Vibrio Becomes Lethal to Shrimp Aquaculture

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Introduction

Exploitation of marine living resources, including fisheries, is already at its peak. Yet the protein demand on our planet keeps increasing. Our oceans cannot supply the demand we require. Hence, aquaculture is the most feasible alternative that can fill the gap in the seafood supply. It has the highest protein retention and the lowest greenhouse gas emissions compared with other animal proteins such as chicken, pork, and beef (Global Seafood Alliance, 2019).

Shrimp farming is a rapidly growing aquaculture sector that is in demand in the world market for its high nutritional value and taste. The shrimp industry becomes an important source for the livelihood of many people in developing countries. However, this industry in India has been at a crossroads in recent times due to several reasons: production constraints, climate change, emerging diseases, etc. Production costs for shrimp farming are increasing steadily, mainly due to increasing electricity charges, feed costs, labour charges, land lease costs, etc. Rapid global climate change is proving to be another significant factor causing major problems in shrimp farming. The diel temperature fluctuations (early morning and afternoon) have been more than 14 !, which is reason enough for the health deterioration of the animals. The sharp rise in temperature, sudden cloudburst, and lack of water or increase in salinity due to low rainfall are economic losses to numerous farmers. Emerging disease is also a major problem in shrimp farming. Shrimps lack cell-mediated immunity or immune memory. Apart from viral infections, bacterial infections caused by members of the genus *Vibrio* have become lethal. These bacterial diseases are emerging as a serious problem in the industry.

Vibrio – opportunistic pathogen

Vibrio is an opportunistic pathogen, and can be devastating for the early life stages of shrimp development as well as in the culture pond (Goulden et al., 2012a; De Schryver et al., 2014; Kumar et al., 2017). These days, *Vibrio* is used as a microbial indicator for climate change (Baker-Austin et al., 2017). Though many strains of *Vibrio* are non-virulent, selected strains can cause higher mortality. Some virulent strains produce lethal toxins such as cysteine protease, haemolysins, Vibriocins, Pir A and Pir B, Vhvp1 and Vhvp2, and antibacterial effectors produced by the Type 6 Secretion System (T6SS) that can damage the intestinal epithelial cell lining, which consequently facilitates the infiltration of opportunistic bacteria into other tissues and organs of the body (Liu and Lee, 1999; Montero and Austin, 1999; Soonthornchai et al., 2010, Zou et al, 2020, Liu et al, 2023).

Zoae-2 syndrome and vibriosis

In the years 2017–18, we collected Zoea-2 syndrome samples from 3 coastal lines (Chennai-Pondicherry coast, Nellore-Babaatla coast, Kakinada-Tuni coast). These coasts have the highest number of hatcheries in India. In the collected samples, *Vibrio* was isolated and identified to establish the species associated with Zoea-2 syndrome. We found that the collected samples were positive for *Vibrio alginolyticus*. *Vibrio alginolyticus* is aggravated as a disease when it is present along with other *Vibrio* spp. such as *Vibrio harveyi*, *V. parahaemolyticus*, *V. campbellii*, *V. mimicus*. Kumar et al (2017) reported rapid proliferation of V. alginolyticus in the shrimp hatcheries.

Upon careful observation at the shrimp diseases, we found that *Vibrio* has becoming primary infection unlike secondary infection as in the past years.

Vibrio – acquiring virulence factors

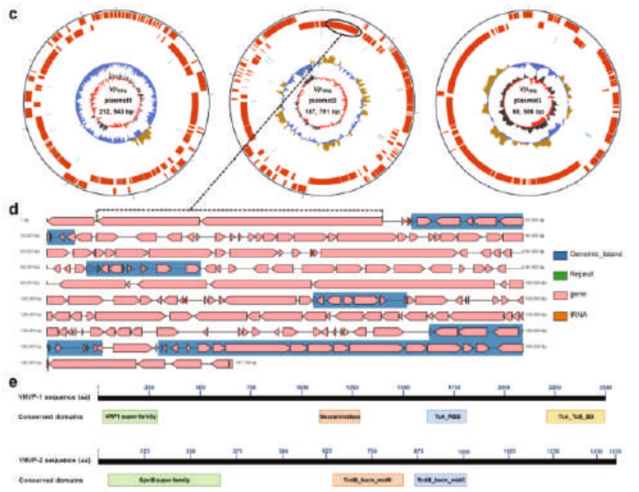
In the last decade, *Vibrio* has become more lethal to shrimp aquaculture because of its acquired virulent factors. These virulent factors were first reported in *Vibrio parahaemolyticus* and later in several other species. It suggests that the virulence factors might be able to transfer via-conjugation among different *Vibrio* spp. These virulence factors are acquired from virulent protein coding plasmids. Acute Hepatopancreatic Necrosis Disease (AHPND) and the highly lethal *Vibrio* disease, Translucent Post-Larvae Disease (TPD) or Glass Post-Larvae Disease (GPD) are examples of such vibrio diseases. Upon looking into available genetic information in the public databases and literature for the virulence factors of the AHPND samples, we found a fraction of samples do have both Pir toxins (Pir A, Pir B virulence factors), TPD virulence factors (vpvh1 and vpvh2). So, these virulence factors look like they are transferable and may cause more than one disease.

