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Minireview

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

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Summary

***Vibrio spp*. thrive in warm water and moderate salinity, and they are associated with aquatic invertebrates, notably crustaceans and zooplankton. At least 12 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 behavioural 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 modelling and are described, namely in the context of prediction of environmental conditions favourable 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

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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 $^{-1}$ml^{-1} 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, faecal 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 unfavourable 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* (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 that 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), molluscs (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 12 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 unfavourable, 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 favourable, 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 *et al*. (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; Mutreja *et al*., 2013).

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 behavioural 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 behaviour patterns, notably those associated with water use, hygiene, food preparation, consumption of raw or undercooked seafood, and other behaviours 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). Behaviour 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 behaviour, 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 favourable for proliferation of *Vibrio spp*. and the diseases they cause.

Vibrio ecology and epidemiology

Approximately 12 *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 Centres for Disease Control and Prevention (CDC) has developed improved *Vibrio* surveillance programs for the United States, 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 United States, *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 (Fig. 1A), with most *Vibrio* infections occurring in July and August (Fig. 1B). *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 (Fig. 1C). Interestingly, most *Vibrio* infections (*ca*. 55%) occur in males, and roughly 75% in the elderly population over the age of 65 years (Fig. 1D). *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 United States 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 United States 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 centres and geographic regions of economic activity in the United States 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, 2021b). 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, 2021b). 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 US$1 billion (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*. (Fig. 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 prokaryotes 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*., 2006). 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 that may promote virulence or resistance factors (Espinoza-Vergara

