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Generalised Additive Model Improves Estimates of *Vibrio* Species Abundance in *Penaeus vannamei* Boone, 1931 Biofloc Production System

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Abstract

Environmental factors influence the abundance of *Vibrio* species in shrimp culture systems. Prediction of the abundance of presumptive *Vibrio* species can help prevent the occurrence of bacterial diseases as this will provide insights about when and which environmental factors to manage. In this study, the parametric linear regression model (LRM) and negative binomial model (NBM), and semiparametric generalised additive model (GAM) were used to identify correlations and predict changes of *Vibrio* abundance with physicochemical and biological water parameters. Water parameters were recorded from three 300 m² biofloc ponds stocked with *Penaeus vannamei* Boone, 1931, at 500 individuals.m⁻³ over four culture run periods. Each culture run lasted for 16 weeks. Imputed data were initially subjected to univariate analysis and Pearson's correlation analysis. The abundance of presumptive *Vibrio* species was found to be highly correlated with alkalinity, pH, and phytoplankton density. GAM performed best among the three models based on Akaike's information criterion (AIC), having the smallest value of 5,743.222 compared to 6,572.014 and 5,857.997 values for ordinary LRM and NBM, respectively. It also had the largest deviance explained statistic with 41.2 % of the deviance reduced by including the predictors compared with ordinary LRM and NBM with only 16.04 % and 14.5 % deviance reduced, respectively. GAM introduced flexibility that predicts the dependent variable better in terms of statistical significance than LRM and NBM. It is important to consider using a semiparametric modelling approach as a tool for aquaculture management.

Keywords: linear regression model, negative binomial model, statistical modelling techniques, abundance of vibrios

Introduction

Bacteria of the genus *Vibrio* are ubiquitous in aquaculture production systems. They cause vibriosis and other associated diseases, posing a major economic threat to shrimp farming (de Souza Valente and Wan, 2021). The abundance of *Vibrio* species is influenced by abiotic factors such as temperature, pH, and salinity, and biotic factors such as microalgae and other organisms (Takemura et al., 2014). One strategy in suppressing the abundance of *Vibrio* species is the application of biofloc technology (BFT). It promotes large groups of heterotrophic microorganisms known as floc. The heterotrophic microorganisms limit the growth of *Vibrio* by competing on space, substrate, and nutrients (Emerenciano et al., 2013; Cadiz et al., 2016). Despite

the application of BFT, the presence of external stressors such as low dissolved oxygen levels, high temperature, and high total suspended solids can still encourage the proliferation of pathogenic *Vibrio* on *Penaeus vannamei* Boone, 1931, production in intensive biofloc systems (Prangnell et al., 2015). These disturbances are the defining features of many ecological systems and can result in changes in ecosystem structure and function (Folke et al., 2004; Dudgeon et al., 2006).

The correlations between environmental factors and *Vibrio* abundance have to be investigated for effective shrimp health management. It is important to establish the leading factors that drive *Vibrio* abundance. Parameters known to show significant correlations with *Vibrio* abundance can be used to

predict when a disease outbreak might occur. Statistical modelling can help investigate and predict the relationship between environmental factors and *Vibrio* abundance (Selven and Philip, 2012). The role of the environmental factors and their interaction with organisms has been recognised in the literature using both parametric and semiparametric models (Ma and Xu, 2015; Fernandez-Zapico et al., 2017). Semiparametric modelling is a recent technique that introduces flexibility to parametric models. It is a statistical model with parametric and nonparametric components. Parametric models often use stringent distributional assumptions such as normality and homoscedasticity of error terms and heavily rely on the assumption that the relationships between the dependent and independent variables are linear and monotonic. Violations of these assumptions lead to biased estimates and unreliable predictions using these models. A semiparametric approach, such as the generalised additive model (GAM), can be used to model both linear and nonlinear relationships without any of the distributional assumptions used in a linear model (Sun and Kumbhakar, 2013). GAM provides a more realistic approach to modelling growth curves and higher prediction accuracy in the spatio-temporal distribution and abundance of aquatic species in relation to predictor variables such as oceanographic or environmental conditions (Olsen et al., 2006; Ligas et al., 2015; Wang et al., 2020; Yusop et al., 2020).

