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REVIEW

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Host, pathogen and the environment: the case of *Macrobrachium rosenbergii*, *Vibrio parahaemolyticus* and magnesium

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Abstract

Macrobrachium rosenbergii is well-known as the giant freshwater prawn, and is a commercially significant source of seafood. Its production can be affected by various bacterial contaminations. Among which, the genus *Vibrio* shows a higher prevalence in aquatic organisms, especially *M. rosenbergii*, causing food-borne illnesses. *Vibrio parahaemolyticus*, a species of *Vibrio* is reported as the main causative of the early mortality syndrome. *Vibrio parahaemolyticus* infection in *M. rosenbergii* was studied previously in relation to the prawn's differentially expressed immune genes. In the current review, we will discuss the growth conditions for both *V. parahaemolyticus* and *M. rosenbergii* and highlight the role of magnesium in common, which need to be fully understood. Till date, there has not been much research on this aspect of magnesium. We postulate a model that screens a magnesium-dependent pathway which probably might take effect in connection with *N*-acetylglucosamine binding protein and chitin from *V. parahaemolyticus* and *M. rosenbergii*, respectively. Further studies on magnesium as an environment for *V. parahaemolyticus* and *M. rosenbergii* interaction studies will provide seafood industry with completely new strategies to employ and to avoid seafood related contaminations.

Keywords: *Macrobrachium rosenbergii*, *Vibrio parahaemolyticus*, Magnesium, GbpA, Chitin

Background

Macrobrachium rosenbergii is a freshwater prawn species of which there is a considerable production range when compared to *Macrobrachium nipponense* (information sourced from http://www.fao.org/fishery/culturedspecies/Macrobrachium_rosenbergii/en). Seafood is affected by several bacteria, and the major factors affecting bacterial survival in sea water are: absence of required nutrients, presence of toxic substances in sea water, presence of bacteriophages, adsorption of bacteria and their sedimentation, the harmful action of the sunlight, utilization of bacteria as food by not only protozoa, but other predators and competitive, antagonistic effects of the microorganism [1].

There are a wide range of bacteria such as *Vibrio cholerae*, *Escherichia coli* 0157:H7, Shigella, *Campylobacter jejuni*, Leptospirosis, Salmonella, *Helicobacter pylori*, Legionella and the *Mycobacterium avium* complex reported from contaminated water (information sourced from http://www.cdc.gov/healthyswimming) [2, 3]. However, mostly *Vibrio* species are pathogenic to marine organisms. Previously, pathogenicity of *Vibrio anguillarum*, *Vibrio anginolyticus*, *Vibrio panaeicida*, *V. vulnificus*, *Vibrio harveyi*, and *Vibrio salmonicida* was observed in the population of fish and other marine organisms such as eel [4, 5]. Those associated with coral reef bleaching were *Vibrio campbellii*, *Vibrio shiloi*, *V. harveyi* and *Vibrio fortis*. These *Vibrios* are a real cause of concern especially in the aquaculture industry [6].

In terms of aquatic food borne diseases, various virulence factors highlight *Vibrio vulnificus*, *Vibrio parahaemolyticus*, and *V. cholerae* considerably important. The factors primarily include the capsular polysaccharide,

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lipopolysaccharide, cytotoxins and flagellum [7, 8]. While *V. parahaemolyticus* and *V. cholerae* are mostly related to oysters, causing gastroenteritis [9]. *Vibrio vulnificus* was observed to cause primary septicemia not only in marine populations [10], but also in humans. Most cases of infection were reported due to the consumption of seafood [11], especially shellfish [12–22]. *Vibrio vulnificus* was reported to have caused high fatality rates due to its invasiveness associated with soft-tissue infection and severe sepsis [8]. This species was reported in an encapsulated form, which most commonly occurs in clinical isolates rather than environmental isolates [17].

Other species such as *Vibrio fluvialis*, *Vibrio mimicus*, *Vibrio alginolyticus*, *Photobacterium damsel* (*Vibrio damsela*), *Vibrio metschnikovii*, *Vibrio cincimnatiensis*, *Vibrio fuenisii* and *Vibrio hollisae* are also known to be pathogenic [23, 24]. These can cause severe infections to environmental specimens as well as human. *Vibrio parahaemolyticus* in particular was identified as a cause of food-borne illnesses [25], and is associated with the consumption of crab [26]. It was also associated with seafood contamination ranging from crustacean, molluscan shellfish to the giant water prawn. *Vibrio parahaemolyticus* was previously studied of its infection in *M. rosenbergii*, with the latter's expressed immune genes [27]. Studies even reported *N*-acetylglucosamine binding protein in other species of *Vibrio*. It was shown to have the ability to bind chitinaceous structures such as the outer covering of crustaceans [28–30]. Several studies on GbpA in relation to *Vibrio* show GbpA as an attachment factor to the host chitin (the exoskeleton of crustaceans is called a carapace and consists of chitin) [28, 30, 31]. There are no studies yet on the aspect of GbpA in *V. parahaemolyticus* in particular, and its attachment to chitin of *M. rosenbergii*. The yet unmapped factors of *V. parahaemolyticus* are involved in triggering bacteria to possibly enter the prawns (*M. rosenbergii*) which are our concern in the present review article.

The farming of *M. rosenbergii* in modern times started in the early 1960′s (http://www.fao.org/docrep/005/y4100e/y4100e04.htm#P193_35649). It was during this time, *M. rosenbergii* require brackish water conditions for its survival, though being found as a freshwater prawn [32]. However, *V. parahaemolyticus* was observed in both brackish and fresh water [33]. From the above, the water conditions required by the prawn and bacteria appear quite similar. Hence, the term "conditions for growth" which precisely defines the effect of environmental factors cannot be ruled out in such studies. Therefore, the implication of dealing with host and the pathogen in connection with the environment is conferred by considering *M. rosenbergii, V. parahaemolyticus,* and magnesium. Based on this, a preliminary designed experiment was conducted by us in our lab at University of Malaya and the work is currently under communication as a research article. Our current review hypothesis the possible rhythmic roles that *V. parahaemolyticus* GbpA and *M. rosenbergii* chitin play in the presence of a magnesium environment which could indeed be very useful in not only farming of prawn, but also in future aquaculture research.

