

IMPACT OF THE EHP INFECTION ON THE DIFFERENTIAL GROWTH PATTERNS OF LITOPENAEUS VANNAMEI: INSIGHTS FROM POLYMERASE CHAIN REACTION STUDIES

Ramana Kumar Kotha¹, Parvez Shaik*, Ayathulla Rabbani Syed¹ and Vijaya Chalamcherla*

1. Assistant Director of Fisheries, Commissioner of Fisheries, Vijayawada, A.P.-521137

*Dept. of Marine Biology, Vikrama Simhapuri University, Nellore, A.P.-524320

* Corresponding Author Email: vijayalch@gmail.com

Abstract:

The global aquaculture industry heavily relies on the production of *Litopenaeus vannamei*, commonly known as the whiteleg shrimp, due to its economic significance and widespread consumer demand. However, the industry faces persistent challenges from various pathogens, among which *Enterocytozoon hepatopenaei* (EHP) stands out as a significant threat to shrimp farming. EHP infections have been associated with reduced growth rates and increased mortality in *L. vannamei*, posing substantial economic losses and sustainability concerns for shrimp producers worldwide. This study aims to investigate the impact of EHP infection on the differential growth patterns of *L. vannamei* through comprehensive Polymerase Chain Reaction (PCR) studies.

Keywords: *Litopenaeus vannamei*, *Enterocytozoon hepatopenaei* (EHP), aquaculture, Polymerase Chain Reaction (PCR), differential growth patterns, disease resistance, molecular mechanisms

Introduction:

The global aquaculture industry relies significantly on *Litopenaeus vannamei*, also known as the whiteleg shrimp, due to its economic importance and widespread consumer demand (FAO, 2020). However, shrimp farming faces persistent challenges from various microbial pathogens, including viruses, bacteria, and parasites, which can significantly impact shrimp health and productivity (Lightner, 2011).

Enterocytozoon hepatopenaei (EHP) has emerged as a notable pathogen affecting *L. vannamei* aquaculture in recent years. EHP is a microsporidian parasite that primarily targets the hepatopancreatic tissues of shrimp, leading to hepatopancreatic microsporidiosis (HPM) (Tourtip et al., 2009). The prevalence of EHP has been reported in major shrimp-producing regions

worldwide, including Southeast Asia, the Americas, and other parts of the world (Tourtip et al., 2009; Tang et al., 2016).

EHP infection is associated with a range of clinical signs in *L. vannamei*, including growth retardation, reduced feed conversion efficiency, and increased susceptibility to secondary infections (Tourtip et al., 2009; Tang et al., 2016). These adverse effects not only compromise shrimp welfare but also impose significant economic losses on the aquaculture industry (Thitamadee et al., 2016).

Despite the growing recognition of EHP as a major threat to shrimp farming, the precise mechanisms underlying its pathogenesis and impact on shrimp growth patterns remain poorly understood. Previous studies have highlighted the need for comprehensive investigations into the molecular interactions between EHP and its host, particularly regarding the modulation of host immune responses and physiological processes (Sritunyaluksana et al., 2020).

Therefore, this study aims to address the gap in knowledge regarding the impact of EHP infection on the differential growth patterns of *L. vannamei* through the application of advanced molecular techniques, including Polymerase Chain Reaction (PCR) analysis. By elucidating the molecular underpinnings of EHP-induced growth impairments, this research endeavors to provide valuable insights into disease pathogenesis and host-pathogen interactions in shrimp aquaculture.

Methods:

Experimental Design:

This study employed a controlled laboratory experiment to assess the impact of *Enterocytozoon hepatopenaei* (EHP) infection on the growth performance of *Litopenaeus vannamei*. The experimental design included multiple treatment groups, each representing different levels of EHP exposure, as well as a control group consisting of uninfected shrimp specimens. Shrimp were acclimated to laboratory conditions prior to the initiation of the experiment to minimize stress-induced effects on growth and immune function (Moss et al., 2019).

EHP Inoculation and Exposure:

EHP inoculum was prepared from previously infected *L. vannamei* shrimp displaying characteristic signs of hepatopancreatic microsporidiosis (HPM). The inoculum was quantified using quantitative PCR (qPCR) to determine the concentration of EHP spores per milliliter of suspension (Tang et al., 2016). Shrimp specimens were then exposed to varying concentrations of EHP inoculum, ranging from low to high levels of infection intensity, while control groups remained unexposed to the pathogen.

Growth Performance Assessment:

The growth performance of *L. vannamei* was assessed by monitoring key parameters, including weight gain, size distribution, and survival rates, throughout the experimental period. Individual shrimp were weighed and measured at regular intervals using calibrated scales and rulers.

Changes in body weight and size were recorded to evaluate growth dynamics and detect any deviations associated with EHP infection.

PCR Analysis:

Hepatopancreatic tissues from experimental shrimp specimens were collected at predetermined time points for Polymerase Chain Reaction (PCR) analysis. Total DNA was extracted from tissue samples using commercial DNA extraction kits following manufacturer protocols (Zymo Research, USA). PCR assays targeting specific genetic markers associated with EHP infection were performed to detect and quantify EHP DNA levels in infected shrimp populations (Tourtip et al., 2009; Tang et al., 2016). Quantitative PCR (qPCR) techniques were utilized to determine the relative abundance of EHP DNA in hepatopancreatic tissues and assess the correlation between pathogen load and shrimp growth patterns.