This paper aims to compare the predictive abilities of GAM with backfitting algorithm with ordinary linear regression model and negative binomial model to presumptive *Vibrio* abundance in BFT aquaculture setting.

Materials and Methods

Culture conditions

Whiteleg shrimp (*P. vannamei*) was reared in 300 m² ponds with a depth of 1.8 m for 16 weeks in Carcar Prawn Farm, Carcar City, Cebu, Philippines, over a four-culture run period. Rearing water conditions were uniformly stabilised at normal levels in BFT before stocking *P. vannamei* at 500 individuals.m⁻³. The limited water exchange system utilised molasses as the carbon source and its supplementation was adjusted based on 15 mL.L⁻¹ floc level (Ebeling et al., 2006). Water management practices involved the application of lime and probiotics to adjust alkalinity and ammonia levels, respectively. Shrimp were fed twice daily with commercial shrimp feeds at the rate of 10 % body mass with consequent adjustments based on weekly growth sampling.

Monitoring of physicochemical and biological water parameters

Physicochemical properties of water such as dissolved oxygen, temperature, pH, salinity, and

transparency were recorded daily. Methyl orange alkalinity (M.O. alkalinity), ammoniacal-nitrogen (NH₃-N) and nitrite-nitrogen (NO₂-O) were measured (Fortes and Pahila, 1992) every other day. Phytoplankton and zooplankton densities determination (Prescott, 1962; Martinez et al., 1975) and bacterial enumeration were done every other day. Total bacterial and presumptive *Vibrio* counts were determined using dilution and spread plate techniques on nutrient agar supplemented with NaCl following the method of Reilly (1982) as cited in Janeo et al. (2009) and on thiosulfate citrate bile salt sucrose agar (TCBS), respectively. Green and yellow colonies were enumerated as presumptive *Vibrio* isolates (Okoh et al., 2015). The plates were incubated for 24 h at 30 °C. Aseptic techniques were strictly observed in carrying out bacteriological analysis.

Data pre-processing

Imputation is the first statistical technique used in this study with the purpose of filling in missing values. The data contained a few missing at random (MAR) and mostly missing not at random (MNAR) values in chemical and biological parameters of water because sampling is done every other day, and when the laboratory is closed due to holidays. The imputed data set can provide inferential parameter estimates and fill in missing data without distorting the distribution of the original dataset (Kang, 2013). Univariate analysis was used to describe, summarise, and find patterns in each water parameter based on central tendencies and dispersion. Each parameter was plotted against presumptive *Vibrio* count as *Vibrio* abundance. Plotting the two variables using the locally weighted scatterplot smoothing (LOESS) graph gave the summary of the actual data on the behaviour of *Vibrio* abundance, whether increasing or decreasing, for instance. Pearson's correlation analysis was used to know whether there was a presence of multicollinearity among all the water parameters. The presence of multicollinearity would dictate whether certain parameters could be clustered, reducing the number of variables used for modelling.

Parametric and semiparametric modelling

Two types of parametric models were used for comparison. The ordinary LRM summarises relationships between two continuous variables and the NBM accounts for possible over-dispersion in the data set (Linton, 2017). In the semiparametric GAM, the function derived from each graph generated from the GAM fit, regardless of parametric, was used to create the model. The gam function in the mgcv package (v1.8-17; Wood, 2011) in R was used to estimate the parametric components' coefficients and for smoothing the nonparametric components' functions. Akaike's information criterion (AIC) was the

index used to compare the three models based on the “goodness-of-fit” (Fabozzi et al., 2014). Statistical analyses were done using SPSS[®]20 and freeware R, an open-source statistical programming language and the environment by R Core Team (2013).

