

Integration of zootechnical and ecological factors to evaluate the physiological well-being of *Penaeus vannamei* on farms connected to Lake Maracaibo, Venezuela

Integración de factores zootécnicos y ecológicos para evaluar el bienestar fisiológico de *Penaeus vannamei* en fincas conectadas al lago de Maracaibo, Venezuela

Integração de fatores zootécnicos e ecológicos para avaliar o bem-estar fisiológico de *Penaeus vannamei* em fazendas conectadas ao Lago Maracaibo, Venezuela

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Abstract

The semi-intensive commercial shrimp farming in Lake Maracaibo, Venezuela, is affected by eutrophication processes resulting from excess nutrients, which favor the proliferation of microalgae and the deterioration of water quality. There is limited information on the relationship between production variables, fluctuations in planktonic communities, and their impact on the physiological state of the shrimp. Therefore, the objective of this study was to analyze the relationship between production and ecological variables with the Relative Condition Index (RCI) as a physiological indicator of *Penaeus vannamei* during a ten-week grow-out cycle. Production and ecological variables were monitored weekly, including qualitative and quantitative analyses of phytoplankton and zooplankton. Data were evaluated using Pearson correlation, variable selection with VIF and FCR, logistic regression with Firth's bias reduction, and deviance analysis. Shrimp exhibited a final average RCI of 1.36 ± 0.92 and an average FRC of 1.81 ± 0.89 . The phytoplankton community was dominated by Cyanobacteria (57.8%), followed by Chlorophyta (32.4 %) and Heterokontophyta (9.1 %), while zooplankton was primarily represented by copepods (69.9 %). However, the model showed a positive association between RCI and the abundance of *Chlorella* sp. and barnacle nauplii, and a negative relationship with *Mesocyclops* sp. These results suggest that the nutritional and functional quality of plankton may be more relevant than its total abundance, constituting a bioindicator of physiological well-being and a potential supplementary food source.

Resumen

El cultivo semiintensivo comercial de camarón en el Lago de Maracaibo, Venezuela, se ve afectado por procesos de eutrofización derivados del exceso de nutrientes, los cuales favorecen la proliferación de microalgas y el deterioro de la calidad del agua. Existe escasa información sobre la relación entre las variables productivas, las fluctuaciones de las comunidades planctónicas y su impacto sobre el estado fisiológico del camarón. Por ello, el objetivo de este estudio fue analizar la relación entre variables productivas y ecológicas con el Índice de Condición Relativa (ICR) como indicador fisiológico de *Penaeus vannamei* durante un ciclo de engorde de diez semanas. Se monitorearon semanalmente variables productivas y ecológicas, incluyendo análisis cualitativos y cuantitativos de fitoplancton y zooplancton. Los datos fueron evaluados mediante correlación de Pearson, selección de variables con VIF y AIC, regresión logística con reducción de sesgo de Firth y análisis de devianza. Los camarones presentaron un ICR promedio final de $1,36 \pm 0,92$ y un ICA promedio de $1,81 \pm 0,89$. La comunidad fitoplanctónica estuvo dominada por Cyanobacteria (57,8 %), seguida por Chlorophyta (32,4%) y Heterokontophyta (9,1 %), mientras que el zooplancton estuvo representado principalmente por copépodos (69,9 %). Sin embargo, el modelo mostró una asociación positiva entre el ICR y la abundancia de *Chlorella* sp. y nauplios de cirrípedos, y una relación negativa con *Mesocyclops* sp. Estos resultados sugieren que la calidad nutricional y funcional del plancton puede tener mayor relevancia que su abundancia total, constituyendo un bioindicador del bienestar fisiológico y una potencial fuente alimenticia complementaria.

Palabras clave: eutrofización, fitoplancton, zooplancton, ICR, bioindicador.

