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Effects of Global Warming on *Vibrio* Ecology

LUIGI VEZZULLI,[1] ELISABETTA PEZZATI,[1] INGRID BRETTAR,[2] MANFRED HÖFLE,[2] and CARLA PRUZZO[1]

[1]Dipartimento di Scienze della Terra, dell'Ambiente e della Vita (DISTAV), University of Genoa, 16132 Genoa, Italy; [2]Department of Vaccinology and Applied Microbiology – Microbial Diagnostics, Helmholtz Centre for Infection Research, D 38124 Braunschweig, Germany

ABSTRACT *Vibrio*-related infections are increasing worldwide both in humans and aquatic animals. Rise in global sea surface temperature (SST), which is approximately 1°C higher now than 140 years ago and is one of the primary physical impacts of global warming, has been linked to such increases.
In this chapter, major known effects of increasing SST on the biology and ecology of vibrios are described. They include the effects on bacterial growth rate, both in the field and in laboratory, culturability, expression of pathogenicity traits, and interactions with aquatic organisms and abiotic surfaces. Special emphasis is given to the effect of ocean warming on *Vibrio* interactions with zooplankters, which represent one of the most important aquatic reservoirs for these bacteria.
The reported findings highlight the biocomplexity of the interactions between vibrios and their natural environment in a climate change scenario, posing the need for interdisciplinary studies to properly understand the connection between ocean warming and persistence and spread of vibrios in sea waters and the epidemiology of the diseases they cause.

INTRODUCTION

Vibrios are considered as one of the most abundant culturable bacteria present in the oceans. They are found ubiquitously in a great variety of aquatic environments, from estuary and coastal waters to the deep sea. Vibrios are metabolically versatile microbes, preferring an attached and possibly biofilm existence, in association with animals, plants, and abiotic substrates (1). Habitat preferences have been characterized reasonably well for several *Vibrio* species and relationships have been established between the presence of certain species and selected environmental factors such as sea surface temperature (SST) and salinity (2). With few exceptions, vibrios grow preferentially in warm (>18°C), low salinity (<2.5% NaCl) waters, and exhibit strong seasonality, being most abundant during summer months when waters are warmer (3, 4, 5). As a consequence, the concentration of these bacteria in seawater and filter feeder organisms, such as oysters (representing a common vehicle of *Vibrio* transmission to humans), reaches the highest points at these times, posing the greatest threat to human health (6, 7, 8, 9).

Vibrio-related infections associated with the consumption of contaminated water and seafood are increasing worldwide. In the US, *Vibrio parahaemolyticus* and *Vibrio vulnificus* infections have increased since 2000, while the relative rates of infections from other major foodborne pathogens (e.g., *Salmonella*, *Campylobacter*, and enterotoxigenic *Escherichia coli)* have decreased (10, 11, 12). The number of cholera cases reported to the World Health Organization continues to rise and, for 2011 alone, a total of 589,854 cases and 7,816 deaths were reported by 58 countries. A rise in SST over the past decades is blamed for the observed increase in the incidence of *Vibrio* infections in humans (13). Warming of the climate system is unequivocal,

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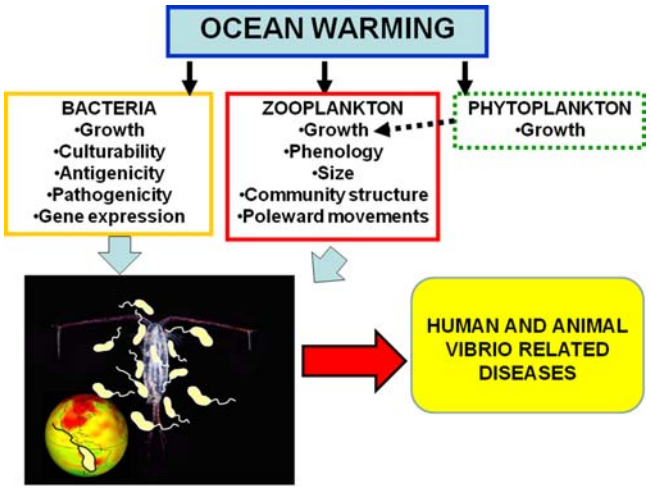
Correspondence: Carla Pruzzo, carla.pruzzo@unige.it

with evidence from observations of increases in global average temperatures by nearly 0.8°C since the late 19th century and approximately 0.2°C/decade over the past 25 years (14). It has been estimated that global SST is approximately 1°C higher now than 140 years ago and is one of the primary physical impacts of climate change (15).