Results

Data pre-processing

Vibrio abundance plotted against physicochemical and biological parameters using locally weighted scatterplot smoothing known as the LOESS method revealed possible parametric and nonparametric forms of the data. Figure 1a depicts a sigmoidal curve

where *Vibrio* abundance initially increases slowly, then goes exponentially but eventually approaches a steady state when plotted against dissolved oxygen. Figure 1b shows that *Vibrio* species frequently occur in temperatures between 28 °C to 30 °C. A positively inclined line observed in Figures 1c and 1d suggests a direct relationship between *Vibrio* abundance with salinity and pH. In contrast, Figures 1e and 1f suggest an indirect relationship between *Vibrio* abundance to alkalinity and ammonia levels. *Vibrio* abundance initially increases but then eventually decreases as nitrite level increases in Figure 1g. *Vibrio* abundance and water transparency seem to have no relationship, as indicated by the horizontal line in Figure 1h.

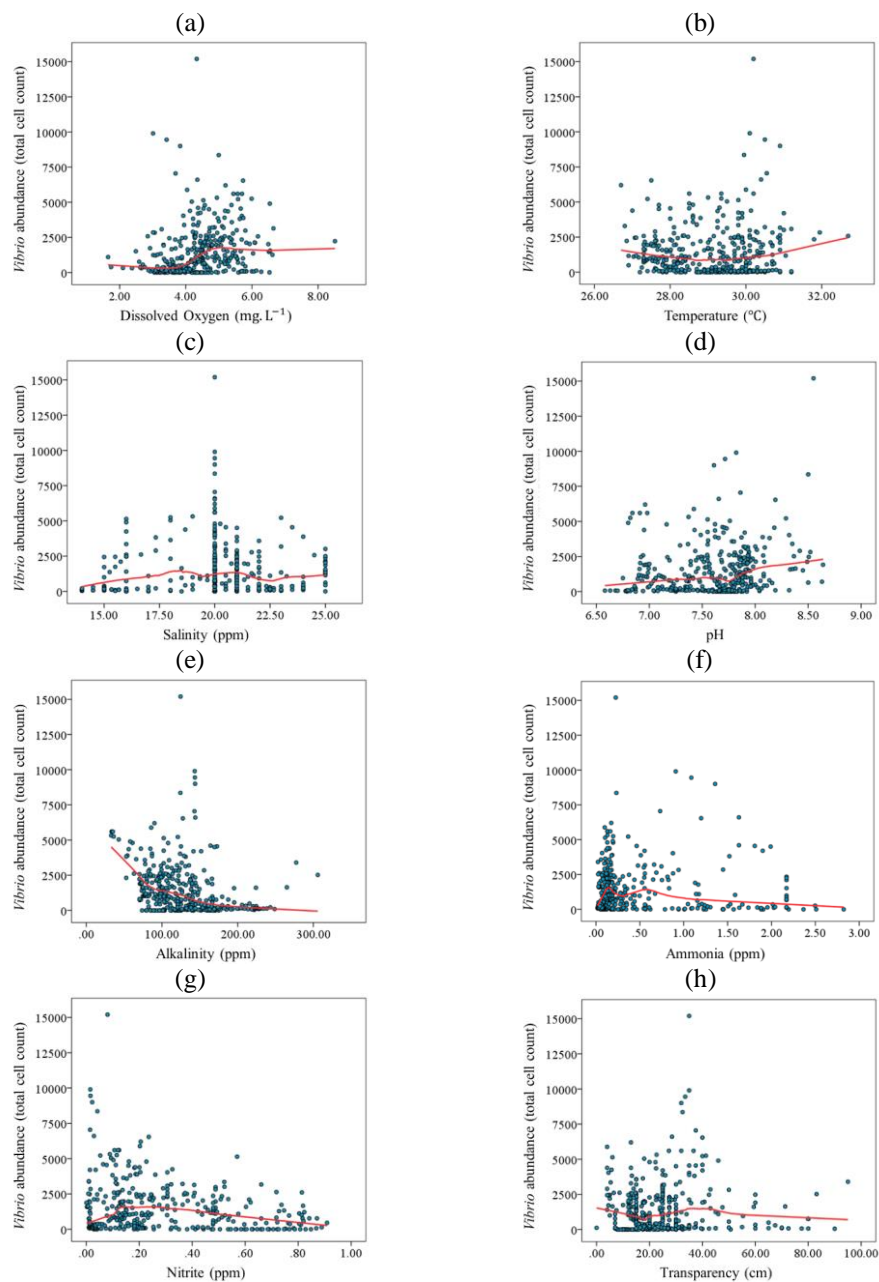


Fig. 1. Scatterplot of *Vibrio* abundance (total cell count) against physicochemical parameters: (a) dissolved oxygen, (b) temperature, (c) salinity, (d) pH, (e) alkalinity, (f) ammonia, (g) nitrite, and (h) transparency of water samples from *Penaeus vannamei* biofloc ponds. Fitted locally weighted scatterplot smoothing (LOESS) regression curves (red line) show nonlinear and nonmonotonic relationships between variables.

Major groups of phytoplankton identified from the water samples were green algae (86 %) and blue-green algae (10 %), while major groups of zooplankton identified were dinoflagellates (79 %) and barnacle larva (13 %). Figure 2a shows that *Vibrio* abundance decreases as phytoplankton count increases. Meanwhile, Figure 2b suggests no possible relationship between *Vibrio* abundance and zooplankton count, as indicated by the horizontal line. A summary of the mean and standard deviation of the physicochemical and biological parameters of water measured during the four culture runs of *P. vannamei* in biofloc ponds are given in Table 1.

