



Mathematical Modeling to Estimate the Effect of Probiotics on the Growth of *Andinoacara rivulatus* in a Biofloc System

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ABSTRACT

Andinoacara rivulatus, known as vieja azul, is a species native to Ecuador with great ecological and productive relevance. However, its population has declined due to overexploitation and habitat degradation. The incorporation of probiotics in fish farming is an important advancement, with multiple potential benefits, since it favors both fish growth and the stability of the aquatic ecosystem. The present study evaluated the influence of probiotics on the growth of *Andinoacara rivulatus* and water quality in a Biofloc system, using mathematical modeling to analyze biometric relationships. An experiment with five treatments (T1-T5) was developed in a biofloc system, under a triplicate factorial design, where fish were kept for 30 days. Probiotics with different compositions were applied, and biometric and physicochemical parameters of the water were monitored. The results indicated that the T5 treatment promoted the greatest growth of the fish, with a median height of 3.4cm, compared to 1.1cm in the control (T1). Likewise, the weight-length and weight-perimeter opercular relationship was higher in T5, coinciding with better feed conversion. In contrast, the T3 treatment optimized water quality, registered lower concentrations of TAN ($0.99 \pm 0.11\text{mg/L}$) and BOD ($9.12 \pm 0.20\text{mg/L}$), compared to the control treatment, whose TAN was $1.38 \pm 0.08\text{mg/L}$ and BOD ($12.43 \pm 0.95\text{mg/L}$). In addition, T3 presented the highest electrical conductivity ($175.62 \pm 0.81\mu\text{S/cm}$), suggesting higher microbial activity in the system. Mathematical modeling allowed characterizing growth using allometric equations, determining that T5 favored positive growth, while T3 contributed to the sustainability of the ecosystem. It is concluded that, although T5 optimizes zootechnical performance, T3 is more efficient in maintaining adequate physicochemical parameters. Therefore, probiotic selection should consider both fish development and the stability of the aquatic environment.

INTRODUCTION

Fish is one of the most available and affordable sources of complementary animal protein (Mehar *et al.*, 2022). In general, it is a widely recommended food in human diets due to its high nutritional quality, which undoubtedly contributes to the nutritional and food security of the population. In recent years, consumption of fishery and aquaculture products has grown worldwide at an average annual rate of 3.1%, with an average per

capita fish intake of 20.20kg/ year (Hebsale *et al.*, 2023). Ecuador is among the 25 largest fish producers, as a result of the high diversity of wild freshwater fish that it harbors (Medina *et al.*, 2024). In the country, artisanal fishing gear techniques are the most widely used, although they often lack adequate management and regulation. This condition negatively impacts the reproduction and quality of native species and, consequently, their survival (Gonzalez *et al.*, 2021).

Andinoacara rivulatus, recognized as Vieja Azul, is a native species that is mainly cultivated and consumed in rural areas in and around the province of Los Ríos (Fabara, 2018). The species exhibits omnivorous reproductive behavior and deposits its eggs on fixed surfaces, with no evidence of providing oral shelter for its larvae (Caez *et al.*, 2019). It is also on the verge of extinction as a result of several factors, the most predominant being habitat contamination, overexploitation and the appearance of predatory species that are not native to the area. Despite its relevance in the production sector, the study on the influence of culture systems on production yield remains limited (Gonzalez Angón, *et al.*, 2021). The implementation of controlled aquaculture systems could prevent their extinction and promote the sustainable development of aquaculture.

The incorporation of probiotics in fish farming is an important development with multiple potential benefits. It promotes beneficial microbiota that support intestinal and liver health by acting as a supplement, improves feed intake efficiency and accelerates growth. It also balances water quality parameters and strengthens the immune system against pathogens (Gaffar *et al.*, 2023). Probiotics are live microbial assemblages introduced into the gastrointestinal tract with food or water, promoting good health by improving internal microbial balance and suppressing harmful microorganisms. Incorporating probiotics in culture media or with a supplemental diet provides additional nutrition and their secreted enzyme increases feed utilization, ultimately reducing feed cost. In addition, probiotics help maintain water parameters within an optimal range, especially balancing nitrate and ammonia, as well as reducing environmental stress (El-Saadony *et al.*, 2021).