The scarcity of baseline and epidemiological information at a global level challenges the possibility of assessing the real significance of the recent spate of *Vibrio*-associated diseases and to properly determine its link with ocean warming. Moreover, difficulty in both carrying out rigorous temporal and spatial sampling over a large scale and translating laboratory or on-deck experiments to the natural environment (16) greatly affects the chance of understanding real consequences of ocean warming on *Vibrio* spp. occurrence and spread in coastal waters. Hereinafter, major known effects of increasing SST on *Vibrio* spp. biology and ecology are described. Figure 1 shows most relevant effects of ocean warming on vibrios and zooplankters that represent important environmental reservoir for these bacteria (16, 17). In addition, recently published studies carried out in the southern North Sea are summarized as they provided the first experimental evidence for a positive and significant relationship between SST and *Vibrio* occurrence over a multidecadal time scale (17).

FIGURE 1 Simplified scheme of the response of bacteria and zooplankton to ocean warming. Ocean warming deeply influences bacterial growth and biology (e.g., culturability, antigenicity, pathogenicity, and gene expression) and zooplankton development, phenology, size, structure, and poleward movements. An increasing SST also triggers algal blooms that in turn promote an increase in zooplankters feeding on algae. Consequences of all the above include a higher association of vibrios to zooplankters (also due to a temperature-dependent higher expression of specific ligands), introduction of new *Vibrio* species/clones in new areas, and spread of associated human and animal diseases. doi:10.1128/microbiolspec.VE-0004-2014.f1



VIBRIO INFECTIONS IN HUMANS AND AQUATIC ORGANISMS: RELATIONSHIPS WITH INCREASING SST

It is becoming more and more evident that, although different factors linked to both climate and human activities are involved in driving the incidence of *Vibrio*-related diseases, the increase in SST is one of the major triggering determinants in most examined scenarios. A geographical transition in terms of disease into non-endemic regions can be observed, especially in temperate and cold regions of the world, which are the most affected by global warming (18, 19). *V. parahaemolyticus* O6: K18 infections occurred in Alaska in 2004 in coincidence with the arrival of warm waters that have exceeded the limit of 40 degrees latitude, usually not crossed during the summer (11). Satellite images have identified the Pacific coast of the US as the most likely origin of these warm waters. A similar scenario was observed during an outbreak of infection by *V. parahaemolyticus* in Galicia in 1999 (19). The emergence of the cases has been associated with a new serotype (O4: K11), and was coincident with the arrival of warm tropical waters in the coast of Galicia, and their permanence in the region. In northwest European countries, the number of reported *Vibrio*-related infections associated with recreational bathing has increased within the last decades. For instance, during the hot summer of 2006, wound infections linked to contact with Baltic and North Sea waters were reported from Germany (*V. vulnificus*) (20), southeast Sweden (*V. cholerae* non-O1/O139) (21), The Netherlands (*V. alginolyticus*) (22), and Denmark (*V. alginolyticus* and *V. parahaemolyticus*) (23). In Peru, climate events, such as El Niño, have been implicated in the geographical expansion of the *V. parahaemolyticus* outbreaks in 1997 to 1998, as a result of the southward shift of warm, low salinity water surges (11). Where cholera is endemic, cases tend to demonstrate distinct seasonal trends (24, 25). These patterns are strongly related to the ecology of *V. cholerae* in the environment, where high numbers are observed during times of warm-water temperatures and zooplankton blooms (24). A study by Huq et al. (26) showed that water temperature is directly correlated with cases of cholera in Bakerganj, the southernmost site included in the study, having the most direct influence

from the Bay of Bengal, where most of the initial cases of cholera occurred, including the outbreak of the then newly recognized serotype O139 in 1992 (27). A detailed analysis of existing data indicated that the interannual occurrence of cholera in Bangladesh, and the area surrounding the Bay of Bengal, is related to El Niño-Southern Oscillation (ENSO) (28, 29). In South America, where the disease has become endemic since its reemergence in 1991, cases are concentrated in the austral summer months (January and February) (30, 24). A Poisson regression model has been used to analyze the possible association between the cholera rates in southeastern Africa and the annual variability of air temperature and SST at regional and hemispheric scales for the period 1971 to 2006. The results showed that the annual mean air temperature and SST at the local scale, as well as anomalies at hemispheric scales, had a significant impact on the cholera incidence during the study period (31). The use of remote sensing imagery to gather data on SST and sea surface height uncovered a significant correlation of temperature with cholera outbreaks in Bangladesh and in South America (32).

