for *glp*, *gyrB*, *mdh*, *metG*, *purM*, *dtdS*, *lysA*, *pntA*, *pyrC*, and *tnaA*), including ST621-ST628 (**Table 5**). Core genome phylogeny was used investigate genomic relatedness of *V. vulnificus* isolates from this study with established lineages, representing isolates recovered from a wide range of geographical and ecological sources (López-Pérez et al., 2019). All strains clustered into four distinct groups (C1 to C4). C1 and C2 are significantly divergent lineages including much of the clinical isolate diversity. By comparison, C3 and C4 indicate high clonality. Nine isolates were phylogenetically joined in C1, and three in C2. However, there was no distinct clustering pattern linking sample location, source of isolation, or virulence.

Phylogenetic relatedness of *Vibrio parahaemolyticus* isolates

The nine *V. parahaemolyticus* isolates (three from oyster and six from water) were assigned to eight MLST profiles (using alleles for *dnaE*, *gyrB*, *recA*, *dtdS*, *pntA*, *pyrC*, and *tnaA*), including ST16, ST564, ST896, ST3121, and ST3361-ST3364, and one isolate was assigned to clonal complex 49 (**Figure 16B** and **Table 6**). Based on the core genome phylogeny of *V. parahaemolyticus* isolates from this study and established phylogenetic and biogeographic patterns of strains from North America (Miller et al., 2021), distinct population structures were observed, with at least nine well-supported

clades. Four clades (ST36, ST636, ST65, and ST3) comprised clinical sources. Three clades (ST676, ST1151, and ST735) were from oysters and the remaining (cluster S3 and ST32/ST34) were of both clinical and environmental origin. Four isolates (OYST-50, CCO1A-12, CC1B-23, and WBS2B-19) composed a clade of primarily environmental isolates. Like *V. vulnificus* phylogeny, no distinct pattern clustering pattern was observed linking *V. parahaemolyticus* phylotype from FGC.

Genetic characterization of the *Vibrio spp.*

All isolates examined in this study carried homologous genes coding for ARG, VFs, and MGEs (**Figure 17**). Overall, the *Vibrio spp.* showed similar patterns of resistance, with minor variation in multi-drug and biocide resistance, namely *msba,* the *vex* operon (*vexA*, *vexB*, *vexD*, *vexE*, *vexF, vexH*, and *vexK*), and Tetracycline resistance encoded by *tet*(34) and *tet*(35). Interestingly, *V. parahaemolyticus* isolates carried *carB* while *V. vulnificus* carried *varG*, both coding resistance to Beta-lactams. One isolate, CC1B-23, carried genes associated with Fosfomycin resistance.

Many VF homologs were detected in the *Vibrio spp.* isolates, namely genes coding for outer membrane adhesion factors, flagella, stress response, and biofilm formation. T3SS (type 1) and TLH/LDH was detected in all *V. parahaemolyticus* strains, while the type six secretion system (T6SS) and RTX toxin were detected in *V. vulnificus*. Major integrative conjugative elements (ICEs) encoding VFs and ARGs were not detected but several insertion sequences (ISs), transposase genes flanked by two inverted repeats, were detected. Overall, ISs were more common in strains recovered from water, compared to those from oysters. IS3 and IS5 insertion sequence

families were common in both *V. parahaemolyticus* and *V. vulnificus*, while the IS4 family of insertion sequences were detected only in *V. vulnificus*.

Metagenomic data analysis

Microbiome community profile

SMS using DNA prepared from Sterivex concentrated and APW enriched water samples generated an average of 18M (min = 13M; max = 21M) and 27M (min = 20M; max = 37M) paired reads across raw sequence read libraries, with a mean of 9M and 18M million unique paired reads, respectively. Following core gene metagenomic profiling, most reads were unclassified, while Sterivex concentrated and APW enriched water samples generated on average 300K (min = 181K; max = 405K) and 1M (min = 720K; max = 1.4M) unique paired reads. Measures of alpha diversity (species richness, Shannon, and Simpson) are shown in **Table 7**. Overall, Sterivex concentrated water samples revealed significantly higher alpha diversity compared to APW enriched samples. Bacteria, archaea, fungi, protists, and viruses identified by DNA metagenomics are listed in **Figure 18**, representing microbial taxa relative abundance (RA).

Proteobacteria were most abundant, followed by *Actinobacteria* detected in all samples. *Pseudoalteromonas* was the most abundant genus detected. Major differences were observed with respect to method, i.e., Sterivex concentrated vs. APW enriched. RA of *Proteobacteria* increased substantially after APW enrichment. Other differences were the dominance of *Bacteroidetes* in Sterivex concentrated samples and occurrence

of *Fusobacteria* in APW enriched samples. Genus level differences were also observed relative to method, with increased RA of *Vibrio* following enrichment (**Figure 18B**). Vibrios were not detected in Sterivex concentrated samples, whereas *Prochlorococcus*, *AVDB_g*, *Pelagibacter*, and *Pseudomonas* were dominant. APW enriched samples revealed increased RA of *AB062844_g, Propionigenium*, and *Photobacterium*, with *Villovirus* as the dominant viral genus.

Pathogenic *Vibrio spp.*

Members of the genus *Vibrio* were detected after APW enrichment of the water samples and the *Vibrio spp.* are listed in **Figure 18C**. Similar profiles were observed at all sample locations and many members of the genus *Vibrio* were detected, including a number of species considered opportunistic and/or pathogenic. *V. alginolyticus*, *V. diabolicus*, and *V. parahaemolyticus* were the most abundant in all samples, followed by other members of the *V. harveyi* clade, including *V. harveyi*, *V. owensii*, *V. campbellii*, *V. natriegens*, and *V. rotiferianus*. Other pathogenic *Vibrio spp.* included *V. vulnificus*, *V. fluvialis*, and *V. furnissi* in all samples. *V. cholerae* group and *V. mimicus* were detected in CK and WBS samples and *V. metschnikovii* and *V. cincinnatiensis* were detected only in a single sample from CK.

Detection of fungi, protists, and viruses

Whereas bacteria and archaea predominated, fungi, protozoa, and viruses were also present (**Table 9**). *Chlorophyta* were dominant in Sterivex samples but not

detected in APW enriched samples. Similarly, *Chrysochromulina ericina virus* was detected only in the Sterivex concentrated water samples. The protist *Minutocellus polymorphus* and *Vibrio* phages, notably *Vibrio virus Kappa*, were dominant in APW enriched water samples. It is worth noting *Vibrio virus CTXphi*, a phage encoding *V. cholerae* cholera toxin production, was detected in one sample from the CK station.

Community resistome, virulome, and mobilome

Genes coding for antimicrobial resistance, virulence, and mobile genetic elements, namely ICEs, were found at all stations (**Figure 19**). Only a few ARGs were detected in Sterivex concentrated samples, namely Tetracycline resistance, compared to APW enrichment (**Figure 19A**). Genes encoding resistance to major antibiotic classes, including Tetracycline, Quinolone, Fosfomycin, and Beta-lactam, were detected at all stations, including Trimethoprim resistance genes at CK, and Phenicol resistance genes at WBS.

VFs of non-*Vibrio* origin (**Figure 19B**) were common in Sterivex concentrated samples, and *Vibrio spp*. VFs (**Figure 19C**) in APW enriched samples. Of non-*Vibrio* VFs, those from *Mycobacterium spp.* were most common across all locations, notably in the Sterivex concentrated samples. VFs of pathogenic *Vibrio spp.*, namely *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, were detected at all stations and at increased abundance following APW enrichment. *V. parahaemolyticus* associated VFs, namely T3SS (type 1), were most common. *V. vulnificus* VFs included, RTX toxin, VVH heme receptors, flagella, LOS, and autoinducers and *V. cholerae* VFs included RTX toxin, MARTX toxins, and T6SS.

Compared to Sterivex concentrated samples, APW enrichment allowed for detection of additional ICEs (**Figure 19D**). ICEs originating in *V. cholerae*, *V. alginolyticus*, and *V. parahaemolyticus* were most prevalent, but ICEs originating in *Proteus mirabilis* were also common. Notably, two closely related ICEs, SXT and R391, respectively associated with *V. cholerae* clinical isolates and *Providencia rettgeri* were detected at all stations and at increased abundance in the CK and WBS samples.

Discussion

Climate change is reported to have effects in most regions of the world and more so in coastal communities, notably through overall warming trends, altered precipitation patterns, and changes in jet streams and ocean currents. Climate change is also associated with increased frequency and intensity of severe weather events (Capstick et al., 2022). Furthermore, climate change has the potential to alter intensity and behavior of hurricanes, a notable example of which is Hurricane Ian, ranked the fifth strongest storm to hit the USA mainland (**Figure 14A**), associated with a catastrophic storm surge and extreme rainfall. Hurricane Ian exemplified the more intense, slower-moving, and wetter signature commonly associated with a current generation of Atlantic storms influenced by climate warming (Espinel et al., 2022). In the days following Ian, an increase in cases of vibriosis was recorded by the FDH, including 38 cases and 11 vibriosis-associated deaths attributed to the storm (case fatality rate of *ca.* 28.9%) (Sodders et al., 2023). Between September 28, 2022, and

October 9, 2022, *V. vulnificus* infections were most common, but *V. cholerae* non-O1/non-O139, *V. parahaemolyticus*, and *V. fluvialis* were also confirmed by FDH (Sodders et al., 2023). Of the 11 deaths, nine were *V. vulnificus* and one *V. cholerae* non-O1/non-O139. Compared to Hurricane Irma which made landfall in 2017, six storm-related vibriosis cases were documented in FL, compared to 38 after Ian. There was a lower storm surge during Irma compared to Ian (Sodders et al., 2023) but in comparison with other vibriosis outbreaks caused by environmental catastrophes in the U.S., the outbreak associated with Ian is notable because of the large number of hurricane-attributable cases occurring during a short period of time. In addition to storm surge, time series analysis for SST and chlorophyll (**Figure 14C**) suggests some parameters of the coastal aquatic environment changed significantly during and after Ian, favoring growth and proliferation of *Vibrio spp*.

Detection and characterization of pathogenic *Vibrio spp.* from the environment has been ongoing in the Chesapeake Bay since the 1960's (Lovelace et al., 1968; Kaneko and Colwell, 1973, 1974, 1977; Colwell et al., 1977, 1977; Kaper et al., 1981; Wright et al., 1996; Zo et al., 2009; Banakar et al., 2011). *Vibrio spp.* are native to and thrive in warm water with moderate salinity (Singleton et al., 1982; Brumfield et al., 2021b), and pathogenic *Vibrio spp.* have been shown to be present in sediment during unfavorable environmental conditions, even when not detectable in water samples (Kaper et al., 1981; Brumfield et al., 2023). A recent culture-based investigation of *V. parahaemolyticus* and *V. vulnificus* in the Chesapeake Bay reaffirmed environmental predictors for these bacteria and documented their long-term increase and extended seasonality, notably during the fall (Brumfield et al., 2023). Specifically, critical

environmental parameter thresholds were determined for SST (25 °C), pH (8), DO (5 to 10 mg/L), and salinity (10 to 15 PPT) whereby increased to maximum abundance of *Vibrio spp.* occurs. In areas with high salinity profiles, pathogenic vibrios are known to proliferate rapidly during heavy rainstorms that reduce the salinity and favor growth of these bacteria, as demonstrated in the French Mediterranean (Esteves et al., 2015), Chesapeake Bay (Shaw et al., 2014a), and Northern Gulf Coast (Motes et al., 1998). Salinity of the FGC is demonstrably higher than that of the Chesapeake Bay. However, considering the impact of Hurricane Ian's heavy rainfall on salinity and other environmental parameters recorded in the FGC, including temperature, pH, and DO (**Table 8**), these values are within the range considered optimal for pathogenic vibrios to proliferate in the environment. In addition, chlorophyll serves as an important predictor of *Vibrio* abundance by indicating density of phytoplankton populations, thus serving as an indicator of subsequent proliferation of zooplankton, which feed on phytoplankton (Colwell, 1996; Lobitz et al., 2000; Constantin de Magny et al., 2008; Jutla et al., 2013b). Here, it was observed that chlorophyll concentrations increased drastically during and after Hurricane Ian which would, by proxy, indicate increased zooplankton abundance with which *Vibrio* are associated.

Cultures of *V. parahaemolyticus* and *V. vulnificus* isolated from water and oyster samples collected from the FGC subjected to WGS provided a core gene phylogeny analysis (**Figure 15**) that matched previously proposed clades for *Vibrionaceae* (Sawabe et al., 2013), whereby each species formed coherent clusters within taxonomic subclades, in agreement with recent phylogenetic analysis (Jiang et al., 2022). Species specific core gene phylogeny was also done (**Figure 16**), with

subspecies clustering obtained for *V. vulnificus* (López-Pérez et al., 2019) and *V. parahaemolyticus* (Miller et al., 2021). It was hypothesized that isolates from a single location would cluster with other strains from that location. However, distinct clustering patterns were not observed that linked strain phylogeny, source of isolation, or virulence capability, instead providing evidence for multiple clonal populations in circulation simultaneously. López-Pérez and colleagues (2019), did a pangenome and phenotypic analysis that yielded two markedly different lifestyles with differentiated phylogenetic clusters, indicating commensal (C2) and bloomer (C1) ecotypes, with differences in carbohydrate utilization, defense systems, and chemotaxis. All *V. vulnificus* isolates from the study reported here fell into these two clusters. Based on the findings of Miller and colleagues (2021), who performed WGS analysis to describe phylogenetic and biogeographic patterns of *V. parahaemolyticus* from North America, the FGC isolates in this study can be characterized as multiple sequence types, providing additional evidence indicating a hub of genetic variability of *V. parahaemolyticus* along the Gulf Coast.

Unlike clinical isolates, environmental *V. parahaemolyticus* strains generally do not encode primary VFs, such as thermostable direct hemolysin (*tdh*) and thermostable direct-related hemolysin (*trh*) (DePaola et al., 2000; Drake et al., 2007; Letchumanan et al., 2014; Raghunath, 2015). However, it has been estimated that up to *ca*. 27% of *V*. *parahaemolyticus* clinical isolates do not encode *tdh* and/or *trh* either (Jones et al., 2012), suggesting possible presence of other virulence factors. Furthermore, *tdh*/*trh* negative *V. parahaemolyticus* strains can cause severe infections in marine fish (Colwell and Grimes, 1984) and shrimp (Prithvisagar et al., 2021), with

significant economic burden and aquaculture loss (Freitag et al., 2022). *V. parahaemolyticus* isolated in this study did not carry *tdh* and/or *trh* but did have additional VFs, notably coding for the T3SS. ARGs were also detected in all *V. parahaemolyticus* strains isolated (**Figure 17**). Interestingly, no single virulence gene has been identified that distinguishes pathogenic and non-pathogenic *V. vulnificus* (Baker-Austin and Oliver, 2018). It is worth noting that RTX toxin, which promotes cytotoxicity and enhances survival of the bacterium during infection (Lee et al., 2007; Lo et al., 2011), was detected in all *V. vulnificus* isolated in this study and T6SS, a pilin apparatus contributing to biofilm formation, adherence to epithelial cells, and virulence (Paranjpye and Strom, 2005), was detected in three of the FGC isolates.

Vibrio spp. have been shown to enter a protective state, namely viable but nonculturable (VBNC), whereby the cells become metabolically dormant. That is, VBNC cells cannot be cultured using standard laboratory media, yet are detectable using molecular genetic methods (Colwell, 2000). Historically, detection of *Vibrio spp.* in the environment using culture methods had been challenging because *Vibrio spp.* in the VBNC state complicating detection and resulting in severe under representation of total *Vibrio* populations. VBNC cells have been shown to have increased resistance to thermal, low salinity, and acidic inactivation, suggesting this state plays a role in survival during adverse environmental conditions (Nowakowska and Oliver, 2013). Advances in molecular methods for microbial detection, identification, and characterization, notably next generation sequencing and metagenomics, allow researchers to investigate more completely the mode of emergence and transmission of pathogenic agents that would have otherwise gone undetected (Brumfield et al., 2020c).

SMS is an effective molecular surveillance tool that allows bacterial, archaeal, viral, fungal, and protozoan microbiome community members to be identified and characterized. Demonstration of its value for microbial source tracking and wastewater surveillance linked to climate has been successful (Brumfield et al., 2022a).

Traditional culture-based methods using selective media allowed successful recovery of *V. parahaemolyticus* and *V. vulnificus* from FGC samples. However, SMS of water samples concentrated using Sterivex filters was effective in profiling the microbiome, as shown for drinking water by Brumfield and colleagues (2020b). Here, SMS of Sterivex concentrated water samples successfully profiled the community composition of environmental water samples but yielded an insufficient number of reads to be able to determine the total numbers of the *Vibrio spp.* with confidence. SMS of APW enriched samples allowed detection of many pathogenic species but not quantification because of enrichment. Nevertheless, *V. cholerae* and *V. fluvialis*, species of *Vibrio* confirmed to be the cause of deaths in FL linked to Hurricane Ian (Sodders et al., 2023), were detected. Furthermore, WGS and SMS allowed characterization of the VFs, ARGs, and MGEs, along with multiple ARGs in the isolates of *Vibrio spp.* (**Figure 17A**) and in the microbiomes (**Figure 19A**).

In summary, climate conditions associated with the growth of and rapid response to environmental signals by *Vibrio spp*. make them a valuable microbial indicator of the impact of a changing global climate on public health (Colwell, 1996; Vezzulli et al., 2012, 2015; Baker-Austin et al., 2017; Froelich and Daines, 2020; Brumfield et al., 2021b, 2023; Vezzulli, 2022; Archer et al., 2023). Between 2009 and 2022, a long-term increase in the number of confirmed *V. vulnificus* infections in the

State of FL was documented (**Figure 14D**), the outbreak of vibriosis following Hurricane Ian being a traumatic example of the correlation of climate/weather processes and public health. By employing satellite remote sensing, changes in the coastal aquatic environment can be coupled with WGS and metagenomic analysis to develop predictive risk models for *Vibrio spp.* and related pathogens. Such strategies will be critical as climate change accelerates over time.

Acknowledgements

This research was funded by the National Institute of Environmental Health Sciences, National Institutes of Health (R01ES030317A), the National Science Foundation (OCE1839171, CCF1918749, and CBET1751854), and the National Aeronautics and Space Administration (80NSSC20K0814 and 80NSSC22K1044).

Tables

Table 4: Primers, PCR parameters, and reference strains used in this study.

[a] Isolate synonym of N16961

[b] Isolate characterized previously (Chen et al., 2017)

[c] Isolate characterized previously (Stine et al., 2000)

Table 4: Primers, PCR parameters, and reference strains used in this study.

Description (Reference)	Oligonucleotide name	Sequence (5'-3')	PCR product size (bp)	Annealing temp (°C)	Reference strain used in this study
Vibrio genus 16S rRNA (Polz et al., 2004)	567F	GGCGTAAAGCGCATGCAGGT	120	55	*V. cholerae* ATCC 39315[a]
	680R	GAAATTCTACCCCCCTCTACAG			
toxR of *V. parahaemolyticus* (Vp), *V. cholerae* (Vc), and *V. vulnificus* (Vv) (Bauer and Rørvik, 2007)	UtoxF	GASTTTGTTTGGCGYGARCAAGGTT			
	vptoxR	GGTTCAACGATTGCGTCAGAAG	297 (*Vp*)	60	*V. parahaemolyticus* ATCC 17803
	vctoxR	GGTTAGCAACGATGCGTAAG	640 (*Vc*)	55	*V. cholerae* ATCC 39315
	vvtoxR	AACGGAACTTAGACTCCGAC	435 (*Vv*)	55	*V. vulnificus* ATCC 27562
Total and hemolysin-producing *V. parahaemolyticus via tlh*, *tdh*, and *trh* (Bej et al., 1999)	L-tl	AAAGCGGATTATGCAGAAGCACTG	450 (*tlh*)	58	*V. parahaemolyticus* ATCC 17803
	R-tl	GCTACTTTCTAGCATTTTCTCTGC			
	L-tdh	GTAAAGGTCTCTGACTTTTGGAC	269 (*tdh*)	58	*V. parahaemolyticus* TX2103 (*tdh*+/*trh*-)[b]
	R-tdh	TGGAATAGAACCTTCATCTTCACC			
	L-trh	TTGGCTTCGATATTTTCAGTA	500 (*trh*)	58	*V. parahaemolyticus* AQ4037 (*tdh*-/*trh*+)[b]
	R-trh	CATAACAAACATATGCCCATTTCCG			
V. vulnificus hemolysin *vvhA* (Panicker et al., 2004)	L-vvh	TTCCAACTTCAAACCGAACTATGAC	205	58	*V. vulnificus* ATCC 27562
	R-vvh	ATTCCAGTCGATGCGAATACGTTG			
Toxigenic *V. cholerae* O1 and O139 via *ctxA*, *rfb*-O1, and *rfb*-O139 (Hoshino et al., 1998)	O139F2	AGCCTCTTTATTACGGGTGG	449 (O139)	55	*V. cholerae* MO10 (O139)[c]
	O139R2	GTCAAACCCGATCGTAAAGG			
	O1F2	GTTTCACTGAACAGATGGG	192 (O1)	55	*V. cholerae* ATCC 39315[a]
	O1R2-2	GGTCATCTGTAAGTACAAC			
	VCT1	ACAGAGTGAGTACTTTGACC	308 (*ctxA*)	55	*V. cholerae* ATCC 39315[a]
	O139F2	AGCCTCTTTATTACGGGTGG	449 (O139)	55	*V. cholerae* MO10 (O139)[c]

Table 5: *Vibrio vulnificus* accession numbers and MLST profiles.

				MLST										
Isolate	**Source**	**Location**	**BioSample Accession**	***glp***	***gyrB***	***mdh***	***metG***	***purM***	***dtdS***	***lysA***	***pntA***	***pyrC***	***tnaA***	**ST**
CCO1A-11	oyster	CC	SAMN35557952	8	95	2	24	8	39	28	105	33	2	621
CCO1A-81	oyster	CC	SAMN35557953	8	95	2	24	8	39	28	105	33	2	621
CCO1B-90	oyster	CC	SAMN35557954	5	18	54	16	17	8	87	19	18	7	622
CK1A-14	water	CK	SAMN35557955	92	4	24	5	15	29	2	1	16	159	623
CK1A-40	water	CK	SAMN35557956	92	4	24	5	15	29	2	1	16	159	623
CK1A-46	water	CK	SAMN35557957	13	95	125	7	95	188	65	119	152	9	624
CK1A-8	water	CK	SAMN35557958	39	27	127	88	22	34	2	1	73	58	625
CK2B-37	water	CK	SAMN35557959	28	3	22	5	30	41	26	28	67	160	626
CK2B-58	water	CK	SAMN35557960	28	3	22	5	30	41	26	28	67	160	626
CKO-35	oyster	CK	SAMN35557961	63	1	90	25	96	217	200	36	43	13	627
WBS2A-88	water	WBS	SAMN35557962	157	1	3	14	97	218		1	153	161	
WBS2B-2	water	WBS	SAMN35557963	1	3	37	4	36	8	70	4	108	54	628

Table 6: *Vibrio parahaemolyticus* accession numbers and MLST profiles.

				MLST									AA-MLST							
Isolate	**Source**	**Location**	**BioSample Accession**	***dnaE***	***gyrB***	***recA***	***dtdS***	***pntA***	***pyrC***	***tnaA***	**ST**	**clonal complex**	**p*dnaE***	**p*gyrB***	**p*recA***	**p*dtdS***	**p*pntA***	**p*pyrC***	**p*tnaA***	**pST**
CC1A-27	water	CC	SAMN35557943	31	106	135	74	37	212	54	564	no	1	1	1	1	1	1	1	1
CC1B-23	water	CC	SAMN35557944	22	424	3	631	230	461	14	3361	no	2	1	1	1	2	1	4	
CCO1A-12	oyster	CC	SAMN35557945	10	104	551	68	26	568	2	3362	no	2	1	1	1	1	2	1	5
CK1A-7	water	CK	SAMN35557946	354	57	3	75	168	37	46	3363	no	1	1	1	1	1	2	1	3
CK2B-83	water	CK	SAMN35557947	12	13	15	22	3	1	12	16	no	2	1	1	1	1	23	1	111
OYST-50	oyster	pooled	SAMN35557948	10	104	551	68	26	568	2	3362	no	2	1	1	1	1	2	1	5
WBS2B-138	water	WBS	SAMN35557949	42	134	99	79	4	41	51	3364	no	2	1	1	1	1	1	1	2
WBS2B-19	water	WBS	SAMN35557950	28	17	21	119	20	23	24	896	49	1	1	1	1	3	1	1	18
WBSO1B-30	oyster	WBS	SAMN35557951	283	88	31	355	53	45	13	3121	no	1	1	1	1	1	1	1	1

Table 7: Sequencing statistics and diversity indices.

Method/Sample	Accession no.	Richness	Shannon	Simpson
CC_S	SRR24799355	376.00	4.29	0.95
CK_S	SRR24799352	276.00	4.10	0.94
WBS_S	SRR24799349	397.77	4.25	0.94
CC1A_APW	SRR24799357	109.00	2.49	0.76
CC2B_APW	SRR24799356	118.00	2.50	0.76
CK1A_APW	SRR24799357	215.89	3.16	0.91
CK2A_APW	SRR24799353	174.00	3.12	0.90
WBS1A_APW	SRR24799351	153.00	2.76	0.87
WBS2A_APW	SRR24799350	153.00	2.93	0.88

Table 8: Environmental parameters recorded during sample collection.

Parameter	Cutthroat Clams[a]	Clam Key[a]	White Booth Seafood[a]
Water Temperature (°C)	25.4/24.8 (25.1)	24.6/24.4 (24.5)	24.5/24.4 (24.45)
pH	8.00/8.07 (8.035)	7.83/7.85 (7.84)	7.79/7.90 (7.845)
DO (mg/L)	6.0/6.1 (6.05)	6.2/5.3 (5.75)	6.1/5.2 (5.65)
Salinity (PPT)	30.36/30.60 (30.48)	19.74/19.75 (19.745)	19.84/19.84 (19.84)

[a] Environmental parameters presented as: surface recording/bottom recording (mean).

Table 9: Other taxa detected, listed as relative abundance (%).

Phylum	Genus	Species	Cutthroat Clams			Clam Key Seafood			White Booth Seafood		
			CC_S	CC1A_APW	CC2B_APW	CK_S	CK1A_APW	CK2A_APW	WBS_S	WBS1A_APW	WBS2A_APW
Viruses											
Virus no name P	*Bracovirus*	*Cotesia glomerata bracovirus*	-	-	-	-	-	-	0.00	-	-
Uroviricota	*Igirivirus*	*Synechococcus T7-like virus S-TIP37*	-	-	-	-	-	-	0.10	-	-
Uroviricota	*Longwoodvirus*	*Vibrio virus Kappa*	-	-	-	-	0.05	0.02	-	0.04	0.18
Phixviricota	Virus no name G	*Chrysochromulina ericina virus*	0.11	-	-	0.70	-	-	0.93	-	-
Hofneiviricota	*Affertcholeramvirus*	*Vibrio virus CTXphi*	-	-	-	-	0.10	-	-	-	-
Hofneiviricota	*Fibrovirus*	*Vibrio virus fs1*	-	-	-	-	<0.01	-	-	-	-
Hofneiviricota	*Villovirus*	*Vibrio virus Vf33*	-	-	-	-	-	-	<0.01	-	-
Protists											
Bacillariophyta	*Minutocellus*	*Minutocellus polymorphus*	-	1.99	6.62	-	8.33	5.67	-	0.65	0.59
Algae											
Chlorophyta	*Nannochloris*	*Nannochloris sp. X1*	0.04	-	-	-	-	-	-	-	-
Chlorophyta	*Picochlorum*	*Picochlorum sp. BH-2019*	0.08	-	-	-	-	-	0.09	-	-
Chlorophyta	*Picochlorum*	*Picochlorum sp. 'soloecismus'*	0.05	-	-	0.06	-	-	0.05	-	-
Chlorophyta	*Picochlorum*	*Picochlorum sp. SENEW3*	0.10	-	-	2.75	-	-	4.05	-	-
Chlorophyta	*Picochlorum*	*Picochlorum oklahomense*	0.13	-	-	-	-	-	0.13	-	-

Figures

Figure 14: Area of study in Florida Gulf Coast.

(A) Path of Hurricane Ian and confirmed *V. vulnificus* infections in FL at the county level for 2022 (Florida Department of Health, 2023). Counties reporting *V. vulnificus* deaths and number are indicated. Track, timeline, and windspeed of Hurricane Ian, indicated by diamonds, were retrieved from the International Best Track Archive for Stewardship (IBTrACS) project (Knapp et al., 2010). The outlined red box indicates area of sampling locations. **(B)** Map of sampling locations, indicated by red diamonds. Scale bar corresponds to distance according to World Map Data from Natural Earth (Massicotte and South, 2023). **(C)** Percent change in a time series analysis to determine potential impact of anomalous weather events before (July 24, 2022 – September 23, 2022), during (September 24, 2022 – September 30, 2022), and after (October 1, 2022 – November 30, 2022) Hurricane Ian, for sea surface temperature (SST) and chlorophyll (CHL). **(D)** Confirmed *V. vulnificus* cases and deaths between 2008 and 2022. Correlations among axis variables were respectively generated using Kendall's tau method.

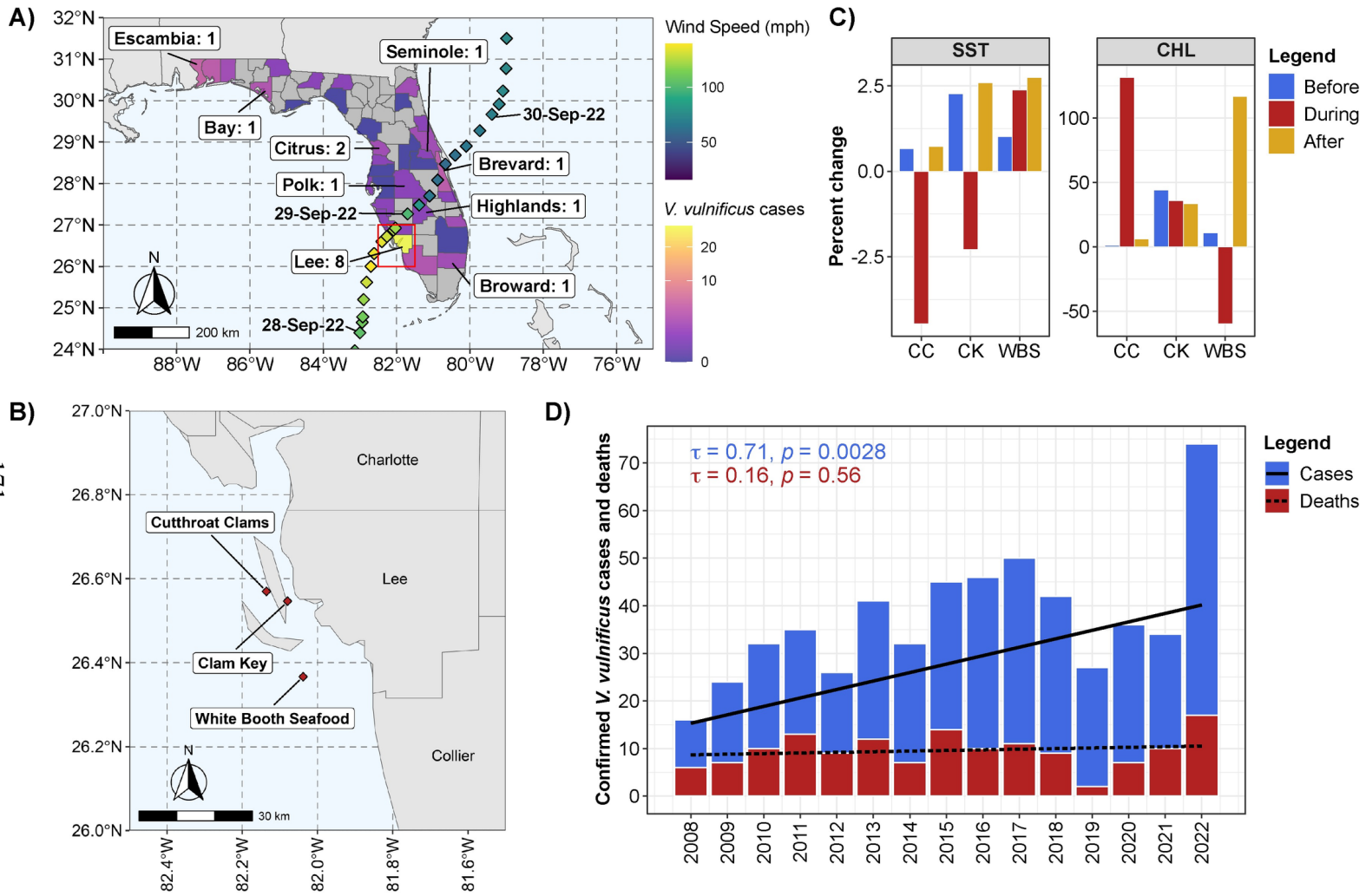

Figure 14: area of study in Florida Gulf Coast.

Figure 15: Phylogenetic relatedness of *Vibrionaceae spp*.

Codon tree was created using 344 coding sequences (*ca.* 7.13×10^4 amino acids in length) shared by all genomes with the LG model. *Shewonella oneidensis* MR-1 was used as an outgroup to root the tree. Bootstrap values are shown as percentages. *Vibrio spp.* isolates collected during this study are shown in blue. Trees were built using the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) software package (Brettin et al., 2015) and Randomized Axelerated Maximum Likelihood (RAxML) algorithm automatic model selection (Stamatakis, 2014).