In fish and shellfish culture, a wide range of genera of Gram-positive bacteria are commonly used as probiotics. Although any individual strain of these bacteria brings productivity to the culture, the synergy of two or more strains generates a superior increase in yield. *Bacillus coagulans* species has been used as dietary supplementation in Pacific white shrimp culture, generating higher growth performance and body composition in shrimp, as well as higher survival rate against the pathogen *V. parahaemolyticus* (Amoah *et al.*, 2019). The combination of *Bacillus* and *Lactobacillus* has also been tested in aquatic ecosystems, where its ability to model the immune response and hematological parameters of host species has been demonstrated (Silva *et al.*, 2021).

The contribution of mathematical models to the biological sciences is an ongoing research topic, and aquaculture is no exception. Mathematical modeling, from the

perspective of education, is presented as a field that explores the competencies, meanings and opportunities that arise from model constructions, as well as the interdisciplinary relationships linked to mathematical training. Consequently, modeling acts as a “translation” tool between the mathematical system and the extramathematical one, simplifying the understanding of complex phenomena (Sepúlveda *et al.*, 2020). However, mathematical modeling should not focus exclusively on the creation of new models, but also on the use, analysis and interpretation of existing models. The international literature reflects a lack of consensus regarding the term mathematical 'model' and its role in science, keeping open the debate on its epistemological status (Gainsburg, 2006).

The objective of this research was to evaluate the influence of probiotics on the growth of *Andinoacara rivulatus* under conditions of a Biofloc system, using mathematical modeling tools to identify and understand the dynamics of development of this little studied species. In addition, the effect of microorganisms integrated in probiotics on water quality in aquaculture systems was evaluated.

MATERIALS AND METHODS

Experimental units

The experimental study was carried out in the private property Vladimir, located in the city of San Isidro, province of Manabí, Ecuador. *Andinoacara rivulatus* fish specimens were used, selected under certain criteria, all with an initial size between 2.00 - 2.50cm.

Experimental design and feeding management

For the execution of the experiment, the fish were distributed in 15 similar glass aquaria, with dimensions of 60 x 44 x 34cm and a capacity of 60 liters, grouped in five groups corresponding to the treatments (T1, T2, T3, T4, T5). Each aquaculture system was inoculated with 5L of biofloc from a previous old blue culture. The carbon/nitrogen ratio was set at 2:1, according to Avnimelech's (1999) guidelines, being calculated and adjusted every two weeks during the thirty days of the study. Aeration and water movement were ensured by diffuser stones connected to a centralized pump.

Feeding of aquatic organisms consisted of two servings per day of commercial balanced feed corresponding to 5% of body weight (9:00 am and 5:00 pm), based on the recommendations of Karunaarachchi *et al.* (2018). The portion was adjusted every fortnight.

Probiotics were added at the same schedule as the balanced feed. Probiotic dosage consisted of 2g/ kg of balanced feed, applied following the protocols established by the suppliers. Details of the composition of probiotics in each treatment are described in Table (1).

Table 1. Composition of probiotics in experimental treatments

Treatment	Composition
T1	It did not include probiotics.
T2	<i>Enterococcus faecium</i> DSM 7134, enterobacteria, yeast fungi.
T3	Dried yeasts and cell wall.
T4	<i>Bacillus subtilis</i> , haematococcus, spirulina, beta-glucans, mannan-oligosaccharides.
T5	<i>Bacillus</i> sp., <i>Enterococcus</i> sp., <i>Pediococcus</i> sp., <i>Thiobacillus</i> sp., <i>Paracoccus</i> sp., pure enzymes.

The measurement of functional traits of the fish, such as height, weight, length and opercular perimeter was carried out at the end of the project (Zamudio *et al.*, 2016).

