

## Ecoepidemiology of *Zoothamnium* sp. as a gill ectoparasite in *Penaeus vannamei* and its relationship with water parameters Ecoepidemiología de *Zoothamnium* sp. como ectoparásito branquial en *Penaeus vannamei* y su relación con los parámetros del agua

Edison Pascal<sup>1</sup>, Helimar Vásquez-Pascal<sup>1,2</sup>, Ellen Acurero<sup>3</sup>, Rafael Yagua<sup>4</sup>

<sup>1</sup> Centro de Biomedicina Molecular "Dr. Humberto Fernández Morán", Instituto Venezolano de Investigaciones Científicas (IVIC), Maracaibo, Venezuela

<sup>2</sup> Facultad de Ciencias Veterinarias, Universidad del Zulia (LUZ), Maracaibo, Venezuela

<sup>3</sup> Facultad de Medicina, Universidad del Zulia (LUZ), Maracaibo, Venezuela

<sup>4</sup> Grupo LAMAR, Maracaibo, Venezuela.

**Correspondencia:** Edison Pascal **E-mail:** [epascal@ivic.gov.ve](mailto:epascal@ivic.gov.ve)

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**ABSTRACT** | The ciliate protozoan *Zoothamnium* sp. acts as an epibiont on the gills and carapace of shrimp and, at high densities, can cause respiratory impairment, molting disorders, reduced growth, and mortality. This study examines the ecoepidemiological relationship between *Zoothamnium* sp. infestation on the gills of *Penaeus vannamei* (mean weight  $12.5 \pm 2.3$  g) and environmental water conditions, to evaluate the influence of aquatic parameters on infection prevalence. The research was conducted on a shrimp farm in the Gulf of Venezuela, near Casigua, in Falcón state. Adult *P. vannamei* shrimp were captured, and their gills were extracted for pathological analysis and identification of *Zoothamnium*. The prevalence of the ciliate was calculated using the standard prevalence formula ( $P = N/Nt \times 100$ ) and correlated with physicochemical water parameters pH, salinity, temperature, and transparency using Pearson's linear correlation test. The results showed that decreasing pH and salinity, along with reduced water transparency, favored the proliferation of the protozoan on the shrimp gills. The strongest correlation was observed between water transparency and prevalence ( $r^2 = 0.891$ ). Water parameters significantly influenced the prevalence of *Zoothamnium*, and a proper control of water quality is essential to mitigate the negative impacts of this protozoan in shrimp cultures, thereby improving health and productivity in aquaculture systems.

### Palabras clave

Ectoparásito  
ciliophora  
prevalencia  
calidad del agua  
acuicultura de camarón

**RESUMEN** | El protozoo ciliado *Zoothamnium* sp. actúa como epibionte en las branquias y el caparazón de los camarones y, en altas densidades, puede causar insuficiencia respiratoria, trastornos de la muda, reducción del crecimiento y mortalidad. Este estudio examina la relación ecoepidemiológica entre la infección por *Zoothamnium* sp. en las branquias de *Penaeus vannamei* (peso medio  $12,5 \pm 2,3$  g) y las condiciones ambientales del agua, con el objetivo de evaluar la influencia de los parámetros acuáticos sobre la prevalencia de la infección. La investigación se realizó en una finca camaronera en el Golfo de Venezuela, cerca de Casigua, en el estado Falcón. Se capturaron camarones adultos de *P. vannamei* y se extrajeron las branquias para su análisis patológico e identificación de *Zoothamnium*. La prevalencia del ciliado se calculó utilizando la fórmula estándar de prevalencia ( $P = N/Nt \times 100$ ) y se correlacionó con los parámetros físico-químicos del agua, pH, salinidad, temperatura y transparencia, mediante la prueba de correlación lineal de Pearson. Los resultados mostraron que la disminución del pH y la salinidad, junto con la reducción de la transparencia del agua, favorecieron la proliferación del protozoario en las branquias de los camarones. La correlación más fuerte se observó entre la transparencia del agua y la prevalencia ( $r^2 = 0,891$ ). Los parámetros acuáticos influyeron significativamente en la prevalencia de *Zoothamnium*, por lo que el control adecuado de la calidad del agua es esencial para mitigar los impactos negativos de este protozoario en los cultivos de camarón, mejorando así la salud y la productividad en sistemas acuícolas.