In spite of numerous reports suggesting that SST increase is one of the most important drivers of cholera and other human vibriosis worldwide, other potential climate-driven factors must be taken into account (e.g., droughts and floods, sea surface height, salinity, pH, and river discharge). This is suggested by recent observations that vibriosis have increased significantly in the USA in the last decade (12, 33) and also in areas where long-term coastal temperature trends are weakly cooling or not significant {South Atlantic Bight [$-0.1°C \pm 0.3°C$ (100 years)$^{-1}$] and off Florida [$-0.3°C \pm 0.2°C$ (100 years)$^{-1}$]} (34).

In marine animals, several disease outbreaks have been associated with a rise in SST and climatic events such as ENSO (28). This is clearly evident among corals, which are known to bleach in response to a range of environmental stresses (35). For instance, the coral bleaching of 1998 on the Great Barrier Reef was the most geographically extensive and severe in recorded history, causing significant mortality of coral reefs worldwide (35, 36). The stress for many of these coral reef systems seems to be the result of long-term exposure to unusually high water temperatures as a consequence of a prolonged ENSO event. Such climate-mediated, physiological stresses may compromise host resistance and increase frequency of opportunistic diseases due, among others, to vibrios (35). In the Mediterranean Sea, coral bleaching of *Oculina patagonica* was promoted by *Vibrio shiloi* when SST rose above 25°C (37). Another example comes from studies on mass mortality events of gorgonians (*Paramuricea clavata*) in the Mediterranean Sea (38). Phylogenetic and phenotypic analysis of *Vibrio* isolates associated with healthy and diseased *P. clavata* colonies collected during mortality episodes showed that these bacteria are significantly more abundant in diseased than in healthy corals, including the species *Vibrio coralliilyticus*, which is only found in diseased organisms. Results of infection studies conducted in aquaria suggested that long-lasting high temperature values and trophic conditions can predispose gorgonians to infections by vibrios, whose concentration in ambient water is triggered by the temperature increase. Other studies conducted to define environmental determinants affecting the capability of *Vibrio harveyi* to induce mortality in a wide variety of marine animal species showed a strong positive correlation between sea temperature and abalone mortality caused by this pathogen on the basis of 5 years' worth of data (2003 to 2007) (39).

Overall, predicted global warming leading to long-lasting hot summer periods represents a major threat to the survival of invertebrates in temperate areas due to increased sensitivity to pathogenic/opportunistic infections coupled with an increased concentration of vibrios in surrounding waters and an increased expression of virulence factors (40). It is hypothesized that the effects of climate change on both aquatic animals and (opportunistic) pathogens will be superimposed onto the effects of other stressors in ecosystems, such as those deriving from human activity (e.g., pollution, shipping, fishing and recreational activities), leading to habitat and biodiversity loss (28).

EFFECT OF INCREASING SST ON *VIBRIO* GROWTH AND BIOLOGY: FIELD AND *IN VITRO* EVIDENCE

The density of vibrios is related to seasonal variations and maximal *Vibrio* concentration is recorded in water above a temperature of approximately 10°C to 17°C, depending on species (41). Field studies conducted worldwide over the last 40 years have shown such a relationship (42, 43, 44, 45). As an example, results from different studies conducted over the period from 1995 to 2004 along the central-northern Italian Adriatic coast (46, 47, 48, 49) showed that culturable vibrios (mainly the species *Vibrio alginolyticus*) were abundant in this area during the warmest months, when SST ranged from about 18°C to about 25°C. They were not present or were present in very low concentration during winter.

