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Interactions of *Vibrio* spp. with Zooplankton

MARTINA ERKEN,[1] CARLA LUTZ,[2] and DIANE McDOUGALD[3]

[1]Center for Marine Bio-Innovation, School of Biotechnology and Biomolecular Science, University of New South Wales, Sydney, NSW 2052, Australia; [2]Singapore Centre on Environmental Life Sciences Engineering, Nanyang Technological University, Singapore; [3]ithree Institute, University of Technology, Sydney, Australia; and Singapore Centre on Environmental Life Sciences Engineering, School of Biological Sciences, Nanyang Technological University, Singapore; and Center for Marine Bio-Innovation, School of Biotechnology and Biomolecular Science, University of New South Wales, Sydney, NSW 2052, Australia

ABSTRACT Members of the genus *Vibrio* are known to interact with phyto- and zooplankton in aquatic environments. These interactions have been proven to protect the bacterium from various environmental stresses, serve as a nutrient source, facilitate exchange of DNA, and to serve as vectors of disease transmission. This review highlights the impact of *Vibrio*-zooplankton interactions at the ecosystem scale and the importance of studies focusing on a wide range of *Vibrio*-zooplankton interactions. The current knowledge on chitin utilization (i.e., chemotaxis, attachment, and degradation) and the role of these factors in attachment to nonchitinous zooplankton is also presented.

INTRODUCTION

Bacteria in the genus *Vibrio* are natural inhabitants of aquatic environments. They are found in freshwater, estuarine and marine environments and are present in the benthos and in the water column. As a result, *Vibrio* spp. interact with higher organisms within the aquatic biosphere. Interactions of *Vibrio* spp. with representatives of the zooplankton community are of particular interest as these zooplankton have been proven to protect attached *Vibrio* spp. from various environmental stresses, serve as a nutrient source, facilitate exchange of DNA, and to serve as vectors of disease transmission (e.g., 1, 2, 3, 4, 5).

Vibrio spp. have been found to be associated with many higher organisms including fish (6), corals (7), sponges (8), hydroids (9), crabs (10), mollusks (11), and protozoa (12) (for a review of reservoirs for *Vibrio cholerae* see reference 13). Despite the majority of these associations being benign or beneficial, the majority of current research usually focuses on pathogenic *Vibrio* spp. For example, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *V. cholerae* cause severe human diseases such as diarrhea, septicemia, and cholera (e.g., 14, 15, 16), while *Vibrio coralliilyticus* and *Vibrio tubiashii* are causative agents of disease in commercially and ecologically important organisms such as oysters and corals (e.g., 17, 18).

As common bacterioplankton in aquatic environments, *Vibrio* spp. interact with planktonic animals (zooplankters) of many different phylogenetic affiliations and with different surface characteristics and compositions. The numerous adaptations that have evolved in *Vibrio* spp. that allow for surface colonization and intracellular associations with individual zooplankters reflect the diversity of zooplankton. The best-studied association of a *Vibrio* sp. with zooplankters is that of *V. cholerae* and crustaceans such as copepods. The role of zooplankton in cholera transmission is well established, as is the role of chitin as a carbon source for *Vibrio* spp. in the oligotrophic environment of the oceans (e.g., 4, 19, 20, 21).

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Correspondence: Diane McDougald, d.mcdougald@unsw.edu.au

To understand better which environmental factors promote the persistence of these microorganisms in the environment, it is necessary to understand their ecological niche, including interactions/adaptations that may lead to disease events. In this review, we present current knowledge on the interactions of *Vibrio* spp. with higher organisms. We also review the impact of *Vibrio*-zooplankton interactions at the ecosystem scale and highlight the importance of studies focusing on a wide range of *Vibrio*-zooplankton interactions, with a focus on recently published studies (within the last 5 years). The current knowledge on chitin utilization (i.e., chemotaxis, attachment, and degradation) and the role of these factors in attachment to nonchitinous zooplankton is also presented.

ECOLOGICAL INTERACTIONS OF *VIBRIO* SPP. WITH ZOOPLANKTON

The interactions of zooplankton and *Vibrio* spp. on a global and/or ecosystem scale are influenced by many different abiotic and biotic factors such as temperature, salinity, nutrients, and competing or predatory organisms. Just as *Vibrio* spp. have different niche preferences, there are many zooplankters that, although they share certain characteristics, occupy very diverse niches. To begin to understand the complex and diverse interactions of zooplankters and *Vibrio* spp. it is important to identify the different groups that comprise the zooplankton.

Zooplankton Taxa

Plankton are biota suspended in a water body and although some are able to swim, they are not able to move against water currents (22). In general, plankton consists of microscopic to macroscopic organisms of all trophic levels. Their sizes range from the micrometer scale to centimeters. Plankton includes organisms as small as phages and other viruses (<0.2 μm, femtoplankton), bacteria (0.2 to 2 μm, picoplankton), and nano-flagellates (2 to 20 μm, nanoplankton). Larger plankters include flagellates and ciliates (20 to 200 μm, microplankton), rotifers, nauplii larvae, fish larvae, and copepods and cladocerans (0.2 to 20 mm, mesoplankton) of which many are visible to the naked eye (23). All planktonic organisms that are able to carry out photosynthesis to generate energy, such as autotrophic protists and cyanobacteria, are referred to as phytoplankton.

These planktonic communities are interconnected by complex and diverse bottom-up and top-down controls. Nutrient availability, temperature, intra- and interspecies competition and predation shape the planktonic community from the smallest viruses to larger organisms (such as jellyfish or cladocerans) and also impact the larger nekton communities (organisms that can swim against the current). All planktonic bacteria recycle nutrients by feeding on dissolved and particulate organic matter (DOM and POM, respectively) from exudates of phytoplankton, nutrients released by "sloppy feeding" of predators or exoskeletons of chitinous zooplankton (24) (Fig. 1). Many bacteria, including most *Vibrio* spp., are chitinolytic, that is, they are able to obtain their nitrogen (N) and carbon (C) by degradation of chitin (2, 25).

The term zooplankton includes a large collection of heterotrophic groups, including but not limited to crustaceans such as copepods, water fleas (*Daphnia* spp.) and brine shrimp (e.g., *Artemia* spp.), nauplius larvae, rotifers, heterotrophic protists, and jellyfish (Fig. 2). Freshwater, coastal, and marine water zooplankton communities differ in some respects but most zooplankton taxa appear everywhere, just in different abundances (22). The most important zooplankters in these environments are protists and crustaceans such as copepods and cladocerans. Many invertebrates, such as annelids and chaetognaths, and the larvae of fish, echinoderms, mollusks, and arthropods, are also abundant members of the zooplankton community.

The smallest representatives of the zooplankton community are flagellates, amoebae, and ciliates. These heterotrophic protists, or protozoa, are an important part of the planktonic community (Fig. 2D). These unicellular, eukaryotic organisms are essential components of the microbial loop, and thus, are necessary for nutrient recycling and for controlling and shaping bacterial abundances in pelagic environments (26). *Vibrio* spp. have been shown to interact with flagellates, ciliates and amoebae in attached as well as in suspended communities (e.g., 12, 27, 28, 29, 30, 31).

Representatives of the phylum Rotifera are found mostly in freshwater environments, with some occurring in brackish water and only 100 taxa that are exclusively marine (Fig. 2E) (22). These small (100 to 500 μm) metazoans prey on protozoans, bacteria, yeasts and microalgae and they are themselves preyed upon by larger plankton (32). *Brachionus* spp. play an important role in *Vibrio* spp. ecology in Bangladesh, where the presence of this rotifer was significantly correlated with the presence of toxigenic strains of *V. cholerae* and with outbreaks of cholera (33).

Representatives of the phylum Arthropoda are the most abundant taxa of the zooplankton. Although

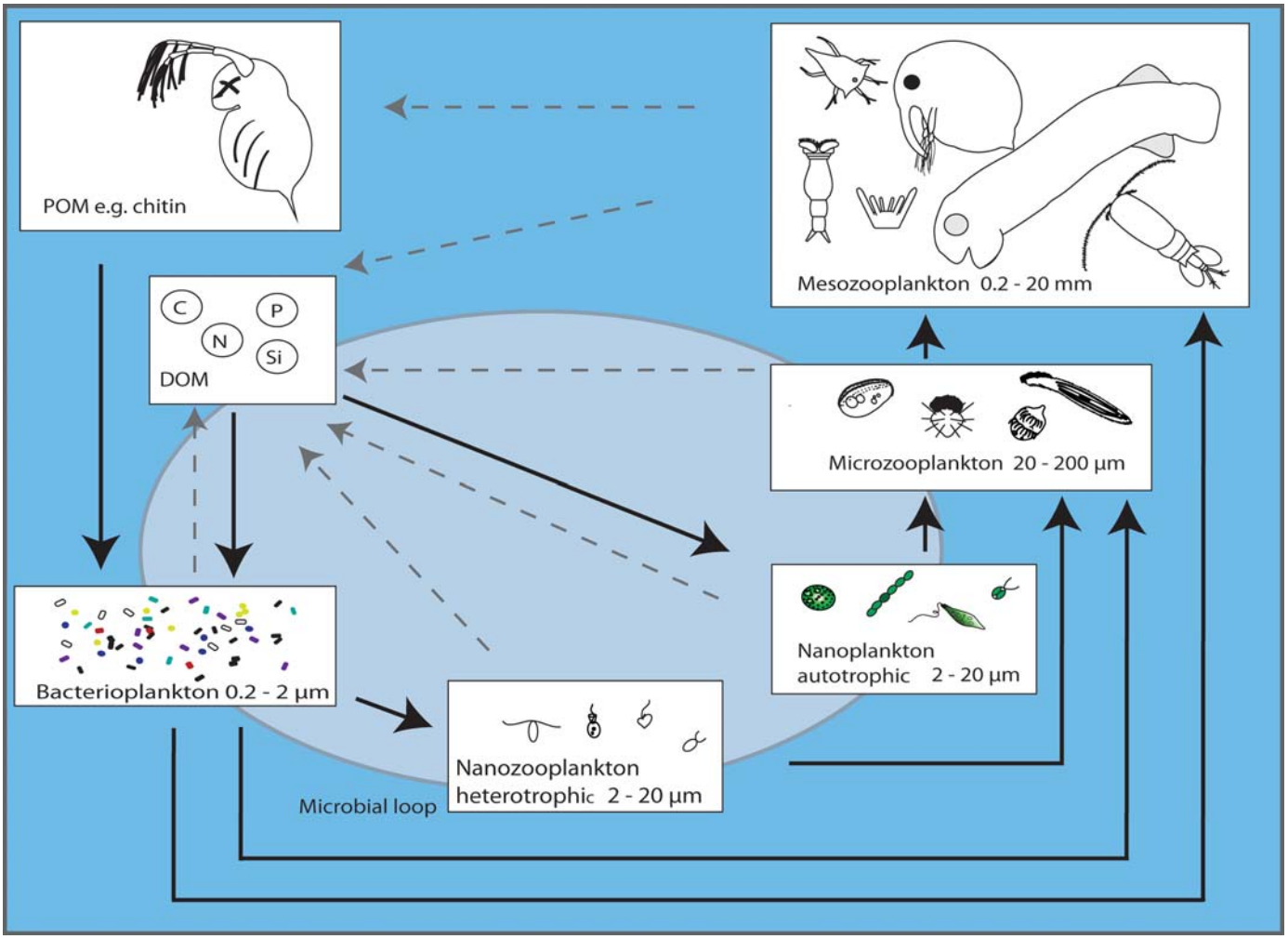


FIGURE 1 Food web interactions of planktonic organisms. Bacteria take up dissolved and particular organic matter (DOM and POM, respectively). Heterotrophic protists ingest bacteria within the planktonic environment and are themselves preyed upon by larger predatory protists and metazoans. Carcasses and fecal matter of these organisms contribute to the DOM and POM bacteria utilize as nutrients. Black arrows indicate direct uptake for nutrients; gray arrows indicate contribution to the pool. doi:10.1128/microbiolspec.VE-0003-2014.f1

