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ARTICLE

RESEARCH ARTICLE

Influence of dietary protein levels and biofloc system on the nursery performance of *Macrobrachium rosenbergii*

Los efectos de los niveles de proteína en la dieta y la tecnología biofloc en la fase de cría del langostino gigante de agua dulce *Macrobrachium rosenbergii*

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Abstract: *Aim of study:* To assess the performance of the giant freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879) during the nursery phase under different culture systems (water exchange - WES and biofloc - BFS) and varying crude protein levels, using high-density culture conditions (2,500 post-larvae m^{-3}). *Area of study;* This study was conducted at the Federal Rural University of Pernambuco, in Brazil. *Material and methods:* The experimental design followed a 2 x 2 factorial arrangement, in which the initial variable corresponded to the type of rearing system, while the second one referred to the dietary crude protein content (35% and 40%), yielding in the combinations WES 35, WES 40, BFS 35, and BFS 40. *Main results:* The results demonstrated that the BFS 35 combination exhibited the lowest basic variable production cost and a substantial reduction in water footprint, ten times lower than the WES combinations. Furthermore, the BFS 35 system achieved a notable improvement in net benefits (2,876 USD 1000 m^{-3}), which was approximately twice that of the WES 35 (1,441 USD 1000 m^{-3}) and nearly three times that of the WES 40 (1,006 USD 1000 m^{-3}). *Research highlights:* The BFS 35 system can significantly reduce both the water footprint and the crude protein content in commercial feed, while simultaneously enhancing economic returns. These results suggest that the biofloc system with a 35% protein level is a viable and more sustainable option for the nursery phase of *M. rosenbergii*, offering a promising alternative to traditional systems.

Keywords: culture systems; high stocking density; production cost reduction; productive parameters; water saving.

Resumen: *Objetivo del estudio:* Se evaluó el rendimiento del langostino gigante de agua dulce *Macrobrachium rosenbergii* (De Man, 1879) durante la fase de cría en diferentes sistemas de cultivo (sistema de renovacion de agua - WES y sistema de biofloc - BFS) y variando los niveles de proteína bruta, utilizando condiciones de cultivo de alta densidad (2.500 postlarvas por m^{-3}). *Área del estudio*: Se llevó a cabo en Federal Rural University of Pernambuco, in Brazil. *Material y métodos:* El diseño experimental adoptado fue un arreglo factorial (2 x 2), donde el primer factor fue el tipo de sistema de cultivo, y el segundo fue el nivel de proteína del alimento (35% y 40%), resultando en las combinaciones WES 35, WES 40, BFS 35, y BFS 40. *Resultados principales:* Los resultados demostraron que la combinación BFS 35 presentaba el coste variable básico de producción más bajo y una reducción sustancial de la huella hídrica, diez veces inferior a la de las combinaciones WES. Además, el BFS 35 logró una mejora notable en los beneficios netos (2.876 USD 1000 m^{-3}), lo que representó aproximadamente el doble que el WES 35 (1.441 USD 1000 m^{-3}) y casi el triple que el WES 40 (1.006 USD 1000 m^{-3}). *Aspectos destacados de la invesitgación:* Por lo tanto, concluimos que el BFS 35 en la fase de vivero de *M. rosenbergii* puede reducir significativamente tanto la huella hídrica como el contenido de proteína bruta en los piensos comerciales, al tiempo que mejora los beneficios económicos. Estos resultados sugieren que el sistema biofloc con un nivel de proteína del 35% es una opción viable y más sostenible para la fase de vivero de *M. rosenbergii*, ofreciendo una alternativa prometedora a los sistemas tradicionales.

Palabras clave: ahorro de agua; alta densidad de población; parámetros productivos; sistemas de cultivo; reducción de costes de producción.

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Supplementary information ↓

1. INTRODUCTION

Since the 1990s, freshwater prawn farming has experienced substancial expansion, with the giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879), being the most commercially significant species. This is due to well-managed breeding techniques, high fecundity rate, inert feed adaptability, high disease resistance, and meat quality (Lobão, 1996; Gupta *et al.*, 2007; Mohanty, 2010; New *et al.*, 2010). However, despite all the benefits, heterogeneous individual growth is a major concern that hampers the profitability of farming the giant freshwater prawn (Karplus & Barki, 2019). To minimize the effects of body size heterogeneity on aggressive behavior and survival, production is typically structured into three phases: hatchery, nursery, and grow-out. The hatchery phase focuses in the initial larval development until metamorphosis into post-larvae. The nursery phase can be further subdivided into nursery I, covering the first 15 days after metamorphosis, and nursery II, lasting up to 60 days post-metamorphosis. However, nursery II may be omitted if post-larvae are directly stocked into ponds for the grow-out phase (Valenti & Flickinger, 2020).