Macrobrachium rosenbergii lifecycle

Macrobrachium rosenbergii resides in the tropical environments of the freshwater (http://www.fao.org/docrep/005/y4100e/y4100e04.htm#P193_35649), but is influenced by the areas of brackish water. The female prawn bears a gelatinous mass underneath and between the fourth pair of its walking legs. It is here that the male prawn deposits the sperm. After a few hours of mating, eggs are laid and are fertilized by the sperm. "Berried Females" is the terminology used for females carrying the eggs [34]. During the course of embryo development, the eggs remain constantly adhered to the female. It is during this time that the females migrate towards estuaries as the larvae cannot survive in fresh water for more than 2 days. The eggs hatch in brackish water where the salinity ranges from approximately nine parts per thousand (ppt) to 19 ppt [34], and they exist as free-swimming larvae at this stage.

The changes in phase from a larval to a post larval stage is very crucial in a prawn's life cycle as it grows by the process of moulting (http://www.thefishsite.com/articles/464/moulting-and-behaviour-changes-in-freshwater-prawn/). It undergoes around 11 moults to transform into post larvae. These moults represent a process of metamorphosis. This stage is a critical part of a prawn's life cycle as the old exoskeleton is replaced by a new soft exoskeleton underneath. It is here that the *M. rosenbergii* absorbs water into the tissue to increase in size (http://www.thefishsite.com/articles/464/moulting-and-behaviour-changes-in-freshwater-prawn/). Hence, the environmental conditions play a significant role in *M. rosenbergii* to enhance its ability to grow into an adult or to alter its chances of survival.

Vibrio genomes and distribution

Vibrios are widely distributed in marine environments and are easily adaptable to changes. Hence, these bacteria are considered significant for elucidating correlation between genome evolution and adaptation [35]. 16S rRNA sequence is the basis on which the *Vibrio* species are largely classified within the Vibrionaceae family. To establish the DNA patterns of epidemiological interest, which are associated with the pathogenicity of the strain and to record correlation of diseases among bacteria with

specific strains, serotyping was identified as one of the useful markers [36]. Further, the distribution and emergence of pathogenic bacterial strains, the prediction of events [37, 38] through construction of models, and the identification of evolutionary relationships were also done by multi-locus sequence typing/analysis, serogroup association and comparative genomics [39]. For example, with the potential pathogenicity of *V. cholerae, V. parahaemolyticus*, and the association of their serogroups, the specificity of the serogroups was correlated [36, 40, 41]. Studies on comparative genomics of *Vibrio* dealt with the phylogeny of 86 species of *Vibrio* and nine house-keeping genes primarily targeting biodiversity and genome evolution [42]. However, comparative genomic analysis among both the pandemic and non-pandemic Vibrios distributed worldwide has to glean into the bacterial adaptation, evolution as well as antibiotic resistance. Such studies have dealt with the role of integrons in *Vibrio* species for which genes comprise of approximately 1–3 % of the genome [43], genome plasticity shaped by HGT and comparative analysis of pandemic and non-pandemic species [44, 45]. Considering the above studies, the distribution of *Vibrio* in different environmental conditions could be a significant factor responsible for its evolution, resistance, virulence and adaptation.

Growth conditions of the host and pathogen

Vibrio parahaemolyticus growth conditions

Vibrio parahaemolyticus causes wound and nosocomial infections, abdominal pain, diarrhoea, nausea, vomiting and gastroenteritis [26, 46–48].

Temperature and growth

Vibrio parahaemolyticus is a Gram-negative bacterium which is curved and rod-shaped. It is a non-spore forming bacterium whose high motility is due to a polar flagellum. By a mechanism called swarming, these bacteria migrate across semi-solid surfaces [49] with the help of their lateral flagella. Throughout the world, inshore marine waters are the primary area where the distribution of *V. parahaemolyticus* is in abundance. It is mostly an inhabitant of estuarine marine water. The effect of seasons on *V. parahaemolyticus* has reported that *V. parahaemolyticus* in a small number was isolated from among sediment samples of marine water, but was not detected during the period of winter (i.e., November–March) in the Chesapeake Bay seawater [50]. *Vibrio parahaemolyticus* is proposed to multiply when there is an increase in temperature i.e., by re-introduction of the microorganism into the sea water or by living in the marine sediments throughout the winter [51].

The temperature ranging from 35 to 39 °C [52] are the optimal conditions for the growth of *V. parahaemolyticus*. Though the doubling time of *V. parahaemolyticus* is as little as 5 min [53], under optimal conditions this organism has a generation time of less than 20 min. Hence, *V. parahaemolyticus* is most prevalently observed in a suitable environment in the course of the warm season. In peaking summer, it causes food borne outbreaks as it exhibits mesophilism [54, 55]. Though the count of *V. parahaemolyticus* in seafood which is freshly harvested are rather lower than the dose of infection predicted [56], the rapid multiplying ability of this bacterium at suitable temperatures shows its presence in food, is enough to cause a disease.

Salinity

Vibrio parahaemolyticus has an important need for its multiplication and living conditions, which is salinity. *V. parahaemolyticus* encounters salinity concentrations in the marine environment typically ranging between 0.8 and 3 % [57]. With optimal levels ranging between 1 and 3 %, *V. parahaemolyticus* can thrive very well in different concentrations of sodium chloride, i.e., between 0.5 and 10 % based on laboratory studies [58].

Metals

Apart from salinity, the capacity of the organism to utilize, tolerate and thrive in marine conditions is affected by several different concentrations of metal ions present. *V. parahaemolyticus* isolates are found to survive in 300 mM magnesium (approximately 73,941 ppm), a condition which is considered as toxic to various other microorganisms. This is an example from severely polluted coastal waters in some parts of India [59]. *Vibrio parahaemolyticus* survival rates under several conditions can be improved by the increase in its ability to utilize magnesium. A 5.5 kb plasmid in the bacterium is said to carry genes responsible for bacterial resistance to increased magnesium concentrations [59]. Injured or thermally treated *V. parahaemolyticus* cells show increased uptake of magnesium, which indicates a possible higher requirement for magnesium not only for the stability and repair [60] of its ribosomes, but also its cell membrane.

