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Cracking the glass: a look into the mysterious post larval disease affecting aquaculture

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Abstract

Translucent Post-Larvae Disease (TPD), also known as Post-Larval Glass Disease (PLGD), poses a serious threat to shrimp aquaculture, particularly affecting Penaeus vannamei post-larvae. First identified in 2020 in China and Vietnam, it is caused by a virulent Vibrio parahaemolyticus strain (Vp-JS20200428004-2), distinct from AHPND, and often co-infected with Baishivirus. Symptoms include glassy transparency, empty guts, and lethargy. Diagnosis involves clinical signs, molecular tests, and VHVP-2 primers. The disease leads to economic loss and reduced exports. Effective control relies on early detection, improved biosecurity, and targeted treatment. PLGD highlights the urgent need for regional cooperation and sustainable management strategies.

Kewwords : Post-larval disease, *Vibrio parahemolyticus*, shrimp disease, Aquaculture

Introduction

An unusual and troubling phenomenon has emerged in the shrimp farming industry, which is worth billions globally. Minuscule shrimp larvae, merely a few days old, are inexplicably becoming transparent, halting their feeding, and dying en masse. This puzzling ailment was later revealed as Post Larval Glass Disease (PLGD). First identified in 2020 in China and Vietnam, this rapidly spreading condition is now a major concern for hatcheries and shrimp farmers across Asia. This report will delve into the characteristics of PLGD, its causative agent, its impact on shrimp farming, and the broader implications for disease management and sustainability in the aquaculture industry.

What is post-larval glass disease?

As of 2020, Post Larval Glass Disease (PLGD), also called Translucent Post-Larvae Disease (TPD), has been reported to cause high-severity mortality in shrimp hatcheries globally, including in China and Southeast Asia, usually in association with *Penaeus vannamei* (Pacific white shrimp). PLGD gained immediate notoriety when shrimp farms in southern China reported rapid, massive mortality of post-larvae within 72 hours of stocking. Affected shrimp were reported to have a glassy transparency, pale or colourless internal organs, an empty digestive tract, and slow movement. The visual status of the shrimp experience earned them the unofficial industry term "glass post-larvae". Mortality rates can reach 80–100% within 24 - 48 hours, with a critical mortality period of 3–5 days post-stocking. Post-larval stages 2 to 4 (shortly after moulting) are the most susceptible and are essential for early identification and treatment action.

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Major cause: *Vibrio parahaemolyticus*

The disease has been closely associated with a novel and pathogenic strain of *Vibrio parahaemolyticus* (Vp-JS202004280042). This strain differs genetically from the strains causing acute hepatopancreatic necrosis disease (AHPND), which also affects the hepatopancreas. In challenge studies, VJS202004280042 caused 100% mortality in shrimp post-larvae in 40 hours at a bacterial density of 1.83×10^6 CFU/mL. Moribund shrimp were clinically similar in presentation to natural PLGD outbreaks, and similar midgut lesions and hepatopancreas lesions were observed as with natural outbreaks. The pathogen was reisolated from experimental infections, confirming the pathogenicity associated with this strain. Its rapid lethality has prompted some researchers to refer to PLGD as Highly Lethal Vibrio Disease (HLVD). Complicating the situation is confirmation of the presence of a virus belonging to the Marnaviridae family (tentatively named Baishivirus) in shrimp showing PLGD clinical signs. The implications of this virus remain to be determined; however, preliminary evidence suggests it may interact either by enhancing the severity of the disease or in conjunction with bacteria. Environmental factors and microbial dysbiosis also have a large role in disease initiation and progression. Most PLGD disease outbreaks are correlated with water quality issues and dysbiosis in microbiota. We see this in the gut and environment of infected shrimp. When studies of the post-larvae have been conducted, the findings have shown that pathogenic Vibrio species dominate the gut. At the same time, potential beneficial organisms such as the genus *Ralstonia* have decreased. It is worth noting that the gut microbiota of shrimp and the microbes in ponds closely reflected each other at the outbreak, indicating compromised integrity of the ecosystem from cross-contamination.