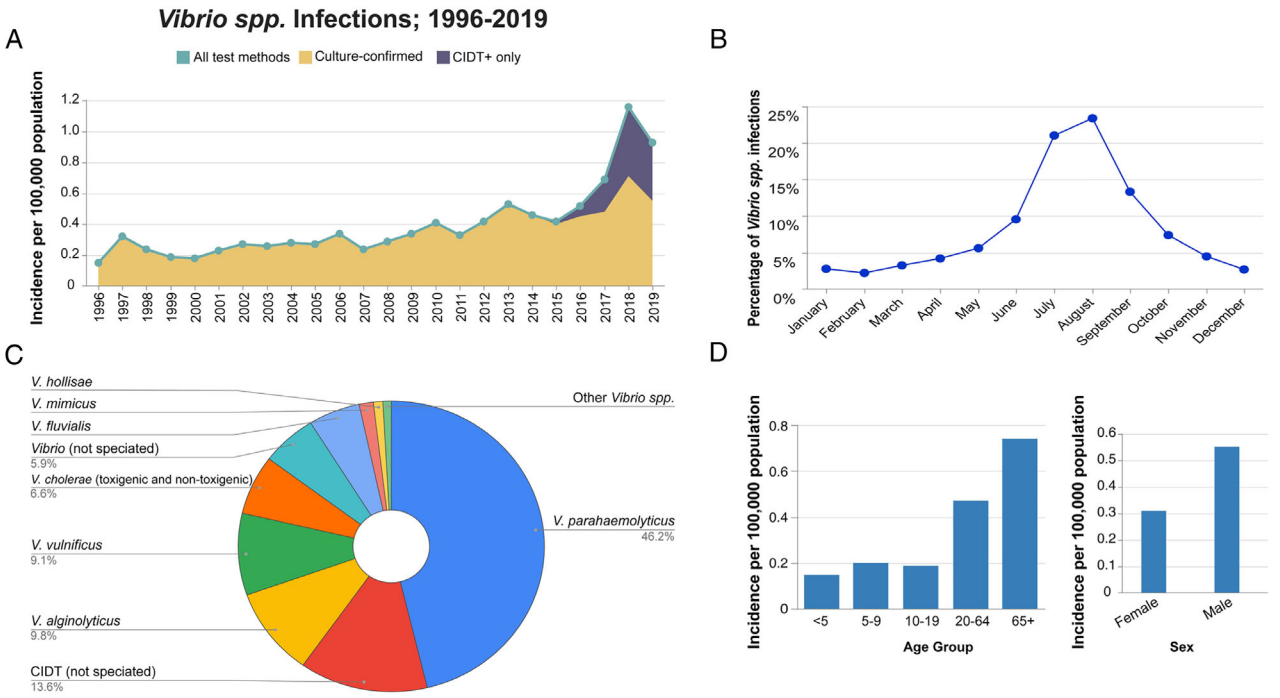

Fig. 1. Pathogen surveillance of infections caused by pathogenic *Vibrio spp*., 1999–2019 (CDC, 2021). Graphs were created using the Foodborne Diseases Active Surveillance Network (FoodNet) Fast to display data for *Vibrio* infections. Where indicated, data are presented as number of infections per 100 000 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).

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 *et al*. (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 unfavourable 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

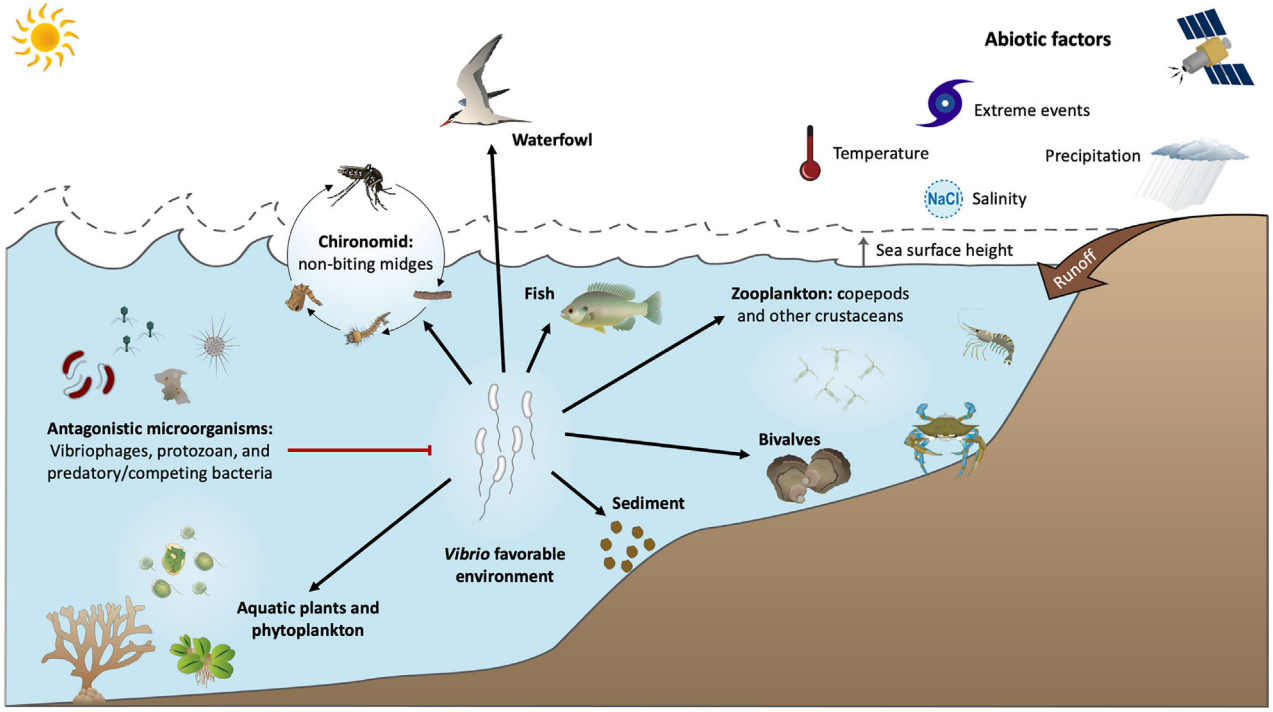

Fig. 2. Interactions of *Vibrio* spp. and their natural environment, adapted from the study by Sakib *et al*. (2018).
Shown are biotic and abiotic factors influencing incidence and distribution of *Vibrio spp*. in the natural environment. Black arrows indicate potential reservoirs, e.g., zooplankton (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).

