OPEN ACCESS

Spanish Journal of Agricultural Research
22 (3), e0606, 9 pages (2024)
ISSN-L: 1695-971X, eISSN: 2171-9292
https://doi.org/10.5424/sjar/2024223-20549

RESEARCH ARTICLE

Fish feeding rate affects the productive performance of whiteleg shrimp and lebranche mullet integrated culture using biofloc technology

Ana C. S. Pereira[1], Esmeralda C. Legarda[1], Mateus A. Martins[1]*, Claudia Machado[1], Caio C. F. Magnotti[2], Felipe N. Vieira[1] and Luis A. V. Arana[1]

[1] Laboratório de Camarões Marinhos, Departamento de Aquicultura, Universidade Federal de Santa Catarina, Rua dos Coroas, 503, CEP 88061-600, Barra da Lagoa, Florianópolis, Santa Catarina, Brazil. [2] Laboratório de Piscicultura Marinha, Departamento de Aquicultura, Universidade Federal de Santa Catarina, Rua dos Coroas, 503, CEP 88061-600, Barra da Lagoa, Florianópolis, Santa Catarina, Brazil.

***Correspondence** should be addressed to Mateus Aranha Martins: m.aranha.martins@gmail.com.

Abstract

Aim of study: To evaluate the effects of different fish feeding rates on the growth performance, water quality, and water microbiology in the integrated culture of lebranche mullet (*Mugil liza*) and whiteleg shrimp (*Penaeus vannamei*) using biofloc technology.

Area of study: Southern Brazil.

Material and methods: A 46-day experiment was performed to assess four feeding rates (0%, 1%, 2%, and 3% of mullet biomass) with four replicates per treatment. Shrimp were fed according to a feeding table. Eight-hundred L tanks were used for shrimp culture, while 90 L tanks were utilized for mullet culture, employing recirculation between the tanks through a submerged pump (Sarlo-Better 650 L hour^{-1}).

Main results: Water quality variables and water microbiology, evaluated through bacterial counts, were unaffected by the fish feeding rates ($p>0.05$). Regarding growth performance, while shrimp were unaffected ($p>0.05$), mullet final mean weight, biomass, daily growth coefficient, and yield significantly increased with higher fish feeding rates ($p<0.05$). Mullet feed conversion ratio and survival were not influenced by the feeding management ($p>0.05$). Furthermore, linear regression models for the overall system productivity showed a positive correlation with the fish feeding rate. As the fish feeding rate increased, the yield of the integrated culture system also increased ($p<0.05$).

Research highlights: These findings emphasize the importance of considering appropriate feeding rates to maximize the productivity and overall performance of integrated aquaculture systems using biofloc technology.

Additional keywords: BFT; growth performance; integrated multitrophic aquaculture; *Mugil liza*; *Penaeus vannamei*; water microbiology; water quality.

Abbreviations used: BFT (biofloc technology); CFU (colony forming units); IMTA (integrated multitrophic aquaculture); TAN (total ammonia nitrogen); TCBS (thiosulfate-citrate-bile salts-sucrose agar); TSA (trypticase soy agar); TSS (total suspended solids).

Citation: Pereira, ACS; Legarda, EC; Martins, MA; Machado, C; Magnotti, CCF; Vieira, FN; Arana, LAV (2024). Fish feeding rate affects the productive performance of whiteleg shrimp and lebranche mullet integrated culture using biofloc technology. Spanish Journal of Agricultural Research, Volume 22, Issue 3, e0606. https://doi.org/10.5424/sjar/2024223-20549

Received: 20 Jun 2023. **Accepted**: 25 Apr 2024. **Published**: 23 May 2024.

Introduction

It is expected that aquaculture will supply the future demand for aquatic foodstuffs due to the combination of a stagnated capture fisheries production and an increase in human consumption as a result of increases in both global population and per capita aquatic food products consumption (FAO, 2022). Moreover, it has been emphasized that to provide for this through conventional semi-intensive and intensive systems, such as those employing earthen ponds and water renewal, could create conflicts with other uses of land and water resources, particularly in the coast where human population is concentrated (Avnimelech, 2008), in addition to coastal aquaculture already being heavily criticized by environmental movements due to the destruction of mangroves (Lacerda et al., 2021).

