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ORIGINAL ARTICLE

A study on the effect of feed load on mortality of Pacific white shrimp *Litopenaeus vannamei*

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Abstract

In recirculating aquaculture systems (RAS), understanding the relationship between feed load and its impact on water quality and overall shrimp health is important. This study utilised an indoor marine RAS for the intensive culture of Pacific white shrimp *Litopenaeus vannamei*. The shrimp were stocked into the grow-out system at an average body weight of 0.25 g and a stocking density of 347 shrimp/m^3. Water quality parameters of temperature, salinity, total dissolved solids, pH, and dissolved oxygen (DO) were measured twice daily. Total ammonia nitrogen (TAN), nitrite and nitrate were measured once weekly or when needed. Sampling was conducted to calculate the daily feed ratio based on the total estimated biomass. Mortality was recorded as a means of evaluating the overall shrimp health. The data obtained were analysed using Pearson correlation (r) analysis and multi-linear regression with a significant difference accepted $p < 0.05$. Correlation (r) established relationships among the water quality parameters, feed load and mortality. The TAN level of 24.20 mg/L was recorded when the feed load increased by 81.2% resulting in the mortality of 40% of shrimp. A negative correlation between TAN and DO resulted in a synergistic effect causing a massive consumption of DO in the water, reducing its availability to the shrimp and leading to a drastic change in the shrimp's behaviour. Overfeeding can lead to an accumulation of uneaten feed and waste, causing ammonia spikes and oxygen depletion in the water. Monitoring and adjusting feed rates accordingly can help maintain optimal water conditions for shrimp growth and health. Therefore, it is essential to use appropriate feed rates in recirculating systems because feed load can influence water quality parameters that can be detrimental to shrimp culture.

KEYWORDS
ammonia, mortality, nitrite, oxygen consumption, Pacific white shrimp, RAS

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1 | INTRODUCTION

The demand for seafood is increasing globally, but the wild harvest is insufficient to meet the growing demand. Aquaculture can help to bridge the gap in which seafood production facilities can utilise the best management practices and acceptable technologies to optimise production and reduce the overall costs to satisfy the rapidly growing customer demand (Mateka et al., 2015). Pacific white shrimp *Litopenaeus vannamei* culture is one of the fastest growing industries in aquaculture with a global production of 4.9 million tons in 2020 (Espinoza-Ortega et al., 2023). The success of an aquaculture enterprise depends on its ability to optimise shrimp health through proper management of the aquatic environment and critical water quality parameters, especially in recirculating aquaculture systems (RAS). These systems were developed for intensive production where water availability is limited. It offers low or zero water exchange, controlled inputs and generally requires less area than traditional open earthen ponds (Ray et al., 2017).

A good water quality condition is necessary for the survival and growth of shrimp since the entire life process is completely dependent on the quality of its environment. The complexity of the aquatic environment is based on the multiple water quality parameters. Each water quality parameter is important, but the interrelationship and combination of these parameters influence the health of all cultured animals (Timmons & Ebeling, 2010). The quality of water in a system can deteriorate due to waste accumulation, which can be impacted by the total feed load. The feed load is the total amount and frequency of feed added to the culturing environment (Ruiz et al., 2020). Monitoring and managing the feed load is crucial to maintaining a healthy environment and preventing any negative impacts on water quality. In RAS systems, about 75% of the nitrogen accumulation is due to feed, in the form of total ammonia nitrogen (TAN) (56.2%), faeces (13.8%) and non-consumed feed (5%) (Deng et al., 2020). Excess feeding can lead to increases in solid waste and nitrifying load, which affects the overall water quality (Rojas-Tirado et al., 2018; Zhou et al., 2018). Studies have indicated that the diverse microbial community can maintain a healthy environment in RAS through bio-filtration (Zhao et al., 2020). The process of nitrification is utilised in RAS facilities to control and regulate ammonia levels. In aquaculture systems, ammonia and nitrite are crucial parameters that impact on survival and growth of white leg shrimp. In the biofilters, nitrification occurs through aerobic respiration where ammonia is oxidised to nitrites and nitrates by nitrifying bacteria. This process involves two steps: ammonia-oxidising bacteria which oxidise ammonia to nitrite, followed by nitrite-oxidising bacteria (NOB) which oxidise nitrite to nitrate. It is important to note that during the nitrification process for every gram of ammonia converted, dissolved oxygen (DO) and alkalinity are consumed from the water (Wu et al., 2024). Alkalinity or inorganic carbon in RAS is a measure of the water-buffering capacity and ability to prevent pH fluctuations. Hence, the addition of carbonates is necessary to maintain the system's stability, and the most commonly used buffer is sodium bicarbonate since it is safe and dissolves easily and rapidly (Jafari et al., 2024). Therefore, there must be sufficient DO, inorganic carbon and retention time to facilitate the decomposition of the nitrifying biomass (Medinilla et al., 2020). It is essential to maintain a balance between feed loads and stocking density rates in relation to the availability of DO through aeration for recirculating systems.

Strategies to improve feeding must be used to optimise production and reduce waste since discharge nutrients in shrimp farms are generated from feed (Espinoza-Ortega et al., 2023). As feed constitutes almost half of the total production cost in a shrimp farm, it is crucial to determine the ideal feed rate for shrimp farm management. Therefore, the feed conversion ratio (FCR) is a measure of efficient feed conversion that uses the ratio of feed weight to animal weight gain. Overfeeding or underfeeding can increase the ratio, which is undesirable. The aim is to ensure that the maximum amount of feed is consumed while producing the least amount of waste (Arnold et al., 2016). Excess feed can directly affect the physicochemical properties of water through leaching loss, residual and undigested feed that can affect the overall shrimp health and increase the cost of managing water quality (Espinoza-Ortega et al., 2023; Lindholm-Lehto, 2023). The relationship between feed load or biomass and water quality parameters is crucial for maintaining optimal shrimp health in sustainable aquaculture systems. In cases where there is no monitoring of feed consumption, it is difficult to adjust the feed ration and prevent overfeeding (Ullman et al., 2019). This can lead to the production of detrimental parameters that can directly affect overall shrimp health and survival if it exceeds regulatory limits. This study will examine the effects of the increase in feed load on various water quality parameters during a production cycle and identify the parameters that are responsible for shrimp mortality. Understanding how feed load affects water quality is crucial for optimising shrimp production and reducing mortality rates. By analysing the relationship between these factors, aquaculture farmers can make informed decisions to improve overall production efficiency.