spending only part of their life in water (i.e., meroplanktonic), the larvae and eggs of some insects are important components of the planktonic community of fresh and brackish water; chironomids, or nonbiting midges, may be the most abundant insects in these environments (34). Their eggs are covered in a gelatinous mass, which can span a large area near the shore. In marine, as well as fresh and brackish water, the crustacean subclass of Copepoda and the order of Cladocera are the best-known representatives of the chitinous zooplankton. Although formerly classified as its own taxon, nauplii larvae are now known to be the larval stages of some crustaceans such as krill (22).

The crustacean class of copepods consists of roughly 10,000 species. These organisms can reach such high numbers in the plankton that they are probably the most abundant metazoan group on earth (22, 35) (Fig. 2B). There are 10 taxonomic orders of which nine are present in marine environments. The three major groups are the Calanoida, Cyclopoida and Harpacticoida, having an average size range of 1 to 2 mm. Copepods are generally more abundant in coastal and upwelling areas, and many are also found in sediment. Because copepods have on average five nauplii stages and molt on average a further five times from copepodite to the adult form, they produce a massive amount of chitin. Copepods play

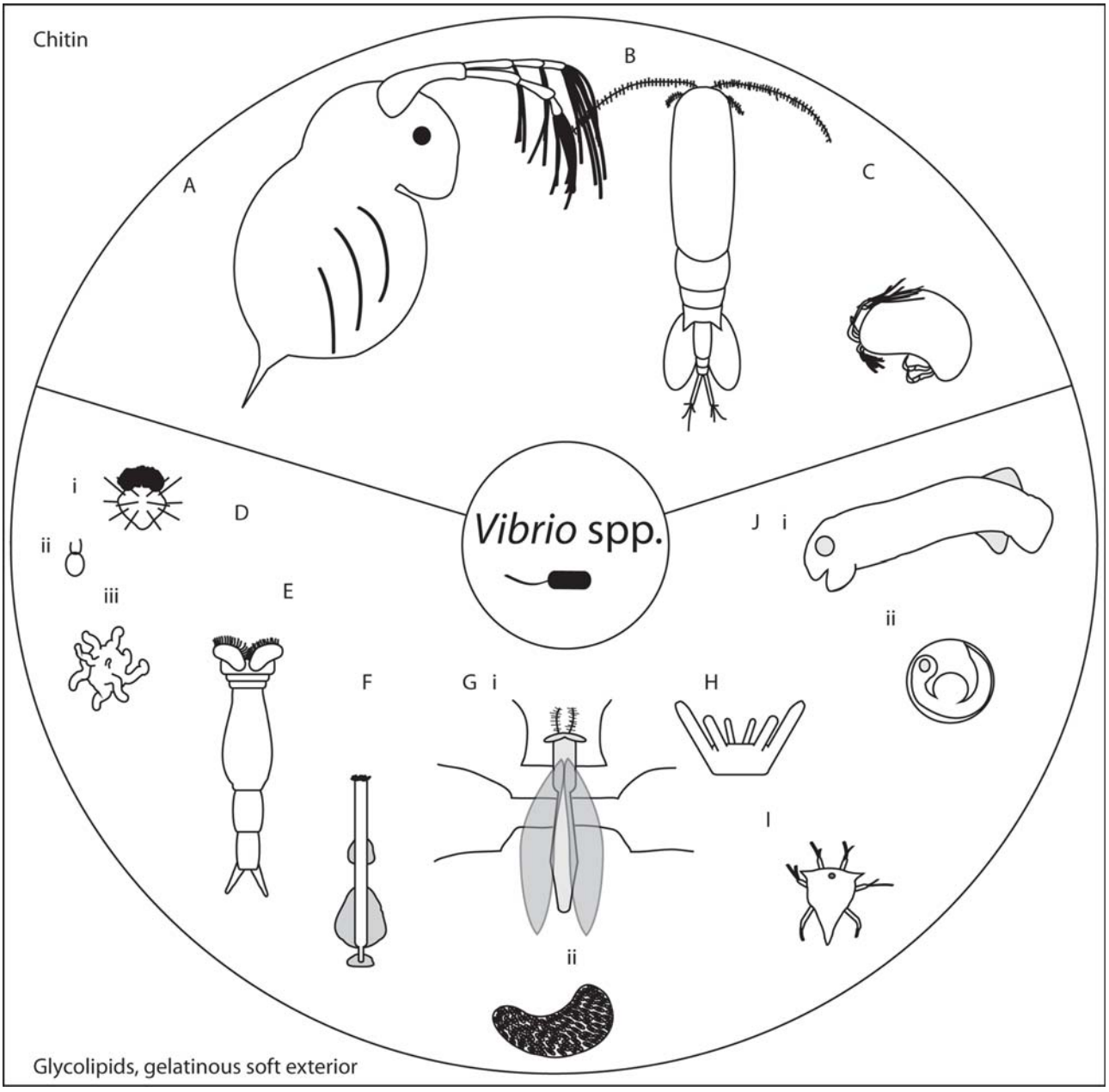


FIGURE 2 Common zooplankters with which *Vibrio* spp. interact. *Vibrio* spp. (especially *V. cholerae*) colonize crustaceans, such as cladocerans (A), copepods (B), and ostracods (C). *Vibrio* spp. have also been shown to interact with gelatinous and soft tissue zooplankters, and protozoa (D; i. ciliate, ii. flagellate, iii. amoeba), rotifers (E), chaetognaths (F), chironomids (G; i. adult, ii. egg masses), echinoderm pluteus larvae (H) , nauplius larvae (I), fish larvae (J i.), and fish eggs (J ii.) (12, 19, 33, 45, 64, 66, 76, 131). Please note that the images are not to scale. doi:10.1128/microbiolspec.VE-0003-2014.f2

an important role in the ecosystem as they graze on phytoplankton and smaller zooplankton and are themselves preyed upon by fish larvae (Fig. 1).

Cladocera is a suborder of the crustacean class Brachinopoda (Fig. 2A) (22). Their body shape can vary greatly but most have a flattened appendage (36). While they are mostly found in freshwater, there are also marine groups and some that tolerate high salinity. Their habitat is mostly the plankton of lakes and ponds where they are important links in the food web as they prey on

bacteria and protists, and thus, transport nutrient up to higher trophic levels (Fig. 1). The cladocerans have an average size of 1 to 2 mm with some growing up to 5 to 6 mm in length.

Bacterial Attachment to Zooplankton: a Mechanism for Dispersal

As mentioned above, it is well established that bacteria, including vibrios, are able to attach to zooplankton (37). In particular, the chitinous zooplankters and their fecal pellets are hot spots of microbial activity in oligotrophic environments (38). Attachment to zooplankton can protect bacteria from various stresses, including high temperatures, UV and biocide treatments used for sanitation purposes, that suspended bacteria may not survive (25, 39).

Grossart et al. (40) referred to bacteria as "hitchhikers" that use the larger zooplankton for fast dispersal. Many zooplankters use diurnal vertical migration to avoid predation pressure (41), and thus, bacteria that attach to these zooplankters can disperse in the water column much faster than on their own accord (40). In Chesapeake Bay, USA, genetically diverse *V. cholerae* were found at the same sampling site, whereas genetically identical *V. cholerae* were found at multiple sites (42), which the authors suggested was due to bacteria being transported by water currents. As plankton organisms cannot move against currents, the "hitchhiker" hypothesis would be consistent with the observed distribution of *V. cholerae* and it is plausible as a dispersal strategy for *Vibrio* spp. At the same location, Heidelberg et al. (43) used fluorescent oligonucleotide direct counts to investigate the seasonal variation of bacteria attached to zooplankton. Up to 40% of the bacterial cells in the water column were attached to various zooplankton taxa and reached $9.6\pm23.3\times10^5$ bacteria per zooplankter (43). Interestingly, there was no correlation between bacterial taxa having the highest abundances in the water column and those that were attached to the zooplankton. Chironomid adults, larvae, and their gelatinous egg masses have been shown to harbor non-O1/non-O139 *V. cholerae* cells (44, 45). Adult chironomids that were captured in the air were found to carry *V. cholerae*, and thus, may play a role in the dispersal of this bacterium from one body of water to another. Although only non-cholera causing serogroups were identified, the potential for dispersal of O1/O139 strains of *V. cholerae* should not be underestimated.

Interactions of *Vibrio* spp. with Zooplankton

V. cholerae, the causative agent of cholera, a disease that is still at epidemic proportions in many countries where seasonal outbreaks of cholera occur, is also known to attach to zooplankton. Thus, zooplankton may act as a vector for toxigenic strains, enhancing disease transmission (46, 47, 48, 49, 50). Recent studies have focused on the interactions of *Vibrio* spp. with different zooplankton taxa and the reasons for the increase in *Vibrio* abundances that occur with changing zooplankton community composition, as well as in response to environmental parameters such as temperature and salinity. Strong correlations between the occurrence of *Vibrio* spp. and temperature, salinity, chlorophyll *a*, rainfall, and dissolved oxygen have been reported repeatedly (51, 52, 53, 54, 55, 56, 57), and such measures, therefore, have significant potential for the use as early warning and monitoring systems to predict outbreaks of cholera.