Ensuring high productivity and maintaining optimal rearing conditions for this species requires frequent water exchange, either partial or total, which results in a substancial volume of effluents rich in nutrients and organic matter being discharged into natural ecosystems (Naranjo *et al.*, 2012). Thus, alternative systems that can substantially reduce the water exchange in aquaculture, such as the biofloc system (Fróes *et al.*, 2013), are useful for developing sustainable production. Biofloc system presents a potencial solution to this issue and has emerged due to its advantages over conventional clear water systems, including the ability to enhance stocking densities and minimize the water footprint through water reuse and nutrients recycling (Avnimelech, 2009; Felix *et al.*, 2020). These benefits are primarily derived from the metabolic activities of chemoautotrophic and heterotrophic bacteria. Chemoautotrophs oxidize nitrogenous compounds, such as ammonia and nitrite into nitrate, thereby mitigating nitrogen toxicity in the system. Simultaneously, under conditions of sufficient organic carbon availability, heterotrophic bacteria assimilate inorganic nitrogen, particularly ammonia, into microbial biomass through biosynthetic pathways, effectively contributing to nitrogen removal and enhanced water quality control (El-Sayed, 2021; Hosain *et al.*, 2021). The macro aggregates develop (biofloc) can act as an additional source of natural nutrition for fish and crustaceans and may contain high protein levels in their dry matter (30% to 45%), depending on the specific conditions of the applied system (Avnimelech, 2009; Felix *et al.*, 2020).

Utilizing biofloc as protein-rich live feed contributes to lowering production expenses, as feeding during the nursery phase typically relies on commercial formulated diets containing between 36% and 40% crude protein (Marques & Moraes-Valenti, 2012). Consequently, incorporating biofloc as a dietary component can help mitigate the substantial expenses related to feed, which may account for up to half of the overall production costs (Felix *et al.*, 2020).

Nonetheless, the viability of giant freshwater prawn farming relies not only on its environmental impact but also on its economic feasibility, which can be improved by increasing stocking density-a particularly challenging task for this species due to its aggressive behavior linked to competition for food and territory (Silva & Arruda, 2015). This study aimed to assess two culture systems (water exchangeand biofloc and different crude protein levels (35% and 40%), under high stocking densities for *M. rosenbergii* juveniles during the nursery phase to determine the most cost effective and environmentally sustainable approach.

2. MATERIAL AND METHODS

2.1. Experimental conditions

The experiment was carried out at the Aquaculture Station of the Federal Rural University of Pernambuco (UFRPE) over a 21-day period, following a 2 x 2 factorial design. The study evaluated a water exchange system (WES) and a biofloc system (BFS), along with different crude protein contents in the commercial feed (35% and 40%), resulting in the treatment groups WES 35, WES 40, BFS 35, and BFS 40, each with four replicates. The 16 experimental setups comprised rectangular tanks with a working volume of 40 liters (0.2 m^2), thermostats set at 27 ˚C, and constant aeration, all covered with mesh to prevent animal escape. A rectangular polyethylene screen (0.2 x 0.05 m) was included as a substrate and shelter, increasing the available surface area by 5% in all experimental units.

For the WES, each experimental unit received freshwater that had been previously chlorinated (10 ppm), dechlorinated with ascorbic acid, and subjected to a 50% water exchange every three days (Braga *et al.*, 2023). The BFS underwent a 40-day maturation period prior to the experiment, following the methodology described by Felix *et al.* (2020), with 30 L (75%) of matured biofloc added to each unit along with 10 L (25%) of freshwater, maintaining settleable solids at 9 mL L^{-1}. If this threshold was exceeded, settling chambers were introduced to regulate the concentration of suspended solids. The quantification of settleable solids was performed using Imhoff cones (Avnimelech, 2009). To compensate for water losses due to evaporation, weekly replenishments were conducted, along with the addition of organic sugarcane molasses as a carbon source whenever total ammonia nitrogen surpassed 0.8 mg L^{-1} in the BFS, ensuring a stable C:N ratio of 6:1. The quantity of carbohydrate (wet weight) required to achieve this ratio in each experimental unit was calculated using the equation:

$$\Delta \text{ carbohydrate} = \left[(\Delta \text{N} \ \times \text{C/N}) \times \%\text{C}^{-1}\right]$$

where ΔN corresponds to the amount of nitrogen present in the system, and % C^{-1} indicates the proportion of organic carbon in the carbon source, 0.37 in the case of molasses.