2 | METHODOLOGY

This study was conducted utilising an indoor marine RAS located at Seafood Industry Development Company Limited (SIDC) in Trinidad and Tobago. Treated tap water from the municipal supply was mixed with commercial Crystal Sea marine mix salt (Marine Enterprises International). This mixture, with an initial salinity of 32 g/L was used in both the quarantine and growth-out systems. Approximately 50,000 specific pathogen-free pacific white shrimp, 10-day-post-larvae (PL10) were transported from a hatchery in Florida, USA to the Marine Aquaculture Facility at the SIDC. The PL10s were acclimated, and approximately 7143 PLs were distributed into each tank in the quarantine marine recirculating system (QRAS) at a stocking density of 12,755 shrimp/m^3 for 30 days. The QRAS, as shown schematically in Figure 1, comprised seven 560-L rectangular polyethylene tanks (Figure 1–'G') supplied with air from a 1.5 HP blower (Sweetwater) with 8 ft of Aero-TubeTM aeration tubing (Colorite Plastics Co.) in each tank. The filtration system comprised of a 680-L polyethylene settler (Figure 1–'F'), a 680-L polyethylene sump (Figure 1–'A'), a 110-W

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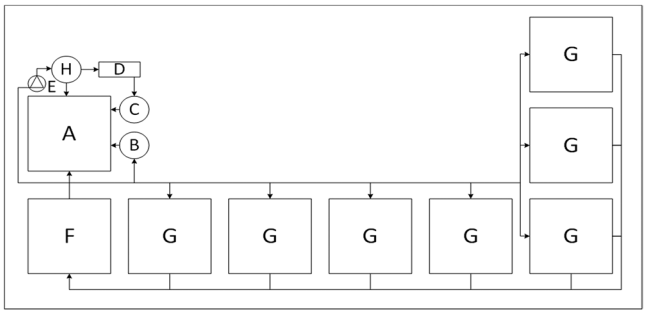

FIGURE 1 A schematic diagram of the quarantine recirculating system used for the study. Key components are (A) sump, (B) foam fractionator, (C) moving bed bioreactor, (D) UV steriliser, (E) pump, (F) settler, (G) tanks, and (H) sand filter. The arrows indicate the direction of water flow.

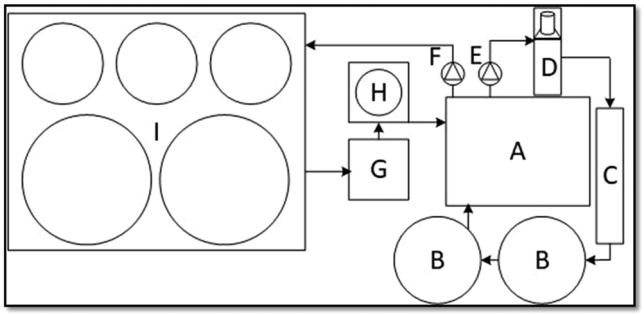

FIGURE 2 A schematic diagram of the marine recirculating aquaculture systems (MRAS) used for the study. Key components are (A) sump 2, (B) moving bed bioreactor, (C) UV steriliser, (D) foam fractionator, (E) pump 1, (F) pump 2, (G) sump 1 (H) mechanical filter, and (I) production tanks. The arrows indicate the direction of water flow.

ultraviolet steriliser (Emperor Aquatics, Figure 1–'D') and a 680-L rectangular polyethylene tank moving bed bioreactor (MBBR, Figure 1–'C') filled with approximately 0.27 m^3 of Kaldnes K1 media (Kaldnes Inc.) and seeded with nitrifying bacteria (Proline). A marine pump 1.0 HP high efficiency (Sweetwater, Figure 1–'E') was used to recirculate water in the filtration system and tanks, a 0.04-m^3 sand filter (Pentair Aquatic Ecosystems, Figure 1–'H') filled with 45 kg of sand and a 18 LPM foam fractionator (Figure 1–'B') was used for particulate removal as shown in Figure 1 (Gabbadon et al., 2014).

The PL10s were exposed to a 12:12 light-day cycle with marine blue LED lights controlled by digital timers positioned 2 m above the quarantine tanks. The PL10s were hand-fed ad libitum for the duration of quarantine with Zeigler AP100 commercial larval (Zeigler) ration containing a minimum of 50.0% crude protein and 12% crude fat. The diet was supplemented with recently hatched brine shrimp nauplii *Artemia salina*, microalgae *Nannochloropsis* sp. and *Tetraselmis* sp. (Pentair Aquatic Ecosystems), and rotifers *Brachionus plicatilis* (Reed Mariculture Inc.) due to their high macro- and micronutrient levels, to improve survival, growth and stability (Kandathil Radhakrishnan et al., 2020).

2.1 | Marine recirculating aquaculture systems (MRAS)

After the quarantine period, the shrimp were transferred and stocked into a grow-out system at an average body weight of 0.25 ± 0.02 g. This system, as shown schematically in Figure 2, consisted of three 7500-L circular polyethylene tanks and two 30,000-L fibreglass tanks (Figure 2–'I') supplied with air from a 5 HP blower (Sweetwater) via rubber-polyethylene grid diffusers (Pentair Aquatic Ecosystems). The water treatment comprised a 1020-L fibreglass rectangular sump 1 (Figure 2–'G'), a 7500-L circular polyethylene sump 2 (Figure 2–'A') and a 600 W, 1283 LPM ultraviolet steriliser (Emperor Aquatics, Figure 2–'C') to reduce the bacterial load. A 1500 LPM drum filter with 60μ screen (Faivre Drum Filter 2-80 Series, Figure 2–'H'), and a 265 LPM foam fractionator (RK2, Figure 2–'D') to remove large and small particles. Two MBBR of 2200 L each (Figure 2–'B') filled with 40% Kaldnes K1 media as seeded with bacteria as described previously in the quarantine phase. Two marine pumps, a 0.5 HP high efficiency (Sweetwater, Figure 2–'E') used to recirculate the water in the filtration system and a 2 HP Wave II high speed (W. Lim Corporation, Figure 2–'F') was used to recirculate the water volume of 82,500 L in the production tanks as shown in Figure 2 (Gabbadon et al., 2014). After transferring into the grow-out systems, the shrimps were fed ad libitum and transitioned to a larger feed size of 600–900 μm, shrimp feed containing a minimum of 42% crude protein and 9.5% crude fat (GISIS SA, Ecuador) because the optimum dietary protein requirement for *L. vannamei* size between 0.09 and 2.2 g is estimated to be 46.7%. When the juveniles achieved 3.0 g, they were fed a diet that consisted of 42%–35% crude protein and a minimum of 9% crude fat (GISIS SA, Ecuador) for the duration of the 150-day production cycle as reported by other studies the optimal protein requirement ranges between 20% and 40% (Lee & Lee, 2018). Sodium bicarbonate was added weekly to the system at a rate of 250 g for every kg of feed to compensate for the loss of inorganic carbon during the nitrification process and buffer the pH (Timmons & Ebeling, 2010).