In this regard, super-intensive closed systems can be an alternative to increase aquaculture production with greater efficiency in the use of water and land, through techniques such as biofloc technology and recirculating aquaculture systems (Sun et al., 2023). Biofloc technology (BFT), in particular, has been studied extensively for the culture of whiteleg shrimp [*Penaeus* (*Litopenaeus*) *vannamei*] (Samocha, 2019; Emerenciano et al., 2022; Khanjani et al., 2023), the most produced crustacean worldwide (FAO, 2022). However, a problem faced in aquatic animal production – particularly in more intensive systems in comparison with extensive ones that rely on natural productivity – is the fact that cultured animals assimilate a low percentage of the feed nutrients provided to them, about 39.1% of nitrogen and 35.0% of phosphorus in the case of marine shrimp (Silva et al., 2013), with the rest being converted into wastes that can damage both the cultured animals and the environment. The integration of additional species with different feeding habits to a main fed species receives the name of integrated multitrophic aquaculture (IMTA) and can help alleviate this problem (Khanjani et al., 2022). In this strategy, organic extractive (e.g. bivalves and fish) and inorganic extractive (e.g. macroalgae and terrestrial plants) use the wasted nutrients from the main species to grow, allowing for a greater and diversified overall production (Chopin et al., 2008).

The application of integrated cultures to shrimp rearing using BFT has been evaluated with different species, e.g. Nile tilapia (*Oreochromis niloticus*) and lebranche mullet (*Mugil liza*) (Poli et al., 2019; Legarda et al., 2021), and different system configurations, e.g. both species in the same tank or in separate ones (Holanda et al., 2020, 2022). One aspect of such system relates to how the secondary species, whose aim is to consume the wastes of the primary one, is to be fed. In fish monoculture, different feeding strategies, such as underfeeding, have been employed as a means to optimize feed use as regards it effects on animal growth performance and waste production (Ali et al., 2010; Cavalcante et al., 2017). In integrated cultures, considering that the role of the added species is to take advantage of the greatest amount of waste as possible, rates of supplementary feeding can affect the performance of the fish and, consequentially the overall system performance (Silva et al., 2022). For instance, when Nile tilapia was integrated to whiteleg shrimp culture using biofloc technology, the use of supplementary feeding for the fish allowed for higher fish and system yields without affecting water microbiology and sludge production (Silva et al., 2022), whereas, to the best of our knowledge, such evaluation of feeding rates when mullet was integrated has not been published so far. Therefore, the aim of this study was to evaluate the effects of different fish feeding rate levels when lebranche mullet was cultured in integration with whiteleg shrimp in biofloc technology as regards animal and overall system productive performance, water quality and water microbiology.

Material and methods

The experimental work lasted 46 days and was carried out at the Marine Shrimp Laboratory, of the Federal University of Santa Catarina (UFSC), located in Barra da Lagoa, in Florianópolis, Santa Catarina. This study was approved by the Ethics Committee on the Use of Animals of UFSC, through the protocol number 5718250220.

Biological material

Shrimp (*Penaeus vannamei*) post-larvae, acquired from the commercial laboratory AQUATEC Ltda., located in Rio Grande do Norte, were used. The shrimp were kept in a biofloc system in the Marine Shrimp Laboratory until the beginning of the experiment.

Fish of the species *Mugil liza*, from the Marine Fish Farming Laboratory of UFSC, located in Barra da Lagoa, in Florianópolis, Santa Catarina, were used. The fish were kept in a biofloc system in the Marine Shrimp Laboratory and, for the initial stocking in the experimental units, a pre-selection of fish size was made.

Experimental design and system management

Four experimental groups consisting of different fish feeding rates were evaluated with four replicates in a completely randomized design: 0%, 1%, 2% and 3% of fish biomass. The shrimp feeding was unchanged in between treatments.