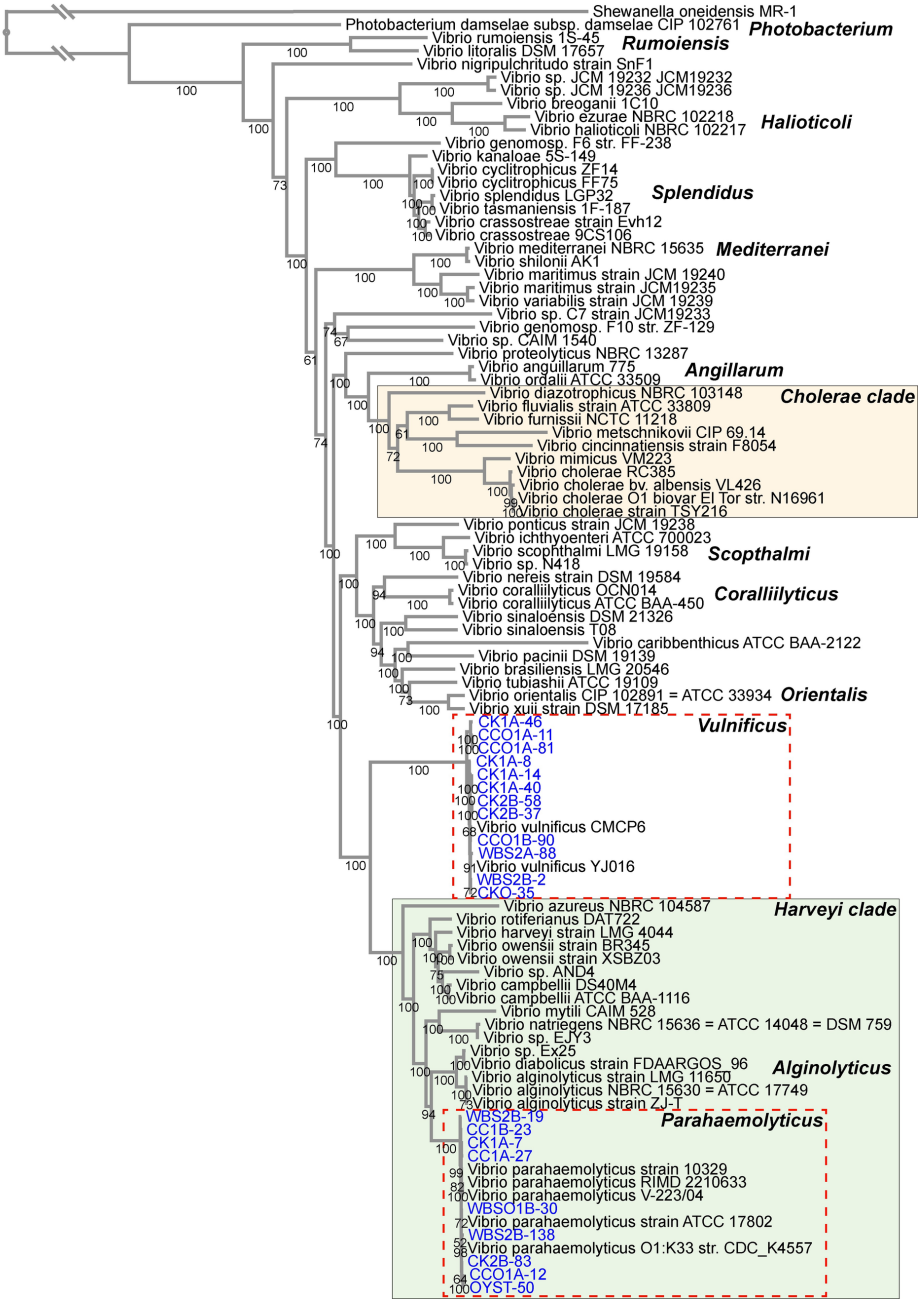

Figure 15: Phylogenetic relatedness of *Vibrionaceae spp*.

Figure 16: Phylogenetic relationships of *Vibrio vulnificus* and *Vibrio parahaemolyticus*.

(A) *V. vulnificus* codon tree was created was created using 982 coding sequences (*ca.* 3.02×10^5 amino acids in length) shared by all genomes with the JTT model. **(B)** *V. parahaemolyticus* codon tree was created was created using 1,000 coding sequences (*ca.* 3.88×10^5 amino acids in length) shared by all genomes with the HIVB model. Bootstrap values are shown as percentages. *Vibrio spp.* isolates collected during this study are shown in blue. Trees were built using the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) software package (Brettin et al., 2015) and Randomized Axelerated Maximum Likelihood (RAxML) algorithm automatic model selection (Stamatakis, 2014).

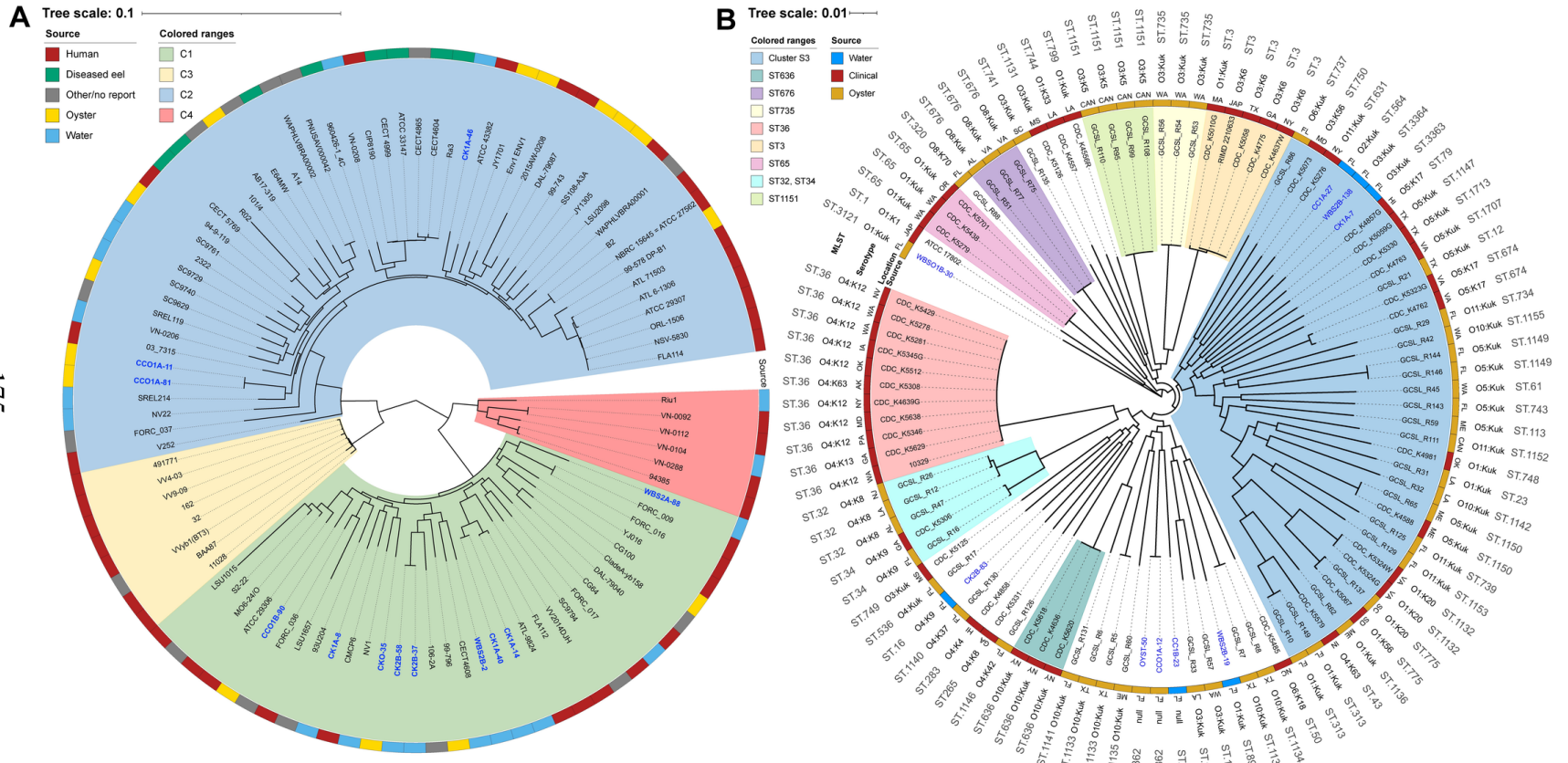

Figure 16: Phylogenetic relationships of *Vibrio vulnificus* and *Vibrio parahaemolyticus*.

Figure 17: Genomic characterization of *Vibrio spp*. isolates.

Assembled contigs were characterized to determine carriage of **(A)** antimicrobial drug, biocide and metal resistance determinants using the Microbial Ecology Group Antimicrobial Resistance (MEGARes) database (Doster et al., 2020), **(B)** virulence factors the Virulence Factor Database (VFDB) (Liu et al., 2018), and **C)** MGEs the Mobile Genetic Element database (MGEdb) (Johansson, 2023), and ABRicate (Seemann, 2020) with identity and coverage thresholds set to 60%.

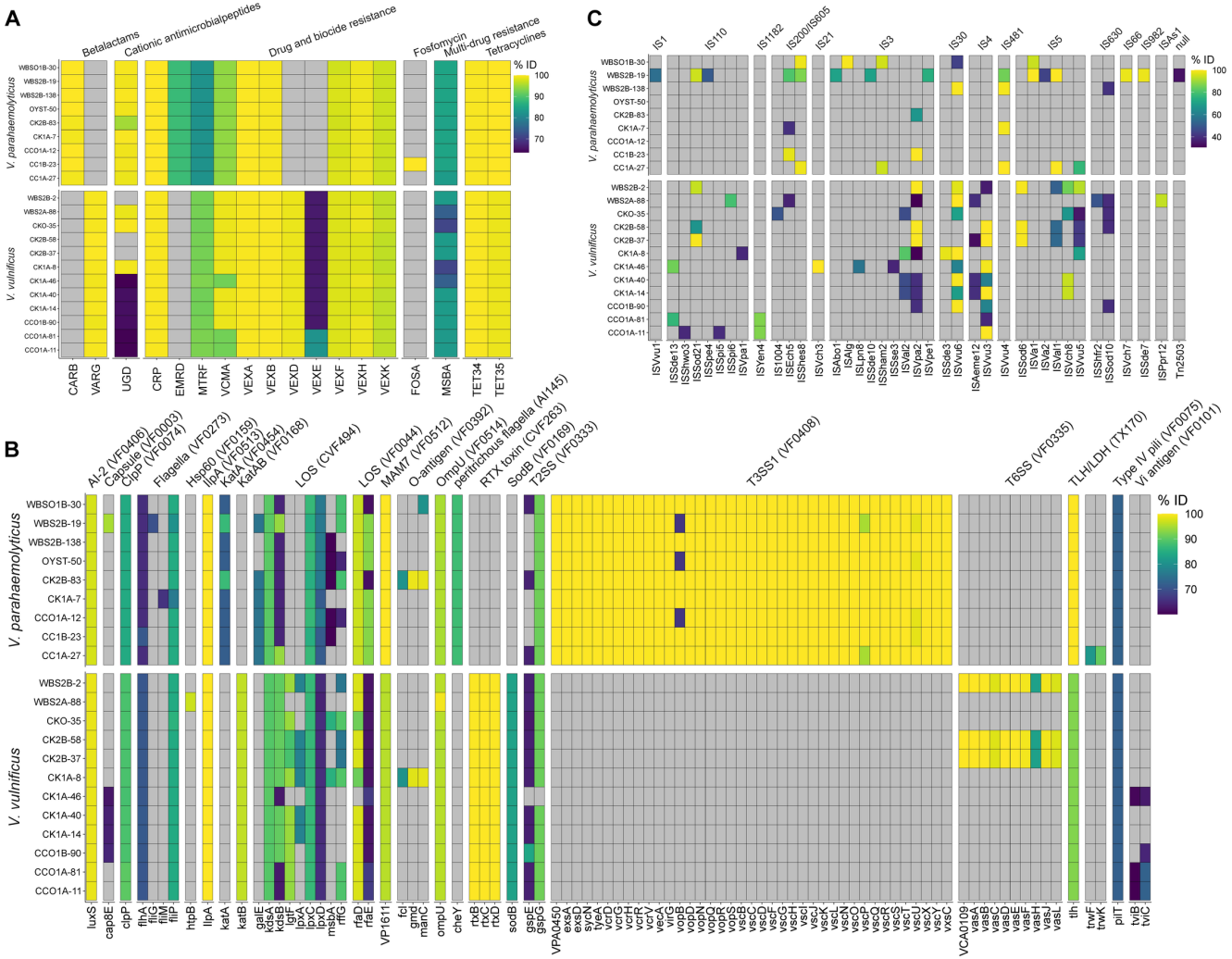

Figure 17: Genomic characterization of *Vibrio spp*. isolates.

Figure 18: Microbial community composition.

Metagenomic community profiling was done using up-to-date bacterial core gene (UBCG) algorithm (Chalita et al., 2020) with EzBioCloud database (Yoon et al., 2017). **(A)** Stacked bar plot showing relative sequencing read abundance of most abundant phyla. **(B)** Stacked bar plot showing relative sequencing read abundance of 20 most abundant genera. **(C)** Heatmap showing $\log_{10}$(relative abundance) *Vibrio spp.* Dendrogram shows k-means clustering of species detected and sampling location.

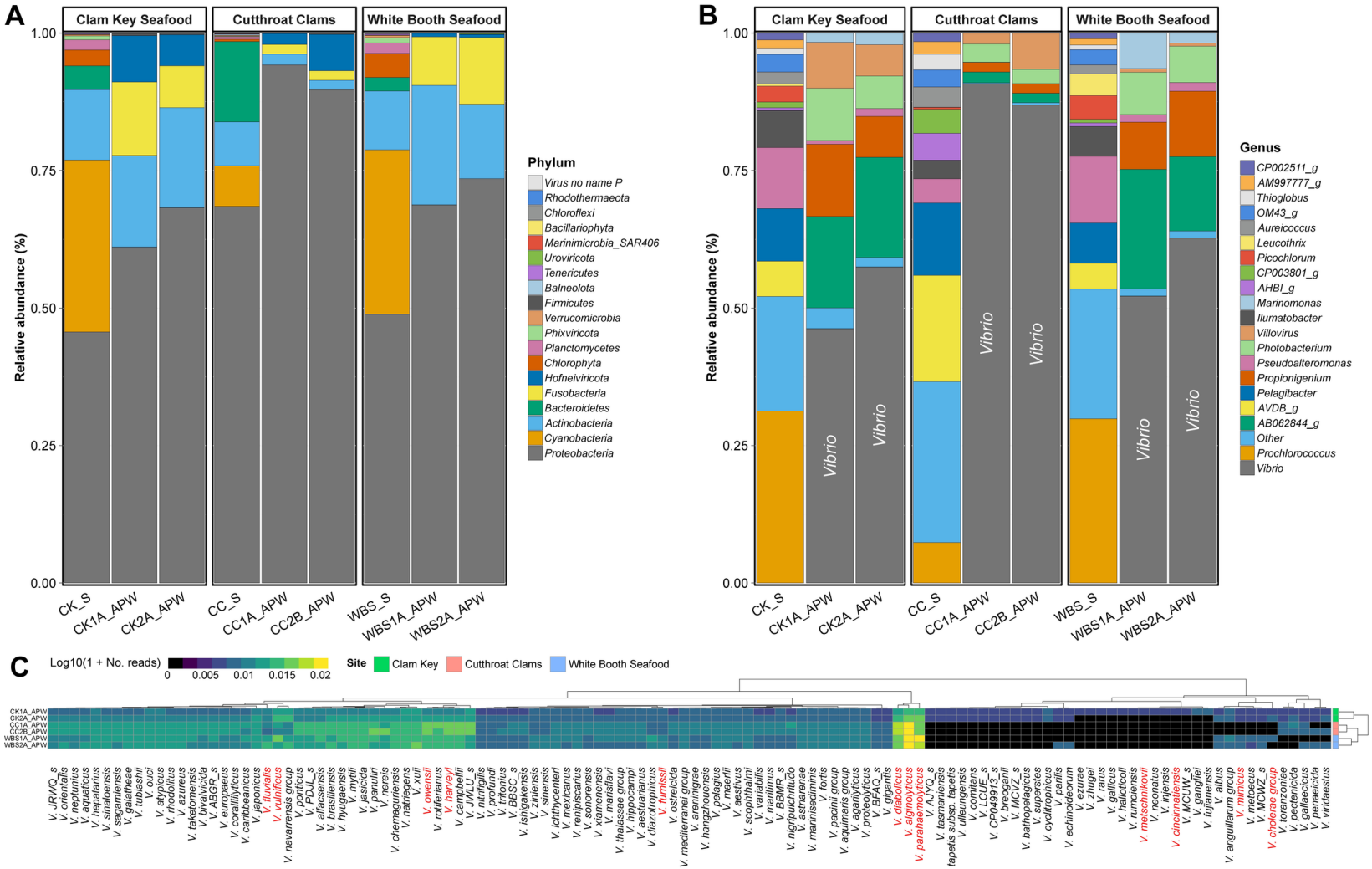

Figure 18: Microbial community composition.

Figure 19: Microbiome community characteristics.

Presence of **(A)** Antimicrobial resistance genes using the NCBI National Database of Antibiotic Resistant Organisms (NDARO) database (NCBI, 2023), **(B)** non-*Vibrio* virulence factors and **(C)** *Vibrio spp.* Virulence factors using the Virulence Factor Database (Liu et al., 2018), and **(D)** integrative and conjugative elements using the Mobile Genetic Element Database (Johansson, 2023). These were determined by mapping metagenomic sequencing reads to the respective databases using bowtie2 (Langmead and Salzberg, 2012) with the "--very-sensitive" option and a Phred quality score threshold of 33.

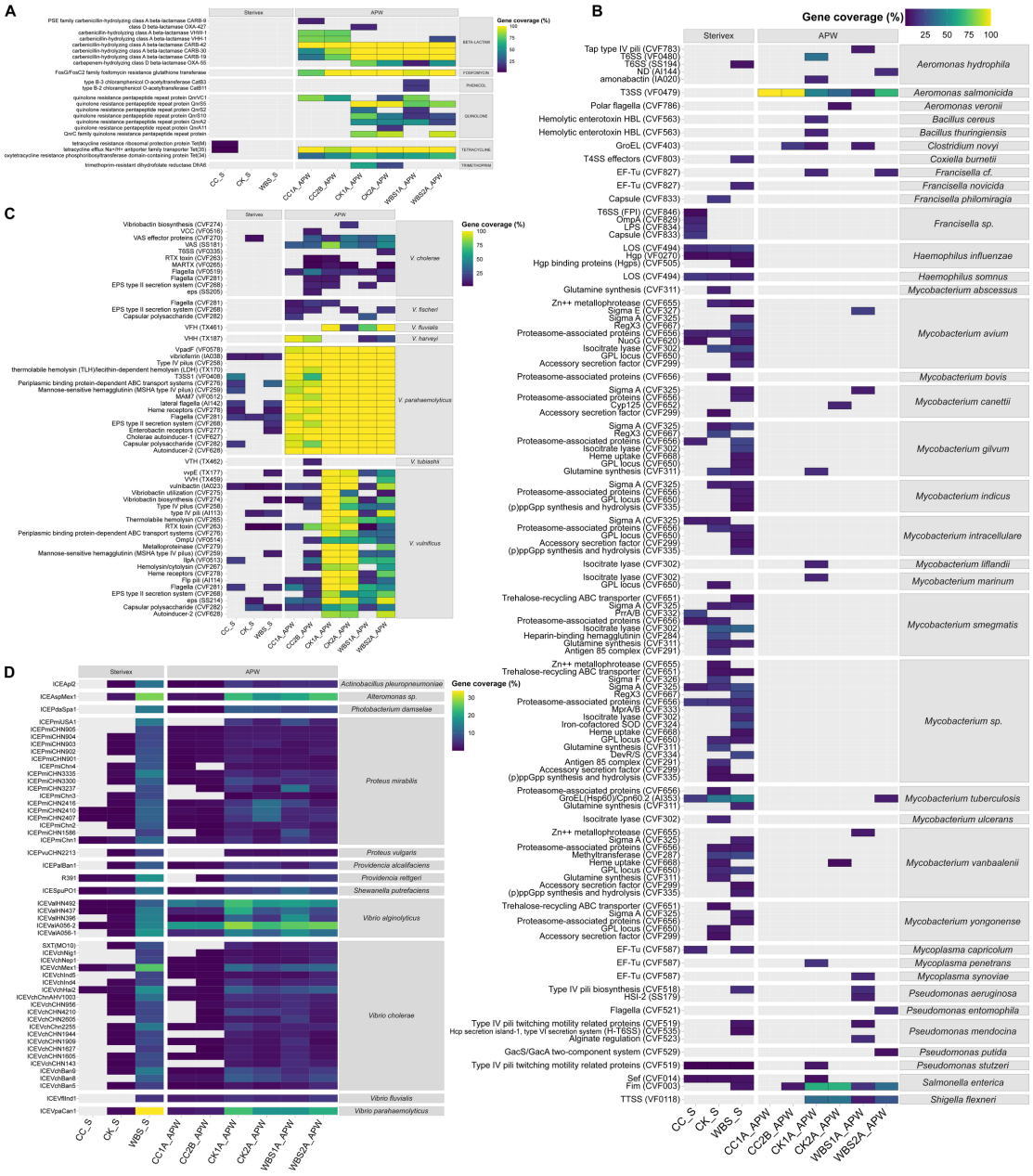

Figure 19: Microbiome community characteristics.

Chapter 5: Discussion

Molecular detection of pathogens

Humans have long understood that climate is associated with epidemic diseases. The Greek physician Hippocrates was among the first to document observational evidence connecting climate (air and water), land phenology (soil), and occurrence of disease in human populations (Hippocrates of Cos, BCE400). However, it was not until the 19th century that the role microorganisms play in disease transmission was understood. Traditionally, microbial communities have been defined using culture dependent methods to detect and enumerate microorganisms. However, it is estimated that the vast majority of prokaryotic genospecies remain uncultured (Sleator et al., 2008), and genomes of uncultured microorganisms encode a largely untapped reservoir of novel metabolites and metabolic processes (Simon and Daniel, 2011). Accordingly, the field of metagenomics has developed rapidly and effectively obviates the need to isolate and culture microorganisms by utilizing the genetic material of a sample to identify the functional gene composition. This major accomplishment has allowed detailed comparison and exploration of microbial ecology, including the community profiles of complex ecosystems such as drinking water (Brumfield et al., 2020b, 2020a), urban watersheds (Brumfield et al., 2021a), wastewater (Brumfield et al., 2022a), and gut of insects (Brumfield et al., 2022b).

Since the emergence of metagenomics, whereby DNA is sequenced directly from environmental samples, sequencing for microbial identification has evolved to include a variety of approaches. The polymerase chain reaction (PCR) is one of the fundamental methods currently being used for taxonomic identification. PCR-based metagenomics is now routine in gene prospecting by direct amplification of specific

genes (Kotik, 2009) or colony PCR to screen metagenomic libraries (Itoh et al., 2014). Moreover, PCR amplification of specific genes is used to evaluate microbial species diversity based on sequence composition. The use of 16S ribosomal RNA (rRNA) genes that occur in one or more copies in most bacterial and archaeal genomes is widely recognized as the "Gold Standard" for prokaryotic identification. The 16S rRNA gene sequencing method generally employs universal PCR primers to amplify hypervariable regions of the 16S rRNA gene that infer taxonomic identification by bioinformatic alignment against various 16S rRNA sequence databases.

With advances being made in sequencing technologies, the cost of sequencing has decreased drastically in recent years, and shotgun metagenomic sequencing (SMS) is an attractive alternative to study all of the genes in all microorganisms present in a sample, independent of culture. Instead of targeting specific genomic markers, the SMS method utilizes total DNA extraction and acts to shear extracted DNA into fragments that are independently sequenced and aligned to achieve total taxonomic identification by utilizing reference genomes in genomic databases. While both 16S rRNA sequencing and SMS have application in microbiome research, SMS has been shown to yield greater microbial resolution, increased accuracy, and allow identification of more genera of bacteria, archaea, viruses, eukaryote, and putative functional genes that would have gone undetected using 16S rRNA sequencing (Shah et al., 2011; Tessler et al., 2017; Brumfield et al., 2020c; Durazzi et al., 2021).

Historically, detection of *Vibrio spp.* in the environment has been difficult since *Vibrio spp.* are often in the unculturable state, i.e., viable but nonculturable (VBNC) (Colwell et al., 1985; Roszak and Colwell, 1987; Colwell and Huq, 1994; Colwell,

2000). In **Chapter 4**, traditional culture-based methods allowed successful recovery of *Vibrio parahaemolyticus* and *Vibrio vulnificus* from samples of coastal water collected along the Florida Gulf. However, only a limited number of subspecies/strains and no additional *Vibrio spp.* could be cultured, hence detected. In addition, both PCR and SMS were employed to detect of pathogenic *Vibrio spp.* While the 16S rRNA marker for the genus *Vibrio*, was successfully detected in all samples via PCR, successful detection of species-specific markers, e.g., *tlh* and *vvhA*, and virulence factors was minimal. By contrast, SMS analysis of water samples provided insight with respect to human health, notably detection of approximately 12 pathogenic *Vibrio spp.*, including *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*, and *V. fluvialis*, along with virulence and antibiotic resistance genes and mobile genetic elements. Detection of these species that are naturally occurring in the environment is notable since they have been confirmed to be the cause of vibriosis and deaths in FL linked to Hurricane Ian (Sodders et al., 2023). Hence, it is concluded that SMS is a valuable microbiological tool for detection and characterization of pathogens in the environment and, when coupled with environmental data, can comprise an infectious disease early warning system useful for public health.

Climate change and infectious disease

In recent years, the world has experienced an apparent increase in the emergence and re-emergence of infectious diseases. There are many factors (anthropogenic and naturogenic) involved in the emergence of infectious diseases. For

example, human settlements are continually expanding into new geographic regions, replacing pristine forests with land intended for urban development and agriculture which increases the opportunity for human interaction with different animal species. Similarly, unprecedented biodiversity loss has been suggested to increase the risk of human exposure to new and established zoonotic pathogens (Keesing and Ostfeld, 2021). Hence, climate change is increasingly becoming a major cause of concern (Wu et al., 2016; Baker et al., 2021). Global and regional climatic variability have the potential to impact the emergence, distribution, and prevalence of infectious (vector-, water- or air-borne) diseases, resulting in a significant public health burden. Previous studies have established relationships between environmental variables and waterborne diseases (WBD) (Colwell, 1996; Lobitz et al., 2000; Brumfield et al., 2021b, 2023; Usmani et al., 2021, 2023). However, the direct and indirect impacts of enhanced climate variability on distribution and transmission of infectious diseases remain a subject of active inquiry.

There has been an observed warming period since 1971 accompanied by changes in precipitation patterns globally (Räisänen, 2022). Changes in climatic processes have significantly impacted the environment in certain geographic areas, with evidence suggesting a change in the spatial distribution of pathogens (Hutchins et al., 2019; El-Sayed and Kamel, 2020). That is, with a changing environment, pathogenic microorganisms are capable of proliferating in areas where these organisms were not previously found. A prime example of this phenomenon with respect to WBD is the significant geographic expansion of pathogenic *Vibrio spp.*, a finding corroborated by their impact on public health, namely increased numbers of reported

vibriosis infections in humans and also aquaculture loss (Colwell, 1996; Vezzulli et al., 2012, 2015; Baker-Austin et al., 2017; Froelich and Daines, 2020; Brumfield et al., 2021b, 2023; Vezzulli, 2022; Archer et al., 2023). Therefore, a key motivation for the work reported here was to ascertain impacts of climate variability on prevalence and distribution of climate modulated WBDs, using *Vibrio spp.* as a model taxon, where growth of these pathogens is linked with their environmental niche. Specifically, the impact of environmental factors on occurrence of pathogenic *Vibrio spp.* was investigated in the Chesapeake Bay, Maryland, during 2009 to 2012 and 2019 to 2022 (**Chapter 3**). In addition, samples were collected from areas in Florida significantly impacted by Hurricane Ian, with the focus on detection and characterization of pathogenic *Vibrio spp.* circulating in the environment and elucidating environmental changes associated with anomalous weather events that favor growth of these organisms (**Chapter 4**).

Geophysical connections of waterborne diseases

WBD can include a variety of infection types and causative agents, e.g., viruses, protozoa, fungi, and bacteria, which cause a range of symptoms such as diarrhea, gastroenteritis, wound infections, fever, neurological disorders, and septicemia (**Table 10**). Exposure of humans to WBD occurs primarily by consumption or contact with contaminated water containing the causative agent. However, some WBD can also be acquired through person-to-person contact, notably via the fecal-oral route whereby microscopic fecal particles contaminate water and food. Thus, access to clean and safe

water for drinking, cooking, recreation, sanitation, hygiene, and industry are important to sustaining community health. Unfortunately, many developing countries do not have proper water, sanitation, and hygiene (WASH) infrastructure, especially in rural areas. In 2017, more than two billion people did not have access to basic sanitation, including *ca.* 785 million people without access to basic water services and *ca.* 884 million people lacking safe drinking water (WHO, 2017). Diarrhea is currently the fifth-leading cause of death among children under the age of five, and nearly 88% of diarrhea-associated deaths are due to inadequate WASH conditions (CDC, 2020). Developed countries, have adopted filtration and disinfection treatment methods for drinking water, significantly reducing incidence of disease associated with contaminated water and mortality rates. However, aging infrastructure, chlorine-tolerant and biofilm-related pathogens, and increased recreational water use have created challenges. In the United States, it is estimated that between 12 and 19 million people contract WBDs annually (Trtanj et al., 2016), incurring U.S. $3.33 billion in direct healthcare costs (Collier et al., 2021). Collier and colleagues (2021) presented the most recent estimate of the burden of WBD in the U.S. (2000-2015), showing otitis externa and norovirus infection were the most common illnesses and biofilm-associated pathogens, including non-tuberculosis *Mycobacterium spp*. (NTM), *Pseudomonas spp*., and *Legionella spp*., the most common cause of hospitalization and death. However, *Vibrio spp.* infections, notably wound infections, are an emerging concern in coastal areas of the U.S. eastern seaboard (Archer et al., 2023).

Incidence of WBD is generally more common during the summer since increased water temperatures provide conditions favorable for proliferation of many

infectious agents. Additionally, there is an increased risk for recreational and occupational exposure to infectious WBD during summer months. Marine heatwaves, analogous episodes in the oceans, are caused by anomalous ocean heating at the surface and are expected to exacerbate in a warming climate (Smale et al., 2019), causing irreversible impacts on marine ecosystems (Holbrook et al., 2019). During the past half-century, ocean warming and increased nutrient discharges have been driving marine deoxygenation globally and dead zones near the coast have multiplied 10-fold, causing suffocation of marine life and severe ecological disruption (Breitburg et al., 2018). In the environment, solar ultraviolet radiation (UV) is a major factor for natural inactivation of pathogens and maintaining homeostasis in the environment (Williamson et al., 2017). Recently, it has been suggested that release of terrestrially-derived dissolved organic material and browning of surface waters reduce the potential for solar UV inactivation of pathogens and increase exposure to infectious diseases in humans and wildlife (Williamson et al., 2017). Hence, changes that occur in the aquatic environment can have a significant impact on the abundance and distribution of marine microorganisms and the impact of ocean warming on the emergence and spread of environmental microbial pathogens is rightfully a developing concern (Colwell, 1996; Harvell et al., 2002; Baker-Austin et al., 2013; Vezzulli, 2022; Archer et al., 2023; Brumfield et al., 2023).

Due to their high growth rate and rapid response to environmental signals, members of the genus *Vibrio*, marine bacteria that occur naturally in aquatic and marine habitats globally, have been proposed as a microbial indicator of a changing global climate (Colwell, 1996; Vezzulli et al., 2012, 2015; Baker-Austin et al., 2017; Froelich

and Daines, 2020; Brumfield et al., 2021b, 2023; Vezzulli, 2022; Archer et al., 2023). Estuarine waters, generally warm and brackish, provide ideal environmental conditions for proliferation of pathogenic *Vibrio spp*. (**Chapter 1**) and have warmed at twice the rate of the open oceans and atmosphere (Scanes et al., 2020). Over the past five decades, extensive datasets have been generated with respect to incidence and behavior of pathogenic vibrios in the ocean and estuarine environments (Berk et al., 1976; Huq et al., 1983, 1996; Colwell and Huq, 1994; Colwell, 1996; Mutreja et al., 2011; Hasan et al., 2012; Johnson et al., 2012; Vezzulli et al., 2016; Baker-Austin et al., 2017; Chen et al., 2017; Martinez-Urtaza et al., 2017; Parveen et al., 2020). Importantly, results of these studies demonstrated a transition during the 1980s, namely *Vibrio spp*. become increasingly numerous members of the bacterial community, a transition found to correspond with sea surface warming (*ca*. 0.5°C) and increased incidence of marine-borne *Vibrio* diseases, particularly at higher latitudes. Higher temperatures can also act as selective pressure for strains with higher virulence and/or antimicrobial resistance potential or elevated expression of factors that allow more effective host invasion and environmental fitness. That is, as water temperature increased, the increase was found to be correlated with human exposure to specific pathogenic *Vibrio spp*., i.e., risk of infection, most notably for vulnerable groups, e.g., elderly, low-income households, or immunocompromised individuals.

In **Chapter 3**, analysis of samples collected between 2009 and 2022 and comparison of the data with results of research done in the Chesapeake Bay since the 1960's, a long-term increase in *Vibrio spp.* numbers was observed, with evidence suggesting extended seasonality for these bacteria, particularly during the fall months

of the year. Alarmingly, evidence for increased *Vibrio spp*. abundance in the Chesapeake Bay area is corroborated by a long-term increase in *Vibrio spp.* infections in Maryland between 2006 and 2019, especially from *V. vulnificus* water-associated wound infections (Morgado et al., 2023). In addition, between 2009 and 2022, a long-term increase in the number of confirmed *V. vulnificus* infections in the State of FL was documented (**Chapter 4, Figure 14D**). These observations support claims by Archer et al. (2023), whereby *V. vulnificus* infections are demonstrably increasing in the eastern U.S. It should also be noted that in terms of distribution and prevalence, incidence of cholera has increased globally in recent years (**Chapter 2**), primarily attributed to environmental transmission linked to parameters and anomalous weather events caused by climate change (Colwell, 1996; Lobitz et al., 2000; Jutla et al., 2011, 2015b; Usmani et al., 2023).

Since the 1960s, the world has been plagued by the seventh cholera pandemic, resulting in millions of deaths each year (WHO, 2022b). It has been established that climate is a primary driver of cholera (Colwell, 1996; Lobitz et al., 2000; Pascual et al., 2000; Jutla et al., 2011). Cholera outbreaks occur in two dominant forms: epidemic, characterized by sporadic/rampant occurrence of cases, or endemic, defined as cases occurring annually at a continuous level. The dominant hypothesis for epidemic cholera is related to conditions when the air temperature is anomalously high, and excess rainfall occurs with insufficient and/or damaged (WASH) infrastructure in the region (**Chapter 2, Figure 6**). Human populations will then be at higher risk of exposure to the causative agent and disease (Colwell, 1996; Jutla et al., 2013b, 2015b; Usmani et al., 2021). Endemic cholera has been shown to occur in coastal regions where *V.*

cholerae is constant throughout the year, even at low abundance, and circulating in the aquatic environment. Generally, environmental factors influencing endemic cholera will result in a seasonal peak in recurrence of the disease (Alam et al., 2006b, 2011; Akanda et al., 2009). Sustained epidemic cholera can become endemic in regions demonstrating favorable environmental conditions, with potential for enhanced and continued exposure to, and transmission of, *V. cholerae*.