2.2 | Water quality monitoring data

Water quality parameters for salinity, temperature, DO and total dissolved solids (TDS) were measured twice daily using a YSI Multiparameter Instrument (YSI Professional Plus), and pH was measured with a pH tester (Oakton Instruments). These parameters were taken directly from the production tanks. TAN, nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) were measured once weekly in the morning or wherever there were any changes observed in the shrimp behaviour. A sample of 1 L was randomly collected from a production tank and further analysed using the Salicylate method and Nesslerization method for TAN, Diazotization method for NO_2-N and cadmium reduction for NO_3-N using a SMART3 Colorimeter (LaMotte). Unionised ammonia (UIA) levels in water were calculated by using the data obtained

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TABLE 1 The average body weight obtained during the production cycle.

Day	Number of shrimps	ABW (g)	% Increase	No of days among samples	Daily growth rate (g)
1	1225	0.25			
20	1159	0.34	34.00	20.00	0.00
33	956	1.31	291.79	13.00	0.08
46	1054	2.30	74.96	13.00	0.08
55	679	3.86	68.13	9.00	0.17
70	864	10.20	164.19	15.00	0.42
81	848	11.21	9.94	11.00	0.09
111	998	19.85	77.00	30.00	0.29
117	822	21.53	8.47	6.00	0.28
124	1020	22.94	6.55	7.00	0.20
130	1003	30.95	55.93	6.00	1.85
148	748	33.17	54.06	18.00	0.65

for TAN, pH and temperature at the time the sample was taken. The calculations were based on the percentage of dissociation produced by Bower and Bidwell in 1978 (Alcaraz et al., 1997). Equations for calculating the fraction of dissociation (f_s) for UIA were based on Whitfield (1974) (see, e.g. Florida Department of Environmental Protection, 2001). Equation (1) gives the equation for f_s in mol/L:

$$f_s = \frac{1}{10^{(pKa,s\ -pH)} + 1} \quad (1)$$

where p*K*a, *s* is the ionised constant of ammonium ions in seawater in mol/L given by

$$pKa,\ s = 0.901821 + \frac{2729.92}{(T + 273.2)} + (0.1552 - 0.0003142T)\, I \quad (2)$$

where *I* is the molal ionic strength in mol/L,

$$I = \frac{19.973S}{1000 - 1.2005109S} \quad (3)$$

and *T* is temperature (°C) and *S* is salinity (g/L).

2.3 | Shrimp body weight, feed rate and mortality data

Shrimp were sampled randomly, and their wet weights were recorded using a bench scale (30 kg ± 0.10 g) (Ohaus). The growth was evaluated as the daily growth rate (DGR) expressed in grams, described by the following formula (Fóes et al., 2016):

$$DGR = \frac{W_f - W_f}{t} \quad (4)$$

where W_f is the final weight (g), W_i is the initial weight (g), and *t* is the time between measurements (days).

Table 1 displays the average body weight collected on various days throughout the production cycle as well as the percentage growth and DGR in grams. These data were used in conjunction with the initial number of shrimps stocked in each tank in the system to calculate the total biomass (T_{Bio}) based on an estimated survival rate of 80% of the entire system as described in the formula and expressed in kg:

$$T_{Bio} = \frac{ABW\left(\frac{T_s}{100} \times 80\right)}{1000} \quad (5)$$

where ABW is the average body weight in (g), and T_S is the total number of shrimps in the system.

The daily feed (DF) was calculated based on the T_{Bio} of the shrimp obtained. Feed was supplied to the tanks at a rate of 10% of the biomass during the entire cycle as described in the formula and expressed in kg:

$$DF = \frac{T_{Bio}}{100} \times 10 \quad (6)$$

where T_{Bio} is the total biomass in kg.

The DF was weighed, recorded and divided into six equal portions, which were then fed at regular intervals throughout the day, according to standard procedures (see, e.g. Espinoza-Ortega et al., 2023). The system was monitored daily for mortalities, if any dead shrimps were noticed in the production tanks it was removed, counted and recorded.

All readings collected for water quality parameters, growth and mortality are presented in Figures 3–6. The data presented in Figure 3 illustrate the changes in salinity, TDS, pH and mortality throughout the production cycle. Salinity was gradually lowered and maintained at approximately 26 g/L. However, from day 95, the salinity was gradually increased to 30 g/L. The measurements of temperature, nitrite, nitrate and mortality during the production cycle are shown in Figure 4. The temperature ranged from 25.2 to 27.17°C, whereas nitrite was below 5 mg/L for the majority of the cycle except for day 96 when it peaked at 10.06 mg/L. Nitrate followed a similar pattern, remaining below 25 mg/L for most of the cycle, with an increase on day 95

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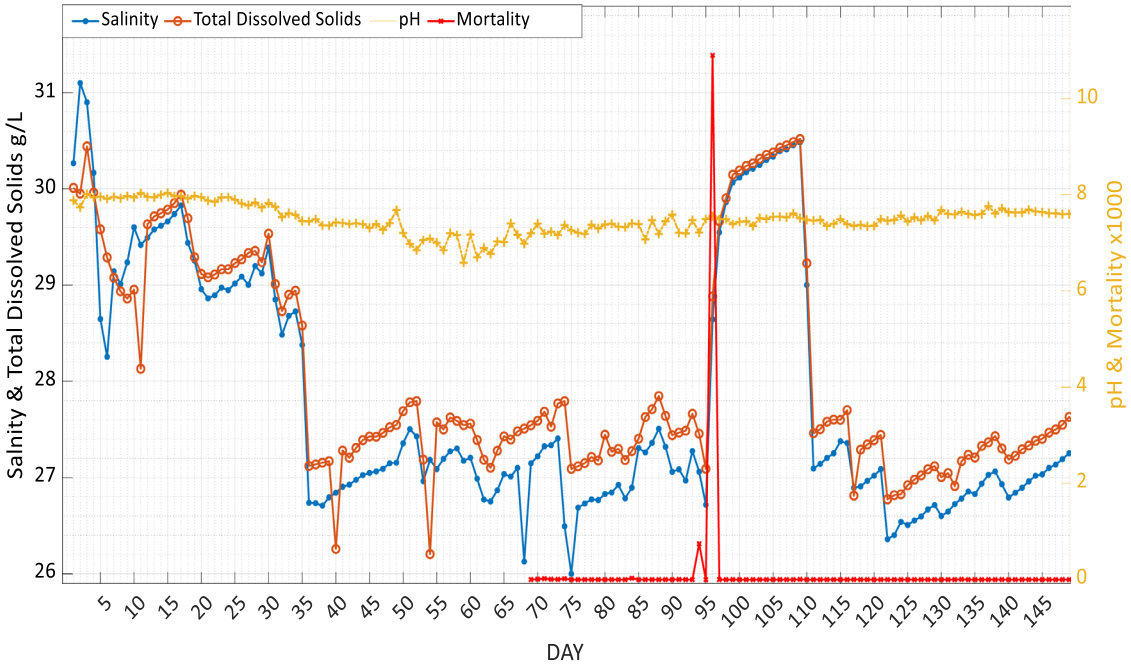

