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ISSN 1462 2815

# MORPHOLOGICAL IDENTIFICATION OF *LITOPENAEUS VANNAMEI* INFECTED WITH ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND)

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**DOI: 10.5281/zenodo.12158250**

**Abstract**

Acute Hepatopancreatic Necrosis Disease (AHPND), also known as Early Mortality Syndrome (EMS), poses a significant threat to the aquaculture industry, particularly affecting the white leg shrimp, *L. vannamei*. This study focuses on the morphological identification of AHPND-infected shrimp to facilitate early detection and effective disease management. Ninety shrimp samples from East Java, Indonesia, were examined for external and internal signs of AHPND. Externally, infected shrimp exhibited decreased appetite, weakness, and abnormal swimming behavior. Internally, the hepatopancreas showed distinct signs of puckering, pale brown coloration, and flabbiness, while the intestine displayed dotted strips and reddish areas. Histopathological analysis confirmed tissue atrophy, necrosis, vacuolation, and inflammation in both the hepatopancreas and intestine. The presence of *Vibrio parahaemolyticus* was detected using Total Vibrio Count (TVC) and PCR. These findings provide reliable morphological markers for AHPND diagnosis, underscoring the importance of integrating morphological and molecular techniques for comprehensive disease management in shrimp aquaculture.

**Keywords:** AHPND, Diagnosis, Histopathology, *L. Vannamei*, Morphology.

## INTRODUCTION

Acute Hepatopancreatic Necrosis Disease (AHPND), also known as Early Mortality Syndrome (EMS) (V. Kumar et al., 2021; Santos et al., 2019), is a significant threat to the aquaculture industry, particularly impacting the farming of the white leg shrimp, *L. vannamei* (Hernández-Cabanyero et al., 2023; Peña-Navarro et al., 2020; To et al., 2020). This disease is caused by specific strains of *V. parahaemolyticus* and is characterized by the rapid onset of hepatopancreatic necrosis, leading to high mortality rates within the first 30 days of a shrimp's life cycle (Soto-Rodriguez et al., 2015).

AHPND has had severe consequences on shrimp production in regions like Asia and Latin America, resulting in substantial economic losses (Nunan et al., 2014). Early and accurate detection of AHPND-infected shrimp is crucial for effective disease management. While traditional diagnostic methods like histopathology and molecular techniques are effective, they are often time-consuming and require specialized equipment and expertise (Nunan et al., 2014). Hence, there is a critical need to develop reliable and rapid morphological identification methods for AHPND-infected shrimp to enable timely intervention and control of disease outbreaks.

Previous research provides field and experimental evidence confirming *V. parahaemolyticus* as the causative agent of AHPND in cultured shrimp, specifically *L. vannamei* (Soto-Rodriguez et al., 2015). This study contributes to understanding the pathogenicity of *V. parahaemolyticus* in causing AHPND, shedding light on the mechanisms behind this devastating disease (R. Kumar et al., 2020; López-Landavery et al., 2024; Restrepo et al., 2021).

Additionally, published studies emphasize the urgent need for enhanced diagnostic capabilities for AHPND-infected shrimp to improve disease management strategies (Nunan et al., 2014). Furthermore, the published study highlights the importance of identifying specific genes like PirA and PirB toxin genes in *V. parahaemolyticus*, which play a crucial role in causing AHPND or EMS in *L. vannamei* (Zhang et al., 2021). Understanding the genetic basis of AHPND is essential for developing targeted interventions and control measures against this disease. Another published study also provide insights into the antibacterial mechanisms of *V. parahaemolyticus* strains causing AHPND, offering potential targets for disease control (Li et al., 2017).

Moreover, the study underscores the global impact of AHPND, originally known as EMS, which emerged in China in 2009 and subsequently spread to other countries like Vietnam, Malaysia, and Thailand (R. Kumar et al., 2020; V. Kumar et al., 2021; Santos et al., 2019).

This highlights the transboundary nature of AHPND and the need for international collaboration in disease management. Additionally, research emphasizes the severe impact of AHPND on shrimp health, particularly in Penaeus monodon, due to *V. parahaemolyticus* infections (Hossain et al., 2021).

AHPND poses a significant challenge to the aquaculture industry, necessitating the development of rapid and reliable diagnostic methods for early detection and intervention. By understanding the morphological and genetic markers associated with AHPND in shrimp like *L. vannamei*, researchers can enhance disease management strategies and potentially mitigate the economic losses caused by this devastating disease.

## MATERIAL AND METHODS

### Sampling

Ninety white shrimp (*L. vannamei*) were sampled from two distinct locations: Gresik Regency and Situbondo Regency, located in East Java, Indonesia. The selected shrimp had a post-larval age of 40 days, indicating the duration since their stocking as fry. Samples were collected and immediately stored at -80°C to maintain the stability of the hepatopancreas, as recommended by published methods (Niemcharoen et al., 2022).