The experimental units were composed of a tank with a useful volume of 800 L, in which the shrimps were stocked, connected by recirculation to a tank with a useful volume of 90 L, used for the stocking of the mullets (Fig. 1). The water was pumped from the shrimp tank to the fish tank by a Sarlo-Better 650 L $hour^{-1}$ pump, returning to the shrimp tank by gravity, keeping the system in recirculation 24 hours a day. Each 800 L tank was equipped with a central aeration ring, while the 90 L tanks each contained four porous stones connected to an aeration system, in order to

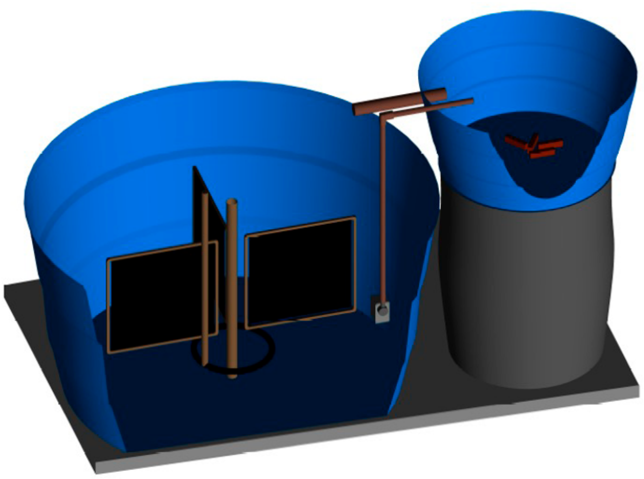

Figure 1. Experimental unit used in the experiment to evaluate different fish feeding rates in the integrated culture of whiteleg shrimp (*Penaeus vannamei*) and lebranche mullet (*Mugil liza*) for 46 days using biofloc technology. From Silva et al. (2022).

keep dissolved oxygen levels within the appropriate range for the species and prevent sedimentation of solids in the tanks. The tanks were located in a greenhouse with natural photoperiod.

Shrimp (2.36 ± 0.02 g) were stocked under a density of 375 animals m^{-3} (300 animals $tank^{-1}$), whereas fish (7.06 ± 0.65 g) were stocked under a density of 167 animals m^{-3} (15 animals $tank^{-1}$).

In each experimental unit, 800 L of mature biofloc water (0.47 mg L^{-1} TAN, 0.16 mg L^{-1} N-NO_2^-, alkalinity 226 mg $CaCO_3$ L^{-1}, 9.44 mg L^{-1} N-NO_3^-, 9.6 mg L^{-1} P-PO_4^{3-}) from a shrimp culture of the Marine Shrimp Laboratory, and 90 L of seawater, pumped from Barra da Lagoa beach, totalling 89.89% of inoculum in each experimental unit. In addition, the 800 L tanks were equipped with four artificial substrates composed of Needlona®, oriented vertically, corresponding to 80% of the tank surface area. To maintain the adequate water temperature for the culture of both species, each experimental unit was equipped with an 800 W titanium heater, located in the shrimp tanks.

The shrimp were fed four times a day (8:30 a.m., 11:30 a.m., 2:00 p.m. and 5:30 p.m.), initially with the Guabitech feed (1 mm), with 45% crude protein, until the morning of the seventh day of experiment, when they started to be fed with the Guabi Poti Guaçu feed (1.6 mm), which contains 35% crude protein. In treatments 1%, 2% and 3%, the mullets were fed twice a day (11:30 a.m. and 4:00 p.m.) with Nutripiscis starter 0.8 mm, which contains 40% crude protein. In these treatments, 1, 2 and 3% of the fish biomass of feed were offered. Fish from treatment 0% were not fed. The adjustment of the amount of shrimp feed was made according to the feeding table developed by Van Wyk (1999) through weekly biometrics, and fortnightly for fish.

Alkalinity was corrected with calcium hydroxide when the measured values were below 120 mg L^{-1} (Furtado et al., 2011). The amount of calcium hydroxide was calculated using 5 to 20% of the amount of feed supplied to the experimental unit on the previous day.

