ABANICO VETERINARIO E-ISSN 2448-6132 abanicoveterinario@gmail.com
https://abanicoacademico.mx/revistasabanico-version-nueva/index.php/abanico-veterinario

Abanico Veterinario. January-December 2025; 16:1-21. http://dx.doi.org/10.21929/abavet2025.1
Case study. Received: 17/07/2024. Accepted:10/02/2025. Published: 06/04/2025. Code: e2024-40.
https://www.youtube.com/watch?v=2TFB9Rujlu4

Factors affecting the density of *Vibrio parahaemolyticus* in the hepatopancreas of shrimp *Penaeus vannamei* in production units in Northwest Mexico

Factores que afectan la densidad de *Vibrio parahaemolyticus* en el hepatopáncreas del camarón *Penaeus vannamei* en unidades de producción al Noroeste de México

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ABSTRACT

Vibrio parahaemolyticus acts as an opportunistic and primary pathogen in shrimp production units, causing Acute Hepatopancreatic Necrosis Disease (AHPND) and the much more virulent postlarvae glass disease (HLVD) than the AHPND strain. However, the variables that determine the density of this pathogen in production units are not yet clear. For this reason, the aim of this study was to determine the physical, chemical and biological variables that favour its development in the hepatopancreas of *Penaeus vannamei* shrimp, in the water and sediment of culture ponds. The research was carried out in commercial farms located on the northwest coast of Mexico. At each farm, water, sediment and shrimp samples were collected for bacteriological analysis (CHROMAgar *Vibrio* plate seeding), water quality (nitrite NO_2^-, nitrate NO_3^-, ammonium NH_4^+, phosphate PO_4^{3-} and alkalinity $CaCO_3$), organic matter and pH (in sediment), as well as measurements of salinity, temperature, dissolved oxygen, pH (in water) and transparency. To analyze the association of physical, chemical and biological variables with the density of *V. parahaemolyticus* in the shrimp hepatopancreas, a Generalized Linear Model (GLM) was constructed. The model that best explained the data had an explained variance of 34.85%. Bacteriological examination showed that the northern and southern zones showed the highest average density of *V. parahaemolyticus* in the shrimp hepatopancreas. The model revealed a significant effect ($P < 0.05$) on the density of *V. parahaemolyticus* in culture pond water and sediment, culture cycle, dissolved oxygen, salinity, pH in culture water, NH_4^+ and alkalinity. The evidence found for the effect of some of the factors on the density of *V. parahaemolyticus* in the shrimp hepatopancreas suggests constant monitoring to avoid densities detrimental to shrimp health.

Keywords: shrimp farms, environmental variables, generalized linear model, *Vibrio parahaemolyticus*.

RESUMEN

El *Vibrio parahaemolyticus* actúa como patógeno oportunista y primario de las unidades de producción camaronícola, lo que ocasiona la Enfermedad de la Necrosis Hepatopancreática Aguda (AHPND, por sus siglas en inglés) y la Enfermedad de las postlarvas de cristal (HLVD) mucho más virulenta que la cepa AHPND. Sin embargo, las variables que determinan la densidad de este patógeno en las unidades de producción no son todavía claras. Por tal motivo, el objetivo del presente trabajo fue determinar las variables físicas, químicas y biológicas que favorecen su desarrollo en el hepatopáncreas del camarón *Penaeus vannamei*, en el agua y en el sedimento de los estanques de cultivo. La investigación se realizó en granjas comerciales localizadas en la costa del noroeste de México. En cada granja se recolectaron muestras de agua, sedimento y camarón para análisis bacteriológico (siembra en placa con CHROMAgar *Vibrio*), calidad de agua (nitritos NO_2^-, nitratos NO_3^-, amonio NH_4^+, fosfatos PO_4^{3-} y alcalinidad $CaCO_3$), materia orgánica y pH (en sedimento), así como mediciones de salinidad, temperatura, oxígeno disuelto, pH (en agua) y transparencia. Para analizar la asociación de las variables físicas, químicas y biológicas con la densidad de *V. parahaemolyticus* en el hepatopáncreas del camarón, se construyó un Modelo Lineal Generalizado (MLG). El modelo que mejor explicó los datos presentó una devianza explicada del 34.85%. El examen bacteriológico arrojó que la zona norte y la zona sur mostraron la mayor densidad promedio de *V. parahaemolyticus* en el hepatopáncreas del camarón. El modelo reveló un efecto significativo ($P<0.05$) en la densidad de *V. parahaemolyticus* en el agua y en el sedimento de los estanques de cultivo, ciclo de cultivo, oxígeno disuelto, salinidad, pH en el agua de cultivo, NH_4^+ y alcalinidad. La evidencia encontrada del efecto de algunos de los factores sobre la densidad de *V. parahaemolyticus* en el hepatopáncreas del camarón sugieren un monitoreo constante para evitar densidades perjudiciales para la salud del camarón.

Palabras clave: granjas de camarón, variables ambientales, modelo lineal generalizado, *Vibrio parahaemolyticus.*

INTRODUCTION

The bacterium *Vibrio parahaemolyticus* inhabits coastal marine environments and estuarine areas (Cariani *et al.*, 2012); it is found free in suspension or associated with suspended particles, such as: sediment (Heitmann *et al*., 2005), plankton (Turner *et al*., 2013), fish (Aliaga *et al*., 2010), bivalve mollusks (Rodríguez-Camacho *et al*., 2014), and crustaceans. In the latter, it usually lodges in the exoskeleton of shrimp because chitin is used by the bacteria as a nutrient (Dulanto, 2013).

