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Enterocytozoon hepatopenaei infection, environmental characteristics, shrimp health status, and microorganisms abundance in intensive Pacific white shrimp farming

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ABSTRACT

Enterocytozoon hepatopenaei (EHP) is a highly contagious pathogen causing hepatopancreatic microsporidiosis in shrimp, resulting in slow growth and economic losses in aquaculture. This study examines environmental factors, shrimp health, and microorganism abundance in healthy and EHP-infected Pacific white shrimp ponds. Samples from two ponds (one healthy and one EHP-infected) across two cycles were analyzed for water physicochemical parameters (salinity, temperature, dissolved oxygen, pH, ammonium, nitrite, total organic matter, phosphate, and alkalinity), bacterial and phytoplankton abundance, *Vibrio* population in the shrimp body, and shrimp health indicators (hepatopancreatic lipid vacuole count, necrosis, and tubule constriction). Our analysis revealed that EHP infection was observed between days of culture (DOC) 46-60 confirmed by PCR analysis. The EHP-infected pond exhibited higher pH levels at DOC 40-60 compared to the healthy pond, along with higher nitrite concentration and alkalinity. Additionally, the EHP pond had lower phytoplankton abundance and phosphate concentration than the healthy pond. The EHP pond also showed higher total bacterial count, total *Vibrio* count, and a higher percentage of *Vibrio* compared to the healthy pond. Shrimp from the EHP pond displayed a lower lipid vacuole count in the hepatopancreas, higher necrosis level in the hepatopancreas, and more hepatopancreatic tubule constrictions compared to those from the healthy pond. These findings indicated metabolic issues in the shrimp from the EHP pond, resulting in slow growth and lower production indices compared to the healthy pond. These compelling results emphasize the critical need to understand and address the impact of EHP infection on shrimp health and aquaculture productivity.

INTRODUCTION

The Pacific white shrimp (*Penaeus vannamei*) is a significant species in global aquaculture due to its rapid growth rates and high market value. This species represents 52.9% of global shellfish aquaculture production, with a production level of 9.4 million tons **(FAO, 2020)**. Intensive shrimp farming operations have expanded significantly to meet the growing demand for shrimp, driving advancements in aquaculture technologies and practices. Despite these advancements, the health and productivity of Pacific white shrimp remain vulnerable to a range of pathogens **(Selvin *et al.*, 2015)**. High stocking density and feed input in intensive shrimp farming lead to several problems, including environmental and physiological stress, often related to disease outbreaks **(Raveendra *et al.*, 2018)**. *Enterocytozoon hepatopenaei* (EHP) is an emerging pathogen that currently poses a particularly challenging threat **(Amelia *et al.*, 2020)** and causes hepatopancreatic microsporidiosis (HPM) **(Aranguren *et al.*, 2017)**. This species replicates in the epithelial cells of hepatopancreatic tubules and causes cell lysis **(Tourtip *et al.*, 2009; Tang *et al.*, 2016)**. The main clinical signs of EHP infection include variation in shrimp sizes, lethargy, empty midguts, reduced growth rates, and impaired feed conversion **(Aranguren *et al.*, 2017)**.

The first report of EHP was in Thailand in 2004 as a microsporidian infecting the black tiger shrimp (*Penaeus monodon*) **(Chayaburakul *et al.*, 2004)**. Subsequently, it affected Pacific white shrimp and caused huge economic losses in several countries, including China, Indonesia, Vietnam, Thailand, Malaysia, India, Venezuela, and Korea **(Tangprasittipap *et al.*, 2013; Rajendran *et al.*, 2016; Tang *et al.*, 2016; Aranguren *et al.*, 2017; Tang *et al.*, 2017; Kim *et al.*, 2021)**. The economic losses caused by EHP were about USD 567 62 million in India and USD 232 million in Thailand **(Shinn *et al.*, 2018; Patil *et al.*, 2021)**. This situation suggests that it is necessary to conduct a comprehensive study to manage and mitigate EHP infection in shrimp farming.

Understanding and effectively managing EHP infections requires an understanding of the pathogen, environmental conditions, and microbial communities within shrimp farming systems. A complex interplay of environmental characteristics and microbial communities influences the prevalence and impact of EHP infections in intensive shrimp farming systems. Factors such as water quality, temperature, salinity, and oxygen levels significantly affect shrimp health and the dynamics of EHP infection. The disease can be caused by various factors, including poor aquaculture practices, low-quality shrimp seed, inadequate water quality, and lack of disease control knowledge among shrimp farmers **(Mello *et al.*, 2011; Velmurugan *et al.*, 2015; Aranguren *et al.*, 2017; Rakasiwi & Albastoni, 2017)**. The infection of EHP can occur in a shrimp pond with a salinity > 2 ppt, and its prevalence and severity increase at a salinity of 30 ppt **(Aranguren *et al.*, 2021a)**. Additionally, the abundance and diversity of microorganisms in the aquatic environment may exacerbate or mitigate the effects of EHP. Crustaceans primarily rely on non-specific immune systems to resist environmental stress and pathogenic infection; therefore, microflora in crustaceans play a vital role in their growth and development **(Smith & Chisholm, 1992; Rungrassamee *et al.*, 2016)**. A previous study revealed that the abundance of intestinal flora in the EHP-infected group was lower than that of the healthy group. Moreover, the high EHP load group is dominated harmful bacterial genera, which may lead to other bacterial diseases attacking the EHP-infected group **(Li**

***et al.*, 2024)**. Understanding these interactions is crucial for developing effective management strategies to control EHP and enhance shrimp health.

The study aims to investigate the relationships between EHP infection, environmental characteristics, and the health status of white shrimp in intensive farming systems. By examining how environmental variables and microbial abundance influence EHP infections, the study seeks to identify key factors that contribute to shrimp health and disease management. The findings from this study are expected to provide valuable insights for aquaculture practitioners, enabling them to implement more effective strategies to prevent and manage EHP infections and improve the overall sustainability of shrimp farming operations.

MATERIALS AND METHODS

Sampling site and sample collection

The sampling sites were the intensive shrimp ponds of PT. Margasari Jaya located in Situbondo, East Java, Indonesia. The shrimp were cultured in different ponds with an average area of 2000 m^2. The sampling was conducted over two production cycles using the shrimp seed purchased from Ayen Hatchery, PT. Ndaru Laut and Agape Hatchery, Indonesia, with a stocking density range of 166-191 shrimp m^{-3} as presented in Table 1. The farming management practices followed the standard operating procedures established in PT. Margasari Jaya. At the start of each production cycle, all ponds were assumed to be normal. However, after a pond exhibited symptoms of the EHP infection, ponds were grouped as normal (A3) and EHP (A1) ponds.

Samples collected from each pond consisted of shrimp and rearing water. Six Pacific white shrimp were randomly taken from each pond. Furthermore, water samples were collected using a composite sampling method by taking 5 L water samples from each pond **(Kurniawinata *et al.*, 2021)**. The observation of shrimp and water samples was immediately executed *in situ* and *ex-situ* in the laboratory in less than 2 hours. Sampling was performed every 10 days during the production cycles.

Table 1. The stocking information in the sampling ponds

Stocking information	**Cycles**	**Ponds**	
		A1	**A3**
Sources	1	Ayen Hatchery	Ayen Hatchery
	2	PT. Ndaru Laut	Agape Hatchery
Stocking density (shrimp m^{-3})	1	191	191
	2	166	166

Analysis of physicochemical water quality parameters

The water quality parameters measured included salinity, temperature, dissolved oxygen level (DO), water pH, ammonium, nitrite, total organic matter (TOM), phosphate, and alkalinity. Water temperature and DO were measured on-site with a portable multiprobe meter, while salinity and water pH were measured on-site using a hand refractometer and a pH meter, respectively. Ammonium, nitrite, and phosphate were measured in the laboratory using test kits, while alkalinity and TOM were measured in the laboratory through the titration method.

Analysis of shrimp's health status

The health of the shrimp was assessed by counting the lipid vacuoles in the hepatopancreas, and observing any hepatopancreatic necrosis and hepatopancreatic tubule constriction. This assessment was conducted every 10 days during each production cycle. To observe the health indicators, a wet mount of the shrimp's hepatopancreas was prepared by placing a section of it on a glass slide with a few drops of physiological solution. The wet mount was then examined under a microscope at 40x magnification **(Gomes *et al.*, 2011)**.

Measurement of the bacterial population

The bacterial population measured consisted of water total bacterial count, water total *Vibrio* count, dominance of *Vibrio* in the rearing water, total *Vibrio* count in the shrimp, total yellow colony *Vibrio* count in the shrimp, and total green colony *Vibrio* count in the shrimp. The bacterial population was observed every 10 days during each production cycle.