Vibrio parahaemolyticus capability to survive magnesium or any metal ion at high concentrations outcompetes other microorganisms of seawater for its own survival and growth in the presence of these ions.

Macrobrachium rosenbergii growth conditions

The optimal range for prawn larvae to survive is 28–31 °C. It was observed that a salinity of <10 % ppt would be ideal for hatcheries for freshwater prawn [32]. Though calcium shows an important role in the formation of the exoskeleton (http://www.thefishsite.com/

articles/464/moulting-and-behaviour-changes-in-fresh-water-prawn/), it is the conditions which are favourable for the "survival" of larvae which stands of primary importance. There were reports which described magnesium as an important component in the environment for prawn survival. One such previous literature explains the requirement of the magnesium in juvenile prawns [61]. A recent article [62] describes the effects of salinity with the use of artificial sea water. Here, it clearly explains the role of magnesium in the survival rates of post larvae. Taking an example of the effect of an acidic environment in the presence of aluminium, an increase in the magnesium ion (Mg^{++}) was observed showing its importance in the survival stages of the post larvae [63]. The composition of water which are good for prawn hatcheries are said to be 10–27 parts per million (ppm) magnesium in freshwater, 1250–1345 ppm magnesium in seawater and 460–540 ppm magnesium in brackish water [32].

These features and conditions show how important is the magnesium ion for the survival of larvae which undergo a very critical "moulting stage" before reaching the post-larval stage.

N-acetylglucosamine-binding protein, chitin and *Vibrio parahaemolyticus*

N-acetylglucosamine-binding protein was reported in *Vibrio cholerae* [30, 31] with its property to bind to epithelial cell surfaces and chitin in the host's exoskeleton. The probable interactions of the *V. parahaemolyticus* GbpA (Additional file 1) was estimated from STITCH 3 [64] interaction database as shown in Fig. 1. Figure 1 even shows the protein-chemical interactions of GbpA with chitin. The role of prawn chitin was previously studied with the ecology of toxigenic *V. cholerae* and cholera transmission [29, 65–70]. In few studies it was even observed that *V. parahaemolyticus* gets absorbed onto chitin particles and was dependent on several factors such the ions and the pH of seawater [71]. Whereas, this was not observed in other bacteria such as *E. coli* or *Pseudomonas flourescens* [71]. This shows how significant environment could be for bacteria to attach to the chitin of prawn, i.e., in the present scenario *V. parahaemolyticus* to the carapace of *M. rosenbergii*. The effect of GbpA attachment to chitin could be of potential hypothetical interest as previous studies showed that a type IV pili of *V. parahaemolyticus* mediates the attachment to chitin [72]. An increase in the bacterial count in the presence of both chitin flakes and phosphate-buffer saline [73], but not in the presence of *N*-acetylglucosamine, starch and casein could probably support the link between the host and pathogen. This is explained with GbpA in relation to chitin in the presence of environmental magnesium further in the review. Bacteria such as *V. fluvialis*, *V. parahaemolyticus*, *V. alginolyticus*, *V. mimicus*, *Listonella anguillarum* and *Aeromonas hydrophila* were found to be capable of utilizing chitin as a sole source of nutrient in river as well as marine waters [74]. This study shows, there could be probable interactions between GbpA and chitin of the host and pathogen. All these above mentioned factors could support the importance of GbpA and chitin as biomolecular counterparts from the bacteria and prawn, respectively.

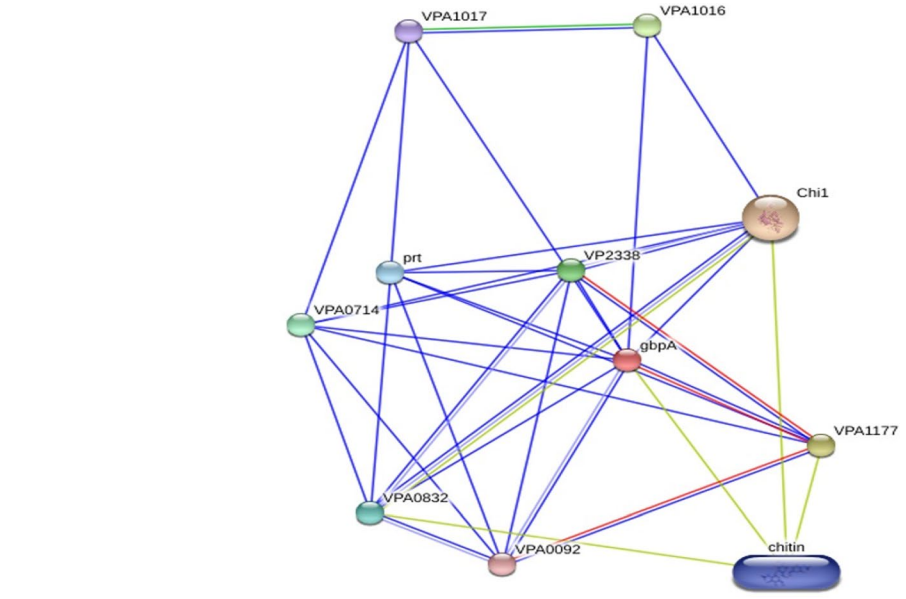
Macrobrachium rosenbergii and *V. parahaemolyticus* appear to share a common magnesium environment

Vibrio parahaemolyticus has several virulence factors with which it can survive aquatic organisms, especially the giant fresh water prawn, *M. rosenbergii* [75].

The growth conditions of *M. rosenbergii* in the environment can be studied in depth to understand the adaptation correlation of *V. parahaemolyticus* to *M. rosenbergii*. Studies show that *M. rosenbergii* survival in different media compositions was observed with variations in NaCl, KCl and $MgCl_2 + MgSO_4$ [54].