The main factors influencing the occurrence and distribution of *V. parahaemolyticus* in seawater are temperature, salinity, nutrient availability, and fluctuations in plankton concentration (Hernández-Ayón *et al.,* 2003; Tantillo *et al*., 2004). Therefore, an imbalance in physical, chemical, and biological factors can cause the proliferation of *V. parahaemolyticus*, which is an opportunistic, facultative anaerobic, Gram-negative pathogenic bacterium responsible for high mortality rates (Cañigral-Cárcel, 2011). One of the diseases caused by *V. parahaemolyticus* is acute hepatopancreatic necrosis disease (AHPND), which has been reported in Asia and in various shrimp species, such as *Penaeus monodon*. The first outbreaks of this species were reported in 2009 in southern China, causing massive mortalities of up to 100% in crops (Kumar *et al.*, 2021). Another highly lethal disease caused by *Vibrio* (HLVD), known as “glass

postlarvae disease" (GPD) or "translucent postlarvae disease" (TPD), first appeared in 2019 and the causative agent has been identified as virulent strains of *V. parahaemolyticus* (Vp $_{HLVD}$) (Zou *et al.*, 2020; Yang *et al.*, 2023). Infection with Vp $_{HLVD}$ severely damages the hepatopancreatic tubule and midgut epithelium of infected shrimp, causing acute and massive mortality in shrimp postlarvae. Although both HLVD and AHPND are caused by vibrios and share similar clinical signs, Vp $_{HLVD}$ is much more virulent than Vp $_{AHPND}$ (Yang *et al.*, 2023).

In Mexico, there is no evidence of HLVD infection, but it is known that the first outbreaks of AHPND occurred in 2013, affecting *Penaeus vannamei* production in the northeast region, mainly in the states of Sonora, Sinaloa, and Nayarit (Galaviz-Silva *et al*., 2021), with estimated losses of up to 65 % in approximately 1,500 farms covering an area of 92 962 ha (Nunan *et al*., 2014). Additionally, it is important to note that the generation time of Vibrio is between 10 and 12 minutes, benefiting from the availability of nutrients in its environment and warm temperatures (> 35 °C), low salinities (< 8 UPS), and alkaline pH (> 7.5) (Zamora-Pantoja *et al.*, 2005). Therefore, some authors mention that the above-mentioned factors would be the main causes of the increase in the occurrence and distribution of this pathogen in seawater (Hernández-Ayón *et al*., 2003; Tantillo *et al.,* 2004). According to Lai *et al.* (2015), this disease occurs when shrimp are under stress or when their immune system is depressed, causing significant economic losses to the shrimp industry. It should be noted that the strains of *V. parahaemolyticus* that cause AHPND in Mexico show genetic differences from Asian strains and have developed resistance to commonly used antibiotics such as oxytetracycline and sulfacloropyridazine-trimethoprim (Galaviz-Silva *et al*., 2021). These findings highlight the importance of monitoring and managing *Vibrio* populations in shrimp aquaculture.

Fourteen years after the emergence of AHPND worldwide, this disease continues to affect the shrimp farming industry. Despite this, there are no reports of *in situ* research confirming the conditions that enhance the development of this microorganism, nor using a Generalized Linear Model (GLM). Nevertheless, predictions have been made and correlations identified for the abundance of *Vibrio* species using the parametric linear regression model (LRM) and the negative binomial model (NBM), as well as the generalized additive semiparametric model (GAM) (Dequito *et al.,* 2022). The only studies that have analyzed the effect of AHNPD on commercial shrimp farms are those by Estrada-Pérez *et al*. (2019) and Estrada Pérez *et al*. (2020) using multiple regression models with a stock model and a bioeconomic model, respectively. In the present study, we evaluated the different physical, chemical, and biological variables that could be contributing to the increase in the densities of this pathogen in the hepatopancreas of *Penaeus vannamei* shrimp, farmed in production units located in northwestern Mexico. In addition, the association of physical, chemical, and biological variables of coastal water bodies on the coastline of northwestern Mexico and of the water in the cultivation

ponds of shrimp farms with the increase in the density of *V. parahaemolyticus* in the hepatopancreas of *Penaeus vannamei* shrimp, using Generalized Linear Models (Nelder & Wedderburn, 1972) as an analysis tool. The purpose of the above is to generate scientific bases about the behavior of *V. parahaemolyticus* in shrimp culture ponds, which will contribute to decision making by the producer for the control and management of the pathogen in his culture facilities.

MATERIAL AND METHODS

Sample collection and measurement of environmental variables

A database of coastal water bodies was obtained, as well as from shrimp farms located in northwestern Mexico, specifically along the coastline of the state of Sinaloa (Figure 1) during 2017, in which ten samples were taken from 26 farms in the first cycle (February-June) and 18 in the second (July-November). The sampling areas were: northern zone: Ahome and Guasave; north-central zone: Angostura and Navolato; central zone: Eldorado and Cospita; south-central zone: Elota; and southern zone: Mazatlán.

During the first 30 days of cultivation, sampling was carried out weekly, as this was considered the critical stage (Hong *et al.,* 2016). Subsequently, sampling was carried out every two weeks.