In addition to *Vibrio spp*., transmission of many other WBD infectious agents is impacted by climate change (**Table 11**). Climate change caused an increase in frequency and duration of extreme weather events such as hurricanes, tsunamis, earthquakes, and tornados (Watson et al., 2007; Honda et al., 2015). During extreme weather events, vulnerable populations are at higher risk of exposure to infectious agents. Honda and colleagues (2015) provided an hypothesis that linked a rise in NTM lung disease and natural disasters following observed geographic overlap of NTM infections and disaster frequencies in the U.S. As average sea surface temperatures rise, some regions could stay warm for longer periods throughout the year. Since the causative agents of many WBD, e.g., *Salmonella spp*. (Akil et al., 2014), Norovirus (Rohayem, 2009), *Campylobacter spp*. (D'Souza et al., 2004), and enterotoxigenic *Escherichia coli* (Kann et al., 2022), exhibit seasonal patterns of growth, a changing climate that alters temperature and rainfall likely will induce extended seasonality of these microorganisms, with serious economic and public health implications. Another consequence of climate variability is melting arctic sea ice, which has emerged as an important driver of marine and terrestrial ecological dynamics as well as pathogen and disease transmission (Turner et al., 2005; Post et al., 2013; Motesharrei et al., 2016).

Under the hypothesis proposed here, temperature impacts growth and survival of microorganisms in the environment, while rainfall has the potential to influence dissemination of the agents. However, severe drought can promote water scarcity, potentially exposing the population to contaminated water. Heavy rainfall and flooding can have a negative impact on sanitation by causing overflows of sewers and treatment facilities and failure of septic systems. Extended periods of intense rainfall can saturate the ground and mobilize subsurface pathogens that may be present in soil, particularly relevant in agricultural areas (Semenza, 2020). In addition, stormwater runoff can lead to surface water contamination by accumulation of microbiological and chemical pollutants on land during dry weather periods and subsequent transport into nearby waterways (Brumfield et al., 2021a). Drinking water-related infections are also expected to increase because of anomalous precipitation events. In a retrospective survey between 1997 and 2009, Chhetri and colleagues (2017) identified a significant increase in cryptosporidiosis and giardiasis following extreme precipitation events, which was exacerbated when extreme precipitation followed a dry period. In 2019, the CDC (CDC, 2019c) suggested a link between the number of reported *Vibrio vulnificus* infections and hurricanes, storm surges, and coastal flooding. A dire example of this phenomenon was the sharp increase in the number of confirmed *V. vulnificus* infections and deaths linked to Hurricane Ian in 2022 (**Chapter 4, Figure 14A**). Interestingly, investigation of environmental parameters acquired using satellite remote sensing data and long-term time series analysis showed changes in sea surface temperature and chlorophyll concentration during and after Hurricane Ian in the Gulf coast of Florida favoring growth of *Vibrio spp*. (**Chapter 4, Figure 14C**). Other notable examples of

WBD outbreaks linked to extreme weather events, namely anomalous rainfall, include salmonellosis incidence in Georgia, U.S. (Lee et al., 2019) and *Escherichia coli* O157:H7 and *Campylobacter jejuni* in Walkerton, Ontario (Auld et al., 2004). These observations, show that temperature and precipitation changes are primary environmental parameters for understanding WBD transmission.

Under current climatic variability projections, extreme heat and precipitation are expected to increase in many parts of the world in the coming decades (Gronlund et al., 2019), and these changes are associated with cascading events of WBD (Semenza, 2020), including spread and emergence of human disease globally along with serious economic implications. While some aquatic microorganisms have infectious potential in humans, these microbes, including *Vibrio spp*., play critical environmental roles in maintaining life on earth, e.g., carbon and nitrogen cycling, and cannot be eradicated. Semenza (2020) presented the associated risk of WBD infection as a function of environmental exposure (individual, community, and WASH) and vulnerability (demographic, income, and public health), and suggested WBD management must include the capricious nature of climate change, in addition to exposure and vulnerability. Therefore, climatic-driven early warning systems are essential to public health and for informing decision-makers and those individuals whose risk of infection is high.

Built infrastructure

Climate variability is expected to accelerate over the next few decades, which increases the likelihood of extreme climate events and their increased frequency and intensity. Though change in temperature and rainfall patterns ascribable to climate variability supports the incidence and distribution of infectious diseases, quantifying its effect on an outbreak is an evolving topic. Insufficient data makes it difficult to demonstrate the impact of climate variability on disease outbreaks and requires further study to understand the relationship between climate and disease sufficiently to intervene in the adverse impact of infectious diseases on the population. Since so many pathogens are of environmental origin and simultaneously play a role in maintaining life on earth, the most effective intervention to mitigate the impact of infectious diseases on a population is the combination of risk predictive modeling and public built infrastructure (transportation and WASH). However, under the futuristic scenarios of climate change, the most vital public built infrastructure holds the potential to become the most prominent adaptation tool as well. The Sixth Assessment Report (AR6) of the Intergovernmental Panel on Climate Change (IPCC, 2021) suggested, in all climate change scenarios, that global surface temperature will increase at least until 2050; in this situation, mitigation alone would not be of great help. There will be the need for adaptation which can only be achieved through easy access to WASH infrastructure (**Figure 20**).

Access to safe drinking water and basic sanitation services are considered basic human rights since they are essential to maintaining healthy livelihoods. However, it is estimated that *ca*. 30% of people lack access to safe drinking water globally, and *ca*.

60% do not have access to safely managed sanitation facilities (WHO, 2017). Access to proper WASH infrastructure is financially dependent, and there are remarkable differences in access to these services within regions and countries. These inequalities in WASH are a global concern and have been addressed by the United Nations (UN) as the sixth goal of the 2030 Sustainable Development Agenda (United Nations, 2015). The common priority between safe drinking water and sanitation facilities services is minimizing human exposure to fecal pathogens and disease transmission, given the well-documented linkages between contaminated drinking water and poor sanitation and hygiene to transmission of diseases (notable examples include cholera, hepatitis A, dysentery, typhoid, polio, malaria, dengue, influenza, and COVID-19). For most regions, when there is abundant access to water services, there are nearly as abundant sanitation services; inversely, lack of water services also conditions lack of sanitation. In these regions, the risk of WBD transmission is high, namely because the lack of water services increases exposure of humans to pathogens. In these regions, climate change exacerbates the problem as accessibility to water resources may be compromised. A natural phenomenon like hurricane or flood events might damage existing WASH infrastructure and expose people to contact with contaminated water.

Predictive intelligence and early warning

In addition to access to proper WASH infrastructure, predicative intelligence can serve as a viable measure for disease mitigation through early warning of impeding infection risk (**Chapter 2**). To develop effective predictive models for environmental

transmission, ground truth observations are needed to ascertain factors that influence occurrence and growth of microorganisms and train the models. Extensive datasets have been generated over the past five decades on incidence and behavior of toxigenic *V. cholerae* and non-cholera *Vibrio spp*. in the ocean and estuarine environments through cross-sectional studies and also some long-term retrospective work, namely in the Bay of Bengal, Arabian Sea, Baltic Sea, Gulf of Mexico, and Chesapeake Bay. In addition, the work presented here contributes to confirming and refining environmental parameter thresholds, e.g., seasonality of occurrence, temperature, salinity, pH, chlorophyll, and dissolved oxygen, for pathogenic *Vibrio spp*. (**Chapters 3 and 4**). These studies show that *Vibrio spp.* are bacteria naturally occurring in the aquatic environment that, under favorable conditions will replicate rapidly and, hence, are highly responsive to environmental stimuli and adaptable to changing environmental parameters (**Chapter 1**).

To effectively employ early warning systems, near-real-time Earth observations are needed as model input for subsequent anticipatory decision-making frameworks. However, ground surveillance is costly and labor intensive, hence, not always possible especially in remote areas or those impacted by natural (**Chapter 4**) or anthropogenic disasters (Usmani et al., 2022). As a result, predictive intelligence using data for satellites, microbiology, and sociology have been proposed as an effective data source, particularly in areas where ground surveillance is not a viable option (Colwell, 1996). Cholera predictive modeling, demonstrated for Haiti (Khan et al., 2017), Zimbabwe (Jutla et al., 2015b), Nepal (Khan et al., 2018), Ukraine (Usmani et al., 2022), and Yemen (Usmani et al., 2023), has the potential, when coupled with both individual and

large-scale intervention early in outbreaks, to contribute to reduction in case fatality rates (Akanda et al., 2012). Similar models have been proposed for *V. vulnificus* in the Chesapeake Bay (Jacobs et al., 2014) and non-cholera vibrios in the Baltic Sea (Semenza et al., 2017). However, it should be noted that ground observations of non-cholera vibrios have been limited essentially to only a few locations, with most studies conducted in developed countries. Future modeling efforts for non-cholera vibrios would also benefit from inclusion of sociological and behavioral factors related to contact with the aquatic environment, such as recreational and occupational activities, that influence contact with waterborne pathogens.

Summary and major conclusions

Epidemiological surveys coupled with environmental surveillance have suggested that sporadic climatic events have significantly impacted the distribution and occurrence of infectious diseases, namely in geographic regions where infectious agents have been found to be naturally occurring in the environment. Climate change-driven emergence and distribution of infectious agents will undoubtedly affect disease prevalence, potentially translating disease outbreaks into endemic diseases and possibly even cascading towards global pandemics. Available evidence suggests that the spatial distribution and abundance of pathogenic *Vibrio spp.* in waters is increasing, notably in coastal regions closer to the poles. The rise in *Vibrio spp.* population numbers is correlated with an increase in the number of confirmed *Vibrio spp.* infections, namely wound infections in developed nations and cholera in regions

lacking access to proper WASH infrastructure. Mitigating the emergence and distribution of infectious diseases is time-sensitive, which promotes use of remote sensing data. However, predictive modelling for global application relies on ground truth observations from multiple regions to train algorithms. Results of this research contribute to the overall understanding of the environmental niche preference for pathogenic *Vibrio spp.*, specifically in the Chesapeake Bay and Florida Gulf coastal waters, as a natural reservoir, and document long-term increase and extended seasonality of pathogenic *Vibrio spp.* in the Chesapeake Bay. In addition, results of the research show the benefit of combining the results of genetic analysis (e.g., SMS and whole genome sequencing), environmental data gathering, and remote sensing as a useful public health tool to proactively detect and characterize environmental pathogens, notably vibrios. Moving forward, atmospheric, hydrologic, space, and earth scientists will need to join with microbiologists and the public health community to develop effective systems for predicting outbreaks and, ultimately, to create an adaptive environment for mitigation of infectious diseases.

Tables

Table 10: Most common waterborne diseases.

Pathogen	Species	Illness
Bacteria		
Shigella	*S. dysenteriae*; *S. flexneri*	Gastroenteritis; Dysentery
Vibrio	*Vibrio cholerae*	Cholera
	Noncholera *Vibrio spp*.	Gastroenteritis; wound infections; septicemia
Salmonella	*Salmonella typhi*	Typhoid
Mycobacterium	*M. avium* complex; Non-tuberculosis *Mycobacterium* (NTM)	Respiratory illness; skin infections
Viruses		
Reoviridae	Rotavirus	Gastroenteritis
Caliciviridae	Norovirus	Gastroenteritis

Table 11: Waterborne disease association with temperature and precipitation.

References are provided in "Appendix A."

Geophysical Parameter	Species	References	Key findings
Temperature	*Shigella spp.*	(1–5)	Positive association
	Vibrio cholerae	(6–13)	
	Noncholera *Vibrio spp.*	(6, 14–18)	
	Salmonella typhi	(19, 20)	
	NTM	(21)	Varies, depending on species and geographic region
	Norovirus	(22–24)	
	Rotavirus	(25–29)	Negative association
Precipitation	*Vibrio cholerae*	(6–13, 30)	Positive association
	Noncholera *Vibrio spp.*	(15, 31–35)	
	Salmonella typhi	(19, 20)	
	Norovirus	(22, 36)	
	NTM	(21)	Varies, depending on species and geographic region
	Shigella spp.	(1–4)	Varies, depending on spatial scales
	Rotavirus	(25–29)	

Figures

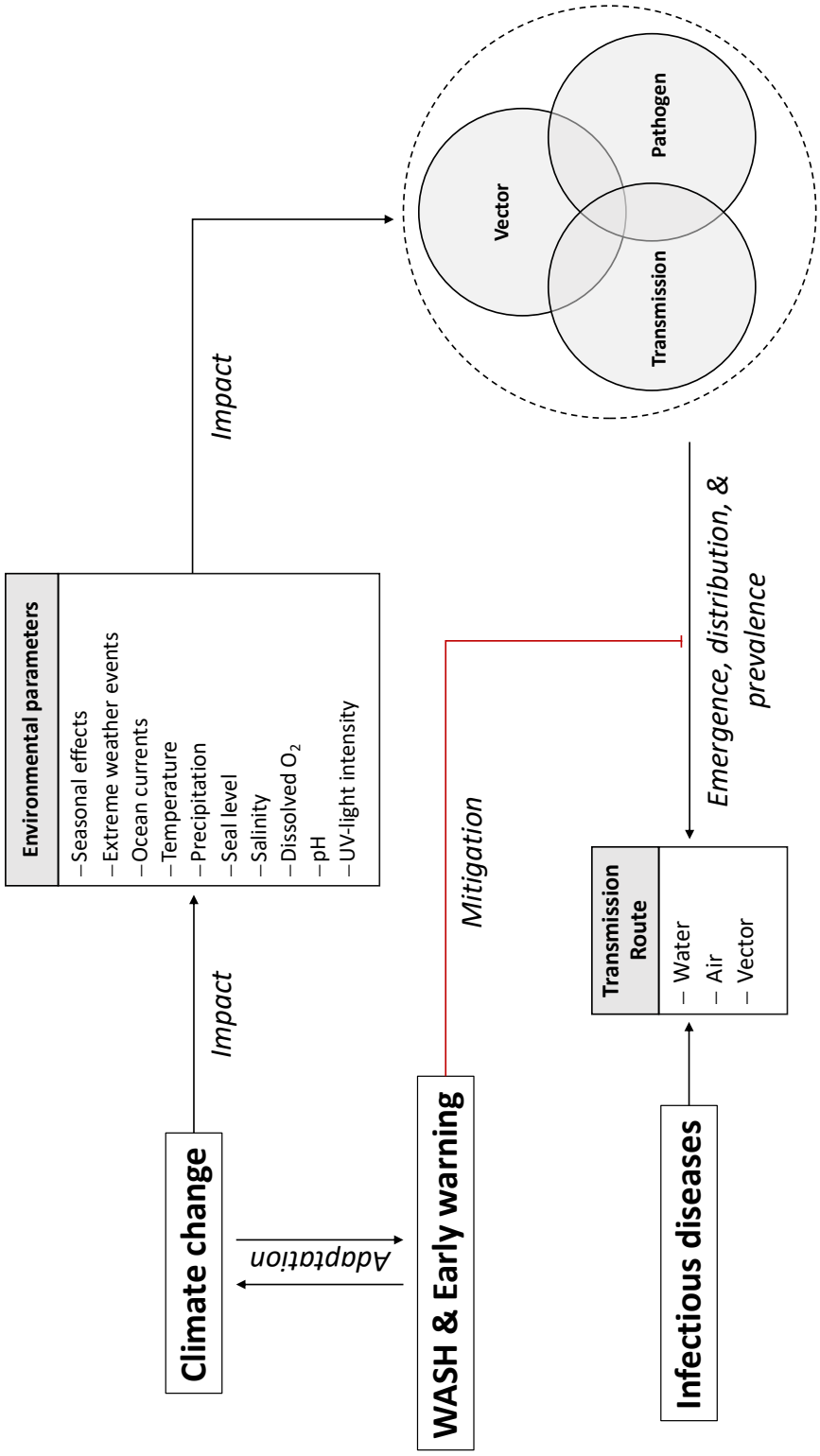

Figure 20: Transmission and mitigation of infectious disease in an era of climate change.

Bibliography

Abanto, M., Gavilan, R.G., Baker-Austin, C., Gonzalez-Escalona, N., Martinez-Urtaza, J., 2020. Global Expansion of Pacific Northwest Vibrio parahaemolyticus Sequence Type 36. Emerg. Infect. Dis. 26, 323–326. https://doi.org/10.3201/eid2602.190362

Acharya, K., Blackburn, A., Mohammed, J., Haile, A.T., Hiruy, A.M., Werner, D., 2020. Metagenomic water quality monitoring with a portable laboratory. Water Res. 184, 116112. https://doi.org/10.1016/j.watres.2020.116112

Acker, J.G., Leptoukh, G., 2007. Online analysis enhances use of NASA Earth science data. Eos Trans. Am. Geophys. Union 88, 14–17. https://doi.org/10.1029/2007EO020003

Akanda, A., Aziz, S., Jutla, A., Hua, A., Alam, M., Ahsan, G., Colwell, R., 2018. Satellites and cell phones form a cholera early-warning system. EOS Hydrol. Cryosphere Earh Surf. https://doi.org/10.1029/2018EO094839

Akanda, A.S., Jutla, A.S., Gute, D.M., Evans, T., Islam, S., 2012. Reinforcing cholera intervention through prediction-aided prevention. Bull. World Health Organ. 90, 243–244. https://doi.org/10.2471/BLT.11.092189

Akanda, A.S., Jutla, A.S., Islam, S., 2009. Dual peak cholera transmission in Bengal Delta: A hydroclimatological explanation. Geophys. Res. Lett. 36, L19401. https://doi.org/10.1029/2009GL039312

Akil, L., Ahmad, H.A., Reddy, R.S., 2014. Effects of climate change on Salmonella infections. Foodborne Pathog. Dis. 11, 974–980.

Alam, M., Islam, A., Bhuiyan, N., Rahim, N., Hossain, A., Khan, G.Y., Ahmed, D., Watanabe, H., Izumiya, H., Faruque, A.G., others, 2011. Clonal transmission, dual peak, and off-season cholera in Bangladesh. Infect. Ecol. Epidemiol. 1, 7273.

Alam, M., Kasan, N.A., Sadique, A., Bhuiyan, N.A., Ahmed, K.U., Nusrin, S., Nair, G.B., Siddique, A.K., Sack, R.B., Sack, D.A., Huq, A., Colwell, R.R., 2006a. Seasonal cholera caused by Vibrio cholerae serogroups O1 and O139 in the coastal aquatic environment of Bangladesh. Appl. Environ. Microbiol. 72, 4096–4104. https://doi.org/10.1128/AEM.00066-06

Alam, M., Kasan, N.A., Sadique, A., Bhuiyan, N.A., Ahmed, K.U., Nusrin, S., Nair, G.B., Siddique, A.K., Sack, R.B., Sack, D.A., Huq, A., Colwell, R.R., 2006b. Seasonal cholera caused by Vibrio cholerae serogroups O1 and O139 in the coastal aquatic environment of Bangladesh. Appl. Environ. Microbiol. 72, 4096–4104. https://doi.org/10.1128/AEM.00066-06

Albert, M.J., 1994. Vibrio cholerae O139 Bengal. J. Clin. Microbiol. 32, 2345.

Albertini, M.C., Accorsi, A., Teodori, L., Pierfelici, L., Uguccioni, F., Rocchi, M.B., Burattini, S., Citterio, B., 2006. Use of multiparameter analysis for Vibrio

alginolyticus viable but nonculturable state determination. Cytometry A 69, 260–265.

Ali, M., Nelson, A.R., Lopez, A.L., Sack, D.A., 2015. Updated global burden of cholera in endemic countries. PLoS Negl. Trop. Dis. 9, 1–13. https://doi.org/10.1371/journal.pntd.0003832

Almada, A.A., Tarrant, A.M., 2016. Vibrio elicits targeted transcriptional responses from copepod hosts. FEMS Microbiol. Ecol. 92, fiw072.

Almagro-Moreno, S., Taylor, R.K., 2013. Cholera: Environmental Reservoirs and Impact on Disease Transmission. Microbiol. Spectr. 1, OH-0003-2012. https://doi.org/10.1128/microbiolspec.OH-0003-2012

Andrews, S.C., 2019. FastQC [WWW Document]. URL https://github.com/s-andrews/FastQC (accessed 10.2.19).

Andrews, W.H., Hammack, T.S., 2019. BAM Chapter 1: Food sampling/preparation of sample homogenate [WWW Document]. Bacteriol. Anal. Man. URL https://www.fda.gov/food/laboratory-methods-food/bam-chapter-1-food-samplingpreparation-sample-homogenate (accessed 4.28.23).

APHA, 2012. Standard Methods for the Examination of Water and Wastewater, 22nd ed. American Public Health Association.

Archer, E.J., Baker-Austin, C., Osborn, T.J., Jones, N.R., Martínez-Urtaza, J., Trinanes, J., Oliver, J.D., González, F.J.C., Lake, I.R., 2023. Climate warming and increasing Vibrio vulnificus infections in North America. Sci. Rep. 13, 3893.

Auld, H., MacIver, D., Klaassen, J., 2004. Heavy rainfall and waterborne disease outbreaks: the Walkerton example. J. Toxicol. Environ. Health A 67, 1879–1887. https://doi.org/10.1080/15287390490493475

Azarian, T., Ali, A., Johnson, J.A., Jubair, M., Cella, E., Ciccozzi, M., Nolan, D.J., Farmerie, W., Rashid, M.H., Sinha-Ray, S., Alam, M.T., Morris, J.G., Salemi, M., 2016. Non-toxigenic environmental Vibrio cholerae O1 strain from Haiti provides evidence of pre-pandemic cholera in Hispaniola. Sci. Rep. 6, 36115. https://doi.org/10.1038/srep36115

Aziz, S., Pakhtigian, E.L., Akanda, A.S., Jutla, A., Huq, A., Alam, M., Ashan, G.U., Colwell, R.R., 2021. Does improved risk information increase the value of cholera prevention? An analysis of stated vaccine demand in slum areas of urban Bangladesh. Soc. Sci. Med. 272, 113716.

Baker, R.E., Mahmud, A.S., Miller, I.F., Rajeev, M., Rasambainarivo, F., Rice, B.L., Takahashi, S., Tatem, A.J., Wagner, C.E., Wang, L.-F., Wesolowski, A., Metcalf, C.J.E., 2021. Infectious disease in an era of global change. Nat. Rev. Microbiol. 20, 1–13. https://doi.org/10.1038/s41579-021-00639-z

Baker-Austin, C., Jenkins, C., Dadzie, J., Mestanza, O., Delgado, E., Powell, A., Bean, T., Martinez-Urtaza, J., 2020a. Genomic epidemiology of domestic and

travel-associated Vibrio parahaemolyticus infections in the UK, 2008–2018. Food Control 115, 107244. https://doi.org/10.1016/j.foodcont.2020.107244

Baker-Austin, C., Oliver, J.D., 2018. Vibrio vulnificus: new insights into a deadly opportunistic pathogen. Environ. Microbiol. 20, 423–430. https://doi.org/10.1111/1462-2920.13955

Baker-Austin, C., Oliver, J.D., Alam, M., Ali, A., Waldor, M.K., Qadri, F., Martinez-Urtaza, J., 2018. Vibrio spp. infections. Nat. Rev. Dis. Primer 4, 8. https://doi.org/10.1038/s41572-018-0005-8

Baker-Austin, C., Stockley, L., Rangdale, R., Martinez-Urtaza, J., 2010. Environmental occurrence and clinical impact of Vibrio vulnificus and Vibrio parahaemolyticus: a European perspective. Environ. Microbiol. Rep. 2, 7–18. https://doi.org/10.1111/j.1758-2229.2009.00096.x

Baker-Austin, C., Trinanes, J., Gonzalez-Escalona, N., Martinez-Urtaza, J., 2017. Non-Cholera Vibrios: The Microbial Barometer of Climate Change. Trends Microbiol. 25, 76–84. https://doi.org/10.1016/j.tim.2016.09.008

Baker-Austin, C., Trinanes, J., Martinez-Urtaza, J., 2020b. The new tools revolutionizing Vibrio science. Environ. Microbiol. 22, 4096–4100. https://doi.org/10.1111/1462-2920.15083

Baker-Austin, C., Trinanes, J.A., Salmenlinna, S., Löfdahl, M., Siitonen, A., Taylor, N.G., Martinez-Urtaza, J., 2016. Heat wave–associated vibriosis, Sweden and Finland, 2014. Emerg. Infect. Dis. 22, 1216.

Baker-Austin, C., Trinanes, J.A., Taylor, N.G.H., Hartnell, R., Siitonen, A., Martinez-Urtaza, J., 2013. Emerging Vibrio risk at high latitudes in response to ocean warming. Nat. Clim. Change 3, 73–77. https://doi.org/10.1038/nclimate1628

Banakar, V., Constantin de Magny, G., Jacobs, J., Murtugudde, R., Huq, A., Wood, R.J., Colwell, R.R., 2011. Temporal and spatial variability in the distribution of Vibrio vulnificus in the Chesapeake Bay: a hindcast study. EcoHealth 8, 456–467. https://doi.org/10.1007/s10393-011-0736-4

Barbieri, E., Falzano, L., Fiorentini, C., Pianetti, A., Baffone, W., Fabbri, A., Matarrese, P., Casiere, A., Katouli, M., Kühn, I., Möllby, R., Bruscolini, F., Donelli, G., 1999. Occurrence, Diversity, and Pathogenicity of Halophilic Vibrio spp. and Non-O1 Vibrio cholerae from Estuarine Waters along the Italian Adriatic Coast. Appl. Environ. Microbiol. 65, 2748 LP – 2753. https://doi.org/10.1128/AEM.65.6.2748-2753.1999

Bartlett, A., Padfield, D., Lear, L., Bendall, R., Vos, M., 2022. A comprehensive list of bacterial pathogens infecting humans. Microbiology 168, 001269.

Barua, D., 1992. History of Cholera. Plenum Books Corperation, New York, NY.

Basedow, S.L., McKee, D., Lefering, I., Gislason, A., Daase, M., Trudnowska, E., Egeland, E.S., Choquet, M., Falk-Petersen, S., 2019. Remote sensing of zooplankton swarms. Sci. Rep. 9, 686.

Basu, A., Garg, P., Datta, S., Chakraborty, S., Bhattacharya, T., Khan, A., Ramamurthy, S., Bhattacharya, S., Yamasaki, S., Takeda, Y., others, 2000. Vibrio cholerae O139 in Calcutta, 1992-1998: incidence, antibiograms, and genotypes. Emerg. Infect. Dis. 6, 139.

Bauer, A., Rørvik, L.M., 2007. A novel multiplex PCR for the identification of Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus. Lett. Appl. Microbiol. 45, 371–375. https://doi.org/10.1111/j.1472-765X.2007.02195.x

Bej, A.K., Patterson, D.P., Brasher, C.W., Vickery, M.C.L., Jones, D.D., Kaysner, C.A., 1999. Detection of total and hemolysin-producing Vibrio parahaemolyticus in shellfish using multiplex PCR amplification of tl, tdh and trh. J. Microbiol. Methods 36, 215–225. https://doi.org/10.1016/S0167-7012(99)00037-8

Berk, S.G., Colwell, R., 1981. Transfer of mercury through a marine microbial food web. J. Exp. Mar. Biol. Ecol. 52, 157–172.

Berk, S.G., Colwell, R.R., Small, E.B., 1976. A study of feeding responses to bacterial prey by estuarine ciliates. Trans. Am. Microsc. Soc. 514–520.

Bhishagratna, K.K., 1963. Susruta: An English Translation of the Sushruta Samhita Based on Original Sanskrit Text with a Full and Comprehensive Introduction, Additional Texts, Different Readings, Notes, Comparative Views, Index, Glossary and Plates. Chowkhamba Sanskrit series office.

Bhuiyan, N.A., Ansaruzzaman, M., Kamruzzaman, M., Alam, K., Chowdhury, N.R., Nishibuchi, M., Faruque, S.M., Sack, D.A., Takeda, Y., Nair, G.B., 2002. Prevalence of the pandemic genotype of Vibrio parahaemolyticus in Dhaka, Bangladesh, and significance of its distribution across different serotypes. J. Clin. Microbiol. 40, 284–286. https://doi.org/10.1128/jcm.40.1.284-286.2002

Bian, S., Jia, Y., Zhan, Q., Wong, N.-K., Hu, Q., Zhang, W., Zhang, Y., Li, L., 2021. VPsero: rapid serotyping of Vibrio parahaemolyticus using serogroup-specific genes based on whole-genome sequencing data. Front. Microbiol. 12, 620224.

Blackwell, K.D., Oliver, J.D., 2008. The ecology of Vibrio vulnificus, Vibrio cholerae, and Vibrio parahaemolyticus in North Carolina Estuaries. J. Microbiol. 46, 146–153. https://doi.org/10.1007/s12275-007-0216-2

Böer, S.I., Heinemeyer, E.-A., Luden, K., Erler, R., Gerdts, G., Janssen, F., Brennholt, N., 2013. Temporal and spatial distribution patterns of potentially pathogenic Vibrio spp. at recreational beaches of the German north sea. Microb. Ecol. 65, 1052–1067. https://doi.org/10.1007/s00248-013-0221-4

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinforma. Oxf. Engl. 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bowers, J.C., Lüdeke, C.H.M., Jones, J.L., Hastback, W., DeRosia-Banick, K., Carey, D.H., 2014. Abundance of Vibrio cholerae, V. vulnificus, and V. parahaemolyticus in Oysters (Crassostrea virginica) and Clams (Mercenaria

mercenaria) from Long Island Sound. Appl. Environ. Microbiol. 80, 7667–7672. https://doi.org/10.1128/aem.02820-14

Boyd, E.F., Cohen, A.L. V, Naughton, L.M., Ussery, D.W., Binnewies, T.T., Stine, O.C., Parent, M.A., 2008. Molecular analysis of the emergence of pandemic Vibrio parahaemolyticus. BMC Microbiol. 8, 110.

Boyd, F.E., Moyer, K.E., Shi, L., Waldor, M.K., 2000. Infectious CTXΦ and the Vibrio Pathogenicity Island Prophage in Vibrio mimicus: Evidence for Recent Horizontal Transfer between V. mimicus and V. cholerae. Infect. Immun. 68, 1507–1513. https://doi.org/10.1128/IAI.68.3.1507-1513.2000

Breitburg, D., Levin, L.A., Oschlies, A., Grégoire, M., Chavez, F.P., Conley, D.J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., 2018. Declining oxygen in the global ocean and coastal waters. Science 359, eaam7240. https://doi.org/10.1126/science.aam7240

Brettin, T., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Olsen, G.J., Olson, R., Overbeek, R., Parrello, B., Pusch, G.D., Shukla, M., Thomason 3rd, J.A., Stevens, R., Vonstein, V., Wattam, A.R., Xia, F., 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci. Rep. 5, 8365. https://doi.org/10.1038/srep08365

Broberg, C.A., Calder, T.J., Orth, K., 2011. Vibrio parahaemolyticus cell biology and pathogenicity determinants. Microbes Infect. 13, 992–1001. https://doi.org/10.1016/j.micinf.2011.06.013

Bross, M.H., Soch, K., Morales, R., Mitchell, R.B., 2007. Vibrio vulnificus infection: diagnosis and treatment. Am. Fam. Physician 76, 539–544.

Broza, M., Gancz, H., Kashi, Y., 2008. The association between non-biting midges and Vibrio cholerae. Environ. Microbiol. 10, 3193–3200. https://doi.org/10.1111/j.1462-2920.2008.01714.x

Brumfield, K.D., Carignan, B.M., Ray, J.N., Jumpre, P.E., Son, M.S., 2017. Laboratory techniques used to maintain and differentiate biotypes of vibrio cholerae clinical and environmental isolates. J. Vis. Exp. 2017. https://doi.org/10.3791/55760

Brumfield, K.D., Carignan, B.M., Son, M.S., 2018a. Genotypic and phenotypic assays to distinguish Vibrio cholerae biotype, in: Sikora, A.E. (Ed.), Methods in Molecular Biology: Vibrio Cholerae. Humana Press, New York, NY, pp. 11–28. https://doi.org/10.1007/978-1-4939-8685-9_2

Brumfield, K.D., Carignan, B.M., Son, M.S., 2018b. Laboratory culturing techniques and maintenance of Vibrio cholerae, in: Sikora, A.E. (Ed.), Methods in Molecular Biology: Vibrio Cholerae. Humana Press, New York, NY, pp. 1–9. https://doi.org/10.1007/978-1-4939-8685-9_1

Brumfield, K.D., Chen, A.J., Gangwar, M., Moiz, U., Hasan, N.A., Jutla, A.S., Huq, A., Colwell, R.R., 2023. Environmental Factors Influencing Occurrence of

Vibrio parahaemolyticus and Vibrio vulnificus. Appl. Environ. Microbiol. 0, e00307-23. https://doi.org/10.1128/aem.00307-23

Brumfield, K.D., Cotruvo, J.A., Leddy, M.B., Colwell, R.R., Huq, A., 2020a. Using Metagenomic Analysis to Assess Water Quality. J. AWWA 112, 84–87. https://doi.org/10.1002/awwa.1618

Brumfield, K.D., Cotruvo, J.A., Shanks, O.C., Sivaganesan, M., Hey, J., Hasan, N.A., Huq, A., Colwell, R.R., Leddy, M.B., 2021a. Metagenomic Sequencing and Quantitative Real-Time PCR for Fecal Pollution Assessment in an Urban Watershed. Front. Water 3, 626849. https://doi.org/10.3389/frwa.2021.626849

Brumfield, K.D., Hasan, N.A., Leddy, M.B., Cotruvo, J., Rashed, S.M., Colwell, R.R., Huq, A., 2020b. A Comparative Analysis of Drinking Water Employing Metagenomics. PLoS ONE 15, e0231210. https://doi.org/10.1371/journal.pone.0231210

Brumfield, K.D., Huq, A., Colwell, R.R., Olds, J.L., Leddy, M.B., 2020c. Microbial resolution of whole genome shotgun and 16S amplicon metagenomic sequencing using publicly available NEON data. PLOS ONE 15, e0228899.