Elevated SST can also have an indirect effect on *Vibrio* growth in coastal waters, as suggested for the first time by Cockburn and Cassanos (50). Sunlight, temperature, and nutrients all influence the growth of phytoplankton and aquatic plants, which, in turn, alter the dissolved O_2 and CO_2 content and, therefore, the pH of the surrounding water (24). It was proposed that in hot, dry weather, algae could raise the pH of the water so high that *V. cholerae* is favored over other organisms and reach infectious dose levels (50).

Field observations are consistent with results of *in vitro* experiments showing that temperature is one of the main factors influencing growth rates of vibrios. For instance, in *V. cholerae*, an exponential relationship in growth rate was observed for the interval from 15°C to 37°C (51). In *V. vulnificus*, cells growing at 23°C and 13°C doubled every 3 h and 13.1 h, respectively (52). In *Vibrio splendidus*, a 4- to 6-fold decrease in bacterial growth was observed as temperatures decreased from 15°C to 0°C (53). Besides temperature, salinity and pH affect the growth rate of vibrios, as in the case of *V. cholerae* for which optimal salinity for growth is between 0.5% and 2.5%. However, it can also exist and prosper in freshwater, with sufficient dissolved organic matter present, and can grow at salinities values up to 4.5% (2).

In addition to cell growth rate, SST influences bacterial culturability, antigenicity, and pathogenicity. In coastal water, during winter months, when SST dips below the preferred range, most vibrios can enter a dormant state known as "viable but nonculturable" (VBNC) (54, 55). Other factors, both chemical and environmental, which have been reported to induce the VBNC state, are diverse and numerous. They include nutrient starvation, elevated or lowered osmotic concentrations, oxygen concentrations, heavy metals, and even exposure to white light (56). *In vitro* and *in vivo* studies showed that VBNC cells continue to be metabolically active, retain pathogenic potential but do not grow and do not form a colony on traditional culture media (57). VBNC bacteria can be detected in biological samples by culture-independent methods only (e.g., immunofluorescence, PCR) (58, 59, 60). Under favorable conditions (such as a rise in temperature), reversion from the VBNC to the culturable state can take place (56, 61).

Concerning antigenicity, microcosm experiments have shown that temperature and salinity are important factors in *V. cholerae* conversion from non-O1 to O1 serogroup (62). Serogroup conversion was shown to be consequent to chitin-induced natural transformation occurring within a community of bacteria living on a chitin surface (63). Increased expression of known and putative virulence-associated traits was correlated with increased temperature in human (*V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus)* and animal *(V. coralliilyticus*, *V. shiloi*) species (64, 65, 66). At high summer seawater temperatures, *V. shiloi*, the causative agent of bleaching of *O. patagonica*, produces an adhesin that allows it to adhere to a beta-galactoside-containing receptor in the coral mucus, penetrate into the coral epidermis, multiply intracellularly, differentiate into a VBNC state, and produce toxins that inhibit photosynthesis and lyse the symbiotic zooxanthellae (40). In *V. cholerae*, temperature regulates transcription of *toxT*, a gene encoding the regulatory protein ToxT that directly activates transcription of virulence factors, including cholera toxin (CT) and the toxin-coregulated pilus (TCP) (67).

EFFECT OF INCREASING SST ON *VIBRIO* INTERACTIONS WITH LIVING AND NONLIVING MARINE SUBSTRATES

The ecology of vibrios in coastal waters is intimately coupled with their attachment to biotic and abiotic surfaces; these interactions are also important for *Vibrio* transmission from the aquatic environment to humans (24). Vibrios are found associated with protozoa, plankton organisms (e.g., copepods), plants, bivalves, fishes, water birds, and a variety of abiotic substrates, such as detrital chitin and inorganic particles (68). Among variables affecting such interactions, temperature seems to play an important role. For instance, it has been shown that attachment of *V. alginolyticus* to chitin particles progressively increases from about 65% to about 80% of the inoculum as temperature increases from 5°C to 20°C (69). A study conducted by Long et al. (70) showed that interspecies antagonistic interactions influence particle colonization by *V. cholerae*. Autochthonous bacteria appear to become less inhibitory against this pathogen at elevated temperatures such as those measured during ENSO and monsoonal events. It is hypothesized that SST increase due to changes in global climate can reduce competitiveness from other autochthonous microbes, contributing to increasing abundance and geographic spread of *V. cholerae*. In this frame, another study conducted by Rypien et al. (71) on bacteria-bacteria antagonism in a coral *Montastrea annularis*-associated microbial community, reported that several potential pathogens are present in the microbial community of apparently healthy corals, and a measurable percentage (about 12%) of isolates is able to inhibit the

growth of the coral pathogen *V. shiloi*. Elevated temperatures generally diminished levels of antagonistic interactions among coral-associated bacteria (71) suggesting that, in this case too, ocean warming might contribute to disease onset, reducing the inhibitory activity exerted by harmless bacteria towards pathogens.