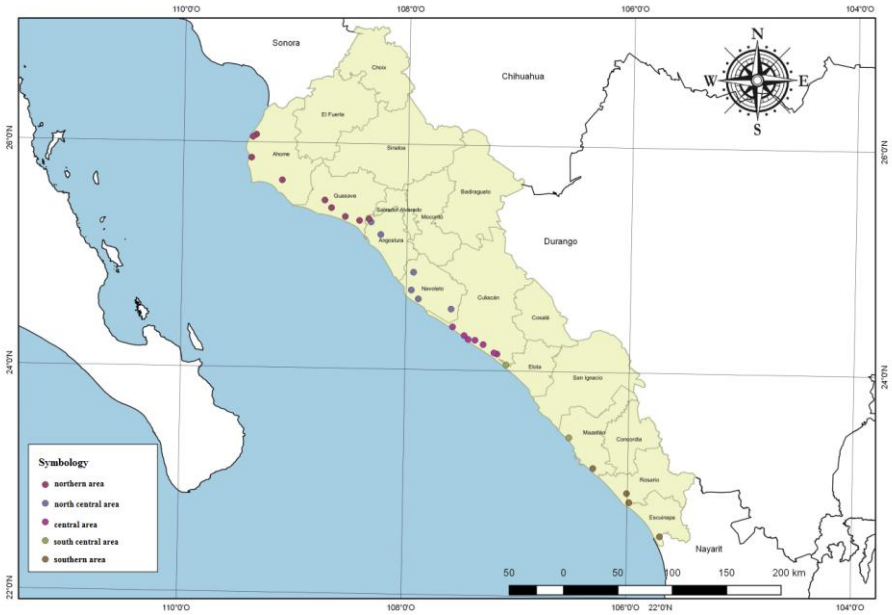

Figure 1. Study area and sampling points

Samples of organisms, water, and sediment from each aquaculture production unit were obtained from a randomly selected pond at the inlet and outlet gates. For the organism samples, 15 shrimp with feeding disorders and physiological alterations were selected (Soto-Rodríguez *et al*., 2010). For fresh and bacteriological analysis, the shrimp were kept alive and placed in ice chests with pond water to be transported to the

laboratory of the State Committee for Aquaculture Health of Sinaloa (CESASIN) where they were processed. Water samples were taken between 20 cm below the surface and 20 cm above the bottom. For bacteriological analysis, the samples were transferred to the aforementioned laboratory, using CHROMagar *Vibrio* plates for bacterial culture, and the results were subsequently quantified and expressed in units of volume (CFU/ml) or mass (CFU/g) (Soto-Rodríguez *et al*., 2015). Sediment samples for organic matter and pH analysis of the pond culture were collected with a sediment probe. Organic matter (OM) was evaluated in accordance with NOM-021-SEMARNAT-2000 using the Walkley & Black (1934) method through the oxidation of organic carbon (OC). In each sampling, data on salinity (S), temperature (T, °C), oxygen (OD, mg L^{-1}), and pH were recorded using a VITAL SINE SR6 handheld refractometer, an YSI-55-12 oximeter, and an YSI pH 10A potentiometer, respectively, and transparency (Tp, cm) using a Secchi disk.

Concentrations of nitrites (NO_2^-), nitrates (NO_3^-), ammonium (NH_4^+), and phosphates (PO_4^{3-}) were determined using the procedures described in the LYSA water analysis manual, based on the principle of Strickland & Parsons (1972). Alkalinity (Alc) was measured by the colorimetric method described by Hanna, using a HI755 manual colorimeter.

Generalized linear model and statistical analysis

To perform an exploratory analysis of the data, measures of central tendency were obtained for the physicochemical variables, nutrients, organic matter, sediment pH, and bacteriology (Figure 2). In order to determine the association between the different variables with respect to the density of *V. parahaemolyticus* (CFU/g) in the hepatopancreas of shrimp, Generalized Linear Models (GLM) (Nelder & Wedderburn, 1972) were adjusted using the base package of the R Core Team 2021 programming language.