Resumo

A carcinicultura comercial semi-intensiva no Lago Maracaibo, Venezuela, é afetada por processos de eutrofização resultantes do excesso de nutrientes, que favorecem a proliferação de microalgas e a deterioração da qualidade da água. Há pouca informação sobre a relação entre variáveis de produção, flutuações nas comunidades planctônicas e seu impacto no estado fisiológico do camarão. Portanto, o objetivo deste estudo foi analisar a relação entre variáveis de produção e ecológicas com o Índice de Condição Relativa (ICR) como indicador fisiológico de *Penaeus vannamei* durante um ciclo de cultivo de dez semanas. As variáveis de produção e ecológicas foram monitoradas semanalmente, incluindo análises qualitativas e quantitativas de fitoplâncton e zooplâncton. Os dados foram avaliados utilizando correlação de Pearson, seleção de variáveis com VIF e AIC, regressão logística com redução de viés de Firth e análise de deviance. O camarão apresentou um ICR médio final de $1,36 \pm 0,92$ e um ICA médio de $1,81 \pm 0,89$. A comunidade fitoplanctônica foi dominada por cianobactérias (57,8 %), seguidas por clorófitas (32,4 %) e heterocontófitas (9,1 %), enquanto o zooplâncton foi representado principalmente por copépodes (69,9 %). No entanto, o modelo revelou uma relação positiva entre o índice de condição relativa (ICR) e a abundância de *Chlorella* sp. e náuplios de cracas, e uma relação negativa com *Mesocyclops* sp. Esses resultados sugerem que a qualidade nutricional e funcional do plâncton pode ser mais relevante do que sua abundância total, constituindo um bioindicador de bem-estar fisiológico e um potencial fonte alimentar suplementar.

Palavras-chave: eutrofização, fitoplâncton, zooplâncton, ICR, bioindicador.

Introduction

The semi-intensive shrimp farms established in Lake of Maracaibo are supplied with water from the lake, which is characterised by eutrophic conditions (Redfield, 1958); therefore, the eutrophic condition of the farming systems is implicit, and this fact poses a challenge for maintaining the organisms in the aquaculture systems (Martínez *et al.*, 2021). Eutrophication generates massive algal blooms that are intensified by the inefficient use of fertilisers in the systems, leading to changes in plankton, a decrease in dissolved oxygen, acidification of the environment, accumulation of nitrates and ammonium, and a general deterioration in the quality of the rearing environment, compromising the growth and health of the shrimp (Qiao *et al.*, 2020). Thus, lake eutrophication, combined with poor aquaculture management practices, affects the growth of organisms, leads to mass mortality and prolongs production cycles (Martínez *et al.*, 2021).

In this context, the inclusion of zootechnical variables such as body condition scores is useful for assessing the physiological well-being of shrimp. The feed conversion ratio (FCR) is a key metric for determining the efficiency of nutrient biotransformation into somatic growth, whilst the relative condition index (RCI) is an indicator of physiological status (Berry *et al.*, 2019). The adoption of the relative condition index (RCI) in intensive aquaculture management systems provides relevant information for optimising production cycles, nutritional planning and the operational organisation of farmed organisms (Bonilla-Flórez *et al.*, 2017). Furthermore, the structure and stability of the planktonic community have been associated with better rearing conditions and an adequate physiological state, indirectly influencing indicators of body performance and production efficiency. In this context, the joint monitoring of the RCI, the feed conversion ratio (FCR) and planktonic dynamics allows for a comprehensive assessment of the organisms' welfare and growth, facilitating the design of adaptive management strategies and the mitigation of potential environmental impacts (Lyu *et al.*, 2021).