Water quality

Temperature and dissolved oxygen were measured twice a day (morning and afternoon), with a YSI pro20 oximeter both in the shrimp tanks as well as in the fish tanks, while the remaining variables were measured in the shrimp tanks only. Twice a week, pH was measured with a Thermo Scientific Orion Star A211 pHmeter. Salinity was measured twice a week with the Ecosense EC300A digital conductivity meter. Alkalinity was measured twice a week using the titrimetric method. Total ammonia nitrogen (Grasshoff et al., 1983) and nitrite-N (Strickland & Parsons, 1972) were measured twice a week. Nitrate-N was measured at the beginning, middle and end of the experiment, according to the method of Strickland & Parsons (1972). Total suspended solids (TSS) were measured twice a week (APHA et al., 2005).

Dissolved oxygen (shrimp tank: 5.34±0.47 mg L^{-1}; fish tank: 5.05±0.60 mg L^{-1}), temperature (shrimp tank: 29.5±1.3°C; fish tank: 29.6±1.4°C) and salinity (33.9±1.4 g L^{-1}) remained within ranges commonly used to successfully rear whiteleg shrimp (Van Wyk & Scarpa, 1999) and *Mugil* spp. (Legarda et al., 2019, 2021).

Productive performance

At the end of the experiment, the final population of shrimps and mullets was counted and weighted. The following variables were calculated with the resulting values:

$$Final\ biomass^{s,f,i}\ (g) = \sum Final\ animal\ weight$$

$$Final\ mean\ weight^{s,f}\ (g) = \frac{Final\ biomass}{Final\ number\ of\ animals}$$

$$^{[1]}Daily\ growth\ coefficient^{s,f}\ (\%\ day^{-1}) = \frac{\left(Wf^{\frac{1}{3}} - Wi^{\frac{1}{3}}\right)}{t} * 100\ \text{a}$$

$$Weekly\ weight\ gain^{s}\ (g\ week^{-1}) = $$
$$= \frac{Final\ mean\ weight - Initial\ mean\ weight}{t} * 7$$

$$Survival^{s,f}\ (\%) = \frac{Final\ number\ of\ animals}{Initial\ number\ of\ animals} * 100$$

$$Feed\ conversion\ ratio^{s,f,i} = \frac{Feed\ input}{Final\ biomass - Initial\ biomass}$$

$$Yield^{s,f,i} (kg\ m^{-3}) = \frac{Final\ biomass}{Tank\ volume}$$

[1] Glencross et al. (2007)

where *s* denotes variables used in the case of shrimp, *f* those in the case of fish and *i* those in the case of the integrated system as a whole. W_i and W_f denote the initial and final individual weights, respectively.

Water microbiology

For the quantification of total heterotrophic bacteria and *Vibrio* spp., 10-mL water samples from each tank were collected at the beginning and end of the experiment using sterile assay tubes with screw caps. A serial dilution using 1-mL samples was performed using tubes filled with 9 mL of sterile saline solution (3% NaCl), after which 100 µL aliquots of the homogenized contents were seeded in Petri dishes containing trypticase soy agar (TSA) and thiosulfate-citrate-bile salts-sucrose agar (TCBS) for viable heterotrophic bacteria and *Vibrio* spp., respectively. The seeded Petri dishes were incubated in a bacteriological stove at 30°C for 24 h and the colonies counted through colony forming units (CFU mL^{-1}) plate counting.

Statistical analysis

Data on water quality and zootechnical performance of organisms were tested for homoscedasticity and normality through Levene and Shapiro-Wilk tests, respectively, for subsequent application of ANOVA. When significant differences were found, the Tukey test was used to compare the means between treatments. When the data did not meet the assumptions for application of ANOVA, the Kruskal-Wallis nonparametric test was used. Linear regressions were also performed on the productive performance variables. The analyses were carried out with the software Statistica (TIBCO) and jamovi (The jamovi project, 2022) at a significance level of 5%.