Brumfield, K.D., Leddy, M., Usmani, M., Cotruvo, J.A., Tien, C.-T., Dorsey, S., Graubics, K., Fanelli, B., Zhou, I., Registe, N., Dadlani, M., Wimalarante, M., Jinasena, D., Abayagunawardena, R., Withanachchi, C., Huq, A., Jutla, A., Colwell, R.R., 2022a. Microbiome Analysis for Wastewater Surveillance during COVID-19. mBio 0, e00591-22. https://doi.org/10.1128/mbio.00591-22

Brumfield, K.D., Raupp, M.J., Haji, D., Simon, C., Graf, J., Cooley, J.R., Janton, S.T., Meister, R.C., Huq, A., Colwell, R.R., Hasan, N.A., 2022b. Gut microbiome insights from 16S rRNA analysis of 17-year periodical cicadas (Hemiptera: Magicicada spp.) Broods II, VI, and X. Sci. Rep. 12, 16967. https://doi.org/10.1038/s41598-022-20527-7

Brumfield, K.D., Usmani, M., Chen, K.M., Gangwar, M., Jutla, A.S., Huq, A., Colwell, R.R., 2021b. Environmental parameters associated with incidence and transmission of pathogenic Vibrio spp. Environ. Microbiol. 23, 7314–7340.

Camacho, A., Bouhenia, M., Alyusfi, R., Alkohlani, A., Naji, M.A.M., de Radiguès, X., Abubakar, A.M., Almoalmi, A., Seguin, C., Sagrado, M.J., Poncin, M., McRae, M., Musoke, M., Rakesh, A., Porten, K., Haskew, C., Atkins, K.E., Eggo, R.M., Azman, A.S., Broekhuijsen, M., Saatcioglu, M.A., Pezzoli, L., Quilici, M.L., Al-Mesbahy, A.R., Zagaria, N., Luquero, F.J., 2018. Cholera epidemic in Yemen, 2016–18: an analysis of surveillance data. Lancet Glob. Health 6, e680–e690. https://doi.org/10.1016/S2214-109X(18)30230-4

Capstick, S., Thierry, A., Cox, E., Berglund, O., Westlake, S., Steinberger, J.K., 2022. Civil disobedience by scientists helps press for urgent climate action. Nat. Clim. Change 12, 773–774.

Carignan, B.M., Brumfield, K.D., Son, M.S., 2016. Single nucleotide polymorphisms in regulator-encoding genes have an additive effect on virulence gene expression in a Vibrio cholerae clinical isolate. mSphere 1, e00253-16. https://doi.org/10.1128/mSphere.00253-16

Cash, R.A., Music, S.I., Libonati, J.P., Snyder, M.J., Wenzel, R.P., Hornick, R.B., 1974. Response of man to infection with Vibrio cholerae. I. Clinical, serologic, and bacteriologic responses to a known inoculum. J. Infect. Dis. 129, 45–52.

CDC, 2005. Vibrio illnesses after Hurricane Katrina--multiple states, August-September 2005. MMWR Morb. Mortal. Wkly. Rep. 54, 928–931.

CDC, 2012. Principles of Epidemiology in Public Health Practice [WWW Document]. URL https://www.cdc.gov/csels/dsepd/ss1978/lesson1/section11.html (accessed 10.14.19).

CDC, 2016. Foodborne Diseases Active Surveillance Network (FoodNet). [WWW Document]. URL https://www.cdc.gov/foodnet/reports/data/incidence-trends.html (accessed 10.2.20).

CDC, 2019a. Cholera and Other Vibrio Illness Surveillance (COVIS) [WWW Document].

CDC, 2019b. Vibrio Species Causing Vibriosis [WWW Document]. URL https://www.cdc.gov/vibrio/faq.html (accessed 4.28.23).

CDC, 2019c. Vibrio vulnificus & Wounds [WWW Document]. URL https://www.cdc.gov/vibrio/wounds.html (accessed 6.14.23).

CDC, 2020. Disease Threats and Global WASH Killers: Cholera, Typhoid, and Other Waterborne Infections [WWW Document]. URL https://www.cdc.gov/healthywater/global/WASH.html (accessed 6.14.23).

CDC, 2021. FoodNet Fast [WWW Document]. Foodborne Dis. Act. Surveill. Netw. FoodNet. URL https://wwwn.cdc.gov/foodnetfast/ (accessed 6.30.21).

CDC, 2022a. Recommendations for the Use of Antibiotics for the Treatment of Cholera [WWW Document]. URL https://www.cdc.gov/cholera/treatment/antibiotic-treatment.html (accessed 1.20.23).

CDC, 2022b. Vaccines [WWW Document]. Cholera - Vibrio Cholerae Infect. URL https://www.cdc.gov/cholera/vaccines.html (accessed 1.27.23).

CDC, 2023. Waterbone Disease in the United States [WWW Document]. Waterborne Dis. Outbreak Surveill. Report. URL https://www.cdc.gov/healthywater/surveillance/burden/index.html (accessed 1.27.23).

Ceccarelli, D., Chen, A., Hasan, N.A., Rashed, S.M., Huq, A., Colwell, R.R., 2015. Non-O1/Non-O139 Vibrio cholerae carrying multiple virulence factors and V.

cholerae O1 in the Chesapeake Bay, Maryland. Appl. Environ. Microbiol. 81, 1909–1918. https://doi.org/10.1128/AEM.03540-14

Ceccarelli, D., Hasan, N.A., Huq, A., Colwell, R.R., 2013. Distribution and dynamics of epidemic and pandemic Vibrio parahaemolyticus virulence factors. Front. Cell. Infect. Microbiol. 3, 1–9. https://doi.org/10.3389/fcimb.2013.00097

Chalita, M., Ha, S., Kim, Y.O., Oh, H.-S., Yoon, S.-H., Chun, J., 2020. Improved metagenomic taxonomic profiling using a curated core gene-based bacterial database reveals unrecognized species in the genus Streptococcus. Pathogens 9, 204.

Chamanrokh, P., Colwell, R.R., Huq, A., 2022. Loop-mediated isothermal amplification (LAMP) assay for rapid detection of viable but non-culturable Vibrio cholerae O1. Can. J. Microbiol. 68, 103–110.

Chao, A., Lee, S.-M., 1992. Estimating the number of classes via sample coverage. J. Am. Stat. Assoc. 87, 210–217.

Che, Y., Yang, Y., Xu, X., Břinda, K., Polz, M.F., Hanage, W.P., Zhang, T., 2021. Conjugative plasmids interact with insertion sequences to shape the horizontal transfer of antimicrobial resistance genes. Proc. Natl. Acad. Sci. 118, e2008731118. https://doi.org/10.1073/pnas.2008731118

Chen, A.J., Hasan, N.A., Haley, B.J., Taviani, E., Tarnowski, M., Brohawn, K., Johnson, C.N., Colwell, R.R., Huq, A., 2017. Characterization of pathogenic Vibrio parahaemolyticus from the Chesapeake Bay, Maryland. Front. Microbiol. 8, 1–10. https://doi.org/10.3389/fmicb.2017.02460

Chen, Y., Stine, O.C., Badger, J.H., Gil, A.I., Nair, G.B., Nishibuchi, M., Fouts, D.E., 2011. Comparative genomic analysis of Vibrio parahaemolyticus : serotype conversion and virulence. BMC Genomics 12, 294. https://doi.org/10.1186/1471-2164-12-294

Chhetri, B.K., Takaro, T.K., Balshaw, R., Otterstatter, M., Mak, S., Lem, M., Zubel, M., Lysyshyn, M., Clarkson, L., Edwards, J., others, 2017. Associations between extreme precipitation and acute gastro-intestinal illness due to cryptosporidiosis and giardiasis in an urban Canadian drinking water system (1997–2009). J. Water Health 15, 898–907.

Chinazzi, M., Davis, J.T., Ajelli, M., Gioannini, C., Litvinova, M., Merler, S., Pastore y Piontti, A., Mu, K., Rossi, L., Sun, K., others, 2020. The effect of travel restrictions on the spread of the 2019 novel coronavirus (COVID-19) outbreak. Science 368, 395–400.

Chowdhury, A., Ishibashi, M., Thiem, V.D., Tuyet, D.T.N., Van Tung, T., Chien, B.T., von Seidlein, L., Canh, D.G., Clemens, J., Trach, D.D., Nishibuchi, M., 2004. Emergence and Serovar Transition of Vibrio parahaemolyticus Pandemic Strains Isolated during a Diarrhea Outbreak in Vietnam between 1997 and 1999. Microbiol. Immunol. 48, 319–327. https://doi.org/10.1111/j.1348-0421.2004.tb03513.x

Chowdhury, F.R., Nur, Z., Hassan, N., von Seidlein, L., Dunachie, S., 2017. Pandemics, pathogenicity and changing molecular epidemiology of cholera in the era of global warming. Ann. Clin. Microbiol. Antimicrob. 16, 1–6.

Chun, J., Haley, B.J., Chertkov, O., Nair, G.B., Jeon, Y.-S., Colwell, R.R., Munk, A.C., Taviani, E., Grim, C.J., Choi, S.Y., Hasan, N.A., Kim, D.W., Huq, A., Lee, J.-H., Detter, J.C., Bruce, D.C., Lee, J.H., Brettin, T.S., Walters, R.A., Challacombe, J.F., Han, C.S., Saunders, E., Meincke, L., 2009. Comparative genomics reveals mechanism for short-term and long-term clonal transitions in pandemic Vibrio cholerae. Proc. Natl. Acad. Sci. 106, 15442–15447. https://doi.org/10.1073/pnas.0907787106

Collier, S.A., Deng, L., Adam, E.A., Benedict, K.M., Beshearse, E.M., Blackstock, A.J., Bruce, B.B., Derado, G., Edens, C., Fullerton, K.E., others, 2021. Estimate of burden and direct healthcare cost of infectious waterborne disease in the United States. Emerg. Infect. Dis. 27, 140.

Colwell, R., Brayton, P., Grimes, D., Roszak, D., Huq, S., Palmer, L., 1985. Viable but non-culturable Vibrio cholerae and related pathogens in the environment: implications for release of genetically engineered microorganisms. Nat. Biotechnol. 3, 817–820.

Colwell, R., Grimes, D., 1984. Vibrio diseases of marine fish populations. Helgoländer Meeresunters. 37, 265–287.

Colwell, R.R., 1996. Global Climate and Infectious Disease: The Cholera Paradigm. Science 274, 2025 LP – 2031. https://doi.org/10.1126/science.274.5295.2025

Colwell, R.R., 2000. Viable but nonculturable bacteria: a survival strategy. J. Infect. Chemother. 6, 121–125.

Colwell, R.R., 2006. A global and historical perspective of the genus Vibrio, in: The Biology of Vibrios. Wiley Online Library, pp. 1–11.

Colwell, R.R., Brayton, P., Herrington, D., Tall, B., Huq, A., Levine, M.M., 1996. Viable but non-culturable Vibrio cholerae O1 revert to a cultivable state in the human intestine. World J. Microbiol. Biotechnol. 12, 28–31. https://doi.org/10.1007/BF00327795

Colwell, R.R., Huq, A., 1994. Vibrios in the environment: viable but nonculturable Vibrio cholerae, in: Vibrio Cholerae and Cholera. Wiley Online Library, pp. 117–133.

Colwell, R.R., Huq, A., Chowdhury, M.A., Brayton, P.R., Xu, B., 1995. Serogroup conversion of Vibrio cholerae. Can. J. Microbiol. 41, 946–950. https://doi.org/10.1139/m95-131

Colwell, R.R., Huq, A., Islam, M.S., Aziz, K.M.A., Yunus, M., Khan, N.H., Mahmud, A., Sack, R.B., Nair, G.B., Chakraborty, J., 2003. Reduction of cholera in Bangladeshi villages by simple filtration. Proc. Natl. Acad. Sci. 100, 1051–1055.

Colwell, R.R., Kaper, J., Joseph, S., 1977. Vibrio cholerae, Vibrio parahaemolyticus, and other vibrios: occurrence and distribution in Chesapeake Bay. Science 198, 394–396.

Colwell, R.R., Seidler, R.J., Kaper, J., Joseph, S.W., Garges, S., Lockman, H., Maneval, D., Bradford, H., Roberts, N., Remmers, E., Huq, I., Huq, A., 1981. Occurrence of Vibrio cholerae serotype O1 in Maryland and Louisiana estuaries. Appl. Environ. Microbiol. 41, 555–558.

Colwell, R.R., Spira, W.M., 1992. The ecology of Vibrio cholerae, in: Cholera. Springer, pp. 107–127.

Conner, J.G., Teschler, J.K., Jones, C.J., Yildiz, F.H., 2016. Staying Alive: Vibrio cholerae's Cycle of Environmental Survival, Transmission, and Dissemination. Microbiol. Spectr. 4, 10.1128/microbiolspec.VMBF-0015–2015. https://doi.org/10.1128/microbiolspec.VMBF-0015-2015

Constantin de Magny, G., Colwell, R.R., 2009. Cholera and climate: a demonstrated relationship. Trans. Am. Clin. Climatol. Assoc. 120, 119.

Constantin de Magny, G., Murtugudde, R., Sapiano, M.R.P., Nizam, A., Brown, C.W., Busalacchi, A.J., Yunus, M., Nair, G.B., Gil, A.I., Lanata, C.F., Calkins, J., Manna, B., Rajendran, K., Bhattacharya, M.K., Huq, A., Sack, R.B., Colwell, R.R., 2008. Environmental signatures associated with cholera epidemics. Proc. Natl. Acad. Sci. 105, 17676 LP – 17681. https://doi.org/10.1073/pnas.0809654105

Cuadrat, R.R.C., Sorokina, M., Andrade, B.G., Goris, T., Dávila, A.M.R., 2020. Global ocean resistome revealed: Exploring antibiotic resistance gene abundance and distribution in TARA Oceans samples. GigaScience 9, giaa046. https://doi.org/10.1093/gigascience/giaa046

Davis, B.J.K., Jacobs, J.M., Davis, M.F., Schwab, K.J., DePaola, A., Curriero, F.C., 2017. Environmental Determinants of Vibrio parahaemolyticus in the Chesapeake Bay. Appl. Environ. Microbiol. 83, e01147-17. https://doi.org/10.1128/AEM.01147-17

De, R., Mukhopadhyay, A.K., Dutta, S., 2020. Metagenomic analysis of gut microbiome and resistome of diarrheal fecal samples from Kolkata, India, reveals the core and variable microbiota including signatures of microbial dark matter. Gut Pathog. 12, 32. https://doi.org/10.1186/s13099-020-00371-8

De Silva, B.C.J., Hossain, S., Dahanayake, P.S., Kang, T., Heo, G., 2019. Vibrio spp. from Yesso scallop (Patinopecten yessoensis) demonstrating virulence properties and antimicrobial resistance. J. Food Saf. e12634. https://doi.org/10.1111/jfs.12634

Deeb, R., Tufford, D., Scott, G.I., Moore, J.G., Dow, K., 2018. Impact of Climate Change on Vibrio vulnificus Abundance and Exposure Risk. Estuaries Coasts 41, 2289–2303. https://doi.org/10.1007/s12237-018-0424-5

DeLoney-Marino, C.R., Wolfe, A.J., Visick, K.L., 2003. Chemoattraction of Vibrio fischeri to Serine, Nucleosides, and N-Acetylneuraminic Acid, a Component of Squid Light-Organ Mucus. Appl. Environ. Microbiol. 69, 7527 LP – 7530. https://doi.org/10.1128/AEM.69.12.7527-7530.2003

DeLuca, N.M., Zaitchik, B.F., Guikema, S.D., Jacobs, J.M., Davis, B.J.K., Curriero, F.C., 2020. Evaluation of remotely sensed prediction and forecast models for Vibrio parahaemolyticus in the Chesapeake Bay. Remote Sens. Environ. 250, 112016. https://doi.org/10.1016/j.rse.2020.112016

DePaola, A., Kaysner, C.A., Bowers, J., Cook, D.W., 2000. Environmental investigations of Vibrio parahaemolyticus in oysters after outbreaks in Washington, Texas, and New York (1997 and 1998). Appl. Environ. Microbiol. 66, 4649–4654.

DePaola, A., Ulaszek, J., Kaysner, C.A., Tenge, B.J., Nordstrom, J.L., Wells, J., Puhr, N., Gendel, S.M., 2003. Molecular, serological, and virulence characteristics of Vibrio parahaemolyticus isolated from environmental, food, and clinical sources in North America and Asia. Appl. Environ. Microbiol. 69, 3999–4005.

Deshayes, S., Daurel, C., Cattoir, V., Parienti, J.-J., Quilici, M.-L., de La Blanchardière, A., 2015. Non-O1, non-O139 Vibrio cholerae bacteraemia: case report and literature review. Springerplus 4, 1–9.

Deter, J., Lozach, S., Derrien, A., Véron, A., Chollet, J., Hervio-Heath, D., 2010. Chlorophyll a might structure a community of potentially pathogenic culturable Vibrionaceae. Insights from a one-year study of water and mussels surveyed on the French Atlantic coast. Environ. Microbiol. Rep. 2, 185–191. https://doi.org/10.1111/j.1758-2229.2010.00133.x

Dinsdale, E.A., Edwards, R.A., Hall, D., Angly, F., Breitbart, M., Brulc, J.M., Furlan, M., Desnues, C., Haynes, M., Li, L., McDaniel, L., Moran, M.A., Nelson, K.E., Nilsson, C., Olson, R., Paul, J., Brito, B.R., Ruan, Y., Swan, B.K., Stevens, R., Valentine, D.L., Thurber, R.V., Wegley, L., White, B.A., Rohwer, F., 2008. Functional metagenomic profiling of nine biomes. Nature 452, 629–632. https://doi.org/10.1038/nature06810

Doni, L., Oliveri, C., Lasa, A., Di Cesare, A., Petrin, S., Martinez-Urtaza, J., Coman, F., Richardson, A., Vezzulli, L., 2023. Large-scale impact of the 2016 Marine Heatwave on the plankton-associated microbial communities of the Great Barrier Reef (Australia). Mar. Pollut. Bull. 188, 114685.

Dorman, M.J., Domman, D., Uddin, M.I., Sharmin, S., Afrad, M.H., Begum, Y.A., Qadri, F., Thomson, N.R., 2019. High quality reference genomes for toxigenic and non-toxigenic Vibrio cholerae serogroup O139. Sci. Rep. 9, 5865. https://doi.org/10.1038/s41598-019-41883-x

Doster, E., Lakin, S.M., Dean, C.J., Wolfe, C., Young, J.G., Boucher, C., Belk, K.E., Noyes, N.R., Morley, P.S., 2020. MEGARes 2.0: a database for classification of antimicrobial drug, biocide and metal resistance determinants in

metagenomic sequence data. Nucleic Acids Res. 48, D561–D569. https://doi.org/10.1093/nar/gkz1010

Drake, S.L., DePaola, A., Jaykus, L.-A., 2007. An overview of Vibrio vulnificus and Vibrio parahaemolyticus. Compr. Rev. Food Sci. Food Saf. 6, 120–144.

D'Souza, R.M., Becker, N.G., Hall, G., Moodie, K.B., 2004. Does ambient temperature affect foodborne disease? Epidemiology 86–92.

Duan, J., Su, Y., 2005. Occurrence of Vibrio parahaemolyticus in Two Oregon Oyster-growing Bays. J. Food Sci. 70, M58–M63. https://doi.org/10.1111/j.1365-2621.2005.tb09047.x

Durazzi, F., Sala, C., Castellani, G., Manfreda, G., Remondini, D., De Cesare, A., 2021. Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota. Sci. Rep. 11, 1–10.

Dziejman, M., Serruto, D., Tam, V.C., Sturtevant, D., Diraphat, P., Faruque, S.M., Rahman, M.H., Heidelberg, J.F., Decker, J., Li, L., Montgomery, K.T., Grills, G., Kucherlapati, R., Mekalanos, J.J., 2005. Genomic characterization of non-O1, non-O139 Vibrio cholerae reveals genes for a type III secretion system. Proc. Natl. Acad. Sci. 102, 3465–3470. https://doi.org/10.1073/pnas.0409918102

Eiler, A., Johansson, M., Bertilsson, S., 2006. Environmental influences on Vibrio populations in northern temperate and boreal coastal waters (Baltic and Skagerrak Seas). Appl. Environ. Microbiol. 72, 6004–6011. https://doi.org/10.1128/AEM.00917-06

Eilers, H., Pernthaler, J., Glöckner, F.O., Amann, R., 2000. Culturability and In situ abundance of pelagic bacteria from the North Sea. Appl. Environ. Microbiol. 66, 3044–3051. https://doi.org/10.1128/aem.66.7.3044-3051.2000

Einarsdóttir, J., Passa, A., Gunnlaugsson, G., 2001. Health education and cholera in rural Guinea-Bissau. Int. J. Infect. Dis. 5, 133–138. https://doi.org/10.1016/S1201-9712(01)90087-6

Eisenberg, M.C., Kujbida, G., Tuite, A.R., Fisman, D.N., Tien, J.H., 2013. Examining rainfall and cholera dynamics in Haiti using statistical and dynamic modeling approaches. Epidemics 5, 197–207. https://doi.org/10.1016/j.epidem.2013.09.004

El-Sayed, A., Kamel, M., 2020. Climatic changes and their role in emergence and re-emergence of diseases. Environ. Sci. Pollut. Res. 27, 22336–22352.

Emch, M., Feldacker, C., Yunus, M., Streatfield, P.K., DinhThiem, V., Canh, D.G., Ali, M., 2008. Local Environmental Predictors of Cholera in Bangladesh and Vietnam. Am. J. Trop. Med. Hyg. 78, 823–832. https://doi.org/10.4269/ajtmh.2008.78.823

EPA, 1997. In Vitro Determination of Chlorophyll-a and Pheophytin-a in Marine and Freshwater Algae by Fluorescence [WWW Document]. Method 4450. URL

https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=309417 (accessed 4.3.23).

Epstein, P.R., Colwell, R.R., Ford, T.E., 1993. Marine ecosystems. J. Onwhyn.

Espinel, Z., Nogueira, L.M., Gay, H.A., Bryant, J.M., Hamilton, W., Trapido, E.J., Shepherd, J.M., Galea, S., Shultz, J.M., 2022. Climate-driven Atlantic hurricanes create complex challenges for cancer care. Lancet Oncol. 23, 1497–1498.

Espinoza-Vergara, G., Hoque, M.M., McDougald, D., Noorian, P., 2020. The Impact of Protozoan Predation on the Pathogenicity of Vibrio cholerae. Front. Microbiol. 11, 17. https://doi.org/10.3389/fmicb.2020.00017

Esteves, K., Hervio-Heath, D., Mosser, T., Rodier, C., Tournoud, M.-G., Jumas-Bilak, E., Colwell, R.R., Monfort, P., 2015. Rapid proliferation of Vibrio parahaemolyticus, Vibrio vulnificus, and Vibrio cholerae during freshwater flash floods in French Mediterranean coastal lagoons. Appl. Environ. Microbiol. 81, 7600 LP – 7609. https://doi.org/10.1128/AEM.01848-15

Europen Environment Agency, 2012. Climate change, impacts and vulnerability in Europe 2012. Copenhagan, Denmak. https://doi.org/10.2800/66071

Fan, J.J., Shao, C.P., Ho, Y.C., Yu, C.K., Hor, L., 2001. Isolation and characterization of a Vibrio vulnificus mutant deficient in both extracellular metalloprotease and cytolysin. Infect. Immun. 69, 5943–5948.

FAO, 2020. The State of World Fisheries and Aquaculture. Rome. https://doi.org/10.4060/ca9229en

Faruque, A.S.G., Malek, M.A., Khan, A.I., Huq, S., Salam, M.A., Sack, D.A., 2004. Diarrhoea in elderly people: aetiology, and clinical characteristics. Scand. J. Infect. Dis. 36, 204–208.

Faruque, S.M., Mekalanos, J.J., 2003. Pathogenicity islands and phages in Vibrio cholerae evolution. Trends Microbiol. 11, 505–510. https://doi.org/10.1016/j.tim.2003.09.003

Faruque, S.M., Mekalanos, J.J., 2012. Phage-bacterial interactions in the evolution of toxigenic Vibrio cholerae. Virulence 3, 556–565. https://doi.org/10.4161/viru.22351

Faruque, S.M., Naser, I.B., Islam, M.J., Faruque, A.S.G., Ghosh, A.N., Nair, G.B., Sack, D.A., Mekalanos, J.J., 2005. Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. Proc. Natl. Acad. Sci. U. S. A. 102, 1702–1707. https://doi.org/10.1073/pnas.0408992102

Faruque, Shah M., Rahman, M.M., Asadulghani, N., Nasirul, I.N., Mekalanos, J.J., 1999. Lysogenic Conversion of Environmental Vibrio mimicus Strains by CTXΦ. Infect. Immun. 67, 5723–5729. https://doi.org/10.1128/IAI.67.11.5723-5729.1999

Faruque, S.M., Sack, D.A., Sack, R.B., Colwell, R.R., Takeda, Y., Nair, G.B., 2003. Emergence and evolution of Vibrio cholerae O139. Proc. Natl. Acad. Sci. 100, 1304–1309.

Faruque, Shah M, Siddique, A., Saha, M.N., Asadulghani, Rahman, M.M., Zaman, K., Albert, M.J., Sack, D.A., Sack, R.B., 1999. Molecular characterization of a new ribotype of Vibrio cholerae O139 Bengal associated with an outbreak of cholera in Bangladesh. J. Clin. Microbiol. 37, 1313–1318.

FDA, 2005. Pathogenic Vibrio parahaemolyticus in Raw Oysters [WWW Document]. URL https://www.fda.gov/food/cfsan-risk-safety-assessments/quantitative-risk-assessment-public-health-impact-pathogenic-vibrio-parahaemolyticus-raw-oysters (accessed 1.4.21).

FDA, 2017. BAM Media M190: Vibrio vulnificus Agar (VVA) [WWW Document]. URL https://www.fda.gov/food/laboratory-methods-food/bam-media-m190-vibrio-vulnificus-agar-vva (accessed 3.16.23).

FDA, 2022. Fish and Fishery Products Hazards and Controls [WWW Document]. URL https://www.fda.gov/food/seafood-guidance-documents-regulatory-information/fish-and-fishery-products-hazards-and-controls (accessed 4.20.23).

Federspiel, F., Ali, M., 2018. The cholera outbreak in Yemen: lessons learned and way forward. BMC Public Health 18, 1338. https://doi.org/10.1186/s12889-018-6227-6

Florida Department of Health, 2023. Vibrio infections [WWW Document]. URL https://www.floridahealth.gov/diseases-and-conditions/vibrio-infections/index.html (accessed 3.10.23).

Freitag, A., Ellett, A., Burkart, H., Jacobs, J., 2022. Estimating the Economic Burden of Vibrio parahaemolyticus in Washington State Oyster Aquaculture: Implications for the Future. J. Shellfish Res. 40, 555–564.

Froelich, B.A., Daines, D.A., 2020. In hot water: effects of climate change on Vibrio–human interactions. Environ. Microbiol. 22, 4101–4111. https://doi.org/10.1111/1462-2920.14967

Froelich, B.A., Noble, R.T., 2016. Vibrio bacteria in raw oysters: managing risks to human health. Philos. Trans. R. Soc. B Biol. Sci. 371, 20150209.

Frölicher, T.L., Fischer, E.M., Gruber, N., 2018. Marine heatwaves under global warming. Nature 560, 360–364. https://doi.org/10.1038/s41586-018-0383-9

Fujino, T., 1951. A report of bacterial examination on the shirasu food poisoning. Jpn J Soc Infect Dis 25, 11–12.

Fujino, T., 1953. From Pasteurella parahaemolytica to Vibrio parahaemolyticus. Pp13-37 T Fujino Fukumi Hed Vibrio Parahaemolyticus Isseido Publ. Co Tokyo.

Fukushima, H., Seki, R., 2004. Ecology of Vibrio vulnificus and Vibrio parahaemolyticus in brackish environments of the Sada River in Shimane Prefecture, Japan. FEMS Microbiol. Ecol. 48, 221–229. https://doi.org/10.1016/j.femsec.2004.01.009

Fykse, E.M., Skogan, G., Davies, W., Olsen, J.S., Blatny, J.M., 2007. Detection of Vibrio cholerae by real-time nucleic acid sequence-based amplification. Appl. Environ. Microbiol. 73, 1457–1466. https://doi.org/10.1128/AEM.01635-06

Gavilan, R.G., Caro-Castro, J., Blondel, C.J., Martinez-Urtaza, J., 2023. Vibrio parahaemolyticus epidemiology and pathogenesis: novel insights on an emerging foodborne pathogen, in: Vibrio Spp. Infections. Springer, pp. 233–251.

Gennari, M., Ghidini, V., Caburlotto, G., Lleo, M.M., 2012. Virulence genes and pathogenicity islands in environmental Vibrio strains nonpathogenic to humans. FEMS Microbiol. Ecol. 82, 563–573. https://doi.org/10.1111/j.1574-6941.2012.01427.x

Gil, A.I., Louis, V.R., Rivera, I.N., Lipp, E., Huq, A., Lanata, C.F., Taylor, D.N., Russek-Cohen, E., Choopun, N., Sack, R.B., others, 2004. Occurrence and distribution of Vibrio cholerae in the coastal environment of Peru. Environ. Microbiol. 6, 699–706.

Givens, C., Bowers, J., DePaola, A., Hollibaugh, J., Jones, J., 2014. Occurrence and distribution of Vibrio vulnificus and Vibrio parahaemolyticus–potential roles for fish, oyster, sediment and water. Lett. Appl. Microbiol. 58, 503–510.

Grim, C.J., Taviani, E., Alam, M., Huq, A., Sack, R.B., Colwell, R.R., 2008. Occurrence and expression of luminescence in Vibrio cholerae. Appl. Environ. Microbiol. 74, 708–715.

Gronlund, C.J., Cameron, L., Shea, C., O'Neill, M.S., 2019. Assessing the magnitude and uncertainties of the burden of selected diseases attributable to extreme heat and extreme precipitation under a climate change scenario in Michigan for the period 2041–2070. Environ. Health 18, 1–17.

Gulig, P.A., de Crécy-Lagard, V., Wright, A.C., Walts, B., Telonis-Scott, M., McIntyre, L.M., 2010. SOLiD sequencing of four Vibrio vulnificus genomes enables comparative genomic analysis and identification of candidate clade-specific virulence genes. Bmc Genomics 11, 512.

Guo, R.H., Lim, J.Y., Tra My, D.N., Jo, S.J., Park, J.U., Rhee, J.H., Kim, Y.R., 2018. Vibrio vulnificus RtxA1 Toxin Expression Upon Contact With Host Cells Is RpoS-Dependent. Front. Cell. Infect. Microbiol. 8, 1–11. https://doi.org/10.3389/fcimb.2018.00070

Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G., 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29, 1072–1075. https://doi.org/10.1093/bioinformatics/btt086

Gutierrez West, C.K., Klein, S.L., Lovell, harles R., 2013. High frequency of virulence factor genes tdh, trh, and tlh in Vibrio parahaemolyticus strains isolated from a pristine estuary. Appl. Environ. Microbiol. 79, 2247–2252. https://doi.org/10.1128/AEM.03792-12

Hacker, J., Kaper, J.B., 2000. Pathogenicity islands and the evolution of microbes. Annu. Rev. Microbiol. 54, 641–679.

Haley, B.J., Chen, A., Grim, C.J., Clark, P., Diaz, C.M., Taviani, E., Hasan, N.A., Sancomb, E., Elnemr, W.M., Islam, M.A., Huq, A., Colwell, R.R., Benediktsdóttir, E., 2013. Vibrio cholerae in an Historically Cholera-Free Country. Env. Microbiol Rep 4, 381–389. https://doi.org/10.1111/j.1758-2229.2012.00332.x.Vibrio

Halpern, M., Izhaki, I., 2017. Fish as Hosts of Vibrio cholerae. Front. Microbiol.

Hammer, O., 2003. Diversity [WWW Document]. URL https://web.archive.org/web/20200217074057/http://folk.uio.no/ohammer/past/diversity.html (accessed 1.26.22).

Han, D., Yu, F., Chen, X., Zhang, R., Li, J., 2019. Challenges in Vibrio parahaemolyticus infections caused by the pandemic clone. Future Microbiol. 14, 437–450. https://doi.org/10.2217/fmb-2018-0308

Han, D., Yu, F., Tang, H., Ren, C., Wu, C., Zhang, P., Han, C., 2017. Spreading of Pandemic Vibrio parahaemolyticus O3:K6 and Its Serovariants: A Re-analysis of Strains Isolated from Multiple Studies. Front. Cell. Infect. Microbiol. 7.

Harris, J., LaRocque, R., Qadri, F., Ryan, E., Calderwood, S., 2012. Cholera. The Lancet 379, 2466–2476. https://doi.org/10.1016/S0140-6736(12)60436-X.Cholera

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., Samuel, M.D., 2002. Climate Warming and Disease Risks for Terrestrial and Marine Biota. Science 296, 2158 LP – 2162. https://doi.org/10.1126/science.1063699

Hasan, J.A., Bernstein, D., Huq, A., Loomis, L., Tamplin, M.L., Colwell, R.R., 1994. Cholera DFA: an improved direct fluorescent monoclonal antibody staining kit for rapid detection and enumeration of Vibrio cholerae O1. FEMS Microbiol. Lett. 120, 143–148. https://doi.org/10.1111/j.1574-6968.1994.tb07021.x

Hasan, N.A., Ceccarelli, D., Grim, C.J., Taviani, E., Choi, J., Sadique, A., Alam, M., Siddique, A.K., Sack, R.B., Huq, A., Colwell, R.R., 2013. Distribution of Virulence Genes in Clinical and Environmental Vibrio cholerae Strains in Bangladesh. Appl. Environ. Microbiol. 79, 5782–5785. https://doi.org/10.1128/aem.01113-13

Hasan, N.A., Choi, S.Y., Li, H., Tallon, L.J., Fraser-Liggett, C.M., Su, Q., Eppinger, M., Alam, M., Cebula, T.A., Furth, K., Huq, A., Cravioto, A., Hoq, M.M., Haley, B.J., Clark, P.W., Colwell, R.R., Prosper, J.B., Chen, A., Hine, E.,

Taviani, E., Ravel, J., 2012. Genomic diversity of 2010 Haitian cholera outbreak strains. Proc. Natl. Acad. Sci. 109, E2010–E2017. https://doi.org/10.1073/pnas.1207359109

Hasan, N.A., Grim, C.J., Lipp, E.K., Rivera, I.N.G., Chun, J., Haley, B.J., Taviani, E., Choi, S.Y., Hoq, M., Munk, A.C., Brettin, T.S., Bruce, D., Challacombe, J.F., Detter, J.C., Han, C.S., Eisen, J.A., Huq, A., Colwell, R.R., 2015. Deep-sea hydrothermal vent bacteria related to human pathogenic Vibrio species. Proc. Natl. Acad. Sci. 112, E2813 LP-E2819. https://doi.org/10.1073/pnas.1503928112

Heidelberg, J.F., Heidelberg, K.B., Colwell, R.R., 2002. Bacteria of the γ-Subclass Proteobacteria Associated with Zooplankton in Chesapeake Bay. Appl. Environ. Microbiol. 68, 5498 LP – 5507. https://doi.org/10.1128/AEM.68.11.5498-5507.2002

Hippocrates of Cos, BCE400. Of The Epidemics [WWW Document]. URL https://classics.mit.edu/Hippocrates/epidemics.1.i.html (accessed 6.13.23).