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 harbour 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 favourable 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*., 2006), 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, the 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*., 2018). 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*., 2013) 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, haemolysins, 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 3 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 endemicform, 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*., 2006). 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; Jutla *et al*., 2013b; Jutla *et al*., 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 *et al*. (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 pre-existing 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 United States (Mead *et al*., 1999) and China (Li *et al*., 2014) since the 1990s. CDC estimates over 45 000 illnesses each year in the United States 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-haemolysis 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 haemolysin (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 haemolysin 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 haemolysin, the thermolabile haemolysin (TLH), which encodes phospholipase A2 (Zhang and Austin, 2005), has been characterized as not causing haemolysis 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 haemolysin 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 non-redundant 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 *et al*. (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*., 2004b; 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 *et al*. (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 *et al*. (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*., 2004a; Rahman *et al*., 2006; Jones *et al*., 2012). Genotyping of non-pandemic strains containing virulence factors remains an open question.

Vibrio vulnificus

Vibrio 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 diarrhoea after consumption of raw or under cooked seafood, primarily oysters where it occurs in large numbers (10^5 g^{-1} or more). *Vibrio 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 post-harvest 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). *Vibrio 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). *Vibrio 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).

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). *Vibrio vulnificus* secreted cytolytic/haemolysin 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 diarrhoea 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 haemolysins, has also been detected in non-cholera *Vibrio spp*. (Nair *et al*., 1988; Ceccarelli *et al*., 2015). In the United States, most cases

of vibriosis have been identified as infections caused by *V. parahaemolyticus*, *V. alginolyticus*, or *V. vulnificus* (Fig. 1C), 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 among *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 1300 *V. alginolyticus* infections in humans were reported in the United States, 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 (Fig. 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 (Hasan *et al*., 2012; Mutreja *et al*., 2013). 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*., 2020, 2021). 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*., 2020), 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 modelling 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 (European 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 *et al*. (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 favours 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 unfavourable 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 suggest a mechanism for dispersal of *Vibrio spp*., evidenced by epidemiological surveys of waterborne diseases (Ruiz *et al*., 2000). Improper holding and transport of shellfish are 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 among *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*., 2006; 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 the risk of infection (Fig. 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*., 2019a, 2019b), 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 behaviour, 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 *et al*. (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 (Fig. 3A) are created from data gathered by large scale sampling in Chesapeake Bay between 2007 and 2010 and forced with the Chesapeake Bay Operational Forecast System (NOAA, 2021a), 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 (Fig. 3B) 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, 2021d) 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 5 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 (Fig. 3C) and salinity (Fig. 3D), 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 5 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 modelling 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 modelling, 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. The 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 modelling and prediction.