Since Colwell and colleagues first described the interactions of *Vibrio* spp. and copepods in the 1970s, studies have focused on the ecology of O1 and O139 *V. cholerae* (19, 51, 58). The correlation between phyto- and zooplankton blooms and cholera outbreaks is now well established [for a recent review on *V. cholerae* interactions with chitinous zooplankton, see Pruzzo et al. (59)]. Here we will review more recent literature on *V. cholerae* (toxigenic and nontoxigenic) interactions with zooplankton.

V. cholerae O1/O139

Crustacean zooplankton, such as copepods and cladocerans, are acknowledged reservoirs of *V. cholerae* in the environment between cholera epidemics (19, 47, 60, 61). The correlation of zooplankton blooms and subsequent cholera epidemics in coastal areas in Bangladesh is well recognized (19, 62, 63). For example, in Bakerganj, Bangladesh, a one log increase in copepod counts was positively associated with cholera cases in three of the four surveillance areas while total counts of phytoplankton were not correlated (48).

In 1996, Huq et al. (4) demonstrated that filtering water through a sari that was folded four times removed 99% of *V. cholerae* cells from the water. This simple method removed the planktonic organisms larger than 20 μm in size (zooplankton and colonial phytoplankton), along with their epibiotic microbial communities; thus, the infectious dose for cholera was not reached. This method was tested in an extensive field trial in Matlab, Bangladesh, to see whether the women responsible for cooking and collecting drinking water would use this method, and whether the incidence of cholera could be reduced. The study, lasting 3 years and involving 65 participating villages, demonstrated that by filtering the water, cholera cases were reduced by 48%

(47). In a follow-up study 5 years later, 31% of the interviewed women who participated in the previous study were still filtering the water, thereby protecting themselves directly and their neighbors indirectly by reducing the overall cases of cholera in the region (49).

In Bakerganj and Mathbaria, Bangladesh, it has been reported that *V. cholerae* was significantly associated with certain members of the zooplankton community (33). The sampling sites were manmade ponds that were heavily used by villagers in the surrounding area for drinking water and other domestic uses. The dominant zooplankton groups, rotifers (34%), nauplii (29.5%), and copepods (22.6%), were repeatedly associated with *V. cholerae* detection and cholera cases. In Mathbaria, the rotifer *Brachionus angularis* was found to be significantly associated with the occurrence of *V. cholerae* O139 and with the gene encoding a cholera toxin subunit, *ctxA*, after a lag of 2 weeks, as well as with *V. cholerae* O1 and *ctxA*, after a lag of 4 weeks. Of the clinical cases of cholerae, 92% were due to *V. cholerae* O1 strains and 8% to O139 strains. The presence of this rotifer was significantly associated with the occurrence of cholera caused by *V. cholerae* O1 in both Mathbaria and Bakerganj. In Bakerganj, all cholera cases were caused by O1 *V. cholerae* and no O139 strains were detected in any of the clinical or zooplankton samples. In addition, in Bakerganj, the presence of the cladocerans *Moina* spp. and *Diphanosoma* spp. were correlated to incidences of cholera caused by *V. cholerae* O1 strains. The authors suggested that the monitoring of these zooplankton species might be useful for prediction of potential cholera outbreaks (33).

In microcosm experiments, *V. cholerae* O1 survived for 7 weeks in estuarine water collected from Mathbaria (50). In microcosms supplemented with chitin chips, *V. cholerae* O1 grew actively for up to 6 months, with the microcosms becoming turbid due to bacterial growth. In addition, chitin flakes were heavily colonized by matrix-enclosed biofilms of *V. cholerae* O1. These bacterial cells remained metabolically active even in highly acidic environments (pH 1.6 to 1.8), demonstrating that biofilm-associated cells were highly resistant to acid stress. This fact may further exacerbate outbreaks of cholera as cells attached to chitinous zooplankton would be resistant to digestion in the human gut due to enhanced acid tolerance.

Although often not detectable by culturing methods due to entry into the viable but nonculturable (VBNC) state (51), molecular techniques have demonstrated that *V. cholerae* is abundant in interepidemic periods in areas where cholera is endemic. In recent years, studies have shown that even in areas where cholera appears sporadically, or in areas where there is no history of cholera epidemics, *V. cholerae* is a natural component of the microbiota (64, 65, 66). In a large-scale study of bacteria attached to zooplankters in Chesapeake Bay, Heidelberg et al. (43) found that *V. cholerae, V. mimicus*, and *V. vulnificus* comprised the largest fraction of attached *Vibrio* and *Photobacterium* species.

Rawlings et al. (61) demonstrated that even within one species of *Vibrio*, there may be differences in preferences of taxa of zooplankters that are used for attachment. It was observed using microcosm experiments that *V. cholerae* O1 El Tor and O139 Bengal strains attached differentially to two different copepods (*Acartia tonsa* and *Eurytemora affinis*). These copepods were the dominant species in Chesapeake Bay (67) and were abundant in the Bay of Bengal. Other studies have shown that *V. cholerae* is often associated with *A. tonsa* (19, 65, 68). Although these two copepods both belong to the order Calanoida they have different life cycles and physical properties. *E. affinis* is demersal (attaches to substrates) and planktonic at different stages of its life cycle, whereas *A. tonsa* is holoplanktonic (planktonic for its entire life cycle). One study demonstrated that both *V. cholerae* O1 and O139 strains attached in higher abundances to *A. tonsa* than to *E. affinis*, while *V. cholerae* O1 attached in higher abundance than O139 to both copepods. The authors suggest that this may explain the dominance of *V. cholerae* O1 over O139 strains in cholera cases in rural areas of Bangladesh (61).

A. tonsa was shown to harbor VBNC *V. cholerae* in estuarine and marine regions in Argentina (68), and these VBNC cells reverted to the pathogenic state under favorable conditions. In estuarine and coastal regions in Mexico, *V. cholerae* O1 but not O139 strains were positively correlated with *A. tonsa* (65). Interestingly, *V. cholerae* was not detected in sewage but only in samples from sewage free environments, supporting the suggestion that natural nonpolluted water is the environmental reservoir of *V. cholerae* (69). Similarly, *V. cholerae* was detected off the coast of Peru (70), where *A. tonsa* was identified in the zooplankton fractions; however, no correlation between specific zooplankton taxa and the occurrence of *V. cholerae* could be verified. The authors also noted that although *V. cholerae* attached to zooplankton, the highest abundance, as detected by direct fluorescent antibody assay (DFA), were suspended in the water column, which is in accordance with Louis et al. (71) and Heidelberg et al. (43).

While the majority of the zooplankters that harbor *V. cholerae* are chitinous crustaceans, there are reports of *V. cholerae* associating with nonchitinous zooplankters. For example, *V. cholerae* O1 was shown to be associated with nonchitinous zooplankters in estuarine and continental shelf environments off the Brazilian coast and was detected on planktonic fish eggs, chaetognaths and pluteus larvae of echinoderms, as well as with crustaceans such as the cladocerans, *Penilia avirostris*, *Pleopis schmackeri*, and *Pseudevadne tergestina* (66).

V. cholerae non-O1/non-O139

Most studies on the ecology of *V. cholerae* have focused on toxigenic O1 and O139 serogroups, while less is known about the ecology of non-O1/non-O139 strains, even though these strains are known to cause diarrhea and wound infections (72, 73). In Neusiedler See, a large recreational freshwater lake in Austria where non-O1/non-O139 infections have increased in recent years, the occurrence of non-O1/non-O139 *V. cholerae* was analyzed in relation to several environmental determinants (74). Zooplankton biomass and temperature were significantly correlated with the occurrence of *V. cholerae*. To investigate the impact of zooplankton on *V. cholerae* numbers in Neusiedler See, the authors used the two dominant crustacean zooplankters, the copepod *Arctodiaptomus spinosus* and the cladoceran *Diaphanosoma mongolianum* incubated in microcosms with non-O1/non-O139 *V. cholerae* and a natural mixed bacterial community (64). Surprisingly, and contrary to other studies, the copepods had a significant negative impact on *V. cholerae* non-O1/non-O139 growth rates and growth yield compared to the copepod free controls. The highest abundance of *V. cholerae* in association with copepods was 7×10^3 non-O1/non-O139 bacteria per copepod. Conversely, the cladocerans had a positive impact on non-O1/non-O139 *V. cholerae* abundance, as the number of *V. cholerae* cells increased 1.5 orders of magnitude in cultures with cladocerans and growth rates approximately doubled. Abundance of bacterial biomass increased to 77×10^4 *V. cholerae* non-O1/non-O139 per cladoceran with on average 100 times more *V. cholerae* attached to the cladoceran than to the copepod.

One characteristic that differs between these zooplankton taxa is their lifestyle. While *A. spinosus* appears year round and shows no significant correlation with temperature, *D. mongolianum* appears in higher numbers in the warmer summer months and its occurrence is significantly correlated with increased temperature. The seasonal pattern of *V. cholerae* occurrence and correlation with increased temperature and the high abundance of *V. cholerae* on the cladoceran suggest an important correlation between these two organisms. The presence of a competing bacterial community from the same environment had a significant negative effect on *V. cholerae* non-O1/non-O139 growth rate and yield. When the growth of a *V. cholerae* non-O1/non-O139 strain from a different source was tested under the same conditions, the native Neusiedler See strains grew faster with competitors indicating that native strains have a competitive advantage over nonnative strains (64).

In a 2-year study in Chesapeake Bay, Zo et al. examined environmental determinants for luminescent *V. cholerae* strains isolated from the environment (75). Of 278 strains isolated, 136 carried the *luxA* gene and expressed luminescence, suggesting this to be an important environmental trait. Luminescence was found to be significantly correlated with the presence of a heat-stable enterotoxin and highly seasonal, correlating with environmental parameters such as temperature, chlorophyll *a*, pH, and salinity. The luminescent *V. cholerae* population was shown to be affected by the species-level composition and maturity of the zooplankton population.

Noncholera *Vibrio* spp.

Modern molecular methods make it possible to examine large numbers of environmental samples for microbial abundance, community composition, and diversity. In a recent 1-year study in coastal waters off Georgia, USA, viable counts of vibrios in plankton samples of two size fractions (63 to 200 μm and >200 μm) were determined and it was shown that seasonal changes in the zoo- and phytoplankton community were significantly correlated with culturable *Vibrio* numbers (52). In both plankton fractions, the abundance of copepods was significantly correlated with *Vibrio* spp. abundance. In the >200 μm fraction, the association was positive, whereas in the 63 to 200 μm fraction, the correlation between copepods and *Vibrio* spp. numbers was negative. The authors suggest that this may reflect differences in zooplankton life stages. As crustacean zooplankton go through at least four molting stages, many zooplankters in the smaller fraction were in younger larval stages. These peaked at times when temperatures are not optimal for *Vibrio* spp. growth. The results highlight the independent and important role for plankton composition in explaining seasonal changes in the abundance of *Vibrio* spp.