Parametric and semiparametric modelling

All environmental parameters except for transparency and zooplankton count were observed to follow significant patterns and thus considered variables or

predictors for parametric and semiparametric modelling. Estimated coefficients of the ordinary LRM fitted using the data with imputed values are shown in Table 2. In general, the model generated was significant ($F = 17.480, P < 0.001$) with no evidence of serial autocorrelation (Durbin-Watson statistic = 0.703). The 16.04 % variability in the *Vibrio* count can be accounted by the set of significant predictors. Days of culture ($t = -2.032, P = 0.043$), pH level ($t = 3.896, P < 0.001$), phytoplankton density ($t = -2.027, P = 0.043$), and alkalinity ($t = -6.726, P < 0.001$), are the predictors found to be significant.

The estimated generalised linear model with negative binomial as the random component distribution and logarithm function as the link function was significant ($\chi^2 = 72.093, P < 0.001$). Using this model, approximately 14.5 % of the variability in the dependent variable or changes in *Vibrio* abundance can be explained by the three predictors listed in the table. Consistently, phytoplankton count ($t = -4.611$,

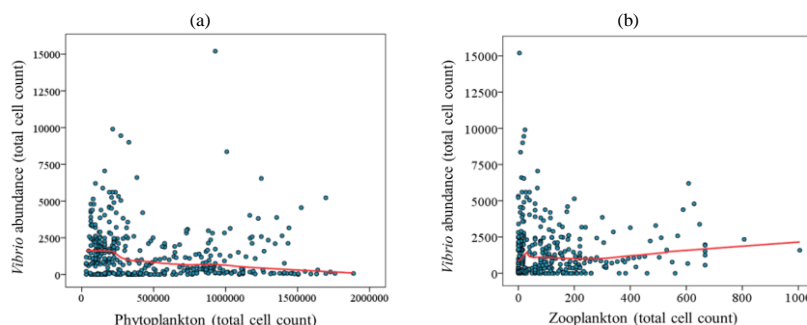


Fig. 2. Scatterplot of *Vibrio* abundance (total cell count) against (a) phytoplankton and (b) zooplankton counts of water samples from *Penaeus vannamei* biofloc ponds. Fitted locally weighted scatterplot smoothing (LOESS) regression curves (red line) show nonlinear and nonmonotonic relationships between variables.