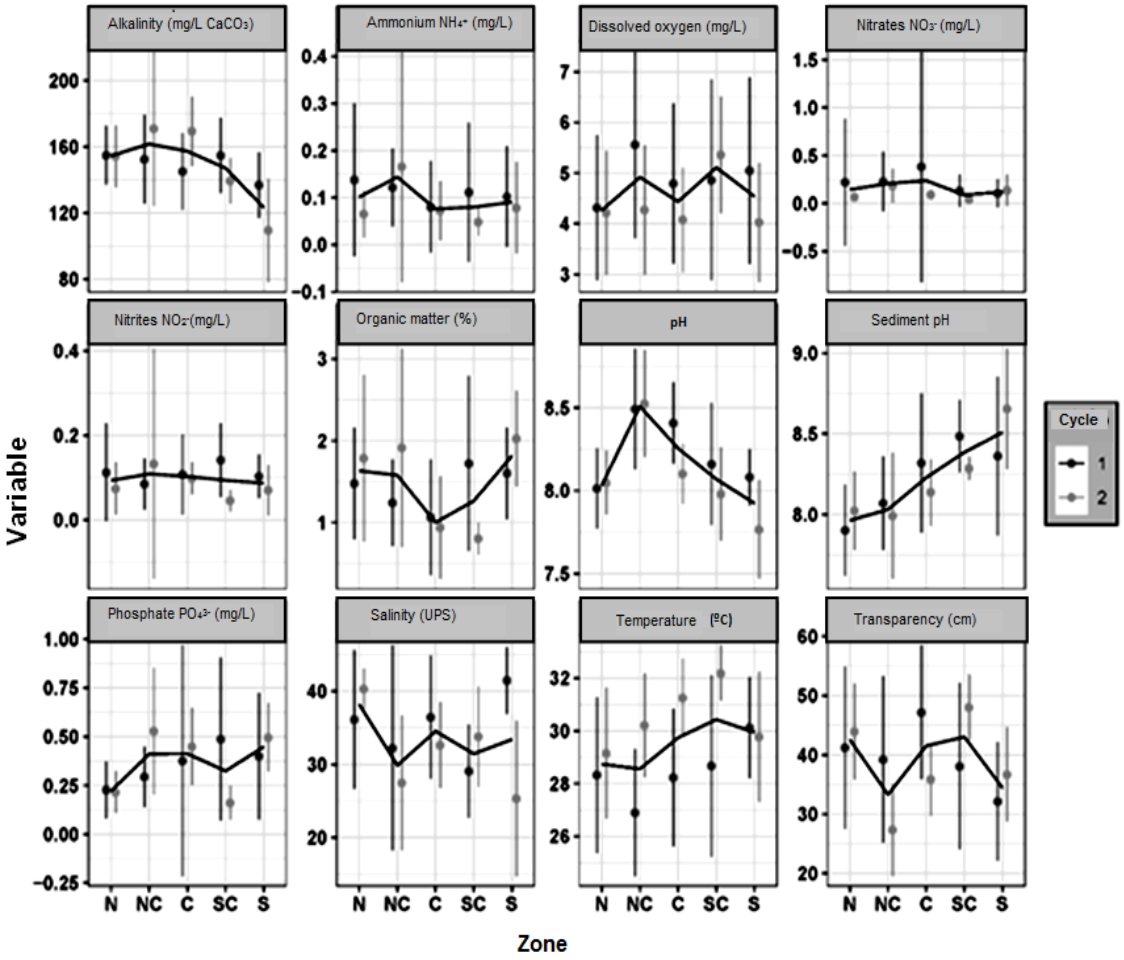

Figure 2. Average ± standard deviation by zone and cultivation cycle of physicochemical and biological variables in shrimp farms in northwestern Mexico. N = North, NC = North Central, C = Central, SC = South Central, and S = South. The black line marks the average between cultivation cycles

To construct the MLGs, T, DO, S, pH, Tp, NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, Alc, OM, pHs, month of cultivation, and density of *V. parahaemolyticus* (CFU/ml) in coastal water bodies and cultivation ponds were established as explanatory variables, as shown in Table 1. Since all explanatory variables were quantitative, a quadratic effect (second-degree polynomial) was added to the model in order to improve the fit of the variables, attempting to better explain the trend of the observed data, which was previously evaluated through exploratory analysis (Sokal & Rohlf, 1986). Since the CFU/g values in the hepatopancreas can only be equal to or greater than zero, a Gamma distribution was assumed in the model error, using the value of x + 1 as the response variable to eliminate the zeros. To assume a Gamma distribution, an inverse link function ($\eta = 1/\mu$) was used. The model selection for the most relevant explanatory variables was performed using the stepwise process with the Akaike criterion as the selection factor

(AIC), choosing as the best model the one with the lowest AIC value (Anderson & Burnham, 2002).

Table 1. Explanatory variables used for the Generalized Linear Model

Variables	Cycle I February-June	Cycle II July-December	Cycle I-II February-December
	Mean ± SD		
Physical and chemical properties of water			
Temperature (ºC)	28.3 ± 2.8	30.2 ± 2.2	29.0 ± 2.8
Oxigen (mg/L)	4.8 ± 1.7	4.2 ± 1.2	4.6 ± 1.6
Salinity (UPS)	35.6 ± 10.0	31.8 ± 9.2	34.2 ± 9.9
pH	8.2 ± 0.3	8.1 ± 0.4	8.2 ± 0.4
Transparency (cm)	40.9 ± 13.4	36.2 ± 9.9	34.2 ± 12.5
Water nutrients (mg/L)			
Nitrates (NO_3^-)	0.242 ± 0.74	0.118 ± 0.125	0.197 ± 0.592
Ammonium (NH_4^+)	0.112 ± 0.139	0.098 ± 0.149	0.107 ± 0.143
Nitrites (NO_2^-)	0.107 ± 0.091	0.094 ± 0.154	0.102 ± 0.118
Phosphates PO_4^{-3})	0.324 ± 0.371	0.406 ± 0.256	0.354 ± 0.335
Alkalinity	149 ± 22	154 ± 38	151 ± 29
Sediment			
OM (%)	1.4 ± 0.7	1.6 ± 1.0	1.5 ± 0.8
pH	8.17 ± 0.41	8.19 ± 0.38	8.19 ± 0.40
Hepatopancreas (UFC/g)			
Densidad de *V. parahaemolyticus*	46102.9 ± 265988.73	13543.8 ± 54802.76	33786.1 ± 212785.82
Bacteriology (UFC/ml) (UFC/g)			
Water- bodies	104.58 ± 781.87	102.46 ± 246.12	103.80 ± 639.82
Sediment- bodies	8256.34 ± 52608.30	12383 ± 35684.82	9765.47 ± 47131.54
Water -ponds	47.44 ± 136.50	87.86 ± 391.54	62.22 ± 260.86
Sediment-ponds	1931.99 ± 5508.59	2856.38 ± 7218.00	2270.04 ± 6197.39

Note: OM, organic matter

RESULTS

Bacteriology

With regard to the presence of *V. parahaemolyticus* in the hepatopancreas of shrimp, it was observed that the highest density of *V. parahaemolyticus* was found in both production cycles in the northern part of the state (Figure 3a). As sampling moved southward, a clear trend toward a decrease in the concentration of *V. parahaemolyticus* was observed. It was also observed that, in the north-central area, there was a higher concentration of bacteria during the month of October and only during the second production cycle (Figure 3b), while in the central and southern areas of the state, the month of September stood out (Figures 3c and d), and in the south-central area, the month of November stood out (Figure 3e).