In a recent study off the coast of Spain, the abundance of *V. parahaemolyticus* in offshore areas was significantly correlated with total zooplankton abundance

(76). Over 80% of *V. parahaemolyticus* biomass in seawater was associated with zooplankton and the virulence-related *trh* gene was detected in 31% of the zooplankton samples. The presence of cnidarians accounted for 51.87% of the *V. parahaemolyticus* variation in abundance, whereas cnidarians represented only roughly 2% of the total zooplankton. In contrast to the importance of copepods in the ecology of *V. cholerae* populations in estuarine areas (46, 59), the results of this study suggest that copepods have a small effect on the offshore occurrence of *V. parahaemolyticus*. Interestingly, the offshore occurrence of *V. parahaemolyticus* was favored by a reduction in primary production, possibly due to grazing pressure by an enhanced abundance of zooplankton.

Other Environmental Factors Affecting the Occurrence of *Vibrio* spp.

As mentioned previously, temperature, salinity, and chlorophyll *a* have major impacts on *Vibrio* spp. dynamics in the natural environment. Increasing temperature and decreasing salinity along with higher chlorophyll *a* levels may result in a higher than usual abundance of potentially pathogenic *Vibrio* spp. In general, algal blooms increase with increasing nutrient concentration and increasing temperatures in spring and autumn in temperate regions, whereas in tropical regions they increase after the rainy/monsoon season (77, 78). These blooms are often followed by zooplankton blooms, which feed on the phytoplankton and reduce the algal abundance (79). In regions where cholera epidemics appear, multiple studies have shown a positive correlation between chlorophyll *a* (as a measurement of phytoplankton blooms), zooplankton and the occurrence of cholera cases [e.g., Bangladesh (57, 80), Africa (81), and South America (70)]. Temperature, salinity, chlorophyll *a*, and nutrients were also demonstrated to be strong indicators of *V. parahaemolyticus* and *V. vulnificus* presence in coastal regions in the USA (53). *Vibrio* spp. dynamics in a tropical marine environment (Arabian Sea) showed that the *Vibrio* spp. density increased with a diatom-dominated phytoplankton assemblage (82).

In a culture-dependent approach in the temperate waters of the North Sea, temperature was significantly correlated with the abundance of *Vibrio* spp., especially *V. parahaemolyticus* that occurred mainly in the summer months (83). Here, plankton-attached *Vibrio* spp. followed the same trend as free-living *Vibrio* spp. A year later the same authors investigated the occurrence of *Vibrio* spp. using a molecular technique (CARD-FISH) in the same region as the previous study (84). Temperature and low salinity were again strong drivers of *Vibrio* spp. abundances. Other environmental parameters, such as nutrients and chlorophyll *a*, were also important and they support the significance of interactions between abiotic and biotic factors in the ecology of *Vibrio* spp.

One study in Chesapeake Bay determined that the frequency of isolation of non-O1 *V. cholerae* was correlated with increased temperature and salinity (42). There was no correlation of *V. cholerae* with phytoplankton; however, there was evidence that *V. cholerae* was associated with zooplankton, although there did not seem to be specific *V. cholerae* genotypes associated with specific zooplankton taxa.

The increasing reports of *Vibrio* spp. interactions with phyto- and zooplankton in the environment highlights the importance of these interactions in the ecology of *Vibrio* spp. and emphasizes the need for more of these types of studies. Such information is necessary for an understanding of the environmental factors that affect the growth and persistence of these organisms in the marine environment.

CELLULAR INTERACTIONS OF *VIBRIO* SPP. WITH ZOOPLANKTON: CHITIN UTILIZATION

We are just beginning to understand, on a global scale, the interactions of *Vibrio* spp. with various taxa of zooplankton. However, the cellular mechanisms of interaction are more fully understood. In the following section, we summarize the current knowledge on the regulation of chitin utilization by *Vibrio* spp.

Chitin is produced by arthropods, fungi, algae, annelids, hydroids, and mollusks and residues can be found in glycoproteins and lipids of intestinal epithelium (85). Chitin is an unbranched polymer of *N*-acetylglucosamine (GlcNAc/NAG) that can be arranged in an antiparallel (α) or parallel (β) fashion; α-chitin is the strongest form and is present in insect cuticles, crab shells, and fungal cell walls (86).

Chitin is the second most abundant organic compound in the environment after cellulose and the most abundant polymer in the ocean (87, 88). In the marine environment, copepods alone produce billions of tons of this polymer and it is estimated that 10^{11} tons are produced annually (89). This results in a continuous rain of chitin to the ocean floors as "marine snow"; however, sediments in the oceans contain only trace amounts. The discrepancy between the amount of chitin produced in the marine environment and the amount found in

ocean sediments may be due to the large number of marine bacteria that are chitinolytic (90). Marine waters are limited in nutrients and the ability to utilize chitin is highly advantageous for marine microorganisms.

Chitin utilization by bacteria is achieved by the ability of microorganisms to (i) sense and locate chitin via chemotaxis; (ii) attach to the chitinous surface; (iii) express enzymes and proteins involved in degradation of chitin to oligosaccharides; (iv) transport oligosaccharides into the cell; and (v) catabolize products to fructose-6-P, acetate, and NH_3 (Fig. 3) (91). Chitin metabolism appears to be a conserved phenotype of *Vibrio* spp. and genes for degradation of chitin are present in all of the sequenced members of the Vibrionaceae, which may explain in part their ubiquity in coastal marine environments (92).

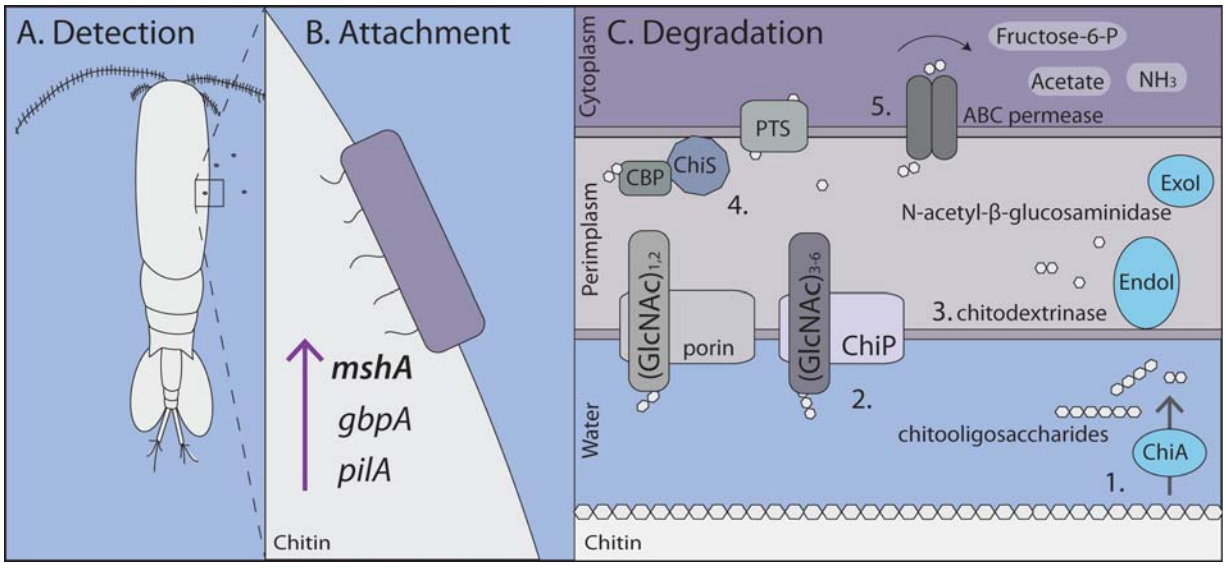
Chemotaxis Towards Chitin

Migration towards preferred, or away from detrimental environments, is known as chemotaxis. In bacteria, this is achieved by adjusting swimming direction and speed in response to chemical gradients (93). Chemotaxis is well described for *Vibrio furnissii*, which possesses at least two independent inducible receptors that recognize (GlcNAc)n (n=2 to 4) and chemotax towards chitin oligomers (GlcNc)n (n=1 to 6) but not to oligosaccharides (94). Taxis by *V. furnissii* is inhibited by Krebs cycle intermediates and is therefore linked to the nutritional status of the cell. *Vibrio fischeri* is attracted by GlcNAc and $(GlcNAc)_2$ as well as to N-acetylneuraminic acid, a component of the squid light organ mucus (95, 96). The enzyme chitin oligosaccharide deacetylase (COD), catalyzes the degradation of $(GlcNAc)_2$ to produce 4-O-(N-acetyl-b-d-glucosaminyl)-d-glucosamine (GlcNAc-GlcN). Hirano et al. (97) showed that *Vibrio* spp. that express COD (*V. parahaemolyticus* and *Vibrio alginolyticus*) are chemotactic towards GlcNAc-GlcN, while those that do not harbor the COD gene (*V. furnissii* and *Vibrio nereis*) are not. Thus, while all *Vibrio* species investigated to date are chitinolytic, they differ in the chemotactic responses, probably related to differences in their environmental niches.

Mechanisms for Attachment to Chitin

Vibrios possess a diverse array of mechanisms for attachment to different biotic and abiotic surfaces. Adhesion to surfaces may be both specific and nonspecific, with nonspecific adhesion mediated by proteins and

FIGURE 3 Utilization of chitin by *Vibrio* spp. **(A)** Chemotaxis towards chitin occurs when chitin oligosaccharides are detected by two independent receptors. **(B)** Attachment to chitin occurs via GbpA, MshA pilus, or chitin-regulated pilus encoded by *pilA*. **(C1)** Attachment to chitin leads to extracellular secretion of chitinases such as ChiA, which degrade chitin polymer to chitooligosaccharides. **(C2)** These enter the periplasm through specific porins such as ChiP and nonspecific porins. The chitooligosaccharides are hydrolyzed by various enzymes into GlcNAc and $(GlcNAc)_2$ **(C3)** and are transported into the cytoplasm **(C4 and C5)**. The oligosaccharides are further phosphorylated into the final products acetate, NH_3, and fructose-6-P **(C5)**. doi:10.1128/microbiolspec.VE-0003-2014.f3



exopolysaccharides (91). The diversity of mechanisms for adhesion reflect the diversity of zooplankton surface composition and associated materials such as molted exoskeletons, egg sacs, and fecal matter. There is evidence that membrane proteins are important for attachment of a number of *Vibrio* spp. (*V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. nerei*, *Vibrio metschnikovii*, *Vibrio anguilllarum* and *Vibrio splendidus*) and that environmental isolates adhere to chitin better than clinical isolates (98). Attachment to copepods is less efficient than attachment to chitin particles, probably due to the waxy covering on the copepod exoskeleton. GlcNAc-specific lectins have been reported in *Vibrio harveyi*, *V. furnissii*, and *Vibrio damsela* (99, 100) and chitin-binding proteins (CBP) have been reported in *V. harveyi, V. alginolyticus*, and *V. cholerae* (99, 101, 102). Attachment and continued adhesion to chitin requires continuous protein synthesis, at least for *V. furnissii*, which indicates that this association is an active one (100).