Planktonic communities in ponds are essential both as a natural food source and because they can induce changes in the aquatic environment; this makes them suitable for use as indicator organisms in water quality assessment (Samosir *et al.*, 2021). Their response to variations in dissolved oxygen or nutrient load provides key metrics for trophic diagnosis and the operational sustainability of the production system (Gómez *et al.*, 2020). A direct and positive correlation has been documented between the abundance of microalgae such as *Chlorella vulgaris* and the production performance of *P. vannamei*. Various studies demonstrate that high concentrations of this chlorophyte optimise yield, survival and physiological condition, as well as significantly improving the feed conversion ratio (Eissa *et al.*, 2023; Hudson, 2024). Furthermore, regarding the contribution of zooplankton, *Artemia* nauplii remain fundamental in larviculture; their high energy density and essential fatty acid profile establish them as the optimal nutritional source for the early stages of *P. vannamei* (Van Stappen *et al.*, 2024). Despite the importance of plankton as a bioindicator, knowledge of planktonic communities in the aquaculture facilities of Lake of Maracaibo is limited compared to other regions such as Brazil and Ecuador (Casé *et al.*, 2008; Neto *et al.*, 2009; Patricio *et al.*, 2023; Delgado *et al.*, 2024).

At present, there is a knowledge gap regarding the relationship between production variables and fluctuations in planktonic communities, and their potential impact on the physiological condition of shrimp. This has limited the ability to implement management strategies based on production and ecological indicators that would optimise production and mitigate negative impacts. From the perspective of the overall functioning of intensive farming systems, the aim of this study is to analyse the relationship between productive and ecological planktonic variables and the physiological condition of *P. vannamei* during the grow-out cycle.

Materials and methods

Study area

The study was conducted at a shrimp farm located in the municipality of Rosario de Perijá, on the western shore of Lake of Maracaibo. The area has a tropical climate with a bimodal rainfall pattern, with peaks between May and October and troughs between January and April, and an average annual rainfall of 1057 mm. The water temperature ranges between 26 and 28 °C, which favours the aquaculture production of *P. vannamei* in the region (Lozada and Graterol, 2003) (Figure 1).

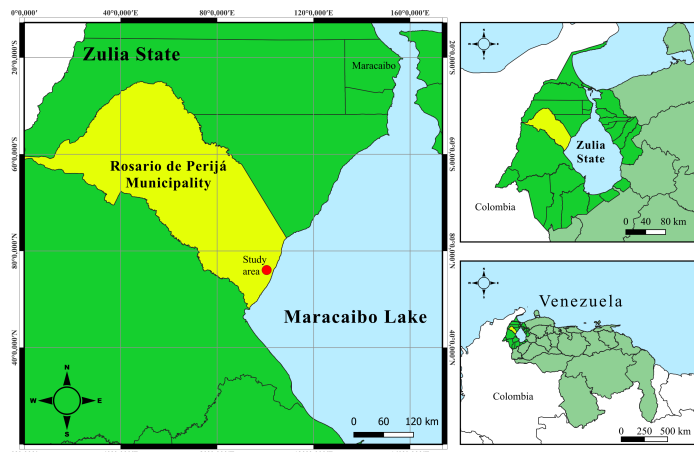


Figure 1. Geographical location of the aquaculture farm on Lake of Maracaibo, Venezuela.

Sampling design

Seven sampling points were established: two control points, one outside the farming system known as the ‘Lake station’ and one inside the system located at the sluice gate, known as the ‘Canal station’; and a block comprising five ponds filled and stocked simultaneously, ensuring uniform conditions for the study. The sampling period ran from 13 April to 14 June 2024 and covered exclusively the fattening phase of *P. vannamei*. Sampling was carried out weekly, with collections taking place in the morning (Cremen *et al.*, 2007; Lyu *et al.*, 2021) (Figure 2).

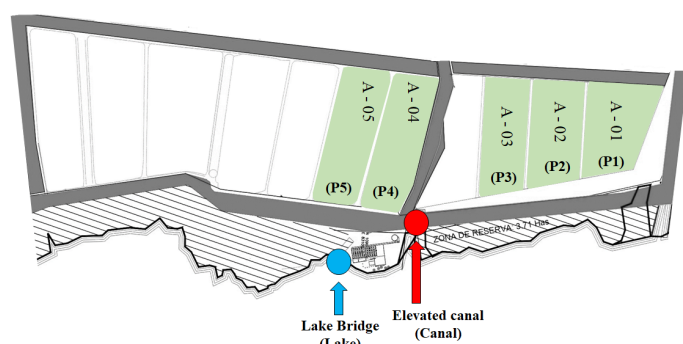


Figure 2. Spatial distribution of sampling points in the culture ponds (A01–A05), the channel and the lake used during the study.