The water total bacterial count and water total *Vibrio* count were determined using the total plate count method with trypticase soy agar (TSA) + 3% NaCl and thiosulfate citrate bile salts sucrose (TCBS) agar, respectively. Water samples from each pond (0.1 mL) were serially diluted into 0.9 mL physiological solution and spread onto Petri dishes containing TSA + 3% NaCl or TCBS agar. The plates were then incubated at room temperature for 24 hours, and the resulting colonies were counted. For the total *Vibrio* count in the shrimp, whole shrimp bodies were crushed, and 0.1 g of the crushed shrimp body was used as a sample. The crushed shrimp body was serially diluted into 0.9 mL physiological solution and plated onto TCBS agar. The plates were then incubated at room temperature for 24 hours, and the resulting colonies were counted and grouped into two categories: yellow colony *Vibrio* and green colony *Vibrio*.

Observation of phytoplankton abundance

The phytoplankton samples were collected from the rearing water of each pond using a plankton net with a mesh size of 50 µm. The samples were then stored in 100 mL sampling bottles, and 8-10 drops of lugol were added to preserve the phytoplankton.

Observations of phytoplankton abundance were conducted every 10 days during the production cycle. Phytoplankton abundance was counted using a Sedgwick-Rafter counting cell. A water sample (1 mL) was dripped onto a Sedgwick-Rafter counting cells using a pipette. The sample was observed under a microscope at 100x magnification. Furthermore, the identification of phytoplankton was done by referring to plankton identification books, including **Davis (1955)**, **Yamaji (1979)**, and **Tomas (1997)**. Phytoplankton were identified under a microscope with a magnification of 400x.

Analysis of shrimp’s growth and production performance

The shrimp's growth was monitored from days of culture (DOC) 1 to 30 using corner pond sampling, and from DOC 31 until shortly before harvesting using net sampling. For corner pond sampling, shrimp were randomly collected using corner nets placed in each pond, while net sampling involved capturing shrimp from each pond using a net. Shrimp growth was measured by average weight gain, and production performance was assessed by analyzing harvesting data from cycles 1 and 2. Harvesting data included biomass, final weight, size, feed conversion ratio (FCR), and survival rate (SR).

Detection of *Enterocytozoon hepatopenaei* using polymerase chain reaction technique

Total DNA was extracted from the hepatopancreas using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol and the DNA extracts were stored at -20°C until further analysis. The primers and polymerase chain reaction (PCR) cycle conditions used in this study were based on the research of **Tang *et al.* (2016)**. The forward primer (F) sequence is 5’-GCC TGA GAG ATG GCT CCC ACG T-3’, and the reverse primer (R) sequence is 5'-GCG TAC TATA CCC CAG AGC CCG A-3'. The PCR conditions were as follows: pre-heat (94°C, 3 minutes), followed by 35 cycles of denaturation (94°C, 30 seconds), annealing (60°C, 30 seconds), and elongation (72°C, 30 seconds), followed by a final extension step at 72°C (5 minutes). The PCR products were then separated using agarose gel electrophoresis 1% and visualized under UV light. The size of the amplified DNA fragments was estimated using a 100 bp DNA ladder.

Data analysis

Data management was performed using Microsoft Excel 2021. The collected data were analyzed using descriptive statistics.

RESULTS

Clinical signs

Enterocytozoon hepatopenaei infection was detected in the sampling ponds on day 46 (cycle 1) and day 76 (cycle 2). The infected shrimp exhibited several clinical signs, including loss of appetite, white feces floating in the rearing water, soft carapace, pale hepatopancreas with a low count of lipid vacuoles in the hepatopancreas, and high constriction of hepatopancreatic tubules. The shrimp from the normal ponds exhibited no abnormalities or symptoms during the production cycles.

Polymerase chain reaction analysis

The detection of EHP was conducted in two phases. The first phase was performed at the beginning of shrimp seed stocking, and the second phase was carried out when clinical signs of EHP infection in shrimp became apparent. Test results showed that the PCR test results were negative when shrimp seeds were stocked and no clear clinical signs were observed. However, in the second test, two shrimp samples exhibiting clear clinical signs showed a positive result for EHP, amplified at 510 bp (Fig. 1).

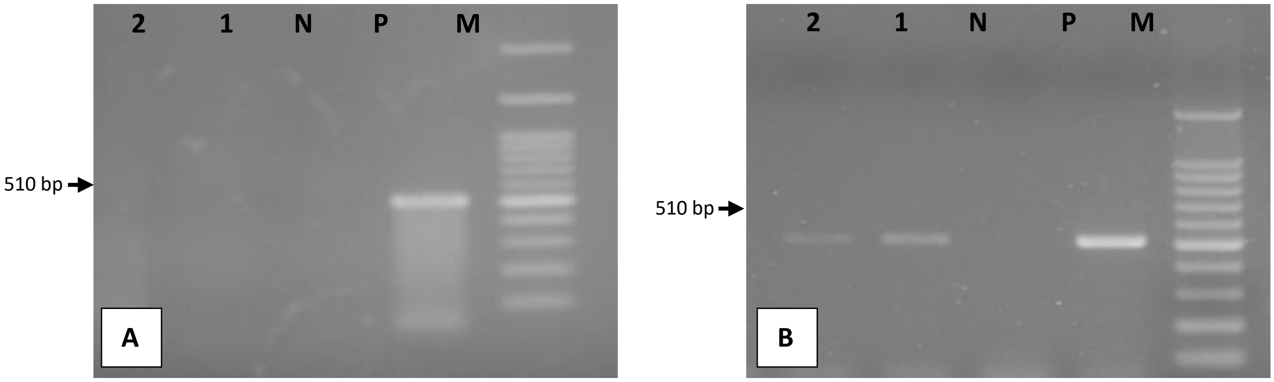


Fig. 1. The polymerase chain reaction test results for *Enterocytozoon hepatopenaei*. A. First stage and B. second stage. (M) 100 bp marker, (P) *Enterocytozoon hepatopenaei* positive control 510 bp, (N) Negative control, (1) Sample 1, and (2) Sample 2

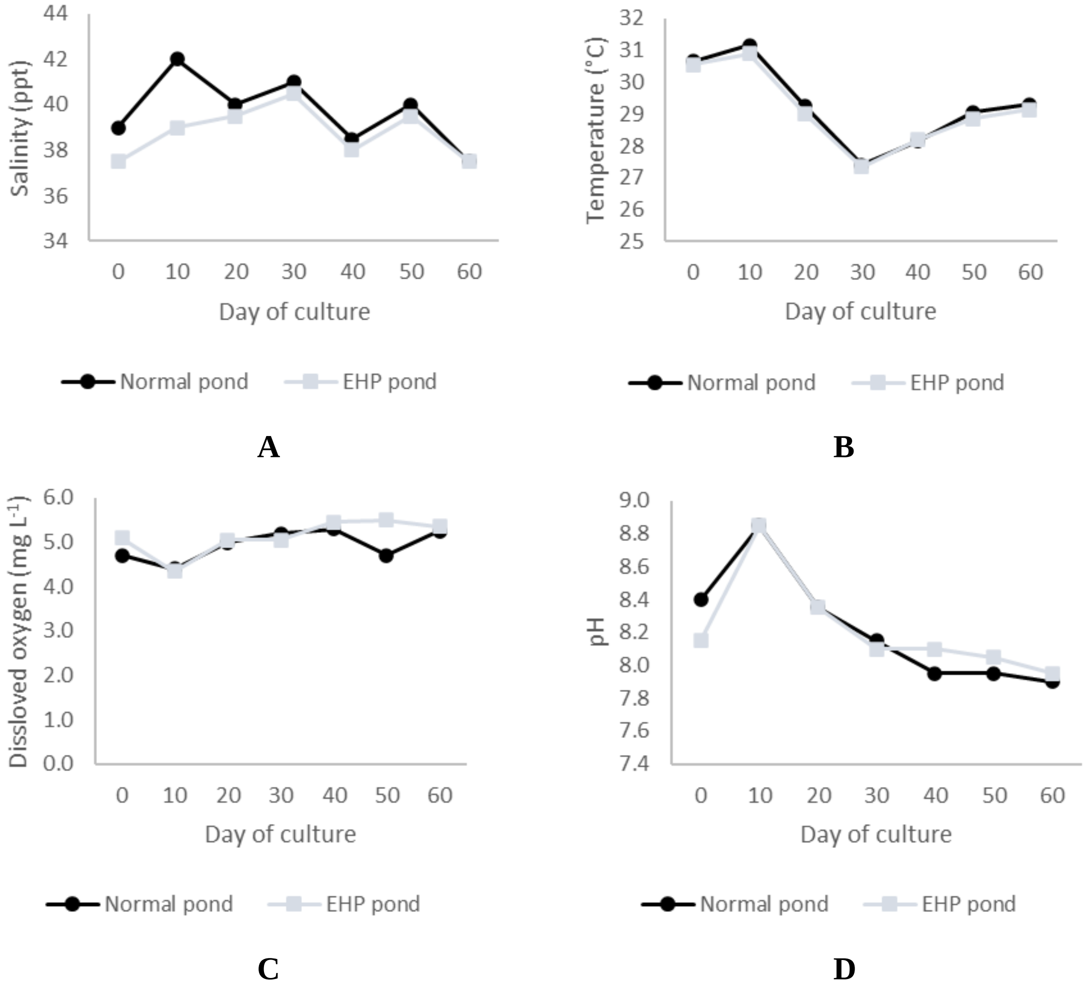
Environmental characteristics

The environmental features of the sampling sites in this study were determined based on the physico-chemical water quality parameters of each pond observed. Each parameter was compared to the standards set by the Ministry of Marine Affairs and Fisheries of the Republic of Indonesia in PERMEN No. 75/PERMEN-KP/2016, presented in Table 2.