FIGURE 3 Salinity, total dissolved solids (TDS), pH and mortality measured during the production cycle. Showing the trends observed among these parameters and their effects on mortality throughout the 150-day cycle.

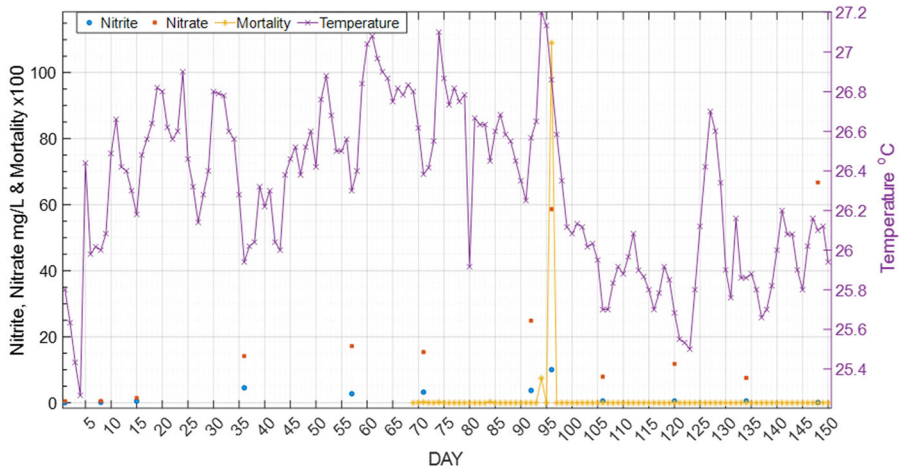

FIGURE 4 Temperature, nitrites, nitrates and mortality measured during the production cycle. Showing the relationship observed between nitrite and temperature and the effect of nitrite on mortality throughout the 150-day cycle.

of 58.66 mg/L and then reaching its maximum levels of 66.76 mg/L on day 148. The water quality parameters of DO and TAN in relation to mortality are presented in Figure 5. DO was maintained above 4.5 mg/L for most of the cycle except for day 95 when it dropped to 2.05 mg/L. TAN was below 2.5 mg/L for most of the cycle. However, from day 92 to day 97 TAN increased above 2.5 mg/L and spiked to a maximum of 24.20 mg/L. Finally, there was an increase in the feed biomass until day 94, after which it decreased due to the reduction in the shrimp biomass caused by mortality. This is shown in Figure 6, which represents the daily increase in the amount of feed added to the system.

2.4 | Statistical data analysis

Exploratory data analysis was used on each dataset to identify general patterns in the data. All parametric tests were conducted under the assumption of normal distribution, equal sample size and variance homogeneity. The Shapiro–Wilks test was used to assess normality (Zhang et al., 2011). Pearson correlation (r) was then used to determine any relationship among the water quality parameters, feed load and mortality. This was then followed by a multi-regression analysis to determine which water quality parameter factor had the most impact on the mortality of the Pacific white shrimp and how these parameters

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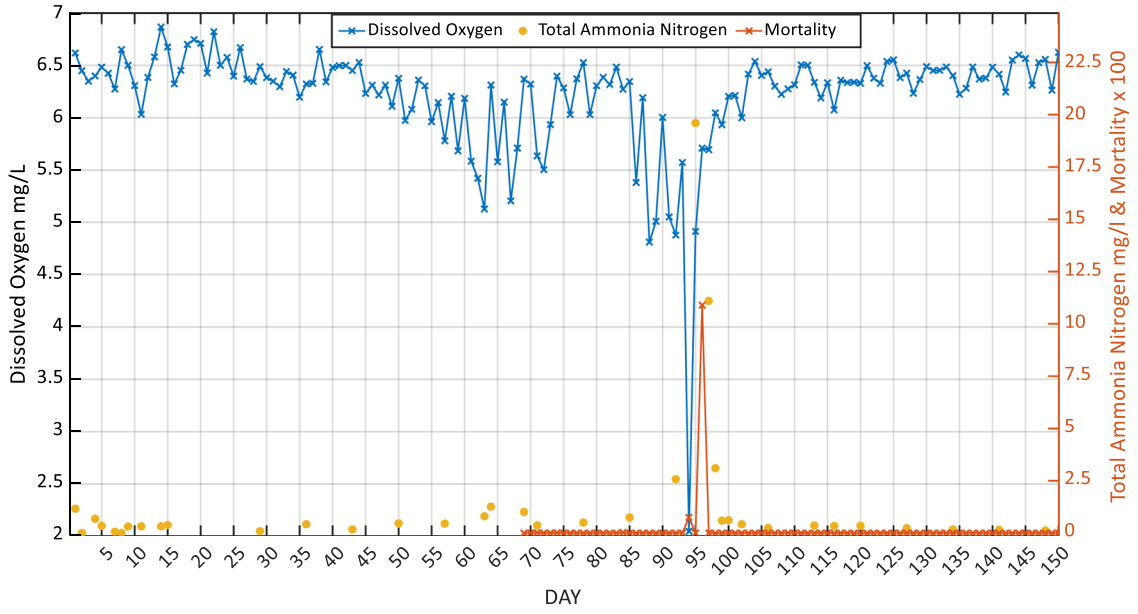

FIGURE 5 Dissolved oxygen, total ammonia nitrogen and mortality measured during the production cycle. Showing the relationship observed between total ammonia nitrogen (TAN) and dissolved oxygen (DO) and their impact on mortality throughout the 150-day cycle.