2.2. Prawn stocking and feed management

Post-larvae of *M. rosenbergii* (PL10, 10 days post-metamorphosis – with a mean weight of 0.01g) were sourced from a commercial hatchery, Acquamarão, located in Goiana, Pernambuco, Brazil. The post-larvae underwent a two-week acclimation period in five fiberglass tanks (0.8 m^3) at a stocking density of 1 PL L^{-1} using freshwater that had been previously chlorinated (10 ppm of active chlorine) and dechlorinated, with strong and continuous aeration for 24 hours. A 50% water exchange was performed every three days to keep total ammonia nitrogen and nitrite nitrogen within optimal levels for rearing.

During this acclimated phase, post-larvae were provided with a commercial shrimp feed containing 45% crude protein and 9.5% lipids (0.6 and 0.8 mm, Wean, ADM Animal Nutrition Company, Brazil). Feeding initially followed an 11% daily ratio, distributed in four meals per day (8 a.m., 11 a.m., 2 p.m., and 5 p.m.) which was gradually reduced to 9% of body weight. Throughout the experimental period, prawns were fed four times daily (8 a.m., 11 a.m., 2 p.m., and 5 p.m.) using commercial diets with 40% crude protein and 9.0% lipids (WES 40 and BFS 40) or 35% crude protein and 7.5% lipids (WES 35 and BFS 35) (Density 40 CR2, Camanutri 35J, ADM Animal Nutrition Company, Brazil). These feeds were chosen based on market availability and practical applicability, allowing for the identification of the most cost-effective solution. Initially, a 30% daily feeding rate was implemented, which was progressively reduced to 9% of biomass. Following acclimation, post-larvae were counted, weighed (0.08 ± 0.01g), and stocked in each experimental unit at a density of 2,500 PL m^{-3}, corresponding to 100 post-larvae per unit.

2.3. Water quality and water footprint

To monitor water quality, parameters such as temperature (˚C), pH, and dissolved oxygen (mg L^{-1}) were measured twice daily (9 a.m. and 4 p.m.), using a multiparameter AK88 probe (AKSO, Rio Grande do Sul, Brazil). Weekly water samples were taken from each experimental unit to analyze total ammonia nitrogen (TAN mg L^{-1}; Vanselow, 1940), nitrite nitrogen (N-NO_2 mg L^{-1}; Strickland and Parsons, 1972), nitrate nitrogen (N-NO_3 mg L^{-1}; Strickland & Parsons, 1972), orthophosphate (PO_4^{3-} mg L^{-1}; Aminot and Chaussepied, 1983), total alkalinity ($CaCO_3$ mg L^{-1}; APHA, 1995; which was adjusted with sodium bicarbonate to maintain levels close to 150 mg L^{-1} $CaCO_3$, Felix, *et al.*, 2020), total hardness (mg L^{-1} $CaCO_3$; APHA, 1995), and settleable solids (mL L^{-1}; Avnimelech, 2009).

During the experimental period, the total water consumption was recorded (m^3), and the water footprint was determined following the methodology described by Braga *et al.* (2023). The water footprint was calculated as the ratio between the total water used and the final biomass produced.

2.4. Feed and Biofloc Proximate Composition

The commercial feed used in the experiment, along with the final biofloc samples (collected from the surface and subsequently filtered using a 50 µm mesh to concentrate the flocs), underwent ingredient composition analysis following the official AOAC (2000) methodology (Table 1). Crude protein content was determined using the Kjeldahl method (N x 6.25), while lipid extraction was performed using the Soxhlet method. Moisture content was assessed through gravimetric analysis by drying samples in an oven with air circulation at 105 °C until constant weight was reached, followed by incineration at 550 °C in a muffle furnace for 5 hours for ash content determination.

Table 1. Proximate composition (% dry matter) of the biofloc and feed used in the experiment with *Macrobrachium rosenbergii*

	Biofloc		**Feed**	
	35%	**40%**	**35%**	**40%**
Dry matter	19.17	16.19	87.37	86.88
Ash	31.57	25.73	14.18	11.49
Crude protein	35.46	40.24	35.97	40.91
Ether extract	4.04	4.10	9.50	10.33

2.5. Prawn performance

Feeding was adjusted weekly based on biomass measurements, following biometric assessments, and initially set at 30% of the total biomass (Ballester *et al.*, 2017). During harvesting, post-larvae were weighed using a digital scale (BL3200H, Shimadzu, Kyoto – Japan of 0.01g precision) and counted to determine performance parameters, such as:

mean final weight (g) = final biomass (g) / final number of individuals;

weight gain (g): WG = (final weight – initial weight);

specific growth rate (% day^{-1}): SGR = [100* (ln final weight – ln initial weight) / culture time)]

feed conversion ratio: FCR = (amount of feed supplied / biomass gain)

survival (%): S = [100*(final population / initial population)]

productivity (g m^{-3}): P = (final biomass / volume of the experimental unit)