Water quality analysis

Five sampling points were designated for each Biofloc system, where aliquots of water were collected at two specific times: at the beginning and at the end of the experiment. Water quality was evaluated using electronic equipment, which allowed the measurement of dissolved oxygen, biochemical oxygen demand, total ammoniacal nitrogen, total nitrogen, phosphate concentration and electrical conductivity (APHA, 2005).

Mathematical modeling

The characterization of growth in fish was carried out through non-linear models that estimated allometry. The mathematical models applied modeled the relationship between the evolution of one variable with respect to another variable, starting from the function

$$Y = aX^b$$

Equation 1. Allometry function

Where:

Y = dependent variable;

X = independent variable;

a = nodal increment;

b = growth parameter.

Given $b=1$, the growth is called isometric; if b is greater than 1, allometric growth corresponds to a positive increase; whereas, if b is less than 1, allometric growth reflects a negative increase.

The biometric relationships addressed in the study were:

Length-weight

The length-weight relationship of the fish was estimated using a potential regression that links a linear measure, such as the total length of the fish, to its weight or volume. The analysis was based on the following equation:

$$WT = a * L^b$$

Equation 2. Length-weight ratio.

Where:

WT = total weight of the fish (g);

a = is a regression constant equivalent to the condition factor;

L = total length (cm);

b = allometric coefficient.

Opercular perimeter-weight

It was analyzed how the opercular perimeter is related to the weight of the fish, allowing to determine the isometry or allometry through the following formula:

$$Po = \left(\frac{WT}{a} \right)^{\frac{1}{b}}$$

Equation 3. Opercular perimeter-weight ratio

Where:

Po = opercular perimeter of the fish (cm);

WT = total weight of the fish (g);

a = proportionality constant equivalent to the condition factor;

b = allometric coefficient.

Data analysis

The biometric data obtained in the experiment were submitted to the software R studio ver. 4.4.2. software for graphical representation. The biometric relationships weight-total length and weight-opercular perimeter were plotted by scatter plot. The heights of each treatment were examined using the boxplot technique.

Additionally, water quality parameters were studied by analysis of variance (ANOVA). For the comparison of means between treatments, Duncan's test was applied with a significance level of $P < 0.05$. All statistical analyses were performed using SPSS software, version 26.0.0.0 (IBM Corp., New York, USA).

RESULTS AND DISCUSSION

1. Modeling of biometric relationships

Fig. (1) represents the relationship between total length and weight of aquatic organisms subjected to different treatments (T1 to T5). For the formation of the scatter plot, the treatments were represented by a specific color and shape, indicated as follows: T1, organized by red colored circle; T2, conformed by blue colored triangle; T3,

presented by green colored square; T4, visualized by orange colored circle; and T5, consolidated by purple colored triangle.