N-acetylglucosamine-Binding Protein A

One well-studied mechanism facilitating association of *Vibrio* spp. with chitin is the GlcNAc-binding protein A (GbpA). This protein is important for binding to the chitinous exoskeletons of zooplankton, such as copepods and cladocerans, as well as to egg sacs and fecal matter (103, 104) (Fig. 3). The dual ability of GbpA to attach to both chitin and mucilage may explain how *Vibrio* spp. can be detected on the surface of many functionally different types of zooplankton (33, 44, 45, 66). GbpA-encoding genes have been detected in all *V. cholerae* strains tested (clinical and environmental). as well as some pathogenic *Vibrio* species including *V. alginolyticus*, *V. vulnificus* and *V. parahaemolyticus* (104). A mutation of GbpA in *V. cholerae* resulted in a 10-fold reduction in binding to *Daphnia* exoskeletons (103).

Interestingly, the GbpA mutant was also deficient for binding to human epithelial cells. This is due to the fact that GlcNAc is a modification of glycoproteins and lipids present on intestinal epithelia; therefore, GbpA plays an important role in facilitating human infection and disease. Within the human host, *V. cholerae* attaches to the intestinal epithelium via secreted GbpA (103, 105), and a *gbpA* mutant was shown to be attenuated for colonization using a mouse model of infection (103). The expression of *gbpA* is increased in the presence of intestinal mucin (106) and it is expressed at low cell density, along with other virulence factors such as cholera toxin. At high cell density, the quorum sensing regulated proteases, HapA and PrtV, act to degrade GbpA, and thereby, release *V. cholerae* from the intestinal epithelium (107). This mechanism allows *V. cholerae* to replicate within the human host and then disperse when cell numbers are high via induced diarrhea, facilitating the spread of disease.

An examination of the structure of the GbpA protein revealed that the protein is made up of four functional binding domains (108): two chitin-binding domains that have specificity for different GlcNAc oligomers, mucin and epithelial cells, and two domains that interact with the surface of *V. cholerae*. Thus, it is likely that once secreted, GbpA binds to chitin via domains 1 and 4 and then adheres to the *V. cholerae* cell via domains 2 and 3.

Mannose-Sensitive Hemagglutinin Pilus

In addition to the secreted GbpA protein, the mannose-sensitive hemagglutinin (MSHA) pilus has been shown to be important for attachment to several members of the zooplankton community (109). This particular adhesion system has been suggested to play an important role in the environment, as the majority of clinical and environmental strains of *V. cholerae* (98.8% in one study) possess *mshA* genes (110). Mutation of *mshA* reduced attachment of *V. cholerae* to *Daphnia* sp. 30-fold compared to the wild type (20) and the mutant was shown to be less adhesive towards the exoskeleton and egg sac of plankton than the *gbpA* mutant. This indicates that the MSHA pilus is more important for attachment to plankton than GbpA (104). This bacterium possesses another type IV pilus, the chitin-regulated pilus (ChiRP) encoded by *pilA*, whose expression is induced by GlcNAc. ChiRP facilitates bacterial cell-to-cell interactions (20), thereby enhancing the stability of the attached cells. In *V. parahaemolyticus* the MSHA pilus is important for attachment to abiotic surfaces as well as to chitin (111). Therefore, *Vibrio* spp. have a variety of mechanisms for attachment to zooplankton, and the differences in these mechanisms probably reflects differences in the environmental niches that these organisms inhabit.

Chitin Degradation and Utilization

Microarray analysis of *V. cholerae* has shown that GlcNAc induces the expression of genes involved in chemotaxis and adherence to chitin, transport and assimilation of products of chitin catabolism (20). This study identified three classes of chitin-regulated genes: class I genes respond to chitin oligosaccharides but not to GlcNAc, class II genes respond to GlcNAc and class III genes respond to $(GlcN)_2$. In *V. cholerae* and

V. furnissii, the genes responsible for chitin catabolism are stringently regulated by a sensor kinase, ChiS, and by catabolite repression (89, 112). *V. cholerae* mutants lacking cAMP and CRP are unable to colonize chitin or use chitin as a carbon source (112). When externally provided cAMP was added, there was enhanced colonization of chitin beads. Catabolite repression of the chitin catabolic cascade ensures that the chitinolytic pathway is repressed when other preferred food sources are present (91).

The chitinolytic cascade involves the extracellular degradation of chitin by secreted chitinases that degrade chitin polymers to chitooligosaccharides (Fig. 3). There are multiple chitinases; for example, genome analysis has identified seven chitinases in the *V. cholerae* genome (106), while *V. harveyi* is thought to secrete 10 chitinases (86). The ChiA chitinase is thought to be the most active in the environment (92). $(GlcNAc)_{1,2}$ produced by chitin degradation is thought to enter the periplasm by an ABC-type transporter, while $(GlcNAc)_{3–6}$ is transported by a specific chitoporin (ChiP) (89, 92, 113) (Fig. 3). The chitin oligosaccharides are hydrolyzed in the periplasm by a membrane-bound chitodextrinase (EndoI) and an exoenzyme (N-acetyl-ß-glucosaminidase; ExoI) (114, 115) to GlcNAc and $GlcNAc_2$ (2). Chitodextrinase degrades oligomers to di- and trisaccharides while the ß-GlcNAcidase hydrolyzes the GlcNAc termini from the oligomers. GlcNAc is then transported from the periplasm to the cytosol and phosphorylated by a phosphenolpyruvate:glycose phosphotransferase system (PTS) while $(GlcNAc)_2$ is transported through an ABC-type permease. (116). $(GlcNAc)_2$ in the periplasm binds to a chitin-binding protein (CBP) that is associated with the sensor/kinase ChiS, causing the release of ChiS from the CBP and activation of ChiS (89). Activated ChiS regulates approximately 50 genes, most of which are involved in chitin catabolism (20, 117). Once in the cytosol, the intermediates are phosphorylated and the final products are fructose-6-P, acetate, and NH_3 (118).

Analysis of 54 strains from 32 taxa revealed that chitin degradation genes were almost universally conserved, and that all of the strains were able to grow on GlcNAc, the majority grew on crab shell, squid pen and had *chiA* genes, although these genes were highly divergent (92). These data indicate that chitin utilization is likely to be a core phenotype for vibrios and may in part explain their ubiquitous distribution. Amazingly, *V. furnissii* cultures have been shown to utilize twice the total cell mass (dry weight) per hour of chitin (2) and no degradative products are released into the medium so that all the carbon and nitrogen are utilized by the cell mass.

Chitin-Induced Competence

Chitin not only serves as a carbon and nitrogen source for *Vibrio* spp. but it also induces competence and acquisition of foreign DNA via horizontal gene transfer (HGT). Seitz and Blokesch (119) recently published an extensive review on natural transformation in Gram-negative bacteria including a thorough section on natural competence and transformation of vibrios. Therefore, only a short summary of the most important points will be presented here.

Although chitin-induced competence has been mostly studied in *V. cholerae*, there is evidence that it is a common response in Vibrionaceae. Successful chitin-based natural transformation of *V. vulnificus* (120), *V. parahaemolyticus* (121), and *V. fischeri* (122) have been reported and whole genome studies give evidence that HGT in *V. cholerae* is widespread in the natural environment (123). For example, studies have highlighted the importance of chitin-induced transformation and HGT in the conversion of nontoxigenic to toxigenic serogroups of *V. cholerae* (124, 125) and carbotype conversion of *V. vulnificus* (126), indicating that chitin-induced transformation may play a large role in the acquisition of new genes in the environment.

In *V. cholerae*, $(GlcNAc)_2$, produced by chitin degradation, activates the transcription and translation of *tfoX* (127), encoding a protein required for natural competence (127, 128). TfoX upregulates competence genes (119), which are involved in the production of a type IV pilus complex, four chitinases (including ChiA-1 and ChiA-2), and a chitoporin (127, 128). The degradation of chitin (as discussed above) leads to increased concentrations of $(GlcNAc)_2$, the smallest chitin polymer that induces competence in *V. vulnificus* (126) and in *V. cholerae* (127). Although GlcNAc is present in other sources, such as glycolipids and polysaccharides, the dimer $(GlcNAc)_2$ is only derived from chitin degradation.

In *V. cholerae*, there is integration of quorum sensing and competence as quorum sensing has been shown to regulate the switch from extracellular DNA degradation to DNA uptake (129). At high cell densities, such as those attained on the surface of zooplankton, the expression of the transcriptional regulator of the quorum sensing system, *hapR*, leads to the positive regulation of a subset of competence genes (119, 128). HapR represses the expression of *dns*, a DNase, which at low cell densities is responsible for degradation of extracellular DNA, and thus, repression of natural transformation (130). HapR also upregulates the expression of ComEA and ComEC, which are required for DNA uptake (129).

Chitin-induced competence is also under catabolite repression with cAMP and CRP being required for the expression of *pilA* and *comEA* (112, 129). The PTS-dependent transport of preferred carbon sources has been shown to repress natural transformation and this may be partly due to the delay in activation of the quorum sensing regulator, HapR. Thus, in *Vibrio* spp. competence is regulated through the integration of the quorum sensing system, catabolite repression, and chitin degradation pathways. This integration serves to repress chitin colonization and utilization when other preferred carbon sources are present and delays competence until a high cell density has accumulated.

CONCLUDING REMARKS

Vibrio spp. are indigenous inhabitants of the marine environment and members of the genus have been isolated worldwide. *Vibrio* spp. abundance is often correlated with abiotic factors such as temperature and salinity. This correlation is closely related with changes in the abundance of different members of the phyto- and zooplankton communities. For example, *Vibrio* spp. may specifically attach to the chitinous surfaces of zooplankters to obtain nutrients and to form resistant biofilms. As a consequence, the zooplankton become agents of transmission of *Vibrio* spp., which is particularly important for pathogenic strains. Furthermore, zooplankters become foci for genetic exchange, which is known to be essential for the conversion of nontoxigenic *V. cholerae* strains into toxigenic strains. In order to fully understand the factors that drive the occurrence of these important bacterioplankton, it is necessary to understand how their abundance is affected by the phyto- and zooplankton communities and how these communities drive the seasonal patterns of *Vibrio* spp. in the environment. These insights may provide important information that could lead to the ability to predict the occurrence of these microbes in marine and estuarine ecosystems.