Planktonic community in *P. vannamei* culture

Phytoplankton samples were taken in triplicate in the morning, between 8:00 and 10:00 am, to ensure consistency in light conditions and biological activity. Phytoplankton was collected at a depth of between 30 and 50 cm using a rod fitted at its distal end with a 350 mL plastic collector. The sample was fixed *in situ* by adding 1 mL of 1 % concentrated Lugol’s solution (Cremen *et al.*, 2007). Samples for the identification and quantification of zooplankton were collected by filtering 5 L of water using a plastic collector of the same volume, employing a plankton net with a mesh size of 150 μm . The samples were fixed *in situ* with 4 % formaldehyde (Casé *et al.*, 2008). The samples were labelled and stored in the dark until subsequent analysis in the laboratory.

For phytoplankton quantification, a 0.1 mm deep Neubauer haemocytometer was used (Martínez *et al.*, 2021), and for zooplankton quantification, a 1 mL Sedgewick-Rafter counting chamber was used; in both cases, quantification was performed in triplicate (Picapedra *et al.*, 2020). Observations and counts were carried out using a high-resolution Carl Zeiss® binocular microscope.

The taxonomic identification of zooplankton was carried out using the taxonomic keys of Bradford-Grieve (2002) and Ávila-Parga *et al.* (2022), validated against the World Register of Marine Species (WoRMS, 2023) database. Meanwhile, for the taxonomic identification of phytoplankton, recognised taxonomic keys were used, including those proposed by Yacubson (1972, 1974a, 1974b, 1974c), validated against the updated database by Guiry and Guiry (2024).

Estimation of production variables

Production variables were measured weekly during the fattening phase in five ponds within a semi-intensive system. The following metrics were calculated: harvest density (individuals.m⁻²), weight gain (g), survival (%), yield per hectare (kg.ha⁻¹) and the Feed Conversion Ratio (FCR) (Domingos and Vinatea, 2008). To calculate the Relative Condition Index (RCI) of *P. vannamei* shrimp, the model by Peig and Green (2010). The scalar mass of each individual was estimated using the formula ($M_i = P_i (LT_0 / LT_i)^b$), where P_i is the individual weight, LT_0 is the average length calculated as the sum of the lengths divided by the total number of individuals, and LT_i is the length of the individual; where an RCI value greater than 1 indicates good growth and lower values indicate insufficient growth. For greater accuracy, the population standard deviation of the RCI was also calculated to assess variability in population growth.

In addition, the total abundance of phytoplankton and zooplankton was correlated with production variables through weekly comparative analyses, assessing their potential influence on growth, survival, ICA and relative condition index during the culture. Differences between tanks for the FCR were assessed using one-way ANOVA (or Kruskal-Wallis, as appropriate), using a significance level of $\alpha = 0.05$.

RCI Modelling vs. Productive Factors

Collinearity Assessment: using Pearson’s correlation, the Survival variable was removed as it showed a strong correlation ($r \approx 0.9$) with harvest density and ICA. The step VIF function (pedometrics package, Samuel-Rosa, 2022) was then used to select a subset of predictor variables that maintained a Generalised Variance Inflation Factor (GVIF) below the collinearity threshold. Values of $GVIF \geq 4$ and $GVIF \geq 10$ were considered indicative of correlation issues or serious correlation issues, respectively.

Handling of Complete Separation: Due to a problem of complete separation in the influence of the FRC variable on the RCI, the FRC variable was recategorised into two levels (Low and High) using quantile-based intervals (the ‘cut2’ function, Harrell, 2025).

Model Fitting: The final model was fitted using Firth's Bias-Reduced Logistic Regression (Firth, 1993). This is a penalised maximum likelihood method that was employed to mitigate bias in the logistic regression estimators, a recommended practice in the presence of issues such as separation or scattered data.