2.6. Basic variable production costs in the prawn nursery

To assess the primary variable costs of production during this experiment, partial expenses and net benefit (gross income – inputs) were recorded throughout the 21-day trial period (Laine *et al.*, 2024): the amounts of sodium bicarbonate (0.96 USD kg^{-1}) used for alkalinity correction, molasses (1.0 USD kg^{-1}) used for nitrogen control, post-larvae acquisition price (28.96 USD by 1,000 PL), feed supplied (0.96 USD kg^{-1} – 35% crude protein and 1.17 USD kg^{-1} – 40% crude protein), a sale price of juvenile prawns (35.13 USD by 1,000 prawn), considering 1,000 m^2 x 1 m = 1,000 m^3. In this evaluation, electric equipment was not taken into account.

The total energy consumption required to ensure adequate oxygen levels for each tank was estimated by summing the energy needed to meet the oxygen demand of both bacteria and prawns, as well as that required for water exchange throughout the experiment. The average oxygen demand of bacteria in the tanks (g O_2 bacteria) was calculated based on the oxygen requirements of chemoautotrophic and heterotrophic bacteria necessary for the removal of one gram of TAN (Ebeling *et al.*, 2006) as:

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$$\text{kg } O_2 \text{ bacteria} = \text{Feed supply tank} \times \text{C.P} \times 0.16 \times 0.75 \times 4.18 \text{ (chemoautotrophic)}$$

$$\text{kg } O_2 \text{ bacteria} = \text{Feed supply tank} \times \text{C.P} \times 0.16 \times 0.75 \times 4.71 \text{ (heterotrophic)}$$

where:

4.18 and 4.71 – amount in g O_2 necessary to remove 1 g of TAN.

C.P – crude protein.

0.16 – amount of N in the crude protein.

0.75 – On average 75% of the total feed-N ends up in the water (ammonification of unused feed + excretion by culture species) (Piedrahita, 2003).

Next, the O_2 required for the prawn biomass in the tank (g O_2 prawn) for the entire period of the experiment was estimated as follows:

$$\text{kg } O_2 \text{ prawn} = \text{APB kg} \times 0.4 \text{ kg } O_2/\text{ h/kg} \times 24 \text{ h} \times 21 \text{ d}$$

where:

APB is the average prawn biomass per tank in kg.

0.4 kg is the average O_2 consumption per hour per kg of prawn (Boyd, 1990)

Thus, the overall oxygen requirement (kg O_2 total) is kg O_2 total = kg O_2 bacteria + kg O_2 prawn.

Following the methodology proposed by Boyd (1998), the total oxygen demand (g O_2 total) was used to estimate the required energy consumption (Kwh). Initially, this was calculated based on the standard aeration efficiency (SAE) and subsequently adjusted using the oxygen transfer rate (OTR):

Kwh SAE = Kg O_2 total / 2.2, where 2.2 represents the average kg of O_2 SAE for *paddle wheels* typically used on ponds and Kwh OTR = Kwh SAE / 0.40, where 0.40 is a correction factor applied to conditions similar to those in the experiment, assuming a minimum dissolved oxygen level of 5 mg L^{-1} and a water temperature close to 27 °C.

$$\text{Cost aeration} = \text{Kwh OTR} \times 0.13\text{USD}$$

The pumping cost for water exchange in WES considered a water exchange of 50% of the tank volume per day for 8 hours (estimate per 1000 m^3 – 83.34 m^3 / hour, 4 CV = 2.94 kw), while in BFS a replacement of evaporation losses 15 m^3 / day / 2 hours (7.5 m^3 / hour, 0.5 CV = 0.37 kw) was assumed.

$$\text{Pumping cost WES} = 11.03 \times 8\text{h} \times 21 \text{ days} \times 0.13 \text{ USD}$$

$$\text{Pumping cost BFS} = 2.20 \text{ kw} \times 8\text{h} \times 21 \text{ days} \times 0.13 \text{ USD}$$

The cost of energy for aeration was set at USD 0.13 per Kwh, with an exchange rate of BRL 1.00 = USD 5.18. Additionally, the water volume utilized for water exchanges in the WES and losses due to evaporation in the BFS was recorded.

2.7. Statistical analyses

The data were tested for normality using the Shapiro-Wilk test and for homogeneity of variances with the Levene test. Subsequently, water parameters data were analyzed using repeated measures variance analysis, while performance metrics and basic variable production costs were assessed through two-factor variance analysis. When significant differences ($P < 0.05$) were detected, the Tukey test was applied for multiple comparisons.

