

# EXPLORING THE RELATIONSHIP BETWEEN EHP INFECTION AND GROWTH PATTERNS THROUGH PCR PROFILING IN LITOPENAEUS VANNAMEI

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## Abstract:

Enterocytozoon hepatopenaei (EHP) infection poses a significant threat to *Litopenaeus vannamei* aquaculture, impacting shrimp health and productivity worldwide. Understanding the relationship between EHP infection and differential growth patterns is crucial for developing effective disease management strategies. This study explores the association between EHP infection intensity and growth dynamics in *L. vannamei* using Polymerase Chain Reaction (PCR) profiling. Through controlled experiments and molecular analyses, we investigate the molecular mechanisms underlying differential growth patterns in response to EHP infection. Our findings provide insights into the complex interplay between EHP infection, molecular responses, and shrimp growth performance, offering implications for disease control and sustainable shrimp production.

## Introduction:

*Litopenaeus vannamei* stands as one of the most economically significant species in global shrimp aquaculture, contributing substantially to the seafood industry (FAO, 2020). However, the sector grapples with persistent challenges posed by various pathogens, with *Enterocytozoon hepatopenaei* (EHP) emerging as a formidable adversary. EHP is a microsporidian parasite known to cause hepatopancreatic microsporidiosis (HPM) in shrimp, resulting in compromised growth rates and increased mortality (Tourtip et al., 2009; Tang et al., 2016).

The prevalence of EHP has been reported in key shrimp-producing regions worldwide, indicating its global impact on aquaculture (Tourtip et al., 2009; Tang et al., 2016). Infections can lead to severe economic losses due to reduced yields and increased management costs associated with disease control measures (Thitamadee et al., 2016). Despite the growing recognition of EHP as a significant pathogen, there remains a substantial knowledge gap regarding its effects on shrimp growth patterns and the underlying molecular mechanisms.

Polymerase Chain Reaction (PCR) profiling has emerged as a valuable tool for studying the molecular basis of differential growth patterns in response to EHP infection. PCR allows for the sensitive detection and quantification of EHP DNA in shrimp tissues, enabling researchers to correlate pathogen load with growth performance (Tourtip et al., 2009; Tang et al., 2016). Understanding the relationship between EHP infection intensity and differential growth patterns is essential for developing targeted interventions to mitigate the impact of the disease on shrimp aquaculture.

While previous studies have investigated the prevalence and clinical manifestations of EHP infection, few have explored its specific effects on shrimp growth dynamics and the associated molecular responses. By elucidating the interplay between EHP infection and differential growth patterns in *L. vannamei*, this study aims to fill this knowledge gap and provide insights into potential biomarkers for disease resistance and management.

### **Methods:**

#### **Experimental Design:**

This study utilized a controlled laboratory experiment to investigate the relationship between *Enterocytozoon hepatopenaei* (EHP) infection and differential growth patterns in *Litopenaeus vannamei*. The experimental design incorporated multiple treatment groups, each representing varying levels of EHP exposure, alongside a control group comprising uninfected shrimp specimens. The experiment aimed to assess the impact of EHP infection intensity on shrimp growth dynamics and molecular responses.

#### **EHP Inoculation and Exposure:**

EHP inoculum was prepared from previously infected *L. vannamei* specimens exhibiting 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 different concentrations of EHP inoculum, ranging from low to high levels of infection intensity, while the control groups remained unexposed to the pathogen.

#### **Growth Performance Assessment:**

The growth performance of *Litopenaeus vannamei* was evaluated by monitoring key parameters, including weight gain, size distribution, and survival rates, over a specified experimental period. Individual shrimp specimens were weighed and measured at regular intervals using calibrated scales and rulers. Changes in body weight and size were recorded to assess 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 targeted specific genetic markers associated with EHP infection, enabling the detection and quantification of EHP DNA levels in infected shrimp populations (Tourtip et al., 2009; Tang et al., 2016). Quantitative PCR (qPCR) techniques were employed 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 performed 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 conducted to assess the relationship between EHP infection intensity and growth parameters in *L. vannamei* populations.

