

Updated roles of the gut microbiota in exploring shrimp etiology, polymicrobial pathogens, and disease incidence

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ABSTRACT

Litopenaeus vannamei is the most extensively cultured shrimp species globally, recognized for its scale, production, and economic value. However, its aquaculture is plagued by frequent disease outbreaks, resulting in rapid and massive mortality. etiological research often lags behind the emergence of new diseases, leaving the causal agents of some shrimp diseases unidentified and leading to nomenclature based on symptomatic presentations, especially in cases involving co- and polymicrobial pathogens. Comprehensive data on shrimp disease statuses remain limited. In this review, we summarize current knowledge on shrimp diseases and their effects on the gut microbiome. Furthermore, we also propose a workflow integrating primary colonizers, “driver” taxa in gut networks from healthy to diseased states, disease-discriminatory taxa, and virulence genes to identify potential polymicrobial pathogens. We examine both abiotic and biotic factors (e.g., external and internal sources and specific-disease effects) that influence shrimp gut microbiota, with an emphasis on the “holobiome” concept and common features of gut microbiota response to diverse diseases. After excluding the effects of confounding factors, we provide a diagnosis model for quantitatively predicting shrimp disease incidence using disease common-discriminatory taxa, irrespective of the causal agents. Due to the conservation of functional genes used in designing specific primers, we propose a practical strategy applying qPCR-assayed abundances of disease common-discriminatory functional genes. This review updates the roles of the gut microbiota in exploring shrimp etiology, polymicrobial pathogens, and disease incidence,

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offering a refined perspective for advancing shrimp aquaculture health management.

Keywords: Shrimp disease; Gut microbiota; Polymicrobial pathogens; Diagnosis model; Disease common-discriminatory taxa; Disease prediction

INTRODUCTION

Litopenaeus vannamei has emerged as one of the most successful aquaculture species due to its low food conversion ratio, contributing significantly to the “Blue Revolution”. Over the past 40 years, its production has increased from less than 0.075 million tons in 1980 to over 7.3 million tons in 2021 (FAO, 2023). This remarkable achievement is partially attributed to the development of large-scale production facilities, high-density farming practices, and intensive feeding regimens, which have also created stressful conditions that facilitate the frequent occurrence and rapid spread of diverse infectious diseases (Table 1). Although antibiotics are commonly applied as prophylactic and therapeutic agents, their overuse and abuse has led to bacterial resistance, increased disease susceptibility, and the emergence of new diseases (Lu et al., 2021; Rodgers & Furones, 2009; Sha et al., 2024a; Zhou et al., 2019a). Vaccination represents an alternative strategy for controlling infectious diseases; however, its efficacy is often limited in juvenile shrimp due to their inability to develop long-term acquired immunity (Falaise et al., 2016). Importantly, shrimp diseases cause rapid and massive mortality within days, often without noticeable symptoms at onset stage of disease (Kumar et al., 2020; Thitamadee et al., 2016; Xiong et al., 2017a). Several methods are currently used to evaluate disease risk in shrimp

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Table 1 Summary of shrimp diseases, potential causative agents, and typical symptoms

Disease name	Causative agent	Typical symptoms	References
White spot disease	White spot syndrome virus	Sharp reduction in food consumption, listlessness, loose cuticle, white spots 0.5–2.0 mm in diameter	Liu et al., 2009
Taura syndrome	Taura syndrome virus	Soft cuticle, empty guts, extreme lethargy, and expansion of reddish chromatophores	Brock, 1997
Yellow head disease	Yellow head virus	Nuclear pyknosis and heterokaryosis, bleached or pale-yellow cephalothorax, discoloration of underlying hepatopancreas	Boonyaratpalin et al., 1993
Tetrahedral baculovirosis	<i>Baculovirus penaei</i>	Whitish midguts in severe infections. Polyhedral occlusion bodies	Couch, 1974
Spherical baculovirosis	Monodon baculovirus	Hypertrophied nuclei with single or multiple eosinophilic occlusion bodies, chromatin diminution and margination	Chen et al., 1989
Infectious hypodermal and hematopoietic necrosis (IHHN)	IHHN virus	Buff-colored spots at carapace and abdominal plates, bluish, necrotic, melanized cuticle in serious disease	Lightner et al., 1983
Infectious myonecrosis (IMN)	IMN virus	Opaque skeletal muscle	Lightner et al., 1983
White tail disease	<i>Macrobrachium</i> nodavirus	Tails exhibiting white lesions	Arcier et al., 1999
Hepatopancreatic parvovirus disease	Hepatopancreatic parvovirus	Slowed growth rate	Chong & Loh, 1984
Hemocyste iridescent virus disease	Hemocyste iridescent virus	Hepatopancreatic atrophy with color fading, empty stomach and guts, and soft shell	Qiu et al., 2017
Running mortality syndrome	Nodavirus	Enlarged nuclei in hepatopancreas, coagulative muscle necrosis, whitening muscle, slow growth and softening shell	Zhang et al., 2014
Abdominal segment deformity disease	Unknown retrovirus-like agent	Enlarged or twisted abdominal segments, either laterally and/or dorsoventrally, sometimes opaque muscles	Sakaew et al., 2008
Slow growth syndrome	Laem Singh virus and a cryptic integrase containing element	Slow growth and coefficients of size variation > 35%	Chayaburakul et al., 2004
Acute hepatopancreatic necrosis disease	<i>Vibrio anguillarum</i> , <i>Vibrio parahaemolyticus</i>	Sloughing tubule epithelial cells, pale hepatopancreas, massive and rapid mortality	Tran et al., 2013
Necrotizing hepatopancreatitis	<i>Rickettsia</i> -like bacterium	Reduced feeding activity, empty guts, pale hepatopancreas	Chayaburakul et al., 2004
“Bright-red” syndrome	<i>Vibrio harveyi</i>	Lethargy, anorexia, flaccid body, multifocal reddish discoloration spots on abdominal cuticle, melanized erosions around spots	Soto-Rodriguez et al., 2010
Luminous vibriosis	<i>Vibrio campbellii</i>	Symptoms include fluorescent, less food consumption and high mortality	Wang et al., 2015
White feces syndrome	Uncertain <i>Vibrio</i>	White-golden gut contents and white fecal strings	Durai et al., 2015
Black gill disease	<i>Fusarium solani</i> , <i>Fusarium moniliforme</i> , <i>Aspergillus awamori</i>	Changes from “gill discoloration” to “blackened gill” with disease progression, with subsequent death	Hatai & Egusa, 1978
Balantidiasis	<i>Zoothamnium</i>	Reduction in feeding and growth. Increased risk of secondary bacterial infections and massive mortality	Nurlatiffah et al., 2018
Hepatopancreatic microsporidiosis	<i>Enterocytozoon hepatopenaei</i>	Severely stunted growth	Chayaburakul et al., 2004
Amoebic gill disease	Amphizoic amoeba	Reduced appetite, lethargy, respiratory distress, eroded carapace, and blackened gill	Han, 2019
“Cotton shrimp-like” disease	Uncertain fungi	Reduced growth, atrophic hepatopancreas, empty digestive tract, and inactivity. Soft shell with slightly white, opaque muscle	Zhou et al., 2019b
Blue body syndrome	Unknown	Slow growth, reduced or no feed intake, thin, partially or fully blue bodies	Liang et al., 2020

aquaculture, including water quality indices, *Vibrio* species density, pathogen diagnostics, as well as artificial intelligence and machine learning techniques (Bohara et al., 2024; Ferreira et al., 2011; MacAulay et al., 2022). However, these approaches are predominantly qualitative, rather than quantitative, and lag behind the detection of early stages of disease onset stage. Therefore, there is an urgent need to establish quantitative strategies for predicting the incidence of shrimp disease.