For non-parametric data, the Kruskal-Wallis test was used to assess weight gain and survival. When significant differences were found, Dunn's post hoc test with Holm-Bonferroni correction was applied.

The Friedman test was conducted for orthophosphate, total ammonia nitrogen, and nitrite, followed by Conover's multiple comparison test with Holm-Bonferroni. All analyses were performed at a 5% significance level using Statistica 12.5 software (StatSoft, 2014).

3. RESULTS

3.1. Water quality parameter and system performance

The water quality parameters are presented in Table 2. No significant differences were observed among the treatments for temperature, dissolved oxygen, and pH throughout the experiment, as these variables were not significantly affected by the rearing systems or crude protein levels, or their interactions (Table 2). However, regarding the nitrogenous compounds, TAN and N-NO_3 concentrations were higher in the BFS compared to the WES, regardless of the crude protein levels. At the same time, N-NO_2 concentration was significantly higher in the WES, with no significant effect ($P > 0.05$) of the protein level.

Table 2. Water quality variables of juvenile *Macrobrachium rosenbergii* reared in different culture systems (water exchange system - WES and biofloc system - BFS) and crude protein levels

Water quality variables	Combinations				Interaction		
	WES 35	WES 40	BFS 35	BFS 40	Culture system	Crude protein	CS x CP
Temperature (°C)	27.29 ± 0.94	27.82 ± 0.83	27.86 ± 0.72	28.04 ± 0.74	ns	ns	ns
Dissolved oxygen (mg L^{-1})	6.16 ± 0.19	6.05 ± 0.18	6.19 ± 0.15	6.10 ± 0.19	ns	ns	ns
pH	8.15 ± 0.22	7.97 ± 0.10	8.13 ± 0.05	8.07 ± 0.06	ns	ns	ns
Total ammonia-N (mg L^{-1})	0.40 ± 0.23^{b}	0.49 ± 0.36^{b}	1.39 ± 0.54^{a}	1.39 ± 0.54^{a}	*	ns	ns
Nitrite-N (mg L^{-1})	0.16 ± 0.17^{a}	0.16 ± 0.23^{a}	0.04 ± 0.04^{b}	0.03 ± 0.02^{b}	*	ns	ns
Nitrate-N (mg L^{-1})	4.45 ± 2.74^{b}	4.49 ± 2.70^{b}	12.47 ± 7.21^{a}	11.41 ± 8.71^{a}	*	ns	ns
Total alkalinity (mg $CaCO_3$ L^{-1})	122.87 ± 46.49^{b}	126.75 ± 37.99^{b}	145.00 ± 40.79^{a}	150.00 ± 25.11^{a}	*	ns	ns
Total hardness (mg $CaCO_3$ L^{-1})	37.66 ± 10.43^{b}	40.98 ± 17.32^{b}	62.19 ± 16.75^{a}	64.96 ± 12.15^{a}	*	ns	ns
Orthophosphate (mg L^{-1})	0.50 ± 0.21^{b}	0.70 ± 0.63^{b}	6.60 ± 0.92^{a}	7.41 ± 0.95^{a}	*	*	ns
Settleable solids (mL L^{-1})	-	-	14.91 ± 2.11^{a}	15.84 ± 0.99^{a}	-	-	-

Results are averages of four replicates per combination ± standard deviation. Mean values of horizontal lines with different exponents differed significantly ($P < 0.05$). Water exchange system with 35% feed (WES 35); water exchange system with 40% feed (WES 40); biofloc system with 35% feed (BFS 35); biofloc system with 40% feed (BFS 40); culture system (CS); crude protein (CP); Ns - there was no significant effect of factors; * Effect of the factor at 5% significance level.

During the final week of the experiments, sodium bicarbonate was added for total alkalinity adjustments in the different culture systems, with a total of 18.00g, 19.60g, 39.00g, and 70.00g required for BFS 35, BFS 40, WES 35, and WES 40, respectively. The total hardness results indicated that the water used in the study was classified as soft, with low concentrations of calcium and magnesium ions. Settleable solids remained below the recommended threshold of 20 mL L^{-1}. Orthophosphate concentrations exhibited significant differences ($P < 0.05$) between culture systems and crude protein levels, with the BFS combinations presenting higher values.

By the end of the experiment, approximately 120 L (0.12 m^3) of pre-treated freshwater had been used to compensate for evaporation losses in all experimental units of the BFS, whereas approximately 1,120 L

(1.12 m^3) had been utilized for periodic water exchange in the WES tanks. The calculated water footprint values corresponded to 0.17 m^3 kg^{-1} for BFS and 1.76 m^3 kg^{-1} for WES. These values were significantly influenced by the culture systems, crude protein levels, and their interactions ($P < 0.05$) (Figure 1).