Table 1. Descriptive statistics of the physicochemical and biological parameters of water measured during the four culture runs of *Penaeus vannamei*. Number of sampling points (n), mean, and standard deviation (SD) are shown. This does not include the imputed data.

Variables	Run 1			Run 2			Run 3			Run 4		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Temperature (°C)	25	29.22	1.03	32	28.57	1.53	21	29.37	1.65	33	28.95	0.86
Transparency (cm)	20	17.52	17.26	32	22.98	12.79	21	37.62	19.05	33	27.24	20.96
Salinity (ppt)	25	17.89	1.91	32	20.04	0.20	23	16.83	2.89	33	21.82	1.29
pH	25	7.67	0.18	32	7.81	0.46	23	7.45	0.59	33	7.49	0.35
D.O. (ppm)	25	4.61	0.75	32	4.83	0.80	21	3.47	0.60	33	4.17	1.05
Alkalinity (ppm)	17	109.59	33.06	25	116.47	91.97	14	196.36	48.93	20	157.91	35.54
Ammonia (ppm)	21	0.52	0.81	21	0.47	0.63	14	0.74	0.84	20	0.31	0.55
Nitrite (ppm)	17	0.21	0.19	22	0.25	0.19	13	0.14	0.17	18	0.35	0.35
Phytoplankton (total cell count in '000)	18	225.73	166.61	22	407.27	436.44	14	879.78	632.73	20	836.80	328.69
Zooplankton (total cell count)	18	145.78	169.63	22	769.32	2060.20	14	7.14	10.75	17	96.47	100.56
<i>Vibrio</i> abundance (total cell count)	25	1912.44	2546.52	32	2114.69	2160.75	23	171.17	142.23	33	360.91	448.00

Table 2. Parameter estimates for the ordinary linear regression^a between *Vibrio* abundance and the set of predictors. Estimated coefficients (B) with their corresponding standard errors (SE), t and P - values are shown. Time, phytoplankton count, and alkalinity negatively affect *Vibrio* abundance, while pH level positively affects *Vibrio* abundance.

Predictors	B	SE(B)	t	P - value
Intercept	- 2,865.000	1714.944	- 1.671	0.096
Time	- 5.399	2.657	- 2.032	0.043
pH level	855.332	219.564	3.896	<0.001
Phytoplankton ^b	- 182.185	89.878	- 2.027	0.043
Alkalinity	- 14.278	2.123	- 6.726	<0.001

^aR² = 0.1604, F = 17.48, P < 0.001; D - W = 0.703; AIC = 6,572.104

^bPhytoplankton count.

P < 0.001) and alkalinity (t = - 7.055, P < 0.001) are observed to be negatively associated with *Vibrio* abundance. Specifically, an increase in phytoplankton count decreases *Vibrio* abundance by 42.7 %, while an increase in the alkalinity decreases *Vibrio* abundance by 1.2 % (Table 3). On the other hand, pH level is positively associated with *Vibrio* count (t = 6.676, P < 0.001). There is a corresponding increase of 346.3 % in the *Vibrio* count for every increase in the pH level. The intercept is observed to be insignificant (t = - 0.590, P = 0.555).

The estimated generalised linear model with negative binomial as the random component distribution and logarithm function as the link function was significant ($\chi^2 = 72.093$, P < 0.001). Using this model, approximately 14.5 % of the variability in the dependent variable or changes in *Vibrio* abundance can be explained by the three predictors listed in the table. Consistently, phytoplankton count (t = - 4.611, P < 0.001) and alkalinity (t = - 7.055, P < 0.001) are observed to be negatively associated with *Vibrio* abundance. Specifically, an increase in phytoplankton count decreases *Vibrio* abundance by 42.7 %, while an increase in the alkalinity decreases *Vibrio* abundance by 1.2 % (Table 3). On the other hand, pH level is positively associated with *Vibrio* count (t = 6.676, P < 0.001). There is a corresponding increase of 346.3 % in the *Vibrio* count for every increase in the pH level. The intercept is observed to be insignificant (t = - 0.590, P = 0.555).