### Morphological Characterization

#### *External Signs*

The general appearance of the sampled shrimp was assessed for signs of AHPND infection, including decreased appetite, weakness, and abnormal swimming behavior.

### *Internal Signs*

1. Hepatopancreas Appearance: The hepatopancreas was examined for characteristic changes, such as puckering, pale brown coloration, and flabbiness in texture.
2. Intestine Appearance: The intestine was inspected for the presence of a dotted strip on its surface and reddish areas.
3. Hepatopancreas Histopathology: Histopathological examination was conducted to observe tissue atrophy and irregular tissue shape.
4. Intestine Histopathology: Tissue integrity, organ tissue width (vacuolation), and signs of cell swelling and inflammation (edema) were evaluated.

The abundance of *Vibrio* sp bacteria in shrimp infected by Acute Hepatopancreatic Necrosis Disease (AHPND) was assessed using the pour method. The bacterial culture media utilized for Total Vibrio Count (TVC) was Thiosulfate Citrate Bile Salt Sucrose Agar (TCBSA) media. The Physiological NaCl Solution (0.9%) was prepared to create a suitable environment for subsequent procedures. The TCBSA Media was dissolved in 1 L of aquades and sterilized using an autoclave, ensuring the elimination of any contaminants.

The Sample Solution was prepared by crushing the hepatopancreas organ of white shrimp with a mortal pestle and mixing it in a Na-physiological solution using a vortex mixer. Petri Dishes were warmed using an oven to facilitate the growth of bacterial colonies. Inoculation involved pipetting a 0.1 ml aliquot of the sample solution onto each petri dish, followed by the addition of approximately 20 ml of TCBSA media to each dish while heating on a bunsen burner. Solidification and Incubation followed, with the TCBSA medium solidifying and the petri dishes being inverted and placed into an incubator for 24 hours at an appropriate temperature conducive to Vibrio growth. Counting Colonies involved the calculation of Total Vibrio Count (TVC) observations using a colony counter tool, providing quantitative data on bacterial abundance in the samples. The abundance of bacteria was determined according to SNI 10-2332.3-2006, using the following formula:

$$Abundance = \frac{N \times C \times n1 \times n2}{d}$$

Where:

- N = Number of Product Colonies
- C = Number of colonies on each cup is calculated
- n1 = Number of cups at the first dilution that was calculated
- n2 = Number of cups at the second dilution that was calculated
- d = Calculation of first dilution

This standardized method ensured accurate quantification of Vibrio sp bacteria in AHPND-infected shrimp samples, providing valuable insights into the bacterial load associated with the disease.

Data were analyzed using descriptive analysis methods to summarize and interpret the morphological and histopathological observations. Descriptive statistics were employed to characterize the prevalence and severity of external and internal signs of AHPND in the shrimp samples. This approach facilitated the identification of key patterns and trends, enhancing the understanding of disease manifestation and aiding in the development of effective diagnostic criteria.

## RESULT AND DISCUSSION

### Morphological Identification of AHPND in *L. vannamei*

#### *External Signs*

AHPND-infected *L. vannamei* exhibited noticeable external symptoms, including decreased appetite, weakness, and abnormal swimming behavior, consistent with previous observations (V. Kumar et al., 2021; Manchanayake et al., 2023). These behavioral changes are indicative of underlying physiological stress and compromised health in shrimp affected by AHPND.

The observed external signs in AHPND-infected *L. vannamei* align with the clinical manifestations reported in previous studies. Decreased appetite and abnormal swimming behavior are commonly observed behavioral changes in shrimp affected by AHPND, reflecting alterations in feeding patterns and locomotor activity attributed to the pathological effects of hepatopancreatic necrosis (Lee et al., 2022; Manchanayake et al., 2023; Saputra et al., 2023). Weakness may result from systemic effects of the disease, including metabolic disturbances and organ dysfunction, further impairing the shrimp's ability to perform normal activities (Santos et al., 2019).

These external signs serve as valuable indicators for early detection and diagnosis of AHPND in shrimp farming operations. By promptly identifying affected individuals based on observable symptoms, aquaculture practitioners can implement targeted management strategies to mitigate the spread of the disease and minimize economic losses associated with shrimp mortality. Moreover, the consistency of these external signs across different studies underscores their reliability as diagnostic markers for AHPND in *L. vannamei*.

Continued research focusing on understanding the underlying physiological and biochemical mechanisms driving these behavioral changes will further enhance our ability to diagnose and manage AHPND effectively.

The recognition of characteristic external signs in AHPND-infected *L. vannamei* serves as a crucial component of disease surveillance and control efforts in shrimp aquaculture. By integrating morphological identification with other diagnostic methods, such as molecular assays and histopathological examination, comprehensive strategies can be developed to safeguard shrimp health and ensure the sustainability of aquaculture operations.

