Effects of Water Preparation Systems on Microbial Dynamics and AHPND Severity in *Litopenaeus vannamei* Culture

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Abstract

The Pacific white shrimp (*Litopenaeus vannamei*) is a major species in global aquaculture, but its production is constrained by bacterial diseases such as Acute Hepatopancreatic Necrosis Disease (AHPND). This field study evaluated the effects of different water preparation and management systems on microbial dynamics, water quality, disease severity, and production performance in commercial shrimp farms along the Persian Gulf coast of Iran. Five farming systems were compared: traditional reservoir filtration (T1), mechanical drum filtration (T2), lined ponds without reservoir (T3), earthen ponds (T4), and small lined ponds with intermediate storage and chlorination (T5). The results showed significant differences among treatments. T2 and T5 exhibited lower Vibrio and total heterotrophic bacterial loads, improved dissolved oxygen levels, and reduced ammonia and nitrite concentrations. In contrast, T4 showed the highest bacterial densities, poorest water quality, and lowest shrimp survival (25%), whereas T5 achieved the highest survival rate (80%). PCR analysis confirmed the presence of pirA and pirB toxin genes of Vibrio parahaemolyticus across all systems; however, disease severity varied depending on environmental conditions. These findings indicate that pathogen presence alone does not determine AHPND outcomes, and that microbial control and environmental stability are critical determinants of disease severity. Integrated water treatment strategies, including mechanical filtration, sedimentation–disinfection reservoirs, and lined pond systems, were associated with reduced microbial loads and improved production performance. Overall, effective water preparation and biosecure management are essential for mitigating AHPND and enhancing sustainability in intensive shrimp farming systems.

Keywords: *Litopenaeus vannamei;* AHPND; water treatment systems; microbial dynamics; shrimp aquaculture; biosecurity.

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Introduction

The Pacific white shrimp *Litopenaeus vannamei* represents one of the most important species in global aquaculture, contributing substantially to animal protein supply and the economic development of coastal regions. According to the Food and Agriculture Organization (FAO), global farmed shrimp production exceeded 6.8 million tons in 2022, with *L. vannamei* accounting for the dominant share of this production (FAO, 2024). In Iran, shrimp aquaculture has expanded rapidly along the southern and northern coastal provinces, including Bushehr, Hormozgan, Khuzestan, Golestan, and Sistan and Baluchestan, becoming a significant contributor to export revenues and rural employment. However, the sustainability of this industry is increasingly threatened by emerging infectious diseases and environmental instability in intensive farming systems.

Among these diseases, Acute Hepatopancreatic Necrosis Disease (AHPND) is considered one of the most destructive bacterial diseases affecting shrimp aquaculture. First reported in China in 2009, the disease rapidly spread throughout Asia and subsequently to the Americas, causing severe production losses (Tran *et al*., 2013; Kumar *et al*., 2021). AHPND is caused by specific strains of *Vibrio parahaemolyticus* carrying the pVA1 plasmid encoding the PirA and PirB binary toxins. These toxins induce extensive necrosis in the hepatopancreatic tubules of shrimp, leading to anorexia, lethargy, and mortality rates approaching 100% during the early culture stages (Kumar *et al*., 2021). In Iran, outbreaks of AHPND have been reported since 2022, particularly in southern shrimp farming regions (Bushehr and Hormozgan provinces), resulting in significant reductions in production (Pazir *et al*., 2025).

In addition to AHPND, other emerging diseases threaten shrimp aquaculture. Hepatopancreatic microsporidiosis (HPM) caused by *Enterocytozoon hepatopenaei* (EHP) leads to severe growth retardation and economic losses despite relatively low mortality (Tourtip *et al*., 2009; Govindasamy *et al*., 2023; Le *et al*., 2026). Similarly, Translucent Post-larvae Disease (TPD), first reported in China in 2019, causes extremely high mortality in post-larval shrimp and is associated with hypervirulent strains of *Vibrio parahaemolyticus* (Jia *et al*., 2024; Dinh-Hung *et al*., 2025). These diseases share common ecological drivers, particularly poor water quality, accumulation of organic matter in pond sediments, and proliferation of opportunistic bacterial communities (Chainark *et al*., 2025).

In traditional earthen pond systems, bottom sediments often act as reservoirs for pathogens, including vibrios and EHP spores. Organic matter derived from uneaten feed and feces accumulates in sediments, creating favorable conditions for bacterial growth and increasing the risk of disease outbreaks (Boyd, 2000; Kumar *et al*., 2025). Environmental stressors such as fluctuations in dissolved oxygen, ammonia, and pH further exacerbate microbial instability and disease susceptibility in shrimp populations.