Table 2. The water quality standard of the rearing water in the intensive shrimp pond (PERMEN No. 75/PERMEN-KP/2016)

Parameters	**Units**	**Ranges**
Temperature	°C	28-30
Salinity	ppt	26-32
pH		7.5-8.5

Dissolved oxygen	mg L^{-1}	> 4
Alkalinity	mg L^{-1}	100-150
Organic matter	mg L^{-1}	≤ 90
Nitrite	mg L^{-1}	≤ 1
Phosphate	mg L^{-1}	0.1-5
Total *Vibrio* count	CFU mL^{-1}	≤ 1 x 10^3



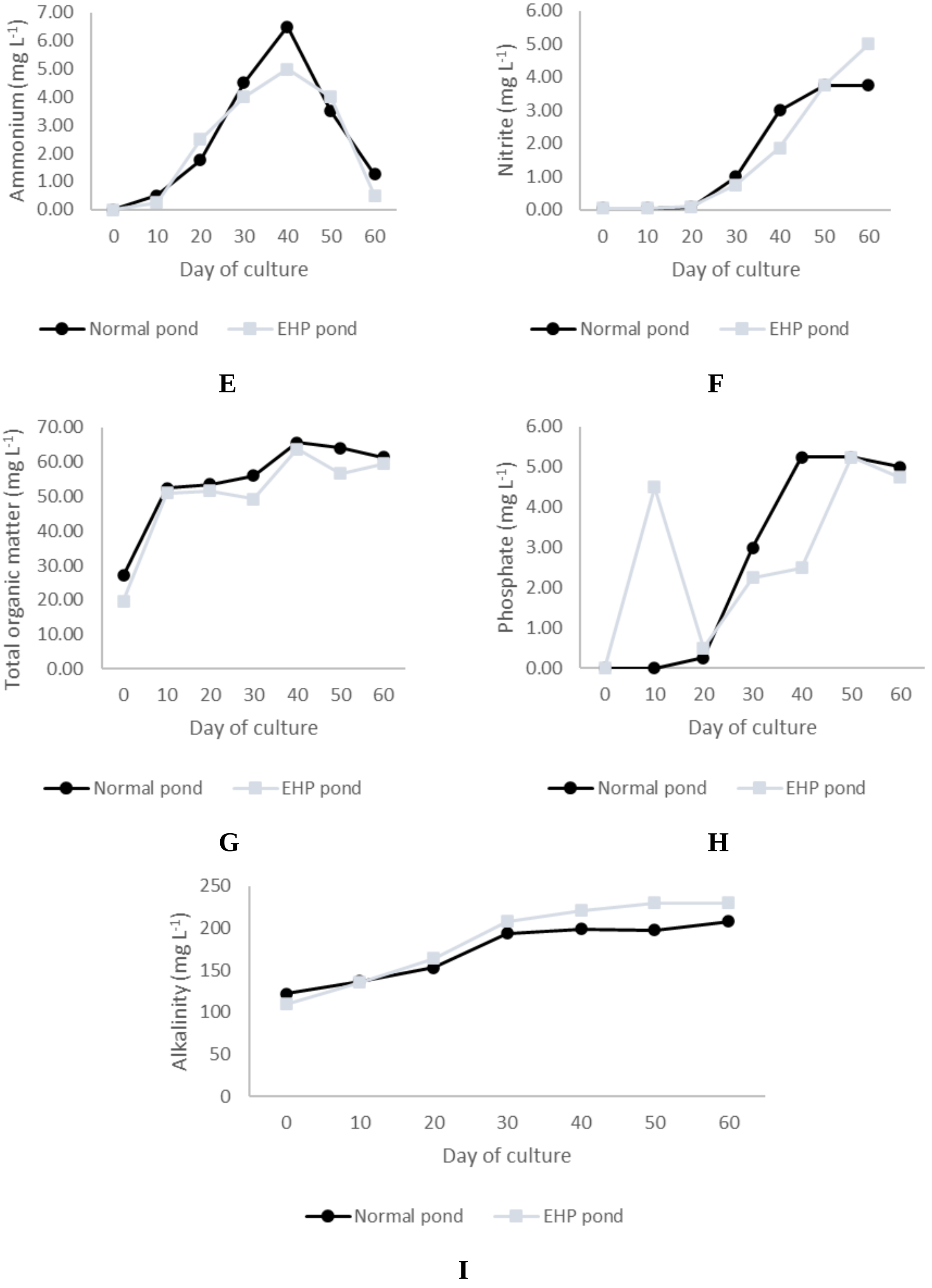
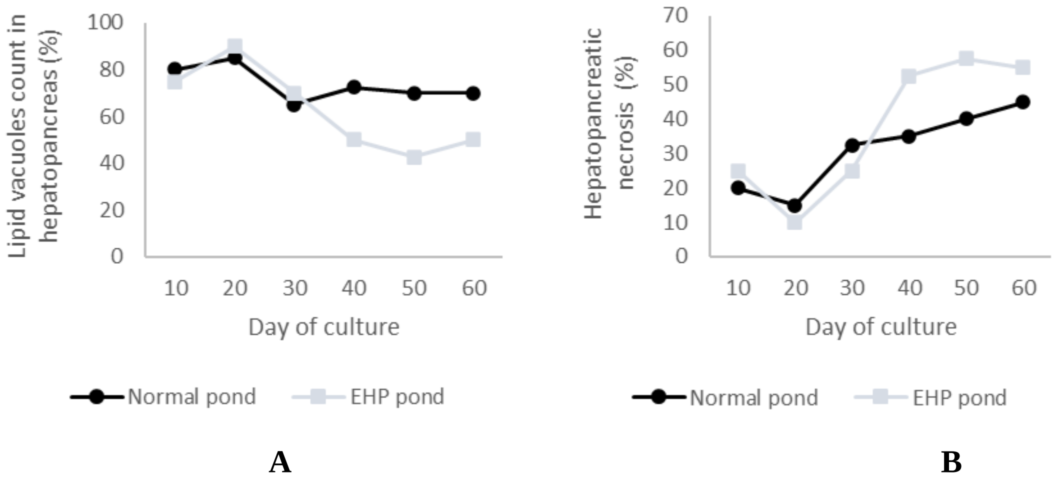


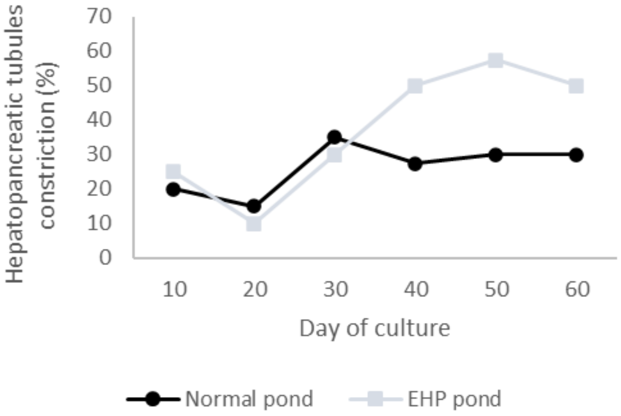
Fig. 2. Physico-chemical water quality parameters of normal and *Enterocytozoon hepatopenaei*-infected ponds. A. Salinity; B. Temperature; C. Dissolved oxygen; D. pH; E. Ammonium; F. Nitrite; G. Total organic matter; H. Phosphate; I. Alkalinity

The environmental characteristics of the sampling sites showed several parameters that were higher than the standard. These parameters included salinity, pH, nitrite, phosphate, and alkalinity. Other parameters such as temperature, dissolved oxygen, and total organic matter fell within the suggested ranges (Fig 2). The sampling sites had high salinity since the beginning of the production cycles. Additionally, high pH values were detected in the normal and EHP ponds at DOC 10, with the EHP ponds showing higher pH values than those from DOC 40 to 60. Nitrite concentration increased with the culture duration, surpassing the standard values since DOC 40. The EHP ponds exhibited higher nitrite concentrations than normal ponds, with the peak concentration found at DOC 60. Furthermore, EHP ponds showed high phosphate concentrations since DOC 10, while it was only detected in normal ponds since DOC 40. Overall, EHP ponds had lower phosphate concentrations compared to normal ponds. The alkalinity levels increased with the farming duration, exceeding the standard values since DOC 20. EHP ponds showed higher alkalinity levels than normal ponds.