RCI Modelling vs. Species Abundance

The relationship between the abundance of phytoplankton and zooplankton species and the RCI was analysed using binary logistic regression, which enabled the identification of species predictive of the physiological status of the shrimp. Variable (species) selection was carried out in three stages:

Starting from a saturated model, the step VIF function (pedometrics package) was used to obtain an initial set of predictor species with low collinearity (specifically, GVIF < 4).

With the preselected species, the step AIC function (MASS package, Venables and Ripley, 2002) was employed. This function performs a stepwise (backward) variable selection based on the Akaike Information Criterion (AIC), seeking the most parsimonious model that optimises the trade-off between goodness of fit and complexity.

Finally, a deviance analysis was carried out using the chi-square test to assess the significance of the final predictors. Only those species that significantly reduced the residual deviance were included in the final model.

The estimated coefficients of the final model were converted into odds ratios (OR) with their respective confidence intervals. The discriminatory power of the logistic regression model was assessed using Tjur's R^2 (or discrimination coefficient), a measure of the model's ability to distinguish between the two classes of the response variable (Tjur, 2009). All statistical analyses were performed using the statistical programming language R (R Core Team, 2024).

Results and discussion

Correlation between production variables and the RCI

The shrimp exhibited an average Relative Condition Index (RCI) of 1.36 ± 0.92 during the study period, a value suggesting a favourable physiological condition according to the ranges reported for organisms in the fattening phase under semi-intensive systems, where values close to or above 1 are typically associated with individuals exhibiting adequate somatic performance and relative growth (Prajapati and Ujjania, 2021). The RCI showed variability between tanks (Figure 3).

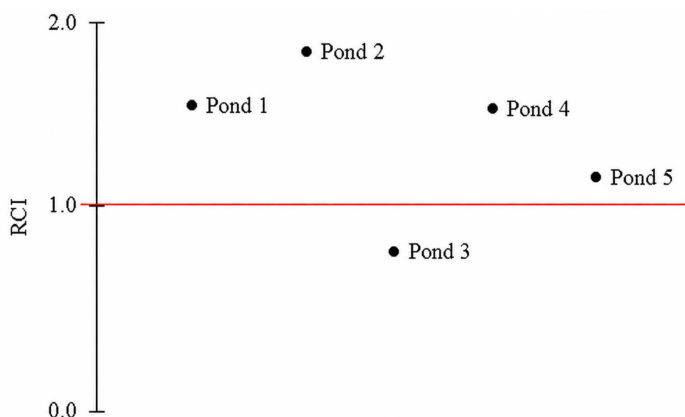


Figure 3. Comparison of the Relative Condition Index (RCI) of white shrimp across the ponds evaluated.

Tank 2 had the highest average value ($RCI = 1.81 \pm 1.16$), followed by tanks 1 and 4 (1.54 ± 0.94 and 1.51 ± 1.01 , respectively). Pool 5 recorded an intermediate RCI (1.14 ± 0.63), whilst pool 3 had the lowest mean value (0.81 ± 0.45). Although no statistically significant differences were detected between means ($p > 0.05$), the observed variation suggests a differential trend in the relative condition of the organisms across experimental units. As the RCI reflects the relationship between weight and size, values below 1 may indicate lower accumulation of relative body biomass or differences in individual growth under specific rearing conditions.

When evaluating production values spatially, it can be seen that the lowest RCI was recorded in the third pond and, consequently, the lowest yield/ha⁻¹; however, contrary to expectations, the shrimp in this pond showed the highest weight gain (Figure 4). The fourth pond achieved the highest yield, followed by the fifth pond. These indices appeared to contradict the findings regarding the RCI of the ponds; however, this discrepancy can be explained by survival patterns. Furthermore, the third pond recorded the highest survival rate, which led to the lowest FRC, as was the case with the fifth pond. Conversely, the low yield was associated with the reduced RCI observed previously.