The gut microbiota plays an indispensable role in maintaining shrimp fitness and health by aiding in digestion, vitamin synthesis, immunity regulation, toxin degradation, and pathogen suppression (Cornejo-Granados et al., 2018; Holt et al., 2021; Sha et al., 2024b; Xiong, 2018). As such, the gut microbiota acts as a central hub, integrating environmental exposure with genetic and immune signals to influence shrimp fitness. In healthy shrimp, optimal balance of gut symbionts provides numerous benefits, while an imbalanced gut microbiota increases disease risk (Holt et al., 2021; Xiong, 2018). Researches on human and terrestrial animal

microbiomes have established cause-and-effect relationships, demonstrating that a healthy gut microbiome influences the colonization, growth, and virulence of pathogens, thereby affecting disease outcomes (Bäumler & Sperandio, 2016; Kamada et al., 2013). However, comparable studies on the causal roles of the gut microbiota in aquatic invertebrates such as shrimp are scarce (Sha et al., 2024b). High-throughput sequencing (HTS) and metagenomics have provided increasing evidence that the gut microbiomes of diseased shrimp differ significantly from those of healthy individuals (Holt et al., 2021; Mao et al., 2023; Sha et al., 2022b), a condition known as “dysbiosis”. This suggests that sensitive gut commensals could serve as reliable indicators for diagnosing shrimp health. Growing evidence suggests that the extent of deviation from the healthy gut microbiota may be linearly correlated with the severity of shrimp disease (Chen et al., 2017; Lu et al., 2023; Xiong et al., 2017a). Consequently, gut bacterial indicators could be used to diagnose the onset and severity of shrimp diseases.

The causal links between gut microbiota and shrimp health

remain relatively unexplored compared to the extensive research conducted on humans. Advances in sequencing technologies have significantly enhanced our understanding of the interactions between shrimp health and their gut microbiota. Nevertheless, much remains to be discovered regarding the shared and unique responses of the gut microbiome to diverse shrimp diseases. Recent reviews have highlighted external factors influencing shrimp microbiota (Cornejo-Granados et al., 2018; Holt et al., 2021) and diagnostic approaches ranging from traditional visual inspection and histopathology to portable sequencing and artificial intelligence for early disease detection (Bohara et al., 2024; MacAulay et al., 2022). In contrast, this review focuses on the current state of shrimp diseases and their effects on the gut microbiota. Going a step further, we conclude the common features of gut microbiota responses to diverse shrimp diseases and their potential applications in predicting disease incidence. While the concept of co- and polymicrobial pathogens is accepted in human and veterinary medicine (Frisan, 2021), it has received little attention in aquaculture. Given the challenges of identifying polymicrobial pathogens using Koch's postulates, we propose an integrated workflow that incorporates diverse ecological features to identify potential polymicrobial pathogens.

CURRENT STATE OF SHRIMP DISEASES

One of the primary challenges in shrimp aquaculture is the frequent occurrence of disease outbreaks, leading to rapid and massive mortality (Seibert & Pinto, 2012; Thitamadee et al., 2016). Over the past two decades, scientific research on shrimp diseases has intensified in response to the global expansion of shrimp production. Shrimp are susceptible to infections from a wide range of pathogens across microbial kingdoms, including viruses, bacteria, fungi, and parasites, throughout the entire aquaculture cycle (Table 1). Despite periodic updates from the World Organization for Animal Health (WOAH) on the status on shrimp diseases, a comprehensive understanding is still lacking. The following section provides a brief summary of current knowledge on shrimp diseases and their impact on the gut microbiome.

Viral pathogens

Approximately 20 viruses have been identified as causative agents of shrimp diseases, including members of the Parvoviruses, Baculoviruses, Picornaviruses, Toga-like viruses, as well as several newly identified viral families (Arulmoorthy et al., 2020). Among these, white spot syndrome virus (WSSV) poses the most significant threat to shrimp health globally, leading to substantial production losses (Flegel, 2006; Liu et al., 2009). WSSV, an ovaloid, double-stranded DNA virus with a lipid envelope (120–150 nm×270–290 nm), infects the nuclei of mesodermal- and ectodermal-derived tissues, resulting in reduced food intake and lethargy in infected shrimp (Pradeep et al., 2012). Intriguingly, WSSV can attach to abiotic microplastics, thereby prolonging their survival and virulence under varying water pH and temperatures (Shan et al., 2023). This persistence in abiotic hosts may contribute to the recurrence of WSSV infections. Since its first report in the early 1990s, global economic losses attributed to WSSV have been established at approximately \$15 billion USD, increasing at an alarming rate of \$1 billion USD per year and accounting for a 10% loss in global shrimp production (Panchal et al., 2021). Currently,

there is no effective strategy to prevent and control this disease. WSSV infection significantly alters the composition of the shrimp gut microbiota, leading to enrichment in Proteobacteria and Fusobacteria phyla, as well as pathogenic bacteria belonging to the *Arcobacter* and *Flavobacterium* genera (Wang et al., 2019). While gut bacterial diversity and richness remain unaffected, WSSV infection increases the heterogeneity of gut bacterial communities (de Souza Valente et al., 2020). Additionally, prior WSSV infection predisposes shrimp to subsequent *Vibrio campbellii* infections, resulting in more rapid and severe mortality compared to *V. campbellii* alone (Phuoc et al., 2008).

In addition to WSSV, yellow head virus (YHV), specifically genotype one among six genetically related genotypes, is a rod-shaped, enveloped positive-sense single-stranded RNA virus (40–60 nm×150–200 nm). YHV induces persistent and severe disease in shrimp (Boonyaratpalin et al., 1993), although major outbreaks have not been reported in recent years. YHV has been detected exclusively in outdoor ponds, but not in covered ponds, suggesting an airborne mode of transmission (Thitamadee et al., 2016). However, potential carriers and non-aquatic insects have tested negative for YHV, casting doubt on the airborne vector hypothesis. The rapid dissemination of YHV may be attributed to the cannibalistic behavior of YHV-infected individuals (Tuyen et al., 2014). The initial source of YHV infection remains unclear, but the close genetic relationship among YHV genotypes suggests that it may naturally infect wild penaeid or palemonid shrimp (Wijegoonawardane et al., 2008). Surprisingly, limited research has been conducted on the response of the shrimp gut microbiota to YHV infection.

Decapod iridescent virus 1 (DIV1), a recently identified icosahedral linear double-stranded DNA virus, induces stunted growth and 100% cumulative mortality in shrimp (Qiu et al., 2017). DIV1 infection reduces the abundance of potentially beneficial populations of Bacteroidetes, Firmicutes, and Actinobacteria, while significantly enriching pathogenic bacteria such as *Vibrio* and *Photobacterium*. These microbial shifts elevate the risk of secondary bacterial infections. Furthermore, the proliferation of pathogenic bacteria triggers the expression of NF-κB inhibitors, which suppress the TLR-mediated immune response, exacerbating DIV1 infection (Liao et al., 2023).