Shrimp health status

The shrimp in EHP ponds showed a lower count of lipid vacuoles in the hepatopancreas and higher levels of hepatopancreatic necrosis and increased constriction of hepatopancreatic tubules compared to shrimp in normal ponds. Since DOC 40, the shrimp in EHP ponds experienced a decrease in lipid vacuole count in the hepatopancreas, an increase in hepatopancreatic necrosis, and an increase in hepatopancreatic tubule constriction (Fig. 3).





C

Fig. 3. The shrimp's health status in normal and *Enterocytozoon hepatopenaei*-infected ponds. A. Lipid vacuoles count in the hepatopancreas; B. Hepatopancreatic necrosis levels; C. Hepatopancreatic tubules constriction levels

Microorganisms abundance

Our study observed different types of microorganisms, including phytoplankton and bacteria. Overall, the ponds with EHP infection had lower phytoplankton abundance than normal ponds. Specifically, the phytoplankton abundance in EHP ponds decreased significantly at DOC 20 (see Fig. 4). The most dominant class of phytoplankton at the sampling site was Diatom, followed by Cyanophyta, Chlorophyta, and Dinoflagellates. Additionally, the EHP ponds were found to have a lower proportion of Diatoms and a higher proportion of Cyanophyta compared to the normal ponds (refer to Table 3).

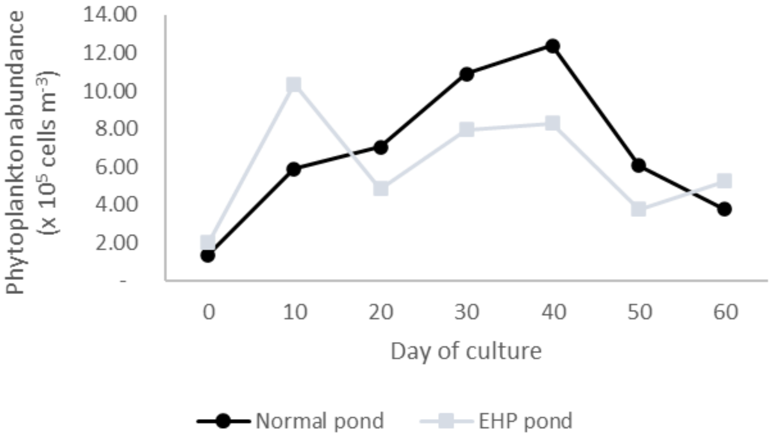
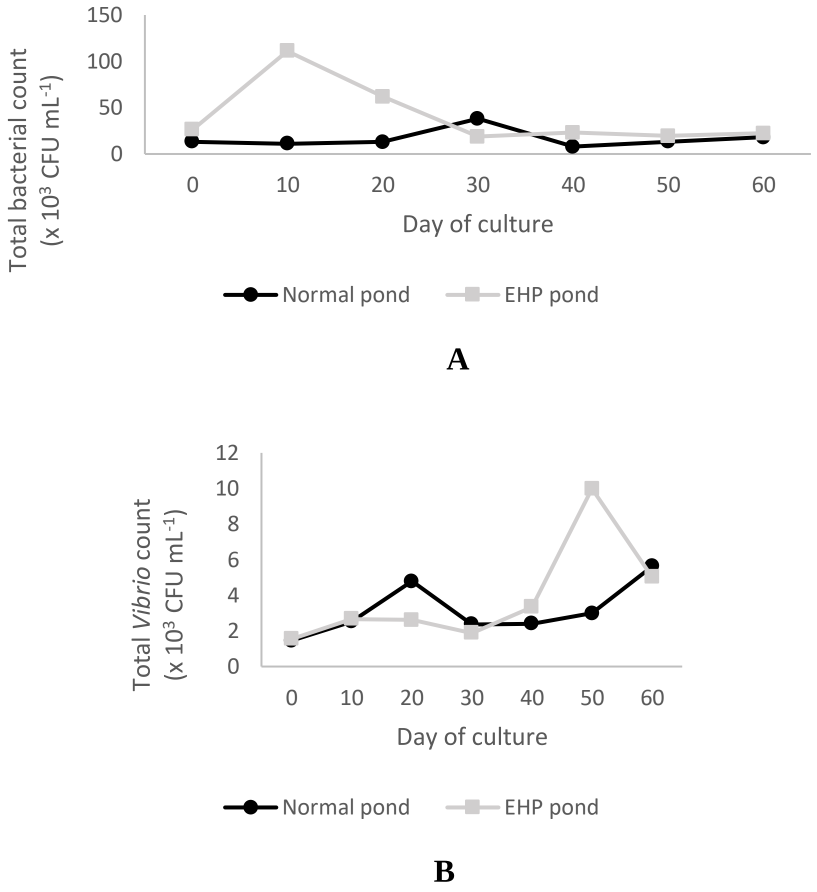


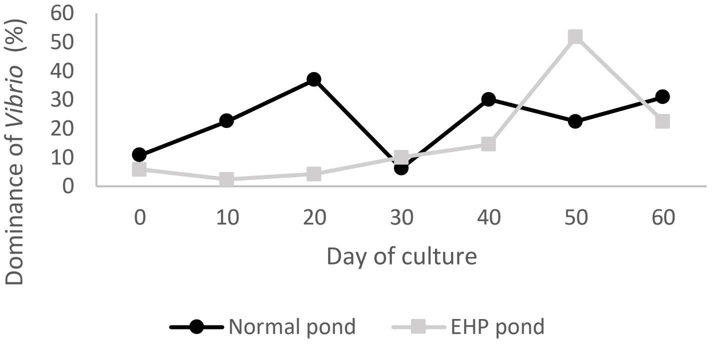
Fig. 4. The phytoplankton abundance in normal and *Enterocytozoon hepatopenaei*-infected ponds

Table 3. The percentage of Diatom, Chlorophyta, Cyanophyta, and Dinoflagellates in normal and *Enterocytozoon hepatopenaei*-infected ponds

Classes	**Normal ponds**	**EHP ponds**
Diatom	75%	74.5%
Chlorophyta	3.5%	3%
Cyanophyta	15.5%	16%
Dinoflagellates	5.5%	5.5%

The water in the ponds with EHP infection had a higher total bacterial count than in normal ponds. Additionally, the EHP ponds showed a significant increase in total bacterial count on DOC 10. Overall, the rearing water in EHP ponds had a higher total *Vibrio* count and dominance of *Vibrio* compared to the normal ponds, with the peak occurring on DOC 50 (see Fig. 5).

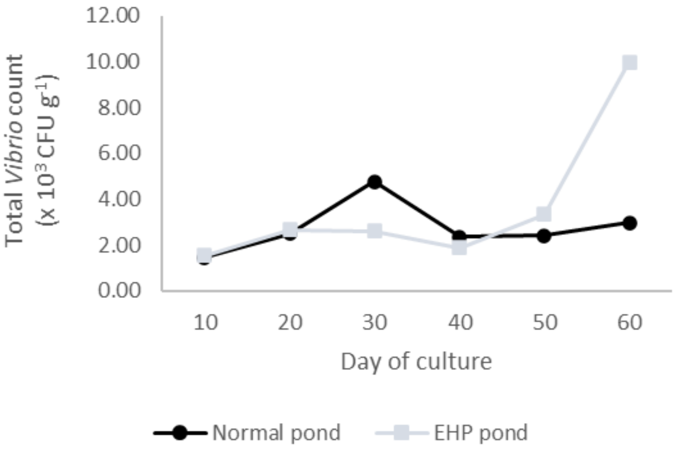




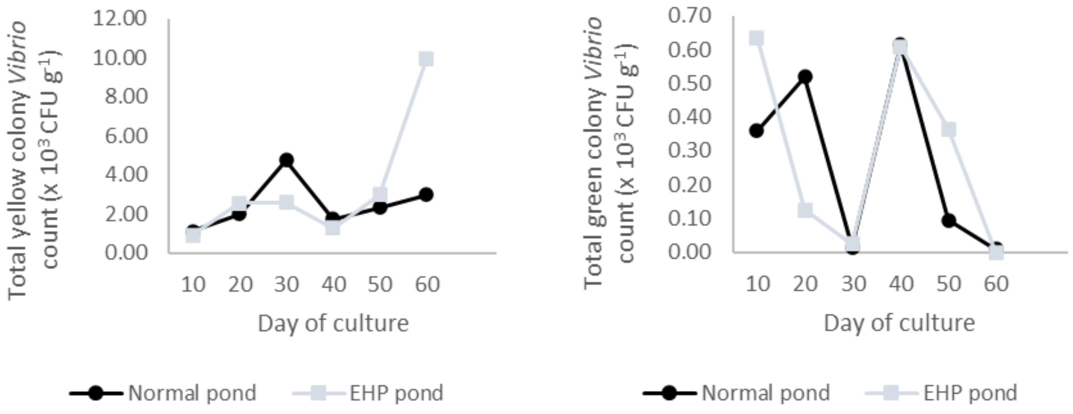
C

Fig. 5. The bacterial population in the rearing water of normal and *Enterocytozoon hepatopenaei*-infected ponds. A. Water total bacterial count; B. Water total *Vibrio* count; C. Dominance of *Vibrio* in the water

In ponds affected by EHP infection, the shrimp had a higher total *Vibrio* count than those in normal ponds. The total *Vibrio* count in the whole body of shrimp raised in EHP-infected ponds showed an increasing trend from DOC 20 to 60, with the peak occurring on DOC 60. This trend was also observed in the total yellow colony *Vibrio* count, while the total green colony *Vibrio* count showed drastic fluctuation patterns from DOC 30 onwards (see Fig. 6).