## INTRODUCTION

The culture of the Pacific white shrimp (*Penaeus vannamei* Boone 1931) has experienced exponential growth in recent decades, becoming one of the most relevant economic activities in tropical and subtropical regions (Galaviz *et al.* 2016). However, this development has been accompanied by the proliferation of epibionts and pathogens, which impact shrimp health and reduce the yield of intensive farming systems (Cabrera 2012). Among these pathogens, ciliate protozoans of the genus *Zoothamnium* have been identified as common ectoparasites on the gills of *P.*

*vannamei*, where they negatively impact host physiology, primarily by altering gas exchange and inducing oxidative stress (Cabrera 2012, Gutiérrez 2011).

*Zoothamnium* sp. is a colonial protozoan that adheres to shrimp gills via a contractile stalk, allowing it to withstand water currents. However, massive colonization can induce a series of detrimental effects on gill tissue, including delamination of the cuticle, respiratory obstruction, and increased vulnerability to secondary infections (Valdez *et al.* 2010). Furthermore, the presence of *Zoothamnium* on shrimp gills can significantly reduce respiratory capacity, affecting the organism's growth and overall health (Limonta *et al.* 2017).

Despite histopathological studies that have documented structural damage in the gill tissues of infected shrimp, the molecular mechanisms responsible for the adhesion and tissue damage induced by *Zoothamnium* remain largely unknown. It has been suggested that these processes could be mediated by surface proteins and degradative enzymes secreted by the protozoan, while the immune response of *P. vannamei*, based primarily on its innate immune system, might be insufficient to control the proliferation of *Zoothamnium* (Cabrera 2012, Martínez 2002).

In addition to the biological factors involved, the environmental context plays a crucial role in the ecology and epidemiology of *Zoothamnium* in farming systems. It has been demonstrated that physico-chemical water parameters, such as pH, salinity, temperature, and transparency, significantly influence the prevalence and spread of this protozoan in shrimp (Galaviz *et al.* 2016). Variations in these parameters can influence both the metabolic activity of *Zoothamnium* and the capacity of *P. vannamei* to resist colonization, underscoring the importance of understanding the ecoepidemiological relationship between ectoparasites and aquatic environmental conditions.

This study aims to characterize the interactions between the protozoan *Zoothamnium* sp. and the gills of *P. vannamei*, focusing on the ecoepidemiological relationship with aquatic culture parameters. Through pathological and ecoepidemiological analyses, we intend to evaluate the impact of environmental conditions on the prevalence of this ectoparasite. This approach will contribute to a deeper understanding of aquatic pathology associated with epibionts and will enable the development of management strategies to mitigate the impact of *Zoothamnium* sp. in shrimp cultures.

## MATERIALS AND METHODS

### Study area

The present study was conducted on a shrimp farm from the Gulf of Venezuela, near the town of Casigua, in Falcón state. This area is representative of intensive *Penaeus vannamei* aquaculture systems in the region, where environmental and culture conditions are key for the development of epibionts and pathogens. While water conditions on the farm are maintained within controlled parameters, variations in physico-chemical parameters such as pH, salinity, and water transparency favor the colonization of ectoparasites like *Zoothamnium* sp., which provided an ideal context for this study (Cabrera 2012, Pascal & Vásquez 2024).

### Animal capture

Adult *P. vannamei* shrimp, aged between 3 and 6 months, were selected from the farm's culture ponds. The specimens had an average weight of  $12.5 \pm 2.3$  g, corresponding to juveniles in the final fattening phase, a critical stage where biomass density and organic matter accumulation increase the risk of epibiont colonization (Limonta *et al.* 2017). The shrimp were captured using seine nets on the farm and immediately transported to the laboratory for analysis. To ensure that the sampled shrimp were representative of the population in the study area, 100 individuals were randomly collected each month over four months, from June through September. This period was chosen as it corresponds to the late rainy-to-early dry season transition in the Gulf of Venezuela, during which progressive fluctuations in temperature, salinity, and organic load typically create favorable ecoepidemiological conditions for the proliferation of ciliate protozoans such as *Zoothamnium* sp. The shrimp were euthanized by anoxia induction, ensuring animal welfare during the process (Martínez 2002).