In summary, viral infections frequently disrupt the composition of shrimp gut microbiota, increasing susceptibility to secondary bacterial infections (Liao et al., 2023; Shan et al., 2023). However, the specific alterations in bacterial populations appear to be dependent on the viral pathogen involved.

Bacterial pathogens

Vibriosis is the most prevalent and severe bacterial disease endangering shrimp aquaculture, caused by gram-negative *Vibrio* bacteria such as *V. harveyi*, *V. parahaemolyticus*, *V. vulnificus*, and *V. anguillarum* (Kumar et al., 2020; Li et al., 2022; Shen et al., 2021a). These diseases are typically elicited by external stressors and, if left untreated, can result in rapid and extensive mortality. Although many *Vibrio* species are opportunistic pathogens, certain strains, such as *V. harveyi* and *V. parahaemolyticus*, can act as acute pathogens (Dhar et al., 2019; Zhou et al., 2012). The *V. harveyi* clade comprises luminous bacteria commonly detected in coastal and marine waters, as well as in the skin and gut of aquatic

organisms (Ina- Salwany et al., 2019; Vandenberghe et al., 2003). *Vibrio harveyi* infections can cause multiple shrimp diseases, including “bright-red syndrome” (Soto-Rodriguez et al., 2010), “bacterial white tail disease” (Zhou et al., 2012), and luminescent vibriosis (Mandal & Das, 2018; Wang et al., 2015). In addition, *V. parahaemolyticus* carrying the *pirAB* gene can cause acute hepatopancreatic necrosis disease (AHPND) (Han et al., 2015; Tran et al., 2013) and red disease in shrimp (Jayasree et al., 2006). In addition to *Vibrio*, other pathogenic genera have been reported, such as *Shewanella algae*, which causes black spot disease (Cao et al., 2018), *Acinetobacter venetianus*, which causes red leg disease (Huang et al., 2020a), and *Streptocephalus sirindhornae* and *Branchinella thailandensis*, which cause black disease (Saejung et al., 2011). Notably, some *Vibrio* species, such as *V. hepatarius* and *V. diabolicus*, are non-pathogenic and serve as probiotics that protect shrimp from infection (Ramirez et al., 2022; Restrepo et al., 2021). Consequently, traditional monitoring of *Vibrio* species density may erroneously suggest an elevation of disease risk.

Gut bacterial communities in shrimp are disrupted upon exposure to *V. harveyi*, with amplicon sequence variants (ASVs) from the Harveyi and Vulnificus clades becoming enriched (Angthong et al., 2023). Interestingly, the resilience of the gut microbiota can mitigate shrimp mortality during *V. harveyi* infection (Rungrassamee et al., 2016). *Vibrio* strains carrying the pVA1 plasmid, which encodes the binary *pirAB* toxin genes, are the causal agents of shrimp AHPND (Han et al., 2015). The virulent plasmid pVA1 harbors a post-segregational killing system, enabling its stable inheritance and dissemination among *Vibrio* species (Kumar et al., 2020) and non-*Vibrio* and non-pathogenic strains, such as *Algoriphagus* species, via conjugative transfer (Muthukrishnan et al., 2019). These molecular characteristics partially explain the difficulty in controlling shrimp AHPND. Indeed, AHPND poses a serious threat to the shrimp industry worldwide, often causing extensive or complete mortality within days (Kumar et al., 2020). The occurrence of AHPND substantially reduces α -diversity and the stability of the gut bacterial community, while increasing the variation coefficient and ecological dysbiosis indices compared to healthy shrimp (Dong et al., 2021). Of note, the gut microbiota exhibits different responses to infection by AHPND-causing *V. parahaemolyticus* (Vp) compared to common pathogenic Vp. Specifically, AHPND-causing Vp infection results in rapid shifts in the gut bacterial community, with enrichment of *Photobacterium* and *Vibrio* genera. In contrast, common Vp infection increases the abundance of gut *Motilimonas* and *Ruegeria* genera (Chang et al., 2023). Furthermore, variations in the bacterioplankton community in rearing water correlate with the virulence of Vp (Aguilar-Rendón et al., 2022). Similarly, elevated nutrient levels enhance the virulence of AHPND-causing Vp (Li et al., 2022), leading to quicker dysbiosis in the gut microbiota and earlier and higher mortality in shrimp compared to AHPND-causing Vp precultured at oligotrophic condition (Sha et al., 2022a). Intriguingly, the gut microbiota responds linearly to increasing shrimp AHPND severity, enabling accurate diagnosis of progressed stages of AHPND using gut indicators (Lu et al., 2023; Shen et al., 2021a).

Fungal pathogens

Fungi are prevalent in aquatic environments, with approximately 500 species isolated from marine and estuarine habitats, including several that act as opportunistic pathogens

of shrimp (Karunasagar et al., 2004). Fungal infections typically result in chronic and steady losses, causing lower mortality rates compared to viral and bacterial infections (Paulraj et al., 2016). Common pathogenic fungi include *Lagenidium callinectes* and *Sirolopidium* species (Karunasagar et al., 2004), which preferentially infect larval shrimp. These pathogens enter larvae through antennae, eyestalks, and body tissues, ultimately causing death. Fungal spores and mycelia are detectable in targeted tissues, particularly the gills and appendages, rather than in the shrimp gut and hepatopancreas (Karunasagar et al., 2004). Endophytic *Fusarium* species, known plant pathogens producing fumonisins and trichothecenes that affect human and animal health (Munkvold, 2017), are also causal agents of shrimp black gill disease (BG), leading to chronic mortality over prolonged infections (Khoa et al., 2004). Research on the effects of fungal pathogens on the shrimp gut microbiota is limited compared to studies on viral and bacterial infections. Some studies indicate that fungal infections alter gut microbiota composition. For example, shrimp with BG harbor higher levels of the pathogenic fungus *Candida*, and reduced abundances of *Didymella* and *Filobasidium* in the gut (Li et al., 2019). However, gut microbial diversity remains comparable between healthy and BG-affected cohorts (Song et al., 2022). Although many fungi are saprophytic or parasitic, certain strains, such as *Aspergillus niger* and *A. fumigatus* are beneficial for shrimp growth, health, and antifungal immunity (Brzezinska & Jankiewicz, 2012; Li et al., 2012; Zhang et al., 2023).