Hiyoshi, H., Kodama, T., Iida, T., Honda, T., 2010. Contribution of Vibrio parahaemolyticus virulence factors to cytotoxicity, enterotoxicity, and lethality in mice. Infect. Immun. 78, 1772–1780. https://doi.org/10.1128/IAI.01051-09

Hoffman, S., Maculloch, B., Batz, M., 2015. Economic burden of major foodborne illnesses acquired in the United States.

Holbrook, N.J., Scannell, H.A., Sen Gupta, A., Benthuysen, J.A., Feng, M., Oliver, E.C.J., Alexander, L.V., Burrows, M.T., Donat, M.G., Hobday, A.J., Moore, P.J., Perkins-Kirkpatrick, S.E., Smale, D.A., Straub, S.C., Wernberg, T., 2019. A global assessment of marine heatwaves and their drivers. Nat. Commun. 10, 2624. https://doi.org/10.1038/s41467-019-10206-z

Hollis, D., Weaver, R., Baker, C., Thornsberry, C., 1976. Halophilic Vibrio species isolated from blood cultures. J. Clin. Microbiol. 3, 425–431.

Honda, J.R., Bernhard, J.N., Chan, E.D., 2015. Natural disasters and nontuberculous mycobacteria: a recipe for increased disease? Chest 147, 304–308.

Honda, S.I., Goto, I., Minematsu, I., Ikeda, N., Asano, N., Ishibashi, M., Kinoshita, Y., Nishibuchi, M., Honda, T., Miwatani, T., 1987. Gastroenteritis due to Kanagawa Negative Vibrio parahaemolyticus. The Lancet 329, 331–332. https://doi.org/10.1016/S0140-6736(87)92062-9

Honda, T., Ni, Y., Miwatani, T., 1988. 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. 56, 961–965.

Hori, M., Nakayama, A., Kitagawa, D., Fukushima, H., Asai, H., Kawai, Y., Okuchi, K., 2017. A case of Vibrio vulnificus infection complicated with fulminant

purpura: gene and biotype analysis of the pathogen. JMM Case Rep. 4, e005096.

Hoshino, K., Yamasaki, S., Mukhopadhyay, A.K., Chakraborty, S., Basu, A., Bhattacharya, S.K., Nair, G.B., Shimada, T., Y, Y.T., 1998. Development and evaluation of a multiplex PCR assay for rapid detection of toxigenic Vibrio cholerae O1 and O139. FEMS Immunol. Med. Microbiol. 20, 201–207.

Hounmanou, Y.M., Sit, B., Fakoya, B., Waldor, M.K., Dalsgaard, A., 2022. Genomic and Phenotypic Insights for Toxigenic Clinical Vibrio cholerae O141. Emerg. Infect. Dis. 28, 617.

Hu, D., Liu, B., Feng, L., Ding, P., Guo, X., Wang, M., Cao, B., Reeves, P.R., Wang, L., 2016. Origins of the current seventh cholera pandemic. Proc. Natl. Acad. Sci. 113, E7730 LP-E7739. https://doi.org/10.1073/pnas.1608732113

Hunt, D.E., David, L.A., Gevers, D., Preheim, S.P., Alm, E.J., Polz, M.F., 2008. Resource Partitioning and Sympatric Differentiation Among Closely Related Bacterioplankton. Science 320, 1081 LP – 1085. https://doi.org/10.1126/science.1157890

Huq, A., Colwell, R.R., Rahman, R., Ali, A., Chowdhury, M.A., Parveen, S., Sack, D.A., Russek-Cohen, E., 1990. Detection of Vibrio cholerae O1 in the aquatic environment by fluorescent-monoclonal antibody and culture methods. Appl. Environ. Microbiol. 56, 2370 LP – 2373.

Huq, A., Sack, R.B., Nizam, A., Longini, I.M., Nair, G.B., Ali, A., Morris Jr, J.G., Khan, M.N.H., Siddique, A.K., Yunus, M., Albert, M.J., Sack, D.A., Colwell, R.R., 2005. Critical factors influencing the occurrence of Vibrio cholerae in the environment of Bangladesh. Appl. Environ. Microbiol. 71, 4645–4654. https://doi.org/10.1128/AEM.71.8.4645-4654.2005

Huq, A., Small, E.B., West, P.A., Huq, M.I., Rahman, R., Colwell, R.R., 1983. Ecological relationships between Vibrio cholerae and planktonic crustacean copepods. Appl. Environ. Microbiol. 45, 275–283.

Huq, A., West, P.A., Small, E.B., Huq, M.I., Colwell, R.R., 1984. Influence of water temperature, salinity, and pH on survival and growth of toxigenic Vibrio cholerae serovar O1 associated with live copepods in laboratory microcosms. Appl. Environ. Microbiol. 48, 420–424.

Huq, A., Xu, B., Chowdhury, M.A.R., Islam, M.S., Montilla, R., 1996. A Simple Filtration Method To Remove Plankton-Associated Vibrio cholerae in Raw Water Supplies in Developing Countries. Appl. Environ. Microbiol. 62, 2508–2512.

Hutchins, D.A., Jansson, J.K., Remais, J.V., Rich, V.I., Singh, B.K., Trivedi, P., 2019. Climate change microbiology—problems and perspectives. Nat. Rev. Microbiol. 17, 391–396.

Igbinosa, E.O., Okoh, A.I., 2008. Emerging Vibrio species: an unending threat to public health in developing countries. Res. Microbiol. 159, 495–506. https://doi.org/10.1016/j.resmic.2008.07.001

Igbinosa, E.O., Okoh, A.I., 2010. Vibrio fluvialis: an unusual enteric pathogen of increasing public health concern. Int. J. Environ. Res. Public. Health 7, 3628–3643.

Imamura, D., Morita, M., Sekizuka, T., Mizuno, T., Takemura, T., Yamashiro, T., Chowdhury, G., Pazhani, G.P., Mukhopadhyay, A.K., Ramamurthy, T., Miyoshi, S.I., Kuroda, M., Shinoda, S., Ohnishi, M., 2017. Comparative genome analysis of VSP-II and SNPs reveals heterogenic variation in contemporary strains of Vibrio cholerae O1 isolated from cholera patients in Kolkata, India. PLoS Negl. Trop. Dis. 11, 1–14. https://doi.org/10.1371/journal.pntd.0005386

IPCC, 2021. Climate Change 2021: The physical Science Basis [WWW Document]. URL https://www.ipcc.ch/report/sixth-assessment-report-working-group-i/. (accessed 6.14.23).

Islam, M Sirajul, Zaman, M.H., Islam, M Shafiqul, Ahmed, N., Clemens, J.D., 2020. Environmental reservoirs of Vibrio cholerae. Vaccine 38, A52–A62. https://doi.org/10.1016/j.vaccine.2019.06.033

Itoh, N., Isotani, K., Makino, Y., Kato, M., Kitayama, K., Ishimota, T., 2014. PCR-based amplification and heterologous expression of Pseudomonas alcohol dehydrogenase genes from the soil metagenome for biocatalysis. Enzyme Microb. Technol. 55, 140–150. https://doi.org/10.1016/j.enzmictec.2013.10.012

Jacobs, J.M., Rhodes, M., Brown, C.W., Hood, R.R., Leight, A., Long, W., Wood, R., 2014. Modeling and forecasting the distribution of Vibrio vulnificus in Chesapeake Bay. J. Appl. Microbiol. 117, 1312–1327. https://doi.org/10.1111/jam.12624

Jahangir Alam, M., Tomochika, K.I., Miyoshi, S.I., Shinoda, S., 2002. Environmental investigation of potentially pathogenic Vibrio parahaemolyticus in the Seto-Inland Sea, Japan. FEMS Microbiol. Lett. 208, 83–87. https://doi.org/10.1111/j.1574-6968.2002.tb11064.x

Janda, J.M., Newton, A.E., Bopp, C.A., 2015. Vibriosis. Clin. Lab. Med. 35, 273–288.

Jerez, S.A., Plaza, N., Bravo, V., Urrutia, I., Blondel, C.J., 2022. The Vibrio Type III Secretion System 2 is not restricted to the Vibrionaceae and encodes differentially distributed repertoires of effector proteins. bioRxiv 2022–08.

Jiang, C., Tanaka, M., Nishikawa, S., Mino, S., Romalde, J.L., Thompson, F.L., Gomez-Gil, B., Sawabe, T., 2022. Vibrio Clade 3.0: New Vibrionaceae evolutionary units using genome-based approach. Curr. Microbiol. 79, 1–15.

Johansson, M., 2023. MGEdb - Mobile Genetic Element database [WWW Document]. URL https://pypi.org/project/MGEdb/ (accessed 3.16.23).

Johnson, C.N., 2013. Fitness factors in vibrios: a mini-review. Microb. Ecol. 65, 826–851.

Johnson, C.N., Bowers, J.C., Griffitt, K.J., Molina, V., Clostio, R.W., Pei, S., Laws, E., Paranjpye, R.N., Strom, M.S., Chen, A., Hasan, N.A., Huq, A., Noriea, N.F., Grimes, D.J., Colwell, R.R., 2012. Ecology of vibrio parahaemolyticus and vibrio vulnificus in the coastal and estuarine waters of Louisiana, Maryland, Mississippi, and Washington (United States). Appl. Environ. Microbiol. 78, 7249–7257. https://doi.org/10.1128/AEM.01296-12

Johnson, C.N., Flowers, A.R., Noriea III, N.F., Zimmerman, A.M., Bowers, J.C., DePaola, A., Grimes, D., 2010. Relationships between environmental factors and pathogenic vibrios in the northern Gulf of Mexico. Appl. Environ. Microbiol. 76, 7076–7084.

Jolley, K.A., Bray, J.E., Maiden, M.C.J., 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res. 3, 124. https://doi.org/10.12688/wellcomeopenres.14826.1

Jones, E.H., Feldman, K.A., Palmer, A., Butlera, E., Blythe, D., Mitchell, C.S., 2013. Vibrio infections and surveillance in Maryland, 2002–2008. Public Health Rep. 128, 537–545.

Jones, J.L., Ludeke, C.H.M., Bowers, J.C., Garrett, N., Fischer, M., Parsons, M.B., Bopp, C.A., DePaola, A., 2012. Biochemical, serological, and virulence characterization of clinical and oyster Vibrio parahaemolyticus isolates. J. Clin. Microbiol. 50, 2343–2352. https://doi.org/10.1128/JCM.00196-12

Jones, M.K., Oliver, J.D., 2009. Vibrio vulnificus: Disease and pathogenesis. Infect. Immun. 77, 1723–1733. https://doi.org/10.1128/IAI.01046-08

Joseph, S.W., Colwell, R.R., Kaper, J.B., 1982. Vibrio Parahaemolyticus and Related Halophilic Vibrios. CRC Crit. Rev. Microbiol. 10, 77–124. https://doi.org/10.3109/10408418209113506

Julie, D., Solen, L., Antoine, V., Annick, D., Dominique, H.-H., 2010. Ecology of pathogenic and non-pathogenic Vibrio parahaemolyticus on the French Atlantic coast. Effects of temperature, salinity, turbidity and chlorophyll a. Environ. Microbiol. 12, 929–937.

Jutla, A., Akanda, A., Griffiths, J., Colwell, R., Islam, S., 2011. Warming oceans, phytoplankton, and river discharge: implications for cholera outbreaks. Am. J. Trop. Med. Hyg. 85, 303–308. https://doi.org/10.4269/ajtmh.2011.11-0181

Jutla, A., Akanda, A., Islam, S., 2013a. A framework for predicting endemic cholera using satellite derived environmental determinants. Environ. Model. Softw. 47, 148–158. https://doi.org/10.1016/j.envsoft.2013.05.008

Jutla, A., Akanda, A., Unnikrishnan, A., Huq, A., Colwell, R., 2015a. Predictive time series analysis linking Bengal cholera with terrestrial water storage measured from Gravity Recovery and Climate Experiment sensors. Am. J. Trop. Med. Hyg. 93, 1179–1186. https://doi.org/10.4269/ajtmh.14-0648

Jutla, A., Aldaach, H., Billian, H., Akanda, A., Huq, A., Colwell, R., 2015b. Satellite Based Assessment of Hydroclimatic Conditions Related to Cholera in Zimbabwe. PLOS ONE 10, e0137828.

Jutla, A., Whitcombe, E., Hasan, N., Haley, B., Akanda, A., Huq, A., Alam, M., Sack, R.B., Colwell, R., 2013b. Environmental factors influencing epidemic cholera. Am. J. Trop. Med. Hyg. 89, 597–607. https://doi.org/10.4269/ajtmh.12-0721

Jutla, A.S., Akanda, A.S., Islam, S., 2010. Tracking cholera in coastal regions using satellite observations. JAWRA J. Am. Water Resour. Assoc. 46, 651–662.

Jutla, A.S., Akanda, A.S., Islam, S., 2012. Satellite remote sensing of space–time plankton variability in the Bay of Bengal: Connections to cholera outbreaks. Remote Sens. Environ. 123, 196–206.

Kahler, A.M., Haley, B.J., Chen, A., Mull, B.J., Tarr, C.L., Turnsek, M., Katz, L.S., Humphrys, M.S., Derado, G., Freeman, N., Boncy, J., Colwell, R.R., Huq, A., Hill, V.R., 2015. Environmental surveillance for toxigenic vibrio cholerae in surface waters of haiti. Am. J. Trop. Med. Hyg. 92, 118–125. https://doi.org/10.4269/ajtmh.13-0601

Kaneko, T., Colwell, R.R., 1973. Ecology of Vibrio parahaemolyticus in Chesapeake Bay. J. Bacteriol. 113, 24–32. https://doi.org/10.1128/JB.113.1.24-32.1973

Kaneko, T., Colwell, R.R., 1974. Distribution of Vibrio parahaemolyticus and related organisms in the Atlantic Ocean off South Carolina and Georgia. Appl. Microbiol. 28, 1009–1017.

Kaneko, T., Colwell, R.R., 1977. The annual cycle of Vibrio parahaemolyticus in Chesapeake Bay. Microb. Ecol. 135–155.

Kann, S., Hartmann, M., Alker, J., Hansen, J., Dib, J.C., Aristizabal, A., Concha, G., Schotte, U., Kreienbrock, L., Frickmann, H., 2022. Seasonal Patterns of Enteric Pathogens in Colombian Indigenous People—A More Pronounced Effect on Bacteria Than on Parasites. Pathogens 11, 214.

Kanrar, S., Dhar, A.K., 2018. Complete genome sequence of a novel mutant strain of Vibrio parahaemolyticus from Pacific white shrimp (Penaeus vannamei). Genome Announc. 6, e00497-18.

Kaper, J., Lockman, H., Colwell, R., Joseph, S., 1979. Ecology, serology, and enterotoxin production of Vibrio cholerae in Chesapeake Bay. Appl. Environ. Microbiol. 37, 91–103.

Kaper, J., Remmers, E., Lockman, H., Colwell, R., 1981. Distribution of Vibrio parahaemolyticus in Chesapeake Bay during the summer season. Estuaries 4, 321–327.

Kaper, J.B., Morris, J.G.J., Levine, M.M., 1995. Cholera. Clin. Microbiol. Rev. 8, 48–86. https://doi.org/10.1128/CMR.8.1.48-86.1995

Kaysner, C., DePaola, A., Jones, J., 2019. BAM Chapter 9: Vibrio [WWW Document]. Bacteriol. Anal. Man. URL https://www.fda.gov/food/laboratory-methods-food/bam-chapter-9-vibrio (accessed 4.28.23).

Keesing, F., Ostfeld, R.S., 2021. Impacts of biodiversity and biodiversity loss on zoonotic diseases. Proc. Natl. Acad. Sci. 118, e2023540118.

Kemp, K.M., Westrich, J.R., Alabady, M.S., Edwards, M.L., Lipp, E.K., 2018. Abundance and Multilocus Sequence Analysis of Vibrio Bacteria Associated with Diseased Elkhorn Coral. Appl. Environ. Microbiol. 84, e01035-17. https://doi.org/10.1128/AEM.01035-17

Khan, R., Aldaach, H., McDonald, C., Alam, M., Huq, A., Gao, Y., Akanda, A.S., Colwell, R., Jutla, A., 2019a. Estimating cholera risk from an exploratory analysis of its association with satellite-derived land surface temperatures. Int. J. Remote Sens. 40, 1898–4909. https://doi.org/10.1080/01431161.2019.1577575

Khan, R., Anwar, R., Akanda, S., McDonald, M.D., Huq, A., Jutla, A., Colwell, R., 2017. Assessment of Risk of Cholera in Haiti following Hurricane Matthew. Am. J. Trop. Med. Hyg. 97, 896–903. https://doi.org/10.4269/ajtmh.17-0048

Khan, R., Thanh, N.H., Shisler, J., Lin, L.-S., Jutla, A., Colwell, R., 2018. Evaluation of Risk of Cholera after a Natural Disaster: Lessons Learned from the 2015 Nepal Earthquake. J. Water Resour. Plan. Manag. 144, 4018044. https://doi.org/10.1061/(ASCE)WR.1943-5452.0000929

Khan, R., Usmani, M., Akanda, A., Palash, W., Gao, Y., Huq, A., Colwell, R., Jutla, A., 2019b. Long-Range River Discharge Forecasting Using the Gravity Recovery and Climate Experiment. J. Water Resour. Plan. Manag. 145, 06019005. https://doi.org/10.1061/(asce)wr.1943-5452.0001072

Khouadja, S., Roque, A., Gonzalez, M., Furones, D., 2022. Vibrio pathogenicity island and phage CTX genes in Vibrio alginolyticus isolated from different aquatic environments. J. Water Health 20, 1469–1478.

Khoury, P., 2023. Cholera is back but the world is looking away. bmj 380, 141. https://doi.org/10.1136/bmj.p141

Kierek, K., Watnick, P.I., 2003. The Vibrio cholerae O139 O-antigen polysaccharide is essential for Ca2+-dependent biofilm development in sea water. Proc. Natl. Acad. Sci. 100, 14357 LP – 14362. https://doi.org/10.1073/pnas.2334614100

Kim, J.Y., Lee, J.L., 2017. Correlation of Total Bacterial and Vibrio spp. Populations between Fish and Water in the Aquaculture System. Front. Mar. Sci.

Kimes, N.E., Grim, C.J., Johnson, W.R., Hasan, N.A., Tall, B.D., Kothary, M.H., Kiss, H., Munk, A.C., Tapia, R., Green, L., Detter, C., Bruce, D.C., Brettin, T.S., Colwell, R.R., Morris, P.J., 2012. Temperature regulation of virulence

factors in the pathogen Vibrio coralliilyticus. ISME J. 6, 835–846. https://doi.org/10.1038/ismej.2011.154

Kimsey, H.H., Nair, G.B., Ghosh, A., Waldor, M.K., 1998. Diverse CTXφs and evolution of new pathogenic Vibrio cholerae. The Lancet 352, 457–458.

Kirpich, A., Weppelmann, T.A., Yang, Y., Ali, A., Morris Jr., J.G., Longini, I.M., 2015. Cholera Transmission in Ouest Department of Haiti: Dynamic Modeling and the Future of the Epidemic. PLoS Negl. Trop. Dis. 9, e0004153.

Klein, S.L., Gutierrez West, C.K., Mejia, D.M., Lovell, C.R., 2014. Genes similar to the vibrio parahaemolyticus virulence-related Genes tdh, tlh, and vscC2 Occur in Other vibrionaceae species isolated from a pristine estuary. Appl. Environ. Microbiol. 80, 595–602. https://doi.org/10.1128/AEM.02895-13

Knapp, K.R., Kruk, M.C., Levinson, D.H., Diamond, H.J., Neumann, C.J., 2010. The international best track archive for climate stewardship (IBTrACS) unifying tropical cyclone data. Bull. Am. Meteorol. Soc. 91, 363–376.

Koley, H., Senoh, M., Barman, S., Takahashi, T., Ramamurthy, T., Takeda, Y., Hamabata, T., Yamasaki, S., Mukhopadhyay, A.K., Ghosh-Banerjee, J., Chatterjee, S., Nair, G.B., Asakura, M., 2010. Cholera Toxin Production by the El Tor Variant of Vibrio cholerae O1 Compared to Prototype El Tor and Classical Biotypes. J. Clin. Microbiol. 48, 4283–4286. https://doi.org/10.1128/jcm.00799-10

Koralage, M.S.G., Alter, T., Pichpol, D., Strauch, E., Zessin, K.-H., Huehn, S., 2012. Prevalence and molecular characteristics of Vibrio spp. isolated from preharvest shrimp of the North Western Province of Sri Lanka. J. Food Prot. 75, 1846–1850. https://doi.org/10.4315/0362-028X.JFP-12-115

Kotik, M., 2009. Novel genes retrieved from environmental DNA by polymerase chain reaction: Current genome-walking techniques for future metagenome applications. J. Biotechnol. 144, 75–82. https://doi.org/10.1016/j.jbiotec.2009.08.013

Koton, Y., Gordon, M., Chalifa-Caspi, V., Bisharat, N., 2015. Comparative genomic analysis of clinical and environmental Vibrio vulnificus isolates revealed biotype 3 evolutionary relationships. Front. Microbiol.

Krantz, G.E., Colwell, R.R., Lovelace, E., 1969. Vibrio parahaemolyticus from the blue crab Callinectes sapidus in Chesapeake Bay. Science 164, 1286–1287.

Kulikov, A.S., Prjibelski, A.D., Tesler, G., Vyahhi, N., Sirotkin, A.V., Pham, S., Dvorkin, M., Pevzner, P.A., Bankevich, A., Nikolenko, S.I., Pyshkin, A.V., Nurk, S., Gurevich, A.A., Antipov, D., Alekseyev, M.A., Lesin, V.M., 2012. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. J. Comput. Biol. 19, 455–477. https://doi.org/10.1089/cmb.2012.0021

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359. https://doi.org/10.1038/nmeth.1923

Lattos, A., Bitchava, K., Giantsis, I.A., Theodorou, J.A., Batargias, C., Michaelidis, B., 2021. The Implication of Vibrio Bacteria in the Winter Mortalities of the Critically Endangered Pinna nobilis. Microorganisms 9, 922. https://doi.org/10.3390/microorganisms9050922

Le Roux, F., Blokesch, M., 2018. Eco-evolutionary Dynamics Linked to Horizontal Gene Transfer in Vibrios. Annu. Rev. Microbiol. 72, 89–110. https://doi.org/10.1146/annurev-micro-090817-062148

Lee, D., Chang, H.H., Sarnat, S.E., Levy, K., 2019. Precipitation and Salmonellosis Incidence in Georgia, USA: Interactions between Extreme Rainfall Events and Antecedent Rainfall Conditions. Environ. Health Perspect. 127, 97005. https://doi.org/10.1289/EHP4621

Lee, J.-H., Kim, M.-W., Kim, B.-S., Kim, S.-M., Lee, B.-C., Kim, T.-S., Choi, S.-H., 2007. Identification and characterization of the Vibrio vulnificus rtxA essential for cytotoxicity in vitro and virulence in mice. J. Microbiol. 45, 146–152.

Letchumanan, V., Chan, K.G., Lee, L.H., 2014. Vibrio parahaemolyticus: A review on the pathogenesis, prevalence, and advance molecular identification techniques. Front. Microbiol. 5, 1–13. https://doi.org/10.3389/fmicb.2014.00705

Letelier, R.M., Bidigare, R.R., Hebel, D. V, Ondrusek, M., Winn, C.D., Karl, D.M., 1993. Temporal variability of phytoplankton community structure based on pigment analysis. Limnol. Oceanogr. 38, 1420–1437.

Letunic, I., Bork, P., 2021. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. 49, W293–W296. https://doi.org/10.1093/nar/gkab301

Levade, I., Saber, M.M., Midani, F.S., Chowdhury, F., Khan, A.I., Begum, Y.A., Ryan, E.T., David, L.A., Calderwood, S.B., Harris, J.B., LaRocque, R.C., Qadri, F., Shapiro, B.J., Weil, A.A., 2021. Predicting Vibrio cholerae Infection and Disease Severity Using Metagenomics in a Prospective Cohort Study. J. Infect. Dis. 223, 342–351. https://doi.org/10.1093/infdis/jiaa358

Levitus, S., Antonov, J.I., Boyer, T.P., Baranova, O.K., Garcia, H.E., Locarnini, R.A., Mishonov, A. V, Reagan, J.R., Seidov, D., Yarosh, E.S., 2012. World ocean heat content and thermosteric sea level change (0–2000 m), 1955–2010. Geophys. Res. Lett. 39, L10603.

Levy, S., 2018. ECDC Vibrio Map Viewer: Tracking the Whereabouts of Pathogenic Species. Environ. Health Perspect. 126, 34003. https://doi.org/10.1289/EHP2904

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., Data, G.P., Sam, T., 2009. The Sequence Alignment/Map format and SAMtools 25, 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Li, Y., Wang, Y., Fu, L., Gao, Y., Zhao, H., Zhou, W., 2017. Aerobic-heterotrophic nitrogen removal through nitrate reduction and ammonium assimilation by marine bacterium Vibrio sp. Y1-5. Bioresour. Technol. 230, 103–111. https://doi.org/10.1016/j.biortech.2017.01.049

Li, Y., Xie, X., Shi, X., Lin, Y., Qiu, Y., Mou, J., Chen, Q., Lu, Y., Zhou, L., Jiang, M., Sun, H., Ma, H., Cheng, J., Hu, Q., 2014. Vibrio parahaemolyticus, Southern Coastal Region of China, 2007-2012. Emerg. Infect. Dis. 20, 685–688. https://doi.org/10.3201/eid2004.130744

Liao, Y., Li, Y., Wu, S., Mou, J., Xu, Z., Cui, R., Klena, J.D., Shi, X., Lu, Y., Qiu, Y., Lin, Y., Xie, X., Ma, H., Li, Z., Yu, H., Varma, J.K., Ran, L., Hu, Q., Cheng, J., 2015. Risk Factors for Vibrio parahaemolyticus Infection in a Southern Coastal Region of China. Foodborne Pathog. Dis. 12, 881–886. https://doi.org/10.1089/fpd.2015.1988

Lipp, E.K., Huq, A., R., C.R., 2002. Effects of Global Climate on Infectious Disease: the Cholera Model. Clin. Microbiol. Rev. 15, 757–770. https://doi.org/10.1128/CMR.15.4.757-770.2002

Lipp, E.K., Rivera, I.N., Gil, A.I., Espeland, E.M., Choopun, N., Louis, V.R., Russek-Cohen, E., Huq, A., Colwell, R.R., 2003. Direct detection of Vibrio cholerae and ctxA in Peruvian coastal water and plankton by PCR. Appl. Environ. Microbiol. 69, 3676–3680.

Lipp, E.K., Rodriguez-Palacios, C., Rose, J.B., 2001. Occurrence and distribution of the human pathogen Vibrio vulnificus in a subtropical Gulf of Mexico estuary BT - The Ecology and Etiology of Newly Emerging Marine Diseases, in: Porter, J.W. (Ed.), . Springer Netherlands, Dordrecht, pp. 165–173. https://doi.org/10.1007/978-94-017-3284-0_15

Liston, J., 1990. Microbial hazards of seafood consumption. Food Technol. USA.

Liu, B., Zheng, D., Jin, Q., Chen, L., Yang, J., 2018. VFDB 2019: a comparative pathogenomic platform with an interactive web interface. Nucleic Acids Res. 47, D687–D692. https://doi.org/10.1093/nar/gky1080

Lo, H.-R., Lin, J.-H., Chen, Y.-H., Chen, C.-L., Shao, C.-P., Lai, Y.-C., Hor, L.-I., 2011. RTX toxin enhances the survival of Vibrio vulnificus during infection by protecting the organism from phagocytosis. J. Infect. Dis. 203, 1866–1874.

Lobitz, B., Beck, L., Huq, A., Wood, B., Fuchs, G., Faruque, A.S.G., Colwell, R., 2000. Climate and infectious disease: Use of remote sensing for detection of Vibrio cholerae by indirect measurement. Proc. Natl. Acad. Sci. 97, 1438 LP – 1443. https://doi.org/10.1073/pnas.97.4.1438

López-Pérez, M., Jayakumar, J.M., Haro-Moreno, J.M., Zaragoza-Solas, A., Reddi, G., Rodriguez-Valera, F., Shapiro, O.H., Alam, M., Almagro-Moreno, S., 2019. Evolutionary model of cluster divergence of the emergent marine pathogen Vibrio vulnificus: From genotype to ecotype. MBio 10, e02852-18.

Love, D.C., Kuehl, L.M., Lane, R.M., Fry, J.P., Harding, J., Davis, B.J.K., Clancy, K., Hudson, B., 2020. Performance of cold chains and modeled growth of Vibrio parahaemolyticus for farmed oysters distributed in the United States and internationally. Int. J. Food Microbiol. 313, 108378. https://doi.org/10.1016/j.ijfoodmicro.2019.108378

Lovelace, T., Tubiash, H., Colwell, R., 1968. Quantitative and qualitative commensal bacterial flora of Crassostrea virginica in Chesapeake Bay. Proc. Natl. Shellfish. Assoc. 58, 82–87.

Lowenhaupt, E., Huq, A., Colwell, R.R., Adingra, A., Epstein, P.R., 1998. Rapid detection of Vibrio cholerae O1 in west Africa. Lancet Lond. Engl. https://doi.org/10.1016/s0140-6736(05)78094-6

Luo, Y., Wang, H., Liang, J., Qian, H., Ye, J., Chen, L., Yang, X., Chen, Z., Wang, F., Octavia, S., others, 2021. Population structure and multidrug resistance of non-O1/non-O139 Vibrio cholerae in freshwater rivers in Zhejiang, China. Microb. Ecol. 1–15.

Luo, Y., Ye, J., Payne, M., Hu, D., Jiang, J., Lan, R., 2022. Genomic Epidemiology of Vibrio cholerae O139, Zhejiang Province, China, 1994–2018. Emerg. Infect. Dis. 28, 2253–2260.

Lutz, C., Erken, M., Noorian, P., Sun, S., McDougald, D., 2013. Environmental reservoirs and mechanisms of persistence of Vibrio cholerae. Front. Microbiol. 4, 1–15. https://doi.org/10.3389/fmicb.2013.00375

Maiden, M.C., Bygraves, J.A., Feil, E., Morelli, G., Russell, J.E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K., Caugant, D.A., Feavers, I.M., Achtman, M., Spratt, B.G., 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. U. S. A. 95, 3140–3145. https://doi.org/10.1073/pnas.95.6.3140

Maniam, G., Nguyen, E.N., Milton, J.S., 2020. Acquisition of Cholera Within the United States. J. Investig. Med. High Impact Case Rep. 8, 2324709620904204–2324709620904204. https://doi.org/10.1177/2324709620904204

Martinelli Filho, J.E., Colwell, R.R., Queiroz, A.F., Rivera, I.N., Lopes, R.M., 2020. Vibrio cholerae O139 attached to zooplankton: reservoir diversity and distribution over an Estuarine-coastal gradient. J. Coast. Res. 95, 92–96.

Martinelli Filho, J.E., Lopes, R.M., Rivera, I.N.G., Colwell, R.R., 2011. Vibrio cholerae O1 detection in estuarine and coastal zooplankton. J. Plankton Res. 33, 51–62. https://doi.org/10.1093/plankt/fbq093

Martínez, J.L., 2013. Bacterial pathogens: from natural ecosystems to human hosts. Environ. Microbiol. 15, 325–333.

Martinez-Urtaza, J., Baker-Austin, C., Jones, J.L., Newton, A.E., Gonzalez-Aviles, G.D., DePaola, A., 2013. Spread of Pacific northwest Vibrio parahaemolyticus strain. N. Engl. J. Med. 369, 1573–1574.

Martinez-Urtaza, J., Bowers, J.C., Trinanes, J., DePaola, A., 2010. Climate anomalies and the increasing risk of Vibrio parahaemolyticus and Vibrio vulnificus illnesses. Food Res. Int. 43, 1780–1790. https://doi.org/10.1016/j.foodres.2010.04.001

Martinez-Urtaza, J., Huapaya, B., Gavilan, R.G., Blanco-Abad, V., Ansede-Bermejo, J., Cadarso-Suarez, C., Figueiras, A., Trinanes, J., 2008a. Emergence of Asiatic Vibrio diseases in South America in phase with El Niño. Epidemiol. Camb. Mass 19, 829–837. https://doi.org/10.1097/EDE.0b013e3181883d43

Martinez-Urtaza, J., Lozano-Leon, A., Varela-Pet, J., Trinanes, J., Pazos, Y., Garcia-Martin, O., 2008b. Environmental Determinants of the Occurrence and Distribution of Vibrio parahaemolyticus in the Rias of Galicia, Spain. Appl. Environ. Microbiol. 74, 265 LP – 274. https://doi.org/10.1128/AEM.01307-07

Martinez-Urtaza, J., Trinanes, J., Gonzalez-Escalona, N., Baker-Austin, C., 2016. Is El Niño a long-distance corridor for waterborne disease? Nat. Microbiol. 1, 16018. https://doi.org/10.1038/nmicrobiol.2016.18

Martinez-Urtaza, J., van Aerle, R., Abanto, M., Haendiges, J., Myers, R.A., Trinanes, J., Baker-Austin, C., Gonzalez-Escalona, N., 2017. Genomic Variation and Evolution of Vibrio parahaemolyticus ST36 over the Course of a Transcontinental Epidemic Expansion. mBio 8, e01425-17. https://doi.org/10.1128/mBio.01425-17

Massicotte, P., South, A., 2023. rnaturalearth: World Map Data from Natural Earth.