Results

Water quality

Water quality variables are presented in Table 1, with no significant differences found between the experimental groups (p>0.05). The treatments had pH values ranging from 7.3 to 8.5, with a mean value higher than 7.9 in all treatments. Mean total ammonia nitrogen values remained below 0.1 mg L^{-1}, and mean nitrite-N values below 1 mg L^{-1}. Alkalinity remained around 150 mg $CaCO_3$ L^{-1} (92–200 mg $CaCO_3$ L^{-1}) in all treatments. The mean values of total suspended solids remained between 500 mg L^{-1} and 600 mg L^{-1} in all treatments. The mean nitrate-N values remained between 16 mg L^{-1} and 20 mg L^{-1}, increasing from an overall mean of 11.61 ± 3.22 mg L^{-1} at the beginning to 23.34 ± 4.33 mg L^{-1} at the end of the experiment.

Productive performance

Table 2 shows the productive performance of both species and the system as a whole. For the shrimp performance, no significant differences between treatments were found (p>0.05). The mean final weight of the shrimp was above 12.0 g. The final shrimp biomass presented mean values above 3.0 kg. Shrimp grew an average of around 1.5 g per week in all treatments. Mean daily growth coefficient values around 2.1 % day^{-1} were observed in all cases. The feed conversion ratio had a mean value close to 1.6. All treatments showed mean survival above 82% and mean yield around 3.9 kg m^{-3}.

For the fish, significant differences were found for final mean weight, final biomass, daily growth coefficient and yield (p>0.05), with each increase in the fish feeding rate resulting in a significant increase in the assessed variable (Table 2). Survival was above 95% in all cases and mean feed conversion ratio mean values in the treatments that received feed ranged between 1.34 and 1.40.

Table 1. Water quality variables of lebranche mullet (*Mugil liza*) and whiteleg shrimp (*Penaeus vannamei)* integrated system using biofloc technology for 46 days under different fish feeding rates.

Variable[1]	Fish feeding rate				p-value[2]
	0%	1%	2%	3%	
pH	7.98 ± 0.34	7.92 ± 0.35	7.92 ± 0.34	7.94 ± 0.36	0.775
TAN (mg L^{-1})	0.07 ± 0.10	0.05 ± 0.07	0.07 ± 0.10	0.04 ± 0.06	0.174
Nitrite-N (mg L^{-1})	0.73 ± 0.73	0.88 ± 1.00	0.75 ± 0.80	0.81 ± 0.77	0.743
Nitrate-N (mg L^{-1})	18.18 ± 6.21	19.21 ± 5.77	16.74 ± 6.66	18.34 ± 5.38	0.793
Alkalinity (mg $CaCO_3$ L^{-1})	149.25 ± 17.88	147.64 ± 17.75	150.68 ± 19.28	142.57 ± 19.79	0.114
TSS (mg L^{-1})	521.98 ± 171.05	561.95 ± 188.73	575.98 ± 189.49	552.91 ± 162.70	0.487

Data presented as mean ± standard deviation. [1] TAN: total ammonia nitrogen. TSS: total suspended solids. [2] p-value for one-way analysis of variance (ANOVA). Statistical significance was set at 5%.

As regards the system performance, similar to the case with the fish, significant increase in final biomass and yield were observed as the fish feeding rate increased ($p<0.05$) (Table 2), with the 3% feeding rate treatment presenting the highest value in comparison with both the 0% and 1% treatments. The system mean feed conversion ratio remained around 1.6 in all experimental groups.

Significant linear regression models were found for some of the fish and system performance variables ($p<0.05$) (Table 3), i.e. fish final mean weight, final biomass, daily growth coefficient and yield, in addition to system final biomass and yield. Fish final mean weight, daily growth coefficient and yield regression models presented R^2 values higher than 0.9 (Fig. 2).

Water microbiology

There were no significant differences between treatments for the counts of total heterotrophic bacteria and *Vibrio* spp. ($p>0.05$) (Fig. 3). The overall values were: total heterotrophic bacteria 5.0 ± 0.7 CFU mL^{-1} (log) and *Vibrio* spp. 3.2 ± 0.6 CFU mL^{-1} (log).