The fertilization envelope of shrimp eggs was observed to thin, when there is a depletion in calcium and magnesium [76]. Embryos in their early stages were shown to require optimal levels of medium including $MgCl_2 + MgSO_4$ for their proper development [77]. The role of magnesium ion in the normal hatching rate or the newly hatched larvae was not shown to be significant [77], but its importance in prawn survival was observed [62].

There are various resistance factors which *V. parahaemolyticus* carry such as: cobalt, zinc, cadmium, and chromium resistance genes [78]. This can also explain its possible survival rate with *M. rosenbergii*, which could have been exposed to toxic substances during its life cycle [79, 80]. During the course of evolution, the bacteria must have acquired these resistance genes on prolonged exposure while surviving together with the host, which is *M. rosenbergii*. The most interesting factor is the tolerance of *V. parahaemolyticus* unlike other bacteria to higher concentrations of magnesium, and its growth under iron-limiting conditions which appears directly proportional to conditions of the prawn larvae survival as mentioned earlier. Various studies on the importance of magnesium in *Vibrio* species support its significance as an environment, which was observed in one scenario where magnesium sulfate could regulate luminescence in *Vibrio fischeri* [81], while in the other, magnesium had a very high impact in promoting flagellation in *Vibrio* [82]. Previously, research was done to check the effect of magnesium ion in protein secretion by magnesium-resistant bacterial strains [59] which indeed shows that magnesium cannot be ruled out in studies on *Vibrio*. Studies



gbpA N-acetylglucosamine-binding protein A; Probably interacts with GlcNAc residues. May promote attachment to both epithelial cell surfaces and chitin (By similarity) (487 aa) *(Vibrio parahaemolyticus)*

Predicted Functional Partners:

		Score
Chi1	chitinase (848 aa)	0.745
VPA1177	putative chitinase A (430 aa)	0.744
VPA1016	multidrug resistance protein D (377 aa)	0.731
VP2338	chitinase (848 aa)	0.685
VPA0714	putative collagenase (705 aa)	0.576
VPA0832	chitodextrinase (1054 aa)	0.575
prt	collagenase; Possesses gelatinolytic activity. Can cause weak haemolysis on blood agar (816 aa)	0.556
chitin	Polysaccharide. (627.6 g/mol)	0.503
VPA1017	AraC/XylS family transcriptional regulator (259 aa)	0.488
VPA0092	spindolin-related protein (392 aa)	0.463

Fig. 1 *Vibrio parahaemolyticus* gbpA protein (GbpA) interactions with chemicals on the STITCH 3 database. The predicted functional partners on the STITCH 3 database are most importantly chitinase, collagenase, multidrug resistance protein D and chitinodextrinase. Chitin is also observed as one of the predicted functional partner to GbpA, which supports GbpA's possible interactions with chitin

even highlighted that the growth of *V. parahaemolyticus* under iron limiting conditions was when the bacteria survived high concentrations of magnesium [83].

Figure 2 is a hypothetical schematic representation which shows magnesium ion as an important link between *V. parahaemolyticus* and *M. rosenbergii*. During the moulting stage of prawn, the prawn often loses a thick moult to regain a transparent exoskeleton (http://www.thefishsite.com/articles/464/moulting-and-behaviour-changes-in-freshwater-prawn/). The figure shows the relation of *V. parahaemolyticus* with the prawn following exuviation in the presence of magnesium. This is conveyed by keeping the magnesium environment constant, i.e., with its levels common to both prawn and bacteria. When a prawn undergoes exuviation, the GbpA of bacteria might probably have greater chances of binding strongly to the sensitive exoskeleton of the prawn. This when compared to the prawn before moulting, its thick exoskeleton might affect the attachment of GbpA to chitin. Here, the binding capacity of GbpA needs to be higher due to a strong layer of chitin containing exoskeleton. This will require further studies to understand the importance of the presence of magnesium to both the host and pathogen.

Conclusion

With regard to food-borne illnesses, *V. parahaemolyticus* contributes significantly to morbidity worldwide [54].

Apart from controlling the severity of bacterial vigour caused by *V. parahaemolyticus*, strategies to control disease spreading through seafood consumption caused by bacteria adapting to aquatic environments are indeed