AHPND vs PLGD

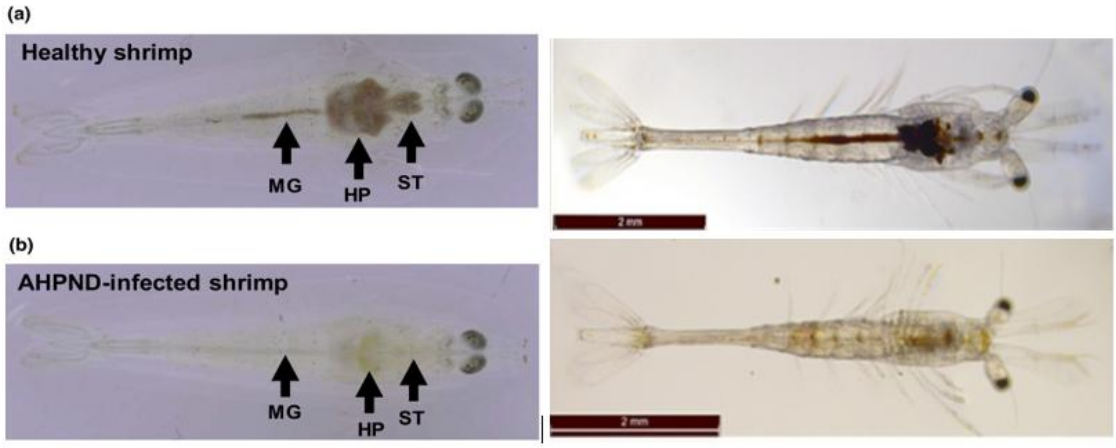

Fig 1. Healthy shrimp (both top) vs AHPND-infected shrimps (bottom left) vs PLGD-infected shrimps (bottom right) (Jia et al., 2024)

Although PLGD and AHPND present similar clinical symptoms in shrimp, recent studies indicate they arise from genetically distinct strains of *V. parahaemolyticus*. The key toxin genes linked to AHPND, namely pirAvp, pirBvp, AP1, and AP2, were completely absent in the strains causing PLGD. Notably, when shrimp were experimentally infected with both

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PLGD and AHPND in a controlled environment, the PLGD-infected group showed considerably higher mortality rates at the same time intervals. This implies that the Vibrio strain responsible for PLGD is an even more deadly variant. Due to its aggressive nature, researchers have suggested renaming it HLVD to emphasise the significant risk it poses to shrimp hatcheries and aquaculture worldwide.

Clinical signs

In the initial phases, the shrimp show signs of anorexia, sluggish movements, and a delayed reaction to external stimuli. Their gills may have a pale-yellow tint, and a significant alteration occurs in the color of the hepatopancreas, changing from dark brown to light brown. More intense symptoms emerge as the situation progresses to the later clinical stage. The gut may appear vacant, and the gill filaments can become swollen and loose. The hepatopancreas becomes necrotic and pale, while a thin and cloudy area stretching from the cephalothorax to the abdomen can be observed. In severely affected shrimp, the symptoms become even more alarming. These shrimps appear white and translucent due to muscle wasting. Their gill tissue becomes fragile and eroded; the hepatopancreas is necrotic and pale. Furthermore, there is glassy degeneration present in the gut, and the stomach remains empty.

Epidemiology

The disease was initially documented in September 2019 in Southern Fujian, China. The first online details about this disease surfaced on the Chinese social media platform WeChat, posted in April 2020. At first, the issue was linked to variations in salinity, bacterial causes (Vibrio), and EHP. Taishan, Guangxi, Fujian, and the Pearl River Delta regions experienced significant impacts.

Susceptible species

Studies on the host range of PLGD indicate that it mainly impacts certain shrimp speci especially during their early developmental phas The most affected species include *Penaeus vannamei* and *Penaeus japonicus*, with post-larvae at stages PL2 and PL4 particularly prone to infection. Conversely, various other crustacean species seem to be resistant to this disease. Specifically, *Macrobrachium rosenbergii* (giant freshwater prawn) and *Procambarus clarkii* (red swamp crayfish) have shown no signs of