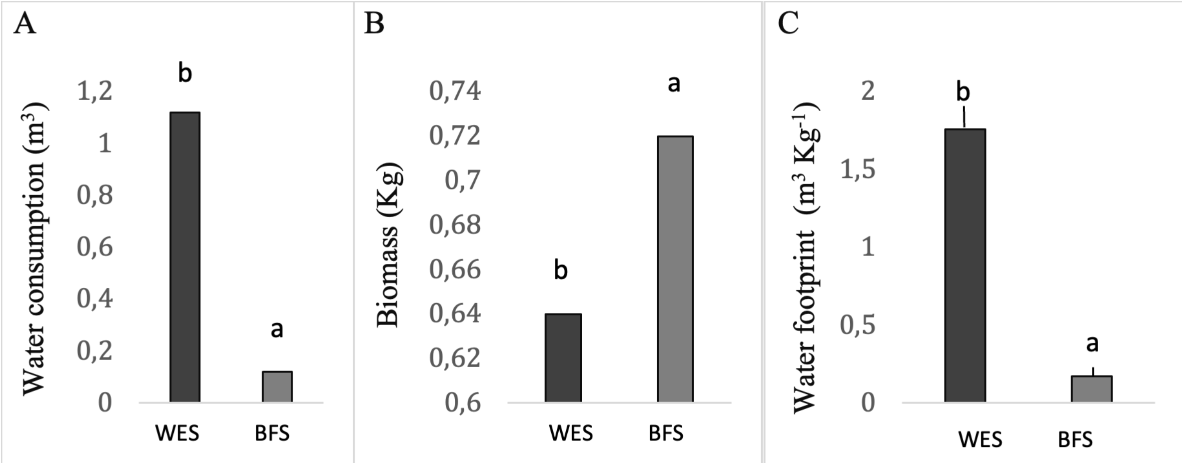

Figure 1. (A) Water consumption (m^3), (B) biomass (Kg), and (C) water footprint (m^3 Kg^{-1}) for the nursery (21 days) of *Macrobrachium rosenbergii* culture in the water exchange system (WES) and biofloc (BFS) system.

3.2. Prawn performance

The performance parameters of the prawns are presented in Table 3. No significant differences ($P >$ 0.05) were observed among the experimental combinations for final weight (g), weight gain (g), specific growth rate (% day^{-1}), feed conversion ratio, survival (%), and productivity (g m^{-3}). Additionally, none of these parameters were significantly affected by the culture system, the crude protein level, or the interaction between these factors ($P > 0.05$).

Table 3. Performance of *Macrobrachium rosenbergii* juveniles reared in different culture systems (water exchange system - WES and biofloc system - BFS) and crude protein levels

Performance variables	Combinations				Interaction		
	WES 35	WES 40	BFS 35	BFS 40	Culture system	Crude protein	CS x CP
Initial weight (g)	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.02	0.08 ± 0.02	ns	ns	ns
Final weight (g)	0.13 ± 0.01	0.14 ± 0.02	0.14 ± 0.01	0.16 ± 0.01	ns	ns	ns
Weight gain (g)	0.06 ± 0.04	0.04 ± 0.01	0.05 ± 0.00	0.06 ± 0.01	ns	ns	ns
Specific growth rate (% day^{-1})	1.06 ± 0.30	1.49 ± 0.73	1.37 ± 0.30	1.96 ± 0.40	ns	ns	ns
FCR	1.39 ± 0.13	1.41 ± 0.39	1.24 ± 0.10	1.32 ± 0.02	ns	ns	ns
Survival (%)	95.00 ± 1.83	96.25 ± 5.56	98.75 ± 2.50	97.75 ± 1.50	ns	ns	ns
Productivity (g m^{-3})	300.69 ± 27.86	342.62 ± 55.99	339.37 ± 24.01	384.56 ± 31.82	ns	ns	ns

Results are means of four replicates per combination ± standard deviation. Water exchange system with 35% feed (WES 35); water exchange system with 40% feed (WES 40); biofloc system with 35% feed (BFS 35); biofloc system with 40% feed (BFS 40); culture system (CS); crude protein (CP); ns - there was no significant effect of the factors; Feed Conversion Ratio (FCR).

3.3. Basic variable production costs

Regarding partial expenses, the BFS 35 exhibited a lower variable production cost, even with the additional requirement of sugar cane molasses to sustain the microbial floc, compared to the WES. The net benefit, calculated as the difference between income and partial expenses, indicated that BFS 35 (2,876 USD 1000 m^{-3}) yielded the highest economic return, followed by WES 35 (1,457 USD 1000 m^{-3}) (Table 4).