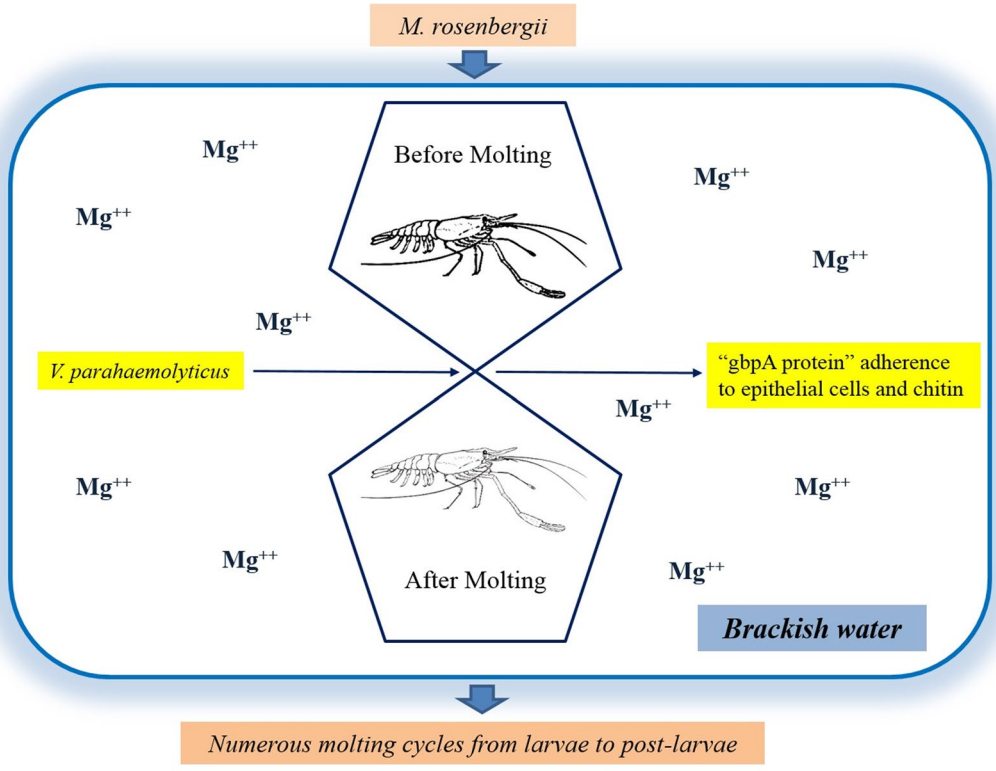


Fig. 2 Hypothetical schematic representation of the probable host–pathogen–environment relationship. *Macrobrachium rosenbergii* undergoes a number of moulting stages through the larvae to post-larvae stage. It is during the intervening phase of moulting that the prawn attains a thin exoskeleton. The presence of magnesium in the environment at this phase might influence favourable conditions for *V. parahaemolyticus* GbpA protein (GbpA) to bind/interact with the epithelial cells, especially the sensitive chitinaceous surface of *M. rosenbergii*

required and needs more attention. This is because, most human populations worldwide are relying on seafood consumption on a daily basis. There are many aquatic organisms which need to be considered for the control of bacterial infections from spreading. The basis of selecting *V. parahaemolyticus* and *M. rosenbergii* in the current review is because of the widely spreading early mortality syndrome (EMS), which is capable of producing a toxin similar to the cholera which can cause life-threatening diarrhoea [84–86].

We think that the utilization of magnesium ion to check any possible interactions between GbpA and carapace (chitin) of the bacteria and prawn, respectively could probably assist us to understand the significance of a magnesium environment. In the present context, as *V. parahaemolyticus* is dealt in relation with *M. rosenbergii*, a giant freshwater prawn of commercial importance, further research based on the aspect of magnesium ion usage by both the prokaryotic or eukaryotic counterparts could help us understand the contamination strategies better. One such strategy could be tweaking the magnesium levels in order to avoid bacteria from entering aquatic organisms. Our review provides the understanding that maintaining magnesium could be important in order to avoid bacteria from multiplying rapidly to infectious levels. Hence, this could help minimize the risk of contamination in the aquaculture systems which might help control food-borne diseases in the long run.

Additional file

Additional file 1. Fasta sequence of the *Vibrio parahaemolyticus* GbpA protein.

Abbreviations

CTXcP: Cholera toxin cP; GbpA: *N*-acetylglucosamine binding protein.

Authors' contributions

ST and SB wrote and edited the manuscript. Both authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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References