Table 1: Zoea syndrome samples and prevalence of Vibrio spp. based on real-time qPCR

Sampling locations for the Zoea syndrome samples	*Vibrio harveyi*	*Vibrio parahaemolyticus*	*Vibrio alginolyticus*	*Vibrio vulnificus*
Pondicherry –Chennai (Sample 1)	**Slight positive (Ct – 32.34)**	**Positive (Ct – 27.43)**	**Positive (Ct – 24.27)**	Negative
Pondicherry –Chennai (Sample 2)	Negative	**Positive (Ct – 29.27)**	**Positive (Ct – 26.57)**	**Slight positive (Ct –34.08)**
Pondicherry–Chennai (Sample 3)	**Positive (Ct – 28.86)**	Negative	**Slight Positive (Ct –32.53)**	Negative
Kakinada coast (Sample 1)	Negative	**Slight positive (Ct – 35.44)**	**Positive (Ct – 28.26)**	**Slight positive (Ct –35.04)**
Ongole	Negative	**Slight positive (Ct – 32.2)**	**Positive (Ct – 26.58)**	Negative
Ongole	Negative	**Positive (Ct – 29.34)**	**Slight positive (Ct-31.22)**	Negative
Kakinada coast (Sample 2)	**Positive (Ct – 32.34)**	Negative	**Positive (Ct – 29.63)**	Negative
Kakinada coast (Sample 3)	**Positive (Ct – 27.34)**	Negative	**Slight Positive (Ct – 30.52)**	Negative

Table 2 : Some the diseases caused by *Vibrio* in both hatchery and in the grow-out ponds.

Disease	Common *Vibrio* species	Known susceptible crustaceans		References
Zoea II Syndrome (larvae)	*V. alginolyticus* *V. campbellii* *V. harveyi* *V. mimicus* *V. parahaemolyticus*	Shrimp	*Penaeus stylirostris* *Penaeus vannamei*	Soto-Rodriguez et al. (2006a, b), Cullar-Anjel et al. (2014) Kumar et al. (2017)
Septic Hepatopancreatic Necrosis Disease (hatchery and grow-out)	*V. alginolyticus* *V. campbellii* *V. harveyi* *V. parahaemolyticus* *V. penaeicida* *V. vulnificus*		*All farmed shrimp*	Morales-Covarrubias and Gomez-Gil (2014) Stern and Sonnenholzner (2014) Morales-Covarrubias et al. (2018)
Luminescent vibriosis (eggs and larvae)	*V. campbellii* *V. harveyi* *V. splendidus*		*Penaeus indicus* *Penacus monodon* *Penaeus vannamei*	Lavilla-Pitogo et al. (1990) Diggles et al. (2000)
Shell disease (juvenile and adults)	Chitinolytic *Vibrio* spp. including *V. alginolyticus*		*Macrobrachium rosenbergii* *Penaeus sp.*	Cook and Lofton (1973) Porter et al. (2001) Vogan etal. (2002)
Acute Hepatopancreatic Necrosis Disease (postlarvae)	AHPND-causing *V. campbellii* AHPND-causing *V. harveyi* AHPND-causing *V. owensii* AHPND-causing *V. parahaemolyticus* (Vpayenp)		*Penaeus monodon* *Penaeus vannamei* *Penaeus chinensis** *Penaeus japonicus**	Yang et al. (2014) Kondoet al. (2015) Liu et al. (2015) Choietal. (2017) Dong et al. (2017a) Muthukrishnan et al. (2019) OIE (2019) Prachumwatet al. (2020)
Summer syndrome (grow-out)	*V.nigripulchritudo*		*Penaeus japonicus* *Penaeus stylirostris*	Goarant et al. (2006) Sakai et al. (2007)