A

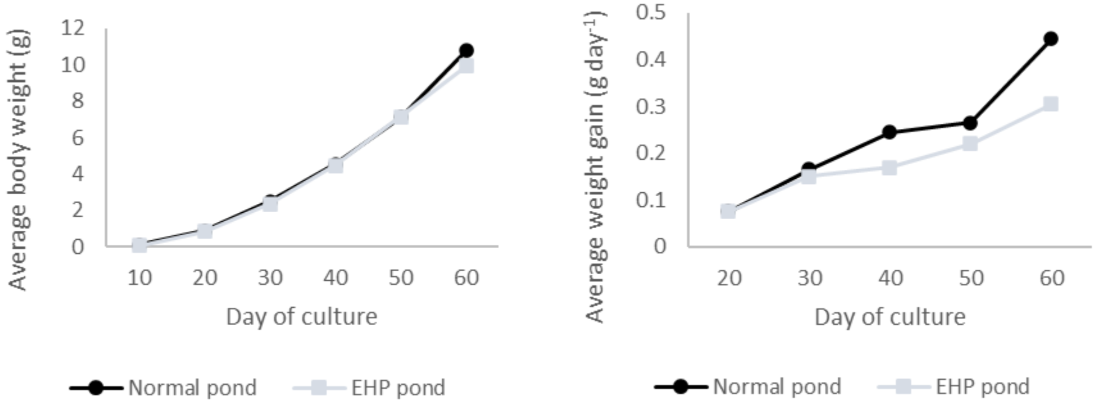


B **C**

Fig. 6. The bacterial population of the shrimp whole body in normal and *Enterocytozoon hepatopenaei*-infected ponds. A. Total *Vibrio* count in the shrimp whole body; B. Total yellow colony *Vibrio* count in the shrimp whole body; C. Total green colony *Vibrio* in the shrimp whole body

Shrimp's growth and production performance

The shrimp in EHP ponds had lower average body weight and weight gain compared to the shrimp in normal ponds (see Fig. 7). This led to lower production performance in EHP ponds compared to normal ponds (refer to Table 4). The shrimp from EHP ponds were harvested on DOC 98 (cycle 1) and 77 (cycle 2), while the shrimp from normal ponds were harvested on DOC 88 (cycle 1) and 91 (cycle 2). Additionally, EHP ponds showed lower shrimp biomass, lower final shrimp weight, smaller shrimp size, higher feed conversion ratio, and lower survival rate compared to the normal ponds.



A **B**

Fig. 7. The shrimp's growth in normal and *Enterocytozoon hepatopenaei*-infected ponds. A. The shrimp's average body weight; B. The shrimp's average gain

Table 4. Production performance of normal and *Enterocytozoon hepatopenaei*-infected ponds

Parameters	Normal pond	EHP pond
Biomass (kg)	3,878.63	3,756.16
Final weight (g shrimp^{-1})	20.09	16.99
Size (shrimp.kg^{-1})	53	59
Feed conversion ratio	1.41	1.44
Survival rate (%)	81.65	72.58

DISCUSSION

The clinical signs of EHP infection observed in this study align with previously documented symptoms in shrimp. These symptoms are primarily characterized by decreased appetite and white feces in the rearing water. The most apparent sign, loss of appetite, corresponds with the diminished feeding activity seen in another EHP study **(Tang *et al.*, 2016)**. Additionally, the observation of white feces, a hallmark of severe infection, points to compromised digestive function, likely due to hepatopancreatic damage **(Salachan *et al.*, 2017)**. Moreover, the shrimp may produce white fecal strings as a response to other causes such as vibriosis, hemocytic enteritis, heavy gregarine, and massive production of spores by EHP plasmodia in the hepatopancreas **(Chaijarasphong *et al.*, 2021)**. Other clinical symptoms in the infected shrimp included a soft carapace, pale hepatopancreas, and a reduction in lipid vacuoles, all indicative of malnutrition and hepatopancreatic dysfunction **(Ibarra-Gámez *et al.*, 2023)**. Lipids are the main components in crustacean hepatopancreas due to their functions as membrane structural building blocks and energy storage substance repositories **(Sánchez-Paz *et al.*, 2007; Ding, 2021)**. The hepatopancreas reserves lipids uptaking from the feed, which needs a mass of mitochondria and sERs **(Vogt, 2019)**. Microsporidian steals ATP from the host cells by transporting them from cytosol or directly binding them to the mitochondria **(Tsaousis *et al.*, 2008; Hacker *et al.*, 2014)**. This mechanism leads to mitochondria damage and decreased energy for lipid absorption and catabolism, ultimately causing a decline in lipid storage and metabolism. Moreover, EHP steals lipids from the host to build its membrane system due to its deficient fatty acid biosynthesis **(Boakye *et al.*, 2017; Ding *et al.*, 2021)**. The severe constriction of the hepatopancreatic tubules and necrosis observed in infected shrimp signifies substantial structural damage to the organ, impairing nutrient absorption and weakening the immune system **(Chayaburakul *et al.*, 2004; Aranguren *et al.*, 2017)**. In contrast, shrimp from non-infected ponds displayed no clinical abnormalities, suggesting effective pond management and a lack of stressors that predispose shrimp to EHP infection.

The PCR test conducted in two stages showed different results. The first stage, when the seeds were stocked, showed a negative result for EHP. However, in the second stage, when clinical symptoms began to appear clearly, the test results showed positive. This indicates the complex dynamics of EHP infection influenced by various factors. First, the sensitivity of PCR detection may not be high enough to detect very low numbers of EHP parasites at the beginning of infection **(Faisal & Pancoro, 2018)**. In the early stages, the number of EHP spores in the shrimp body may still be below the detection limit of PCR. Over time, with increasing intensity of infection, the number of spores will increase significantly so that it can be detected by PCR. Second, environmental factors can affect the growth rate and reproduction of EHP **(Aras *et al.*, 2023)**. Suboptimal environmental conditions can slow down parasite growth, while optimal conditions can accelerate its life cycle. Finally, the shrimp's immune system also plays an important role in controlling EHP infection **(Zhang *et al.*, 2023)**. At the beginning of the infection, the shrimp's immune system may still be able to control parasite growth. However, over time, the shrimp's immune system may weaken or be suppressed by various factors, allowing EHP to multiply rapidly.

In ponds where shrimp are infected with EHP, the hepatopancreas showed a reduction in lipid vacuoles and an increase in necrosis, along with greater constriction of the hepatopancreatic tubules compared to shrimp from non-infected ponds. Previous studies have also shown significant histopathological changes in the hepatopancreas of EHP-infected shrimp, including a decrease in the number of lipid droplets and the presence of spores in the lumen of the hepatopancreatic tubules **(Tourtip *et al.*, 2009; Aranguren *et al.*, 2017)**. The hepatopancreas is a crucial organ for nutrient storage and metabolic regulation in shrimp, and it is greatly impacted by EHP infection, leading to impaired nutritional absorption and energy deficiencies **(Dou *et al.*, 2022)**. The decrease in lipid vacuoles observed in the EHP ponds starting from DOC 40 can be attributed to partial or total dysfunction of the hepatopancreas due to moderate or severe damage within this organ **(Aranguren *et al.*, 2021b)**. The significant increase in hepatopancreatic necrosis and tubule constriction further supports the findings in this study, as these structural changes hinder the shrimp’s ability to properly absorb nutrients and maintain healthy metabolic functions. Such damage is also associated with reduced growth rates in infected shrimp, which is a common outcome of EHP infections. The increased constriction of hepatopancreatic tubules and necrosis observed in shrimp from the EHP ponds may also correlate with the parasite's influence on the shrimp’s hormonal and immune regulation systems. The infection of EHP disrupts the normal function of the hepatopancreas by altering the expression of genes related to metabolism and molting **(Dou *et al.*, 2022)**. This, in turn, impairs the shrimp's overall energy management, leading to the depletion of lipid reserves and further weakening of the organism.