1. Carlucci AF, Pramer D. Factors affecting the survival of bacteria in sea water. Appl Microbiol. 1959;7:388–92.
2. Craun MF, Craun GF, Calderon RL, Beach MJ. Waterborne outbreaks reported in the United States. J Water and Health. 2006;4(Suppl 2):19–30.
3. Pond K. Water recreation and disease. Plausibility of associated infections: aute effects, sequelae and mortality. London: IWA Publishing; 2005.
4. Wang XH, Leung KY. Biochemical characterization of different types of adherence of *Vibrio* species to fish epithelial cells. Microbiology. 2000;146(Pt 4):989–98. doi:10.1099/00221287-146-4-989.
5. Macian MC, Arias CR, Aznar R, Garay E, Pujalte MJ. Identification of *Vibrio* spp. (other than *V. vulnificus*) recovered on CPC agar from marine natural samples. Int Microbiol. 2000;3(1):51–3.
6. Rosenberg E, Kushmaro A, Kramarsky-Winter E, Banin E, Yossi L. The role of microorganisms in coral bleaching. ISME J. 2009;3(2):139–46. doi:10.1038/ismej.2008.104.
7. Ceccarelli D, Hasan NA, Huq A, Colwell RR. Distribution and dynamics of epidemic and pandemic *Vibrio parahaemolyticus* virulence factors. Front Cell Infect Microbiol. 2013;3:97. doi:10.3389/fcimb.2013.00097.
8. Horseman MA, Surani S. A comprehensive review of *Vibrio vulnificus*: an important cause of severe sepsis and skin and soft-tissue infection. Int J Infect Dis. 2011;15(3):e157–66. doi:10.1016/j.ijid.2010.11.003.
9. Jones JL, Ludeke CH, Bowers JC, DeRosia-Banick K, Carey DH, Hastback W. Abundance of *Vibrio cholerae*, *V. vulnificus*, and *V. parahaemolyticus* in oysters (*Crassostrea virginica*) and clams (*Mercenaria mercenaria*) from Long Island sound. Appl Environ Microbiol. 2014;80(24):7667–72. doi:10.1128/AEM.02820-14.
10. Gulig PA, Bourdage KL, Starks AM. Molecular pathogenesis of *Vibrio vulnificus*. J Microbiol. 2005;43(Suppl 1):118–31.
11. Shigenobu S, Watanabe H, Hattori M, Sakaki Y, Ishikawa H. Genome sequence of the endocellular bacterial symbiont of aphids Buchnera sp. APS. Nature. 2000;407(6800):81–6. doi:10.1038/35024074.
12. Centers for Disease Control and Prevention. Vaccines for selected use in international travel: cholera vaccine. Morbid Mortal Weekly Rep. 1978;27:173–4.
13. WHO (ed)Guidelines for Cholera control. 1993. p. 22–3.
14. Besser RE, Feikin DR, Eberhart-Phillips JE, Mascola L, Griffin PM. Diagnosis and treatment of cholera in the United States. Are we prepared? JAMA. 1994;272(15):1203–5.
15. Finelli L, Swerdlow D, Mertz K, Ragazzoni H, Spitalny K. Outbreak of cholera associated with crab brought from an area with epidemic disease. J Infect Dis. 1992;166(6):1433–5.
16. Gilbert DN, Moellering RC, Sande MA. Sanford guide to antimicrobial therapy. 29th ed. Hyde Park: Antimicrobial Therapy, Inc; 1999.
17. Hayat U, Reddy GP, Bush CA, Johnson JA, Wright AC, Morris JG Jr. Capsular types of *Vibrio vulnificus*: an analysis of strains from clinical and environmental sources. J Infect Dis. 1993;168(3):758–62.
18. Hollis DG, Weaver RE, Baker CN, Thornsberry C. Halophilic *Vibrio* species isolated from blood cultures. J Clin Microbiol. 1976;3(4):425–31.
19. Klontz KC, Tauxe RV, Cook WL, Riley WH, Wachsmuth IK. Cholera after the consumption of raw oysters. Ann Intern Med. 1987;107(6):846–8.
20. Lowry PW, Pavia AT, Mcfarland LM, Peltier BH, Barrett TJ, Bradford HB, et al. Cholera in louisiana. widening spectrum of seafood vehicles. Arch Intern Med. 1989;149(9):2079–84. doi:10.1001/archinte.149.9.2079.
21. Mahalanabis D, Molla AM, Sack DA. Clinical management of cholera. In: Barua D, Greenough WB, editors. New York: Plenum Medical Book Company; 1992.
22. Pavia AT, Campbell JF, Blake PA, Smith JD, McKinley TW, Martin DL. Cholera from raw oysters shipped interstate. JAMA. 1987;258(17):2374.
23. Daniels NA, Shafaie A. A review of pathogenic vibrio infections for clinicians. Infect Med. 2000;17(10):665–85.
24. Kim MN, Bang HJ. Detection of marine pathogenic bacterial *Vibrio* species by multiplex polymerase chain reaction (PCR). J Environ Biol. 2008;29(4):543–6.
25. Fujino T, Okuno Y, Nakada D, Aoyama A, Fukai K, Mukai T, et al. On the bacteriological examination of Shirasu-food poisoning. Med J Osaka Univ. 1953;4:299–304.
26. Dadisman TA Jr, Nelson R, Molenda JR, Garber HJ. *Vibrio parahaemolyticus* gastroenteritis in Maryland. I. Clinical and epidemiologic aspects. Am J Epidemiol. 1972;96(6):414–26.
27. Rao R, Bing Zhu Y, Alinejad T, Tiruvayipati S, Lin Thong K, Wang J, et al. RNA-seq analysis of *Macrobrachium rosenbergii* hepatopancreas in response to *Vibrio parahaemolyticus* infection. Gut Pathog. 2015;7:6. doi:10.1186/s13099-015-0052-6.
28. Bhowmick R, Ghosal A, Das B, Koley H, Saha DR, Ganguly S, et al. Intestinal adherence of *Vibrio cholerae* involves a coordinated interaction between colonization factor GbpA and mucin. Infect Immun. 2008;76(11):4968–77. doi:10.1128/IAI.01615-07.
29. Keyhani NO, Roseman S. Physiological aspects of chitin catabolism in marine bacteria. Biochim Biophys Acta. 1999;1473(1):108–22.
30. Wong E, Vaaje-Kolstad G, Ghosh A, Hurtado-Guerrero R, Konarev PV, Ibrahim AF, et al. The *Vibrio cholerae* colonization factor GbpA possesses a modular structure that governs binding to different host surfaces. PLoS Pathog. 2012;8(1):e1002373. doi:10.1371/journal.ppat.1002373.
31. Jude BA, Martinez RM, Skorupski K, Taylor RK. Levels of the secreted *Vibrio cholerae* attachment factor GbpA are modulated by quorum-sensing-induced proteolysis. J Bacteriol. 2009;191(22):6911–7. doi:10.1128/JB.00747-09.
32. Department FFaA. Cultured Aquatic Species Information Programme. *Macrobrachium rosenbergii*. In: New MB, editor. Cultured Aquatic Species Information Programme. Rome: FAO 2004–2016; 2004.
33. Fukushima H, Seki R. Ecology of *Vibrio vulnificus* and *Vibrio parahaemolyticus* in brackish environments of the Sada River in Shimane Prefecture Japan. FEMS Microbiol Ecol. 2004;48(2):221–9. doi:10.1016/j.femsec.2004.01.009.
34. D'Abramo LR, Brunson MW. Biology and Life History of freshwater prawns, No. 483. USA: SRAC publications; 1996.
35. Ceccarelli D, Colwell RR. Vibrio ecology, pathogenesis, and evolution. Front Microbiol. 2014;5:256. doi:10.3389/fmicb.2014.00256.
36. Han H, Wong HC, Kan B, Guo Z, Zeng X, Yin S, et al. Genome plasticity of *Vibrio parahaemolyticus*: microevolution of the 'pandemic group'. BMC Genom. 2008;9:570. doi:10.1186/1471-2164-9-570.
37. Faruque SM, Islam MJ, Ahmad QS, Faruque AS, Sack DA, Nair GB, et al. Self-limiting nature of seasonal cholera epidemics: role of host-mediated amplification of phage. Proc Natl Acad Sci USA. 2005;102(17):6119–24. doi:10.1073/pnas.0502069102.
38. Reen FJ, Almagro-Moreno S, Ussery D, Boyd EF. The genomic code: inferring Vibrionaceae niche specialization. Nat Rev Microbiol. 2006;4(9):697–704. doi:10.1038/nrmicro1476.
39. Chen Y, Stine OC, Badger JH, Gil AI, Nair GB, Nishibuchi M, et al. Comparative genomic analysis of *Vibrio parahaemolyticus*: serotype conversion and virulence. BMC Genom. 2011;12:294. doi:10.1186/1471-2164-12-294.
40. Chowdhury NR, Stine OC, Morris JG, Nair GB. Assessment of evolution of pandemic *Vibrio parahaemolyticus* by multilocus sequence typing. Journal Clin Microbiol. 2004;42(3):1280–2.
41. Wong HC, Liu SH, Chiou CS, Nishibuchi M, Lee BK, Suthienkul O, et al. A pulsed-field gel electrophoresis typing scheme for *Vibrio parahaemolyticus* isolates from fifteen countries. Int J Food Microbiol. 2007;114(3):280–7. doi:10.1016/j.ijfoodmicro.2006.09.024.
42. Sawabe T, Ogura Y, Matsumura Y, Feng G, Amin AR, Mino S, et al. Updating the Vibrio clades defined by multilocus sequence phylogeny: proposal of eight new clades, and the description of *Vibrio tritonius* sp. Nov. Frontiers in microbiology. 2013;4:414. doi:10.3389/fmicb.2013.00414.
43. Rapa RA, Labbate M. The function of integron-associated gene cassettes in *Vibrio* species: the tip of the iceberg. Front Microbiol. 2013;4:385. doi:10.3389/fmicb.2013.00385.