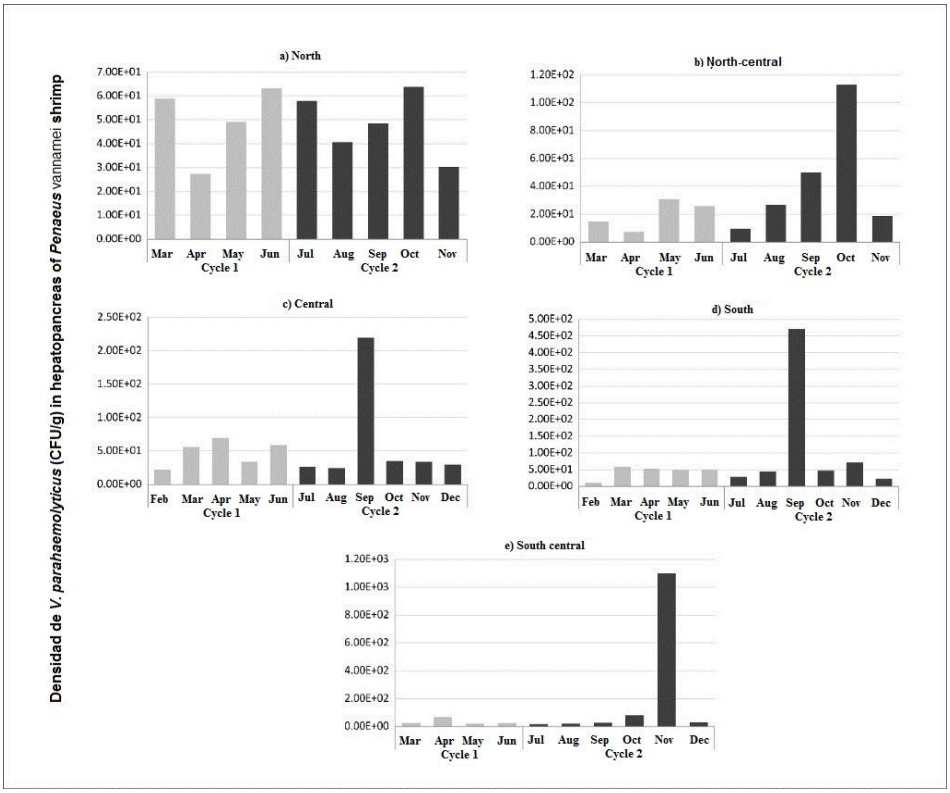

Figure 3. Behavior of *V. parahaemolyticus* density (CFU/g) in farm ponds in different areas.
Northern area a: Ahome and Guasave, north-central area b: Angostura and Navolato, central area c: El Dorado and Cospita, southern area d: Elota, and south-central area e: Mazatlán

Association of physical, chemical, and biological variables with the density of *V. parahaemolyticus* in the hepatopancreas of shrimp

The significant effect ($P<0.05$) of DO, S, pH of the culture water, NO_3^-, NH_4^+, Alc, OM, pHs, month of culture, and density of *V. parahaemolyticus* (CFU/ml) in water bodies and culture ponds explained the phenomenon of *V. parahaemolyticus* density in the hepatopancreas of shrimp with a null deviance of 5895.2, a residual deviance of 3840.2, and an explained deviance of 34.85% (Table 2).

Table 2. Statistical analysis of the physical, chemical, and biological variables that enhance the density of *V. parahaemolyticus* in the hepatopancreas of *Penaeus vannamei* shrimp

	Coefficient	Error		
Variable	**Estimated**	**Standard**	***t* Value**	**P**
(Intercept)	-5.2964	7.7347	-0.6847	0.4940
CFU in water				
(Water bodies)	0.0016	0.0011	1.4304	0.1537
CFU in water (Farm)	**-1.01E-05**	**1.94E-06**	**-5.1911**	**4.05E-07**
CFU in Sediment (Farm)	**-1.33E-08**	**3.41E-09**	**-3.9066**	**0.0001**
Cycle: 2	**-1.7144**	**0.6305**	**-2.7191**	**0.0069**
Month: april	-3.6409	3.8923	-0.9354	0.3503
Month: may	-3.3051	3.8084	-0.8678	0.3862
Month: june	-4.1755	3.8211	-1.0927	0.2754
Month: july	-2.4848	3.8558	-0.6444	0.5198
Month: august	-2.8677	3.8537	-0.7441	0.4574
Month: september	-4.1767	3.8496	-1.0849	0.2788
Month: october	-2.7915	3.8384	-0.7272	0.4676
Month: november	-5.8335	3.9263	-1.4857	0.1384
Month: december	-7.2182	4.2012	-1.7181	0.0868
Dissolved Oxygen (Farm)	**-0.4408**	**0.1553**	**-2.8375**	**0.0048**
Salinity (Farm)	**-0.0039**	**0.0019**	**-2.0667**	**0.0396**
pH in Water (Farm)	**-0.1034**	**0.0394**	**-2.6204**	**0.0092**
Organic matter (**Farm**)	0.3915	0.2429	1.6116	0.1081
pH in Sediment (Farm)	**0.0667**	**0.0358**	**1.8622**	**0.0636**
NH_4 in water (Farm)	**4.4103**	**2.0688**	**2.1317**	**0.0339**
NO_3 in Water (**Farm**)	-2.3772	1.3827	-1.7192	0.0866
Alkalinity in Water (Farm)	**-0.0005**	**0.0001**	**-2.8160**	**0.0052**

Variables in bold P < 0.05

The model indicated that the density of *V. parahaemolyticus* in the hepatopancreas increases as the concentration of bacteria found in coastal waters increases (Figure 4). A similar trend in the concentration of bacteria in sediments and water showed that intermediate concentrations increased the density of *V. parahaemolyticus* in the hepatopancreas; however, concentrations higher than intermediate values showed a decrease (Figure 4).