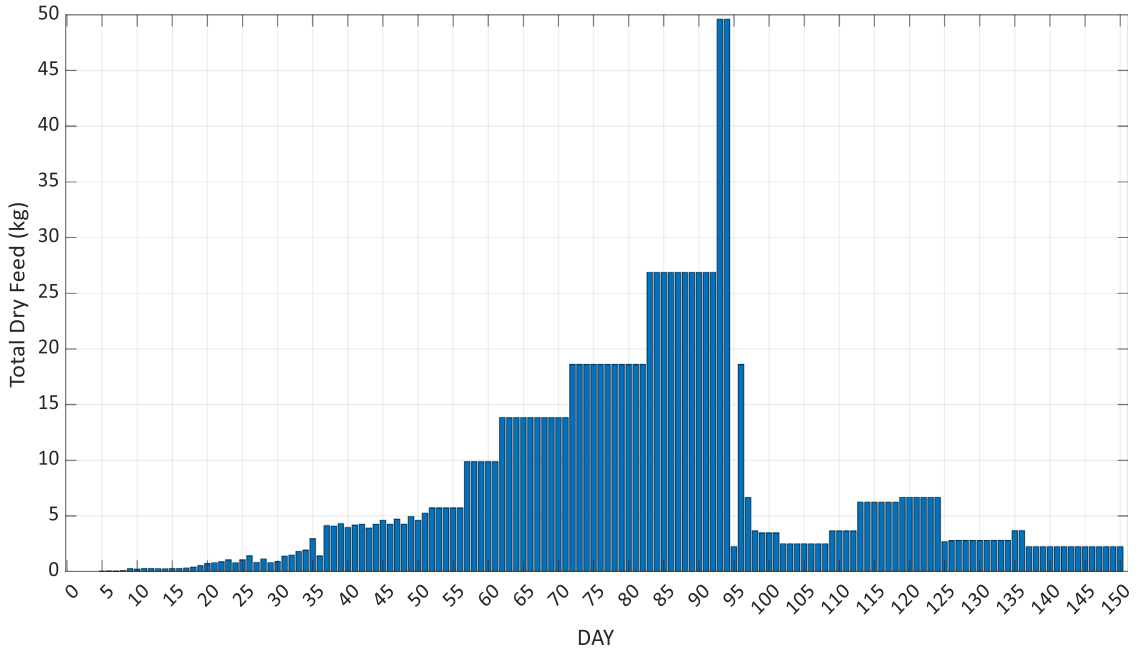

FIGURE 6 The daily dry feed biomass added to the system during the 150-day production cycle. The changes in the daily feed rate mass observed were based on 10% of the total biomass calculated using the average body weight obtained during sampling.

influence each other. Data analyses were conducted using Microsoft Office Excel 365 and all graphs were generated in MATLAB.

3 | RESULTS

The different readings of various water quality parameters and mortality are presented as mean and standard deviation as shown in Table 2. The TAN and nitrite had relatively low averages throughout the production cycle and the maximum mortality recorded during the production was 10,889 shrimp. Significant correlations were observed between water quality parameters, feed load and mortality of the shrimp as shown in Table 3. Negative correlations were noted for a range of water quality parameters such as DO and TAN as compared to a positive relationship between feed load and TAN. The data indicated that a significant relationship was observed among mortal-

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TABLE 2 Water quality data mean with standard deviation along with the maximum and minimum data recorded for the 150-day production cycle.

	TAN (mg/L)	UIA (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	Mortality
Mean	2.00	0.03	2.25	18.91	588
SD	5.19	0.09	2.94	21.78	2432
Median	0.38	0.01	0.56	12.98	4
Max	24.20	0.43	10.06	66.76	10,898
Min	0.00	0.00	0.05	0.53	0

Abbreviations: TAN, total ammonia nitrogen; UIA, unionised ammonia.

TABLE 3 The Pearson correlation coefficient (*r*) observed for the water quality parameters with mortality and feed load during the culture of Pacific white shrimp in a tank-based system.

	Sal	Temp	pH	DO	TDS	TAN	Nitrite	Nitrate	Mort	Feed
Sal	1.000	−0.244	**0.539**	0.189	0.983	−0.091	−0.092	−0.288	−0.187	−0.384
Temp		1.000	−0.401	**−0.490**	−0.233	0.345	0.588	0.121	0.366	0.402
pH			1.000	0.429	0.524	−0.081	−0.431	−0.167	0.123	−0.496
DO				1.000	0.191	**−0.663**	−0.587	−0.300	−0.384	**−0.644**
TDS					1.000	−0.090	−0.081	−0.285	−0.184	−0.383
TAN						1.000	**0.591**	0.399	**0.796**	**0.240**
Nitrite							1.000	0.333	0.566	**0.373**
Nitrate								1.000	0.585	0.169
Mort									1.000	−0.185
Feed										1.000

Note: Significant ($p < 0.05$) correlation coefficient represented in bold.
Abbreviations: DO, dissolved oxygen; Mort, mortality; Sal, salinity; TAN, total ammonia nitrogen; TDS, total dissolved solids; Temp, temperature.

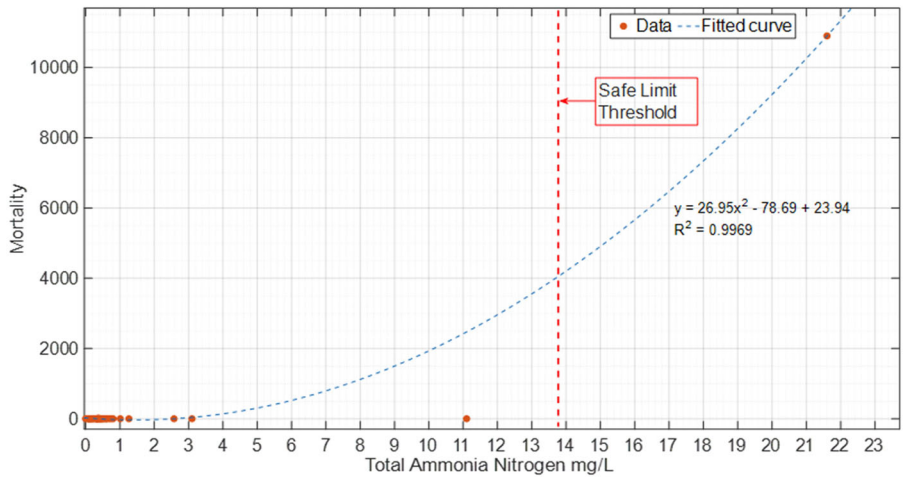

FIGURE 7 Regression line with slope and intercept for total ammonia nitrogen against mortality with the safe limit threshold for *Litopenaeus vannamei* after 48-h exposure based on research by Tu et al. (2022).