One technological approach to mitigate these risks involves the use of High-Density Polyethylene (HDPE) geomembrane liners, which prevent direct interaction between pond water and underlying soils. Lined ponds facilitate improved pond hygiene, enhanced water management, and reduced sediment accumulation. Several studies have demonstrated that lined systems can significantly reduce bacterial loads and improve survival and productivity compared with conventional earthen ponds (Saraswathy *et al*., 2022; Supriatin *et al*., 2024; Kumar *et al*., 2026). By limiting pathogen reservoirs and stabilizing environmental conditions, such systems may also reduce the incidence of diseases such as AHPND, EHP, and TPD.

Despite the growing adoption of lined ponds in major shrimp-producing countries, field-based comparative studies under Iranian environmental conditions remain limited. The Persian Gulf coastal environment is characterized by high salinity, elevated temperatures, and in some areas acid sulfate soils, all of which may influence microbial dynamics and disease risk in shrimp culture systems.

Therefore, the present study aimed to evaluate the effects of different water preparation and pond management systems on microbial dynamics and production performance in *L. vannamei* farming operations in southern Iran. Specifically, the study investigated how different water preparation protocols influence *Vibrio* abundance, total heterotrophic bacterial loads, shrimp survival, growth performance, and production yield under commercial farming conditions. Understanding these relationships is essential for developing improved biosecurity and management strategies to enhance the sustainability of shrimp aquaculture in Iran and similar environments.

Materials and methods

Study area and experimental design

The study was conducted during the 2025 shrimp farming season (May–October) in nine commercial *L. vannamei* farms located in Bushehr Province along the Persian Gulf coast of Iran. The farms were situated within a relatively homogeneous climatic region to minimize environmental variability in water source quality and meteorological conditions.

The investigation followed a comparative field observational design. Farms were categorized according to their pond construction type and source water preparation systems. Across all farms, management variables including post-larvae source, feeding strategy, aeration management, and water quality monitoring protocols were maintained as consistently as possible.

Experimental treatments

Five production systems differing in pond type and water preparation were evaluated, representing common shrimp farming strategies in Bushehr Province (Figs. 1 and 2). Each treatment included three representative ponds, all managed under similar commercial conditions for feeding, aeration, and water exchange to reduce management-related variation (Table 1).

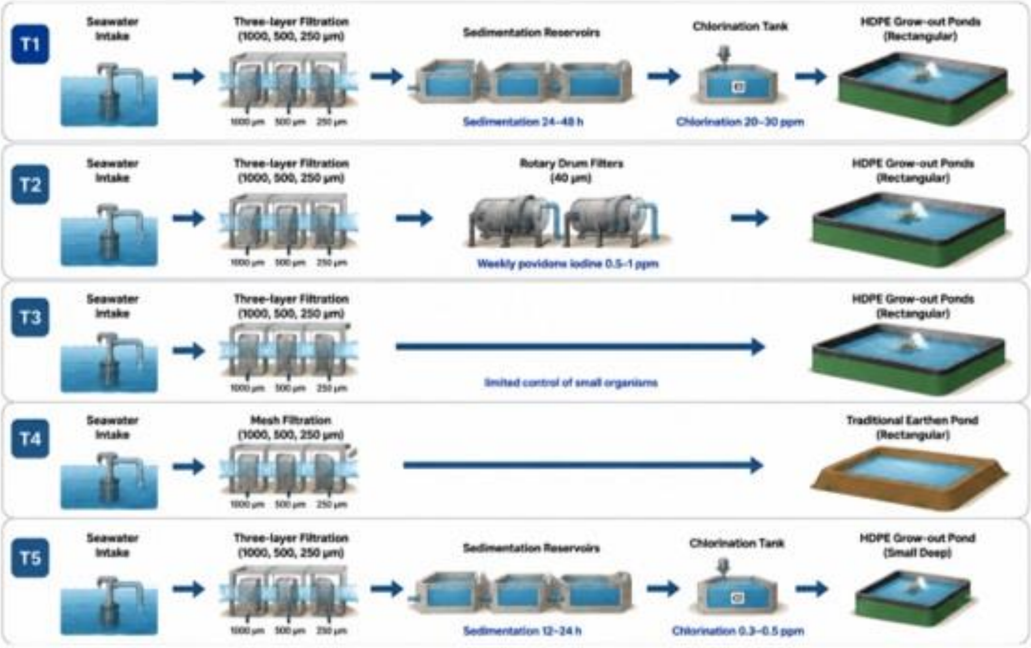

Figure 1: Schematic infographic of the five experimental treatments used in the shrimp culture study, showing pond types and water preparation systems. Treatments included HDPE-lined ponds with sedimentation–chlorination reservoirs (T1), mechanical drum filtration (T2), direct seawater intake after mesh filtration (T3), traditional earthen ponds with mesh filtration (T4), and small HDPE-lined ponds supplied through sedimentation reservoirs and chlorination (T5). Key differences among treatments were related to pond structure and the level of water treatment prior to entering the grow-out ponds.