V. cholerae interaction with copepods deserves particular attention as these zooplankton organisms are the most (or one of the most) important environmental reservoir for the bacterium, favoring its persistence and spread in oceans, and also serving as vector of infection (68). Huq et al. (72) proposed that once cells of *V. cholerae* attach to zooplankton, they are protected from the external environment and begin to proliferate. The chitin exoskeleton of a single colonized copepod has been shown to contain up to 10^4 cells of *V. cholerae* (73), thus providing the required infectious dose for clinical cholera, ranging from 10^4 to 10^{11} bacteria, depending on the strain and the infected host (74). In the VBNC state, bacteria maintain the capability to adhere to chitin particles and copepods, although at a lower degree compared to actively growing controls (75). Interactions between vibrios and copepods are affected by environmental variables. Salinity of 1.5% and temperatures ranging from 25°C to 30°C have been shown to be important in influencing the attachment of *V. cholerae* to copepods (76, 77). A possible explanation for this comes from recent studies (78, 79) evaluating the expression of two major *V. cholerae* adhesins involved in interactions with zooplankters and chitin substrates [N-acetyl glucosamine-binding protein A (GbpA) and mannose-sensitive hemagglutinin (MSHA)] in aquatic microcosms. Experiments conducted at different temperatures (15°C, 20°C, and 25°C), matching the seasonal temperature range of temperate marine environments, showed a significant increase in expression level of both adhesins at 25°C. This suggests that temperature can play a role in promoting *V. cholerae* colonization of environmental surfaces via an enhanced expression of MSHA and GbpA (78, 79).

The recognized biological interaction between vibrios (mainly *V. cholerae*) and zooplankters makes the study of the response of these marine organisms to ocean warming crucial for understanding the effects of climate change on the persistence and spread of *Vibrio* bacteria in the ocean. Conditions favorable for multiplication of copepods and related chitinous zooplankton species are expected to result in an increase in the number of *V. cholerae* and other vibrios in the aquatic environment (2). Changes in abundance and spread of warm-water zooplankton species can be observed as a direct consequence of heating of the ocean's surface waters. Moreover, elevated water temperatures are known to trigger algal blooms that, in turn, promote the increase in zooplankton grazers feeding on algae (80). Other major direct impacts of an increasing SST are manifest as size reduction, earlier timing of important life cycle events, changes in community structure, and poleward movements in the distribution of individual species and assemblages (81, 82, 83, 84). In the eastern North Atlantic and on the European shelf, due to latitudinal movement of warm and tropical waters, strong biogeographical shifts in copepod assemblages occurred during the last 50 years, with a poleward extension of more than 10 degrees latitude (more than 1,000 km) for warm-water species and a decrease in the number of colder water species (82). Allochthonous plankton organisms serve as vehicles for the introduction of new *Vibrio* species/clones in new areas (11, 19, 85). Such invasive microorganisms may become endemic if environmental conditions are favorable for their establishment (11, 85). It must be taken into consideration that differences may exist in the capability of different *Vibrio* species and clones to colonize various zooplankton species and organisms at different life stages. At present, very few comparative measures exist to evaluate this possibility and these are restricted to only a few plankton species under a limited range of the possible environmental conditions (86).

LONG-TERM EFFECTS OF OCEAN WARMING ON THE *VIBRIO* COMMUNITY IN THE SOUTHERN NORTH SEA: A CASE STUDY

Despite the fact that statistically significant empirical relationships have been established between the presence of vibrios and SST and other environmental factors (2), gaps still exist that preclude conclusive evidence regarding the effect of ocean warming on *Vibrio* occurrence and spread. A possible explanation for this is the lack of comparable data, conducted over a large spatial and temporal scale. To fill in this gap, at least in part, a study was recently conducted to analyze long-term relationships between *Vibrio* occurrence and climatic variables through retrospective molecular analysis of samples collected by the Continuous Plankton Recorder (CPR) survey in the North Sea (17), where, in recent years, an unexpected increase in the number of bather infections associated with vibrios was reported (see above, and 20, 21, 22, 23).