Jutla *et al*. (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

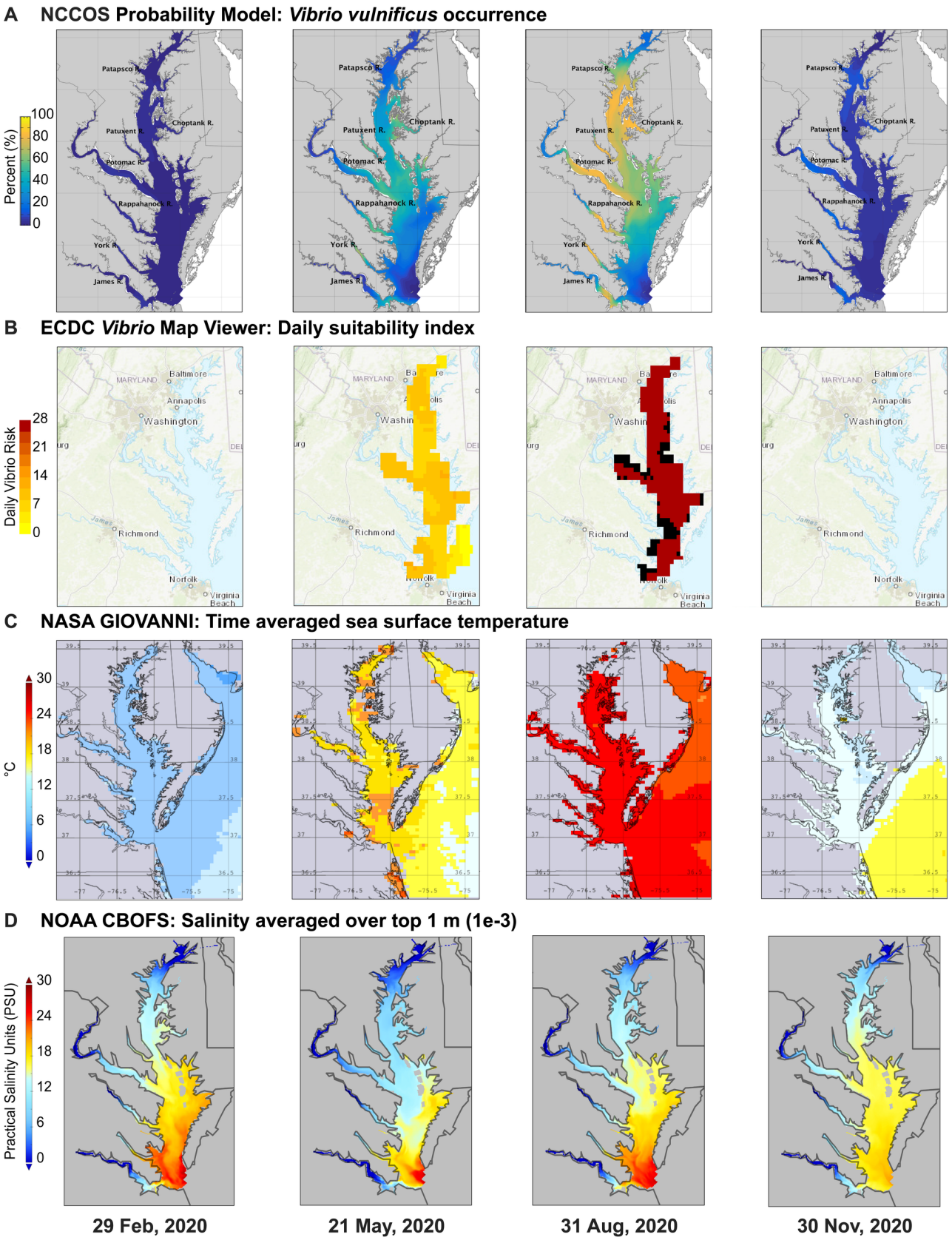

Fig. 3. Legend on next page.

to an outbreak by increasing contact of the population with unsafe water (Jutla *et al*., 2015b). This hypothesis is based on conditions favourable 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 favourable 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 favourable 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 modelling techniques for many parts of the world. However, the use of time series-independent algorithms (Jutla *et al*., 2011; Jutla, *et al*., 2013a; Khan *et al*., 2017; Khan *et al*., 2019b) that employ similarity principles to determine the conditions of favourability 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. parahae molyticus* correlated with water temperature. Water column temperatures between 14 and 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 United States (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

Fig. 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 February; Spring, 21 May; Summer, 31 August; and Fall, 30 November).
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, 2021a) and visualized using NASA Panoply Data Viewer (NASA, 2021).