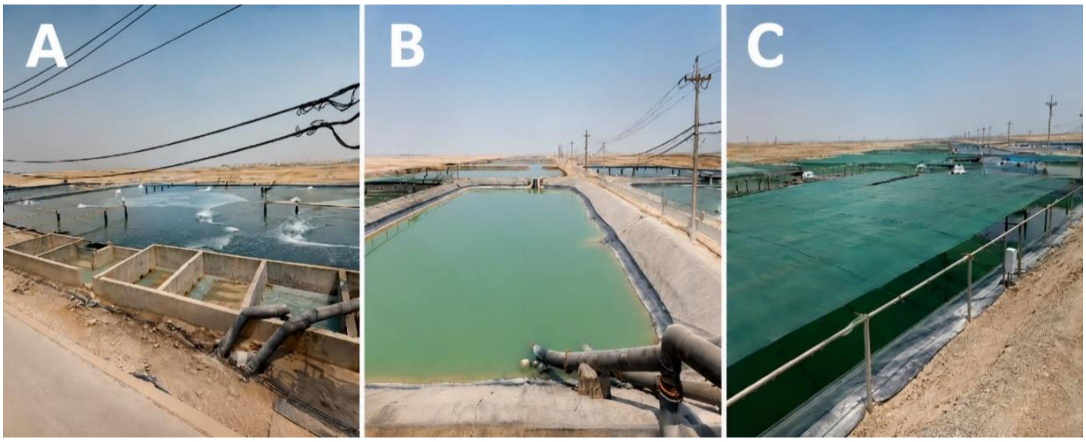

Figure 2: Water preparation and shrimp culture infrastructure in the studied farm: (A) three-layer water filtration system; (B) sedimentation, water storage, and disinfection ponds; and (C) lined shrimp culture ponds equipped with shade nets and water management infrastructure.

Table 1: Characteristics of the evaluated shrimp farming treatments.

Treatment	Pond type	Stocking density (PL m⁻²)	Description
T1	HDPE-lined ponds (0.75–1.0 mm; 0.7–1.0 ha; 1.4–1.6 m depth)	357–428	Traditional reservoir-based water preparation
T2	HDPE-lined ponds (0.75–1.0 mm; 0.7–1.0 ha; 1.4–1.6 m depth)	357–428	Advanced mechanical filtration system
T3	HDPE-lined ponds (0.75–1.0 mm; 0.7–1.0 ha; 1.4–1.6 m depth)	357–428	Lined ponds without intermediate storage
T4*	Traditional earthen ponds (0.7–1.0 ha; 1.4–1.6 m depth)	40–60	Traditional soil ponds
T5	Small HDPE-lined ponds (2000–3000 m²; 3–3.2 m depth)	357–428	Lined ponds with intermediate holding tanks

T4*: The management of the earthen ponds adhered to the prevailing standard operating procedures (SOPs) of the region, which encompassed bottom preparation protocols (specifically sun-drying,

Feeding and biomass management

Feeding strategy was influenced by pond size. In large HDPE-lined ponds (0.7–1 ha), feed was mainly distributed along pond margins, which could lead to uneaten feed accumulation and anaerobic sludge formation. As shrimp later moved toward these areas (around days 25–30), ingestion of contaminated sediments could increase *Vibrio* loads and AHPND risk. In contrast, smaller ponds (2000–3000 m²) allowed uniform feed distribution, improving feed consumption, reducing sludge formation, and promoting more uniform growth. Stocking density in these ponds ranged from 700,000 to 1,000,000 post-larvae per pond (≈333–500 PL m⁻²). Feeding rates were adjusted according to shrimp biomass and water temperature to minimize organic loading in the culture system. Standard feeding practices were applied around the optimal temperature range of 28–29 °C, with feeding rates proportionally modified in response to deviations in water temperature (Avnimelech, 2009, Boyd and Tucker, 1998). Biomass was regulated to maintain pond carrying capacity, with a maximum of 3 kg m⁻³ at 28°C, reduced by 10% for each 1°C increase; at 33–34°C biomass was maintained below 1.5 kg m⁻³. Partial harvesting began on days 70–80. Feeding (35–40% crude protein diet) and aeration (paddlewheel aerators and blowers) were standardized across farms (Boyd, 2000; Saraswathy *et al.*, 2022; Supriatin *et al.*, 2024; Kumar *et al.*, 2026).