The environmental conditions at the sampling sites in this study, as indicated by the physical and chemical water quality parameters, significantly affected the health of shrimp populations, especially with respect to the onset of EHP infection. Several parameters, including salinity, pH, nitrite, phosphate, and alkalinity, were found to surpass the standards set by the Ministry of Marine Affairs and Fisheries of Indonesia (PERMEN No. 75/PERMEN-KP/2016), indicating potential environmental stress that could increase the susceptibility of shrimp to infections **(MMAF, 2016)**. Salinity levels at the sampling sites remained consistently high throughout the production cycles. High salinity is known to increase the prevalence and severity of EHP infection, with a previous study demonstrating that EHP flourishes in environments with salinity as high as 30 ppt **(Aranguren *et al.*, 2021a)**. Elevated pH values were also observed, particularly in EHP-infected ponds, from DOC 40 to 60. Increased pH levels can disrupt the delicate balance of aquatic ecosystems and may lead to stress in shrimp, further compromising their immune systems. The high pH can be caused by water contamination, elevated photosynthesis levels during the afternoon, saline-alkaline water, and red tide **(Li *et al.*, 2021)**. An alkaline environment could trigger various stress responses in physiological status, biochemical levels, and metabolism pathways in crustaceans, leading to immune suppression, oxidative stress, pathogen susceptibility, and low survival rates **(Li & Chen, 2008; Liu *et al.*, 2015; Han *et al.*, 2018; Huang *et al.*, 2018)**. Nitrite levels that exceeded acceptable standards are another cause for concern. Elevated ammonium and nitrite concentrations can be toxic to shrimp and lead to mortality **(Adam *et al.*, 2022)**. High nitrite concentration may be due to improper pond management practices in the intensive culture system **(Pilli, 2022)**. Phosphate concentrations in the EHP-infected ponds were high from the start of the cycle, although the concentrations were lower than in normal ponds. High phosphate levels can promote algal blooms, which can lead to oxygen depletion, further stressing shrimp populations **(Boyd, 2001)**. Additionally, alkalinity, which increased throughout the production cycle, exceeded standard levels from DOC 20. While higher alkalinity levels are generally beneficial as a pH buffer, excessive levels can indicate an imbalance in water chemistry, potentially influencing shrimp health **(Adam *et al.*, 2022)**. Overall, these environmental factors—elevated salinity, pH, nitrite, phosphate, and alkalinity—not only exceeded regulatory standards but also created conditions that may have facilitated the spread and severity of EHP infections.

The recent study examined the abundance of phytoplankton and bacteria in shrimp ponds. An important discovery was that ponds infected with EHP showed a lower abundance of phytoplankton compared to normal ponds, with a significant decrease observed at DOC 20. This reduction in phytoplankton, particularly the dominant Diatoms, was caused by environmental stressors such as high bacterial load and water quality changes, leading to suppressed phytoplankton populations. In contrast, Cyanophyta (blue-green algae) became more prevalent in the EHP-infected ponds, indicating a shift in the microorganism community. The high abundance of Cyanophyta in the shrimp pond suggests high levels of temperature, salinity, ammonia, and orthophosphate **(Mahmudi *et al.*, 2022)**. The bacterial composition of the water also differed between EHP-infected and normal ponds. The EHP ponds exhibited higher total bacterial counts early in the production cycle, with a significant spike at DOC 10. The increased total bacterial count, particularly the dominance of *Vibrio* species, was linked to deteriorating water quality and the presence of pathogens that thrive in stressed environments. *Vibrio* species such as *Vibrio cholerae* and *V. parahaemolyticus* are known to be associated with shrimp diseases like white feces disease (WFD), which has been reported to co-occur with EHP infections **(Cao *et al.*, 2015; Aranguren *et al.*, 2021b)**. In shrimp raised in EHP-infected ponds, the total *Vibrio* count in their whole bodies increased steadily from DOC 20 to DOC 60, peaking at DOC 60. This trend mirrored the overall bacterial dynamics in the pond water and was accompanied by clinical signs of infection, such as pale hepatopancreas and impaired growth. The green colony *Vibrio* count showed significant fluctuations starting from DOC 30, indicative of an unstable microbial environment that could exacerbate disease conditions in the shrimp. Additionally, the functions of the intestinal microflora predicted that most genes related to diseases and environmental information processing were abundant in animals with high EHP loads **(Li *et al.*, 2024)**. The presence of green colonies of *Vibrio* on TCBS plates indicated that the isolate was most likely *V. parahaemolyticus*. There is a synergistic relation between EHP and *V. parahaemolyticus* that led to the manifestation of white feces syndrome **(Aranguren *et al.*, 2021b)**. Additionally, the severe infection of EHP can enhance susceptibility to other bacterial infections like *Vibrio* spp., leading to shrimp mortalities **(Kmmari *et al.*, 2018)**.

The study shows that EHP infection has a long-lasting negative impact on shrimp growth and survival. Shrimp raised in EHP-infected ponds had lower average body weight and weight gain compared to shrimp from non-infected ponds. This resulted in lower overall biomass and production output by the end of the culture cycle. The differing harvest times across the two cycles further highlight the long-term detrimental effects of EHP infection. Shrimp in EHP ponds were harvested later in cycle 1 (DOC 98) but earlier in cycle 2 (DOC 77) compared to the normal ponds, where the shrimp were harvested at DOC 88 and DOC 91, respectively. This suggests that EHP infections worsen growth challenges over time, requiring farmers to adjust their management strategies **(Sankar *et al.*, 2023)**. The EHP infection does not cause immediate death but rather leads to chronic health issues, such as stunted growth **(Kim *et al.*, 2021; WOAH, 2022)**. Shrimp in infected ponds consistently showed lower final weights, smaller sizes, higher FCR, and lower survival rates. The higher FCR in the EHP ponds also indicated inefficiency of nutrient uptake and feed conversion due to hepatopancreatic damage caused by EHP, limiting the shrimp's ability to process nutrients efficiently due to disruptions in the digestion, secretion, absorption, and assimilation in the shrimp **(Kumar *et al.*, 2022)**. This study and other findings showed that EHP infection is associated with slow growth syndromes in shrimp farming. The prolonged damage to the shrimp's hepatopancreas, reduced growth, and higher susceptibility to other pathogens like *Vibrio* species, indicates that EHP infection is a long-term challenge in aquaculture. Vigilant management is required to mitigate its impact on production performance **(Biju *et al.*, 2016; Sankar *et al.*, 2023)**.

The results of this study emphasize the importance of implementing effective management strategies to minimize the impact of EHP on shrimp health. Regular monitoring of the hepatopancreas and prompt action upon detecting histopathological changes can assist in alleviating the parasite's effects on shrimp growth and production. Additionally, maintaining water quality, particularly by controlling bacterial counts and promoting beneficial microbial communities, is crucial in mitigating the impact of EHP and sustaining healthy shrimp populations.

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REFERENCES

Adam, M. A.; Widiastuti, I. M.; Ernawati; Yayan, A. Y.; Insivitawati, E.; Yuliana; Pakaya, R. F.; Soegianto, A. and Khumaidi, A. (2022). Analysis of white feces disease (WFD) cause by *Vibrio* sp. and dinoflagellata in vannamei shrimp (*Litopenaeus vannamei*) in brackishwater culture pond. J. Ilm. Perikan. Kelaut., 14(1): 160-166. https://doi.org/10.20473/jipk.v14i1.26684

Amelia, F.; Halalludin, B.; Naim, S. and Permana, R. (2020). Analisis keanekaragaman genus *Vibrio* pada udang terinfeksi *Enterocytozoon hepatopenaei* (EHP) dengan metode *enterobacterial repetitive intergenic consensus polymerase chain reaction* (ERIC PCR). J. Aquac. Fish Health, 9(3): 238-251. https://doi.org/10.20473/jafh.v9i3.18855

Aranguren, L. F.; Alghamdi, F.; De Belder, K.; Lin, J.; Mai, H. N.; Millabs, J.; Alrehaili, Y.; Alazwari, A.; Algetham, S. and Dhar, A. K. (2021a). The effect of salinity on *Enterocytozoon hepatopenaei* infection in *Penaeus vannamei* under experimental conditions. BMC Vet. Res., 17: 65. https://doi.org/10.1186/s12917-021-02778-0

Aranguren, L. F.; Han, J. E. and Tang, K. F. J. (2017). *Enterocytozoon hepatopenaei* (EHP) is a risk factor for acute hepatopancreatic necrosis disease (AHPND) and septic hepatopancreatic necrosis (SHPN) in the Pacific white shrimp *Penaeus* *vannamei*. Aquaculture, 471: 37-42. https://doi.org/10.1016/j.aquaculture.2016.12.038

Aranguren, L. F.; Mai, H. N.; Cruz-Florez, R.; Marcos, F. L. A.; Alenton, R. R. R. and Dhar, A. K. (2021b). Experimental reproduction of White Feces Syndrome in whiteleg shrimp, *Penaeus vannamei*. PLoS ONE, 16(12): e0261289. https://doi.org/10.1371/journal.pone.0261289