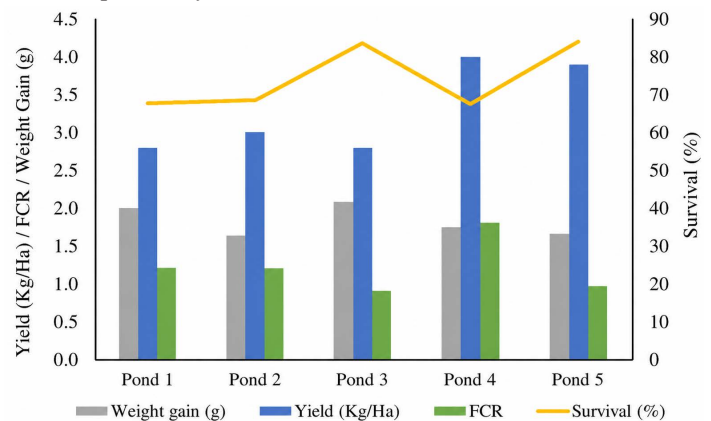


Figure 4. Comparison of the production indices recorded in the evaluated grow-out ponds.

Differences were observed in the behaviour of the production variables between weeks of cultivation (Figure 5).

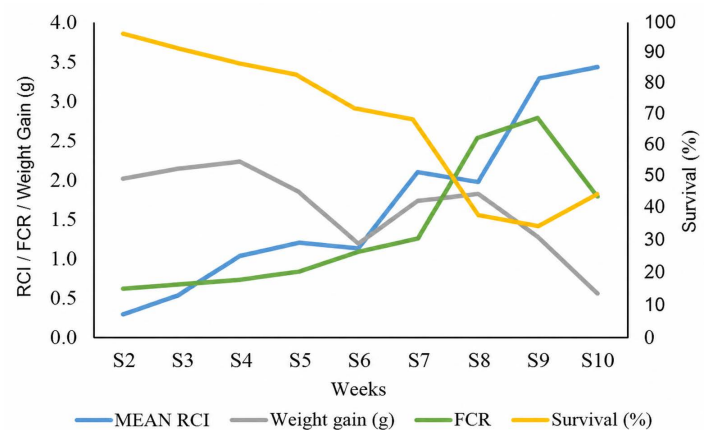


Figure 5. Temporal variation in production variables during the weeks of cultivation.

Weight gain showed relatively consistent values throughout the evaluation period, whilst survival rates varied between weeks, reaching their highest percentages during weeks 2 and 5. The relative

reduction in survival observed at certain stages of the culture could be associated with increases in effective density and intraspecific competition, factors reported as limiting factors for production performance in semi-intensive systems (Zhang *et al.*, 2024). Furthermore, an inverse relationship was observed between survival and FCR, suggesting a possible alteration in feed efficiency associated with stocking density stress and competition for resources (Shirly-Lim *et al.*, 2024). Furthermore, the RCI and FRC showed variability between weeks, which could reflect changes in physiological condition and feed utilisation by the organisms during the fattening period. The seventh and tenth weeks, which coincided with variations in stocking density and survival rates.

Yield.ha⁻¹ remained stable throughout the study, except for decreases observed in the seventh and tenth weeks, which coincided with variations in stocking density and survival rates. These changes were attributed to the carrying capacity of the ponds and the progressive deterioration of water quality, disrupting the ecological balance of the system. An increase in stocking density can induce chronic stress in the shrimp due to overcrowding and environmental deterioration, limiting the efficiency and yield of the culture (Aguilar *et al.*, 2012).

The logistic regression model revealed a moderate negative association (OR = 0.99) between harvest density and the probability of obtaining a favourable RCI, whilst high FRC showed a significant positive association, increasing the probability of obtaining a good RCI by 7.6 times (OR = 7.57). Overall, the model fit was adequate (R² = 0.5). Therefore, increased density was associated with RCI values close to poor condition, and 70% of cases with good physiological condition were associated with a high FRC (Figure 6).