Discussion

Water quality

The increasing input of feed did not have deleterious effects on water quality, with the values of pH, TAN, nitrite-N, nitrate-N and alkalinity remaining within appropriate ranges for shrimp (Van Wyk & Scarpa, 1999; Lin & Chen, 2001) and within values previously employed to successfully rear mullets in BFT (Legarda et al., 2019; Chamorro-Legarda et al., 2020; Holanda et al., 2020; Legarda et al., 2021).

There are studies evaluating the optimal TSS concentration for different species in BFT, such as shrimp *P. vannamei* (400 to 600 mg L^{-1}; Schveitzer et al., 2013), South American catfish (*Rhamdia quelen*) larvae (200 mg L^{-1}; Poli et al., 2015) and pacu (*Piaractus mesopotamicus*) juveniles (>250 mg L^{-1}; Pellegrin et al., 2022). For lebranche mullets (*M. liza*), no such study has been published, but previous studies have successfully cultivated *Mugil* spp. under mean TSS concentrations varying from values as low as 89.79 ± 18.32 mg L^{-1} (Holanda et al., 2020), intermediate values between 300 mg L^1 and 600 mg L^{-1} (Legarda et

Table 2. Productive performance variables of shrimp, fish and the integrated system for mullet (*Mugil liza*) and Pacific white shrimp (*Penaeus vannamei*) cultured in integration in biofloc technology for 46 days under different fish feeding rates.

Variable	Fish feeding rate[1]				p-value[2]
	0%	1%	2%	3%	
Shrimp					
Final mean weight (g)	12.63 ± 0.07	12.70 ± 0.49	12.48 ± 0.37	12.27 ± 0.50	0.472
Final biomass (kg)	3.14 ± 0.07	3.12 ± 0.08	3.15 ± 0.09	3.08 ± 0.12	0.710
Weekly weight gain (g $week^{-1}$)	1.57 ± 0.01	1.57 ± 0.07	1.54 ± 0.05	1.51 ± 0.07	0.419
Daily growth coefficient (% day^{-1})	2.17 ± 0.01	2.18 ± 0.06	2.15 ± 0.04	2.12 ± 0.06	0.302
Survival (%)	83.00 ± 2.33	82.00 ± 4.77	84.25 ± 3.66	83.75 ± 3.20	0.824
Feed conversion ratio	1.63 ± 0.05	1.65 ± 0.06	1.63 ± 0.07	1.68 ± 0.08	0.654
Yield (kg m^{-3})	3.93 ± 0.09	3.90 ± 0.10	3.94 ± 0.12	3.85 ± 0.15	0.710
Fish					
Final mean weight (g)	19.72[a] ± 1.07	25.06[b] ± 1.50	31.20[c] ± 2.54	38.02[d] ± 1.46	<0.001
Final biomass (kg)	0.29[a] ± 0.01	0.36[b] ± 0.02	0.47[c] ± 0.04	0.57[d] ± 0.02	<0.001
Daily growth coefficient (% day^{-1})	0.34[a] ± 0.09	0.76[b] ± 0.12	1.19[c] ± 0.18	1.70[d] ± 0.10	<0.001
Survival (%)	98.33 ± 3.33	96.67 ± 3.85	100 ± 0.00	100 ± 0.00	0.238[3]
Feed conversion ratio	-	1.34 ± 0.45	1.34 ± 0.25	1.40 ± 0.13	0.942
Yield (kg m^{-3})	3.23[a] ± 0.09	4.03[b] ± 0.21	5.20[c] ± 0.42	6.34[d] ± 0.24	<0.001
Integrated system					
Final biomass (kg)	3.44[a] ± 0.07	3.48[ab] ± 0.08	3.62[bc] ± 0.06	3.65[c] ± 0.11	0.007
Feed conversion ratio	1.61 ± 0.04	1.63 ± 0.05	1.60 ± 0.04	1.64 ± 0.06	0.576
Yield (kg m^{-3})	3.86[a] ± 0.07	3.91[ab] ± 0.09	4.07[bc] ± 0.07	4.10[c] ± 0.12	0.007

Data presented as mean ± standard deviation. [1] Different letters across a row indicate significant differences by Tukey's test. Statistical significance was set at 5%. [2] p-value for one-way analysis of variance (ANOVA). [3] Kruskal-Wallis test was employed.