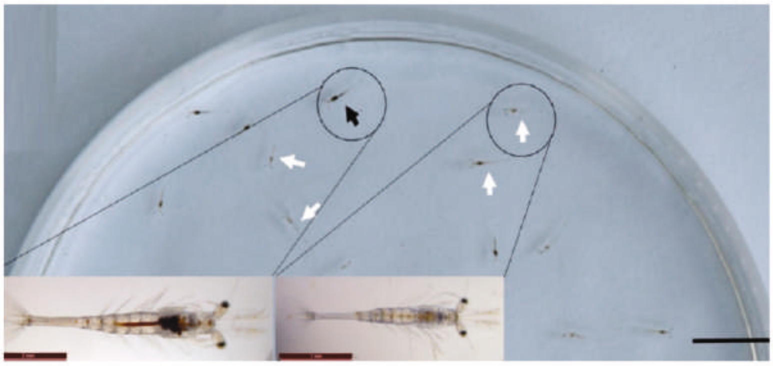


Figure1: *Vibrio* high virulent protein (VHVP) virulence factor (Vhvp 1 & VhVp2) in *Vibrio parahaemolyticus* Plasmid 2 (VP $_{\mathrm{TPD}}$)– Ref –Liu et al, 2023)

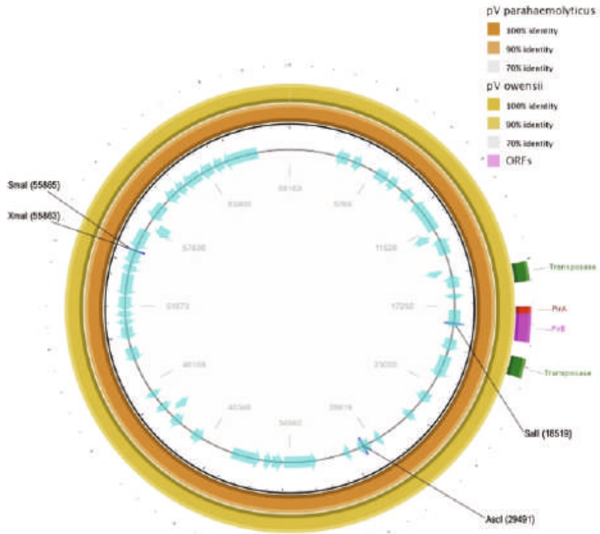


Figure 2: BLAST comparison with the plasmid of *V. parahaemolyticus* (orange) and *V. owensii* (yellow). The transposase genes in green, red and purple represent the *PirA* and *PirB* genes (Restrepo L et,al. 2018)

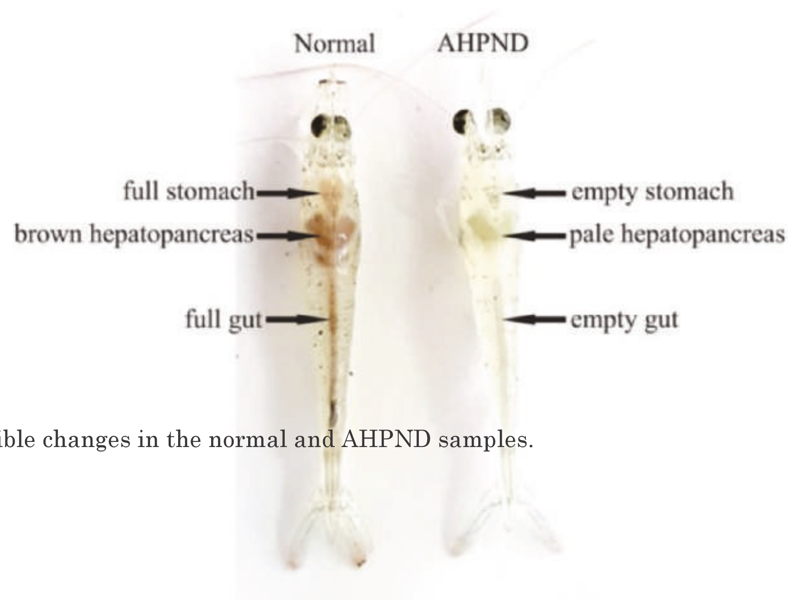


Figure 3: Comparison of visible changes in the normal and AHPND samples.