Parasitic pathogens

Parasites are detrimental to their hosts as they exploit host nutrients for their own utilization, adversely affecting host immunity and growth. Various parasites, particularly protozoa, impact shrimp at different developmental stages (Table 1). Among these, *Enterocytozoon hepatopenaei* (EHP) has emerged as an important pathogen in shrimp aquaculture (Aranguren et al., 2017). EHP is an intracellular and microsporidian parasite that causes shrimp hepatopancreatic microsporidiosis, leading to stunted growth (Chayaburakul et al., 2004). Shrimp size is inversely associated with EHP load (Shen et al., 2021b). Environmental reservoirs can harbor EHP, resulting in recurrent infections in shrimp populations (Raj et al., 2023). EHP infection follows a cyclic pattern, where shrimp with severe infections can regress to milder infection, but never fully recover. This parasite-host interaction impairs shrimp health, leading to stunted growth and low mortality (López-Carvalho et al., 2022). Although EHP infection does not cause acute mortality, it increases shrimp susceptibility to subsequent microbial infections, including *Vibrio* species (Shen et al., 2021a), and WSSV (Suryakodi et al., 2022). The impact of EHP on the shrimp gut microbiota is less understood compared to bacterial and viral pathogens. The gut microbiota is affected by EHP infection severity, with enrichment of *Vibrio*, *Pseudomonas*, and *Bradyrhizobium* species (Shen et al., 2021a). EHP infection progression displaces beneficial microbes with opportunistic/pathogenic bacteria and fungi, depleting energy reserves. Specifically, the late stages of EHP infection are marked by an increase in gut bacterial genera such as *Leadbetterella*, *Aquimarina*, and *Vibrio*, and the fungal genus *Malassezia* (López-Carvalho et al., 2022). EHP infection causes greater dispersion, lower modularity, and a decrease in the proportion of negative associations and keystone taxa within the gut network, resulting in an unstable gut microbiota

(Shen et al., 2022). These alterations, in turn, compromise the capacity of shrimp to combat invading pathogens (Xiong, 2018). Chronic EHP infection progressively disrupts the bacterial communities in the gut and hepatopancreas (López-Carvalho et al., 2022; Shen et al., 2022). Consequently, gut EHP-discriminatory taxa can accurately distinguish healthy shrimp from EHP-infected individuals with infection progression (Shen et al., 2022). It is important to note that not all parasitic protozoa are harmful; some, such as certain phagotrophs, can be commensal or beneficial to shrimp health. For example, gut phagotrophs can directly prey on bacterial pathogens, thereby supporting shrimp health through interkingdom predator-prey interactions (Lu et al., 2022).

Emerging and syndromic diseases

Given the lag in etiological studies, the causal agents of various shrimp diseases are unknown. In such cases, the emerging and syndromic diseases are named based on typical symptoms, such as white feces syndrome (WFS) (Durai et al., 2015), “blue body syndrome” (Liang et al., 2020), and “cotton shrimp-like” disease (Zhou et al., 2019b) (Table 1).

Shrimp WFS typically manifests 50 to 60 days post-inoculation, causing persistent mortality, reducing survival rates by 20% to 30% and decreasing yield by up to 60% (Durai et al., 2015). WFS is characterized by white-golden gut contents and white fecal strings, but its complex etiology remains inconclusive. In this context, efforts devoted to the exploration of the etiological factors of WFS could significantly contribute to the current paradigm. The etiology of WFS has been proposed as a co-infection involving EHP and opportunistic marine pathogens, including *Vibrio* and *Propionigenium* species (Sriurairatana et al., 2014; Subash et al., 2023). Nevertheless, experimental challenges using these pathogens have failed to satisfy Koch’s postulates (Aranguren Caro et al., 2021; Munkongwongsiri et al., 2022). Transplantation of gut microbiota from WFS-affected shrimp to healthy subjects induces WFS in recipients, suggesting a causal role for gut dysbiosis in WFS (Huang et al., 2020b). Shrimp infected with WFS show enrichment of various gut bacteria, such as *Candidatus Bacilloplasma*,

Phascolarctobacterium, and *Vibrio*, but decreased levels of *Paracoccus* and *Lactococcus* based on field and microcosm studies (Boopathi et al., 2023; Hou et al., 2018; Huang et al., 2020b). Both gut bacterial and microeukaryotic disease-discriminatory taxa indicate the progression of WFS in shrimp (Dai et al., 2019a; Xiong et al., 2017a). While these findings collectively suggest that gut microbiota dysbiosis is implicated in WFS etiology, the causal pathogens involved in WFS remain uncertain. Recent studies integrating primary colonizers, “driver” taxa in gut networks from healthy and WFS-affected shrimp, WFS-discriminatory taxa, and encoding virulence genes have identified *V. fluvialis*, *V. coralliilyticus*, and *V. tubiashii* as potential WFS pathogens (Dai et al., 2018; Lu et al., 2020). The causal roles of the three pathogens in shrimp WFS have been further validated through pure culture experiments (Sha et al., 2024b). Given ongoing debates, other pathogens implicated in shrimp WFS cannot be ruled out, meriting further corroborative research.

Based on previous studies, we propose a workflow for identifying potential polymicrobial pathogens (Figure 1). Initially, the taxonomies of the microbial community are assigned by blasting against an up-to-date pathogen database with strict criteria, including 100% similarity (Lo et al., 2023). Due to the high temporal dynamics of the gut microbiota as shrimp age (Figure 2A) (Xiong et al., 2020), age-discriminatory taxa are excluded to account for host age effects (Figure 2B). After this optimization, primary responders, which are absent from healthy individuals and show linear enrichment during disease progression, are extracted from the community. Next, keystone taxa are identified using a causal inference algorithm integrated with dynamic intervention simulation (Wu et al., 2022). The removal of these keystone taxa results in network fragmentation (Dai et al., 2019b). These potential pathogens can serve as biomarkers for accurately diagnosing shrimp health status. Finally, candidate polymicrobial pathogens are confirmed using molecular approaches, such as pathogen-specific virulence genes. The infection order and ratio of the polymicrobial pathogens can be inferred by tracing back to the gut microbiota in disease-progressed individuals (Figure 1). This workflow provides a robust solution for identifying

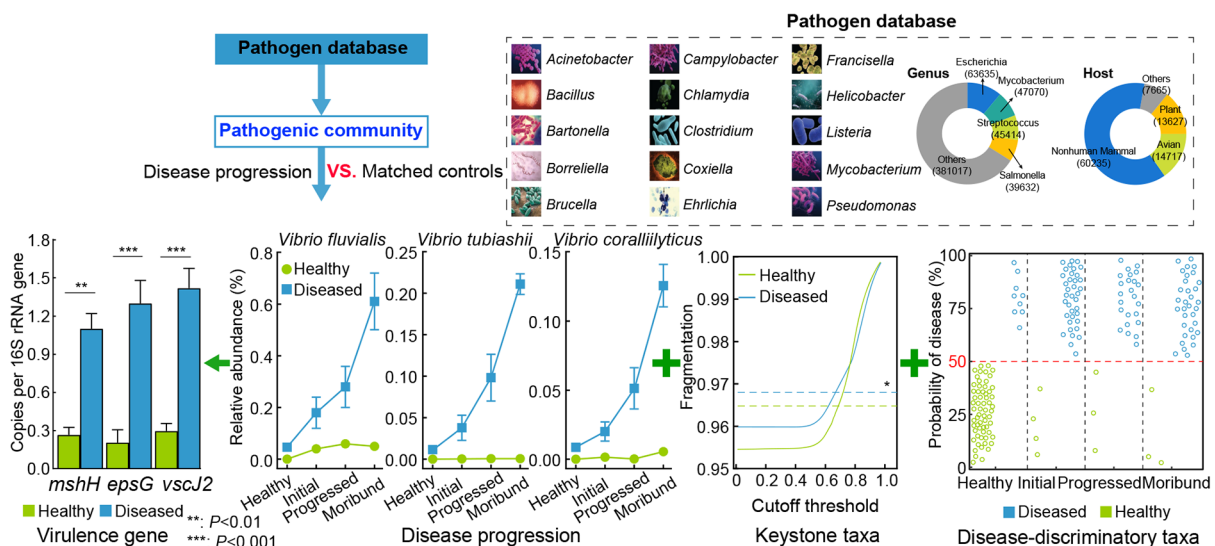


Figure 1 Proposed workflow for identifying potential polymicrobial pathogens

Polymicrobial pathogens are identified based on high similarity with pathogens, linear enrichment over disease progression, keystone taxa, disease-discriminatory taxa, and pathogen-specific virulence genes.