### Gill tissue collection

Once euthanized, the shrimp gills were extracted using sterile forceps. The gills were carefully separated and immediately placed on glass slides with a drop of sterile saline solution (0.9% NaCl) to maintain tissue hydration. Fresh mount preparations were subsequently examined under an optical microscope at 40× magnification to identify *Zoothamnium* sp. colonies attached to the gill surfaces, based on their characteristic morphology, including a contractile stalk and ciliated zooids. This direct pathological examination enabled the rapid detection and evaluation of ectoparasite colonization. (Pascal *et al.* 2024, Reyes 2021, Valdez *et al.* 2010).

### Ectoparasite identification

The identification of *Zoothamnium* sp. on gill tissues was performed through direct pathological examination using fresh mount preparations. Gill samples were observed under an optical microscope at 40× magnification to detect colonies attached to the gill surfaces. Identification was based on the characteristic morphological features of the protozoan, including the presence of a contractile stalk and ciliated zooids associated with gill colonization. These traits are considered diagnostic for the genus *Zoothamnium* and allow reliable identification in fresh preparations. (Pascal 2022, Reyes 2021).

### Prevalence formula

The prevalence of *Zoothamnium* sp. in *P. vannamei* was calculated using the following formula:

$$P = \frac{N}{N_t} \times 100$$

Where  $P$  is Prevalence,  $N$  is the number of hosts with parasites, and  $N_t$  is the total number of hosts examined. The calculation was performed monthly to evaluate the infection dynamics throughout the study period. Prevalence values were recorded monthly from June to September and compared with physico-chemical water parameters to identify potential correlations (Pascal *et al.* 2024, Cabrera 2012, Morales *et al.* 2011).

### Statistical analysis

To evaluate the relationship between the monthly prevalence of *Zoothamnium* sp. and the physicochemical water parameters (pH, salinity, temperature, and transparency), linear regression and correlation analyses were conducted. These analyses aimed to determine the degree to which variations in environmental conditions explained changes in ectoparasite prevalence.

For each physico-chemical parameter, the Pearson correlation coefficient ( $r$ ) was calculated to assess the strength and direction of the linear relationship, the coefficient of determination ( $r^2$ ) was calculated to estimate the proportion of variance in prevalence explained by each parameter, and the  $p$ -value was determined to assess the statistical significance of the relationship, considering a significance level  $p < 0.05$  (Field 2018).

## RESULTS AND DISCUSSION

### Prevalence of *Zoothamnium* sp. in the gill tissue of *P. vannamei* during the study period

Sessile protozoan (Ciliophora) *Zoothamnium* sp. was detected as an ectoparasite on the gill tissue of *P. vannamei*. The prevalence of the protozoan showed a clearly ascending trend throughout the study period, increasing from 18.5% in June to 30.7% in September, with intermediate values of 20.1% in July and 28.9% in August. This temporal progression suggests a strong influence of variable aquatic parameters during these months (Table 1).

**Table 1.** The prevalence (%) represents the frequency of gill infestation by *Zoothamnium* sp. in the sampled population. The "Increase" column calculates the percentage change in prevalence compared to the immediately preceding month, highlighting the acceleration of infestation, particularly in August.

**Tabla 1.** La prevalencia (%) es la frecuencia de infestación branquial por *Zoothamnium* sp. en la población muestreada. La columna "Aumento" calcula el cambio porcentual en la prevalencia con respecto al mes inmediatamente anterior, lo que pone de manifiesto la aceleración de la infestación, especialmente en agosto.