Matsuda, S., Kodama, T., Okada, N., Okayama, K., Honda, T., Iida, T., 2010. Association of Vibrio parahaemolyticus thermostable direct hemolysin with lipid rafts is essential for cytotoxicity but not hemolytic activity. Infect. Immun. 78, 603–610. https://doi.org/10.1128/IAI.00946-09

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

Matz, C., Nouri, B., McCarter, L., Martinez-Urtaza, J., 2011. Acquired type III secretion system determines environmental fitness of epidemic Vibrio parahaemolyticus in the interaction with bacterivorous protists. PloS One 6, e20275. https://doi.org/10.1371/journal.pone.0020275

McDougald, D., Kjelleberg, S., 2006. Adaptive Responses of Vibrios. Biol. Vibrios, Wiley Online Books. https://doi.org/10.1128/9781555815714.ch10

McFall-Ngai, M., 2014. Divining the essence of symbiosis: insights from the squid-vibrio model. PLoS Biol 12, e1001783.

Mead, P.S., Slutsker, L., Griffin, P.M., Tauxe, R. V., 1999. Food-Related Illness and Death in the United States Reply to Dr. Hedberg. Emerg. Infect. Dis. 5, 841–842. https://doi.org/10.3201/eid0506.990625

Michael Beman, J., Arrigo, K.R., Matson, P.A., 2005. Agricultural runoff fuels large phytoplankton blooms in vulnerable areas of the ocean. Nature 434, 211–214. https://doi.org/10.1038/nature03370

Miller, J.J., Weimer, B.C., Timme, R., Lüdeke, C.H., Pettengill, J.B., Bandoy, D.D., Weis, A.M., Kaufman, J., Huang, B.C., Payne, J., others, 2021. Phylogenetic and biogeographic patterns of Vibrio parahaemolyticus strains from North America inferred from whole-genome sequence data. Appl. Environ. Microbiol. 87, e01403-20.

Miyamoto, Y., Kato, T., Obara, Y., Akiyama, S., Takizawa, K., Yamai, S., 1969. In vitro hemolytic characteristic of Vibrio parahaemolyticus: its close correlation with human pathogenicity. J. Bacteriol. 100, 1147–1149.

Mohan Raj, J.R., Vittal, R., Shivakumaraswamy, S.K., Deekshit, V.K., Chakraborty, A., Karunasagar, I., 2019. Presence & mobility of antimicrobial resistance in Gram-negative bacteria from environmental samples in coastal Karnataka, India. Indian J. Med. Res. 149, 290–294. https://doi.org/10.4103/ijmr.IJMR_2088_17

Mora, C., McKenzie, T., Gaw, I.M., Dean, J.M., von Hammerstein, H., Knudson, T.A., Setter, R.O., Smith, C.Z., Webster, K.M., Patz, J.A., others, 2022. Over half of known human pathogenic diseases can be aggravated by climate change. Nat. Clim. Change 12, 869–875.

Morgado, M.E., Brumfield, K.D., Colwell, R.R., Sapkota, A.R., 2023. Vibriosis trends in Maryland, U.S., 2006-2019: Increased incidence of water-associated wound infections and risk of hospitalization. Manuscr. Prep.

Morris Jr., J.G., Calderwood, S.B., Baron, E.L., 2023. Vibrio vulnificus infections [WWW Document]. UpToDate. URL https://www.uptodate.com/contents/vibrio-vulnificus-infections (accessed 4.18.23).

Morrison, S.S., Williams, T., Cain, A., Froelich, B., Taylor, C., Baker-Austin, C., Verner-Jeffreys, D., Hartnell, R., Oliver, J.D., Gibas, C.J., 2012. Pyrosequencing-based comparative genome analysis of Vibrio vulnificus environmental isolates. PLoS One 7, e37553.

Motes, M., DePaola, A., Cook, D., Veazey, J., Hunsucker, J., Garthright, W., Blodgett, R., Chirtel, S., 1998. Influence of water temperature and salinity on Vibrio vulnificus in Northern Gulf and Atlantic Coast oysters (Crassostrea virginica). Appl. Environ. Microbiol. 64, 1459–1465.

Motesharrei, S., Rivas, J., Kalnay, E., Asrar, G.R., Busalacchi, A.J., Cahalan, R.F., Cane, M.A., Colwell, R.R., Feng, K., Franklin, R.S., others, 2016. Modeling sustainability: population, inequality, consumption, and bidirectional coupling of the Earth and Human Systems. Natl. Sci. Rev. 3, 470–494.

Muhling, B.A., Jacobs, J., Stock, C.A., Gaitan, C.F., Saba, V.S., 2017. Projections of the future occurrence, distribution, and seasonality of three Vibrio species in the Chesapeake Bay under a high-emission climate change scenario. GeoHealth 1, 278–296. https://doi.org/10.1002/2017GH000089

Mutreja, A., Kim, D.W., Thomson, N.R., Connor, T.R., Lee, J.H., Kariuki, S., Croucher, N.J., Choi, S.Y., Harris, S.R., Lebens, M., Niyogi, S.K., Kim, E.J., Ramamurthy, T., Chun, J., Wood, J.L.N., Clemens, J.D., Czerkinsky, C., Nair, G.B., Holmgren, J., Parkhill, J., Dougan, G., 2011. Evidence for several waves of global transmission in the seventh cholera pandemic. Nature 477, 462–465. https://doi.org/10.1038/nature10392

Nair, G.B., Colwell, R.R., Alam, M., Sack, D.A., Sack, B.R., Sadique, A., Watanabe, H., Grim, C.J., Ahmed, K.U., Hasan, N.A., Huq, A., Sultana, M., Siddique, K.A., 2007a. Viable but nonculturable Vibrio cholerae O1 in biofilms in the aquatic environment and their role in cholera transmission. Proc. Natl. Acad. Sci. 104, 17801–17806. https://doi.org/10.1073/pnas.0705599104

Nair, G.B., Faruque, S.M., Bhuiyan, N.A., Kamruzzaman, M., Siddique, A.K., Sack, D.A., 2002. New variants of Vibrio cholerae O1 biotype El Tor with attributes of the classical biotype from hospitalized patients with acute diarrhea in Bangladesh. J. Clin. Microbiol. 40, 3296–3299. https://doi.org/10.1128/JCM.40.9.3296-3299.2002

Nair, G.B., Hormazabal, J.C., 2005. The Vibrio parahaemolyticus pandemic. Rev. Chil. Infectologia Organo Of. Soc. Chil. Infectologia 22, 125–130. https://doi.org//S0716-10182005000200002

Nair, G.B., Oku, Y., Takeda, Y., Ghosh, A., Ghosh, R.K., Chattopadhyay, S., Pal, S.C., Kaper, J.B., Takeda, T., 1988. Toxin profiles of Vibrio cholerae non-O1 from environmental sources in Calcutta, India. Appl. Environ. Microbiol. 54, 3180–3182. https://doi.org/10.1128/AEM.54.12.3180-3182.1988

Nair, G.B., Ramamurthy, T., Bhattacharya, S.K., Dutta, B., Takeda, Y., Sack, D.A., 2007b. Global dissemination of Vibrio parahaemolyticus serotype O3:K6 and its serovariants. Clin. Microbiol. Rev. 20, 39–48. https://doi.org/10.1128/CMR.00025-06

Nair, G.B., Ramamurthy, T., Bhattacharya, S.K., Mukhopadhyay, A.K., Garg, S., Bhattacharya, M.K., Takeda, T., Shimada, T., Takeda, Y., Deb, B.C., 1994. Spread of Vibrio cholerae 0139 Bengal in India. J. Infect. Dis. 169, 1029–1034. https://doi.org/10.1093/infdis/169.5.1029

Nair, G.B., Safa, A., Bhuiyan, N.A., Nusrin, S., Murphy, D., Nicol, C., Valcanis, M., Iddings, S., Kubuabola, I., Vally, H., 2006. Isolation of Vibrio cholerae O1 strains similar to pre-seventh pandemic El Tor strains during an outbreak of gastrointestinal disease in an island resort in Fiji. J. Med. Microbiol. 55, 1559–1562. https://doi.org/10.1099/jmm.0.46734-0

Namadi, P., Deng, Z., 2021. Modeling and forecasting Vibrio parahaemolyticus concentrations in oysters. Water Res. 189, 116638.

Namadi, P., Deng, Z., 2023. Optimum environmental conditions controlling prevalence of vibrio parahaemolyticus in marine environment. Mar. Environ. Res. 183, 105828. https://doi.org/10.1016/j.marenvres.2022.105828

Nandi, B., Nandy, R.K., Mukhopadhyay, S., Nair, G.B., Shimada, T., Ghose, A.C., 2012. Rapid Method for Species-Specific Identification of Vibrio cholerae Using Primers Targeted to the Gene of Outer Membrane Protein OmpW 38, 1–11.

NASA, 2021. Panoply net CDF, HDF and GRIB Data Viewer [WWW Document]. URL https://www.giss.nasa.gov/tools/panoply/ (accessed 7.7.21).

NASA, 2023. Moderate Resolution Imaging Spectroradiometer [WWW Document]. URL https://modis.gsfc.nasa.gov/data/ (accessed 5.2.23).

Nasr-Azadani, F., Khan, R., Rahimikollu, J., Unnikrishnan, A., Akanda, A., Alam, M., Huq, A., Jutla, A., Colwell, R., 2017. Hydroclimatic sustainability assessment of changing climate on cholera in the Ganges-Brahmaputra basin. Adv. Water Resour. 108, 332–344. https://doi.org/10.1016/j.advwatres.2016.11.018

Nasr-Azadani, F., Unnikrishnan, A., Akanda, A., Islam, S., Alam, M., Huq, A., Jutla, A., Colwell, R., 2015. Downscaling river discharge to assess the effects of climate change on cholera outbreaks in the Bengal Delta. Clim. Res. 64, 257–274.

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

NCBI, 2023. National Database of Antibiotic Resistant Organisms (NDARO) [WWW Document]. URL https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/ (accessed 3.13.23).

NCCOS, 2018. Vibrio Predictive Models [WWW Document]. URL https://products.coastalscience.noaa.gov/vibrioforecast/default.aspx (accessed 1.4.21).

NCCOS, 2019. Water Guidance - Vibrio vulnificus in Chesapeake Bay [WWW Document]. URL https://products.coastalscience.noaa.gov/vibrioforecast/vulnificus/chesapeake/default.aspx (accessed 1.8.21).

Newton, A., Kendall, M., Vugia, D.J., Henao, O.L., Mahon, B.E., 2012. Increasing rates of vibriosis in the United States, 1996-2010: review of surveillance data from 2 systems. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 54, S391–S395. https://doi.org/10.1093/cid/cis243

NIH, 2009. Cholera linked to climate change, says Colwell [WWW Document]. URL https://www.fic.nih.gov/News/GlobalHealthMatters/Pages/0209_colwell.aspx (accessed 1.27.23).

Nishibuchi, M., 1996. Origin of the pathogenic vibrios in the environment: inference from the studies on the molecular genetics of Vibrio cholerae and Vibrio parahaemolyticus. Nihon Saikingaku Zasshi Jpn. J. Bacteriol. 51, 823.

Nishibuchi, M., Fasano, A., Russell, R.G., Kaper, J.B., 1992. Enterotoxigenicity of Vibrio parahaemolyticus with and without genes encoding thermostable direct hemolysin. Infect. Immun. 60, 3539–3545.

Nishibuchi, M., Kaper, J.B., 1995. Thermostable direct hemolysin gene of Vibrio parahaemolyticus: A virulence gene acquired by a marine bacterium. Infect. Immun. 63, 2093–2099.

NOAA, 2021a. Fast Facts: Economics and Demographics [WWW Document].

NOAA, 2021b. Chesapeake Bay Operational Forecast System [WWW Document]. URL https://tidesandcurrents.noaa.gov/ofs/cbofs/cbofs.html (accessed 7.4.21).

NOAA, 2021c. nowCoast [WWW Document]. URL https://nowcoast.noaa.gov (accessed 7.4.21).

NOAA, 2021d. Global Ocean Heat and Salt Content [WWW Document].

NOAA, 2021e. Fast Facts: Economics and Demographics [WWW Document]. URL (accessed 7.6.21).

Nogales, B., Lanfranconi, M.P., Piña-Villalonga, J.M., Bosch, R., 2011. Anthropogenic perturbations in marine microbial communities. FEMS Microbiol. Rev. 35, 275–298. https://doi.org/10.1111/j.1574-6976.2010.00248.x

Noman, E., Al-Gheethi, A., Radin Mohamed, R.M.S., Talip, B., Al-Sahari, M., Al-Shaibani, M., 2021. Quantitative microbiological risk assessment of complex microbial community in Prawn farm wastewater and applicability of nanoparticles and probiotics for eliminating of antibiotic-resistant bacteria. J. Hazard. Mater. 419, 126418. https://doi.org/10.1016/j.jhazmat.2021.126418

Nordstrom, J.L., DePaola, A., 2003. Improved recovery of pathogenic Vibrio parahaemolyticus from oysters using colony hybridization following enrichment. J. Microbiol. Methods 52, 273–277.

Nowakowska, J., Oliver, J.D., 2013. Resistance to environmental stresses by Vibrio vulnificus in the viable but nonculturable state. FEMS Microbiol. Ecol. 84, 213–222. https://doi.org/10.1111/1574-6941.12052

Oberbeckmann, S., Fuchs, B.M., Meiners, M., Wichels, A., Wiltshire, K.H., Gerdts, G., 2012. Seasonal Dynamics and Modeling of a Vibrio Community in Coastal Waters of the North Sea. Microb. Ecol. 63, 543–551. https://doi.org/10.1007/s00248-011-9990-9

Okuda, J., Ishibashi, M., Abbott, S.L., Janda, J.M., Nishibuchi, M., 1997. Analysis of the thermostable direct hemolysin (tdh) gene and the tdh-related hemolysin (trh) genes in urease-positive strains of Vibrio parahaemolyticus isolated on the West Coast of the United States. J. Clin. Microbiol. 35, 1965–1971.

Okura, M., Osawa, R., Iguchi, A., Arakawa, E., Terajima, J., Watanabe, H., 2003. Genotypic analyses of Vibrio parahaemolyticus and development of a pandemic group-specific multiplex PCR assay. J. Clin. Microbiol. 41, 4676–4682. https://doi.org/10.1128/JCM.41.10.4676-4682.2003

O'Leary, N.A., Wright, M.W., Brister, J.R., Ciufo, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D., Astashyn, A., Badretdin, A., Bao, Y., Blinkova, O., Brover, V., Chetvernin, V., Choi, J., Cox, E., Ermolaeva, O., Farrell, C.M., Goldfarb, T., Gupta, T., Haft, D., Hatcher, E., Hlavina, W., Joardar, V.S., Kodali, V.K., Li, W., Maglott, D., Masterson, P., McGarvey, K.M., Murphy, M.R., O'Neill, K., Pujar, S., Rangwala, S.H., Rausch, D., Riddick, L.D., Schoch, C., Shkeda, A., Storz, S.S., Sun, H., Thibaud-Nissen, F., Tolstoy, I., Tully, R.E., Vatsan, A.R., Wallin, C., Webb, D., Wu, W., Landrum, M.J., Kimchi, A., Tatusova, T., DiCuccio, M., Kitts, P., Murphy, T.D., Pruitt, K.D., 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. 44, D733–D745. https://doi.org/10.1093/nar/gkv1189

Oliver, J.D., 2006. Vibrio vulnificus. Biol. Vibrios, Wiley Online Books. https://doi.org/10.1128/9781555815714.ch25

Oliver, J.D., 2010. Recent findings on the viable but nonculturable state in pathogenic bacteria. FEMS Microbiol. Rev. 34, 415–425. https://doi.org/10.1111/j.1574-6976.2009.00200.x

Oliver, J.D., Roberts, D.M., White, V.K., Dry, M.A., Simpson, L.M., 1986. Bioluminescence in a strain of the human pathogenic bacterium Vibrio vulnificus. Appl. Environ. Microbiol. 52, 1209–1211.

Olson, E., 2022. A rare but dangerous flesh-eating bacteria is infecting Florida residents [WWW Document]. URL https://www.npr.org/2022/10/19/1129865243/flesh-eating-bacteria-florida-floodwater (accessed 11.19.22).

Ottaviani, D., Leoni, F., Serra, R., Serracca, L., Decastelli, L., Rocchegiani, E., Masini, L., Canonico, C., Talevi, G., Carraturo, A., 2012. Nontoxigenic Vibrio parahaemolyticus strains causing acute gastroenteritis. J. Clin. Microbiol. 50, 4141–4143.

Paranjpye, R.N., Strom, M.S., 2005. A Vibrio vulnificus Type IV Pilin Contributes to Biofilm Formation, Adherence to Epithelial Cells, and Virulence. Infect. Immun. 73, 1411 LP – 1422. https://doi.org/10.1128/IAI.73.3.1411-1422.2005

Park, K.-S., Ono, T., Rokuda, M., Jang, M.-H., Okada, K., Iida, T., Honda, T., 2004. Functional characterization of two type III secretion systems of Vibrio

parahaemolyticus. Infect. Immun. 72, 6659–6665. https://doi.org/10.1128/IAI.72.11.6659-6665.2004

Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P., Tyson, G.W., 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. 25, 1043–1055.

Parveen, S., Hettiarachchi, K.A., Bowers, J.C., Jones, J.L., Tamplin, M.L., McKay, R., Beatty, W., Brohawn, K., DaSilva, L.V., DePaola, A., 2008. Seasonal distribution of total and pathogenic Vibrio parahaemolyticus in Chesapeake Bay oysters and waters. Int. J. Food Microbiol. 128, 354–361.

Parveen, S., Jacobs, J., Ozbay, G., Chintapenta, L.K., Almuhaideb, E., Meredith, J., Ossai, S., Abbott, A., Grant, A., Brohawn, K., others, 2020. Seasonal and geographical differences in total and pathogenic Vibrio parahaemolyticus and Vibrio vulnificus levels in seawater and oysters from the Delaware and Chesapeake Bays determined using several methods. Appl. Environ. Microbiol. 86, e01581-20.

Pascual, M., Rodó, X., Ellner, S.P., Colwell, R., Bouma, M.J., 2000. Cholera dynamics and El Nino-southern oscillation. Science 289, 1766–1769.

Paz, S., Bisharat, N., Paz, E., Kidar, O., Cohen, D., 2007. Climate change and the emergence of Vibrio vulnificus disease in Israel. Environ. Res. 103, 390–396. https://doi.org/10.1016/j.envres.2006.07.002

Pollard, M.O., Gurdasani, D., Mentzer, A.J., Porter, T., Sandhu, M.S., 2018. Long reads: their purpose and place. Hum. Mol. Genet. 27, R234–R241.

Pollitzer, R., 1954. Cholera studies: 1. History of the disease. Bull. World Health Organ. 10, 421–461.

Pollitzer, R., Sawaroop, S., Burrows, W., 1959. World Health Organization monograph series ; no. 43.

Polz, M.F., Thompson, J.R., Randa, M.A., Lim, E., Marcelino, L.A., Tomita-Mitchell, A., 2004. Diversity and Dynamics of a North Atlantic Coastal Vibrio Community. Appl. Environ. Microbiol. 70, 4103–4110. https://doi.org/10.1128/aem.70.7.4103-4110.2004

Post, E., Bhatt, U.S., Bitz, C.M., Brodie, J.F., Fulton, T.L., Hebblewhite, M., Kerby, J., Kutz, S.J., Stirling, I., Walker, D.A., 2013. Ecological consequences of sea-ice decline. science 341, 519–524.

Prithvisagar, K.S., Krishna Kumar, B., Kodama, T., Rai, P., Iida, T., Karunasagar, Iddya, Karunasagar, Indrani, 2021. Whole genome analysis unveils genetic diversity and potential virulence determinants in Vibrio parahaemolyticus associated with disease outbreak among cultured Litopenaeus vannamei (Pacific white shrimp) in India. Virulence 12, 1936–1949.

Pruzzo, C., Vezzulli, L., Colwell, R.R., 2008. Global impact of Vibrio cholerae interactions with chitin. Environ. Microbiol. 10, 1400–1410. https://doi.org/10.1111/j.1462-2920.2007.01559.x

Quilici, M.-L., de La Blanchardière, A., Parienti, J.-J., Cattoir, V., Daurel, C., Deshayes, S., 2015. Non-O1, non-O139 Vibrio cholerae bacteraemia: case report and literature review. SpringerPlus 4, 1–9. https://doi.org/10.1186/s40064-015-1346-3

Quinlan, A.R., Hall, I.M., 2010. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 26, 841–842.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Raghunath, P., 2015. Roles of thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) in Vibrio parahaemolyticus. Front. Microbiol. 5, 805.

Rahman, Mokhlasur, Bhuiyan, N.A., Kuhn, I., Ramamurthy, T., Rahman, M, Mollby, R., Nair, G.B., 2006. Biochemical fingerprinting of Vibrio parahaemolyticus by the PhenePlate system: comparison between pandemic and non-pandemic serotypes. Epidemiol. Infect. 134, 985–989. https://doi.org/10.1017/S0950268806006017

Räisänen, J., 2022. Probabilistic forecasts of near-term climate change: verification for temperature and precipitation changes from years 1971–2000 to 2011–2020. Clim. Dyn. 59, 1175–1188.

Ralston, E.P., Kite-Powell, H., Beet, A., 2011. An estimate of the cost of acute health effects from food-and water-borne marine pathogens and toxins in the USA. J. Water Health 9, 680–694.

Ramamurthy, T., 1993. Emergence of novel strain of Vibrio cholerae with epidemic potential in southern and eastern India. Lancet 341, 703–704.

Ramamurthy, T., Pragasam, A.K., Taylor-Brown, A., Will, R.C., Vasudevan, K., Das, B., Srivastava, S.K., Chowdhury, G., Mukhopadhyay, A.K., Dutta, S., others, 2022. Vibrio cholerae O139 genomes provide a clue to why it may have failed to usher in the eighth cholera pandemic. Nat. Commun. 13, 1–10.

Rampersad, S., Tennant, P., 2018. Replication and Expression Strategies of Viruses. Viruses 55–82. https://doi.org/10.1016/B978-0-12-811257-1.00003-6

Randa, M.A., Polz, M.F., Lim, E., 2004. Effects of temperature and salinity on Vibrio vulnificus population dynamics as assessed by quantitative PCR. Appl. Environ. Microbiol. 70, 5469–5476.

Rawlings, T.K., Ruiz, G.M., Colwell, R.R., 2007. Association of Vibrio cholerae O1 El Tor and O139 Bengal with the Copepods Acartia tonsa and Eurytemora affinis. Appl. Environ. Microbiol. 73, 7926–7933.

Redondo-Salvo, S., Fernández-López, R., Ruiz, R., Vielva, L., de Toro, M., Rocha, E.P.C., Garcillán-Barcia, M.P., de la Cruz, F., 2020. Pathways for horizontal gene transfer in bacteria revealed by a global map of their plasmids. Nat. Commun. 11, 3602. https://doi.org/10.1038/s41467-020-17278-2

Reen, F.J., Almagro-Moreno, S., Ussery, D., Boyd, E.F., 2006. The genomic code: inferring Vibrionaceae niche specialization. Nat. Rev. Microbiol. 4, 697–704. https://doi.org/10.1038/nrmicro1476

Rehnstam-Holm, A.-S., Atnur, V., Godhe, A., 2014. Defining the niche of Vibrio parahaemolyticus during pre- and post-monsoon seasons in the coastal Arabian Sea. Microb. Ecol. 67, 57–65. https://doi.org/10.1007/s00248-013-0311-3

Reidl, J., Klose, K.E., 2002. Vibrio cholerae and cholera: out of the water and into the host. FEMS Microbiol. Rev. 26, 125–139. https://doi.org/10.1111/j.1574-6976.2002.tb00605.x

Rohayem, J., 2009. Norovirus seasonality and the potential impact of climate change. Clin. Microbiol. Infect. 15, 524–527.

Rohmer, L., Hocquet, D., Miller, S.I., 2011. Are pathogenic bacteria just looking for food? Metabolism and microbial pathogenesis. Trends Microbiol. 19, 341–348.

Roig, F.J., Sanjuán, E., Llorens, A., Amaro, C., 2010. pilF polymorphism-based PCR to distinguish Vibrio vulnificus strains potentially dangerous to public health. Appl. Environ. Microbiol. 76, 1328–1333.

Rosche, T.M., Yano, Y., Oliver, J.D., 2005. A rapid and simple PCR analysis indicates there are two subgroups of Vibrio vulnificus which correlate with clinical or environmental isolation. Microbiol. Immunol. 49, 381–389.

Roser, M., Ochmann, S., Behrens, H., Ritchie, H., Dadonaite, B., 2014. Eradication of diseases. Our World Data.

Roszak, D., Colwell, R., 1987. Survival strategies of bacteria in the natural environment. Microbiol. Rev. 51, 365–379.

Roy, M.A., Arnaud, J.M., Jasmin, P.M., Hamner, S., Hasan, N.A., Colwell, R.R., Ford, T.E., 2018. A Metagenomic Approach to Evaluating Surface Water Quality in Haiti. Int. J. Environ. Res. Public. Health 15, 2211. https://doi.org/10.3390/ijerph15102211

Ruby, E.G., Urbanowski, M., Campbell, J., Dunn, A., Faini, M., Gunsalus, R., Lostroh, P., Lupp, C., McCann, J., Millikan, D., others, 2005. Complete genome sequence of Vibrio fischeri: a symbiotic bacterium with pathogenic congeners. Proc. Natl. Acad. Sci. 102, 3004–3009.

Ruiz, G.M., Rawlings, T.K., Dobbs, F.C., Drake, L.A., Mullady, T., Huq, A., Colwell, R.R., 2000. Global spread of microorganisms by ships. Nature 408, 49–50. https://doi.org/10.1038/35040695

Sakib, S.N., Reddi, G., Almagro-Moreno, S., 2018. Environmental Role of Pathogenic Traits in Vibrio cholerae. J. Bacteriol. 200, e00795-17. https://doi.org/10.1128/JB.00795-17

Sawabe, Tomoo, Ogura, Y., Matsumura, Y., Feng, G., Amin, A.R., Mino, S., Nakagawa, S., Sawabe, Toko, Kumar, R., Fukui, Y., others, 2013. Updating the Vibrio clades defined by multilocus sequence phylogeny: proposal of eight new clades, and the description of Vibrio tritonius sp. nov. Front. Microbiol. 4, 414.

Scaglione, S., Kliethermes, S., Cao, G., Shoham, D., Durazo, R., Luke, A., Volk, M.L., 2015. The Epidemiology of Cirrhosis in the United States: A Population-based Study. J. Clin. Gastroenterol. 49, 690–696. https://doi.org/10.1097/MCG.0000000000000208

Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R. V, Widdowson, M., Roy, S.L., Jones, J.L., Griffi, P.M., 2011. Foodborne Illness Acquired in the United States — Major Pathogens. Emerg. Infect. Dis. 17, 7–15. https://doi.org/10.3201/eid1701.P11101

Scanes, E., Scanes, P.R., Ross, P.M., 2020. Climate change rapidly warms and acidifies Australian estuaries. Nat. Commun. 11, 1803.

Schwartz, K., Hammerl, J.A., Göllner, C., Strauch, E., 2019. Environmental and Clinical Strains of Vibrio cholerae Non-O1, Non-O139 From Germany Possess Similar Virulence Gene Profiles. Front. Microbiol. 10, 733. https://doi.org/10.3389/fmicb.2019.00733

Scro, A.K., Westphalen, J., Kite-Powell, H.L., Brawley, J.W., Smolowitz, R.M., 2022. The effect of off-bottom versus on-bottom oyster culture on total and pathogenic Vibrio spp. abundances in oyster tissue, water and sediment samples. Int. J. Food Microbiol. 379, 109870.

Seaby, R.M., Henderson, P.A., 2006. Species Diversity and Richness. Pisces Conservation Ltd., Lymington, England.

Seas, C., Miranda, J., Gil, A.I., León-Barúa, R., Patz, J., Huq, A., Colwell, R.R., Sack, R., 2000. New insights on the emergence of cholera in Latin America during 1991: the Peruvian experience. Am. J. Trop. Med. Hyg. 62, 513–517.

Sechi, L.A., Duprè, I., Deriu, A., Fadda, G., Zanetti, S., 2000. Distribution of Vibrio cholerae virulence genes among different Vibrio species isolated in Sardinia, Italy. J. Appl. Microbiol. 88, 475–481.

Seemann, T., 2020. ABRicate [WWW Document]. URL https://github.com/tseemann/abricate (accessed 8.30.22).

Seemann, T., 2022. MLST [WWW Document]. URL https://github.com/tseemann/mlst (accessed 3.13.23).

Semenza, J.C., 2020. Cascading risks of waterborne diseases from climate change. Nat. Immunol. 21, 484–487.

Semenza, J.C., Trinanes, J., Lohr, W., Sudre, B., Löfdahl, M., Martinez-Urtaza, J., Nichols, G.L., Rocklöv, J., 2017. Environmental Suitability of Vibrio Infections in a Warming Climate: An Early Warning System. Environ. Health Perspect. 125, 107004. https://doi.org/10.1289/EHP2198

Shah, N., Tang, H., Doak, T.G., Ye, Y., 2011. Comparing bacterial communities inferred from 16S rRNA gene sequencing and shotgun metagenomics, in: Biocomputing 2011. World Scientific, pp. 165–176.

Shaw, K.S., Jacobs, J.M., Crump, B.C., 2014a. 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. 5, 204.

Shaw, K.S., Rosenberg Goldstein, R.E., He, X., Jacobs, J.M., Crump, B.C., Sapkota, A.R., 2014b. Antimicrobial susceptibility of Vibrio vulnificus and Vibrio parahaemolyticus recovered from recreational and commercial areas of Chesapeake Bay and Maryland Coastal Bays. PloS One 9, e89616.

Shirai, H., Ito, H., Hirayama, T., Nakamoto, Y., Nakabayashi, N., Kumagai, K., Takeda, Y., Nishibuchi, M., 1990. Molecular epidemiologic evidence for association of thermostable direct hemolysin (TDH) and TDH-related hemolysin of Vibrio parahaemolyticus with gastroenteritis. Infect. Immun. 58, 3568–3573.

Sievert, C., 2020. Interactive Web-Based Data Visualization with R, plotly, and shiny. Chapman and Hall/CRC.

Silva, I.P., Carneiro, C. de S., Saraiva, M.A.F., Oliveira, T.A.S. de, Sousa, O.V. de, Evangelista-Barreto, N.S., 2018. Antimicrobial resistance and potential virulence of Vibrio parahaemolyticus isolated from water and bivalve mollusks from Bahia, Brazil. Mar. Pollut. Bull. 131, 757–762. https://doi.org/10.1016/j.marpolbul.2018.05.007

Silva-Valenzuela, C.A., Camilli, A., 2019. Niche adaptation limits bacteriophage predation of Vibrio cholerae in a nutrient-poor aquatic environment. Proc. Natl. Acad. Sci. 116, 1627 LP – 1632. https://doi.org/10.1073/pnas.1810138116

Sime-Ngando, T., 2014. Environmental bacteriophages: viruses of microbes in aquatic ecosystems. Front. Microbiol. 5, 355.

Simon, C., Daniel, R., 2011. Metagenomic analyses: past and future trends. Appl. Environ. Microbiol. 77, 1153–1161. https://doi.org/10.1128/AEM.02345-10

Singh, B., Tyagi, A., Billekallu Thammegowda, N.K., Ansal, M.D., 2018. Prevalence and antimicrobial resistance of vibrios of human health significance in inland saline aquaculture areas. Aquac. Res. 49, 2166–2174. https://doi.org/10.1111/are.13672

Singleton, F., Attwell, R., Jangi, S., Colwell, R., 1982. Effects of temperature and salinity on Vibrio cholerae growth. Appl. Environ. Microbiol. 44, 1047–1058.

Singleton, F.L., Attwell, R.W., Jangi, M.S., Colwell, R.R., 1983. Influence of Salinity, Nutrient Concentration and Temperature on Growth and Survival of Vibrio Choleras in the Aquatic Environment, in: Kuwahara, S., Pierce, N.F. (Eds.), Advances in Research on Cholera and Related Diarrheas. Springer

Netherlands, Dordrecht, pp. 101–112. https://doi.org/10.1007/978-94-009-6735-9_12

Sleator, R.D., Shortall, C., Hill, C., 2008. Metagenomics. Lett. Appl. Microbiol. 47, 361–366. https://doi.org/10.1111/j.1472-765X.2008.02444.x

Smale, D.A., Wernberg, T., Oliver, E.C.J., Thomsen, M., Harvey, B.P., Straub, S.C., Burrows, M.T., Alexander, L.V., Benthuysen, J.A., Donat, M.G., 2019. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nat. Clim. Change 9, 306–312.

Smith, S.D., 2019. phylosmith: an R-package for reproducible and efficient microbiome analysis with phyloseq-objects. J. Open Source Softw. 4, 1442. https://doi.org/10.21105/joss.01442

Sochard, M., Wilson, D., Austin, B., Colwell, R., 1979. Bacteria associated with the surface and gut of marine copepods. Appl. Environ. Microbiol. 37, 750–759.

Sodders, N., Stockdale, K., Baker, K., Ghanem, A., Vieth, B., Harder, T., 2023. Notes from the Field: Vibriosis Cases Associated with Flood Waters During and After Hurricane Ian—Florida, September–October 2022. Morb. Mortal. Wkly. Rep. 72, 497.

Spagnoletti, M., Ceccarelli, D., Rieux, A., Fondi, M., Taviani, E., Fani, R., Colombo, M.M., Colwell, R.R., Balloux, F., 2014. Acquisition and evolution of SXT-R391 integrative conjugative elements in the seventh-pandemic Vibrio cholerae lineage. MBio 5, e01356-14.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stine, O.C., Morris Jr, J.G., 2014. Circulation and transmission of clones of Vibrio cholerae during cholera outbreaks. Curr. Top. Microbiol. Immunol. 379, 181–193. https://doi.org/10.1007/82_2013_360

Stine, O.C., Sozhamannan, S., Gou, Q., Zheng, S., Morris Jr, J.G., Johnson, J.A., 2000. Phylogeny of Vibrio cholerae based on recA sequence. Infect. Immun. 68, 7180–7185.

Strömberg, K.P., Smyth, T.J., Allen, J.I., Pitois, S., O'Brien, T.D., 2009. Estimation of global zooplankton biomass from satellite ocean colour. J. Mar. Syst. 78, 18–27.