ity, TAN and nitrite as shown in the regression analysis presented in Table 4. It was further illustrated in Figure 7 that the majority of the mortality occurred when the TAN exceeded the safe threshold limit of 13.8 mg/L. Similarly, Figure 8 represents the regression analysis for the relationship between nitrite and mortality. The majority of the mortality occurred when the nitrite level was above the safe limit threshold of 10 mg/L. The DF biomass used until day 92 of the cycle had no relation to the impact on TAN. However, the entire cycle demonstrated a signif-

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TABLE 4 Regression analysis of the various water quality parameters and feed load in relation to mortality during the production cycle.

Regression statistics				
Multiple *R*	0.86			
R^2	0.74			
Adjusted R^2	0.72			
Standard error	467.79			
Observations	150			
	Coefficients	**Standard error**	***t* Stat**	***p*-Value**
Intercept	−4041.94	3321.36	−1.22	0.23
Salinity	52.19	132.2	0.39	0.69
Temperature	70.84	108.7	0.65	0.52
pH	57	176.39	0.32	0.75
Dissolved oxygen	156.26	108.7	1.44	0.15
TDS	−28.53	146.79	−0.19	0.85
TAN	126.41	19.54	6.47	**0**
Nitrite	492.88	60.89	8.09	**0**
Nitrate	4.97	6.93	0.72	0.47
Feed	6.82	6.44	1.06	0.29

Note: Significant ($p < 0.05$) coefficient is represented in bold.
Abbreviations: TAN, total ammonia nitrogen; TDS, total dissolved solids.

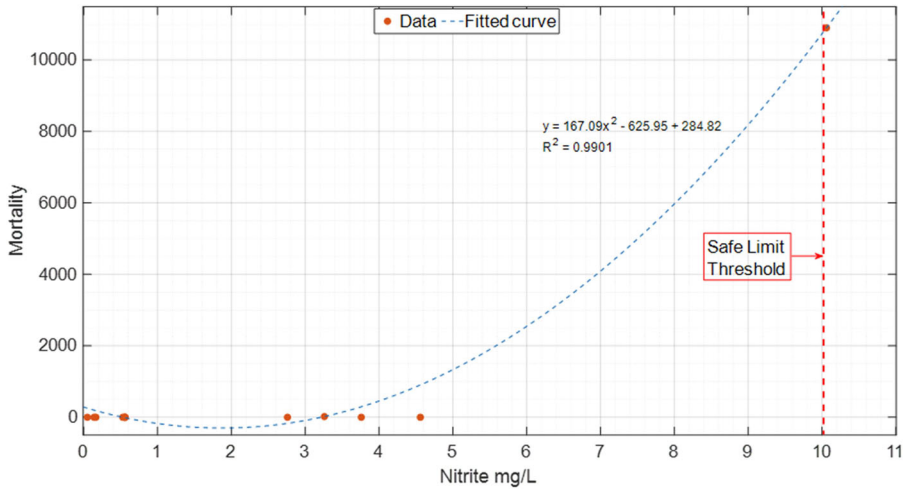

FIGURE 8 Regression line with slope and intercept for nitrite against mortality with the safe limit threshold for *Litopenaeus vannamei* after 48-h exposure based on research by Furtado et al. (2016).

icant relationship between the DF biomass and TAN, as shown in the regression analysis presented in Table 5. Finally, Figure 9 depicts the growth of the shrimp during the production cycle and compares it to a reference cycle where no mortality events occurred. The average body weight of the shrimp increased throughout the production cycle, following a similar trend to the reference data. Mortality was monitored daily during the shrimp production cycle. On day 94, a substantial number of shrimps were jumping erratically out of the production tanks, after this event a high number of mortalities on day 96 occurred.

4 | DISCUSSION

4.1 | Physio-chemical parameters

Pacific white shrimp are euryhaline species, which means that they can tolerate a wide range of salinity from 1 to 50 g/L (Ye et al., 2023). The water quality parameter of salinity was within range throughout this study with a gradual decrease from a maximum of 32.05 g/L to a minimum of 26.00 g/L, averaging at 27.85 ± 1.32 g/L as shown in Figure 3.

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TABLE 5 Regression analysis of the various water quality parameters and feed load in relation to total ammonia nitrogen.

Regression statistics	Until day 92	Total 150 days						
Multiple *R*	0.28	0.42						
R^2	0.08	0.18						
Adjusted R^2	0.00	0.15						
Standard error	0.37	2.49						
Observations	92.00	150.00						
	Production cycle until day 92				Total production cycle 150 days			
	Coefficients	Standard error	*t* Stat	*p*-Value	Coefficients	Standard error	*t* Stat	*p*-Value
Intercept	3.34	3.89	0.86	0.39	−25.50	17.42	−1.46	0.15
Salinity	0.04	0.11	0.40	0.69	−0.30	0.70	−0.42	0.67
Temperature	−0.14	0.13	−1.11	0.27	1.42	0.56	2.52	0.01
pH	−0.09	0.22	−0.43	0.67	0.95	0.93	1.01	0.31
Dissolved oxygen	−0.09	0.13	−0.73	0.47	−2.36	0.54	−4.35	**0.00**
TDS	0.02	0.12	0.14	0.89	0.19	0.78	0.25	0.80
Feed	0.01	0.01	1.45	0.15	−0.13	0.03	−3.99	**0.00**

Note: Significant ($p < 0.05$) coefficient represented in bold.
Abbreviation: TDS, total dissolved solids.