Sampling, microbial analysis, and health assessment

Water samples were collected every 15 days from the mid-water column, and surface sediment samples were also collected for microbial analysis. Total heterotrophic bacteria (THB) were enumerated using standard plate count methods on Tryptic Soy Agar (TSA). *Vibrio* spp. was quantified on Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar. Water samples (100 mL) were processed by membrane filtration, while sediment samples (10 g) were analyzed after serial dilution. Presumptive *Vibrio* colonies were enumerated and expressed as CFU mL^{-1} for water and CFU g^{-1} for sediments. The presence of acute hepatopancreatic necrosis disease (AHPND) was confirmed using nested PCR targeting the *pirA* and *pirB* toxin genes from DNA extracted from the hepatopancreas of symptomatic shrimp (Tran *et al*., 2013; Kumar *et al*., 2021). Shrimp health status was monitored through daily visual inspections for clinical signs of AHPND (lethargy, hepatopancreatic discoloration, and reduced feeding). In addition, necropsy of 50 randomly sampled shrimp per pond was conducted approximately once per month to evaluate pathological changes. Daily mortality was recorded and cumulative mortality was calculated. Water quality parameters, including pH, dissolved oxygen (DO), total ammonia nitrogen (TAN), nitrite, and nitrate, were measured weekly using standard analytical kits (Hach or Merck). Growth performance and production indices were calculated to evaluate shrimp culture performance. The equations used for calculating yield, survival, mortality, feed conversion ratio, and growth parameters are presented in Table 2. Weekly growth rate was calculated according to Kumar *et al*. (2025).

Table 2: Growth performance and production indices used for data analysis.

Parameter	Calculation	Unit
Total biomass yield	Total harvested biomass / Pond area	kg ha^{-1}
Survival rate	(Number of shrimp harvested / Number of shrimps stocked) × 100	%
Cumulative mortality	100 − Survival rate	%
Mean daily mortality	Cumulative mortality / Culture duration (days)	% day^{-1}
Estimated survivors	Survival rate (assuming 100 individuals stocked)	shrimp per 100 stocked
Estimated loss	100 − Estimated survivors	shrimp per 100 stocked
Feed conversion ratio (FCR)	Total feed consumed / Total biomass gain	–
Mean final weight	Total harvested biomass / Number of shrimps harvested	g
Weekly growth rate	(Final mean weight − Initial mean weight)/Culture duration (weeks)	g week^{-1}
Specific growth rate (SGR)	(lnFinalweight−lnInitialweight)/Cultureduration (days)× 100	% day^{-1}

Statistical analysis

All data were expressed as mean±standard deviation (SD). Prior to statistical analysis, the normality of the data distribution was evaluated using the Shapiro–Wilk test, and the homogeneity of variances among treatments was assessed using Levene's test. Differences among the five production systems (T1–T5) in terms of microbial abundance (THB and *Vibrio* spp.), water quality parameters, and production performance indicators (total biomass yield, survival rate, FCR, mean final weight, and weekly growth) were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected ($p<0.05$), Tukey's HSD post-hoc test was applied to identify pairwise differences among treatments. Microbial counts (CFU mL^{-1} and CFU g^{-1}) were log$_{10}$-transformed prior to analysis to satisfy normality assumptions. Relationships between bacterial abundance and water quality parameters were evaluated using Pearson correlation analysis. All statistical analyses were performed using SPSS software (version 26.0, IBM Corp., USA), and statistical significance was accepted at $p<0.05$.

Results

Growth performance of *L. vannamei* differed significantly among treatments (**Error! Reference source not found.**). Shrimp reared in T2 showed the highest final weight and SGR, while T5 exhibited the greatest ADG and weekly growth. In contrast, T4 had the lowest final weight, growth rates, and SGR. Feed conversion ratio was markedly improved in T5, whereas higher FCR values were observed in the other treatments. Survival rate was highest in T5, intermediate in T1 and T2, and significantly reduced in T3 and especially T4. Production performance also varied significantly among treatments (Table 4). The highest biomass was obtained in T2, followed by T5 and T1, whereas T4 produced the lowest biomass. Production yield was greatest in T1 and T2, intermediate in T3, and markedly lower in T5 and particularly T4. Overall, these results indicate that T2 and T5 provided the most favorable conditions in terms of growth, while T1 and T2 yielded the highest production output per unit area.