Month	Prevalence (%)	Increase Relative to Previous Month
June	18.5	-
July	20.1	+1.6%
August	28.9	+8.8%
September	30.7	+1.8%

The sustained increase in the prevalence of *Zoothamnium* sp. (Fig. 1) reached its highest levels towards the end of the culture cycle (September), which may be directly related to the accumulation of organic matter and a progressive deterioration of water quality. As the culture progressed, the shrimp biomass increased, along with the amount of feces, unconsumed feed, and other detritus. This condition may have created an environment conducive to the proliferation of bacteria, which are the primary food source for ciliates like *Zoothamnium* sp. (Schveitzer *et al.* 2013). This eutrophic condition provided an ideal nutritional substrate, allowing the protozoan populations to multiply and disperse more effectively among the hosts.

Furthermore, fluctuations in physico-chemical parameters, such as increased water temperature and a potential decrease in dissolved oxygen, typical of the final months of the culture cycle, can act as chronic stressors for the shrimp. This condition suppresses the shrimp's immune system and increases its susceptibility to parasitic infestations (Xu *et al.* 2021). The associated physiological stress also compromises the molting process, which represents the shrimp's primary defense mechanism for removing epibionts attached to the cuticle and gill surfaces.



**Figure 1.** Ciliate Protozoan *Zoothamnium* sp. on Gill Tissue of *Penaeus vannamei*. Fresh mount preparation (40x) revealing the structural organization of *Zoothamnium* sp. zooids (Z) attached to gill lamellae (GL).

**Figura 1.** Protozoo ciliado *Zoothamnium* sp. en tejido branquial de *Penaeus vannamei*. Preparación en fresco (40x) que revela la organización estructural de los zooides de *Zoothamnium* sp. (Z) adheridos a las lamelas branquiales (GL).

Clinically, severe infestations were associated with lesions in the gill lamellae, manifested by melanization (melanosis) of these respiratory structures. This melanotic response represents an innate immune mechanism in shrimp, in which phenoloxidase catalyzes melanin formation to encapsulate and isolate the parasite, damaged tissue, or secondary pathogens (Pascal *et al.* 2022, Sánchez *et al.* 2016). However, when excessive, this defense response may become detrimental. The accumulation of *Zoothamnium* sp. together with the associated melanization can cause mechanical obstruction of the gills, reduce the surface area available for gas exchange, and induce tissue hypoxia, thereby compromising the overall health of the shrimp and increasing its susceptibility to opportunistic infections.

### **Variation of physicochemical water parameters and their relationship with the proliferation of *Zoothamnium* sp.**

#### ***pH***

Throughout the study period, the water pH showed an acidifying trend, decreasing from 7.9 in June to 7.5 in September. This decline can be attributed to the accumulation of carbon dioxide (CO<sub>2</sub>) and organic acids resulting from respiration and bacterial decomposition of accumulated organic matter, a common phenomenon in intensive culture systems (Galaviz *et al.* 2016). Environmental acidification may have promoted favorable conditions for the colonization of *Zoothamnium* sp., since pH fluctuations have been reported to affect the integrity of the shrimp gill mucus, thereby facilitating the adhesion of epibionts (Valdez *et al.* 2010). Thus, the decrease in pH could have acted synergistically with other stress factors, promoting the increase in the protozoan's prevalence (Table 2).

#### ***Salinity***

Salinity decreased markedly from 35 ppt in June to 29 ppt in September. This reduction, likely associated with freshwater input related to evaporation replacement and culture management practices, represents an important osmotic stress factor for *P. vannamei*. Suboptimal salinity conditions can impair the shrimp's immune system by reducing the effectiveness of defense mechanisms such as phenoloxidase activity and molting frequency, thereby increasing susceptibility to the attachment and proliferation of epibionts (Martínez 2002). Therefore, the observed drop in salinity could have been a determining factor in the increased prevalence of *Zoothamnium* sp., by compromising the host's ability to counteract the infestation.

#### ***Temperature***

The temperature remained relatively stable, with a slight fluctuation from 28.2°C to 28.5°C. While this range is considered optimal for the growth of *P. vannamei*, it is also favorable for the replication of many ciliate protozoans. The metabolic rate of *Zoothamnium* sp. is favored at temperatures around 28-30°C, which can accelerate its life cycle and colonization potential (Cabrera 2012). Although the variation was minimal, the consistency of temperature within this optimal range may have provided a stable condition that sustained the continuous proliferation of the protozoan throughout the trial (Table 2).