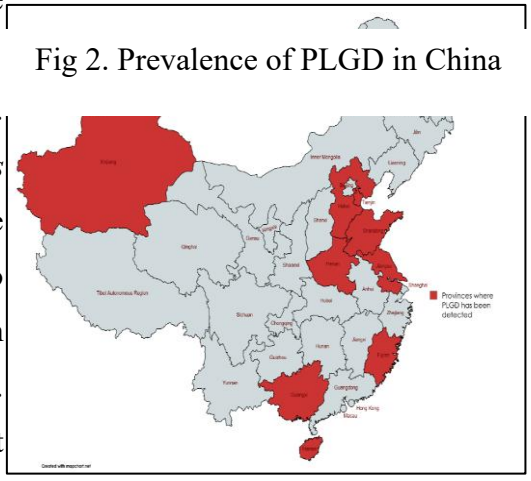

Fig 2. Prevalence of PLGD in China

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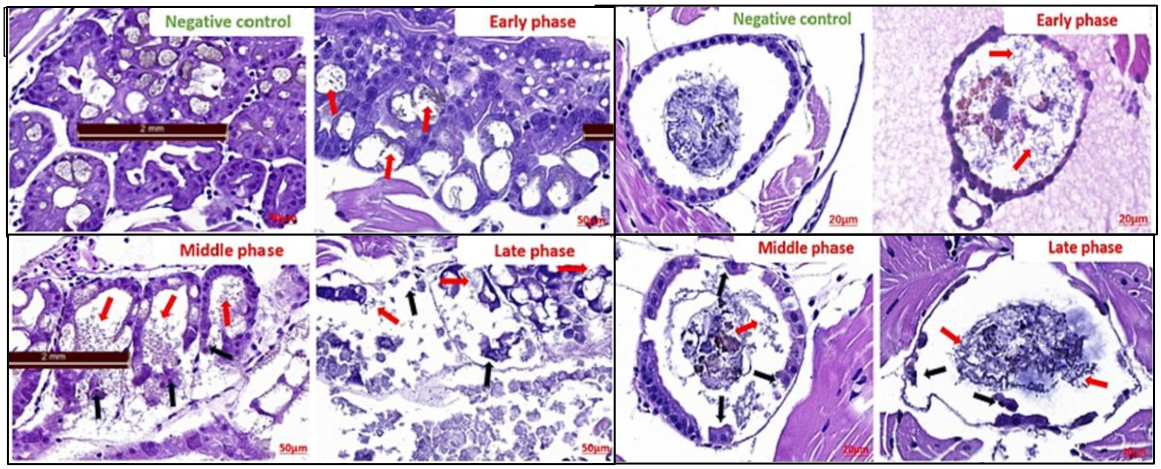
vulnerability. Notably, pathogens associated with PLGD have also been found in non-crustacean organisms like squid and silkworm, indicating the potential for a broader range of carriers. However, wild snail populations have not yielded positive results, highlighting specific species-related constraints in host infection or transmission.

Diagnosis

Diagnosing PLGD involves a combination of clinical observation, differential exclusion, and molecular confirmation. Presumptive diagnosis is often based on visible symptoms such as an empty gut and noticeable discolouration or whitening of the hepatopancreas. However, due to the similarity of clinical signs, PLGD can be easily confused with AHPND. Notably, PLGD tends to cause even higher mortality rates than AHPND. A key step in differential diagnosis is molecular testing, as PLGD-causing Vibrio strains do not possess the AHPND-associated toxin genes pirAvp, pirBvp, AP1, and AP2. Therefore, polymerase chain reaction (PCR) assays targeting these genes can be used to rule out AHPND. Confirmatory diagnosis of PLGD is achieved through PCR amplification using primers specific to the novel virulence gene VHVP-2. The recommended primer set includes VHVP-2-P-F (forward primer: GGAGTATTGGTGGGCTGAAA) and VHVP-2-P-R (reverse primer: GGTAGGCATGGACCGTAAAG), which enable the specific detection of the PLGD-causing bacterial strain.