Table 3: Growth performance of *L. vannamei* cultured under different treatments (mean ± SD).

Treatment	Final weight (g)	ADG (g day^{-1})	Weekly growth (g week^{-1})	SGR (% day^{-1})	FCR	Survival (%)
T1	17.6–26.0^{b}	0.20–0.24^{b}	1.4–1.7^{b}	3.10 ± 0.18^{b}	1.65–2.00^{b}	72.5 ± 2.5^{b}
T2	18.9–28.1^{a}	0.22–0.26^{b}	1.5–1.8^{b}	3.24 ± 0.17^{a}	1.70–2.10^{b}	72.5 ± 2.5^{b}
T3	15.8–25.4^{b}	0.12–0.16^{c}	0.8–1.1^{c}	3.01 ± 0.19^{b}	1.60–1.80^{b}	47.5 ± 2.5^{c}
T4	13.9–23.9^{c}	0.11–0.15^{c}	0.7–1.0^{c}	2.88 ± 0.21^{c}	1.70–2.30^{b}	25.0 ± 5.0^{d}
T5	18.0–26.8^{ab}	0.25–0.28^{a}	1.8–2.0^{a}	3.16 ± 0.18^{ab}	1.25–1.35^{a}	80.0 ± 5.0^{a}

Note: Different superscript letters indicate significant differences ($p<0.05$).

Table 4: Production performance of *L. vannamei* cultured under different treatments.

Treatment	Biomass (kg m^{-3})	Production yield (t ha^{-1} crop^{-1})
T1	2.6 ± 0.3^{b}	35–40^{a}
T2	2.9 ± 0.3^{a}	35–40^{a}
T3	2.3 ± 0.4^{c}	25–35^{b}
T4	1.6 ± 0.3^{d}	2.5–3.0^{c}
T5	2.7 ± 0.3^{b}	10–15^{d}

Note: Different superscript letters within each column indicate significant differences among treatments ($p<0.05$).

Survival and mortality performance

Survival differed significantly among treatments ($p<0.05$;

Table 5). The highest survival was observed in T5 (80.0±5.0%), followed by T1 and T2 (72.5±2.5%), whereas T3 (47.5±2.5%) and especially T4 (25.0±5.0%) showed significantly lower values. Consequently, cumulative mortality ranged from 20% in T5 to 75% in T4. The estimated mean daily mortality rate varied between 0.10% day^{-1} in T5 and 0.38% day^{-1} in T4, with intermediate values in T1 and T2 (0.14% day^{-1}) and T3 (0.26% day^{-1}). In practical terms, this corresponds to an estimated 75–85 survivors per 100 shrimp stocked in T5, compared with only 20–30 survivors per 100 stocked in T4, highlighting the markedly poorer survival performance in T4 relative to the other treatments.

Table 5: Survival and mortality parameters of shrimp cultured in different treatments during the 170–200-day culture period.

Treatment	Survival (%)	Cumulative Mortality (%)	Mean Daily Mortality (% day^{-1})	Estimated Survivors (per 100 stocked)	Estimated Loss (per 100 stocked)
T1	72.5 ± 2.5^{b}	27.5	0.14	72–75	25–28
T2	72.5 ± 2.5^{b}	27.5	0.14	72–75	25–28
T3	47.5 ± 2.5^{c}	52.5	0.26	45–50	50–55
T4	25.0 ± 5.0^{d}	75.0	0.38	20–30	70–80
T5	80.0 ± 5.0^{a}	20.0	0.10	75–85	15–25

Note: Values of survival are presented as mean ± SD derived from.

Water quality parameters

Throughout the 200-day culture period, temperature and salinity remained stable among treatments, with no significant differences detected ($p>0.05$). Dissolved oxygen and pH differed significantly among treatments, with T4 consistently showing the lowest values. In addition, T4 exhibited significantly higher total ammonia nitrogen and nitrite concentrations, along with the lowest water transparency, indicating poorer water quality conditions. By contrast, T2 and T5 generally maintained more favorable water quality, including lower nitrogenous metabolites and higher transparency. Alkalinity remained within a narrow range and did not differ significantly among treatments (Table 6).