Table 4. Economics information of the 21-day experiment of *Macrobrachium rosenbergii* juveniles reared in different culture systems (water exchange system - WES and biofloc system - BFS) and fed different crude protein ratios

			WES 35	WES 40	BFS 35	BFS 40
INCOME	Number of prawns post larvae for stocking	n 1000 m $^{-3}$	2,500,000	2,500,000	2,500,000	2,500,000
	Survival	%	95.0	96.0	98.0	97.0
	Number of sold prawn juvenile	n 1000 m $^{-3}$	2,375,000	2,400,000	2,450,000	2,425,000
	Prawn weight at harvest	g prawn $^{-3}$	0.1300	0.1400	0.1400	0.1600
	Biomass - sold weight	kg 1000 m $^{-3}$	3,088	3,360	3,430	3,880
	Income	**USD 1000 m $^{-3}$**	**83,446**	**84,324**	**86,081**	**85,203**
EXPENSES	Feed intake					
	FCR		1.39	1.41	1.24	1.32
	Feed consume	kg	429	474	425	512
	Feed cost	**USD 1000 m $^{-3}$**	**412.00**	**454.81**	**497.62**	**599.23**
	Molasses intake					
	Molasses consume	Kg	0	0	126	177
	Molasses cost	**USD 1000 m $^{-3}$**	**0.00**	**0.00**	**126.00**	**177.00**
	Sodium Bicarbonate intake					
	Sodium Bicarbonate consume	Kg	173	191	65	64
	Sodium Bicarbonate cost	**USD 1000 m $^{-3}$**	**166.08**	**183.36**	**62.40**	**61.44**
	Postlarve intake					
	Postlarvae consume	**pl 1000 m $^{-3}$**	**2,500,000**	**2,500,000**	**2,500,000**	**2,500,000**
	Postlarvae cost	**USD**	**72,394**	**72,394**	**72,394**	**72,394**
	Aeration cost		8,969.00	10,222.00	10,123.00	11,473.00
	Pump cost		48	48	2	2
	Expenses total	**USD 1000 m $^{-3}$**	81,989	83,302	83,205	84,706
NET BENEFIT	**Net benefit (local currency)**	**USD 1000 m $^{-3}$**	**1,457**	**1,022**	**2,876**	**496**
	Comparison of net benefits					
	WES 35 x WES 40		435.00			
	WES 35 x BFS 35				1,419.00	
	WES 35 X BFS 40		960.83			
	WES 40 X BFS 35				1,854.00	
	WES 40 X BFS 40			526.00		
	BFS35 X BFS 40				2,380.00	

Values calculated in USD / ha (BRL R$ 1.00 = US $4.18); Economic parameters used: Feed cost = 0.96 USD / kg feed, prawn postlarvae cost = 29.00 USD / 1000 juveniles, prawn juveniles price = 38.00 USD / 1000 juveniles, molasses price = 4.83 USD / kg, sodium bicarbonate = 0.96 USD / kg, prawn postlarvae price = 29.00 USD / 1000 postlarvae. Net benefit = gross income – inputs; Comparison of net benefits: presented value means the combination with the highest economic benefit. Water exchange system with 35% feed (WES 35); Clear water exchange system with 40% feed (WES 40); Biofloc with 35% feed (BFS 35); Biofloc with 40% feed (BFS 40); Feed Conversion Ratio (FCR).

4. DISCUSSION

Pond aquaculture has a greater effect on local hydrology, primarily due to enriched nitrogen effluents, which pose a major environmental concern. Effective management strategies to maintain low concentrations of nitrogen compounds are essential for sustainable aquaculture practices. Closed aquaculture technologies, such as recirculation aquaculture systems (RAS), biofloc system (heterotrophic), and symbiotic and aquaponic systems, are alternatives to not only reduce nitrogen effluents but also to meet lower water discharge demand. Global aquaculture water consumption is estimated to be approximately 123 Km^3 (Jiang *et al.*, 2022). Therefore, assessing the water footprint is crucial for evaluating the environmental impact of various aquaculture technologies (Mohanty *et al.*, 2014). This study compared biofloc system and clear water system in terms of water footprint, prawn performance, and basic variable production costs during the nursery I phase of giant freshwater prawn culture. Although expensive, nursery systems have the advantage of evaluating PLs' quality through grading to guarantee that high-standard and uniform PLs will be stocked in grow-out ponds, which is usually associated with a higher survival rate in the final harvest (Quagrainie, 2015).