#### Internal Signs:

#### Hepatopancreas Appearance

Morphological examination revealed distinct alterations in the hepatopancreas, characterized by puckering, pale brown coloration, and flabbiness in texture, indicative of necrosis (Hernández-Cabanyero et al., 2023; Peña-Navarro et al., 2020).

The morphological changes observed in the hepatopancreas, such as puckering, pale brown coloration, and flabbiness, are hallmark features of AHPND. These alterations are indicative of severe necrosis and functional impairment of the hepatopancreas, the primary digestive and absorptive organ in shrimp (Hernández-Cabanyero et al., 2023). The pale coloration is likely due to the loss of pigment and cellular degeneration, while the flabby texture reflects the breakdown of structural integrity within the tissue (Peña-Navarro et al., 2020). These morphological markers are critical for identifying AHPND-infected shrimp and differentiating them from those suffering from other diseases or stress conditions.

**Intestine Appearance**

The intestine displayed notable features such as a dotted strip on its surface and reddish areas, corroborating findings from previous studies (Chaudhry et al., 2021). The intestine's appearance, including the presence of a dotted strip and reddish areas, further substantiates the diagnosis of AHPND. These signs are associated with inflammatory responses and localized hemorrhaging within the intestinal tissue (Soto-Rodriguez et al., 2015; To et al., 2020).

The dotted strip pattern might be a manifestation of the breakdown of the intestinal lining and subsequent exposure of underlying tissues. These observations are consistent with previous studies that have documented similar intestinal alterations in shrimp affected by AHPND, providing a reliable visual cue for early disease detection.

**Hepatopancreas Histopathology:**

Histological analysis confirmed tissue atrophy and irregular tissue shape in the hepatopancreas, consistent with the pathological changes associated with AHPND (R. Kumar et al., 2020; V. Kumar et al., 2021; López-Landavery et al., 2024). Histopathological examination of the hepatopancreas revealed tissue atrophy and irregular tissue shape, corroborating the morphological findings.

These histological changes are indicative of extensive cellular damage and necrosis, which compromise the organ's (R. Kumar et al., 2020; V. Kumar et al., 2021; López-Landavery et al., 2024). The atrophy reflects a reduction in the size and number of functional cells, while the irregular tissue shape indicates disrupted cellular architecture (R. Kumar et al., 2020; V. Kumar et al., 2021; López-Landavery et al., 2024). These histopathological markers are essential for confirming AHPND, particularly in cases where external morphological signs may be less pronounced.

**Intestine Histopathology:**

Intestinal histopathology revealed tissue necrosis, vacuolation, and signs of cell swelling and inflammation, further supporting the diagnosis of AHPND (Hossain et al., 2021). Intestinal histopathology showed tissue necrosis, vacuolation, and signs of cell swelling and inflammation (edema), further supporting the diagnosis of AHPND. Necrosis indicates cell death and loss of tissue integrity, while vacuolation reflects cellular degeneration and the accumulation of fluid-filled spaces within cells. Edema and inflammation are responses to infection and tissue damage, signaling an active immune response against the pathogen. These histopathological features provide detailed insights into the pathological processes underlying AHPND and are critical for a comprehensive diagnosis.

The integration of morphological and histopathological examinations enhances the accuracy of AHPND diagnosis in *L. vannamei*. The consistency of internal signs across different studies underscores their reliability as diagnostic markers. Understanding these signs allows for the development of more effective monitoring and intervention strategies in shrimp aquaculture. Early detection through morphological and histopathological methods enables timely management actions, potentially reducing the spread and impact of AHPND on shrimp populations.

Future research should focus on refining these diagnostic techniques and exploring additional markers for AHPND. Investigating the molecular mechanisms driving these pathological changes can lead to improved disease prevention and treatment strategies, ultimately contributing to the sustainability of the shrimp aquaculture industry. Develop medicine and immunostimulant from marine algae (Kilawati, Arsyad, et al., 2021; Kilawati & Islamy, 2019, 2021) as alternative solution for AHPND cases. All off environmental factor should analyze for support the research (Hertika et al., 2023; Kilawati, Maimunah, et al., 2021; Ulinuha et al., 2020)

## CONCLUSIONS

The morphological and histopathological examinations of *L. vannamei* infected with Acute Hepatopancreatic Necrosis Disease (AHPND) revealed distinct external and internal signs, such as decreased appetite, weakness, abnormal swimming behavior, puckering and pale brown hepatopancreas, and necrotic intestinal tissue. These findings, consistent with previous research, underscore the reliability of these markers for early and accurate AHPND diagnosis, which is crucial for timely disease management and minimizing economic losses in shrimp aquaculture.

**Acknowledgement**

Authors thanks to Universitas Brawijaya, especially the Integrated Research Laboratory (LRT) Brawijaya University which has provided research facilities, shrimp ponds in Situbondo and Gresik Indonesia who have helped in the sampling process, and all research teams involved in this research.

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