#### ***Water transparency***

Water transparency, measured using a Secchi disk, decreased notably from 60 cm to 50 cm, indicating increased turbidity and a higher concentration of suspended particles. This change directly reflects an increase in phytoplankton density, particulate organic matter, and microbial biomass within the water column (Martínez 2002). The eutrophic condition is favorable for *Zoothamnium* sp., as the ciliate feeds on bacteria associated with this organic matter. The reduced transparency is not only a symptom of deteriorating water quality but also an indirect reflection of the conditions that favor the epibiont's trophic base, creating an ideal environment for its population explosion, as observed in August and September (Pascal *et al.* 2024).

**Table 2.** Temporal variation of physicochemical water parameters in the cultivation system of *P. vannamei*. Values are monthly averages. The "Optimal Range" for *Penaeus vannamei* was established based on scientific literature (Wurts 2005, Van Wyk & Scarpa 1999). The arrow ( $\searrow$ ) indicates a decreasing trend and ( $\nearrow$ ) an increasing trend throughout the study period. The initial salinity (35 ppt) was above the upper optimal range, while by the end of the study, the pH (7.5) and transparency (50 cm) were at the lower limit of the recommended range.

**Tabla 2.** Variación temporal de los parámetros fisicoquímicos del agua en el sistema de cultivo de *P. vannamei*. Los valores son promedios mensuales. El "Rango Óptimo" para *Penaeus vannamei* se estableció con base en la literatura científica (Wurts 2005, Van Wyk y Scarpa 1999). La flecha ( $\searrow$ ) indica una tendencia decreciente y ( $\nearrow$ ) una tendencia creciente a lo largo del período de estudio. La salinidad inicial (35 ppm) estaba por encima del rango óptimo superior, mientras que al final del estudio, el pH (7,5) y la transparencia (50 cm) estaban en el límite inferior del rango recomendado.

Parameter	Optimal Range *	June	July	August	September	Trend
pH	7.5 - 8.5	7.9	7.7	7.6	7.5	$\searrow$
Salinity (ppt)	15 - 30	35	33	31	29	$\searrow$
Temperature (°C)	28 - 32	28.2	28.3	28.4	28.5	$\nearrow$
Transparency (cm)	40 - 60	60	57	53	50	$\searrow$

### Mathematical relationship between epidemiological and environmental variables (Ecoepidemiology) through correlation analysis

The results of this study demonstrate a clear relationship between physicochemical water parameters and the increased prevalence of *Zoothamnium* sp. in the gills of *P. vannamei*. The potential implications of these findings within the context of intensive aquaculture are discussed below, with support from existing scientific literature.