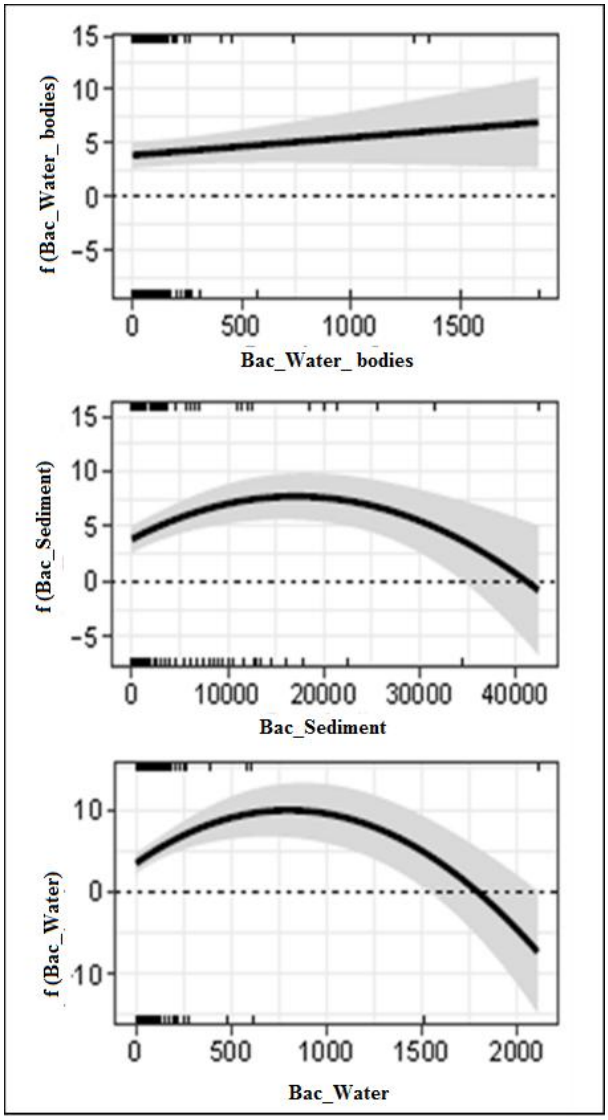

Figure 4. Effect of density (CFU/ml) of *V. parahaemolyticus* (Y) in coastal water bodies (a: X), in pond sediment (b: X), and in aquaculture pond water (c: X)

Regarding the cultivation cycle, cycle 1 favored *V. parahaemolyticus* density more than cycle 2 (Figure 5a). In general, higher values of dissolved oxygen, pH, salinity, and nitrates in the water decreased *V. parahaemolyticus* decreased (Figures 5b, c, d, and h, respectively), contrary to what occurred with organic matter, sediment pH, alkalinity, and ammonium, which, as their values increased, showed higher *V. parahaemolyticus* density (Figures 5e, f, g, e, and i, respectively).

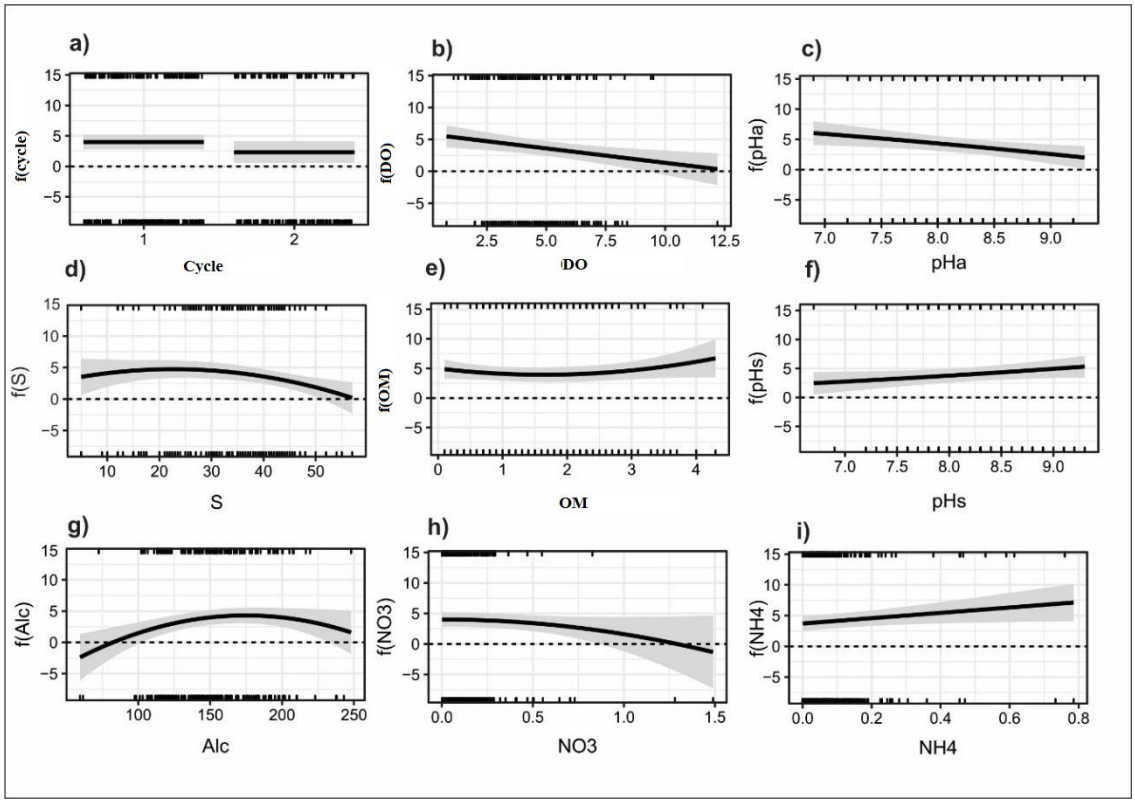

Figure 5. Influence of the culture cycle (a), dissolved oxygen (mg/L) (b), pH in the water (c), salinity (UPS) (d), organic matter (%) (e), pH of the organic matter in the ponds (f), alkalinity (g), NO_3 (h), and NH_4 (i) on the density of *V. parahaemolyticus* (CFU/g) in shrimp hepatopancreas f (Cycle, DO, pHa, S, OM, pHs, Alc, NO_3, NH_4)