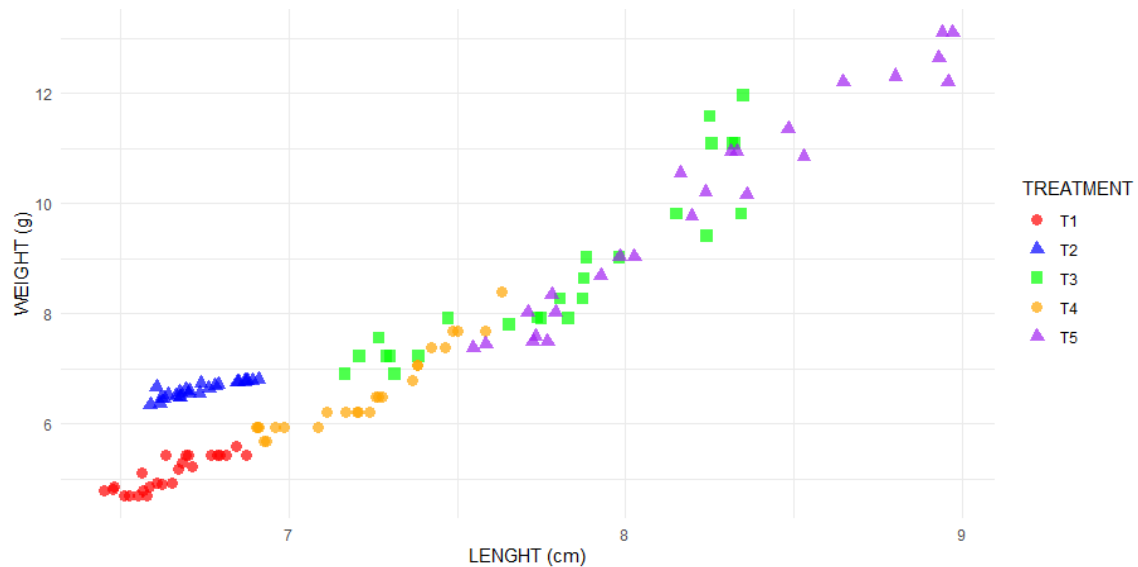


Fig. 1. Scatter plot of weight - length ratio

In terms of distribution, the treatments are scattered along the length and weight axis without a pre-established pattern. Aquatic organisms to which the T1 (red) treatment was applied presented lower length and weight values compared to those of other treatments. On the other hand, organisms subjected to treatment T5 (purple) reached the highest lengths and weights, suggesting that this treatment could be associated with better development in terms of growth. Meanwhile, treatments T2, T3 and T4 show intermediate distributions, with some overlap between them, but with differentiated trends.

Fig. (2) shows a boxplot representing the distribution of the height of the organisms based on the five treatments applied. Each treatment showed variability in the box, whose median (line within the box), interquartile range (box) and extreme values (whiskers) were different. Colors were used to distinguish the treatments: T1 (red), T2 (blue), T3 (green), T4 (orange) and T5 (purple).

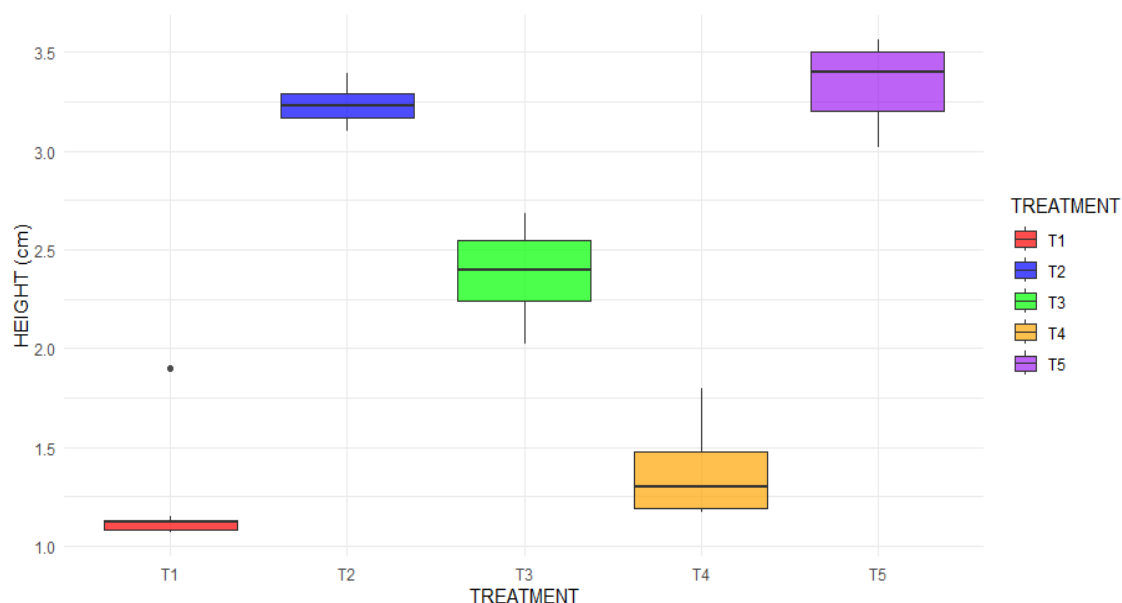


Fig. 2. Boxplot of height by treatment

T1 (red) showed the lowest heights, with a median close to 1.1cm and little dispersion, although with an outlier at 1.9cm. T2 and T5 showed the greatest heights, with medians around 3.2 and 3.4cm, respectively, and moderate dispersion. T3 had an intermediate median of approximately 2.4cm, with a wider range between 2.2 and 2.68cm. T4 had low values with a median of 1.3cm and greater variability than T1, reaching a maximum of 1.8cm.