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REFERENCES

1. Akselman R, Jurquiza V, Costagliola MC, Fraga SG, Pichel M, Hozbor C, Peressutti S, Binsztein N. 2011. *Vibrio cholerae* O1 found attached to the dinoflagellate *Noctiluca scintillans* in Argentine shelf waters. *Mar Biodivers Rec* **3:**e120.

2. Bassler B, Yu C, Lee Y, Roseman S. 1991. Chitin utilization by marine bacteria. Degradation and catabolism of chitin oligosaccharides by *Vibrio furnissii*. *J Biol Chem* **266:**24276–24286.

3. Chowdhury MA, Huq A, Xu B, Madeira FJ, Colwell RR. 1997. Effect of alum on free-living and copepod-associated *Vibrio cholerae* O1 and O139. *Appl Environ Microbiol* **63:**3323–3326.

4. Huq A, Xu B, Chowdhury MA, Islam MS, Montilla R, Colwell RR. 1996. A simple filtration method to remove plankton-associated *Vibrio cholerae* in raw water supplies in developing countries. *Appl Environ Microbiol* **62:**2508–2512.

5. Seitz P, Blokesch M. 2013. Cues and regulatory pathways involved in natural competence and transformation in pathogenic and environmental Gram-negative bacteria. *FEMS Microbiol Rev* **37:**336–363.

6. Haldar S, Maharajan A, Chatterjee S, Hunter SA, Chowdhury N, Hinenoya A, Asakura M, Yamasaki S. 2010. Identification of *Vibrio harveyi* as a causative bacterium for a tail rot disease of sea bream Sparus aurata from research hatchery in Malta. *Microbiol Res* **165:**639–648.

7. Bourne DG, Garren M, Work TM, Rosenberg E, Smith GW, Harvell CD. 2009. Microbial disease and the coral holobiont. *Trends Microbiol* **17:**554–562.

8. Hoffmann M, Fischer M, Ottesen A, McCarthy PJ, Lopez JV, Brown EW, Monday SR. 2010. Population dynamics of *Vibrio* spp. associated with marine sponge microcosms. *ISME J* **4:**1608–1612.

9. Stabili L, Gravili C, Piraino S, Boero F, Alifano P. 2006. *Vibrio harveyi* associated with *Aglaophenia octodonta* (Hydrozoa, Cnidaria). *Microb Ecol* **52:**603–608.

10. Wang W. 2011. Bacterial diseases of crabs: a review. *J Invertebr Pathol* **106:**18–26.

11. Beaz-Hidalgo R, Balboa S, Romalde JL, Figueras MJ. 2010. Diversity and pathogenecity of *Vibrio* species in cultured bivalve molluscs. *Environ Microbiol Rep* **2:**34–43.

12. Abd H, Weintraub A, Sandström G. 2005. Intracellular survival and replication of *Vibrio cholerae* O139 in aquatic free-living amoebae. *Environ Microbiol* **7:**1003–1008.

13. Vezzulli L, Pruzzo C, Huq A, Colwell RR. 2010. Environmental reservoirs of *Vibrio cholerae* and their role in cholera. *Environ Microbiol Rep* **2:**27–33.

14. Broberg CA, Calder TJ, Orth K. 2011. *Vibrio parahaemolyticus* cell biology and pathogenicity determinants. *Microb Infect* **13:**992–1001.

15. Linkous DA, Oliver JD. 1999. Pathogenesis of *Vibrio vulnificus*. *FEMS Microbiol Lett* **174:**207–214.

16. Sack DA, Sack RB, Nair GB, Siddique AK. 2004. Cholera. *Lancet* **363:**223–233.

17. Ben-Haim Y, Thompson FL, Thompson CC, Cnockaert MC, Hoste B, Swings J, Rosenberg E. 2003. *Vibrio coralliilyticus* sp. nov., a temperature-dependent pathogen of the coral *Pocillopora damicornis*. *Int J Syst Evol Microbiol* **53:**309–315.

18. Elston R, Hasegawa H, Humphrey K, Polyak I, Häse C. 2008. Re-emergence of *Vibrio tubiashii* in bivalve shellfish aquaculture: severity, environmental drivers, geographic extent and management. *Dis Aquat Org* **82:**119–134.

19. Huq A, Small EB, West PA, Huq MI, Rahman R, Colwell RR. 1983. Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. *Appl Environ Microbiol* **45:**275–283.

20. Meibom KL, Li XB, Nielsen AT, Wu CY, Roseman S, Schoolnik GK. 2004. The *Vibrio cholerae* chitin utilization program. *Proc Natl Acad Sci USA* **101:**2524–2529.

21. Amako K, Shimodori S, Imoto T, Miake S, Umeda A. 1987. Effects of chitin and its soluble derivatives on survival of *Vibrio cholerae* O1 at low temperature. *Appl Environ Microbiol* **53:**603–605.

22. Suthers I, Rissik D. 2009. *Plankton: A Guide to their Ecology and Monitoring For Water Quality*. Csiro Publishing, Clayton, Australia.

23. Sherr EB, Sherr BF. 2009. Food webs, microbial, p 174–189. *In* Schaechter M (ed), *Encyclopedia of Microbiology*, 3rd ed. Academic Press, Oxford.

24. Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F. 1983. The ecological role of water-column microbes in the sea. *Mar Ecol Prog Ser* **10:**257–263.

25. Nalin D, Daya V, Reid A, Levine M, Cisneros L. 1979. Adsorption and growth of *Vibrio cholerae* on chitin. *Infect Immun* **25:**768–770.

26. Jürgens K, Matz C. 2002. Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. *Ant Leeuw* **81:**413–434.

27. Matz C, McDougald D, Moreno AM, Yung PY, Yildiz FH, Kjelleberg S. 2005. Biofilm formation and phenotypic variation enhance predation-driven persistence of *Vibrio cholerae*. *Proc Natl Acad Sci USA* **102:** 16819–16824.

28. Macek M, Carlos G, Memije P, Ramãrez P. 1997. Ciliate-*Vibrio cholerae* interactions within a microbial loop: an experimental study. *Aquat Microb Ecol* **13:**257–266.

29. Erken M, Weitere M, Kjelleberg S, McDougald D. 2011. *In situ* grazing resistance of *Vibrio cholerae* in the marine environment. *FEMS Microbiol Ecol* **76:**504–512.

30. Worden AZ, Seidel M, Smriga S, Wick A, Malfatti F, Bartlett D, Azam F. 2006. Trophic regulation of *Vibrio cholerae* in coastal marine waters. *Environ Microbiol* **8:**21–29.

31. Rehnstam-Holm AS, Godhe A, Härnström K, Raghunath P, Saravanan V, Collin B, Karunasagar I. 2010. Association between phytoplankton and *Vibrio* spp. along the southwest coast of India: a mesocosm experiment. *Aquat Microb Ecol* **58:**127–139.

32. Wallace RL, Smith HA. 2009. Rotifera, p 689–703. *In* Gene EL (ed), *Encyclopedia of Inland Waters*. Academic Press, Oxford, UK.

33. Constatin de Magny G, Mozumder PK, Grim CJ, Hasan NA, Naser MN, Alam M, Sack RB, Huq A, Colwell RR. 2011. Role of zooplankton diversity in *Vibrio cholerae* population dynamics and in the incidence of cholera in the Bangladesh Sundarbans. *Appl Environ Microbiol* **77:**6125–6132.

34. Armitage PD, Cranston PS, Pinder LCV. 1995. *The Chironomidae: Biology and Ecology of Non-Biting Midges*. Chapman and Hall, London, UK.

35. Harris R. 2001. Copepods, p 512–523. *In* Steele JH, Turekian KK, Thorpe SA (ed), *Encyclopedia of Ocean Sciences*. Academic Press, Oxford, UK.

36. Havel JE. 2009. Cladocera, p 611–622. *In* Gene EL (ed), *Encyclopedia of Inland Waters*. Academic Press, Oxford, UK.

37. Grossart HP, Dziallas C, Tang KW. 2009. Bacterial diversity associated with freshwater zooplankton. *Environ Microbiol Rep* **1:**50–55.

38. Tang KW. 2005. Copepods as microbial hotspots in the ocean: effects of host feeding activities on attached bacteria. *Aquat Microb Ecol* **38:**31–40.

39. Tang KW, Dziallas C, Grossart HP. 2011. Zooplankton and aggregates as refuge for aquatic bacteria: protection from UV, heat and ozone stresses used for water treatment. *Environ Microbiol* **13:**378–390.

40. Grossart HP, Dziallas C, Leunert F, Tang KW. 2010. Bacteria dispersal by hitchhiking on zooplankton. *Proc Natl Acad Sci USA* **107:**11959–11964.

41. Stich HB, Lampert W. 1981. Predator evasion as an explanation of diurnal vertical migration by zooplankton. *Nature* **293:**396–398.

42. Jiang SC, Louis V, Choopun N, Sharma A, Huq A, Colwell RR. 2000. Genetic diversity of *Vibrio cholerae* in Chesapeake Bay determined by amplified fragment length polymorphism fingerprinting. *Appl Environ Microbiol* **66:**140–147.

43. Heidelberg JF, Heidelberg KB, Colwell RR. 2002. Bacteria of the {gamma}-subclass proteobacteria associated with zooplankton in chesapeake bay. *Appl Environ Microbiol* **68:**5498–5507.

44. Broza M, Gancz H, Halpern M, Kashi Y. 2005. Adult non-biting midges: possible windborne carriers of *Vibrio cholerae* non-O1 non-O139. *Environ Microbiol* **7:**576–585.

45. Halpern M, Broza Y, Mittler S, Arakawa E, Broza M. 2004. Chironomid egg masses as a natural reservoir of *Vibrio cholerae* non-O1 and non-O139 in freshwater habitats. *Microb Ecol* **47:**341–349.

46. Colwell RR. 1996. Global climate and infectious disease: the cholera paradigm. *Science* **274:**2025–2031.

47. Colwell RR, Huq A, Islam MS, Aziz KM, Yunus M, Khan NH, Mahmud A, Sack RB, Nair GB, Chakraborty J, Sack DA, Russek-Cohen E. 2003. Reduction of cholera in Bangladeshi villages by simple filtration. *Proc Natl Acad Sci USA* **100:**1051–1055.