The third model considered in this study is the GAM with negative binomial as the count distribution and log as the link function. The estimated model is significant ($\chi^2 = 26.161$, P < 0.001), with 41.2 % of the deviance explained by the model. Consistent with the previous two models, pH level ($\chi^2 = 23.100$, P < 0.001), phytoplankton count ($\chi^2 = 16.140$, P < 0.001), and alkalinity ($\chi^2 = 83.820$, P < 0.001), were found to be related with *Vibrio* count. However, unlike the first two models, the relationship of these three variables with *Vibrio* count is not entirely linear, as manifested in the estimated degrees of freedom (edf) shown in Table 4.

The relationships of these predictors with *Vibrio* count are visualised in Figure 3 by plotting the estimated smoothed functions for pH level, phytoplankton, and alkalinity. It can be observed in Figure 3a that the relationship between *Vibrio* count and pH is not entirely linear, but the overall function is increasing. This means that an increase in the pH level results in an increase in the *Vibrio* count. Large increments can only be observed for 7.5 pH levels and higher. Phytoplankton count has a quadratic relationship with *Vibrio* count, in that, *Vibrio* count decreases at a certain phytoplankton density and increases again as the phytoplankton count increases from 1×10^6 . However, a large confidence band (denoted by the blue-green shaded area) in Figure 3b is noticed in the plot suggesting that the smoothed function is less precise. This is in view of having a small number of observations for a large phytoplankton count value. Alkalinity in Figure 3c shows a downward trend which means *Vibrio* count decreases as alkalinity increases, which is also not entirely linear.

Aside from pH level, phytoplankton, and alkalinity, salinity is also observed to be linearly related to *Vibrio* count. Figure 3d shows a downward linear trend in the *Vibrio* count as the level of salinity increases. Estimating the coefficient for this linear association, Table 3 posted an estimate of - 0.125 (se = 0.046) as the slope of the log of counts. This means that there is a 13.3 % decrease in the *Vibrio* count for every increase in the salinity. It is noted that the intercept is significant using the GAM model (t = 10.055, P < 0.001). When model fits are ranked according to AIC values, the model with the lowest AIC value is considered the "best" model (Fabozzi et al., 2014). Overall, the GAM is the best model, followed by the NBM and the LRM with AIC values of 5,743.222; 5,857.997; and 6,572.104, respectively.

Discussion

Parametric models are the most used statistical approaches in investigating the relationships among

Table 3. Parameter estimates for the negative binomial model^a between *Vibrio* abundance and the set of predictors. Estimated coefficients (B) with their corresponding standard errors (SE), its exponential form, Exp (B), t and P - values are shown. Phytoplankton count and alkalinity negatively affect *Vibrio* abundance, while pH level positively affects *Vibrio* abundance.

Predictors	B	SE (B)	Exp(B)	t	P - value
Intercept	- 0.828	1.404	0.437	- 0.590	0.555
pH Level	1.242	0.186	3.463	6.676	<0.001
Phytoplankton ^b	- 0.355	0.077	0.701	- 4.611	<0.001
Alkalinity	- 0.012	0.002	0.988	- 7.055	<0.001

^aScale parameter = 0.4818; Deviance explained = 14.5 %; $\chi^2 = 72.093$, $P < 0.001$; AIC = 5,857.997

^bPhytoplankton count.

Table 4. Parameter estimates for the semiparametric model^a between *Vibrio* abundance and the set of predictors. Estimated coefficients (B) with their corresponding standard errors (SE), t and P - values are shown. The relationship between *Vibrio* abundance and salinity is linear while it is nonlinear with time, pH level, phytoplankton count, and alkalinity.

	B	SE(B)	t	P - value
Intercept	9.264	0.921	10.055	<0.001
Salinity	- 0.125	0.046	- 2.748	0.006
Smoothed terms	edf	Ref. df	Chi-square	P - value
Time	8.778	8.987	410.160	<0.001
pH level	1.867	1.981	23.100	<0.001
Phytoplankton ^b	1.940	1.996	16.140	<0.001
Alkalinity	1.783	1.951	83.820	<0.001

^aDeviance explained = 41.2 %; $\chi^2 = 26.161$, $P < 0.001$; AIC = 5,743.222

^bPhytoplankton count.