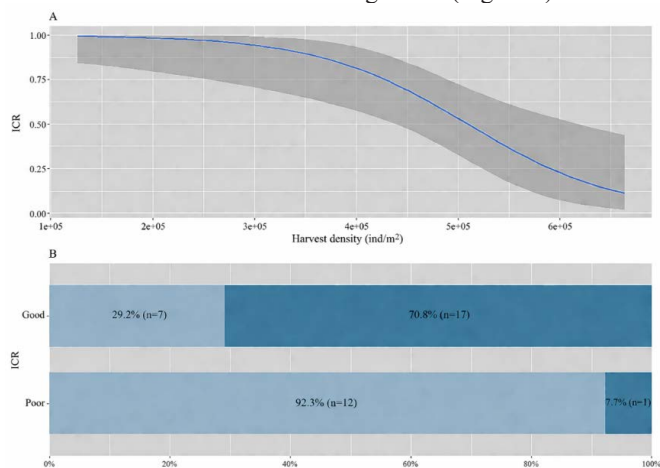


Figure 6. A) Logistic regression curve for predicting RCI categories based on harvest density. B) Relationship between RCI and low and high FRC categories.

The model variables showed that higher FRC values were associated with an increase in the RCI; this relationship can be explained by the fact that feed efficiency directly influences biomass accumulation and the relative body condition of the organisms, given that greater utilisation of feed favours an increase in weight relative to expected size (Le Cren, 1951). A high FRC indicates lower feed loss and greater feed utilisation by the shrimp, as well as greater stability in water quality, which results in an optimisation of the shrimp's physiological condition (Irani *et al.*, 2022). However, in 7.7 % of cases, an inverse relationship was observed: despite a high FRC, the RCI was low. This discrepancy could be attributed to genetic factors or other variables not taken into account.

These variables also have implications for harvest density, as shrimp with a higher RCI have a larger body size and weight, and therefore a higher biomass. The relationship between a population's biomass and the area it occupies is well studied in crustaceans; limited space causes stress in the population, leading it to adapt and reduce its biomass (Rodríguez-Olague *et al.*, 2021). This explains the downward-sloping sigmoid curve between the RCI and density, suggesting that there was either excessive density or unfavourable environmental conditions.

Relationship between planktonic communities and the Relative Condition Index (RCI)

To analyse the relationship between the abundance of phytoplankton and zooplankton species and the Relative Condition Index (RCI), a binary logistic regression was applied, given that the RCI has two possible values: Poor (= 0) and Good (= 1), following a binomial distribution. This approach enabled the identification of which species influenced the physiological condition of the shrimp, providing a clear interpretation of the underlying ecological patterns.

The variable selection process for the phytoplankton community included only the species *Chlorella*, as it showed a positive and significant relationship with high RCI values (OR = 1.08), indicating that an increase in its abundance raises the probability of recording individuals with higher relative condition by 8 %, albeit with weak to moderate discriminatory power (R² Tjur = 0.251) (Figure 7).

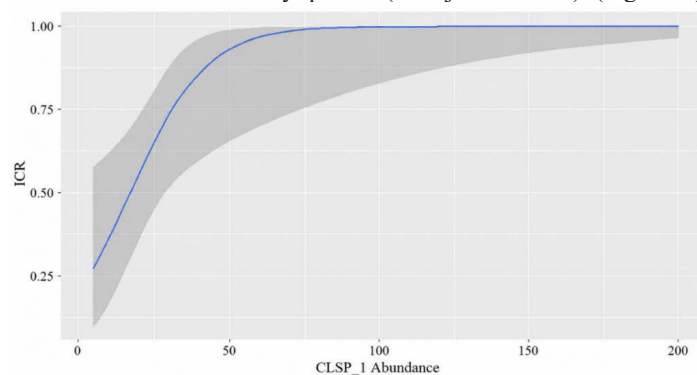


Figure 7. Logistic regression curve of the RCI as a function of *Chlorella* sp. abundance; the grey band represents the 95 % confidence interval.