#### Salinity

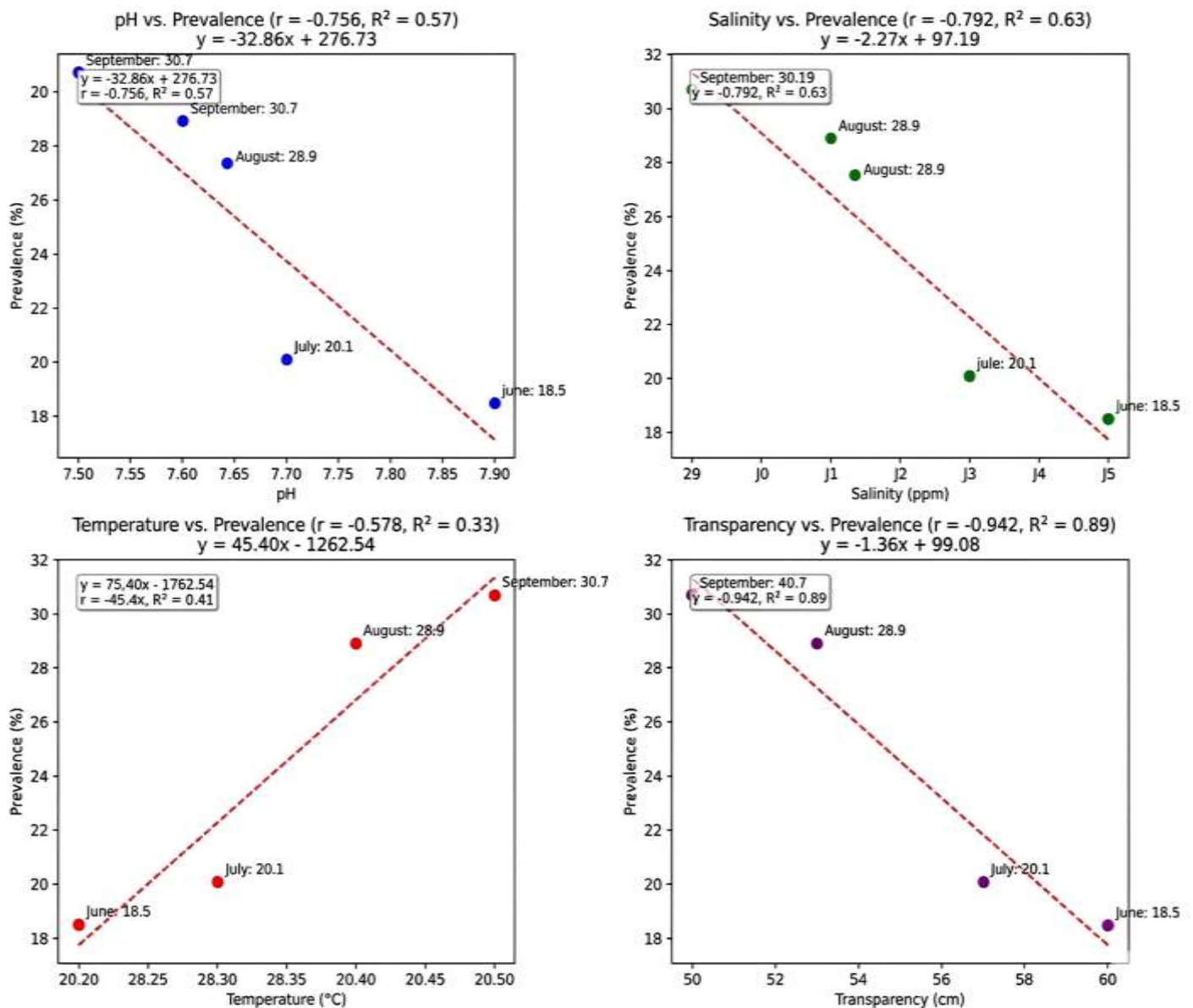
Salinity showed a negative correlation trend ( $r = -0.798$ ;  $p = 0.2$ ), indicating that decreased salinity is associated with increased prevalence of *Zoothamnium* sp. The reduction in salinity can affect the shrimp's osmoregulatory capacity, altering their homeostasis and negatively impacting their immune system (Limonta *et al.* 2017). This decrease in salinity may have favored the proliferation of the protozoan, as shrimp under osmoregulatory stress show a weaker immune response and are more susceptible to infections and epibiont colonization (Martínez 2002).

With a coefficient of determination ( $r^2$ ) of 0.636, the results indicate that 63.6% of the variability in the prevalence of *Zoothamnium* sp. can be explained by fluctuations in water salinity. This finding highlights the importance of monitoring and managing salinity levels in shrimp farming systems, since salinity not only affects shrimp welfare but also influences the dynamics of pathogen infection (Fig. 2).

#### Temperature

Temperature exhibited only slight variation during the study period, ranging from 28.2°C to 28.5°C, and showed a moderate negative correlation trend ( $r = -0.581$ ;  $p = 0.41$ ) with the prevalence of *Zoothamnium* sp. Although no substantial temperature fluctuations were recorded, ciliate protozoans such as *Zoothamnium* sp. are known to develop optimally within a temperature range of approximately 28°C to 30°C (Cabrera 2012). In the present study, water temperature remained within the tolerance limits of *P. vannamei*, which may explain the absence of pronounced effects of this parameter on ectoparasite prevalence. Nevertheless, previous studies have suggested that thermal fluctuations can stimulate pathogen metabolic activity, thereby promoting their proliferation in shrimp tissues (Galaviz *et al.* 2016). The coefficient of determination ( $r^2 = 0.337$ ) indicates that temperature accounted for approximately 33.7% of the variability in *Zoothamnium* sp. prevalence, suggesting that although temperature is a relevant factor, its influence is less pronounced than that of other environmental parameters (Table 3).