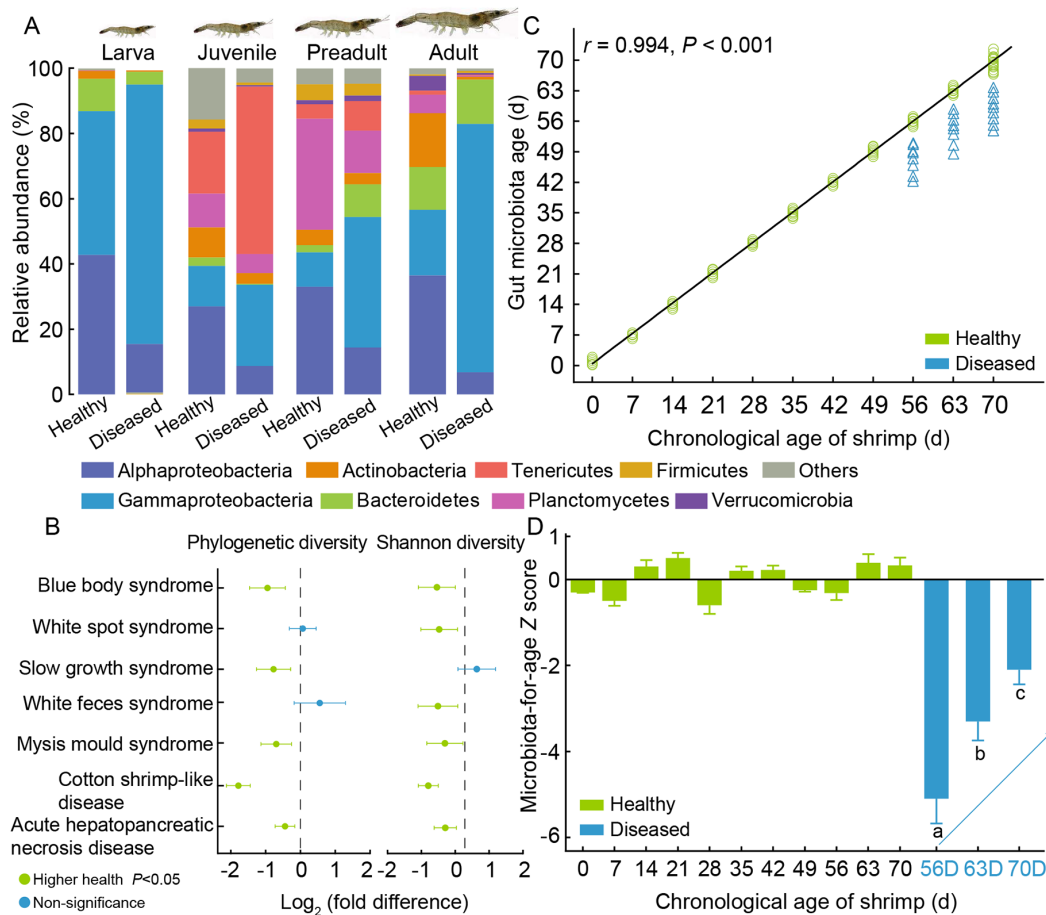


Figure 2 Gut microbiota between shrimp health status and over life stages

A: Comparison of dominant gut bacterial phyla or classes between healthy and diseased shrimp at each life stage. B: Gut microbial diversity showing no consistent change between shrimp health status. C: Regression between actual age of healthy shrimp against predicted age using age-discriminatory taxa profiles. Predicted age of diseased shrimp significantly deviated from linear fit. D: Microbiota maturity expressed as a microbiota-for-age Z score, with mean \pm standard deviation. Differences in microbiota maturity at different ages between healthy (green bar) and diseased (blue bar) shrimp based on *post-hoc* Tukey's multiple comparison.

polymicrobial pathogens (Dai et al. 2018; Lu et al. 2020). The efficacy of this workflow is exemplified in our recent work, which identified *V. fluvialis*, *V. coralliilyticus*, and *V. tubiashii* as the causative pathogens of shrimp WFS (Sha et al., 2024b).

Running mortality syndrome (RMS) leads to persistent low-level mortality throughout the shrimp culture period (Zhang et al., 2014). RMS-affected shrimp are negative for all major WOAH-listed pathogens as well as common pathogens, such as WSSV, YHV, and *Vibrio* species. Moreover, cohabitation of healthy and RMS-affected shrimp does not result in RMS transmission (Alavandi et al., 2019). The incidence of RMS is positively associated with high stocking density, elevated nitrite levels, and increased turbidity, indicating that RMS may be related to pond ecosystem conditions rather than being infectious in nature. However, recent research has identified covert mortality nodavirus (CMNV) as the causal agent of RMS. CMNV infection suppresses the expression of genes involved in digestion, absorption, and growth hormones, which is consistent with the stunted growth observed in shrimp infected with CMNV (Liu et al., 2022).

The two aforementioned diseases reinforce that our understanding of the etiology of newly emerging shrimp diseases is still lacking. Given deteriorating water quality, increasing stocking density, and extreme weather events, this

knowledge gap is likely to persist. However, the identification of causal agents for emerging and syndromic diseases can be achieved through the dedicated efforts of aquatic scientists and the advancement of new technologies.

OVERVIEW OF FACTORS AFFECTING GUT MICROBIOTA OF SHRIMP

Bacterioplankton and shrimp coexist in their rearing environments, forming a mutually beneficial equilibrium characterized by synergistic interactions. The most significant association occurs in the gut, where the microbiota begins to establish from the egg stage, acquiring microbes from the surrounding water, initial feed, and younger individuals (Xiong, 2018; Zhang et al., 2021a). It is increasingly clear that the gut microbiota plays indispensable roles in shrimp fitness, immunity, and health (Holt et al., 2021; Xiong et al., 2019a). Regulating the gut microbiome offers a promising strategy for disease prevention in shrimp aquaculture. However, developing effective approaches requires knowledge of the factors influencing the shrimp gut microbiota. Extensive research has identified various factors affecting the shrimp gut microbiota, including diet (Arce et al., 2021), rearing water salinity (Deris et al., 2022; Hou et al., 2020), temperature (Xiong et al., 2015), nitrite exposure (Huang et al., 2020c), chronic hypoxia (Sun et al., 2020), and stock density (Deng

et al., 2019). In addition to these abiotic variables, biotic factors also strongly influence the gut microbiota, including life stage (Xiong et al., 2020), pathogen exposure (Angthong et al., 2023), disease severity (Xiong et al., 2017a), causal agents (Song et al., 2022), and interkingdom predator-prey interactions (Lu et al., 2022). Meta-analysis has shown that water type (seawater or freshwater) is the primary factor shaping the shrimp gut microbiota, followed by lifestyle (wild-type, farm, or laboratory), life stage, diet, and health status (Cornejo-Granados et al., 2018). Correspondingly, our recent research on marine-cultured shrimp highlighted the impact of life stage and different diseases on shaping the gut microbiota (Mao et al., 2023). While it has been suggested that microbial communities exhibit functional redundancy due to their high diversity (Miki et al., 2014), this redundancy diminishes when disturbances, such as abrupt changes in geochemical variables or host diseases, exceed the buffer capacity of the gut microbiome (Lu et al., 2022; Zhu et al., 2016). Thus, the gut microbiome is frequently altered by disturbances, leading to physiological disorders in shrimp and increased susceptibility to infections (Chen et al., 2017; Holt et al., 2021; Huang et al., 2021; Xiong et al., 2015). These lines of evidence, among others, demonstrate that the shrimp gut microbiota is highly dynamic, rather than static, and is consistently governed by both biotic and abiotic variables.