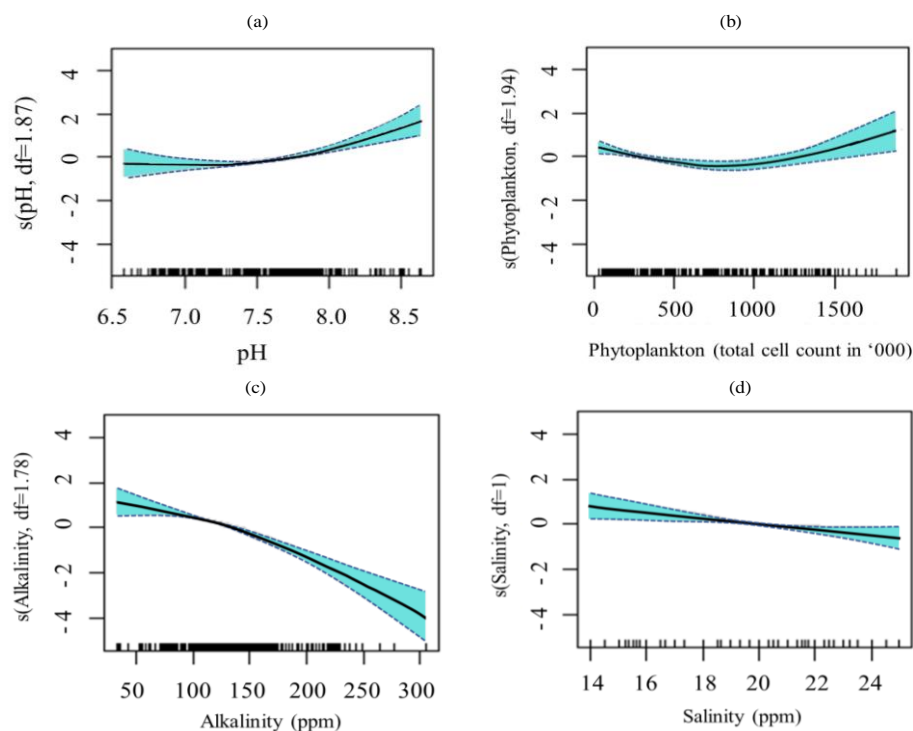


Fig. 3. Results of the generalised additive model (GAM) fitted for *Vibrio* abundance from *Penaeus vannamei* biofloc ponds. Partial response plots from the fitted GAM, showing the additive effects of the covariates on *Vibrio* count: pH level (a), phytoplankton count (b), alkalinity (c), and salinity (d). The solid lines are the smoothing functions and the blue-green shaded areas represent confidence intervals at 95 %.

Vibrio abundance, virulence, host susceptibility, and environmental factors. For instance, Selven and Philip (2012) used analysis of variance (ANOVA), Pearson's correlation analysis, and multiple regression analysis to emphasise the importance of salinity as a significant factor for the virulence of *Vibrio harveyi* in Indian prawn (*Fenneropenaeus indicus* Milne Edwards, 1837) Pérez Farfante, 1969. Azandégbé et al. (2010) concluded that temperature was the main factor influencing the concentration of *Vibrio spp.* and *Vibrio aestuarianus* in the sediment of giant cupped oyster *Crassostrea gigas* (Thunberg, 1793), farms in France by incorporating one-way ANOVA and multiple regression analysis. Pfeffer et al. (2003) indicated that *Vibrio* levels were primarily controlled by temperature, turbidity, and dissolved oxygen levels, estuarine bacteria, and coliforms by conducting extensive multiple regression analysis. Takemura et al. (2014) summarised environmental correlations with *Vibrio* presence and abundance. Temperature and salinity were the strongest predictors, while dissolved oxygen, nitrogen, pH, turbidity, and days of culture (time) had lesser explanatory power.