Su, Y.C., Liu, C., 2007. Vibrio parahaemolyticus: a concern of seafood safety. Food Microbiol. 24, 549–558. https://doi.org/10.1016/j.fm.2007.01.005

Sultana, M., Nusrin, S., Hasan, N.A., Sadique, A., Ahmed, K.U., Islam, A., Hossain, A., Longini, I., Nizam, A., Huq, A., Siddique, A.K., Sack, D.A., Sack, R.B., Colwell, R.R., Alam, M., 2018. Biofilms Comprise a Component of the Annual Cycle of Vibrio cholerae in the Bay of Bengal Estuary. mBio 9, e00483-18. https://doi.org/10.1128/mBio.00483-18

Tack, D.M., Ray, L., Griffin, P.M., Cieslak, P.R., Dunn, J., Rissman, T., Jervis, R., Lathrop, S., Muse, A., Duwell, M., Smith, K., Tobin-D'Angelo, M., Vugia, D.J., Zablotsky Kufel, J., Wolpert, B.J., Tauxe, R., Payne, D.C., 2020. Preliminary Incidence and Trends of Infections with Pathogens Transmitted Commonly Through Food - Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2016-2019. MMWR Morb. Mortal. Wkly. Rep. 69, 509–514. https://doi.org/10.15585/mmwr.mm6917a1

Takemura, A.F., Chien, D.M., Polz, M.F., 2014. Associations and dynamics of vibrionaceae in the environment, from the genus to the population level. Front. Microbiol. 5, 1–26. https://doi.org/10.3389/fmicb.2014.00038

Tamplin, M.L., Gauzens, A.L.L., Huq, A., Sack, D.A. a, Colwell, R.R.R., 1990. Attachment of *Vibrio cholerae* serogroup 01 to zooplankton of Bangladesh waters. Appl. Environ. Microbiol. 56, 1977–1980. https://doi.org/10.1128/AEM.01238-07

Teodoro, J.D., Nairn, B., 2020. Understanding the knowledge and data landscape of climate change impacts and adaptation in the Chesapeake Bay Region: A systematic review. Climate 8, 58.

Tessler, M., Neumann, J.S., Afshinnekoo, E., Pineda, M., Hersch, R., Velho, L.F.M., Segovia, B.T., Lansac-Toha, F.A., Lemke, M., DeSalle, R., Mason, C.E., Brugler, M.R., 2017. Large-scale differences in microbial biodiversity discovery between 16S amplicon and shotgun sequencing. Sci. Rep. 7, 6589. https://doi.org/10.1038/s41598-017-06665-3

Thickman, J.D., Gobler, C.J., 2017. The ability of algal organic matter and surface runoff to promote the abundance of pathogenic and non-pathogenic strains of Vibrio parahaemolyticus in Long Island Sound, USA. PLoS One 12, e0185994.

Thompson, J.R., Polz, M.F., 2006. Dynamics of Vibrio populations and their role in environmental nutrient cycling, in: The Biology of Vibrios. Wiley Online Library, pp. 190–203.

Trinanes, J., Martinez-Urtaza, J., 2021. Future scenarios of risk of Vibrio infections in a warming planet: a global mapping study. Lancet Planet. Health 5, e426–e435.

Trtanj, J., Jantarasami, L., Brunkard, J., Collier, T., Jacobs, J., Lipp, E., McLellan, S., Moore, S., Paerl, H., Ravenscroft, J., Sengco, M., Thurston, J., 2016. Ch. 6: Climate Impacts on Water-Related Illness. The Impacts of Climate Change on Human Health in the United States: A Scientific Assessment. U.S. Global Change Research Program. https://doi.org/10.7930/J03F4MH4

Turnbaugh, P.J., Gordon, J.I., 2008. An Invitation to the marriage of metagenomics and metabolomics. Cell 134, 708–713. https://doi.org/10.1016/j.cell.2008.08.025

Turner, J., Colwell, S.R., Marshall, G.J., Lachlan-Cope, T.A., Carleton, A.M., Jones, P.D., Lagun, V., Reid, P.A., Iagovkina, S., 2005. Antarctic climate change during the last 50 years. Int. J. Climatol. 25, 279–294.

Turner, J.W., Good, B., Cole, D., Lipp, E.K., 2009. Plankton composition and environmental factors contribute to Vibrio seasonality. ISME J. 3, 1082–1092. https://doi.org/10.1038/ismej.2009.50

Turner, J.W., Paranjpye, R.N., Landis, E.D., Biryukov, S. V, González-Escalona, N., Nilsson, W.B., Strom, M.S., 2013. Population structure of clinical and environmental Vibrio parahaemolyticus from the Pacific Northwest coast of the United States. PloS One 8, e55726. https://doi.org/10.1371/journal.pone.0055726

UMCES, 2021. Integration and Application Network [WWW Document]. URL https://ian.umces.edu (accessed 1.8.21).

UMCES, 2022. Standard Operating Procedures for Fluorometric Determination of Chlorophyll α in waters and sediments of Fresh/Estuarine/Coastal Areas [WWW Document]. URL https://www.umces.edu/sites/default/files/Chlorophyll%20Fluoromtretic%20Method%202022-1.pdf (accessed 4.3.23).

Ummenhofer, C.C., Meehl, G.A., 2017. Extreme weather and climate events with ecological relevance: a review. Philos. Trans. R. Soc. B Biol. Sci. 372, 20160135. https://doi.org/10.1098/rstb.2016.0135

United Nations, 2015. Transforming our world: The 2030 Agenda for Sustainable Development [WWW Document]. URL https://sdgs.un.org/publications/transforming-our-world-2030-agenda-sustainable-development-17981 (accessed 6.14.23).

United Nations, 2020. World Population Ageing 2019. New York. https://doi.org/ST/ESA/SER.A/444

United Nations, 2022. Global cholera surge likely accelerated by climate change, warns WHO [WWW Document]. URL https://news.un.org/en/story/2022/12/1131787 (accessed 2.23.23).

United States Census Bureau, 2020. Annual Estimates of the Resident Population: April 1, 2020 to July 1, 2019. [WWW Document].

Urquhart, E.A., Zaitchik, B.F., Waugh, D.W., Guikema, S.D., Del Castillo, C.E., 2014. Uncertainty in Model Predictions of Vibrio vulnificus Response to Climate Variability and Change: A Chesapeake Bay Case Study. PLOS ONE 9, e98256.

Usmani, M., Brumfield, K.D., Jamal, Y., Huq, A., Colwell, R.R., Jutla, A., 2021. A review of the environmental trigger and transmission components for prediction of cholera. Trop. Med. Infect. Dis. 6, 147.

Usmani, M., Brumfield, K.D., Magers, B.M., Chaves-Gonzalez, J., Ticehurst, H., Barciela, R., McBean, F., Colwell, R.R., Jutla, A., 2023. Combating cholera

by building predictive capabilities for pathogenic Vibrio cholerae in Yemen. Sci. Rep. 13, 2255.

Usmani, M., Brumfield, K.D., Magers, B.M., Huq, A., Barciela, R., Nguyen, T.H., Colwell, R.R., Jutla, A., 2022. Predictive Intelligence for Cholera in Ukraine? GeoHealth 6, e2022GH000681. https://doi.org/10.1029/2022GH000681

Vandenberghe, J., Verdonck, L., Robles-Arozarena, R., Rivera, G., Bolland, A., Balladares, M., Gomez-Gil, B., Calderon, J., Sorgeloos, P., Swings, J., 1999. Vibrios Associated with Litopenaeus vannamei Larvae, Postlarvae, Broodstock, and Hatchery Probionts. Appl. Environ. Microbiol. 65, 2592 LP – 2597. https://doi.org/10.1128/AEM.65.6.2592-2597.1999

Verma, J., Bag, S., Saha, B., Kumar, P., Ghosh, T.S., Dayal, M., Senapati, T., Mehra, S., Dey, P., Desigamani, A., Kumar, D., Rana, P., Kumar, B., Maiti, T.K., Sharma, N.C., Bhadra, R.K., Mutreja, A., Nair, G.B., Ramamurthy, T., Das, B., 2019. Genomic plasticity associated with antimicrobial resistance in Vibrio cholerae. Proc. Natl. Acad. Sci. 116, 6226 LP – 6231. https://doi.org/10.1073/pnas.1900141116

Vezzulli, L., 2022. Global expansion of Vibrio spp. in hot water. Environ. Microbiol. Rep. 15, 77–79. https://doi.org/10.1111/1758-2229.13135

Vezzulli, L., Baker-Austin, C., Kirschner, A., Pruzzo, C., Martinez-Urtaza, J., 2020. Global emergence of environmental non-O1/O139 Vibrio cholerae infections linked with climate change: a neglected research field? Environ. Microbiol. 22, 4342–4355. https://doi.org/10.1111/1462-2920.15040

Vezzulli, L., Brettar, I., Pezzati, E., Reid, P.C., Colwell, R.R., Höfle, M.G., Pruzzo, C., 2012. Long-term effects of ocean warming on the prokaryotic community: Evidence from the vibrios. ISME J. 6, 21–30. https://doi.org/10.1038/ismej.2011.89

Vezzulli, L., Colwell, R.R., Pruzzo, C., 2013. Ocean warming and spread of pathogenic vibrios in the aquatic environment. Microb. Ecol. 65, 817–825.

Vezzulli, L., Grande, C., Reid, P.C., Hélaouët, P., Edwards, M., Höfle, M.G., Brettar, I., Colwell, R.R., Pruzzo, C., 2016. Climate influence on Vibrio and associated human diseases during the past half-century in the coastal North Atlantic. Proc. Natl. Acad. Sci. 113, E5062–E5071. https://doi.org/10.1073/pnas.1609157113

Vezzulli, L., Grande, C., Tassistro, G., Brettar, I., Höfle, M.G., Pereira, R.P.A., Mushi, D., Pallavicini, A., Vassallo, P., Pruzzo, C., 2017. Whole-Genome Enrichment Provides Deep Insights into Vibrio cholerae Metagenome from an African River. Microb. Ecol. 73, 734–738. https://doi.org/10.1007/s00248-016-0902-x

Vezzulli, L., Guzmán, C.A., Colwell, R.R., Pruzzo, C., 2008. Dual role colonization factors connecting Vibrio cholerae's lifestyles in human and aquatic environments open new perspectives for combating infectious diseases. Curr. Opin. Biotechnol. 19, 254–259.

Vezzulli, L., Oliveri, C., Borello, A., Gregory, L., Kimirei, I., Brunetta, M., Stern, R., Coco, S., Longo, L., Taviani, E., others, 2021. Aquatic reservoir of Vibrio cholerae in an African Great Lake assessed by large scale plankton sampling and ultrasensitive molecular methods. ISME Commun. 1, 1–4.

Vezzulli, L., Pezzati, E., Brettar, I., Höfle, M., Pruzzo, C., 2015. Effects of Global Warming on Vibrio Ecology. Microbiol. Spectr. 3, 18. https://doi.org/10.1128/microbiolspec.VE-0004-2014

Vezzulli, L., Pezzati, E., Moreno, M., Fabiano, M., Pane, L., Pruzzo, C., 2009. Benthic ecology of Vibrio spp. and pathogenic Vibrio species in a coastal Mediterranean environment (La Spezia Gulf, Italy). Microb. Ecol. 58, 808–818. https://doi.org/10.1007/s00248-009-9542-8

Vezzulli, L., Pruzzo, C., Huq, A., Colwell, R.R., 2010. Environmental reservoirs of Vibrio cholerae and their role in cholera. Environ. Microbiol. Rep. 2, 27–33. https://doi.org/10.1111/j.1758-2229.2009.00128.x

Vincent, A.T., Gauthier, J., Derome, N., Charette, S.J., 2019. The Rise and Fall of Antibiotics in Aquaculture BT - Microbial Communities in Aquaculture Ecosystems: Improving Productivity and Sustainability, in: Derome, N. (Ed.), . Springer International Publishing, Cham, pp. 1–19. https://doi.org/10.1007/978-3-030-16190-3_1

Visick, K.L., McFall-Ngai, M.J., 2000. An Exclusive Contract: Specificity in the Vibrio fischeri-Euprymna scolopes Partnership. J. Bacteriol. 182, 1779 LP – 1787. https://doi.org/10.1128/JB.182.7.1779-1787.2000

Voigt, S., Kemper, T., Riedlinger, T., Kiefl, R., Scholte, K., Mehl, H., 2007. Satellite Image Analysis for Disaster and Crisis-Management Support. IEEE Trans. Geosci. Remote Sens. 45, 1520–1528. https://doi.org/10.1109/TGRS.2007.895830

Waldor, M.K., Ali, A., Baker-Austin, C., Alam, M., Oliver, J.D., Martinez-Urtaza, J., Qadri, F., 2018. Vibrio spp. infections. Nat. Rev. Dis. Primer 4. https://doi.org/10.1038/s41572-018-0005-8

Waldor, M.K., Colwell, R., Mekalanos, J.J., 1994. The Vibrio cholerae O139 serogroup antigen includes an O-antigen capsule and lipopolysaccharide virulence determinants. Proc. Natl. Acad. Sci. 91, 11388 LP – 11392. https://doi.org/10.1073/pnas.91.24.11388

Waldor, M.K., Tschäpe, H., Mekalanos, J.J., 1996. A new type of conjugative transposon encodes resistance to sulfamethoxazole, trimethoprim, and streptomycin in Vibrio cholerae O139. J. Bacteriol. 178, 4157 LP – 4165. https://doi.org/10.1128/jb.178.14.4157-4165.1996

Wang, Q., Miao, Q., Pan, J., Jin, W., Ma, Y., Zhang, Y., Yao, Y., Su, Y., Huang, Y., Li, B., Wang, M., Li, N., Cai, S., Luo, Y., Zhou, C., Wu, H., Hu, B., 2020. The clinical value of metagenomic next-generation sequencing in the microbiological diagnosis of skin and soft tissue infections. Int. J. Infect. Dis. 100, 414–420. https://doi.org/10.1016/j.ijid.2020.09.007

Watson, J.T., Gayer, M., Connolly, M.A., 2007. Epidemics after Natural Disasters. Emerg. Infect. Dis. 13, 1–5.

Watts, N., Amann, M., Arnell, N., Ayeb-Karlsson, S., Belesova, K., Berry, H., Bouley, T., Boykoff, M., Byass, P., Cai, W., 2018. The 2018 report of the Lancet Countdown on health and climate change: shaping the health of nations for centuries to come. The Lancet 392, 2479–2514.

Weill, F., Domman, D., Njamkepo, E., Tarr, C., Rauzier, J., Fawal, N., Keddy, K.H., Salje, H., Moore, S., Mukhopadhyay, A.K., Bercion, R., Luquero, F.J., Koeck, J., Fournier, J., Dougan, G., 2017. Genomic history of the seventh pandemic of cholera in Africa. Science 789, 785–789.

Weill, F.-X., Domman, D., Njamkepo, E., Almesbahi, A.A., Naji, M., Nasher, S.S., Rakesh, A., Assiri, A.M., Sharma, N.C., Kariuki, S., Pourshafie, M.R., Rauzier, J., Abubakar, A., Carter, J.Y., Wamala, J.F., Seguin, C., Bouchier, C., Malliavin, T., Bakhshi, B., Abulmaali, H.H.N., Kumar, D., Njoroge, S.M., Malik, M.R., Kiiru, J., Luquero, F.J., Azman, A.S., Ramamurthy, T., Thomson, N.R., Quilici, M.-L., 2019. Genomic insights into the 2016–2017 cholera epidemic in Yemen. Nature 565, 230–233. https://doi.org/10.1038/s41586-018-0818-3

Wetz, J.J., Blackwood, A.D., Fries, J.S., Williams, Z.F., Noble, R.T., 2014. Quantification of Vibrio vulnificus in an Estuarine Environment: a Multi-Year Analysis Using QPCR. Estuaries Coasts 37, 421–435. https://doi.org/10.1007/s12237-013-9682-4

WHO, 2017. Progress on Drinking Water , Sanitation and Hygiene, Launch version July 12 Main report Progress on Drinking Water , Sanitation and Hygiene. Geneva.

WHO, 2019. Cholera [WWW Document].

WHO, 2022a. Drinking-water [WWW Document]. URL https://www.who.int/news-room/fact-sheets/detail/drinking-water (accessed 1.27.23).

WHO, 2022b. Cholera – Global situation [WWW Document]. URL https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON426 (accessed 1.13.23).

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer New York.

Williamson, C.E., Madronich, S., Lal, A., Zepp, R.G., Lucas, R.M., Overholt, E.P., Rose, K.C., Schladow, S.G., Lee-Taylor, J., 2017. Climate change-induced increases in precipitation are reducing the potential for solar ultraviolet radiation to inactivate pathogens in surface waters. Sci. Rep. 7, 1–12.

Wong, H.C., Wang, P., 2004. Induction of viable but nonculturable state in Vibrio parahaemolyticus and its susceptibility to environmental stresses. J. Appl. Microbiol. 96, 359–366. https://doi.org/10.1046/j.1365-2672.2004.02166.x

Wong, K.C., Brown, A.M., Luscombe, G.M., Wong, S.J., Mendis, K., 2015. Antibiotic use for Vibrio infections: important insights from surveillance data. BMC Infect. Dis. 15, 1–9.

Wood, D.E., Lu, J., Langmead, B., 2019. Improved metagenomic analysis with Kraken 2. Genome Biol. 20. https://doi.org/10.1186/s13059-019-1891-0

Wright, A.C., Hill, R.T., Johnson, J.A., Roghman, M.C., Colwell, R.R., Morris, J.G., 1996. Distribution of Vibrio vulnificus in the Chesapeake Bay. Appl. Environ. Microbiol. 62, 717–724.

Wright, A.C., Morris Jr, J.G., Maneval Jr, D.R., Richardson, K., Kaper, J.B., 1985. Cloning of the cytotoxin-hemolysin gene of Vibrio vulnificus. Infect. Immun. 50, 922–924.

Wu, X., Lu, Y., Zhou, S., Chen, L., Xu, B., 2016. Impact of climate change on human infectious diseases: Empirical evidence and human adaptation. Environ. Int. 86, 14–23.

Xie, J., Bu, L., Jin, S., Wang, X., Zhao, Q., Zhou, S., Xu, Y., 2020. Outbreak of vibriosis caused by Vibrio harveyi and Vibrio alginolyticus in farmed seahorse Hippocampus kuda in China. Aquaculture 523, 735168. https://doi.org/10.1016/j.aquaculture.2020.735168

Xie, Z.Y., Hu, C.Q., Chen, C., Zhang, L.P., Ren, C.H., 2005. Investigation of seven Vibrio virulence genes among Vibrio alginolyticus and Vibrio parahaemolyticus strains from the coastal mariculture systems in Guangdong, China. Lett. Appl. Microbiol. 41, 202–207. https://doi.org/10.1111/j.1472-765X.2005.01688.x

Xu, H.-S., Roberts, N.C., Adams, L.B., West, P.A., Siebeling, R.J., Huq, A., Huq, M.I., Rahman, R., Colwell, R.R., 1984. An indirect fluoresent antibody staining procedure for detection of Vibrio cholerae serovar 01 cells in aquatic environmental samples. J. Microbiol. Methods 2, 221–231. https://doi.org/10.1016/0167-7012(84)90017-4

Xu, M., Cao, C.X., Wang, D.C., Kan, B., Xu, Y.F., Ni, X.L., Zhu, Z.C., 2016. Environmental factor analysis of cholera in China using remote sensing and geographical information systems. Epidemiol. Infect. 144, 940–951. https://doi.org/DOI: 10.1017/S095026881500223X

Yáñez, R., Bastías, R., Higuera, G., Salgado, O., Katharios, P., Romero, J., Espejo, R., García, K., 2015. Amplification of tlh gene in other Vibrionaceae specie by specie-specific multiplex PCR of Vibrio parahaemolyticus. Electron. J. Biotechnol. 18, 459–463. https://doi.org/10.1016/j.ejbt.2015.09.007

Yang, J.H., Mok, J.S., Jung, Y.J., Lee, K.J., Kwon, J.Y., Park, K., Moon, S.Y., Kwon, S.J., Ryu, A.R., Lee, T.S., 2017. Distribution and antimicrobial susceptibility of Vibrio species associated with zooplankton in coastal area of Korea. Mar. Pollut. Bull. 125, 39–44. https://doi.org/10.1016/j.marpolbul.2017.07.054

Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., Chun, J., 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int. J. Syst. Evol. Microbiol. 67, 1613–1617. https://doi.org/10.1099/ijsem.0.001755

Yooseph, S., Nealson, K.H., Rusch, D.B., McCrow, J.P., Dupont, C.L., Kim, M., Johnson, J., Montgomery, R., Ferriera, S., Beeson, K., Williamson, S.J., Tovchigrechko, A., Allen, A.E., Zeigler, L.A., Sutton, G., Eisenstadt, E., Rogers, Y.-H., Friedman, R., Frazier, M., Venter, J.C., 2010. Genomic and functional adaptation in surface ocean planktonic prokaryotes. Nature 468, 60–66. https://doi.org/10.1038/nature09530

Yu, W.T., Jong, K.J., Lin, Y.R., Tsai, S. en, Tey, Y.H., Wong, H. chung, 2013. Prevalence of Vibrio parahaemolyticus in oyster and clam culturing environments in Taiwan. Int. J. Food Microbiol. 160, 185–192. https://doi.org/10.1016/j.ijfoodmicro.2012.11.002

Zeb, S., Shah, M.A., Yasir, M., Awan, H.M., Prommeenate, P., Klanchui, A., Wren, B.W., Thomson, N., Bokhari, H., 2019. Type III secretion system confers enhanced virulence in clinical non-O1/non-O139 Vibrio cholerae. Microb. Pathog. 135, 103645.

Zhang, X., Lin, H., Wang, X., Austin, B., 2018. Significance of Vibrio species in the marine organic carbon cycle—A review. Sci. China Earth Sci. 61, 1357–1368. https://doi.org/10.1007/s11430-017-9229-x

Zhang, X., Lu, Y., Qian, H., Liu, G., Mei, Y., Jin, F., Xia, W., Ni, F., 2020. Non-O1, Non-O139 Vibrio cholerae (NOVC) bacteremia: case report and literature review, 2015–2019. Infect. Drug Resist. 1009–1016.

Zhang, X.H., Austin, B., 2005. Haemolysins in Vibrio species. J. Appl. Microbiol. 98, 1011–1019. https://doi.org/10.1111/j.1365-2672.2005.02583.x

Zhao, Y., He, T., Tu, B., Mao, X., Jiang, J., Jiang, X., Wang, F., Wang, M., Wang, Y., Sun, H., 2022. Death in a farmer with underlying diseases carrying Vibrio cholerae non-O1/non-O139 producing zonula occludens toxin. Int. J. Infect. Dis. 120, 83–87.

Zhelyazkova, M., Yordanova, R., Mihaylov, I., Kirov, S., Tsonev, S., Danko, D., Mason, C., Vassilev, D., 2021. Origin Sample Prediction and Spatial Modeling of Antimicrobial Resistance in Metagenomic Sequencing Data. Front. Genet.

Zhou, Z., Pang, H., Ding, Y., Cai, J., Huang, Y., Jian, J., Wu, Z., 2013. VscO, a putative T3SS chaperone escort of Vibrio alginolyticus, contributes to virulence in fish and is a target for vaccine development. Fish Shellfish Immunol. 35, 1523–1531.

Zo, Y.-G., Chokesajjawatee, N., Grim, C., Arakawa, E., Watanabe, H., Colwell, R.R., 2009. Diversity and seasonality of bioluminescent Vibrio cholerae populations in Chesapeake Bay. Appl. Environ. Microbiol. 75, 135–146.

Zorriehzahra, M.J., Banaederakhshan, R., 2015. Early mortality syndrome (EMS) as new emerging threat in shrimp industry. Adv Anim Vet Sci 3, 64–72.

Appendix A: Table 11 References

1. Zhang Y, Bi P, Hiller JE, Sun Y, Ryan P. 2007. Climate variations and bacillary dysentery in northern and southern cities of China. J Infect 55:194–200.
2. Huang D, Guan P, Guo J, Wang P, Zhou B. 2008. Investigating the effects of climate variations on bacillary dysentery incidence in northeast China using ridge regression and hierarchical cluster analysis. BMC Infect Dis 8:1–9.
3. Li Z, Wang L, Sun W, Hou X, Yang H, Sun L, Xu S, Sun Q, Zhang J, Song H, others. 2013. Identifying high-risk areas of bacillary dysentery and associated meteorological factors in Wuhan, China. Sci Rep 3:3239.
4. Gao L, Zhang Y, Ding G, Liu Q, Zhou M, Li X, Jiang B. 2014. Meteorological variables and bacillary dysentery cases in Changsha City, China. Am J Trop Med Hyg 90:697.
5. Aminharati F, Soltan Dallal MM, Ehrampoush MH, Dehghani-Tafti A, Yaseri M, Memariani M, Rajabi Z. 2018. The effect of environmental parameters on the incidence of Shigella outbreaks in Yazd province, Iran. Water Sci Technol Water Supply 18:1388–1395.
6. Colwell RR. 1996. Global Climate and Infectious Disease: The Cholera Paradigm. Science 274:2025 LP – 2031.
7. Lobitz B, Beck L, Huq A, Wood B, Fuchs G, Faruque ASG, Colwell R. 2000. Climate and infectious disease: Use of remote sensing for detection of Vibrio cholerae by indirect measurement. Proc Natl Acad Sci 97:1438 LP – 1443.
8. Constantin de Magny G, Murtugudde R, Sapiano MRP, Nizam A, Brown CW, Busalacchi AJ, Yunus M, Nair GB, Gil AI, Lanata CF, Calkins J, Manna B, Rajendran K, Bhattacharya MK, Huq A, Sack RB, Colwell RR. 2008. Environmental signatures associated with cholera epidemics. Proc Natl Acad Sci 105:17676 LP – 17681.
9. Reyburn R, Kim DR, Emch M, Khatib A, Von Seidlein L, Ali M. 2011. Climate variability and the outbreaks of cholera in Zanzibar, East Africa: a time series analysis. Am J Trop Med Hyg 84:862.
10. Jutla A, Whitcombe E, Hasan N, Haley B, Akanda A, Huq A, Alam M, Sack RB, Colwell R. 2013. Environmental factors influencing epidemic cholera. Am J Trop Med Hyg 89:597–607.
11. Jutla A, Aldaach H, Billian H, Akanda A, Huq A, Colwell R. 2015. Satellite Based Assessment of Hydroclimatic Conditions Related to Cholera in Zimbabwe. PLOS ONE 10:e0137828.

12. Usmani M, Brumfield KD, Jamal Y, Huq A, Colwell RR, Jutla A. 2021. A review of the environmental trigger and transmission components for prediction of cholera. Trop Med Infect Dis 6:147.

13. Usmani M, Brumfield KD, Magers BM, Chaves-Gonzalez J, Ticehurst H, Barciela R, McBean F, Colwell RR, Jutla A. 2023. Combating cholera by building predictive capabilities for pathogenic Vibrio cholerae in Yemen. Sci Rep 13:2255.

14. Vezzulli L, Brettar I, Pezzati E, Reid PC, Colwell RR, Höfle MG, Pruzzo C. 2012. Long-term effects of ocean warming on the prokaryotic community: Evidence from the vibrios. ISME J 6:21–30.

15. Vezzulli L, Colwell RR, Pruzzo C. 2013. Ocean warming and spread of pathogenic vibrios in the aquatic environment. Microb Ecol 65:817–825.

16. Vezzulli L, Baker-Austin C, Kirschner A, Pruzzo C, Martinez-Urtaza J. 2020. Global emergence of environmental non-O1/O139 Vibrio cholerae infections linked with climate change: a neglected research field? Environ Microbiol 22:4342–4355.

17. Sterk A, Schets FM, de Roda Husman AM, de Nijs T, Schijven JF. 2015. Effect of climate change on the concentration and associated risks of Vibrio spp. in Dutch recreational waters. Risk Anal 35:1717–1729.

18. Brumfield KD, Chen AJ, Gangwar M, Moiz U, Hasan NA, Jutla AS, Huq A, Colwell RR. 2023. Environmental Factors Influencing Occurrence of Vibrio parahaemolyticus and Vibrio vulnificus. Appl Environ Microbiol 0:e00307-23.

19. Dewan AM, Corner R, Hashizume M, Ongee ET. 2013. Typhoid fever and its association with environmental factors in the Dhaka metropolitan area of Bangladesh: a spatial and time-series approach. PLoS Negl Trop Dis 7:e1998.

20. Saad NJ, Lynch VD, Antillón M, Yang C, Crump JA, Pitzer VE. 2018. Seasonal dynamics of typhoid and paratyphoid fever. Sci Rep 8:1–9.

21. Thomson RM, Furuya-Kanamori L, Coffey C, Bell SC, Knibbs LD, Lau CL. 2020. Influence of climate variables on the rising incidence of nontuberculous mycobacterial (NTM) infections in Queensland, Australia 2001–2016. Sci Total Environ 740:139796.

22. Greer AL, Drews SJ, Fisman DN. 2009. Why "winter" vomiting disease? Seasonality, hydrology, and norovirus epidemiology in Toronto, Canada. Ecohealth 6:192–199.

23. Lopman B, Armstrong B, Atchison C, Gray JJ. 2009. Host, Weather and Virological Factors Drive Norovirus Epidemiology: Time-Series Analysis of Laboratory Surveillance Data in England and Wales. PLOS ONE 4:1–10.

24. Lowther JA, Gustar NE, Powell AL, Hartnell RE, Lees DN. 2012. Two-year systematic study to assess norovirus contamination in oysters from

commercial harvesting areas in the United Kingdom. Appl Environ Microbiol 78:5812–5817.

25. Moe K, Shirley JA. 1982. The effects of relative humidity and temperature on the survival of human rotavirus in faeces. Arch Virol 72:179–186.

26. Konno T, Suzuki H, Katsushima N, Imai A, Tazawa F, Kutsuzawa T, Kitaoka S, Sakamoto M, Yazaki N, Ishida N. 1983. Influence of temperature and relative humidity on human rotavirus infection in Japan. J Infect Dis 147:125–128.

27. D'souza R, Hall G, Becker N. 2008. Climatic factors associated with hospitalizations for rotavirus diarrhoea in children under 5 years of age. Epidemiol Infect 136:56–64.

28. Atchison C, Tam C, Hajat S, Van Pelt W, Cowden J, Lopman B. 2010. Temperature-dependent transmission of rotavirus in Great Britain and The Netherlands. Proc R Soc B Biol Sci 277:933–942.

29. Jagai JS, Sarkar R, Castronovo D, Kattula D, McEntee J, Ward H, Kang G, Naumova EN. 2012. Seasonality of rotavirus in South Asia: a meta-analysis approach assessing associations with temperature, precipitation, and vegetation index. PloS One 7:e38168.

30. Hashizume M, Armstrong B, Hajat S, Wagatsuma Y, Faruque AS, Hayashi T, Sack DA. 2008. The effect of rainfall on the incidence of cholera in Bangladesh. Epidemiology 19:103–110.

31. Banakar V, Constantin de Magny G, Jacobs J, Murtugudde R, Huq A, Wood RJ, Colwell RR. 2011. Temporal and spatial variability in the distribution of Vibrio vulnificus in the Chesapeake Bay: a hindcast study. EcoHealth 8:456–467.

32. Vezzulli L, Grande C, Reid PC, Hélaouët P, Edwards M, Höfle MG, Brettar I, Colwell RR, Pruzzo C. 2016. Climate influence on Vibrio and associated human diseases during the past half-century in the coastal North Atlantic. Proc Natl Acad Sci 113:E5062–E5071.

33. Esteves K, Hervio-Heath D, Mosser T, Rodier C, Tournoud M-G, Jumas-Bilak E, Colwell RR, Monfort P. 2015. Rapid proliferation of Vibrio parahaemolyticus, Vibrio vulnificus, and Vibrio cholerae during freshwater flash floods in French Mediterranean coastal lagoons. Appl Environ Microbiol 81:7600 LP – 7609.

34. Hsiao HI, Jan MS, Chi HJ. 2016. Impacts of climatic variability on Vibrio parahaemolyticus outbreaks in Taiwan. Int J Environ Res Public Health 13:19–25.

35. Brumfield KD, Usmani M, Chen KM, Gangwar M, Jutla AS, Huq A, Colwell RR. 2021. Environmental parameters associated with incidence and transmission of pathogenic Vibrio spp. Environ Microbiol 23:7314–7340.

36. Ayukekbong JA, Andersson M, Vansarla G, Tah F, Nkuo-Akenji T, Lindh M, Bergström T. 2014. Monitoring of seasonality of norovirus and other enteric

viruses in Cameroon by real-time PCR: an exploratory study. Epidemiol Infect 142:1393–1402.

July 2023

Curriculum Vitae

Kyle D. Brumfield, M.Sc., Ph.D.

E-mail: KyleDBrumfield@gmail.com
Phone: (301) 466-6530

PERSONAL INFORMATION

Business Address: Maryland Pathogen Research Institute
Department of Cellular Biology and Molecular Genetics
Biosciences Research Building (BLDG #413)
University of Maryland, Room 3239
College Park, Maryland, 20742

Citizenship: United States of America

Place of Residence: Annapolis, MD, USA

Marital Status: Married to Allison Brumfield

INTRODUCTION

With an extensive background spanning nearly 10 years, Dr. Brumfield's expertise lies in the fields of microbial ecology, molecular genetics, and global infectious diseases with a particular focus on water-related health issues. His research revolves around employing molecular and metagenomic techniques to investigate the ecological dynamics of microbial communities, conducting genotypic and phenotypic analyses of clinical and environmental bacterial isolates, notably *Vibrio spp.*, and assessing predictive risk factors for diseases by understanding the influence of environmental parameters on the occurrence and transmission of pathogenic agents under varying climate change scenarios.

EDUCATION

2017 – 2023 **Ph.D., Environmental Molecular Science & Technology**
University of Maryland, College Park, MD
Dissertation: *Climate Change and Vibrio species: Investigation of Environmental Parameters Associated with Occurrence and Transmission*
Advisors: Dr. Rita R. Colwell and Dr. Anwar Huq

2015 – 2017 **M.Sc., Biology**
Concentration: Bacterial Pathogenesis
Plymouth State University, Plymouth, NH
Thesis: *Insights into Genetic Mechanisms Affording Hypervirulence in Vibrio cholerae Outbreak Strains*
Advisor: Dr. Mike S. Son

2011 – 2015 **B.Sc., Biotechnology**
Double minors: Chemistry & Technical Mathematics
Plymouth State University, Plymouth, NH

PROFESSIONAL APPOINTMENTS

2017 – present **Graduate Research Assistant III**, UMD
Cellular Biology and Molecular Genetics
- Actively involved in conducting dissertation research while also overseeing laboratory operations, including safety protocols, data management, and effective communication of research findings.
- Assumed responsibility for onboarding and providing guidance to graduate students within a collaborative laboratory setting.
- Played a direct role in writing research proposals and effectively presenting data through publications and conference presentations.