## pH



**Figure 2.** Scatter plots showing the relationship between the prevalence of *Zoothamnium* sp. on *P. vannamei* and the variation of physicochemical water parameters. The scatter plots display the correlation between water parameters and protozoan prevalence during the sampling period (June to September). Each graph includes trend lines, correlation coefficients ( $r$ ), and coefficients of determination ( $r^2$ ). The analysis reveals strong negative correlations for transparency, salinity, and pH, while temperature showed a weaker relationship. These patterns demonstrate the significant influence of deteriorating water quality on ectoparasite proliferation under ecoepidemiological conditions.

**Figura 2.** Diagramas de dispersión que muestran la relación entre la prevalencia de *Zoothamnium* sp. en *P. vannamei* y la variación de los parámetros fisicoquímicos del agua. Los diagramas de dispersión muestran la correlación entre los parámetros del agua y la prevalencia de protozoos durante el periodo de muestreo (junio a septiembre). Cada gráfico incluye líneas de tendencia, coeficientes de correlación ( $r$ ) y coeficientes de determinación ( $r^2$ ). El análisis revela fuertes correlaciones negativas para la transparencia, la salinidad y el pH, mientras que la temperatura mostró una relación más débil. Estos patrones demuestran la influencia significativa del deterioro de la calidad del agua en la proliferación de ectoparásitos en condiciones ecoepidemiológicas.

Water pH progressively decreased from 7.9 in June to 7.5 in September, exhibiting a negative correlation trend with the prevalence of *Zoothamnium* sp. ( $r = -0.756$ ;  $p = 0.24$ ). Previous studies suggest that a decrease in pH may favor the proliferation of epibiotic protozoans, as many of these organisms have a slightly acidic optimal pH range for their adhesion and growth (Galaviz et al. 2016). It has been demonstrated that certain pathogens, such as *Zoothamnium*

sp., respond positively to lower pH conditions, which could have facilitated their colonization on shrimp gills. Additionally, acidic pH reduces the effectiveness of the shrimp's immune system, favoring the adhesion of epibiotic protozoans (Valdez *et al.* 2010, Fig. 2).

The moderate correlation observed ( $r^2 = 0.572$ ) suggests that approximately 57.2% of the variability in *Zoothamnium* prevalence can be explained by the decrease in pH. This relationship highlights the importance of maintaining pH within optimal ranges for shrimp health and infection prevention, as pH fluctuations can alter the host's immune response, reducing its ability to defend against pathogens.

### Water transparency

Water transparency showed a strong correlation trend with ectoparasite prevalence ( $r = -0.944$ ;  $p = 0.056$ ), suggesting that decreased transparency, likely due to increased levels of organic matter and microorganisms in the water, favors the proliferation of this protozoan. Water turbidity is directly related to the accumulation of detritus, biological waste, and pathogens, which provide nutrients for epibionts like *Zoothamnium* (Pascal *et al.* 2024). In this study, the very strong negative correlation and the coefficient of determination ( $r^2 = 0.891$ ) indicate that 89.1% of the variability in protozoan prevalence can be explained by the decrease in water transparency. These findings emphasize the importance of maintaining good water quality and adequate transparency in culture systems to reduce the proliferation of pathogens and epibionts (Table 3).

**Table 3.** Correlation analysis between the prevalence of *Zoothamnium* sp. and physicochemical water parameters. This table shows the correlation coefficients ( $r$ ), significance values ( $p$ ), and coefficients of determination ( $r^2$ ) between the prevalence of *Zoothamnium* sp. and physicochemical water parameters (pH, salinity, temperature, and transparency) during the sampling period (June to September). The correlation values indicate the relationship between aquatic parameters and protozoan prevalence, while  $r^2$  values reflect the degree of variability explained by each parameter. The  $p$ -values help assess the statistical significance of these relationships.