So far, AHPND and/or TDP cases have not been reported in our country. However, we have observed similar clinical characteristics of these disease, such as lethargy, slow growth, an empty stomach or midgut, and a pale to white atrophied hepatopancreas, etc. in the samples from the hatchery as well as the culture system. Hence, we propose an extensive surveillance program for *Vibrio* toxin as the need of the hour to better understand our current *Vibrio* causing disease status in the Indian shrimp aquaculture industry. The suspected positive samples should be studied at the genetic and genomic level to understand the cause and associated virulence factors. The preventive measures can be enforced if any indication of these diseases is found to help farmers overcome the disease and save them from economic loss.

A comparative table for TPD vs AHPND is given below :

Translucent post-larvae disease (TPD)	Acute Hepatopancreatic Necrosis Disease (AHPND)
Economic loss to 70%–80% coastal shrimp nurseries in China in the spring of 2020	Significant economic losses to shrimp producers in China (2009), Vietnam (2010), Malaysia (2011), Thailand (2012), Mexico (2013) and Philippines (2015).
The disease reported in *Penaeus japonicus, P. vannamei*	The disease can affect *Penaeus monodon, P. vannamei*
Vibrio parahaemolyticus strain (Vp-JS20200428004-2) containing *Vibrio* high virulent protein (VHVP) virulence factor (Vpvh1 & 2) causes the infectious TPD	Causative agent was *Vibrio parahaemolyticus* carrying a plasmid coding for the mortal toxins Pir A, Pir B
Lethal to post-larvae at 4–7 days old (PL4–PL7). The cumulative mortality of the infected post-larvae could reach up to 100% in 3 days in a typical disease case.	100% Mass mortality in cultured ponds has been observed 35 days.
Clinical symptoms are lethargy, erratic swimming behaviour, empty gut and off-white body colour, pale body colour & pale Hepatopancreas	Clinical symptoms include lethargy, slow growth, empty stomach or midgut, and a pale to white atrophied hepatopancreas, with dead shrimp on the pond bottom

Enterocytozoon hepatopenaei (EHP) or White fecal Syndrome in presence of _Vibrio_ spp.

Sometimes, *Vibrio* may also play a pathogenetic role as a secondary infection in *Enterocytozoon hepatopenaei* (EHP) disease and white faecal syndrome. Some countries, such as the United States of America, where shrimp aquaculture is performed in the lower density, get EHP contamination. However, it is not aggravated as a disease unlike in India and other countries where high density aquaculture is performed. In higher density culture, EHP infectious spores become more virulent, along with *Vibrio* and a few other anaerobic bacteria such as *Propionigenium* (Aranguren et al, 2022, GSA, 2024). Specific measures to remove the EHP spores, water and feed management, improve stress free shrimp culture environment help to overcome EHP problems (GSA, 2024). Hence, control over *Vibrio* and pond bottom is indispensable for the management of White faecal syndrome and EHP.

Surveillance and treatment measures

As mentioned earlier, invertebrates do not possess a well-developed immune system, hence, following biosecurity protocols is an important strategy for disease management. Routine surveillance, regular lab monitoring, and following up on the standard operating protocol (SOP) are part of biosecurity management. Farmers must go back to the fundamentals of shrimp aquaculture, and follow the biosecurity protocol without fail. The possible routine check-up protocols to keep the *Vibrio* infection away includes:

- Set up a biosecurity protocol.
- Routine check-ups for brooders, Live feeds: freeze the live feed before use. Nauplii, Post-Larvae, Algae, and Artemia
- In the pond: PL checking before stocking
- Pond water and animal monitoring during the culture period