The CPR archive represents one of the largest spatial-temporal collection of marine biological samples in the

world (http://www.sahfos.ac.uk). The CPR is a plankton sampling instrument designed to be towed from merchant ships, or ships of opportunity, at a depth of approximately 10 m on their normal sailings (87). Water passes through the CPR and plankton is filtered onto a slow-moving band of silk (of 270 μm mesh size) that is covered by a second silk, then spooled into a storage tank containing formaldehyde. On return to the laboratory, the silk is removed from the mechanism and divided into samples. As plankton represents the largest environmental reservoir for vibrios in the aquatic environment, it is reasonable that the CPR system, capturing plankton and particles, also captures a significant fraction of associated vibrios. Based on this rationale, a study was planned for long-term molecular enumeration of vibrios associated with plankton in formaldehyde-fixed CPR samples collected off the Rhine estuary from 1961 to 2006 and off the Humber estuary from 1965 to 2006 (17). All samples analyzed in this study were collected in August along an 18.5 km tow corresponding to 3 m^3 of filtered seawater for each sample. The total area sampled, during the 45 years period, was about 3,000 km^2 for both sites. To analyze formalin-fixed samples, a method was developed to extract good quality genomic DNA to be used for the evaluation of the relative proportion of plankton-associated vibrios (*Vibrio* relative abundance index-VAI) by real-time PCR. VAI was defined as the ratio of *Vibrio* spp. cells to the total number of bacterial cells assessed using genus-specific and universal primers, respectively.

A long-term increase in the relative abundance of vibrios was observed in CPR samples collected off the Rhine estuary that showed a positive and statistically significant correlation with SST; this correlation was not observed in samples collected off the Humber estuary. Such different results for the two areas can be related to the generally higher summer SST values recorded off the Rhine estuary compared to the Humber estuary. In fact, only off the Rhine area did SST in summer periods exceeded 18°C, thus favoring *Vibrio* growth. A nonparametric multiple regression analysis (88), comprising pooled VAI data, the number of total copepods, and phytoplankton color (an index of phytoplankton biomass), calculated for the same areas and period, showed that, in the North Sea, SST and the number of total copepods explained 50% of the variance in the *Vibrio* data ($p<0.05$), with SST alone explaining 45% of the variance. These findings supported evidence from previous studies that an increase in temperature might enhance not only *Vibrio* growth rates but also their capability to attach to and multiply on plankton (76). The analysis of the composition of the bacterial community in selected CPR samples by 16S rDNA pyrosequencing showed that particle-associated vibrios, including the human pathogen *V. cholerae*, have increased in prevalence from 1961 to 2006 in the coastal waters of the southern North Sea (17). These findings provided the first experimental evidence that global warming is having a strong impact on the marine bacterial community.

CONCLUSIONS

Vibrios, for their ubiquity in aquatic environments, flexible physiology, and thermal dependence, represent a very useful model to study the connection between ocean warming and persistence and spread of waterborne pathogens in sea waters and the epidemiology of the diseases they cause. However, in spite of numerous reports, the biocomplexity of interactions between these bacteria and their natural environment in a climate change scenario is still poorly understood. To clarify these aspects, further studies must be planned, including (i) comparative field observations on the occurrence and spread of these bacteria on long temporal and spatial scale (e.g., retrospective analyses and remote sensing measurements). (ii) Studies on *Vibrio* spp. interactions with their aquatic reservoirs, including different species and life stages, both in the laboratory and in the field, in different environmental and climatic scenarios. (iii) Studies on *Vibrio* ecology in less-explored aquatic matrices (e.g., sediment); and (iv) implementation of all the above with results from the analysis of the influence of climate change on host susceptibility and exposure patterns. The success of these interdisciplinary approaches will be also dependent on the creation of an international collaborative research network to develop a freely accessible database that would allow data to be analyzed and interpreted (88, 89). These studies, as a whole, are expected to allow the construction of models and the development of measures to predict, prevent, and control *Vibrio*-related diseases.

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