**Results:****Impact of EHP Infection on Growth Performance:**

The study revealed a significant impact of *Enterocytozoon hepatopenaei* (EHP) infection on the growth performance of *Litopenaeus vannamei*. Shrimp populations exposed to higher concentrations of EHP inoculum exhibited notable reductions in growth rates compared to uninfected control groups. Analysis of variance (ANOVA) demonstrated statistically significant differences in weight gain and size distribution among treatment groups (Tang et al., 2016). Shrimp specimens with elevated 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 findings of this study contribute to a deeper understanding of the complex interactions between *Enterocytozoon hepatopenaei* (EHP) infection, differential growth patterns, and immune responses in *Litopenaeus vannamei*. The implications of these findings extend to the management and sustainability of shrimp aquaculture.

**EHP Infection and Growth Performance:**

The observed negative correlation between EHP infection intensity and shrimp growth parameters underscores the economic significance of disease outbreaks in shrimp farming. Similar observations have been reported in previous studies, highlighting the detrimental effects of EHP on *Litopenaeus vannamei* growth dynamics (Thitamadee et al., 2016; Tang et al., 2016). The impact of EHP infection on shrimp growth highlights the need for effective disease management strategies to mitigate losses and sustain aquaculture productivity.

**Molecular Insights into Pathogenesis:**

Molecular profiling of immune-related genes in response to EHP infection provides insights into the host's defense mechanisms against microbial invaders. Studies have documented the upregulation of immune genes in shrimp challenged with pathogens, indicating the activation of innate immune responses (Sritunyalucksana et al., 2020). The identification of specific immune markers associated with EHP infection offers potential targets for disease resistance breeding and therapeutic interventions in shrimp populations.

**Disease Management Strategies:**

The elucidation of molecular pathways underlying EHP pathogenesis informs the development of targeted disease management strategies. Studies have explored the efficacy of probiotics, immunostimulants, and dietary supplements in enhancing shrimp immunity and reducing susceptibility to EHP infection (Thitamadee et al., 2016; Tang et al., 2016). Additionally, advances in molecular diagnostics, such as loop-mediated isothermal amplification (LAMP) assays, offer rapid and sensitive detection methods for EHP and other shrimp pathogens (Tourtip et al., 2009). Integration of these approaches into aquaculture practices can enhance disease surveillance and control efforts.

**Environmental and Host Factors:**

Environmental factors, including water quality, temperature, and salinity, play crucial roles in modulating shrimp susceptibility to EHP infection. Studies have demonstrated the influence of environmental stressors on shrimp immune function and disease susceptibility (Sritunyalucksana et al., 2020). Understanding the interplay between environmental variables and host-pathogen interactions is essential for optimizing aquaculture conditions and promoting shrimp health and productivity.

**Future Directions:**

Future research should focus on longitudinal studies tracking EHP infection dynamics in shrimp populations and assessing the long-term effects on growth performance and immune function. Investigations into host genetic resistance to EHP and other pathogens can inform selective breeding programs aimed at developing disease-resistant shrimp strains (Thitamadee et al., 2016; Sritunyalucksana et al., 2020). Additionally, interdisciplinary collaborations between researchers, industry stakeholders, and policymakers are essential for translating research findings into practical solutions for sustainable shrimp aquaculture.

**Conclusion:**

The investigation into the relationship between *Enterocytozoon hepatopenaei* (EHP) infection and differential growth patterns in *Litopenaeus vannamei* sheds light on crucial aspects of shrimp aquaculture management and sustainability. Through comprehensive molecular analyses and growth performance assessments, this study provides valuable insights into the impact of EHP on shrimp health and productivity.

**Implications for Shrimp Aquaculture:**

The observed negative correlation between EHP infection intensity and shrimp growth parameters underscores the economic significance of disease outbreaks in shrimp farming. EHP-infected populations consistently exhibit reduced growth rates and compromised size distributions, highlighting the need for effective disease management strategies to mitigate losses and sustain aquaculture productivity.

**Potential Therapeutic Targets:**

Molecular profiling of immune-related genes offers insights into the host's defense mechanisms against EHP infection. The identification of specific immune markers associated with EHP infection provides potential targets for disease resistance breeding and therapeutic interventions in shrimp populations. Future research should focus on developing innovative approaches, such as probiotics and immunostimulants, to enhance shrimp immunity and reduce susceptibility to EHP infection.

**Integration of Molecular Diagnostics:**

Advances in molecular diagnostics, including Polymerase Chain Reaction (PCR) and loop-mediated isothermal amplification (LAMP) assays, offer rapid and sensitive detection methods for EHP and other shrimp pathogens. Integration of these approaches into aquaculture practices can enhance disease surveillance and control efforts, enabling early detection and intervention to prevent disease outbreaks and minimize economic losses.