44. Kirkup BC Jr, Chang L, Chang S, Gevers D, Polz MF. Vibrio chromosomes share common history. BMC Microbiol. 2010;10:137. doi:10.1186/1471-2180-10-137.
45. Rowe-Magnus DA, Guerout AM, Ploncard P, Dychinco B, Davies J, Mazel D. The evolutionary history of chromosomal super-integrons provides an ancestry for multiresistant integrons. Proc Natl Acad Sci U S A. 2001;98(2):652–7. doi:10.1073/pnas.98.2.652.
46. Altekruse SF, Bishop RD, Baldy LM, Thompson SG, Wilson SA, Ray BJ, et al. *Vibrio gastroenteritis* in the US Gulf of Mexico region: the role of raw oysters. Epidemiol Infect. 2000;124(3):489–95.
47. Johnson DE, Weinberg L, Ciarkowski J, West P, Colwell RR. Wound infection caused by Kanagawa-negative *Vibrio parahaemolyticus*. J Clin Microbiol. 1984;20(4):811–2.
48. Yan WX, Dai Y, Zhou YJ, Liu H, Duan SG, Han HH, et al. Risk factors for sporadic *Vibrio parahaemolyticus* gastroenteritis in east China: a matched case–control study. Epidemiol Infect. 2015;143(5):1020–8. doi:10.1017/S0950268814001599.
49. Baumann P, Furniss AL, Lee JV. Genus 1. Vibrio. In: Bergey's manual of systematic bacteriology. Baltimore: Williams and Wilkins Co.; 1984.
50. Colwell RR, West PA, Maneval D, Remmers EF, Elliot EL, Carlson NE. Ecology of pathogenic vibrios in Chesapeake Bay. In: Colwell RR, editor. Vibrios in the environment. Wiley: New York; 1984. p. 367–87.
51. Yeung PS, Boor KJ. Epidemiology, pathogenesis, and prevention of foodborne *Vibrio parahaemolyticus* infections. Foodborne Pathog Dis. 2004;1(2):74–88. doi:10.1089/153531404323143594.
52. Jackson H. Temperature relationships of *Vibrio parahaemolyticus*. In: Fujino T, Sakaguchi G, Sakazaki R, et al., editors. International symposium of *Vibrio parahaemolyticus*. Tokyo: Saikon; 1974. p. 139–45.
53. Barrow GI, Miller DC. Growth studies on *Vibrio parahaemolyticus* in relation to pathogenicity. In: Fujino T, Sakaguchi G, Sakazaki R, et al., editors. International symposium of *Vibrio parahaemolyticus*. Tokyo: Saikon; 1974. p. 205–10.
54. Daniels NA, MacKinnon L, Bishop R, Altekruse S, Ray B, Hammond RM, et al. *Vibrio parahaemolyticus* infections in the United States, 1973–1998. J Infect Dis. 2000;181(5):1661–6. doi:10.1086/315459.
55. Daniels NA, Ray B, Easton A, Marano N, Kahn E, McShan AL 2nd, et al. Emergence of a new *Vibrio parahaemolyticus* serotype in raw oysters: a prevention quandary. JAMA. 2000;284(12):1541–5.
56. Sanyal SC, Sen PC. Human volunteer study on the pathogenicity of *Vibrio parahaemolyticus*. In: Fujino T, Sakaguchi G, Sakazaki R, Takeda Y, editors. International symposium of *Vibrio parahaemolyticus*. Tokyo: Saikon; 1974. p. 227–30.
57. DePaola A, Kaysner CA, Bowers J, Cook DW. Environmental investigations of *Vibrio parahaemolyticus* in oysters after outbreaks in Washington, Texas, and New York (1997 and 1998). Appl Environ Microbiol. 2000;66(11):4649–54.
58. FAO/WHO. Risk assessment of *Vibrio parahaemolyticus* in seafood: interpretative summary and technical report. 2011.
59. Bhattacharya M, Roy SS, Biswas D, Kumar R. Effect of Mg(2+) ion in protein secretion by magnesium-resistant strains of *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus* isolated from the coastal water of Haldia port. FEMS Microbiol Lett. 2000;185(2):151–6.
60. Heinis JJ, Beuchat LR, Boswell FC. Antimetabolite sensitivity and magnesium uptake by thermally stressed *Vibrio parahaemolyticus*. Appl Environ Microbiol. 1978;35(6):1035–40.
61. Akio K, Teshima S, Sasaki M. Requirements of the juvenile prawn for calcium, phosphorous, magnesium, potassium, copper, manganese and iron. Mem Fac Fish. 1984;33(1):63–71.
62. Hangsapreurke K, Thamrongnawasawat T, Powtongsook S, Tabthipwon P, Lumubol P, Pratoomchat B. Embryonic development, hatching, mineral consumption, and survival of *Macrobrachium rosenbergii* (de Man)reared in artificial seawater in closed recirculating water system at different levels of salinity. Mj Int J Sci Tech. 2008;2(3):471–82.
63. Rejeki S. Accumulation of aluminium in the tissue of giant fresh water prawn (*Macrobrachium rosenbergii* de Man) exposed to acidic water contaminated with aluminium salt. J Coast Dev. 2003;6(2):83–95.
64. Kuhn M, Szklarczyk D, Franceschini A, von Mering C, Jensen LJ, Bork P. STITCH 3: zooming in on protein–chemical interactions. Nucleic Acids Res. 2012; 40(Database issue):D876–80. doi:10.1093/nar/gkr1011.