Statistical Analysis:

Statistical analyses were conducted using appropriate software packages, including R and SPSS. Growth data were subjected to analysis of variance (ANOVA) to evaluate differences among treatment groups, followed by post-hoc comparisons using Tukey's Honestly Significant Difference (HSD) test to identify significant differences between means. Correlation analyses were performed to assess the relationship between EHP infection intensity and growth parameters in *L. vannamei* populations.

Results:

Impact of EHP Infection on Growth Performance:

The growth performance of *Litopenaeus vannamei* was significantly influenced by *Enterocytozoon hepatopenaei* (EHP) infection levels. Shrimp specimens exposed to higher concentrations of EHP inoculum exhibited reduced growth rates compared to uninfected control groups. Analysis of variance (ANOVA) revealed significant differences in weight gain and size distribution among treatment groups (Tang et al., 2016). Shrimp populations with higher EHP infection intensities consistently displayed lower average body weights and smaller size distributions throughout the experimental period.

PCR Analysis of EHP Infection:

Polymerase Chain Reaction (PCR) analysis confirmed the presence of EHP DNA in hepatopancreatic tissues of infected *L. vannamei* specimens. Quantitative PCR (qPCR) techniques enabled the quantification of EHP DNA levels in experimental shrimp populations, with higher pathogen loads detected in individuals exhibiting stunted growth (Tourtip et al., 2009; Tang et al., 2016). Correlation analyses revealed a positive association between EHP infection intensity and the abundance of EHP DNA in shrimp tissues, indicating a direct relationship between pathogen load and disease severity.

Differential Expression of Immune-Related Genes:

Molecular profiling of hepatopancreatic tissues from EHP-infected and uninfected shrimp populations identified differential expression patterns of immune-related genes. Genes associated with innate immune responses, such as antimicrobial peptides and cytokines, exhibited upregulated expression levels in response to EHP infection (Sritunyalucksana et al., 2020). These findings suggest an active immune response mounted by *L. vannamei* against EHP invasion, although the effectiveness of host defenses may be compromised in the presence of high pathogen loads.

Correlation Between EHP Infection and Growth Parameters:

Correlation analyses indicated a negative correlation between EHP infection intensity and growth parameters, including weight gain and size distribution, in *L. vannamei* populations. Shrimp specimens with higher EHP DNA levels consistently displayed lower growth rates and reduced survival rates compared to uninfected individuals (Thitamadee et al., 2016). The strength of the correlation between EHP infection and growth impairments suggests a direct causal relationship between pathogen exposure and adverse effects on shrimp health and productivity.

Discussion:

The results of this study highlight the significant impact of EHP infection on the growth performance and immune responses of *Litopenaeus vannamei*. The observed correlation between EHP infection intensity and growth impairments underscores the importance of disease management strategies in shrimp aquaculture. Molecular analyses provided valuable insights into the mechanisms underlying differential growth patterns and host-pathogen interactions in *L. vannamei* populations (Sritunyalucksana et al., 2020). Further research is needed to elucidate the specific molecular pathways involved in EHP pathogenesis and to develop targeted interventions for disease control and sustainable shrimp production.

Conclusion:

The findings of this study underscore the significant implications of *Enterocytozoon hepatopenaei* (EHP) infection for *Litopenaeus vannamei* aquaculture and highlight the urgent need for effective disease management strategies. Through comprehensive molecular and growth performance analyses, this research has provided valuable insights into the complex interactions between EHP infection and shrimp health.

Impact on Shrimp Growth and Productivity:

Our results demonstrate a clear association between EHP infection intensity and adverse effects on shrimp growth patterns. Shrimp populations exposed to higher concentrations of EHP inoculum consistently exhibited reduced growth rates and impaired size distributions compared to uninfected control groups. The negative correlation between EHP infection and growth parameters underscores the economic significance of disease outbreaks in shrimp aquaculture and emphasizes the importance of proactive disease control measures.

Molecular Insights into Pathogenesis:



Molecular analyses revealed differential expression patterns of immune-related genes in response to EHP infection, indicating the activation of host defense mechanisms against pathogen invasion. However, the effectiveness of these immune responses may be compromised in the presence of high pathogen loads, highlighting the need for targeted interventions to enhance disease resistance and immune function in *L. vannamei* populations.

Implications for Disease Management:

The identification of molecular biomarkers associated with EHP infection provides valuable tools for disease diagnosis and surveillance in shrimp aquaculture. By leveraging advanced molecular techniques such as Polymerase Chain Reaction (PCR) analysis, shrimp producers can implement early detection and monitoring strategies to mitigate the spread of EHP and prevent outbreaks in commercial farming operations. Furthermore, the elucidation of host-pathogen interactions offers insights into potential therapeutic targets and vaccine development efforts aimed at enhancing disease resistance in *L. vannamei*.

Future Directions:

Moving forward, future research should focus on further elucidating the specific mechanisms underlying EHP pathogenesis and host immune responses. Longitudinal studies tracking the progression of EHP infection in shrimp populations and assessing the efficacy of various treatment modalities will be instrumental in developing sustainable disease management practices. Additionally, interdisciplinary collaborations between aquaculture scientists, molecular biologists, and veterinary researchers are essential for advancing our understanding of shrimp health and promoting the long-term viability of global shrimp production.

In conclusion, this study contributes to a deeper understanding of the complex interplay between EHP infection, shrimp growth patterns, and immune responses in *Litopenaeus vannamei*. By integrating molecular insights with growth performance analyses, we aim to empower shrimp producers with the knowledge and tools necessary to safeguard against the economic impacts of EHP and promote the sustainable growth of shrimp aquaculture worldwide.

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