Histopathology

Histopathological analysis of the hepatopancreas in shrimp impacted by PLGD shows considerable cellular damage. One notable finding is the existence of detached epithelial cells, a sign of severe tissue disruption (noted by the red arrow in microscopic images). Furthermore, dense clusters of bacterial masses are often visible within the affected tissue (marked by black arrows), indicating significant bacterial colonisation and invasion. These microscopic characteristics offer essential evidence of the pathological consequences of PLGD and assist in distinguishing it from other shrimp diseases at the tissue level (image 1). Similar pathological alterations are observed in both the hepatopancreas and the intestinal tract. In both organs, a defining feature is the detachment of epithelial cells, signalling tissue injury and disruption of barriers (frequently highlighted with a red arrow in microscopic images). Additionally, bacterial masses are regularly seen (indicated by black arrows), emphasising the presence of extensive bacterial colonisation. These microscopic lesions in the gut and hepatopancreas underscore the systemic nature of PLGD and its aggressive effects on crucial digestive and absorptive tissues (Fig. 3)



(Jia et al., 2024)

Economic and Societal Repercussions

The economic burden of diseases like PLGD on the shrimp farming industry is profound and multifaceted, extending beyond direct production losses. The reduction in harvest yield is staggering regarding the financial impact of infectious disease. In India, the harvest yield loss from WSSV was 2.58 tons per hectare per crop, and the harvest yield loss from EHP was 1.80 tons per hectare per crop, contributing to the overall infectious disease-associated yield loss of 0.14 million tons worth US$1.02 billion annually. From a socio-economic impact perspective, the ramifications impacted feed sales, processing, and reduced export revenues. Socio-economically, the impacts were equally pressing, such as increasing unemployment, farmers' debt owing multiple farms, and expensive financial investment in the sector. Aggravating the situation in developing countries where smallholder farmers are more susceptible due to a lack of financial resources to access significant biosecurity measures and loss recovery to limit the risk of PLGD spread via the international movement of post-larvae and broodstock could facilitate PLGD to enter new regions and impact other countries' economies.

Control measures

Effective management and prevention of PLGD require chemical intervention and stringent biosecurity practices. Among tested treatments, polyhexamethylene biguanide hydrochloride (PHMB) has shown promising results, demonstrating high efficacy at a concentration of 1 ppm, with a relative per cent survival (RPS) of 65%. In addition to chemical treatment, maintaining optimal water quality is essential to reduce environmental stress that may predispose shrimp to infection. Implementing strict biosecurity protocols, including disinfection procedures, limiting personnel movement, and using pathogen-free water, is crucial in disease prevention. Furthermore, increased surveillance and early detection are vital

for a prompt response to outbreaks. Regulatory measures such as restricting the movement of shrimp seed and broodstock from affected regions are also recommended to prevent the transboundary spread of this highly lethal Vibrio strain.

Conclusion: New threat to the shrimp industry

The emergence of PLGD, driven by the new and more virulent strain of *V. parahaemolyticus*, Vp-JS20200428004-2, is a pivotal event for the global shrimp aquaculture sector. PLGD, due to its rapid onset, high mortality, and negative productivity impact on hatcheries, indicates the continued susceptibility to emerging pathogens in intensive shrimp farming systems. Like other disease crises (e.g., WSSV, AHPND), PLGD also reminds us that aquaculture is subject to a complex biological system, where pathogens are evolving, in many cases, faster than our ability to prevent and respond to their threats. Such diseases' economic and social impacts do not stop at the farm gate; they have ramifications in supply chains, export markets, and livelihoods in rural areas and significantly impact small-scale farming operations in disease-prone regions. Traditional controls of bacterial pathogens, such as *V. parahaemolyticus*, are becoming less effective, particularly antibiotics, and antimicrobial resistance is now a driving concern in the public sphere. There is an urgent need to consider new pathways and practices to introduce pathogen detection, biosecurity, and health management to protect aquaculture from emerging disease threats. The emergence of PLGD is not merely another event in the history of shrimp diseases; it is an alert that the industry must shift from crisis reaction to an integrated, prevention-based approach.

The future rests in combining science with system-level resilience through: (i) improved biosecurity, (ii) development of genetically robust shrimp lines, (iii) sustainable farming systems, such as biofloc, & RAS, and (iv) developing regional cooperation, early warning systems, and response measures for outbreaks. The industry is on a course heading ever more towards meeting the increasing food demands of the aquaculture industry, and its future survival lies in the fight against diseases, such as PLGD, which is ubiquitous in aquaculture today. As depicted within this publication, we can no longer rely on unquestioning optimism, the 'wonder drug' or reverting to animal husbandry for antibiotic or antimicrobial therapies. We now NEED vigilance, innovation, and most importantly, willful collaboration, coordination and planning for long-term industry sustainability.

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