Koch's postulates have been updated and reformulated to encompass pathogens, local milieu, and susceptible individuals as collective contributors to disease (Sultana et al., 2017; Xiong & Shi, 2024). Under this scenario, it is necessary to consider the shrimp farming system holistically, using the "holobiome" concept, which includes microbiomes in rearing water, sediment, feed, and host, along with their interactions (Gutiérrez-Pérez et al., 2022). Following this logic, disease occurrence can be attributed to an imbalance among pathogens, hosts, and the environment, with the gut microbiota serving as a critical link between the host and the whole system (Engering et al., 2013). Recent research on the shrimp microbiome has thus focused on optimizing the gut microbiome through dietary supplements, probiotics, and prebiotics (Goh et al., 2022). To date, however, available approaches have proven inefficient and unsustainable in the field, despite promising results in microcosm and mesocosm studies. A plausible explanation is that healthy shrimp also exert strong selection on external taxa (Kamada et al., 2013; Xiong, 2018; Xiong et al., 2019a), hindering the successful establishment of applied probiotics. Additionally, as diseases cause a gradual deviation from a healthy state, the pathobiome is expected to evolve, leading to specific symbiont profiles (pathobiomes) at the onset, progression, and moribund stages of shrimp diseases (Lu et al., 2023; Shen et al., 2021a; Xiong et al., 2017a). Addressing this challenge requires the design of therapeutic strategies tailored to shrimp age and disease stage (Xiong et al., 2016). From this perspective, integrative experimental designs are essential to identify the "holobiome" components that significantly influence the shrimp gut microbiome and drive disease progression. Understanding these factors will enable the development of targeted strategies to optimize shrimp cultivation through effective gut microbiome management.

SOURCES OF SHRIMP GUT MICROBIOTA

Aquatic animals are continuously exposed to their rearing water, and shrimp, being filter feeders, are assumed to

acquire their gut commensals primarily from bacterioplankton communities in rearing water (De Schryver & Vadstein, 2014). However, research indicates that the microbial communities in rearing water and shrimp gut are distinct (Chen et al., 2017; Cornejo-Granados et al., 2017; Xiong et al., 2019b). As benthic animals, shrimp also interact with sediment microbial communities, which serve as an important external species pool for the gut microbiota. Compared to rearing water, sediment bacterial communities are more similar to those in the shrimp gut (Cornejo-Granados et al., 2017; Fan et al., 2019; Huang et al., 2021), contributing 36% and 75% of gut symbionts in juveniles and adults, respectively (Zhang et al., 2021a). Interestingly, the gut microbiota in adult shrimp is primarily inherited from the gut commensals of juveniles, forming an internal species pool (Zhang et al., 2021a, 2021b). Indeed, the shrimp gut microbiota is only weakly influenced by the geochemical variables and microbial communities in the rearing water (Xiong et al., 2019b; Zhang et al., 2021b). One possible explanation is that shifts in the gut microbiota lag behind shifts in the bacterioplankton community and geochemical variables. Alternatively, healthy shrimp may be resilient to mild stressors, with their gut microbiota primarily affected by host physiological parameters (Huang et al., 2018). This assertion is supported by observations that the proportion of gut commensals sourced from the ambient bacterioplankton community increases gradually during disease progression, due to compromised selection on surrounding taxa over the same timeframe (Lu et al., 2023; Xiong et al., 2018). Given the open circulatory system of invertebrates, shrimp gut symbionts may also disperse from other organs, such as the hepatopancreas. A high proportion of bacterial taxa are shared between the shrimp gut and hepatopancreas, serving as species pools for each other (Bao et al., 2024). Notably, dramatic changes in rearing conditions, such as elevated temperature and eutrophication, can markedly enhance pathogen virulence, resulting in earlier and higher shrimp mortality (Li et al., 2022; Sha et al., 2022a). Considering that disease results from an imbalance among the host, environmental variables, and ambient microbes (Mallon et al., 2015; Xiong, 2018), it is crucial to systematically explore the microbiomes across rearing water, sediment, and gut in relation to shrimp health status. Such exploration is essential for understanding pathogen transmission and etiological mechanisms.

DIVERSITY AS A BIOMARKER OF DISEASE

Ecologically, a diversity-poor community is more susceptible to invasion by external species compared to a diversity-rich community, known as the diversity-stability relationship (Wang & Loreau, 2014). The rationale behind a positive diversity-stability relationship is that a diverse community confers an insurance effect, where high diversity increases the presence of functionally versatile and redundant species against disturbances, and a negative covariance effect, where losses in some taxa due to disturbance are compensated by gains in others (Cadotte et al., 2012). Given the crucial role of the gut microbiota in antagonizing invading pathogens, numerous studies have compared the gut microbial diversity between healthy and diseased shrimp, revealing a decrease in alpha-diversity in infected shrimp (Shen et al., 2021a; Song et al., 2022; Yao et al., 2018). However, a reduction in diversity is not universally observed in diseased individuals, with some studies reporting comparable (Wang et al., 2019; Xiong et al.,

2017b) or significantly increased diversity in infected shrimp (Zhou et al., 2019b) (Figure 2C). These discrepancies can be attributed to the type (e.g., causal agents) and strength (e.g., disease severity) of disturbance and the metrics used to measure diversity (e.g., weighted Shannon diversity, unweighted observed species). For example, we recently found that gut bacterial diversity is comparable between healthy and diseased shrimp at the disease onset stage (Chen et al., 2017; Lu et al., 2020; Xiong et al., 2017a). As the disease progresses, pathogen colonization outcompetes resident gut microbes, resulting in reduced microbial diversity. However, some resident species may benefit from the ecological niches created by the infection. In addition, diseased shrimp may attenuate their selective effect on external taxa, allowing for the random establishment of surrounding species (Mao et al., 2023; Sha et al., 2022b). Consequently, there is no consistent pattern of decreased gut microbial diversity in diseased shrimp (Figure 2C). Moreover, diversity is strongly affected by sequencing depth, making it challenging to compare diversity across studies. Therefore, declining gut microbial diversity is not a reliable indicator of shrimp health.

COMMON FEATURES OF GUT MICROBIOTA IN RESPONSE TO SHRIMP DISEASES

A growing body of evidence indicates that shrimp diseases significantly alter the gut microbiota, supporting the notion that gut dysbiosis may be a causal agent of shrimp diseases (Anghong et al., 2023; Chen et al., 2017; Cornejo-Granados et al., 2017; Huang et al., 2020b). However, this “chicken-and-egg” dilemma requires sophisticated experiments to disentangle the causes and mechanisms. According to the Anna Karenina principle, all healthy microbiomes are alike, whereas each disease-associated microbiome is unique to the diseased individual (Zaneveld et al., 2017). This principle suggests that host diseases induce heterogeneity and stochasticity in the gut microbiota. Following this logic, extensive studies have compared the heterogeneity of gut microbiota between healthy and diseased shrimp. Generally, shrimp diseases increase beta-dispersion, average variation degree, temporal turnover rate, and dysbiosis indices of the gut microbiota (Lu et al., 2023; Mao et al., 2023; Yao et al., 2018). However, the consistency of these signatures remains controversial. Shrimp with severe disease appear to exhibit a more stable gut microbiota over time compared to healthy controls (Lu et al., 2020; Xiong et al., 2017a). A plausible explanation is that the gut microbiota is not resilient to intense disturbance, such as severe disease.