In this study, the BFS required about 10 times less water than the traditional WES one, which was also observed by studies that compared water recycling and biofloc culture technologies in *M. rosenbergii* culture (Braga *et al.*, 2023) and in tilapia (*Oreochromis niloticus*, Linnaeus, 1758) (Zapata *et al.*, 2017; Lima *et al.*, 2018; Lima *et al.*, 2019). Yet regarding nitrogen compounds, both systems were able to control the total nitrogen in levels considered safe for the freshwater giant prawn, with equivalent final expenses on the acquisition of inorganic (sodium bicarbonate) and organic (molasses) carbon sources.

Even though the biofloc constituted a feed protein source, as it had a proximal composition similar to that recommended by Marques & Moraes-Valenti (2012) for the nursery phase of the giant freshwater prawn, the specific growth rates (SGR) in this study were lower (1.37 - 1.96%) than those (between 0.01 g and 0.10 g and equivalent to SGRs of 5.58 and 6.47% day^{-1}) obtained by Santos *et al.* (2022b), using a stocking density of 263 PL m^{-2} of *M. rosenbergii* for 35 days in a system with clear water and biofloc. This suggests that the prawn performance results may be related to the high stocking density used (2,500 PL m^{-3}), which likely imposed a physiological limit on growth and may have masked the effects of diet or culture system.

The use of an appropriate feeding rate (30% total biomass) likely contributed to the lower FCR (1.24 - 1.41) observed in this study when compared to the values reported by Hosain *et al.* (2021) (2.21 - 4.77 using a feeding rate of 75% total biomass) and Frozza *et al.* (2021) (1.29 - 1.53 using a feeding rate of 7% total biomass), both of whom also used biofloc system and commercial shrimp feed during the nursery phase. Furthermore, maintaining optimum experimental conditions, including water quality, appropriate nursery duration, and increased surface area may have enhanced animal welfare and minimized competition. These factors contributed to the high survival rates, which were comparable to those reported by Santos *et al.* (2022a, 2022b), despite their use of stocking densities 10 times lower (0.8 - 1.0 PL m^{-3} equivalent to 210 - 252 PL m^{-2}) than in the present study, suggesting that higher densities may be feasible.

Despite the growing interest in diversifying aquaculture farming technologies, few studies have focused on evaluating the economic aspects of the nursery phase for giant freshwater prawns (Islam *et al.*, 2023). The implementation of alternative technologies should be accompanied by a cost-benefit analysis to determine investment feasibility and identify potential constraints. Overall, the BFS 35 proved to be economically superior to all other combinations, being almost twice as advantageous in terms of net benefit compared to the second-best option. Islam *et al.* (2023), when adapting traditional farming to the biofloc system, also observed this trend. Even with the added costs of molasses and aeration, BFS 35 outperformed all other combinations in terms of net return, reinforcing its financial viability.

Using feed with a 5% reduction in protein (35%) not only reduced feed costs, allowing a saving of 0.21 USD per kg compared to feed with a higher protein content (40%), but also minimized additional expenses associated with water exchange. These findings are consistent with the role of biofloc as a natural feed supplement that can reduce dependence on high-protein commercial diets, which are typically the most expensive component of aquaculture operations (Pérez-Fuentes *et al.*, 2013). The economic advantages of the BFS 35 could have been even greater if a species-specific feed had been used, as giant freshwater prawn farming currently relies on commercial shrimp feed that lacks appropriate nutrients (Ballester *et al.*, 2018).

5. CONCLUSIONS

The findings of this study indicate that the application of the biofloc system associated with a 35% crude protein diet (BFS 35) during the nursery phase of *M. rosenbergii* constitutes a technically and economically advantageous approach using high stocking densities, as reported by Ly *et al.* (2024). Although no significant differences were observed in the zootechnical performance among the treatments, the BFS 35 combination resulted in the lowest water footprint, approximately ten times lower than in clear water systems, and provided the highest net economic return.

These results suggest that the nutritional contribution of microbial flocs may partially compensate for reductions in dietary protein, enabling a decrease in feed costs without impairing growth or survival. Thus, the use of the BFS 35 strategy represents a feasible alternative to conventional systems, contributing to more sustainable and cost-efficient freshwater prawn production during the nursery phase. These results confirm the initial objective of identifying a cost-effective and environmentally sustainable combination of culture system and dietary protein level for the nursery phase of *M.rosenbergii*. The adoption of the BFS 35 strategy stands out as a promising technological solution for reducing production costs, preserving water resources, and supporting more resilient and sustainable freshwater prawn farming operations.

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Data availability

Data will be available on request.

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Authorship contribution statement

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Competing interests

The authors have declared that no competing interests exist.

Statement on the use of Artificial Intelligence

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