Microbial dynamics

Temporal changes in microbial communities, assessed through total heterotrophic bacteria (THB) and *Vibrio* spp. in both water and sediment, showed clear treatment-dependent patterns over the 200-day culture period (Figure 3). In water, THB counts gradually increased in all treatments as the culture progressed, with more pronounced elevations towards the later phase (approximately days 170–200). Among treatments, T4 consistently exhibited the highest THB and *Vibrio* loads, particularly during the late grow-out phase, whereas T2 maintained the lowest bacterial densities throughout the production cycle. T5 also sustained relatively low microbial abundances, while T1 and T3 showed intermediate values with moderate late-phase increases. A similar pattern was observed in sediment, where both THB and *Vibrio* spp. progressively accumulated over time, with peak levels generally occurring during the last third of the culture period. Sediment from T4 recorded the highest bacterial loads, especially from around day 170 onwards, while T2 and T5 remained significantly lower than the other treatments ($p<0.05$). Overall, across both water and sediment, microbial loads followed a comparable ranking (T2<T5≤T1≈T3<T4), indicating more favorable microbial conditions and better microbial control in T2 and, to a lesser extent, T5, whereas T4 was characterized by persistently elevated bacterial loads towards the end of the culture period.

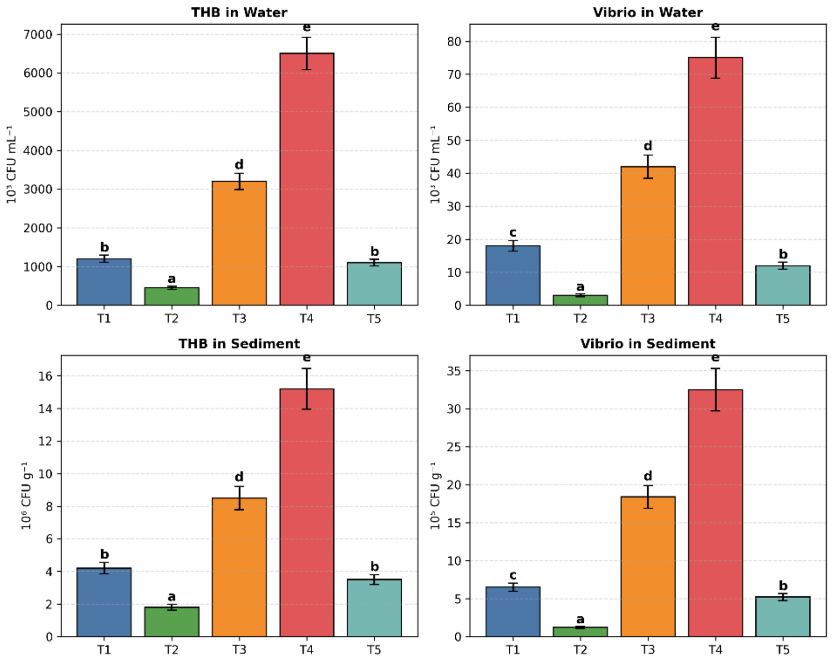

Figure 3: Comparative abundance of total heterotrophic bacteria (THB) and Vibrio in water and sediment across treatments T1–T5. Bars represent mean values, and error bars indicate standard deviation. Different letters above bars denote significant differences among treatments within each microbial indicator (*p*<0.05), whereas bars sharing the same letter are not significantly different.

Table 6: Water quality parameters during the 170–200-day culture period (mean ± SD) with statistical grouping.

Parameter	T1	T2	T3	T4	T5	Significance
Temperature (°C)	29.4 ± 1.1^a	29.3 ± 1.0^a	29.6 ± 1.2^a	29.7 ± 1.3^a	29.4 ± 1.1^a	ns
Salinity (ppt)	38.5 ± 2.1^a	38.2 ± 2.0^a	38.7 ± 2.3^a	39.1 ± 2.4^a	38.4 ± 2.1^a	ns
Dissolved oxygen (mg L^{-1})	5.6 ± 0.7^{ab}	5.9 ± 0.6^a	5.2 ± 0.8^b	4.7 ± 0.9^c	5.7 ± 0.6^a	*
pH	8.05 ± 0.21^a	8.10 ± 0.19^a	8.02 ± 0.23^a	7.92 ± 0.25^b	8.07 ± 0.20^a	*
Total ammonia-N (mg L^{-1})	0.18 ± 0.06^b	0.14 ± 0.05^b	0.23 ± 0.08^b	0.32 ± 0.10^a	0.17 ± 0.06^b	*

Parameter	T1	T2	T3	T4	T5	Significance
Nitrite-N (mg L⁻¹)	0.05 ± 0.02^{b}	0.04 ± 0.02^{b}	0.07 ± 0.03^{b}	0.09 ± 0.04^{a}	0.05 ± 0.02^{b}	*
Alkalinity (mg $CaCO_3$ L^{-1})	165 ± 18^{a}	168 ± 17^{a}	162 ± 19^{a}	158 ± 20^{a}	166 ± 18^{a}	ns
Transparency (cm)	34 ± 6^{a}	36 ± 7^{a}	31 ± 6^{b}	28 ± 5^{c}	35 ± 6^{a}	*

Note: Different superscript letters within each row indicate significant differences among treatments ($p<0.05$). ns = not significant.