According to the holobiont theory, hosts selectively acquire gut commensals from their surrounding environments to improve their fitness (Bordenstein & Theis, 2015). This suggests that the gut microbiota of healthy shrimp is predominantly shaped by deterministic processes, including selection pressures from abiotic environmental variables, biotic host filtering, and interspecies interactions (Cavender-Bares et al., 2009; Nemergut et al., 2013), leading to predictable community structures over space and time. Accordingly, the gut microbiota exhibits a time-similarity decay relationship throughout shrimp development (Xiong et al., 2020). This pattern is fundamental, as deviations from the temporally defined trend are positively associated with disease severity. Conversely, the occurrence of shrimp diseases weakens the relative importance of deterministic processes

governing the gut microbiota, regardless of the causal pathogens (Mao et al., 2023; Sha et al., 2022b). Diseased shrimp typically exhibit a sharp reduction in food consumption (Table 1) and may redirect energy towards immune responses. Consequently, the ability of diseased shrimp to selectively filter external taxa becomes compromised, resulting in increased inter-individual variation and a more stochastic assembly of the gut microbiota.

Although extensive studies have illustrated the effect of environmental and host selection on the gut microbiota, few have explored the role of interspecies interactions during shrimp disease progression (Bäumler & Sperandio, 2016; Lu et al., 2023; Xiong et al., 2018). Characterizing these interactions is critical, as resident gut microbes can directly compete for nutrients or facilitate resistance against invading pathogens (Coyte et al., 2015). The strength of these interspecies interactions is a good predictor of susceptibility to pathogenic disease (Banerjee et al., 2018). In general, gut network stability and complexity are disrupted by shrimp diseases, as evidenced by diverse topological properties, including reduced robustness, modularity and proportion of negative associations, as well as increased vulnerability (Sha et al., 2024b; Xiong et al., 2018; Yao et al., 2018). Among network nodes, gut keystone species exert disproportional roles in sustaining the stability, dynamics, and function of the gut microbiota, making them excellent candidate targets for gut microbiota-based interventions (Dai et al., 2019b; Shetty et al., 2017; Wu et al., 2022). Case studies have shown that one keystone strain can effectively inhibit or eliminate one pathogen, such as *Clostridium scindens* against *C. difficile* (Buffie et al., 2015) and *Bacillus subtilis* against *Staphylococcus aureus* (Piewngam et al., 2018) in humans. Inspired by these findings, we recently design a probiotic consortium that efficiently antagonizes the casual pathogens of shrimp WFS (Sha et al., 2024b). Nevertheless, comparable work in aquaculture species is still limited, warranting further study.

In conclusion, shrimp diseases typically induce dysbiosis, elevated heterogeneity, and stochasticity in the gut microbiota, although the causal relationships require rigorous validation. In addition, shrimp diseases disrupt the stability of the gut microbial network, potentially increasing susceptibility to invading pathogens. These common features across diverse diseases highlight the potential of sensitive gut microbial markers as indicators of shrimp health, irrespective of the causal agents. Early detection of these adverse changes in the gut microbiota is important for predicting and managing the risk of shrimp diseases.

COMMON DISEASE-DISCRIMINATORY TAXA PREDICTING SHRIMP HEALTH STATUS

As discussed above, the shrimp gut microbiota displays common features in response to diverse diseases. The underlying mechanism involves pathogenic infections triggering the production of reactive oxygen species, which facilitate aerobic respiration. Facultative anaerobic bacteria can utilize nonfermentable substrates as carbon sources, enabling them to outcompete obligate anaerobic Bacteroidia for fermentable nutrients (Winter & Bäumler, 2014). Consistent with this mechanism, shrimp diseases frequently increase the abundance of facultative anaerobic Proteobacteria in the gut, while reducing obligate anaerobic Bacteroidia (Mao et al., 2023; Sha et al., 2022b). It is important to note that the shrimp

gut microbiota is affected by diverse biotic and abiotic variables, including shrimp life stage (Xiong et al., 2020), different diseases (Yu et al., 2018a), hypervariable regions (Mao et al., 2023), health status (Anghong et al., 2023; Chen et al., 2017), aquaculture location, and sequencing platform (Cornejo-Granados et al., 2018; Mao et al., 2023) (Figure 3A).

Shrimp age is a robust predictor of variance in the gut microbiota, leading to a predictable microbial assembly as shrimp age (Xiong et al., 2020). The profiles of age-discriminatory taxa in the gut accurately diagnose the chronological age of healthy shrimp. However, the onset of disease can disrupt this defined trend (Figure 2B). Specifically, the microbiota-for-age Z (MAZ) score quantifies the variance in predicted microbiota age across different host age ranges and remains relatively stable in healthy shrimp. In contrast, the MAZ value sharply decreases with the onset of shrimp disease (Figure 2D) (Xiong et al., 2017a). A key challenge is distinguishing gut microbiota features between healthy and

diseased shrimp, considering life stage and other confounding variables (Figure 3). To address this, we propose a workflow for identifying disease common-discriminatory taxa. Initially, a parametric permutational multivariate analysis of variance quantitatively evaluates the effect of various factors on gut microbiota variance, ranking them based on their relative contributions. Subsequently, a random forest model or comparable method, such as statistical logistic regression, identifies indicators characterizing the most influential driving factors, with aquaculture location often being paramount (Figure 3A). To minimize the location effect, the identified location-discriminatory taxa are excluded from the gut microbiota. This process is repeated for subsequent factors until shrimp health status is the primary focus. Finally, disease common-discriminatory taxa serve as biomarkers for diagnosing shrimp health status, independent of the causal agents (Figure 3C). It is also possible to infer potential disease or causal pathogens through disease specific-discriminatory