For zooplankton, *Balanus* nauplii were positively associated with high RCI values (OR = 1.32), whilst *Mesocyclops* sp. showed a significant negative association (OR = 0.23), implying that an increase in its abundance increases the probability of recording individuals with low RCI values by up to 4.35 times. This zooplankton model exhibited moderate discriminatory power (R² Tjur = 0.402), higher than that observed in the phytoplankton model (Figure 8 A and B).

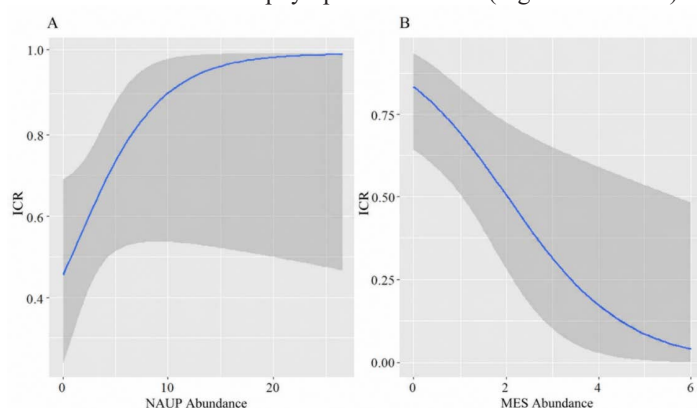


Figure 8. Logistic regression curves for the RCI as a function of the abundance of *Balanus* nauplii (A) and *Mesocyclops* sp. (B).

The positive relationship between the RCI of the shrimp and the presence of *Chlorella* is consistent with the findings of Eissa *et al.* (2023), who demonstrated that the inclusion of *C. vulgaris* in the diet improved the growth and health status of the shrimp, showing that higher concentrations of this microalga resulted in better performance and physiological condition of the farmed organism.

Meanwhile, Hudson (2024) evaluated zootechnical performance and gene expression in *P. vannamei* fed diets supplemented with *Chlorella*, demonstrating an improvement in survival and feed conversion ratio, as well as an increase in final productivity.

As regards the contribution of zooplankton groups, crustacean nauplii such as those of *Artemia*, used in the larviculture of *P. vannamei*, possess high nutritional value, making them an essential component within aquaculture systems as they promote the survival and growth of farmed species (Van Stappen *et al.*, 2024). In this regard, *Balanus* nauplii, in addition to being relatively larger than *Artemia* nauplii, exhibit a nutritional profile comparable to that of copepods, characterised by high levels of EPA, taurine and essential minerals, suggesting potential nutritional value for juveniles in the grow-out phase (Pedro *et al.*, 2025).

On the other hand, the presence of cyclopoids of the genus *Mesocyclops* was linked to an unfavourable RCI. This coincides with their association with poor water quality; these organisms tend to proliferate under eutrophic conditions, when the environment is not optimal for shrimp farming (Jindal and Sharma, 2011). Research conducted by Vidhya *et al.* (2014) showed that the quality of the copepod diet influences their viability as live feed; therefore, it can be inferred that a deficient diet in an eutrophic pond dominated by cyanobacteria negatively affects the viability of copepods, impairing their functional characteristics as high-quality live feed.

Conclusion

The results showed that planktonic and productivity variables are related to the physiological status of *Penaeus vannamei* in semi-intensive systems. The RCI and FRC exhibited weekly fluctuations, which may reflect changes in physiological condition and feed utilisation efficiency during the grow-out period. From a production perspective, the increase in harvest density limited the efficiency and yield of the culture. Furthermore, the planktonic community influenced the physiological status of the shrimp, with *Chlorella* sp. and *Balanus* nauplii standing out for their positive association with high RCI values, whilst *Mesocyclops* sp. showed a negative relationship. These findings suggest that the functional composition of plankton may be more relevant than its total abundance, constituting an important component as a natural food source and as an ecological indicator of physiological performance in semi-intensive farming systems.

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