48. Huq A, Sack RB, Nizam A, Longini IM, Nair GB, Ali A, Morris JG Jr, Khan MN, Siddique AK, Yunus M, Albert MJ, Sack DA, Colwell RR. 2005. Critical factors influencing the occurrence of *Vibrio cholerae* in the environment of Bangladesh. *Appl Environ Microbiol* **71:**4645–4654.

49. Huq A, Yunus M, Sohel SS, Bhuiya A, Emch M, Luby SP, Russek-Cohen E, Nair GB, Sack RB, Colwell RR. 2010. Simple sari cloth filtration of water is sustainable and continues to protect villagers from cholera in Matlab, Bangladesh. *MBio* **1:**pii: e00034-10.

50. Nahar S, Sultana M, Naser MN, Nair GB, Watanabe H, Ohnishi M, Yamamoto S, Endtz H, Cravioto A, Sack RB, Hasan NA, Sadique A, Huq A, Colwell RR, Alam M. 2011. Role of shrimp chitin in the ecology of toxigenic *Vibrio cholerae* and cholera transmission. *Front Microbiol* **2:**260.

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

52. Turner JW, Good B, Cole D, Lipp EK. 2009. Plankton composition and environmental factors contribute to *Vibrio* seasonality. *ISME J* **3:**1082–1092.

53. Johnson CN, Bowers JC, Griffitt KJ, Molina V, Clostio RW, Pei S, Laws E, Paranjpye RN, Strom MS, Chen A, Hasan NA, Huq A, Noriea NF 3rd, Grimes DJ, Colwell RR. 2012. Ecology of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in the coastal and estuarine waters of Louisiana, Maryland, Mississippi, and Washington (United States). *Appl Environ Microbiol* **78:**7249–7257.

54. Johnson C, Flowers A, Noriea N, Zimmerman A, Bowers J, DePaola A, Grimes D. 2010. Relationships between environmental factors and pathogenic vibrios in the Northern Gulf of Mexico. *Appl Environ Microbiol* **76:**7076–7084.

55. Nigro OD, Hou A, Vithanage G, Fujioka RS, Steward GF. 2011. Temporal and spatial variability in culturable pathogenic *Vibrio* spp. in Lake Pontchartrain, Louisiana, following hurricanes Katrina and Rita. *Appl Environ Microbiol* **77:**5384–5393.

56. Eiler A, Gonzalez Rey C, Allen S, Bertilsson S. 2007. Growth response of *Vibrio cholerae* and other *Vibrio* spp. to cyanobacterial dissolved organic matter and temperature in brackish water. *FEMS Microbiol Ecol* **60:**411–418.

57. Lobitz B, Beck L, Huq A, Wood B, Fuchs G, Faruque ASG, Colwell R. 2000. Climate and infectious disease: Use of remote sensing for detection of *Vibrio cholerae* by indirect measurement. *Proc Natl Acad Sci USA* **97:**1438–1443.

58. Kaneko T, Colwell RR. 1973. Ecology of *Vibrio parahaemolyticus* in Chesapeake bay. *J Bacteriol* **113:**24–32.

59. Pruzzo C, Vezzulli L, Colwell RR. 2008. Global impact of *Vibrio cholerae* interactions with chitin. *Environ Microbiol* **10:**1400–1410.

60. Nalin DR. 1976. Cholera, copepods, and chitinase. *Lancet* **2:**958.

61. Rawlings TK, Ruiz GM, Colwell RR. 2007. Association of *Vibrio cholerae* O1 El Tor and O139 Bengal with the Copepods *Acartia tonsa* and *Eurytemora affinis*. *Appl Environ Microbiol* **73:**7926–7933.

62. Huq A, Colwell RR. 1996. Environmental factors associated with emergence of disease with special reference to cholera. *East Mediterr Health J* **2:**37–45.

63. Tamplin M, Gauzens A, Huq A, Sack D, Colwell R. 1990. Attachment of *Vibrio cholerae* serogroup O1 to zooplankton and phytoplankton of Bangladesh waters. *Appl Environ Microbiol* **56:**1977–1980.

64. Kirschner AK, Schauer S, Steinberger B, Wilhartitz I, Grim CJ, Huq A, Colwell RR, Herzig A, Sommer R. 2011. Interaction of *Vibrio cholerae* non-O1/non-O139 with Copepods, Cladocerans and competing bacteria in the large alkaline lake Neusiedler See, Austria. *Microb Ecol* **61:**496–506.

65. Lizarrága-Partida, Mendez-Gómez E, Rivas-Montaño AM, Vargas-Hernández E, Portillo-López A, González-Ramírez AR, Huq A, Colwell RR. 2009. Association of *Vibrio cholerae* with plankton in coastal areas of Mexico. *Environ Microbiol* **11:**201–208.

66. Martinelli Filho JE, Lopes RM, Rivera ING, Colwell RR. 2011. *Vibrio cholerae* O1 detection in estuarine and coastal zooplankton. *J Plankton Res* **33:**51–62.

67. Kimmel DG, Roman MR. 2004. Long-term trends in mesozooplankton abundance in Chesapeake Bay, USA: influence of freshwater input. *Mar Ecol Prog Ser* **267:**71–83.

68. Binsztein N, Costagliola MC, Pichel M, Jurquiza V, Ramírez FC, Akselman R, Vacchino M, Huq A, Colwell R. 2004. Viable but nonculturable *Vibrio cholerae* O1 in the aquatic environment of Argentina. *Appl Environ Microbiol* **70:**7481–7486.

69. Colwell RR, Seidler RJ, Kaper J, Joseph SW, Garges S, Lockman H, Maneval D, Bradford H, Roberts N, Remmers E, Huq I, Huq A. 1981. Occurrence of *Vibrio cholerae* serotype O1 in Maryland and Louisiana estuaries. *Appl Environ Microbiol* **41:**555–8.

70. Gil AI, Louis VR, Rivera IN, Lipp E, Huq A, Lanata CF, Taylor DN, Russek-Cohen E, Choopun N, Sack RB, Colwell RR. 2004. Occurrence and distribution of *Vibrio cholerae* in the coastal environment of Peru. *Environ Microbiol* **6:**699–706.

71. Louis VR, Russek-Cohen E, Choopun N, Rivera IN, Gangle B, Jiang SC, Rubin A, Patz JA, Huq A, Colwell RR. 2003. Predictability of *Vibrio cholerae* in Chesapeake Bay. *Appl Environ Microbiol* **69:**2773–2785.

72. Anderson AM, Varkey JB, Petti CA, Liddle RA, Frothingham R, Woods CW. 2004. Non-O1 *Vibrio cholerae* septicemia: Case report, discussion of literature, and relevance to bioterrorism. *Diagn Microbiol Infect Dis* **49:**295–297.

73. Lukinmaa S, Mattila K, Lehtinen V, Hakkinen M, Koskela M, Siitonen A. 2006. Territorial waters of the Baltic Sea as a source of infections caused by *Vibrio cholerae* non-O1, non-O139: report of 3 hospitalized cases. *Diagn Microbiol Infect Dis* **54:**1–6.

74. Kirschner AK, Schlesinger J, Farnleitner AH, Hornek R, Süss B, Golda B, Herzig A, Reitner B. 2008. Rapid growth of planktonic *Vibrio cholerae* non-O1/non-O139 strains in a large alkaline lake in Austria: dependence on temperature and dissolved organic carbon quality. *Appl Environ Microbiol* **74:**2004–2015.

75. Zo YG, Chokesajjawatee N, Grim C, Arakawa E, Watanabe H, Colwell RR. 2009. Diversity and seasonality of bioluminescent *Vibrio cholerae* populations in Chesapeake Bay. *Appl Environ Microbiol* **75:**135–146.

76. Martinez-Urtaza J, Blanco-Abad V, Rodriguez-Castro A, Ansede-Bermejo J, Miranda A, Rodriguez-Alvarez MX. 2011. Ecological determinants of the occurrence and dynamics of *Vibrio parahaemolyticus* in offshore areas. *ISME J* **6:**994–1006.

77. Wiltshire KH, Manly BF. 2004. The warming trend at Helgoland Roads, North Sea: phytoplankton response. *Helgol Mar Res* **58:**269–273.

78. Assmy P, Smetacek V. 2009. Algal blooms, p 27–41. *In* Schaechter M (ed), *Encyclopedia of Microbiology*, 3rd ed. Academic Press, Oxford, UK.

79. Berger SA, Diehl S, Stibor H, Trommer G, Ruhenstroth M, Wild A, Weigert A, Jäger CG, Striebel M. 2007. Water temperature and mixing depth affect timing and magnitude of events during spring succession of the plankton. *Oecologia* **150:**643–654.

80. Constantin de Magny G, Murtugudde R, Sapiano MR, Nizam A, Brown CW, Busalacchi AJ, Yunus M, Nair GB, Gil AI, Lanata CF, Calkins J, Manna B, Rajendran K, Bhattacharya MK, Huq A, Sack RB, Colwell RR. 2008. Environmental signatures associated with cholera epidemics. *Proc Natl Acad Sci USA* **105:**17676–17681.

81. Bompangue Nkoko D1, Giraudoux P, Plisnier PD, Tinda AM, Piarroux M, Sudre B, Horion S, Tamfum JJ, Ilunga BK, Piarroux R. 2011. Dynamics of cholera outbreaks in Great Lakes region of Africa, 1978–2008. *Emerging Infect Dis* **17:**2026–2034.

82. Asplund ME, Rehnstam-Holm AS, Atnur V, Raghunath P, Saravanan V, Härnström K, Collin B, Karunasagar I, Godhe A. 2011. Water column dynamics of *Vibrio* in relation to phytoplankton community composition and environmental conditions in a tropical coastal area. *Environ Microbiol* **13:**2738–2751.

83. Oberbeckmann S, Wichels A, Wiltshire KH, Gerdts G. 2011. Occurrence of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* in the German Bight over a seasonal cycle. *Ant Leeuw* **100:**291–307.

84. Oberbeckmann S, Fuchs BM, Meiners M, Wichels A, Wiltshire KH, Gerdts G. 2012. Seasonal dynamics and modeling of a *Vibrio* community in coastal waters of the North Sea. *Microb Ecol* **63:**543–551.

85. Dawson MP, Humphrey BA, Marshall KC. 1981. Adhesion: a tactic in the survival strategy of a marine *Vibrio* during starvation. *Curr Microbiol* **6:**195–199.

86. Souza CP, Almeida BC, Colwell RR, Rivera ING. 2011. The importance of chitin in the marine environment. *Mar Biotechnol* **13:**823–830.

87. Martínez JP, Falomir MP, Gozalbo D. 2009. *Chitin: a structural biopolysaccharide, eLS*. John Wiley and Sons, Ltd, Chichester, UK.