AHPND occurrence and associated mortality.

The presence of acute hepatopancreatic necrosis disease (AHPND) was confirmed by nested PCR targeting the *pirA* and *pirB* toxin genes from DNA extracted from the hepatopancreas of symptomatic shrimp. During the culture period, particularly after approximately 30 days of rearing, most samples collected from the ponds of different treatments tested positive for AHPND. However, the severity and impact of infection varied among treatments. Based on the survival rates recorded at the end of the culture period, the lowest impact of the disease was observed in T5, which showed the highest survival, followed by T1 and T2 with moderate survival levels. In contrast, T3 exhibited considerably higher mortality, while T4 showed the most severe impact of the disease, reflected by the lowest survival rate among all treatments. These results indicate that although AHPND infection occurred in most ponds during the culture period, the degree of disease impact differed markedly among treatments (Figure 4).

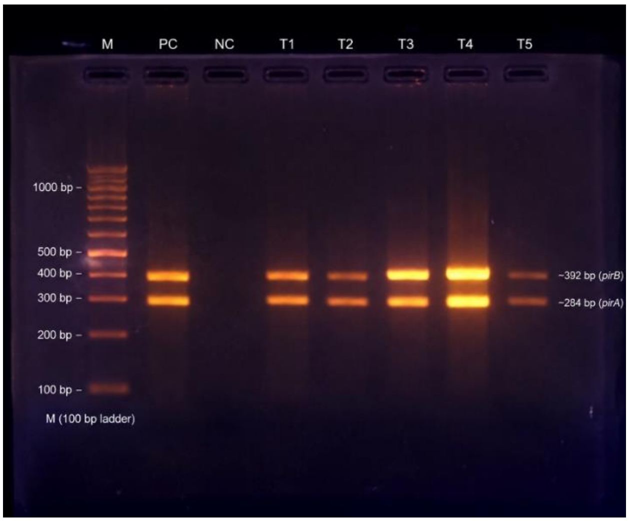

Figure 4: Agarose gel electrophoresis showing nested PCR amplification of pirA and pirB toxin genes associated with AHPND in shrimp hepatopancreas samples collected from different treatments. Lanes include a DNA ladder (M), positive control (+), negative control (−), and samples from treatments T1–T5. The presence of clear target bands in the treatment samples indicates PCR positivity for AHPND, with variation in band intensity among treatments.

Discussion

The results of the present field study indicate that shrimp production performance was strongly influenced by the efficiency of water preparation systems and their ability to regulate microbial dynamics in culture ponds. Although AHPND-associated genes (pirA and pirB) were detected in shrimp samples from most treatments, the severity of disease expression differed substantially among farming systems. This suggests that pathogen presence alone was not the primary determinant of production outcomes. Instead, environmental management and microbial regulation played a decisive role in shaping shrimp health and farm productivity.

Clear differences in total heterotrophic bacteria (THB) and *Vibrio* abundance were observed among treatments, indicating that water preparation acted as a primary ecological filter determining the microbial baseline of the ponds. Treatments utilizing more advanced water preparation protocols, particularly the drum filtration system (T2) and the lined pond system with reservoir management (T5), consistently exhibited lower bacterial loads throughout the culture period. In contrast, treatments relying mainly on simple filtration or lacking an effective reservoir stage (especially T4) showed substantially higher THB and Vibrio densities.

Mechanical filtration systems such as drum filters are capable of removing suspended organic particles and planktonic carriers of bacteria before water enters the culture ponds. By reducing particulate organic matter, these systems limit bacterial substrates and consequently suppress microbial proliferation (Boyd, 2000). Similarly, reservoirs facilitate sedimentation and allow partial microbial stabilization prior to water entering production ponds, which can significantly reduce pathogen introduction into shrimp farming systems (Saraswathy *et al*., 2022; Supriatin *et al*., 2024). The lower microbial abundance observed in T2 and T5 therefore likely reflects the efficiency of these water preparation strategies.

Conversely, inadequate water pre-treatment allows organic matter and microbial propagules to enter ponds, creating favorable conditions for opportunistic bacterial growth. Previous microbiome studies have shown that nutrient-rich aquaculture environments can promote the rapid expansion of heterotrophic bacteria, particularly *Vibrio* species that thrive in organic-rich conditions (Zoqratt *et al*., 2018; Xiong, 2018). The elevated bacterial loads recorded in T4 are therefore consistent with the limited capacity of this system to regulate microbial inputs and organic accumulation.