Aras, A. K.; Fikriyah, A.; Pratiwi, G. A. I. and Nurlita, W. (2023). Prevalensi infeksi EHP (*Enterocytozoon hepatopenaei*) pada udang vaname (*Litopenaeus vannamei*) berdasarkan data surveillance di Bali, Indonesia. Media Akuakultur, 17(2): 59-65. http://dx.doi.org/10.15578/ma.17.2.2022.59-65

Biju, N.; Sathiyaraj, G.; Raj, M.; Shanmugam, V.; Baskaran, B.; Umamaheswari, G.; Kumaresan, G.; Kasthuriraju, K. K. and Chellamma, T. S. R. Y. (2016). High prevalence of *Enterocytozoon hepatopenaei* in shrimps *Penaeus monodon* and *Litopenaeus vannamei* sampled from slow growth ponds in India. Dis. Aquat. Organ., 120: 225-230. https://doi.org/10.3354/dao03036

Boakye, D. W.; Jaroenlak, P.; Prachumwat, A.; Williams, T. A.; Bateman, K. S.; Itsathitphaisarn, O.; Sritunyalucksana, K.; Paszkiewicz, K. H.; Moore, K. A.; Stentiford, G. D. and Williams, B. A. P. (2017). Decay of the glycolytic pathway and adaptation to intranuclear parasitism within *Enterocytozoon microsporidia*. Environ. Microbiol., 19(5): 2077-2089. https://doi.org/10.1111/1462-2920.13734

Boyd, C. E. (2001). Water Quality Standards: Total Phosphorus. https://www.globalseafood.org/advocate/water-quality-standards-total-phosphorus/

Cao, H.; Wen, L.; He, S.; Lu, L.; Yang, X. and Chen, B. (2015). *Penaeus vannamei*, white feces syndrome, *Vibrio cholerae*, pathogenicity, antibiotic susceptibility. Isr. J. Aquac., 67. https://doi.org/10.46989/001c.20685

Chaijarasphong, T.; Mungkongwongsiri, N.; Stentiford, G. D.; Aldama-Cano, D. J.; Thansa, K.; Flegel, T. W.; Sritunyalucksana, K. and Itsathitphaisarn, O. (2021). The shrimp microsporidian *Enterocytozoon hepatopenaei* (EHP): Biology, pathology, diagnostics and control. J. Invertebr. Pathol., 186: 107458. https://doi.org/10.1016/j.jip.2020.107458

Chayaburakul, K.; Nash, G.; Pratanpipat, P.; Sriurairatana, S. and Withyachumnarnkul, B. (2004). Multiple pathogens found in growth-retarded black tiger shrimp *Penaeus monodon* cultivated in Thailand. Dis. Aquat. Organ., 60: 89-96. https://doi.org/10.3354/dao060089

Davis, G. C. (1955). The Marine and Fresh-Water Plankton. Michigan State University Press, Chicago.

Ding, Z. (2021). Lipid metabolism disorders contribute to the pathogenesis of *Hepatospora eriocheir* in the crab *Eriocheir sinensis*. J. Fish Dis., 44(3): 305-313. https://doi.org/10.1111/jfd.13284

Dou, Y.; Zhang, L.; Zhang, S.; Cao, X.; Qiao. Y.; Jiang, G.; Cheng, J.; Wan, X.; Fan, X.; Li, H.; Wang, L.; Shi, W.; Qin, Y.; Sun, X. and Shen, H. (2022). Effect of *Enterocytozoon hepatopenaei* infection on hormonal regulation in Pacific white shrimp *Litopenaeus vannamei*. Preprints, 2022: 2022110291. https://doi.org/10.20944/preprints202211.0291.v1

Faisal, A. F. and Pancoro, A. (2018). Deteksi dini *Enterocytozoon hepatopenaei* (EHP) pada udang vaname (*Litopenaeus vannamei*) menggunakan metode PCR (*polymerase chain reaction*). J. Ris. Akuakultur, 13(3): 267-275. http://dx.doi.org/10.15578/jra.13.3.2018.267-275

FAO. (2020). State of World Fisheries and Aquaculture 2020: Sustainability in Action. Food and Agriculture Organization, Rome.

Gomes, G. B., Domingos, J. A. S., Da Silva, V. A. and Mendes, P. P. (2011). Wet Mount Technique Excellent for Health Monitoring, Not NHP Diagnosis in Shrimp. https://www.globalseafood.org/advocate/wet-mount-technique-excellent-health-monitoring-not-nhp-diagnosis-shrimp/

Hacker, C.; Howell, M.; Bhella, D. and Lucocq, J. (2014). Strategies for maximizing ATP supply in the microsporidian *Encephalitozoon cuniculi*: direct binding of mitochondria to the parasitophorous vacuole and clustering of the mitochondrial porin VDAC. Cell. Microbiol., 16(4): 565-579. https://doi.org/10.1111/cmi.12240

Han, S. Y.; Wang, M. Q.; Wang, B. J.; Liu, M.; Jiang, K. Y. and Wang, L. (2018). A comparative study on oxidative stress response in the hepatopancreas and midgut of the white shrimp *Litopenaeus vannamei* under gradual changes to low or high pH environment. Fish Shellfish Immunol., 76: 27-34. https://doi.org/10.1016/j.fsi.2018.02.001

Huang, W.; Li, H.; Cheng, C.; Ren, C.; Chen, T.; Jiang, X.; Cheng, K.; Luo, P. and Hu, C. (2018). Analysis of the transcriptome data in *Litopenaeus vannamei* reveals the immune basis and predicts the hub regulation-genes in response to high-pH stress. PLoS ONE, 13(12): e0207771. https://doi.org/10.1371/journal.pone.0207771

Ibarra-Gámez, J. C.; Rubio-García, M. F.; Sánchez-Díaz, R.; Casillas-Hernández, R. and Martínez-Ibarra, D. M. (2023). Identification techniques to prevent the current emerging disease hepatopancreatic microsporidiosis in white shrimp *Penaeus vannamei*: an overview. Lat. Am. J. Aquat. Res., 51(1): 1-11. http://dx.doi.org/10.3856/vol51-issue1-fulltext-2693

Kim, B. S.; Jang, G. I.; Kim, S. M.; Kim, Y. S.; Jeon, Y. G.; Oh, Y. K.; Hwang, J. Y. and Kwon, M. G. (2021). First report of *Enterocytozoon hepatopenaei* infection in Pacific whiteleg shrimp (*Litopenaeus vannamei*) cultured in Korea. Animals, 11(11): 3150. https://doi.org/10.3390/ani11113150

Kmmari S.; Rathlavath, S.; Pillai, D. and Rajesh, G. (2018). Hepatopancreatic microsporidiasis (HPM) in shrimp culture: A review. Int. J. Curr. Microbiol. Appl. Sci., 7(1): 3208-3215. https://doi.org/10.20546/ijcmas.2018.701.383

Kumar, T. S.; Praveena, P. E.; Sivaramakrishnan, T.; Rajan. J. J. S.; Makesh, M. and Jithendran, K. P. (2022). Effect of *Enterocytozoon hepatopenaei* (EHP) infection on physiology, metabolism, immunity, and growth of *Penaeus vannamei*. Aquaculture, 553: 738105. https://doi.org/10.1016/j.aquaculture.2022.738105

Kurniawinata, M. I.; Sukenda; Wahjuningrum, D; Widanarni and Hidayatullah, D. (2021). White faeces disease and abundance of bacteria and phytoplankton in intensive Pacific white shrimp farming. Aquac. Res., 52: 5730-5738. https://doi.org/10.1111/are.15449

Li, C. C. and Chen, J. C. (2008). The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus* under low and high pH stress. Fish Shellfish Immunol., 25(6): 701-709. https://doi.org/10.1016/j.fsi.2008.01.007

Li, W.; Hua, S.; Du, Z.; Jiang, H.; Jiang, S.; Yu, M.; Baloch, W.A.; Noonari, S.; Yan, B. and Gao, H. (2024). Interactions between the gut bacterial community of *Exopalaemon carinicauda* and infection by *Enterocytozoon hepatopenaei*. J. Invertebr. Pathol., 204: 108115. https://doi.org/10.1016/j.jip.2024.108115

Li, Z.; Tang, X.; Li, J. and He, Y. (2021). Comparative proteomic and transcriptomic analysis reveals high pH-induced expression signatures of Chinese shrimp *Fenneropenaeus chinensis*. Funct. Integr. Genomics, 21: 299-311. https://doi.org/10.1007/s10142-021-00779-8

Liu, M.; Liu, S.; Hu, Y. and Pan, L. (2015). Cloning and expression analysis of two carbonic anhydrase genes in white shrimp *Litopenaeus vannamei*, induced by pH and salinity stresses. Aquaculture, 448: 391-400. https://doi.org/10.1016/j.aquaculture.2015.04.038

Mahmudi, M.; Musa, M.; Arsad, S.; Lusiana, E. D.; Bunga, A. and Wati, N. A. (2022). Use of phytoplankton to assess water quality of eco-aquaculture system in super-intensive whiteleg shrimp (*Litopenaeus vananmei*) pond. Adv. Anim. Vet. Sci., 10(5): 971-979. https://doi.org/10.17582/journal.aavs/2022/10.5.971.979

Mello, M. V. C.; Aragão, M. E. F.; Torres-Franklin, M. L. P.; Neto, J. M. O. and Guedes, M. I. F. (2011). Purification of infectious myonecrosis virus (IMNV) in species of marine shrimp *Litopenaeus vannamei* in the State of Ceará. J. Virol. Methods, 177(1): 10-14. https://doi.org/10.1016/j.jviromet.2011.05.032

MMAF. (2016). Peraturan Menteri Kelautan dan Perikanan Republik Indonesia Nomor 75/PERMEN-KP/2016 tentang Pedoman Umum Pembesaran Udang Windu (*Penaeus monodon*) dan Udang Vaname (*Litopenaeus vannamei*). Ministry of Marine Affairs and Fisheries, Jakarta.