65. Dalia AB, Lazinski DW, Camilli A. Identification of a membrane-bound transcriptional regulator that links chitin and natural competence in *Vibrio cholerae*. MBio. 2014;5(1):e01028.
66. Nahar S, Sultana M, Naser MN, Nair GB, Watanabe H, Ohnishi M, et al. Role of shrimp chitin in the ecology of toxigenic *Vibrio cholerae* and cholera transmission. Front Microbiol. 2011;2:260. doi:10.3389/fmicb.2011.00260.
67. Nalin DR, Daya V, Reid A, Levine MM, Cisneros L. Adsorption and growth of Vibrio cholerae on chitin. Infect Immun. 1979;25(2):768–70.
68. Sun S, Tay QX, Kjelleberg S, Rice SA, McDougald D. Quorum sensing-regulated chitin metabolism provides grazing resistance to *Vibrio cholerae* biofilms. ISME J. 2015;9(8):1812–20. doi:10.1038/ismej.2014.265.
69. Vezzulli L, Pezzati E, Stauder M, Stagnaro L, Venier P, Pruzzo C. Aquatic ecology of the oyster pathogens *Vibrio splendidus* and *Vibrio aestuarianus*. Environ Microbiol. 2015;17(4):1065–80. doi:10.1111/1462-2920.12484.
70. Williams TC, Ayrapetyan M, Oliver JD. Molecular and physical factors that influence attachment of *Vibrio vulnificus* to chitin. Appl Environ Microbiol. 2015;81(18):6158–65. doi:10.1128/AEM.00753-15.
71. Kaneko T, Colwell RR. Adsorption of *Vibrio parahaemolyticus* onto chitin and copepods. Appl Microbiol. 1975;29(2):269–74.
72. Frischkorn KR, Stojanovski A, Paranjpye R. *Vibrio parahaemolyticus* type IV pili mediate interactions with diatom-derived chitin and point to an unexplored mechanism of environmental persistence. Environ Microbiol. 2013;15(5):1416–27. doi:10.1111/1462-2920.12093.
73. Karunasagar I, Venugopal MN, Karunasagar I, Segar K. Role of chitin in the survival of *Vibrio parahaemolyticus* at different temperatures. Can J Microbiol. 1986;32(11):889–91.
74. Osawa R, Koga T. An investigation of aquatic bacteria capable of utilizing chitin as the sole source of nutrients. Lett Appl Microbiol. 2008;21(5):288–91.
75. Hameed ASS, Rahaman KH, Alagan A, Yoganandhan K. Antibiotic resistance in bacteria isolated from hatchery-reared larvae and post-larvae of *Macrobrachium rosenbergii*. Aquaculture. 2003;217(1–4):39–48.
76. Clark JWH, Lynn JW. A Mg++ dependent cortical reaction in the eggs of Penaeid shrimp. J Exp Zool. 1977;200:177–83.
77. Damrongphol P, Jaroensastraraks P, Poolsanguan B. Effect of various medium compositions on survival and hatching rates of embryos of the giant freshwater prawn *Macrobrachium rosenbergii* cultured in vitro. Fisheries Sci. 2001;67(1):64–70. doi:10.1046/j.1444-2906.2001.00200.x.
78. Permina EA, Kazakov AE, Kalinina OV, Gelfand MS. Comparative genomics of regulation of heavy metal resistance in Eubacteria. BMC Microbiol. 2006;6:49. doi:10.1186/1471-2180-6-49.
79. Lee SW, Najiah M, Wendy W, Zahrol A, Nadirah M. Multiple antibiotic resistance and heavy metal resistance profile of bacteria isolated from giant freshwater prawn (*Macrobrachium rosenbergii*) hatchery. Agric Sci China. 2009;8(6):740–5.
80. Min J, Weiling Z, Qingzhen Y, Xiling D, Zhengguo Z. The toxicity of four heavy metals on *Macrobrachium rosenbergii* postlarva. J. Shanghai Fisheries Univ. 2002;11(3):203–7.
81. Tabei Y, Era M, Ogawa A, Morita H. Effects of magnesium sulfate on the luminescence of *Vibrio fischeri* under nutrient-starved conditions. Biosci Biotechnol Biochem. 2011;75(6):1073–8. doi:10.1271/bbb.100880.
82. O'Shea TM, Deloney-Marino CR, Shibata S, Aizawa S, Wolfe AJ, Visick KL. Magnesium promotes flagellation of *Vibrio fischeri*. J Bacteriol. 2005;187(6):2058–65. doi:10.1128/JB.187.6.2058-2065.2005.
83. Ju CH, Yeung PS, Oesterling J, Seigerman DA, Boor KJ. *Vibrio parahaemolyticus* growth under low-iron conditions and survival under high-magnesium conditions. J Food Prot. 2006;69(5):1040–5.
84. De Schryver P, Defoirdt T, Sorgeloos P. Early mortality syndrome outbreaks: a microbial management issue in shrimp farming? PLoS Pathog. 2014;10(4):e1003919. doi:10.1371/journal.ppat.1003919.
85. Kondo H, Tinwongger S, Proespraiwong P, Mavichak R, Unajak S, Nozaki R, et al. Draft genome sequences of six strains of *Vibrio parahaemolyticus* isolated from early mortality syndrome/acute Hepatopancreatic necrosis disease shrimp in Thailand. Genome Announc. 2014. doi:10.1128/genomeA.00221-14.
86. Yang YT, Chen IT, Lee CT, Chen CY, Lin SS, Hor LI, et al. Draft genome sequences of four strains of vibrio parahaemolyticus, three of which cause early mortality syndrome/acute hepatopancreatic necrosis disease in shrimp in China and Thailand. Genome Announc. 2014. doi:10.1128/genomeA.00816-14.