2017 & 2019 **Graduate Teaching Assistant**, UMD
Principles of Molecular and Cellular Biology (BSCI171)
- Modified existing lesson plans to enhance their effectiveness and align with instructional objectives.
- Facilitated collaborative laboratory sessions, instructing students on the principles and methodologies of molecular biology.
- Assessed and evaluated the performance of approximately 80 students across four sections, providing valuable feedback to support their academic progress.

2015 – 2017 **Graduate Teaching Assistant**, PSU
Biological Science (BI1110)
Microbiology (BI3040)
Microbiology for Nursing Students (BI2340)
- Created comprehensive lesson plans focusing on fundamental concepts in biology/microbiology, fostering student engagement and understanding.

– Facilitated hands-on laboratory activities, guiding approximately 65 students across five sections to enhance their practical skills and comprehension.
– Evaluated student performance through assessments and provided constructive feedback to support their academic growth.
– Concurrently conducted master's thesis research while mentoring undergraduate students within the laboratory, imparting valuable research skills and knowledge.

2014 – 2015 **Undergraduate Researcher**, PSU
– Conducted in-depth investigation into the genotypic and phenotypic attributes of clinical isolates of *Vibrio cholerae*.

2014 **Summer Research Intern**, PSU
– Conducted research to examine the interactions between *Vibrio spp.* and copepods.

EXPERIENCE AND INTERESTS

RESEARCH INTERESTS

Microbiology | molecular biology | microbial ecology | infectious disease | NGS and bioinformatics | Metagenomics | microbial identification and pathogen detection | source tracking | microbial genomics | antibiotic resistance | virulence and survival factors | phenotypic and metabolic characterization | predictive intelligence | environmental determinants | climate change

LABORATORY TECHNIQUES

Experimental design | laboratory management | inventory control | sterile technique | bacterial culture | DNA/RNA/Protein extraction and purification | PCR | real time PCR | primer design | gel electrophoresis | microscopy (light, confocal – Zeiss) | colony blot hybridization | IDEXX | ELISA | western blotting | serotyping | antimicrobial susceptibility testing | library prep & NGS protocols | preparation of solutions/media | general microbiology techniques

TECHNICAL SKILLS

Computer languages (Windows, macOS, Linux) | programing languages (Bash, R, Python) | bioinformatics (genome assembly, annotation, phylogenetic analysis, metagenomics) | database management | cloud computing | text/image processing (MS office, Adobe)

RESEARCH AND SCHOLARLY ACTIVITY

BOOK CHAPTERS

1. **Brumfield, K.D.**, Carignan, B.M. and Son, M.S., 2018. Laboratory Culturing Techniques and Maintenance of Vibrio cholerae. In *Vibrio Cholerae* (pp. 1-9). Humana Press, New York, NY.
2. **Brumfield, K.D.**, Carignan, B.M. and Son, M.S., 2018. Genotypic and phenotypic assays to distinguish Vibrio cholerae biotype. In *Vibrio Cholerae* (pp. 11-28). Humana Press, New York, NY.

FULL TEXT ARTICLES (* denotes co-authorship)

3. Morgado, M.E., **Brumfield, K.D.**, Mitchell, C., Boyel, M., Colwell, R.R., and Sapkota, A.S., 2023. Vibriosis trends in Maryland, U.S., 2006-2019: Increased incidence of water-associated wound infections and hospitalization risk. *Nature Communications*. **Under review**.
4. **Brumfield, K.D.**, Usmani, M., Santiago, S., Singh, K., Gangwar, M., Hasan, N.A., Netherland M. Jr., Deliz, K., Angelini, C., Beatty, N.L., Huq, A., Jutla, A.S., and Colwll, R.R., 2023. Genomic Diversity of *Vibrio spp.* and Metagenomic Analysis of Pathogens in Florida Gulf Coastal Water Following Hurricane Ian. *mBio*. **Under review**.
5. Jutla, A.S., Usmani, M., **Brumfield, K.D.**, Singh, K., McBean, F., Chaves-Gonzalez, J., Gutierrez, A., Gama, S., Huq, A., and Colwell, R.R., 2023. Anticipatory Decision Making for Cholera in Malawi. *mBio*. **Accepted, in press**.
6. Usmani, M., **Brumfield, K.D.**, Mager, B., Zhou, A., Oh, C., Mao, Y., Brow, B., Schmidt, A., Wu, C-Y., Shisler, J., Nguyen, T., Colwell, R.R., and Jutla, A., 2023. Building environmental and sociological predicitive intelligence to understand the seasonal threat of SARS-CoV-2 in human populations. *American Journal of Tropical Medicine and Hygiene*. **Accepted, in press**.
7. **Brumfield, K.D.**, Chen, A.J., Gangwar, M., Usmani, M., Hasan, N.A., Jutla, A., Colwell, R.R., and Huq, A. Environmental factors influencing occurrence of *Vibrio parahaemolyticus* and *Vibrio Vulnificus*. *Applied and Environmental Microbiology*. e00307-23.
8. Usmani, M., **Brumfield, K.D.**, Magers, B.M., Chaves-Gonzalez, J., Ticehurst, H., Sumner, T., Barciela, R., McBean, F., Colwell, R.R., and Jutla, A., 2022. Combating cholera by building predictive capabilities for pathogenic Vibrio cholerae in Yemen. *Scientific Reports*. 13(1), p.2255.
9. **Brumfield, K.D.**, Cox, P., Geyer, J., and Goepp, J., 2023. A taxonomy-agonistic approach to targeted microbiome therapeutics—leveraging principles of systems biology. *Pathogens*, 12(2), p.238.

10. Artman, C., Idegwu, N., **Brumfield, K.D.**, Lai, K., Hauta, S., Falzarano, D., Parreño, V., Yuan, L., Geyer, J.D. and Goepp, J.G., 2022. Feasibility of Polyclonal Avian Immunoglobulins (IgY) as Prophylaxis against Human Norovirus Infection. *Viruses*, *14*(11), p.2371.

11. **Brumfield, K.D.**, Raupp, M.J., Haji, D., Simon, C., Graf, J., Cooley, J.R., Janton, S.T., Meister, R.C., Huq, A., Colwell, R.R. and Hasan, N.A., 2022. Gut microbiome insights from 16S rRNA analysis of 17-year periodical cicadas (Hemiptera: Magicicada spp.) Broods II, VI, and X. *Scientific reports*, *12*(1), pp.1-16.

12. Usmani, M., **Brumfield, K.D.**, Magers, B.M., Huq, A., Barciela, R., Nguyen, T.H., Colwell, R.R. and Jutla, A., 2022. Predictive Intelligence for Cholera in Ukraine?. *GeoHealth*, *6*(9), p.e2022GH000681.

13. **Brumfield, K.D.**, Leddy, M., Usmani, M., Cotruvo, J.A., Tien, C.T., Dorsey, S., Graubics, K., Fanelli, B., Zhou, I., Registe, N., Dadlani, M., Wimalarante, M., Jinasena, D., Abayagunawardena, R., Withanachchi, C., Huq, A., Jutla, A., and Colwell, R.R., 2022. Microbiome analysis for wastewater surveillance during COVID-19. *Mbio*, *13*(4), pp.e00591-22.

14. **Brumfield, K.**, Seo, H., Idegwu, N., Artman, C., Gonyar, L., Nataro, J., Zhang, W., Sack, D., Geyer, J. and Goepp, J., 2022. Feasibility of avian antibodies as prophylaxis against enterotoxigenic escherichia coli colonization. *Frontiers in immunology*, *13*.

15. **Brumfield, K.D.**, Usmani, M., Chen, K.M., Gangwar, M., Jutla, A.S., Huq, A. and Colwell, R.R., 2021. Environmental parameters associated with incidence and transmission of pathogenic Vibrio spp. *Environmental microbiology*, *23*(12), pp.7314-7340.

16. Usmani, M., **Brumfield, K.D.**, Jamal, Y., Huq, A., Colwell, R.R. and Jutla, A., 2021. A review of the environmental trigger and transmission components for prediction of cholera. *Tropical medicine and infectious disease*, *6*(3), p.147.

17. Artman, C.*, **Brumfield, K.D.***, Khanna, S. and Goepp, J., 2021. Avian antibodies (IgY) targeting spike glycoprotein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) inhibit receptor binding and viral replication. *PloS one*, *16*(5), p.e0252399.

18. **Brumfield, K.D.**, Cotruvo, J.A., Shanks, O.C., Sivaganesan, M., Hey, J., Hasan, N.A., Huq, A., Colwell, R.R. and Leddy, M.B., 2021. Metagenomic sequencing and quantitative real-time PCR for fecal pollution assessment in an urban watershed. *Frontiers in water*, *3*, p.626849.

19. Al Malki, A.*, **Brumfield, K.D.***, Tsui, C.K., Anand, A., Rashed, S.M., Ibrahim, E., Al Shamari, H., Huq, A., Colwell, R.R. and Fotedar, R., 2021. Draft Genome Sequences of Seven Vibrio cholerae Isolates from Adult Patients in Qatar. *Microbiology Resource Announcements*, *10*(9), pp.e01489-20.

20. **Brumfield, K.D.**, Cotruvo, J.A., Leddy, M.B., Colwell, R.R. and Huq, A., 2020. Using Metagenomic Analysis to Assess Water Quality. *Journal-American Water Works Association*, *112*(11), pp.84-87.
21. **Brumfield, K.D.**, Hasan, N.A., Leddy, M.B., Cotruvo, J.A., Rashed, S.M., Colwell, R.R. and Huq, A., 2020. A comparative analysis of drinking water employing metagenomics. *PLoS One*, *15*(4), p.e0231210.
22. **Brumfield, K.D.**, Huq, A., Colwell, R.R., Olds, J.L. and Leddy, M.B., 2020. Microbial resolution of whole genome shotgun and 16S amplicon metagenomic sequencing using publicly available NEON data. *PLoS One*, *15*(2), p.e0228899.
23. **Brumfield, K.D.**, Carignan, B.M., Ray, J.N., Jumpre, P.E. and Son, M.S., 2017. Laboratory techniques used to maintain and differentiate biotypes of Vibrio cholerae clinical and environmental isolates. *JoVE (Journal of Visualized Experiments)*, (123), p.e55760.
24. Carignan, B.M.*, **Brumfield, K.D.*** and Son, M.S., 2016. Single nucleotide polymorphisms in regulator-encoding genes have an additive effect on virulence gene expression in a Vibrio cholerae clinical isolate. *Msphere*, *1*(5), pp.e00253-16.

SCIENTIFIC AND CONFERENCE PRESENTATIONS

1. **Brumfield, K.D.**, Huq, A., and Colwell, R.R., 2023. Climate Change and *Vibrio* species: Investigation of Environmental Parameters Associated with Occurrence and Transmission. University of Maryland. July 6.
2. **Brumfield, K.D.**, Gangwar, M., Usmani, M., Jutla, A.S., Huq, A., and Colwell, R.R., 2023. Climate change and *Vibrio spp.* in the Chesapeake Bay, Maryland, USA. Microbe hosted by American Society for Microbiology (ASM). Houston, TX, USA. June 15-19.
3. **Brumfield, K.D.**, Gangwar, M., Usmani, M., Jutla, A.S., Colwell, R.R., and Huq, A., 2023. Climate change and *Vibrio spp.* in the Chesapeake Bay, MD. Oceans and Human Health. May 9-12.
4. **Brumfield, K.D.**, Gangwar, M., Usmani, M., Jutla, A.S., Colwell, R.R., and Huq, A., 2022. Climate change impact on incidence of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in Chesapeake Bay water and oysters. International Society of Bangladesh-affiliated microbiologists (ISBM) Annual Meeting. *Virtual*. November 12-13. **Best presentation award**.
5. **Brumfield, K.D.**, Leddy, M., Usmani, M., Cotruvo, J.A., Tien, C.T., Dorsey, S., Graubics, K., Fanelli, B., Zhou, I., Registe, N., Dadlani, M., Wimalarante, M., Jinasena, D., Abayagunawardena, R., Withanachchi, C., Huq, A., Jutla, A., and Colwell, R.R., 2022. Microbiome analysis for wastewater surveillance during COVID-19 in Maryland, USA. International Conference on Genomics, Nanotech, and Bioengineering-22 (ICGNB-2022). *Virtual*. June 26-28.

6. **Brumfield, K.D.**, Paull, S.H., Hasan, N.A., Spurbeck, R., Thibault, K.M., White, R., Huq, A., and Colwell, R.R., 2022. Speciation of Mosquito Virome: Zoonotic Potential. Microbe hosted by American Society for Microbiology (ASM). Washington, D.C., USA. June 4-8.
7. **Brumfield, K.D.**, Colwell, R.R., and Huq, A., 2021. Microbes and climate change: from *Vibrio spp*. to water safety. Environmental Health Sciences hosted by University of South Carolina. *Virtual*. March 31. **Invited**.
8. **Brumfield, K.D.**, Gangwar, M., Usmani, M., Jutla, A.S., Colwell, R.R., and Huq, A., 2021. Environmental surveillance of *Vibiro* parahaemolyticus and *Vibrio* vulnificus in Chesapeake Bay water and oysters. Oceans and Human Health Annual Meeting. *Virtual*. October 4-6.
9. **Brumfield, K.D.**, Colwell, R.R., Kabbani, N., and Olds, J., 2020. ACE2 binds nicotinic acetylcholine receptors in an evolutionary model of toxin and virus cell convergence. School of Systems Biology hosted by George Mason University. *Virtual.* December 18. **Invited**.
10. **Brumfield, K.D.**, Usmani, M., Chen, K.M., Jutla, A.S., Colwell, R.R., and Huq, A., 2020. Environmental surveillance and prediction of clinically relevant Vibrios in the Chesapeake Bay. Oceans and Human Health Annual Meeting. *Virtual.* October 26-28.
11. **Brumfield, K.D.**, Colwell, R.R., and Huq, A., 2020. Climate change and infectious disease: lessons learned from *Vibrio spp.* Oceans and Human Health Annual Meeting. *Virtual.* October 26-28.
12. **Brumfield, K.D.**, Leddy, M.B., Cotruvo, J.A., Hasan, N.A., Colwell, R.R., and Huq, A., 2020. Pilot study of microbial contamination sources in Rock Creek Park. DC Water Long Term Control Plan. *Virtual*. May 19. **Invited**.
13. **Brumfield, K.D.**, Leddy, M.B., Cotruvo, J.A., Hasan, N.A., Colwell, R.R., and Huq, A., 2020. Gwinnett county microbiome analysis. Gwinnett County Long Term Control Plan. *Virtual*. April 8. **Invited**.
14. **Brumfield, K.D.** and Son, M., 2017. Insights into genetic mechanisms affording hypervirulence in *Vibrio cholerae* outbreak strains. Plymouth State University, NH, USA. April 19.
15. **Brumfield, K.D.** and Son, M.S. 2017. Ubiquity of microorganisms. Microbiology for Nursing Students, BI2340, Plymouth State University, NH. January. **Invited**.
16. **Brumfield, K.D.,** Carignan, B.M., and Son, M.S. 2016. An alternative *Vibrio cholerae* attachment model: Implications of a clinically relevant SNP on enhanced attachment and virulence capabilities. New Hampshire IDeA Network of Biomedical Research Excellence (NH-INBRE) Annual Meeting. Bretton Woods, NH. August 15-16. **Best presentation award**.

17. **Brumfield, K.D.** and Son, M.S. 2016. Introduction to microbiology and the concept of citizen science. Microbiology for Nursing Students, BI2340, Plymouth State University, NH. January. **Invited**.
18. **Brumfield, K.D.,** Carignan, B.M., and Son, M.S. 2015. SNPs in regulator-encoding genes have an additive effect on virulence gene expression in a *Vibrio cholerae* clinical isolate. New Hampshire IDeA Network of Biomedical Research Excellence (NH-INBRE) Annual Meeting. Bretton Woods, NH. August 16-17. **Best presentation award**.

CONFERENCE PROCEEDINGS & POSTER PRESENTATIONS

University of Maryland, 2017 – present

19. Spurbeck, R., **Brumfield, K.D.**, Paull, S.H., Hasan, N.A., Thibault, K.M., White, R., Huq, A., and Colwell, R.R., 2023. Biosurveillance of Vector-Borne Viruses Utilizing Metatranscriptomic Sequencing. Military Health Science Research Symposium. June 23.
20. Gangwar, M., **Brumfield, K.D.**, Usmani, M., Jamal, Y., Jutla, A.S., and Colwell, R.R., 2023. Variability and the odds of total and pathogenic *Vibrio* abundance in Chesapeake Bay. European Geosciences Union (EGU) General Assembly. *Virtual*. April 23-28.
21. Usmani, M., **Brumfield, K.D.**, Magers, B., Wu, C-Y, Nguyen, T.H., Colwell, R.R., and Jutla, A.S., 2022. Thinking Beyond COVID-19: The Geo-Environmental-Sociological-Epidemiological (GESE) Framework for prediction of respiratory infections. American Geosciences Union (AGU) Annual Meeting. Chicago, IL. *Virtual*. December 12-16.
22. Usmani, M., **Brumfield, K.D.**, Huq, A., Colwell, R.R., and Jutla, A.S., 2022. Assessment of earth observation inspired waterborne disease predictive model. International Society of Bangladesh-affiliated microbiologists (ISBM) Annual Meeting. *Virtual*. November 12-13.
23. Usmani, M., **Brumfield, K.D.**, Jamal, Y., Gangwar, M., Colwell, R.R., and Jutla, A.S., 2023. Human health as an indicator of climate change. European Geosciences Union (EGU) General Assembly. *Virtual*. April 23-28.
24. **Brumfield, K.D.**, Usmani, M., Jutla, A., Colwell, R.R., and Huq, A., 2022. Environmental parameters associated with incidence of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in Chesapeake Bay water and oysters. Microbe hosted by American Society for Microbiology (ASM). Washington, D.C., USA. June 4-8.
25. Gangwar, M., Usmani, M., Jamal, Y., **Brumfield, K.D.**, Huq, A., Colwell, R.R., and Jutla, A.S., 2021. Global Prediction of Vibrios: Correlating Environmental Factors and *Vibrio* in Chesapeake Bay. Oceans and Human Health Annual Meeting. *Virtual*. October 4-6.

26. Gangwar, M., Chen, K.M., Usmani, M., **Brumfield, K.D.**, Colwell, R.R., Huq, A., Jutla, A.S., 2020. Global prediction of Vibrios: Exploratory analysis of environmental factors and Vibrios in Cheapeake Bay. Oceans and Human Health Annual Meeting. *Virtual.* October 26-28.

27. **Brumfield, K.D.**, Leddy, M.B., Shanks, O.C., Hey, J., Sivaganesan, M., Hasan, N.A., Colwell, R.R., Huq, A., and Cotruvo, J.A., 2020. Microbial source tracking with metagenomic sequencing and host-associated qPCR in a suburban watershed before and after rainfall. Microbe hosted by American Society for Microbiology (ASM). *Virtual*. June 18-22.

28. **Brumfield, K.D.**, Chen, A., McKay, J., Hiner, S., Colwell, R.R., and Huq, A., 2020. Environmental surveillance of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in Chesapeake Bay water and oysters. Microbe hosted by American Society for Microbiology (ASM). *Virtual*. June 18-22.

29. **Brumfield, K.D.**, Hasan, N.A., Leddy, M.B., Cotruvo, J.A., Rashed, S.M., Colwell, R.R., and Huq, A., 2020. A comparative analysis of drinking water employing metagenomics. American Society for Microbiology Branch Meeting. Washington, D.C., USA. February 21-22.

30. Usmani, M., **Brumfield, K.D.**, Huq, A., Colwell, R. and Jutla, A., 2019. Randomness in the spatiotemporal movement of Vibrio cholerae. American Geosciences Union (AGU) Annual Meeting. San Francisco, CA, USA. December 9-13.

31. Usmani, M., **Brumfield, K.D.**, Huq, A., Colwell, R. and Jutla, A., 2019. Impact of climate variability on randomness of cholera. American Geosciences Union (AGU) Annual Meeting. San Francisco, CA, USA. December 9-13.

32. **Brumfield, K.D.**, Fotedar, R., Malaki, A.A., Rashed, S.M., Anand, A., Marri, M.M., Ramadan, F., Ibrahim, E.B., Colwell, R.R., and Huq, A., 2019. Genomic and Evolutionary Diversity of Travel Associated *Vibrio cholerae* in Qatar. Microbe hosted by American Society for Microbiology (ASM). San Francisco, CA, USA. June 20-25.

33. Usmani, M., **Brumfield, K.D.**, Huq, A., Colwell, R., and Jutla, A., 2019. Impact of climate variability on randomness of cholera. European Geosciences Union (EGU) Annual Meeting. *Virtual*. April 8-12.

34. **Brumfield, K.D.**, Leddy, M.B., Cotruvo, J.A., Hasan, N.A., Colwell, R.R., and Huq, A., 2019. Warwick Mushroom Farm Microbiome Analysis. Warwick County Long Term Control Plan. *Virtual*. February 22.

35. **Brumfield, K.D.**, Leddy, M.B., Cotruvo, J.A., Hasan, N.A., Colwell, R.R., and Huq, A., 2019. DC Water Microbiome Analysis. DC Water County Long Term Control Plan. *Virtual*. February 18.

36. Usmani, M., **Brumfield, K.D.**, Huq, A., Colwell, R. and Jutla, A., 2018. Randomness of Vibrios in the environment. American Geosciences Union (AGU) Annual Meeting. Washington D.C, USA. December 10-14.

37. **Brumfield, K.D.**, Malaki, A.A., Anand, A., Ramadan, F., Rashed, S.M., Ibrahim, E.B., Marri, M.M., Colwell, R.R., Huq, A., and Fotedar, R., 2018. Isolation, Identification, and Characterization of *Vibrio* spp. From Qatar. Marine Estuarine Environmental Sciences (MEES) Colloquium. Frostburg, MD, USA. October 25-26.
38. **Brumfield, K.D.**, Malaki, A.A., Anand, A., Ramadan, F., Rashed, S.M., Ibrahim, E.B., Marri, M.M., Colwell, R.R., Huq, A., and Fotedar, R., 2018. Characterization of *Vibrio* Species Isolated from Marine Environment of Qatar. Microbe hosted by American Society for Microbiology (ASM). Atlanta, GA, USA. June 7-11.

Plymouth State University, 2014 – 2017

39. **Brumfield, K.D.,** Carignan, B.M., and Son, M.S. 2017. An alternative *Vibrio cholerae* attachment model: Implications of a clinically relevant SNP on enhanced attachment and virulence capabilities. Microbiology and Molecular Pathogenesis Program (M2P2) hosted by Geisel School of Medicine at Dartmouth College, Fairlee, VT. February 16-17.
40. **Brumfield, K.D.,** Carignan, B.M., and Son, M.S. 2017. SNPs in regulator-encoding genes have an additive effect on virulence gene expression in a *Vibrio cholerae* clinical isolate. Microbiology and Molecular Pathogenesis Program (M2P2) hosted by Geisel School of Medicine at Dartmouth College, Fairlee, VT. February 16-17.
41. **Brumfield, K.D.,** Carignan, B.M., and Son, M.S. 2016. An alternative *Vibrio cholerae* attachment model: Implications of a clinically relevant SNP on enhanced attachment and virulence capabilities. Microbe hosted by American Society for Microbiology (ASM). Boston, MA. June 16-20.
42. **Brumfield, K.D.,** Carignan, B.M., and Son, M.S. 2016. SNP combinations found in *Vibrio cholerae* clinical isolates have an additive effect on virulence capabilities. Microbe hosted by American Society of Microbiology (ASM). Boston, MA. June 16-20.
43. **Brumfield, K.D.,** Carignan, B.M., and Son, M.S. 2016. An alternative *Vibrio cholerae* attachment model: Implications of a clinically relevant SNP on enhanced attachment and potential virulence capabilities. 2016 Student Showcase of Excellence. Plymouth, NH. May 6.
44. **Brumfield, K.D.,** Carignan, B.M., and Son, M.S. 2016. Clinically identified SNPs in *Vibrio cholerae* regulators have an additive effect on virulence capabilities. 2016 Student Showcase of Excellence. Plymouth, NH. May 6.
45. **Brumfield, K.D.**, Carignan, B.M., and M.S. Son. 2016. Implications of an *frhA* SNP affording enhanced attachment on increased virulence capabilities of *Vibrio cholerae* clinical isolates. Microbiology and Molecular Pathogenesis Program (M2P2) hosted by Geisel School of Medicine at Dartmouth College, Fairlee, VT. February 11-12.

46. **Brumfield, K.D.**, Ceasar, G.J., and M.S. Son. 2015. Implications of an *frhA* SNP affording enhanced attachment on increased virulence capabilities of *Vibrio cholerae* clinical isolates. NH-INBRE External Advisory Committee. Manchester, NH. November 7-9.

47. **Brumfield, K.D**., Carignan, B.M., and M.S. Son. 2015. Implications of an *frhA* SNP affording enhanced attachment on increased virulence capabilities of *Vibrio cholerae* clinical isolates. Graduate Research Symposium. Plymouth State University, Plymouth, NH. November 5.

48. **Brumfield, K.D.**, Ceasar, G.J., Carignan, B.M., and M.S. Son. 2015. The effects of a naturally occurring *frhA* point mutation in *Vibrio cholerae* pathogenesis using a copepod model. New Hampshire IDeA Network of Biomedical Research Excellence (NH-INBRE) Annual Meeting. New Castle, NH. August 6-7.

49. **Brumfield, K.D.**, Carignan, B.M., and Son, M.S. 2015. SNP combinations for in clinical isolates of *Vibrio cholerae* affect virulence capabilities. New Hampshire IDeA Network of Biomedical Research Excellence (NH-INBRE) Annual Meeting. New Castle, NH. August 6-7. **Best poster presentation award**.

50. Swissi, G.S, McEnany, F.F., Ceasar, G.J., **Brumfield, K.D.,** Carignan, B.C., and Son, M.S. 2015. Characterizing the effects of clinically relevant SNPs on metabolic activities and increased virulence capabilities in the human pathogen *Vibrio cholerae.* New Hampshire IDeA Network of Biomedical Research Excellence (NH-INBRE) Annual Meeting. New Castle, NH. August 6-7.

51. Smith, L.L., Dion, A., Freeman, C.J., **Brumfield, K.D**., and C.C. Chabot. 2015. The effects of RNAi on the circa tidal rhythms of *Limulus polyphemus*. 2015 Student Showcase of Excellence. Plymouth, NH. April 24.

52. Swissi, G.S., McEnany, F.B., **Brumfield, K.D**., Ceasar, G.J., and Son, M.S. 2015. Determining the effects of clinically relevant SNPs in regulatory genes on the metabolic activities and virulence capabilities of the human pathogen *Vibrio cholerae.* 2015 Student Showcase of Excellence. Plymouth, NH. April 24.

53. **Brumfield, K.D**., Ceasar, G.J., and Son, M.S. 2015. Developing an *in vitro V. cholerae* model to determine the effects of a clinically relevant SNP in *frhA* on attachment and pathogenicity. 2015 Student Showcase of Excellence. Plymouth, NH. April 24.

54. **Brumfield, K.D**., Ceasar, G.J., and M.S. Son. 2014. Developing a copepod model for in vivo studies involving *Vibrio cholerae* and the potential effects of a point mutation in *frhA* on pathogenicity. New Hampshire IDeA Network of Biomedical Research Excellence (NH-INBRE) Annual Meeting. Whitefield, NH. August 3-4.

55. Barchey-Robinson, K.R., Armstrong, W.R., **Brumfield, K.D**., DeGrace, C.N., van Loon, S.R., Maxner, A.J., and M.S. Son. 2014. Evaluating the effects of clinically relevant point mutations in four regulatory genes on the levels of TcpA and ToxT production. 2014 Student Showcase of Excellence. Plymouth, NH. April 25.
56. **Brumfield, K.D.**, Maxner, A.J., and M.S. Son. 2014. The effects of a naturally occurring *frhA* point mutation in *Vibrio cholerae* pathogenesis using a copepod model. 2014 Student Showcase of Excellence. Plymouth, NH. April 25.
57. Maxner, A.J., Barchey-Robinson, K.R., **Brumfield, K.D**., Armstrong, W.R., DeGrace, C.N., van Loon, S.R., and M.S. Son. 2014. Developing an alternative genetic recombination tool for *Vibrio cholerae*. 2014 Student Showcase of Excellence. Plymouth, NH. April 25.
58. Maxner, A.J., Barchey-Robinson, K.R., **Brumfield, K.D.**, DeGrace, C.N., van Loon, S.R., Armstrong, W.R., and M.S. Son. 2014. Elucidating the role of five genes involved in the increased virulence observed in clinical isolates of *Vibrio cholerae*. Microbiology and Molecular Pathogenesis Program (M2P2) hosted by Geisel School of Medicine at Dartmouth College, Fairlee, VT. February 13-14.

RESEARCH FUNDING

1. "Improved Sensitivity of Metagenomics for Rapid Detection of Pathogens, Virulence, and Antibiotic Resistance" (Supported through Office of the Director of National Intelligence, $210K, Oct 2023 – Oct 2025) Role: Postdoctoral Investigator and Co-PI with Dr. Colwell as Research Advisor; proposal development.
2. "Center for Human Health, OCean BioAerosols and Pathogens (CHOCBAP): Understanding impacts of climate and weather on planktonic processes, marine bioaerosols and clinically relevant pathogens" (Submitted to NIH, multiple R01s & P01, $4.9M) Role: Student Investigator; proposal development.
3. "Diversity of pathogenic *Vibrio spp*. isolated from Chesapeake Bay, MD and Gulf Coast, FL water and oysters." (Submitted to NIEHS, Research Supplement, $80K) Role: Student Investigator; proposal development.
4. "Revealing the Interplay between Anthropogenic Input and Microbiomes in Mosquito and Tick Microbial Ecosystems at Global Scale" (Submitted to NSF, URoL:MIM, $1.2M) Role: Student investigator; proposal development.
5. "Rapid characterization if *Vibrio parahaemolyticus* to identify virulence potential of pandemic and non-pandemic complexes" (Submitted to NSF, STTR, $300K) Role: Co-Investigator; proposal development.

6. "Proactive and Responsive Antibody Treatments of *Vibrio vulnificus* Infections" (Submitted to NSF, STTR, $300K) Role: Co-Investigator; proposal development.
7. "Effects of SNVs affording enhanced virulence capabilities of *Vibrio cholerae* clinical isolates" (Supported through Student Research Advisory Council Grant, PSU, $4K, 2014 – 2015) Role: Student Investigator; proposal development.

CAREER DEVELOPMENT

AD HOC JOURNAL REVIEWER

1. Scientific Reports, Springer Nature
2. Frontiers in Cellular and Infection Microbiology
3. Frontiers in Microbiology
4. Societies, MDPI
5. Tropical Medicine and Infectious Disease, MDPI

PROFESSIONAL AFFILIATIONS

1. Interdisciplinary Society for Applied Microbiomics (2023)
2. International Society of Bangladesh-affiliated microbiologists (2021)
3. American Geophysical Union (2019)
4. American society for Microbiology, Washington DC Branch (2017)
5. American society for Microbiology (2015)
6. The Society for Collegiate Leadership and Achievement (2016)
7. Phi Kappa Phi Honor Society, NH Branch (2013 – 2017)

PROFESSIONAL TRAINING AND WORKSHOPS

1. NGS Workshop: A Deeper Dive into the Cornerstones of NGS Analyses, Trees, and Workflows. American Society for Microbiology. Houston, TX. June 15, 2023.
2. Illumina NextSeq 2000 Training. EzBiome Inc. Gaithersburg, MD. September 20-22, 2022.
3. Standards to Support and Enduring Capability in Wastewater Surveillance for Public Health. Department of Homeland Security and National Institute of Standards and Technology. *Virtual.* June 14-18, 2021.
4. Microbiome Analysis Workshop. University of Maryland School of Medicine Institute for Genome Sciences. College Park, MD. November 10-13, 2020.

5. Microbiome in Health and Disease. Illumina and University of Maryland BioPark. Baltimore, MD. December 11, 2019.
6. Graduate Student Teacher Workshop. University of Maryland Teaching and Learning Transformation Center. College Park, MD. August 25, 2017.
7. Bioinformatics Workshop. Hubbard Center for Genome Studies. Dartmouth College. Plymouth, NH. January 6-7, 2016.
8. Writing for Biomedical Publications Workshop. Dartmouth SYNERGY and Norris Cotton Cancer Center. Hanover, NH. December 11-12, 2015.

HONORS AND AWARDS

1. Best Presentation Award, International Society of Bangladesh-affiliated Microbiologists (2022)
2. Cover Image and Most Downloaded Article, *Environmental microbiology* (2021)
3. University of Maryland Dean's Fellowship (2017)
4. Best Presentation Award, New Hampshier IDeA Network for Biomedical Research Excellence, Dartmouth College (2015 & 2016)
5. Gonfalonier at Commencement, Dept. of Biological Sciences, Plymouth State University (2015)
6. Team Captain, Men's Varsity (NCAA) Ice Hockey, Plymouth State University (2014 – 2015)
7. Massachusetts State Collegiate Athletic Conference, All-Academic Team Award (2011 – 2015, *all semesters*)
8. Outstanding Organic Chemistry Student, Dept. of Atmospheric Science and Chemistry, Plymouth State University (2013)

ADDITIONAL EXPERIENCE

2019 – 2020	**Co-Head Coach, U14 Elite Ice Hockey** Navy Youth Hockey, MD
2015 – 2017	**Assistant Coach, Men's Varsity (NCAA) Ice Hockey** Plymouth State University, NH
2014	**Assistant Coach, U16 Elite Ice Hockey** DD Hockey, PA
2010 – 2011	**Ice Hockey Skills Instructor/Personal Trainer** Athletic Republic, Rochester, NY
2009 – 2010	**Sales Associate** Abercrombie and Fitch, Syracuse, NY

REFERENCES

Dr. Rita Colwell, Distinguished University Professor
University of Maryland
Dept. of Cell Biology & Molecular Genetics
Email: rcolwell@umd.edu

Dr. Anwar Huq, Research Professor
University of Maryland
Maryland Pathogen Research Institute
Email: huq@umd.edu

Dr. Mike Son, Assistant Professor of Microbiology
Plymouth State University
Dept. of Biological Sciences
Email: msson@plymouth.edu