Patil, P. K.; Geetha, R.; Ravisankar, T.; Avunje, S.; Solanki, H. G.; Abraham, T. J.; Vinoth, S. P.; Jithendran, K. P.; Alavandi, S. V. and Vijayan, K. K. (2021). Economic loss due to diseases in Indian shrimp farming with special reference to *Enterocytozoon hepatopenaei* (EHP) and white spot syndrome virus (WSSV). Aquaculture, 533: 736231. https://doi.org/10.1016/j.aquaculture.2020.736231

Pilli, S. (2022). Probiotics in water quality management and disease resistance in *P. vannamei* farms of Razole, East Godavari, Andhra Pradesh. Fish. Aquac. J., 13(5): 308. https://doi.org/10.35248/2150-3508.22.13.308

Rajendran, K. V.; Shivam, S.; Praveena, P. E.; Rajan, J. J. S.; Kumar, T. S.; Avunje, S.; Jagadeesan, V.; Prasad Babu, S. V. A. N. V.; Pandea, A.; Krishnan, A. N.; Alavandi, S. V. and Vijayan, K. K. (2016). Emergence of *Enterocytozoon hepatopenaei* (EHP) in farmed *Penaeus (Litopenaeus) vannamei* in India. Aquaculture, 454: 272-280. https://doi.org/10.1016/j.aquaculture.2015.12.034

Rakasiwi, S. and Albastomi, T. S. (2017). Sistem pakar diagnosa penyakit udang vannamei menggunakan metode *forward chaining* berbasis web. Simetris J. Tek. Ind., Mesin, Elektro Ilmu Komput., 8(2): 647-654. https://doi.org/10.24176/simet.v8i2.1560

Raveendra, M.; Suresh, G.; Nehru, E.; Pamanna, D.; Venkatesh, D.; Kumar, M. Y.; Hameed, A. S. S.; Srilatha, C.; Babu, P. H. and Neeraja, T. (2018). Effect of microsporidian parasite *Enterocytozoon hepatopenaei* (EHP) on pond profitability in farmed Pacific white leg shrimp *Litopenaeus vannamei*. Int. J. Curr. Microbiol. Appl. Sci., 7(5): 1625-1638. https://doi.org/10.20546/ijcmas.2018.705.192

Rungrassamee, W.; Klanchui, A.; Maibunkaew, S. and Karoonuthaisiri, N. (2016). Bacterial dynamics in intestines of the black tiger shrimp and the Pacific white shrimp during *Vibrio harveyi* exposure. J. Invertebr. Pathol., 133: 12-19. https://doi.org/10.1016/j.jip.2015.11.004

Salachan, P. V.; Jaroenlak, P.; Thitamadee, S.; Itsathiphaisarn, O. and Sritunyalucksana, K. (2017). Laboratory cohabitation challenge model for shrimp hepatopancreas microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP). BMC Vet. Res., 13: 9. https://doi.org/10.1186/s12917-016-0923-1

Sankar, G.; Mansingh, V. R. and Gunalan, B. (2023). The impact of *Enterocytozoon hepatopenaei* (EHP) on *Penaeus vannamei* shrimp seed growth in a nursery and growth out system in Andhra Pradesh, South East Coast of India. Mediterr. Aquac. J., 10(1): 23-31. https://doi.org/10.21608/maj.2023.200232.1021

Sánchez-Paz, A.; García-Carreño, F.; Hernández-López, J.; Muhlia-Almazán, A. and Yepiz-Plascencia, G. (2007). Effect of short-term starvation on hepatopancreas and plasma energy reserves of the Pacific white shrimp (*Litopenaeus vannamei*). J. Exp. Mar. Biol. Ecol., 340(2): 184-193. https://doi.org/10.1016/j.jembe.2006.09.006

Selvin, J.; Ninawe, A. S.; Meenatchi, R. and Kiran, G. S. (2015). Control of pathogenic vibrios in shrimp aquaculture using antiinfectives from marine natural products. In: “Nutrición Acuícola: Investigación y Desarrollo.” Cruz-Suárez, L. E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M. G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., Rivas Vega, M. & Miranda Baeza, A. (Eds.). Universidad Autónoma de Nuevo León, San Nicolás de los Garza, pp. 102-141.

Smith, V. J., and Chisholm, J. R. S. (1992). Non-cellular immunity in crustaceans. Fish Shellfish Immunol., 2(1): 1-31. https://doi.org/10.1016/S1050-4648(06)80024-0

Tang, K. F. J.; Aranguren, L. F.; Piamsomboon, P.; Han, J. E.; Maskaykina, I. Y. and Schmidt, M. M. (2017). Detection of the microsporidian *Enterocytozoon hepatopenaei* (EHP) and Taura syndrome virus in *Penaeus vannamei* cultured in Venezuela. Aquaculture, 480: 17-21. https://doi.org/10.1016/j.aquaculture.2017.07.043

Tang, K. F. J.; Han, J. E.; Aranguren, L. F.; White-Noble, B.; Schmidt, M. M.; Piamsomboon, P; Risdiana, E. and Hanggono, B. (2016). Dense populations of the microsporidian *Enterocytozoon hepatopenaei* (EHP) in feces of *Penaeus vannamei* exhibiting white feces syndrome and pathways of their transmission to healthy shrimp. J. Invertebr. Pathol., 140: 1-7. https://doi.org/10.1016/j.jip.2016.08.004

Tangprasittipap, A.; Srisala, J.; Chouwdee, S.; Somboon, M.; Chuchird, N.; Limsuwan, C.; Srisuvan, T.; Flegel, T. W. and Sritunyalucksana, K. (2013). The microsporidian *Enterocytozoon hepatopenaei* is not the cause of white feces syndrome in whiteleg shrimp *Penaeus (Litopenaeus) vannamei*. BMC Vet. Res., 9: 139. https://doi.org/10.1186/1746-6148-9-139

Tomas, C. R. (1997). Identifying Marine Phytoplankton. Academic Press, Massachusetts.

Tourtip, S.; Wongtripop, S.; Stentiford, G. D.; Bateman, K. S.; Sriurairatana, S.; Chavadej, J.; Sritunyalucksana, K. and Withyachumnarnkul, B. (2009). *Enterocytozoon hepatopenaei* sp. nov. (Microsporidia: Enterocytozoonidae), a parasite of the black tiger shrimp *Penaeus monodon* (Decapoda: Penaeidae): Fine structure and phylogenetic relationship. J. Invertebr. Pathol., 102(1): 21-29. https://doi.org/10.1016/j.jip.2009.06.004

Tsaousis, A. D.; Kunji, E. R. S.; Goldberg, A. V.; Lucocq, J. M.; Hirt, R. P. and Embley, M. (2008). A novel route for ATP acquisition by the remnant mitochondria of *Encephalitozoon cuniculi*. Nature, 453: 553-556. https://doi.org/10.1038/nature06903

Velmurugan, S.; Palanikumar, P.; Velayuthani, P.; Donio, M. B. S.; Babu, M. M.; Lelin, C.; Sudhakar, S. and Citarasu, T. (2015). Bacterial white patch disease caused by *Bacillus cereus*, a new emerging disease in semi-intensive culture of *Litopenaeus vannamei*. Aquaculture, 444: 49-54. https://doi.org/10.1016/j.aquaculture.2015.03.017

Vogt, G. (2019). Functional cytology of the hepatopancreas of decapod crustaceans. J. Morphol., 280(9): 1405-1444. https://doi.org/10.1002/jmor.21040

WOAH. (2022). Infection with *Enterocytozoon hepatopenaei* (EHP). World Organization for Animal Health, Paris.

Yamaji, I. (1979). Illustration of the Marine Plankton of Japan. Hoikusha Publishing Co., Ltd., Osaka.

Zhang, L.; Zhang, S.; Qiao, Y.; Cao, X.; Cheng, J.; Meng, Q. and Shen, H. (2023). Dynamic interplay of metabolic and transcriptional responses in shrimp during early and late infection stages of *Enterocytozoon hepatopenaei* (EHP). Int. J. Mol. Sci., 24(23): 16738. https://doi.org/10.3390/ijms242316738