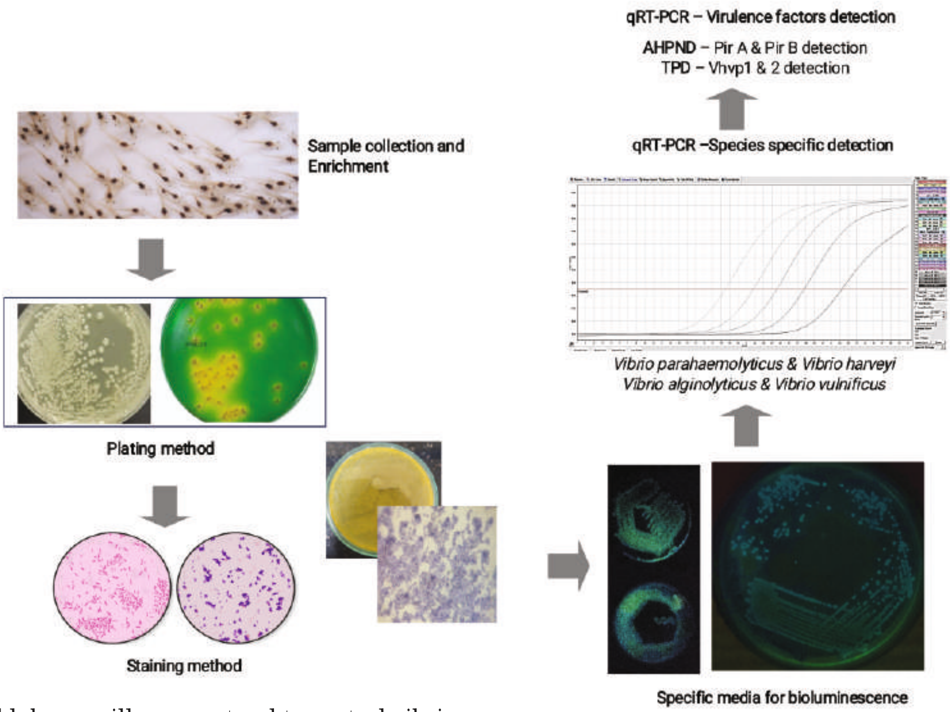


Figure 4: Proposed lab surveillance protocol to control vibrio

A suitable probiotic consortium can be used to control the vibrio growth. Use of postbiotics as short-chain fatty acids (SCFA) and natural anti-vibrio products such as biopolymers: polyhydroxybutyrate can be encouraged to keep *Vibrio* checked in the shrimp aquaculture industry.

Reference

1. A.B. Montero, B. Austin Characterization of extracellular products from an isolate of *Vibrio harveyi* recovered from diseased post-larval *Penaeus vannamei* (Bonne) J. Fish Dis., 22 (5) (1999), pp. 377-386

2. C. Baker-Austin, J. Trinanes, N. Gonzalez-Escalona, J. Martinez-Urtaza Non-cholera vibrios: the microbial barometer of climate change Trends Microbiol., 25 (1) (2017), pp. 76-84

3. De Schryver, P., Defoirdt, T., & Sorgeloos, P. (2014). Early mortality syndrome outbreaks: a microbial management issue in shrimp farming?. *PLoS pathogens*, *10*(4), e1003919.

4. Goulden, E. F., Hall, M. R., Bourne, D. G., Pereg, L. L., & Høj, L. (2012). Pathogenicity and infection cycle of Vibrio owensii in larviculture of the ornate spiny lobster (Panulirus ornatus). *Applied and Environmental Microbiology*, *78*(8), 2841-2849.

5. Kumar, T. S., Vidya, R., Kumar, S., Alavandi, S. V., & Vijayan, K. K. (2017). Zoea-2 syndrome of Penaeus vannamei in shrimp hatcheries. *Aquaculture*, *479*, 759-767.

6. Liu S, Wang W, Jia T, Xin L, Xu T, Wang C, Xie G, Luo K, Li J, Kong J, Zhang Q. 2023. *Vibrio parahaemolyticus* becomes lethal to post-larvae shrimp via acquiring novel virulence factors. Microbiol Spectr 11:e00492-23. https://doi.org/10.1128/spectrum.00492-23

7. P.C. Liu, K.K. Lee Cysteine protease is a major exotoxin of pathogenic luminous *Vibrio harveyi* in the tiger prawn, *Penaeus monodon* Lett. Appl. Microbiol., 28 (6) (1999), pp. 428-430

8. Restrepo, L., Bayot, B., Arciniegas, S. *et al.* PirVP genes causing AHPND identified in a new *Vibrio* species (*Vibrio punensis*) within the commensal *Orientalis* clade. *Sci Rep* 8, 13080 (2018). https://doi.org/10.1038/s41598-018-30903-x

9. W. Soonthornchai, W. Rungrassamee, N. Karoonuthaisiri, P. Jarayabhand, S. Klinbunga, K. Söderhäll, P. Jiravanichpaisal Expression of immune-related genes in the digestive organ of shrimp, *Penaeus monodon*, after an oral infection by *Vibrio harveyi* Dev. Comp. Immunol., 34 (1) (2010), pp. 19-28.