DISCUSSION

Environmental variables play a fundamental role in shrimp development due to their physiological condition and because all biochemical processes have direct effects on food and dissolved oxygen consumption rates, ammonium excretion, and growth (Ferreira *et al*., 2011). In the present study, average temperature, dissolved oxygen, salinity, pH, and transparency were observed for both culture cycles within the optimal range for *P. vannamei* in culture, values consistent with those reported by other authors, such as temperature from 25 °C to 32 °C (Carbajal-Hernández *et al*., 2011; Mateka *et al*., 2015); dissolved oxygen from 4 to 7 mg/L (Alpuche *et al*., 2005); salinity of 15 to 41 UPS (Pérez-Velázquez *et al*., 2007; Rivas-Montaño *et al*., 2018); pH of 6 to 9 (Carbajal-Hernández *et al*., 2013) and transparency of 17 to 45 cm (García-Sánchez *et al*., 2018).

The results of this study showed that *V. parahaemolyticus* in the water supply reached a maximum of 1.35E+04 CFU/ml and, in the southern area, a maximum of 4.94E+03 CFU/ml in pond water. Therefore, it can be presumed that the water supply acts as an inoculum when it enters the cultivation pond. As mentioned by Orellana de Granados & Ayala-Mestanza (2017), these bacteria often find the conditions to grow due to combinations of multiple factors such as high concentrations of organic matter in the bottom, acidic soils, and variation in physicochemical parameters, among others. This proliferation of bacteria can be present in the hepatopancreas and hemolymph of shrimp and cause mortality (Orellana de Granados & Ayala-Mestanza (2017). This is to be expected since the shrimp farming water used in Sinaloa naturally has *Vibrio* sp. densities greater than 10^5 CFU/ml (Soto-Rodríguez *et al.,* 2010). This information is significant when considering acute hepatopancreatic necrosis disease (AHPND), since Soto-Rodríguez *et al.* (2015) mention that among the multiple factors that affect the virulence of *V. parahaemolyticus* is density, as they found that strain M09-04 caused 93 % mortality at a density of 10^5 CFU/ml. With regard to the concentration of *Vibrio* bacteria in the hepatopancreas of shrimp, Orellana de Granados & Ayala-Mestanza (2017) recorded *Vibrio* sp. densities of 1.10E+03 CFU/ml, with mortalities of up to 10% in the first 30 days of culture. In this study, higher concentrations of *V. parahaemolyticus* of 3.00E+06 CFU/g were obtained with maximum mortalities of 81%, demonstrating the impact of *Vibrio* sp. concentrations on mortalities. These results are consistent with Soto-Rodríguez *et al.* (2015), who pointed out that the virulence of *V. parahaemolyticus* strains depends on the dose, with an infectious density threshold of 10^4 CFU/ml; no mortality was observed below that density. Similarly, Soto-Rodríguez *et al.* (2010) estimate reference levels for possible vibriosis in diseased shrimp at a threshold of 1.40E+05 CFU/g of *Vibrio sp*. in the hepatopancreas.

The survival of *Vibrio* spp. in marine environments depends on carbon and energy sources, dissolved oxygen, water pH, salinity, temperature, and starvation (Takemura *et al.,* 2014). Regarding the associations of physicochemical variables, Hung-Sung *et al.* (2001) and Nelapati *et al.* (2012) mention that *V. parahaemolyticus* is a microorganism that has the ability to live and develop in aerobic and anaerobic conditions. However, in the present study, it was observed that at concentrations above 4.6 mg/L and close to 12.5 mg/L of DO, the density of *V. parahaemolyticus* decreases considerably. Concentrations lower than 4.6 mg/L but higher than 2.5 mg/L DO significantly favor an increase in the density of *V. parahaemolyticus* in the hepatopancreas of shrimp. These results are similar to those reported by Van Wyk *et al.* (1999), who suggest that corrective actions should be taken to reduce the density of *V. parahaemolyticus* when DO levels of 3 mg/L or lower are recorded in the culture water.

With regard to salinity, *V. parahaemolyticus* can tolerate a wide range of salinities, but its optimal growth conditions vary. In tropical waters, *V. parahaemolyticus* was found at salinities between 20-35 ppt, with higher densities at 20 ppt (Rivera *et al*., 1989). Experimental studies showed better adaptation at 10 and 20 UPS compared to 39 and 60 UPS (Sami *et al*., 2022); this is consistent with the results of this study, where it was observed that at salinities above 34.2 UPS and close to 50 UPS, the density of *V. parahaemolyticus* decreased, and at salinities of 10-34.2 UPS, the density of the bacteria was higher. Soto-Rodriguez *et al.* (2019) mention that the toxin can be expressed at different salinities and that salinity management in shrimp farming can be an important factor in controlling the infectivity of *V. parahaemolyticus.*

Regarding the pH in the water, it is important to note that studies on the impact of pH on the appearance of *Vibrio parahaemolyticus* in the hepatopancreas of farmed shrimp are scarce, so it is essential to continue with this type of research to clarify the influence of this parameter on the dynamics of *Vibrio parahaemolyticus* density. The results of this research indicate that *V. parahaemolyticus* densities (CFU/g) increased when the pH was lower than 8.2, with the highest density of V. parahaemolyticus occurring at values of 7.0. When the pH was greater than 8.2, the density of Vibrio decreased until a pH of 9.0, where the lowest density was found. These results contrast with the research of Mancilla (2005), who indicates that *V. parahaemolyticus* has the ability to grow in a pH range of 4.8 to 11, with an optimal growth range of 7.5 to 8.8 (FAO/WHO, 2011; Jurquiza, 2014).