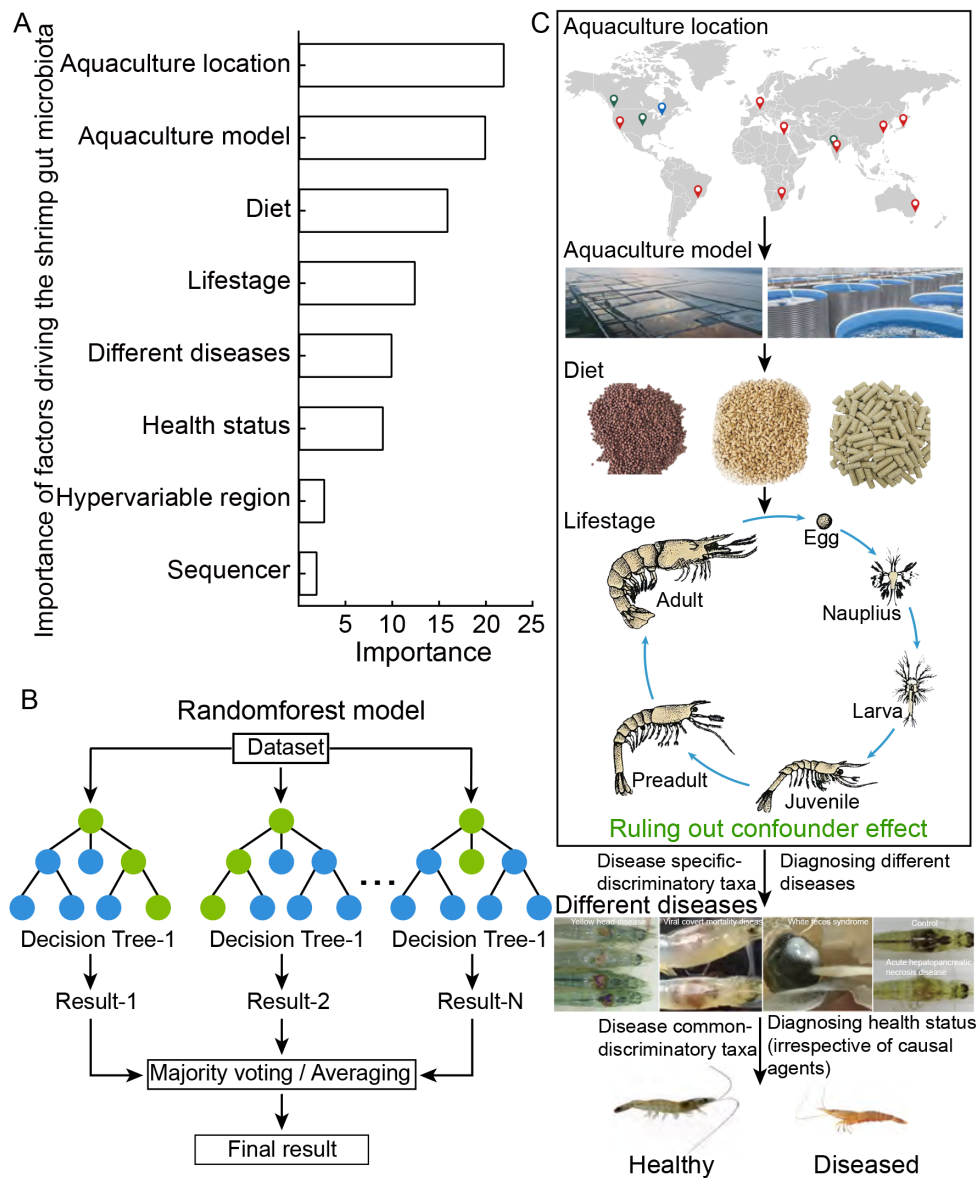


Figure 3 Flowchart screening disease common-discriminatory taxa for diagnosing shrimp health status

A: Quantifying relative importance of factors governing shrimp gut microbiota in descending order. B: Identifying discriminatory taxa for each factor using a random forest model. C: Identifying disease common-discriminatory taxa after progressive removal of effects of factors more important than health status in governing gut microbiota.

taxa, dependent on the causal agents. These optimized procedures can significantly improve diagnostic accuracy, as demonstrated in our recent studies (Mao et al., 2023; Sha et al., 2022b). Current research often compares gut microbiotas between healthy and severely diseased cohorts, leaving uncertainty about whether disease common-discriminatory taxa can predict disease onset. However, case studies provide supportive evidence for this potential (Lu et al., 2023; Shen et al., 2021a; Xiong et al., 2017a), although further validation is needed before practical application.

Disease common-discriminatory taxa are predominantly identified using high-throughput sequencing (HTS), which is time-consuming, expensive, and technically demanding, limiting its application in routine rapid-turnaround disease monitoring and early warning. An attractive solution to these limitations is proposed using quantitative real-time polymerase chain reaction (qPCR) to detect the relative abundances of gut microbial indicators (Yu et al. 2018b). In this case study, using disease-discriminatory phyla, diagnostic accuracy (93.2%) slightly lower than disease-discriminatory taxa (95.1%) and designed specific primers for these phyla for qPCR analysis, showing high consistency between qPCR and HTS assays in diagnosing shrimp WFS (Yu et al., 2018b). Nevertheless, subsequent meta-analyses have reported bacterial taxonomic effects on the diagnostic model, demonstrating that disease biomarkers at the bacterial species level provide the highest diagnostic accuracy (Mao et al., 2023; Sha et al., 2022b). However, due to confounding variables among studies, the identified gut disease-discriminatory taxa rarely overlap. Additionally, the bacterial 16S rDNA gene is insufficient to distinguish bacteria at the species level, except for a few well-studied pathogens. In contrast, functional genes are more conserved than 16S rDNA genes among phylogenetic species that encode specific traits (Yang, 2021). Genes encoding a functional trait share conserved regions for maintaining topological structures and active sites, enabling the design of specific primers, such as those used in qPCR-based SmartChip assays. Following this logic, detecting disease-discriminatory functional genes using qPCR is a promising approach. Considering the rapid decrease in the cost of metagenomic sequencing, researchers can explore the gut functional structures across different shrimp health statuses and during disease progression. Importantly, gut microbiome data should be deposited into publicly accessible databases, allowing researchers to screen for common disease-discriminatory functional genes using meta-analysis in an unbiased manner.

CONCLUSIONS AND PERSPECTIVES

Due to increasing stocking densities and deteriorating water quality, the emergence of new and frequent shrimp diseases is expected. The etiological and molecular mechanisms of these newly emerged diseases remain poorly understood (Table 1), posing significant challenges to shrimp production. It is now recognized that certain infectious diseases are caused by co-infections with polymicrobial pathogens that synergistically disrupt host homeostasis, rather than by a single pathogen. This complexity challenges the traditional “one pathogen, one disease” concept, especially as uncultivable bacteria may significantly contribute to pathogenicity. In addition, Koch’s postulates are difficult to satisfy when the composition and ratios of polymicrobial pathogens are unknown. In this context, ecological

approaches that integrate primary colonizers, keystone taxa, and disease-discriminatory taxa offer a promising alternative for inferring polymicrobial pathogens (Figure 1). Candidate pathogens can be selectively isolated using inferred conditions for subsequent validation (Oberhardt et al., 2015). Shrimp diseases, such as AHPND, generally cause rapid and massive mortality, making early disease prediction crucial for preventing disease progression. Disease common-discriminatory taxa and disease specific-discriminatory taxa hold potentials for predicting disease incidence and inferring causal agents (Figure 2), guiding timely and allopathic treatments. However, current studies typically use static comparisons between diseased and healthy shrimp, resulting in limited data on gut microbiota over the course of the disease and limited confidence in diagnosing disease onset (Yu et al., 2018b). Additionally, abiotic and biotic information is often insufficient when depositing shrimp disease research data into public databases. To address these issues, we propose that detailed relevant data should be collected throughout the course of the disease to construct reliable models for early disease prediction. As our understanding of the gut microbiome and shrimp diseases advances, it becomes increasingly clear that innovative solutions, such as antagonistic probiotic consortia and biomarkers, can be systematically developed to enhance the sustainability of shrimp aquaculture.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS

J.C. and J.B.X. conceptualized the manuscript. J.B.X. and H.N.S. wrote the manuscript. H.N.S. and J.B.X. collected the references and prepared the figures. All authors read and approved the final version of the manuscript.

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