However, in the present study, both parametric and semiparametric models revealed that alkalinity, pH, and phytoplankton count influence *Vibrio* abundance. Generalised additive model (GAM) further revealed that *Vibrio* abundance had a negative linear relationship with salinity. Vibrios prefer alkaline conditions. A unique mechanism of homeostasis allows them to thrive in an environment with higher pH and salinity. Throughout the life cycle of *Vibrio cholerae*, Na⁺ is expected to play a role in the survival and pathogenicity in saline-alkaline environments. *Vibrio cholerae* possesses genes encoding three putative Na⁺/H⁺ antiporters homologous with *V. alginolyticus* and *V. parahaemolyticus* (Padan et al., 2005). Meanwhile, healthy phytoplankton cells can serve as a carbon source for Vibrios and increased species richness can positively affect *Vibrio* abundance. The behaviour of the graph in Figure 2a may be explained by the type of phytoplankton species thriving in the pond. Phytoplankton-associated bacterial communities are strongly affected by phytoplankton bloom in general and by the type of algal species that dominates in the natural bloom (Turner et al., 2009; Peterson et al., 2010; Sison-Mangus et al., 2016).

According to Takemura et al. (2014), the inconsistencies found in the results from different studies might be due to a narrow range of observations. This is true with *P. vannamei* BFT ponds where water parameters are maintained at optimum range such that salinity ranges from 15 to 25 ppt, pH ranges from 7.5 to 8.5, transparency >10 cm and dissolved oxygen >4 ppm (Corre et al., 2015). It is recommended to collect water physicochemical and biological data of a wider range of observations from other *P. vannamei* culture systems. Presumptive *Vibrio* count in this study was enumerated based on

the growth of yellow and green colonies from the commonly used selective medium, TCBS agar (Okoh et al., 2015). A modified TCBS medium can be adopted, such as that of Owens (2019) in which sucrose is added; and Tagliavia et al. (2019) in which the salt composition resembles that of the marine environment to improve the isolation and purification of *Vibrio*.

Although not widely used in aquaculture research, the semiparametric approach is one of the recent methods used in environmental studies. GAMs afford more flexibility in the response function since the predictor variables parametric, semiparametric, and nonparametric regression functions are accepted. It is proved to be competitive and highly promising for more complex data. In addition, GAMs are more adaptive and require less intervention. These have also proved useful for analysing trends in time series. Typically, long series exhibit a nonlinear trend of arbitrary shape in time. One crucial choice is the degree of smoothness of the trend curve, which can be selected using several data-driven methods (Ballesteros-Gomez and Rubio, 2011). Morton et al. (2009) represented the trend as a smoothing spline for easier extrapolation. A method based on the ability to predict a short term into the future was proposed for choosing the smoothing parameter. The choice addressed the purpose at hand and performed very well and avoided the tendency to interpolate data typical of other data-driven methods used to select the smoothing parameter. The following method was applied to stream salinity measurements at Eppalock on the Campaspe River in Victoria, Australia. The semiparametric approach was also applied in the environmental studies of Lu et al. (2014) and Diaby et al. (2015) on forecasting SO₂ and NO_x concentrations for urban air quality assessment and on the temporal evolution of bacterial communities associated with the in-situ wetland-based remediation of a marine shore porphyry copper tailings deposit, respectively.

Conclusion

Vibrio abundance is highly correlated with alkalinity, pH, and phytoplankton density, as revealed by the parametric linear regression model (LRM), negative binomial model (NBM), and generalised additive model (GAM). GAM further showed that *Vibrio* abundance is correlated with salinity. GAM is the best model based on Akaike's information criterion (AIC) values; and the predictors can explain 41.2 % of the variability in the dependent variable. Changes in alkalinity, pH, phytoplankton density, and salinity dictate *Vibrio* abundance. Thus, it is important to monitor these parameters and maintain them at the desired level to achieve effective shrimp health management.

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