Regarding the concentration of organic matter, several authors establish that it can have a direct effect on the health of organisms in culture, since it is capable of modulating bacterial populations and the production of toxic anaerobic metabolites that harm shrimp (Avnimelech & Ritvo, 2003; Nimrat *et al*., 2008). Merchán-Márquez (2017) establishes that the optimal percentage of organic matter in the soil of white shrimp farming ponds is 3 to 5%, since excessive accumulation of organic matter in the soil favors bacterial density. On the other hand, Díaz-Díaz & Arencibia-Carballo (1999), point out that the recommended organic matter values in pond bottom soil are 1.5% to 3%. In the present study, the average OM concentration was 2%, which is within the optimal range reported by other authors. However, it was observed that at concentrations greater than 2% and less than 0.5%, the density of *V. parahaemolyticus* present in the hepatopancreas of shrimp increases considerably, which could be due to the presence of organic nitrogen from the balanced feed present in the sediment, which promotes the formation of new bacterial biomass from its metabolization (Paez-Osuna & Alonso-Rodríguez, 2017), or that, when the concentration of organic matter in the bottom soil of the ponds is very high or excessive (>4%), there will be ideal conditions for microbial development, resulting in high concentrations of bacteria, mainly of the genus *Vibrio* (Díaz-Díaz & Arencibia-Carballo 1999; Nimrat *et al.*, 2008).

Alkalinity refers to base acids such as bicarbonate (HCO_3^-), carbonate (CO_3^{2-}) and hydroxide (OH^-) responsible for neutralizing water. Ferreira *et al.* (2011) mention that the level of alkalinity in culture ponds should not exceed 140 mg/L of $CaCO_3$, which coincides with the results found, as they show that when the level of alkalinity is between 150 and 200 mg/L, the density of *V. parahaemolyticus* in the hepatopancreas of shrimp is higher, while at levels below 140 mg/L and above 250 mg/L, the density of *V. parahaemolyticus* decreases.

With regard to nitrogen compounds (NH_4^+), (NO_2^-) and (NO_3^-), Tseng & Chen (2004), establish that high concentrations of these compounds affect the immune system of shrimp, leaving them susceptible to bacterial proliferation or causing physiological disorders such as the oxidation of hemocyanin to metahemocyanin. Camargo *et al.* (2005) mention that the optimal range for (NO_3^-) is 0.4 to 1.3 mg/L, while for (NO_2^-), Boyd (1995) suggests that an adequate concentration for shrimp development is <0.23 mg/L. However, the results show that at concentrations greater than 0.5 mg/L of NO_3^-, the density of *V. parahaemolyticus* in the hepatopancreas of shrimp decreases considerably, contrary to what occurs with NH_4^+, where the concentration of *V. parahaemolyticus* increases at higher concentrations of NH_4^+. This behavior is similar to that reported by Joseph *et al.* (1982), who established that *V. parahaemolyticus* does not survive in waters with low nutrient concentrations, while Tantillo *et al.* (2004) reported that ammonium salts are the main source of nitrogen for *V. parahaemolyticus*, which is consistent with the present study.

Predicting the abundance of presumed *Vibrio* species can help prevent the onset of bacterial diseases, as it provides information on when and which environmental factors should be managed. Equito *et al*. (2022) used the parametric linear regression model (LRM) and the negative binomial model (NBM), as well as the generalized additive semiparametric model (GAM) to identify correlations and predict changes in *Vibrio* abundance with physicochemical and biological parameters of the water in biofloc ponds with *Penaeus vannamei*. These authors found that the abundance of presumed Vibrio species was highly correlated with alkalinity, pH, and phytoplankton density. In the present study, using the generalized linear model (GLM), it was shown that as salinity, dissolved oxygen, pH, and nitrates in the water increased, the density of Vibrio decreased (with values outside the optimal range for *V. parahaemolyticus*), contrary to what was observed with sediments, where higher pH, alkalinity, and ammonium the density of *Vibrio* increased; concluding that, with an increase in the concentration of bacteria in water and sediment, the model indicated an increase in the concentration of bacteria in the hepatopancreas of shrimp. However, Estrada-Pérez *et al.* (2019) and Estrada-Pérez *et al.* (2020), using a stochastic model of AHPND-infected shrimp production, found in their sensitivity analysis that mortality caused by the disease is significantly related to the salinity and transparency of the pond water. These

differences in the results of the present study are due to the fact that the disease itself was not considered, but rather the density of *V. parahaemolyticus* bacteria.

In this research, temperature, transparency, and NO_2^- and PO_4^{3-} concentration had no effect on the density of *V. parahaemolyticus* (CFU/g) in the hepatopancreas of shrimp. According to the model used, it was indirectly detected that 65.15% of factors affected the density of *V. parahaemolyticus* in the hepatopancreas of shrimp. However, in the present study, it was not possible to measure or consider natural phenomena in the culture environment or management variables, so it is recommended that these be included in future studies to increase predictive capacity. It is concluded that the Generalized Linear model adequately explained the association of various factors with the concentration of *V. parahaemolyticus* in the hepatopancreas of shrimp, and its results are generally consistent with those published in the literature. Furthermore, the results suggest constant and effective monitoring and management to maintain shrimp crops at recommended values, preventing bacterial loads from increasing to levels that affect shrimp health.

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Errata Erratum

https://abanicoacademico.mx/revistasabanico-version-nueva/index.php/abanico-veterinario/errata