**Tabla 3.** Análisis de correlación entre la prevalencia de *Zoothamnium* sp. y los parámetros fisicoquímicos del agua. Esta tabla muestra los coeficientes de correlación ( $r$ ), los valores de significancia ( $p$ ) y los coeficientes de determinación ( $r^2$ ) entre la prevalencia de *Zoothamnium* sp. y los parámetros fisicoquímicos del agua (pH, salinidad, temperatura y transparencia) durante el período de muestreo (junio a septiembre). Los valores de correlación indican la relación entre los parámetros acuáticos y la prevalencia de protozoos, mientras que los valores de  $r^2$  reflejan el grado de variabilidad explicado por cada parámetro. Los valores  $p$  ayudan a evaluar la significancia estadística de estas relaciones.

Parameter	Correlation Coefficient ( $r$ )	$p$ -value	Coefficient of Determination ( $r^2$ )
pH	- 0.75	0.24	0.57
Salinity	- 0.79	0.20	0.63
Temperature	- 0.58	0.41	0.33
Transparency	- 0.94	0.056	0.89

### Implications for intensive aquaculture

The increasing prevalence of the ciliate protozoan *Zoothamnium* sp. in the gills of *P. vannamei* throughout the sampling period highlights the close relationship between environmental conditions and epibiont proliferation in aquaculture production systems. The results obtained suggest that reductions in key parameters such as pH and salinity, together with decreased water transparency, promote ciliate colonization and negatively affect shrimp health and performance.

Effective water quality management, including the stabilization of pH and salinity levels as well as the improvement of water transparency through proper maintenance of aquaculture systems, may be essential for mitigating the proliferation of this ectoparasite and promoting shrimp health in intensive farming conditions.

Nevertheless, this research provides a deeper understanding of the relationship between physico-chemical water parameters and the prevalence of *Zoothamnium* sp. in shrimp farming systems. Appropriate management of these parameters is fundamental to controlling the proliferation of epibiotic pathogens and ensuring a healthy aquatic environment for shrimp in intensive culture systems.

## CONCLUSIONS

The prevalence of *Zoothamnium* sp. in *P. vannamei* increased progressively from 18.5% in June to 30.7% in September, demonstrating a clear temporal progression associated with deteriorating water quality.

Water transparency showed the strongest correlation with protozoan prevalence ( $r^2 = 0.891$ ), followed by salinity ( $r^2 = 0.636$ ) and pH ( $r^2 = 0.572$ ), indicating that reduced transparency, decreased salinity, and acidification are the main environmental drivers favoring *Zoothamnium* colonization.

Pathological analysis confirmed that *Zoothamnium* sp. adhesion causes structural damage to gill lamellae, including cuticle delamination and melanization, compromising the respiratory function and increasing susceptibility to secondary infections.

The innate immune response of *P. vannamei* is insufficient to control *Zoothamnium* epibiosis under suboptimal water quality conditions.

Maintaining optimal water quality conditions (particularly appropriate transparency, salinity, and pH levels) is essential for mitigating the proliferation of *Zoothamnium* and enhancing shrimp health and productivity in intensive aquaculture systems.

## Conflict of interest statement

The authors declare no conflicts of interest.

## Statement on ethical use of living organisms

In this study, good practices in the use of living organisms were adopted through maintaining optimal welfare conditions for *P. vannamei* specimens, in accordance with established ethical protocols for aquaculture research. The capture, transport, and sacrifice of shrimp were conducted using methods that prioritized reducing stress and animal suffering. These practices follow the recommendations of the World Organization for Animal Health (WOAH, 2022) regarding the responsible use of aquatic organisms in scientific research.

## Authorship contribution statement (CrediT)

*Edison Pascal*: Project coordinator, epidemiological calculations and data analysis, organism identification, methodology, and english translation. *Helimar Vásquez-Pascal*: Literature review, manuscript review, methodology. *Ellen Acurero*: Epidemiological calculations and data analysis, methodology. *Rafael Yagua*: Fieldwork, manuscript review.

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