Water quality parameters further supported the link between environmental instability and disease severity. Among the treatments, T4 consistently exhibited the lowest dissolved oxygen levels and the highest concentrations of total ammonia nitrogen (TAN) and nitrite. Such conditions are widely recognized as major physiological stressors in shrimp aquaculture systems.

Elevated ammonia and nitrite concentrations disrupt osmoregulatory processes, reduce feeding activity, and suppress immune responses in penaeid shrimp (Romano and Zeng, 2013). Similarly, insufficient dissolved oxygen can impair metabolic efficiency and increase oxidative stress, weakening the ability of shrimp to resist bacterial infections (Boyd, 2000). Under these conditions, shrimp become more susceptible to opportunistic pathogens, including *Vibrio* species associated with AHPND.

Acute hepatopancreatic necrosis disease is caused by specific *Vibrio* strains harboring plasmid-encoded PirA and PirB toxins that induce severe hepatopancreatic damage (Tran *et al*., 2013; Soto-Rodriguez *et al*., 2015). However, disease expression in commercial ponds is strongly modulated by environmental conditions and host physiological status. Several studies have demonstrated that suboptimal water quality significantly increases mortality during AHPND outbreaks by intensifying physiological stress and facilitating pathogen proliferation (Han *et al*., 2015). The poorer environmental conditions observed in T4 therefore likely amplified the pathogenic effects of AHPND, contributing to the markedly lower survival rates recorded in this treatment.

The progressive increase in THB and *Vibrio* counts toward the later stages of the culture cycle suggests that organic enrichment played a major role in shaping microbial dynamics. In large HDPE-lined ponds, feed was primarily distributed along the pond margins. During the early culture stages (approximately days 25–30), uneaten feed accumulated in these areas and formed anaerobic organic deposits commonly referred to as black sludge. Such organic-rich sediments provide an ideal substrate for the growth of *Vibrio* species and other opportunistic bacteria.

Previous studies have demonstrated that the accumulation of organic matter in pond sediments significantly increases the abundance of *Vibrio* populations and elevates disease risk in shrimp aquaculture systems (Xiong, 2018; Zoqratt *et al*., 2018). These microbial hotspots can act as persistent reservoirs of infection, exposing shrimp to pathogenic bacteria through ingestion of contaminated particles or colonization of the digestive tract.

In contrast, smaller ponds or systems with improved feed distribution and sediment management tend to accumulate less organic waste, resulting in lower microbial loads and more stable pond conditions. This mechanism likely contributed to the lower *Vibrio* abundance and improved survival observed in T5.

Although PCR analysis confirmed the presence of pirA/pirB genes in shrimp from several treatments, the severity of AHPND differed markedly among farming systems. T4 exhibited the highest mortality and the lowest survival rate, whereas T1, T2 and T5 showed considerably better survival despite the molecular detection of the pathogen.

This observation supports the concept that AHPND outbreaks are not solely determined by pathogen presence but rather by the interaction between pathogen abundance, environmental stress, and host susceptibility. When *Vibrio* populations remain relatively low and water quality conditions are stable, shrimp may tolerate low levels of pathogenic bacteria without severe disease expression. However, when microbial loads increase and environmental conditions deteriorate, the likelihood of toxin production and disease progression rises significantly (Tran *et al*., 2013; Han *et al*., 2015).

Therefore, the differential survival observed among treatments in this study likely reflects variations in infection pressure and environmental resilience rather than differences in pathogen exposure alone.

When the results are considered collectively, a clear causal pathway emerges linking farm management practices to production outcomes. Water preparation systems influenced the microbial composition of the ponds and the stability of water quality parameters. These environmental factors determined the magnitude of bacterial proliferation and the severity of AHPND outbreaks, which ultimately affected shrimp survival, biomass accumulation, and production yield.

Treatments with more effective water preparation systems, particularly T2 and T5, maintained lower microbial loads and more stable environmental conditions, leading to improved survival and higher production performance. In contrast, limited water preparation and poorer environmental control in T4 allowed microbial populations to proliferate, exacerbating disease pressure and causing substantial production losses.

In conclusion, shrimp production performance was strongly influenced by the efficiency of water preparation systems and their ability to regulate microbial dynamics and environmental stability. Treatments with improved water management maintained lower *Vibrio* loads, better water quality, and consequently higher survival and biomass production despite the presence of AHPND-associated genes. These findings highlight that effective environmental management and microbial control are critical strategies for mitigating AHPND impacts and improving the sustainability of intensive shrimp farming systems.

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