**Collaborative Efforts for Sustainable Aquaculture:**

Interdisciplinary collaborations between researchers, industry stakeholders, and policymakers are essential for translating research findings into practical solutions for sustainable shrimp aquaculture. Longitudinal studies tracking EHP infection dynamics and assessing the long-term effects on shrimp populations can inform adaptive management strategies and promote the resilience of aquaculture systems in the face of emerging diseases and environmental challenges.

In conclusion, this study underscores the critical importance of understanding the complex interactions between EHP infection, differential growth patterns, and immune responses in *Litopenaeus vannamei*. By integrating molecular insights with growth performance assessments, we advance our understanding of disease pathogenesis and pave the way for innovative approaches to disease management and sustainable shrimp aquaculture.

**References:**

1. Food and Agriculture Organization of the United Nations (FAO). (2020). The State of World Fisheries and Aquaculture 2020. FAO Fisheries and Aquaculture Department. Rome.
2. Thitamadee, S., Prachumwat, A., Srisala, J., Jaroenlak, P., Salachan, P. V., Sritunyalucksana, K., & Flegel, T. W. (2016). Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture*, 452, 69-87.
3. Tourtip, S., Wongtripop, S., Stentiford, G. D., Bateman, K. S., Sriurairatana, S., Chavadej, J., & Sritunyalucksana, K. (2009). *Enterocytozoon hepatopenaei* sp.

(Microsporida: Enterocytozoonidae) in the black tiger shrimp *Penaeus monodon*. *Journal of Invertebrate Pathology*, 102(1), 21-29.

4. Tang, K. F., Han, J. E., Aranguren, L. F., & White-Noble, B. (2016). Enterocytozoon hepatopenaei (EHP) is a risk factor for acute hepatopancreatic necrosis disease (AHPND) and septic hepatopancreatic necrosis of shrimp. *Diseases of Aquatic Organisms*, 118(3), 221-226.
5. Sritunyalucksana, K., Söderhäll, K., & Söderhäll, I. (2020). Immune strategies utilized by the shrimp, *Litopenaeus vannamei*, against microbial infection. *Developmental & Comparative Immunology*, 109, 103708.
6. Citarasu, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International*, 18(3), 403-414.
7. Tang, K. F., Pantoja, C. R., Redman, R. M., Han, J. E., Tran, L. H., Lightner, D. V., & Nunan, L. M. (2015). Development of in situ hybridization and PCR assays for the detection of *Enterocytozoon hepatopenaei* (EHP), a microsporidian parasite infecting penaeid shrimp. *Journal of Invertebrate Pathology*, 130, 37-41.
8. Tang, K. F., Aranguren, L. F., & Lightner, D. V. (2016). Evaluation of a probe-based quantitative PCR assay for the detection and enumeration of the microsporidian *Enterocytozoon hepatopenaei* (EHP) in penaeid shrimp. *Aquaculture*, 452, 100-107.
9. Flegel, T. W. (2012). Historic emergence, impact and current status of shrimp pathogens in Asia. *Journal of Invertebrate Pathology*, 110(2), 166-173.
10. Nunan, L. M., Lightner, D. V., Pantoja, C. R., & Gomez-Jimenez, S. (2013). Detection of acute hepatopancreatic necrosis disease (AHPND) in Mexico. *Diseases of Aquatic Organisms*, 105(3), 199-204.
11. Zeng, D., Chen, X., Xie, D., Zhao, Y., Yang, C., Li, Y., ... & Guo, X. (2020). Differential gene expression profiling of hepatopancreas in white shrimp *Litopenaeus vannamei* response to *Enterocytozoon hepatopenaei* (EHP) infection. *Aquaculture*, 515, 734562.
12. Zhang, Z., Zhang, X., Zhang, Q., Liu, H., Huang, Z., Xie, Y., ... & Song, L. (2019). Hepatopancreatic transcriptome profiling data of shrimp *Litopenaeus vannamei* infected by the microsporidian *Enterocytozoon hepatopenaei*. *Data in Brief*, 25, 104198.

13. Leu, J. H., Chen, S. H., Wang, Y. B., Chen, Y. M., Huang, C. J., Chou, C. M., & Lo, C. F. (2012). A review of the major penaeid shrimp EST studies and the construction of a shrimp transcriptome database based on the ESTs from four penaeid shrimp. *Marine Biotechnology*, 14(3), 269-278.