Regarding growth, treatments T2 and T5 favored a superior development in height, with higher and more consistent values. In contrast, T1 and T4 showed lower growth, with T4 displaying a greater dispersion compared to T1. T3 was in an intermediate position, with a balanced distribution and less dispersed values.

Fig. (3) illustrates the scatter plot showing the relationship between opercular perimeter and weight of fish subjected to the five treatments. Each point represents an individual, and the treatments are differentiated by specific colors and shapes: T1 (red, circles), T2 (blue, triangles), T3 (green, squares), T4 (brown, circles) and T5 (purple, triangles). A general growth trend is observed, in which the increase in opercular perimeter is directly related to an increase in weight, suggesting a positive correlation between both variables.

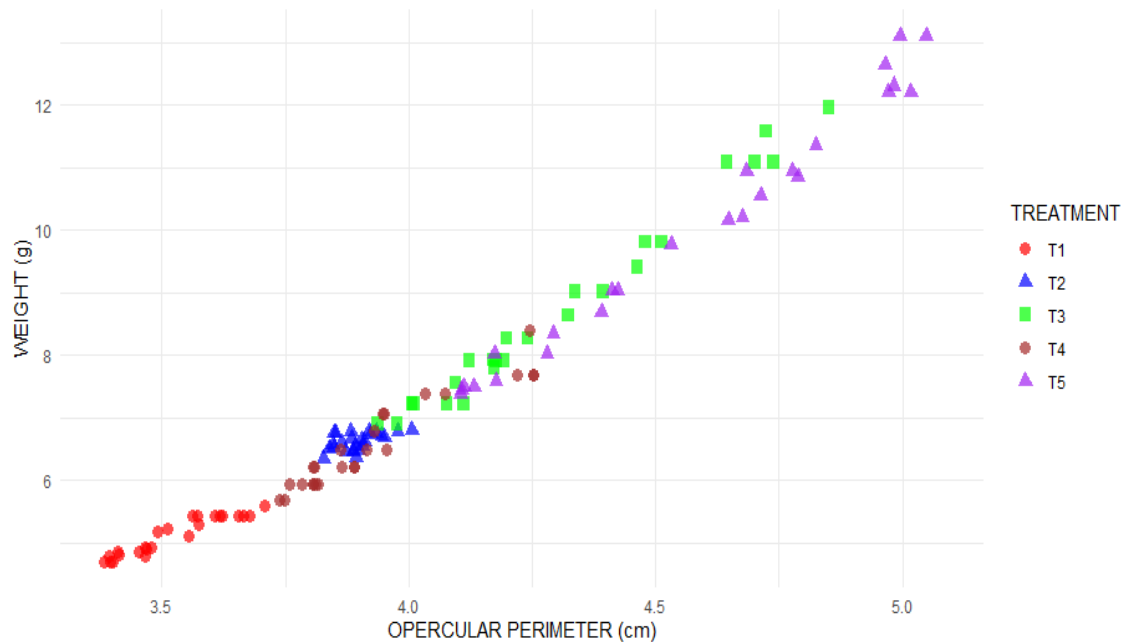


Fig. 3. Scatter plot of weight-opercular circumference ratio

Unlike the other analyses, this graph shows a more compact grouping in the initial treatments (T1 and T2), indicating that under these conditions the organisms show less variability in their opercular dimensions and weight. However, as one progresses through the treatments (T3, T4 and T5), a greater dispersion is observed, which could reflect a differentiated response to the treatment in terms of growth. In particular, T5, whose principle was based on *Bachillus* sp. and *Enterococcus* sp. showed the highest values in both variables, suggesting that this treatment favored a greater development of the organisms compared to the others.