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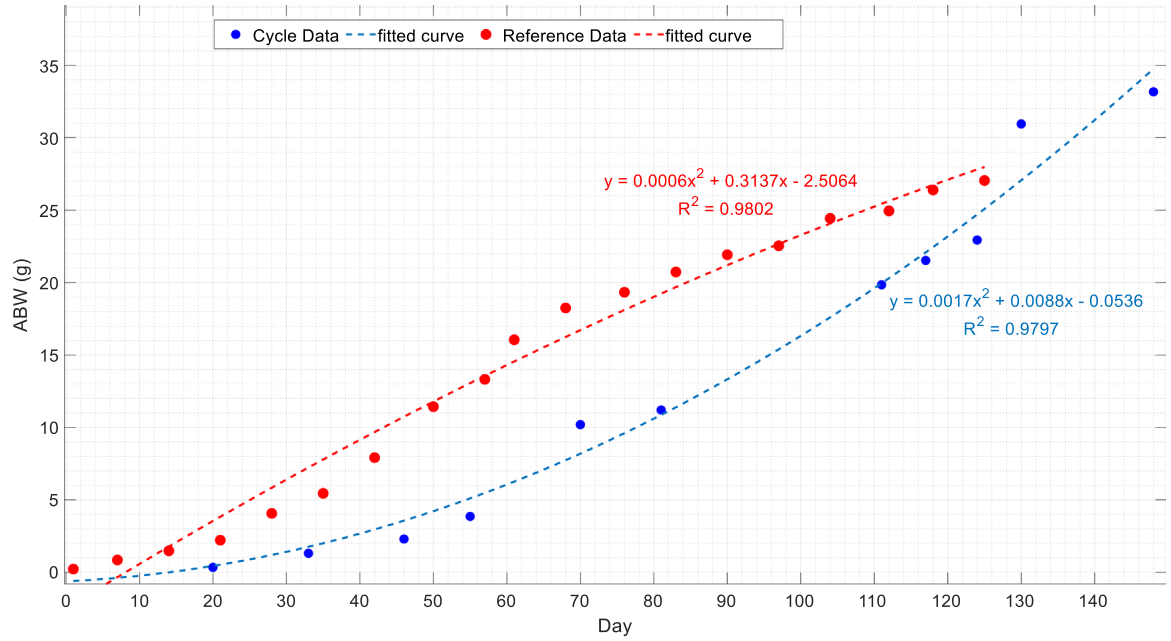

FIGURE 9 Growth curve of Pacific white shrimp for the 150-day production cycle in this study compared to reference data with no mortality event.

However, on day 95, the gradual increase resulted from the addition of the saltwater mixture to replenish the loss caused by the extra cleaning and backwashing of the mechanical filter. The ideal water temperature range for tropical species growth and survival is 20–32°C and for *L. vannamei* ranges between 20 and 33°C (Kır et al., 2023). Therefore, in this study, the temperature was at the optimal range for the culture of the Pacific white shrimp as shown in Figure 4. Overall, the pH level during cultivation was maintained at an average of 7.51 ± 0.29 (max. 8.04 and min. 6.76) as shown in Figure 3. According to Rasyidah et al. (2024), the pH range observed during this study was considered appropriate for white shrimp culture. Ideally, the pH level of the water during cultivation should be maintained between 7.5 and 8.5, with daily fluctuations of no more than 0.5. However, a pH value below 6 or above 9 can inhibit growth, and extremely low or high pH values can lead to the mortality of shrimp (Yessy et al., 2024).

DO is crucial in shrimp culture and in this study, and the levels were maintained at an average of 6.36 ± 0.37 mg/L (max. 6.75 mg/L and min. 2.05 mg/L). The negative correlation found in Table 3 between temperature and DO can be attributed to the fact that DO is more soluble in colder water than in warmer water. However, its solubility can decrease rapidly with increasing water temperature and salinity (Lindholm-Lehto, 2023). The recommended minimum level for DO suitable for culture organisms is above 4 mg/L as suggested by the Global Aquaculture Alliance (Ariadi et al., 2020; Rasyidah et al., 2024), and for white shrimp farming activities, a DO level of 4.1 mg/L is necessary for optimal growth (Ariadi et al., 2019; Madusari et al., 2022). The drop in DO on day 94 to 2.05 mg/L was a result of the increase in TAN as confirmed by the negative correlation obtained between DO and TAN from Pearson's analysis $p < 0.05$ as shown in Table 3. It was also confirmed by the regression analysis for the entire production cycle as shown in Table 5. During the process of nitrifying, a large volume of DO was consumed; therefore, the increase in TAN will lead to an increase in DO consumption as shown in Figure 5 (Ullman et al., 2019). Although alkalinity is also consumed during this process, the addition of sodium bicarbonate provides the necessary inorganic carbon to facilitate nitrification and acts as a buffer for the pH (Kunwong et al., 2024).

4.2 | Nitrogen species

This study analysed the levels of TAN and UIA during the production cycle as shown in Table 2. A strong correlation between TAN and UIA obtained from Pearson's correlation as shown in Table 3 can be attributed to the fact that ammonia can exist in two forms in water ionised (NH_4^+) and unionised (NH_3), and its equilibrium is influenced by temperature, pH and salinity. Ammonia levels above 5 mg/L can cause stress in the animals by utilising more oxygen and affecting their physiological processes. It can also prevent the production and excretion of digesting enzymes in shrimp, which lowers their survivability (Barbieri, 2010; Tu et al., 2022). TAN even at low concentrations can also damage the gill epithelium affecting respiration and can move across the cell membrane, affecting metabolism, osmoregulation, moulting and growth in shrimp (Zhao et al., 2020). According to Tu et al. (2022), shrimp exposed to 13.8 mg/L for 48 h experienced 50% mortality, emphasising the need for monitoring ammonia levels in shrimp aquaculture systems to prevent potential mortality.

Nitrate concentrations fluctuated throughout the production cycle, with the maximum level not exceeding 66.76 mg/L as shown in Figure 4. The nitrate levels in the shrimp culture were found to be optimal as

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per the assessment done by Prates et al. (2024). For long-term exposure, it is considered safe to maintain nitrate levels below 278.91 mg/L. Therefore, the levels were within the acceptable range throughout the cycle. However, the nitrite level exceeded the threshold limit on day 95 based on research by Furtado et al. (2016), which indicated that the tolerance threshold for shrimp culture is ideally between 2.5 and 10 mg/L for salinity above 24 g/L. The significant correlation between temperature and nitrite can be explained by the minimal residual time of nitrite in the aquatic environment under optimal temperature conditions. The increase in temperature can improve the process of nitrification by boosting the activity of NOB in biofilters. Nitrite removal is most effective at a temperature of 25°C (Neissi et al., 2022). The correlation between NO_2-N and TAN concentrations as shown in Table 3 suggests nitrite production through nitrification, where the microbial biomass is assimilated and converted into TAN to NO_2-H and even into the less toxic form NO_3-H (Pimentel et al., 2024).