88. Rinaudo M. 2006. Chitin and chitosan: properties and applications. *Prog Polym Sci* **31:**603–632.

89. Li X, Roseman S. 2004. The chitinolytic cascade in vibrios is regulated by chitin oligosaccharides and a two-component chitin catabolic sensor/kinase. *Proc Natl Acad Sci USA* **101:**627–631.

90. Zobell CE, Rittenberg SC. 1938. The occurrence and characteristics of chitinoclastic bacteria in the sea. *J Bacteriol* **35:**275–287.

91. Keyhani NO, Roseman S. 1999. Physiological aspects of chitin catabolism in marine bacteria. *Biochim Biophys Acta* **1473:**108–122.

92. Hunt DE, Gevers D, Vahora NM, Polz MF. 2008. Conservation of the chitin utilization pathway in the Vibrionaceae. *Appl Environ Microbiol* **74:**44–51.

93. Porter SL, Wadhams GH, Armitage JP. 2011. Signal processing in complex chemotaxis pathways. *Nat Rev Microbiol* **9:**153–165.

94. Bassler BL, Gibbons PJ, Yu C, Roseman S. 1991. Chitin utilization by marine bacteria. Chemotaxis to chitin oligosaccharides by *Vibrio furnissii*. *J Biol Chem* **266:**24268–24275.

95. DeLoney-Marino CR, Wolfe AJ, Visick KL. 2003. Chemoattraction of *Vibrio fischeri* to serine, nucleosides, and N-acetylneuraminic acid, a component of squid light-organ mucus. *Appl Environ Microbiol* **69:**7527–7530.

96. Mandel MJ, Schaefer AL, Brennan CA, Heath-Heckman EA, Deloney-Marino CR, McFall-Ngai MJ, Ruby EG. 2012. Squid-derived chitin oligosaccharides are a chemotactic signal during colonization by *Vibrio fischeri*. *Appl Environ Microbiol* **78:**4620–4626.

97. Hirano T, Aoki M, Kadokura K, Kumaki Y, Hakamata W, Oku T, Nishio T. 2011. Heterodisaccharide N-acetyl-glucosaminyl)-glucosamine is an effective chemotactic attractant for *Vibrio* bacteria that produce chitin oligosaccharide deacetylase. *Lett Appl Microbiol* **53:**161–166.

98. Vezzulli L, Pezzati E, Repetto B, Stauder M, Giusto G, Pruzzo C. 2008. A general role for surface membrane proteins in attachment to chitin particles and copepods of environmental and clinical vibrios. *Lett Appl Microbiol* **46:**119–125.

99. Montgomery MT, Kirchman DL. 1993. Role of chitin-binding proteins in the specific attachment of the marine bacterium *V. harveyi* to chitin. *Appl Environ Microbiol* **59:**373–379.

100. Yu C, Lee AM, Bassler BL, Roseman S. 1991. Chitin utilization by marine bacteria: a physiological function for bacterial adhesion to immobilized carbohydrates. *J Biol Chem* **266:**24260–24267.

101. Pruzzo C, Crippa A, Bertone S, Pane L, Carli A. 1996. Attachment of *Vibrio alginolyticus* to chitin mediated by chitin-binding proteins. *Microbiology* **142:**2181–2186.

102. Tarsi R, Pruzzo C. 1999. Role of surface proteins in *Vibrio cholerae* attachment to chitin. *Appl Environ Microbiol* **65:**1348–1351.

103. Kirn TJ, Jude BA, Taylor RK. 2005. A colonization factor links *Vibrio cholerae* environmental survival and human infection. *Nature* **438:**863–866.

104. Stauder M, Huq A, Pezzati E, Grim CJ, Ramoino P, Pane L, Colwell RR, Pruzzo C, Vezzulli L. 2012. Role of GbpA protein, an important virulence-related colonization factor, for *Vibrio cholerae*'s survival in the aquatic environment. *Environ Microbiol Rep* **4:**439–445.

105. Frederiksen RF, Paspaliari DK, Larsen T, Storgaard BG, Larsen MH, Ingmer H, Palcic MM, Leisner JJ. 2013. Bacterial chitinases and chitin-binding proteins as virulence factors. *Microbiology* **159:**833–847.

106. Bhowmick R, Ghosal A, Das B, Koley H, Saha DR, Ganguly S, Nandy RK, Bhadra RK, Chatterjee NS. 2008. Intestinal adherence of *Vibrio cholerae* involves a coordinated interaction between colonization factor GbpA and mucin. *Infect Immun* **76:**4968–4977.

107. Jude BA, Martinez RM, Skorupski K, Taylor RK. 2009. Levels of the secreted *Vibrio cholerae* attachment factor GbpA are modulated by quorum-sensing-induced proteolysis. *J Bacteriol* **191:**6911–6917.

108. Wong E, Vaaje-Kolstad G, Ghosh A, Hurtado-Guerrero R, Konarev PV, Ibrahim AF, Svergun DI, Eijsink VG, Chatterjee NS, van Aalten DM. 2012. The *Vibrio cholerae* colonization factor GbpA possesses a modular structure that governs binding to different host surfaces. *PLoS Pathog* **8:** e1002373.

109. Chiavelli DA, Marsh JW, Taylor RK. 2001. The mannose-sensitive hemagglutinin of *Vibrio cholerae* promotes adherence to zooplankton. *Appl Environ Microbiol* **67:**3220–3225.

110. Rahman MH, Biswas K, Hossain MA, Sack RB, Mekalanos JJ, Faruque SM. 2008. Distribution of genes for virulence and ecological fitness among diverse *Vibrio cholerae* population in a cholera endemic area: tracking the evolution of pathogenic strains. *DNA Cell Biol* **27:**347–355.

111. Shime-Hattori A, Iida T, Arita M, Park KS, Kodama T, Honda T. 2006. Two type IV pili of *Vibrio parahaemolyticus* play different roles in biofilm formation. *FEMS Microbiol Letters* **264:**89–97.

112. Blokesch M. 2012. Chitin colonization, chitin degradation and chitin-induced natural competence of *Vibrio cholerae* are subject to catabolite repression. *Environ Microbiol* **14:**1898–1912.

113. Keyhani NO, Li XB, Roseman S. 2000. Chitin catabolism in the marine bacterium *Vibrio furnissii*. Identification and molecular cloning of a chitoporin. *J Biol Chem* **275:**33068–33076.

114. Keyhani NO, Roseman S. 1996. The chitin catabolic cascade in the marine bacterium *Vibrio furnissii*: molecular cloning, isolation, and characterization of a periplasmic ß-N-acetylglucosaminidase. *J Biol Chem* **271:**33425–33432.

115. Keyhani N, Roseman S. 1966. The chitin catabolic cascade in the marine bacterium *Vibrio furnissii*: molecular cloning, isolation, and characterization of a periplasmic chitodextrinase. *J Biol Chem* **271:**33414–33424.

116. Bouma CL, Roseman S. 1996. Sugar transport by the marine chitinolytic bacterium *Vibrio furnissii*. *J Biol Chem* **271:**33457–33467.

117. Ghosh S, Rao KH, Sengupta M, Bhattacharya SK, Datta A. 2011. Two gene clusters co-ordinate for a functional N-acetylglucosamine catabolic pathway in *Vibrio cholerae*. *Mol Microbiol* **80:**1549–1560.

118. Suginta W, Chumjan W, Mahendran KR, Janning P, Schulte A, Winterhalter M. 2013. Molecular uptake of chitooligosaccharides through chitoporin from the marine bacterium *Vibrio harveyi*. *PLoS One* **8:**e55126.

119. Seitz P, Blokesch M. 2012. Cues and regulatory pathways involved in natural competence and transformation in pathogenic and environmental Gram-negative bacteria. *FEMS Microbiol Rev* **37:**336–363.

120. Gulig PA, Tucker MS, Thiaville PC, Joseph JL, Brown RN. 2009. USER friendly cloning coupled with chitin-based natural transformation enables rapid mutagenesis of *Vibrio vulnificus*. *Appl Environ Microbiol* **75:**4936–4949.

121. Chen Y, Jianli Dai J, Johnson J. 2010. Genetic analysis of the capsule polysaccharide (K antigen) and exopolysaccharide genes in pandemic *Vibrio parahaemolyticus* O3: K6. *BMC Microbiol* **10:**274.

122. Pollack-Berti A, Wollenberg MS, Ruby EG. 2010. Natural transformation of *Vibrio fischeri* requires *tfoX* and *tfoY*. *Environ Microbiol* **12:**2302–2311.

123. Chun J, Grim CJ, Hasan NA, Lee JH, Choi SY, Haley BJ, Taviani E, Jeon YS, Kim DW, Lee JH, Brettin TS, Bruce DC, Challacombe JF, Detter JC, Han CS, Munk AC, Chertkov O, Meincke L, Saunders E, Walters RA, Huq A, Nair GB, Colwell RR. 2009. Comparative genomics reveals mechanism for short-term and long-term clonal transitions in pandemic *Vibrio cholerae*. *Proc Natl Acad Sci USA* **106:**15442–7.

124. Blokesch M, Schoolnik GK. 2007. Serogroup conversion of *Vibrio cholerae* in aquatic reservoirs. *PLoS Pathog* **3:**e81.

125. Udden SM, Zahid MS, Biswas K, Ahmad QS, Cravioto A, Nair GB, Mekalanos JJ, Faruque SM. 2008. Acquisition of classical CTX prophage from *Vibrio cholerae* O141 by El Tor strains aided by lytic phages and chitin-induced competence. *Proc Natl Acad Sci USA* **105:**11951–11956.

126. Neiman J, Guo Y, Rowe-Magnus DA. 2011. Chitin-induced carbotype conversion in *Vibrio vulnificus*. *Infect Immun* **79:**3195–3203.

127. Yamamoto S, Morita M, Izumiya H, Watanabe H. 2010. Chitin disaccharide (GlcNAc)2 induces natural competence in *Vibrio cholerae* through transcriptional and translational activation of a positive regulatory gene $tfoX^{VC}$. *Gene* **457:**42–49.

128. Meibom KL, Blokesch M, Dolganov NA, Wu CY, Schoolnik GK. 2005. Chitin induces natural competence in *Vibrio cholerae*. *Science* **310:**1824–1827.

129. Lo Scrudato M, Blokesch M. 2012. The regulatory network of natural competence and transformation of *Vibrio cholerae*. *PLoS Genet* **8:** e1002778.

130. Blokesch M, Schoolnik GK. 2008. The extracellular nuclease Dns and its role in natural transformation of *Vibrio cholerae*. *J Bacteriol* **190:**7232.

131. Senderovich Y, Izhaki I, Halpern M. 2010. Fish as reservoirs and vectors of *Vibrio cholerae*. *PLoS One* **5:**e8607.