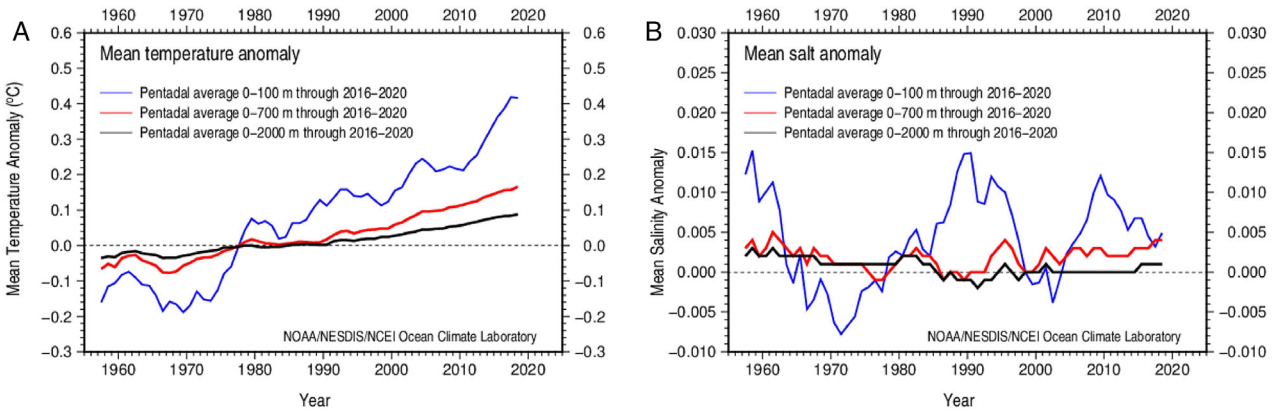

Fig. 4. Global Ocean Heat and Salt Content (1955–2020).
Data distribution for temperature and salinity observations are provided elsewhere (NOAA, 2021c). Pentadal averages are shown through 2016–2020; blue, 0–100 m; red 0–700 m; grey, 0–2000 m.
A. Mean temperature anomaly.
Mean temperature anomalies have previously been detailed by Levitus *et al*. (2012).
B. Mean salt anomaly.
Temperature calculations are extended to keep the fields current and include salinity anomalies.

unfavourable 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 United States 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 *et al*. (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 modelling efforts to be expanded beyond current reach within the United States and Northern Europe.

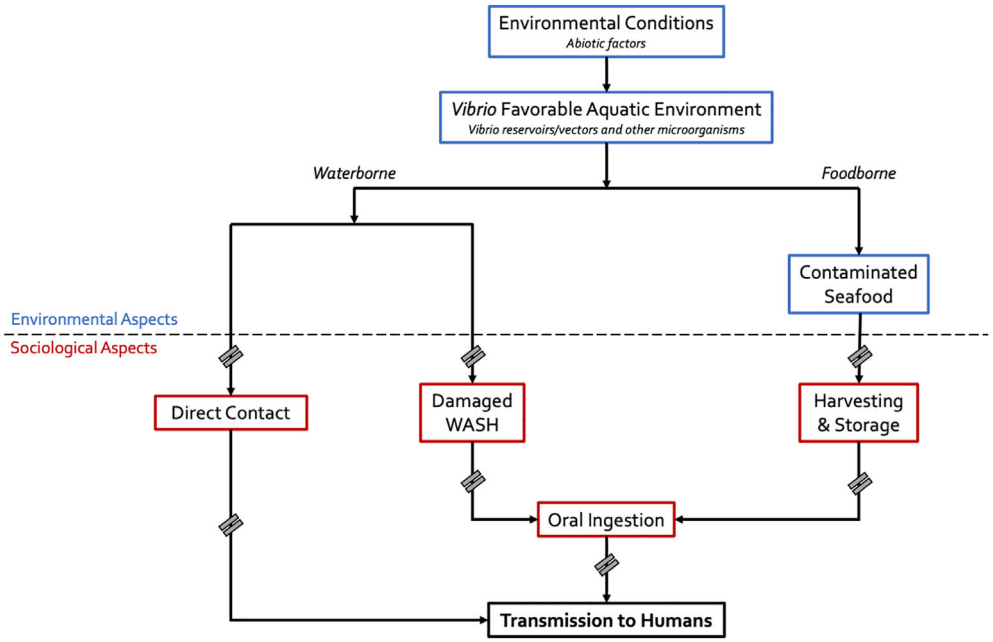

Fig. 5. Environmental and sociological aspects of transmission of *Vibrio spp*.
Pathways of transmission of pathogenic *Vibrio spp*. to humans with respect to environmental (blue) and sociological (red) factors. Disruption of these pathways—informed by early warning– present opportunities for public health intervention. Double slashes represent opportunities for public health intervention to disrupt pathways and reduce likelihood of infection.

It should be pointed out that predictive modelling 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 modelling 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 modelling 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 *et al*. (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 (Fig. 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 (Fig. 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 modellers 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.

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