In addition, T1 and T4 presented a greater overlap of points in the lower part of the graph, indicating that the organisms in these groups have had similar growth in their early stages. However, as opercular perimeter increased, the distribution of treatments became more dispersed, differentiating the groups with better development. This could be related to differences in the physiological response of the organisms to the treatments applied, showing that certain groups reach a higher weight with a larger opercular perimeter.

In general, the results showed that probiotic supplementation has a positive effect on fish growth, although with differences depending on the type of probiotic used. Treatment T5 emerged as the most effective, possibly due to a higher concentration of beneficial bacteria or its ability to improve feed conversion. Further studies could focus on the evaluation of physiological parameters, such as enzymatic efficiency and gut microbiota composition, to better understand the mechanisms behind the differential impact of probiotics on fish growth.

Raslan et al. (2025) reported improved growth performance of the Nile tilapia when fed a basal diet supplemented with probiotics, particularly *Lactobacillus plantarum*. Previous studies have also documented increased growth rates and feed efficiency in fish fed probiotics. Consistent with these indications, our results revealed that the addition of dietary probiotics, specifically the formula combining *Bacillus* sp., *Enterococcus* sp., *Pediococcus* sp., *Thiobacillus* sp., *Paracoccus* sp. and pure enzymes, significantly improved the growth performance of the Nile tilapia, as indicated by weight-length, opercular weight-perimeter and height relationships.

Previously, **Shija et al. (2025)** demonstrated that supplementation with a *Bacillus* strain, specifically *Bacillus amyloliquefaciens* AV5, in the diet of the Nile tilapia positively influenced multiple physiological and biochemical parameters, with more pronounced effects at the highest concentration (G2). Improved growth and survival at G2 suggested increased efficiency in nutrient assimilation, reflected in reduced feed conversion ratio and increased intestinal somatic and hepatosomatic index. In addition, higher enzyme activity in G2, with elevated alkaline and acid phosphatase levels, supported better digestive and immune function, while reduced alanine aminotransferase and aspartate aminotransferase values in this group indicated lower hepatic stress. At the microbiological level, the increased presence of *Bacteroidia* and *Bacillus* in G1 and G2 indicated a possible positive modulation of the intestinal microbiota, favoring the competitive exclusion of potentially pathogenic microorganisms.

2. Proximal water quality analysis

The fish showed a constant growth during the experiment, with no losses reported. The water quality in each aquaculture system, described in Table (2), was analyzed following the reference values of **Boyd and Massaut (1999)**.

Table 2. Water quality parameters in experimental treatments

Treatments	Parameters						
	OD (mg/L)	DBO (mg/L)	TAN (mg/L)	CE (μScm^{-1})	TDS (mg/L)	NH ₃ (ppm)	NO ₂ ⁻ (ppm)
T1	4.71 ± 0.11	12.43 ± 0.95	1.38 ± 0.08	163.19 ± 1.12	103.11 ± 1.23	0.34 ± 0.06	0.09 ± 0.03
T2	4.89 ± 0.62	11.13 ± 0.81	1.21 ± 0.09	164.98 ± 0.92	106.18 ± 0.63	0.29 ± 0.04	0.09 ± 0.02
T3	5.42 ± 0.45	9.12 ± 0.20	0.99 ± 0.11	175.62 ± 0.81	113.72 ± 0.96	0.22 ± 0.03	0.04 ± 0.02
T4	5.15 ± 0.12	9.67 ± 0.11	1.17 ± 0.13	169.51 ± 0.71	108.13 ± 0.32	0.28 ± 0.07	0.04 ± 0.03
T5	5.38 ± 0.38	9.51 ± 0.18	0.91 ± 0.13	173.55 ± 0.75	109.72 ± 0.34	0.22 ± 0.03	0.04 ± 0.03

The water quality of the different aquaculture treatments presented parameters within acceptable limits. It was observed that dissolved oxygen levels were significantly higher in the systems in which the T3 probiotic was applied, followed by the use of the T5 probiotic. This finding suggests that the addition of the microorganisms that compose these probiotics favor the preservation of water quality.