4.3 | Feed load and mortality

Feed load, an important element that influences water quality, is determined by stocking density, feed type and water treatment equipment. It is important to note that an increase in biofilter nitrification capability may lead to higher feed loads, but only if nitrate buildup or poor water quality does not negatively impact shrimp mortality (Lindholm-Lehto, 2023). The gradual increase in feed load at a rate of 10% of the T_{Bio} of the shrimp until day 92 was sufficient for the microbial load to convert the nitrogenous waste and maintain the TAN level below the safe limit threshold according to Tu et al. (2022), obtained from the regression analysis as shown in Table 5. Unfortunately, due to a human error, the DF load was increased using an estimated average body weight of the shrimp on days 93 and 94. This resulted in a significant increase in the feed biomass from 27.37 to 49.60 kg, which represents an 81.2% increase as shown in Figure 5. As a result, this burdened the filtration system, leading to higher levels of TAN due to uneaten feed and excess suspended solids.

Further analysis using Pearson correlation revealed a significant relationship between feed biomass and TAN as shown in Table 3, which is consistent with the research by Zhou et al. (2018), which demonstrated that 60% of feed in an aquaculture system is present in the form of small particles. Their decomposition consumes DO and releases ammonia, which can stress the shrimp and burden the filtration system. The regression analysis shown in Table 5 supported this, indicating that the rise in TAN was caused by an increase in feed biomass. Many problems associated with water quality in RAS are linked to high fish waste, uneaten feed and low DO (Molleda et al., 2007). Therefore, the increase in the TAN was a result of feed load as the coefficient was $p < 0.05$. Through nitrogen excretion, feed protein content can affect the quality of water. In shrimp production systems, the majority of nitrogen intake comes from feeds. Therefore, the primary sources of organic carbon in these systems are feed and shrimp excrement (Deng et al., 2020; Lee & Lee, 2018). This drastic accumulation of solid waste as uneaten or indigestible feed resulted in the large production of ammonia-nitrogen. The organic and inorganic nutrient availability for microbial activity was significantly impacted by feed load, causing a massive spike in TAN.

Throughout the production cycle, the shrimp mortality rate was monitored to evaluate their overall health. On day 94, the shrimp's behaviour was erratic, and a significant amount was observed jumping out of tanks. A total of 725 dead shrimp were removed from the system and the ammonia concentration recorded was 13.93 mg/L with the lowest record for DO being 2.05 mg/L. This was then followed by a record high of 10,898 fatalities on day 96 with the ammonia concentration being 24.20 mg/L. The significant correlation between the mortality and TAN observed in Table 3 was confirmed by the regression analysis indicating that ammonia was one of the significant factors responsible for the mortality as shown in Table 4 and Figure 7. The relationship between mortality and TAN can be considered threshold-driven once exposure exceeds the safe limits (Tu et al., 2022). Second, nitrite levels in the system were also responsible for the elevated mortality rate as indicated by the regression analysis as shown in Figure 8. Although the nitrite level was slightly above the maximum threshold, the synergistic effect of nitrite and ammonia would have lowered the shrimp tolerance level to ammonia toxicity leading to mortality (Schuler et al., 2010). These results not only emphasise the importance of monitoring these factors but also shed light on the potential dangers associated with their levels. After the incident, the feed rate was lowered to 5% of the T_{Bio} of the remaining shrimp for the duration of the cycle to reduce the nitrogenous load on the system.

4.4 | Growth analysis

Shrimp growth monitoring is critical in farm management because it can be used as a production management tool, allowing for expected harvest size, waste output estimates and feed requirements (Zarzar et al., 2023). According to a study by Tanjung et al. (2022), the ideal weekly growth rate of shrimp is between 1.12 and 1.54 g or a DGR of 0.16–0.22 g for intensive culture. Hence, the growth rate in this study was within a good range with the DGR between 0.08 and 0.42 g before the mortality event as shown in Table 1. After a mortality event, the DGR of shrimp increased which can be attributed to research conducted by Kotiya and Vadher (2021), which demonstrated that shrimp growth is inversely proportional to stocking density. Consequently, the significant reduction in stocking density led to an increase in the DGR of shrimp. It was established that the mortality event caused a delay in the shrimp from attaining the target weight of 22 g when compared to a reference data set from a cycle with no mortality event as shown in Figure 8. The shrimp took 124 days to reach the target weight, whereas the reference cycle took 97 days. It is crucial to precisely calculate DF rations since a sudden rise in feed load might result in a significant increase in ammonia and nitrite. This can lead to the microbial community utilising all available resources to break down the nitrogenous waste. As a result, avoiding such circumstances is critical to maintaining a healthy and balanced environment for the shrimp.

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5 | CONCLUSION AND FUTURE WORKS

This study presents the impact of feed load on the mortality of Pacific white shrimp utilised in an indoor marine RAS. The water quality data obtained showed a significant correlation between feed load and the parameters of TAN and nitrite, which would have increased the nitrification process by the microbial community. The mass mortality of approximately 50% of the population was a result of the spike in TAN, as there were significant correlations between TAN and mortality $p < 0.05$, which was confirmed by the regression analysis. The drastic increase in feed load burdened the mechanical and biological filtration leading to a spike in TAN and a drop in DO. During the first 92 days, the water quality parameters were within range for the culture of the Pacific white shrimp. However, only after the feed load increased the production of the detrimental parameters was observed. Therefore, it is important to note that care must be taken when determining the feed rate and calculating the DF ratio for a system because the feed load can affect a wide range of water quality parameters that can influence the overall health of the Pacific white shrimp.

Pacific white shrimp farming is a rapidly growing sector in the aquaculture industry, but concerns such as disease risks, increased costs, water quality and feed management need to be addressed for a successful production. To understand the role of feed load as it affects the aquatic environment, further analysis is needed to determine the rate at which the increase in feed alters the water quality parameters that lead to ammonia toxicity in shrimp culture. This can help reduce feed waste, lower production costs and improve the FCR, leading to a more economically sustainable system.

AUTHOR CONTRIBUTIONS

Fazeel Mohammed: Conceptualization; formal analysis; investigation; methodology; project administration; resources; visualization; writing—original draft; writing—review and editing. **Paul Gabbadon**: Investigation; methodology; project administration; resources. **Tahmina Ajmal and Martin Goodchild**: Formal analysis; visualization; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ETHICS STATEMENT

All procedures conducted in the study adhered to all the applicable guidelines for the culture of Pacific white shrimp.

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