On the other hand, the biochemical oxygen demand reached its lowest value at T3 (9.12 ± 0.20), indicating a lower concentration of biodegradable organic matter in the medium, possibly attributed to a higher consumption of the probiotics by the sent aquatic organisms. In contrast, T1 group, where no growth promoter was applied, recorded the highest BOD (12.43 ± 0.95), suggesting an accumulation of unprocessed organic matter.

Total ammonia nitrogen (TAN) concentration was recorded with the lowest values at T5 ($0.91 \pm 0.13\text{mg/L}$), indicating a higher assimilation of nitrogen by the microbial biomass in this system. On the other hand, T1 had the highest values, with a maximum peak of $1.17 \pm 0.08\text{mg/L}$.

The use of probiotics in aquaculture has generated a growing interest due to their potential to preserve the health and welfare of various aquatic species, while contributing to the reduction of the overuse of antibiotics and synthetic drugs. Among the microbial options available, *Bacillus* strains, whose material is the basis of T4 and T5 treatments, stand out in the literature for their ability to produce compounds with antimicrobial activity, lack toxicity or pathogenicity in fish and form spores, which allows them to resist adverse environmental conditions (**Kewcharoen & Srisapoome, 2019**). In addition, they exhibit quorum sensing inhibition properties, which positions them as a superior probiotic alternative compared to other microorganisms (**Ghosh, 2025**).

Electrical conductivity, used as an indicator of the concentration of dissolved ions and the degree of mineralization in aquaculture systems, was lower in the control treatment ($163.19 \pm 1.12\mu\text{S/cm}$), while treatment T3 recorded the highest value (175.62 ± 0.81). This result indicates a higher accumulation of metabolites and by-products derived from microbial activity, induced by the newly introduced microorganisms.

Finally, nitrite concentrations remained low in all treatments, with the lowest values observed in T3 ($0.04 \pm 0.02\text{ppm}$) and T5 ($0.04 \pm 0.03\text{ppm}$), predisposing to efficient nitrification.

The determination of ideal conditions that enhance the bioremediation action of probiotics in aquaculture sources has been the subject of previous research. **Li and Liu (2024)** explored the potential of *Candida* sp. SW4-6 to reduce nitrite-N and ammonia-N in shrimp ponds. The *Candida* sp. SW4-6 strain showed superior ability to reduce the harmful compounds, achieving complete removal with sucrose as carbon source within a C/N ratio of 15-20. The gene expression study indicated that nitrite reductase was positively regulated after inoculation, with higher expression in the presence of sucrose. In addition, the inevitable influence of parameters such as salinity and temperature on the efficiency of the reduction process was identified.

The analysis of the evaluated treatments revealed that the use of the probiotic T5 promoted fish biometric development to a greater extent, attributed to better feed conversion and more efficient growth compared to the other treatments. However, T3 showed a more favorable effect on the water quality of the aquaculture system, reflected in a greater stability of physicochemical parameters. This differentiation may be due to the bioremediation activity of the microorganisms present in T3, which could favor the degradation of nitrogen compounds or establish more efficient interactions with the microbiota of the system. Thus, while T5 optimized fish growth, T3 improved the sustainability of the aquatic environment, suggesting that probiotic selection should balance both factors.

CONCLUSION

The use of probiotic T5 promoted superior growth in *Andinoacara rivulatus*, reflected in higher indices of weight, length and opercular perimeter compared to the other treatments. This superior zootechnical performance can be attributed to higher feed conversion efficiency and better nutrient assimilation facilitated by the beneficial microbiota present in this probiotic.

Treatment T3 proved to be the most efficient in preserving water quality within the aquaculture system by reducing TAN and nitrite-N concentrations, as well as minimizing biochemical oxygen demand. Consequently, T3 emerges as an ideal strategy to maintain optimal environmental conditions in *Andinoacara rivulatus* culture systems.

The probiotic that showed the highest efficacy in optimizing the biometric ratios of *Andinoacara rivulatus* hatchlings did not coincide with the one that guaranteed the best water quality conditions in aquaculture systems. This indication highlights the relevance of selecting probiotics considering not only the well-being and development of aquatic organisms but also the sustainability and stability of the aquaculture ecosystem.

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