Table 3. Regression analysis of fish and the integrated system for mullet (*Mugil liza*) and Pacific white shrimp (*Penaeus vannamei*) cultured in integration using biofloc technology for 46 days under different fish feeding rates.

Variable	Regression equation	R^2 (adjusted R^2)	p-value[1]
Fish			
Final mean weight (g)	y = 6.12x + 19.33	0.952 (0.949)	<0.001
Final biomass (kg)	y = 0.09x + 0.29	0.853 (0.842)	<0.001
Daily growth coefficient (% day^{-1})	y = 0.465x + 0.302	0.944 (0.940)	<0.001
Survival (%)	-	-	0.173
Feed conversion ratio	-	-	0.736
Yield (kg m^{-3})	y = 1.05x + 3.12	0.957 (0.954)	<0.001
Integrated system			
Final biomass (kg)	y = 0.0875x + 3.425	0.640 (0.614)	<0.001
Feed conversion ratio	-	-	0.503
Yield (kg m^{-3})	y = 0.0825x + 3.87	0.546 (0.513)	0.001

[1] p-value for analysis of variance (ANOVA) omnibus test of the linear regression models. Statistical significance was set at 5%.

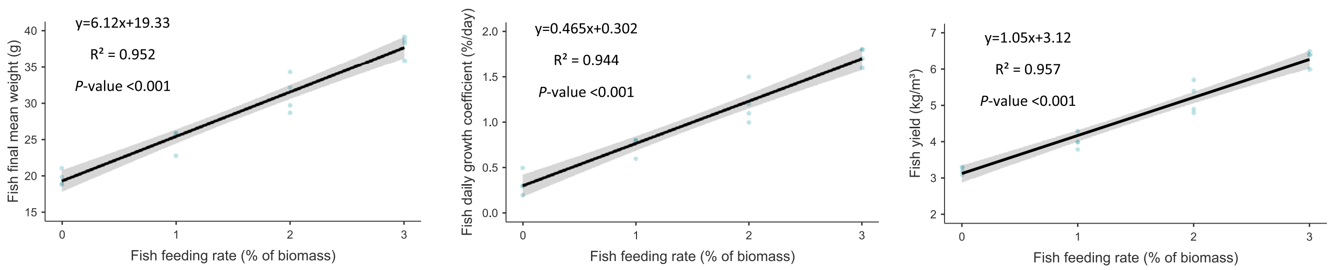

Figure 2. Plots of the linear regression models in which the R^2 was over 0.9, i.e. fish final mean weight, daily growth coefficient and yield, in an experiment evaluating different fish feeding rates in the integrated culture of whiteleg shrimp (*Penaeus vannamei*) and lebranche mullet (*Mugil liza*) for 46 days using biofloc technology.

al., 2019; Chamorro-Legarda et al., 2020; Legarda et al., 2021) and as high as 706.52 ± 252.13 mg L^{-1} (Borges et al., 2020). In the current study, the fish exhibited high survival (above 96% in all treatments) in a situation where the mean TSS concentration varied from 521.98 ± 171.05 mg L^{-1} to 575.98 ± 189.49 mg L^{-1}, suggesting the feasibility of cultivating mullets under these concentrations.

Strategies employed to manage biofloc systems vary, from using different organic carbon sources to stimulate the immobilization of ammonia, to using tactics to hasten the establishment of a nitrifying community (Ebeling et al., 2006). In the case of this study, the use of an inoculum of mature bioflocs, i.e. water from a tank in which nitrification is already occurring, allowed for the maintenance of low concentrations of the most toxic nitrogenous compounds, TAN and nitrite-N, throughout the entire experiment. It can also be highlighted that the nitrate-N concentration increased at the end of the experiment in comparison with the inoculum (from 11.61 ± 3.22 mg L^{-1} to 23.34 ± 4.33 mg L^{-1}), although remaining in concentrations that are not harmful to the cultured animals, where concentrations as high as 177 mg L^{-1} having been reported as acceptable for rearing *P. vannamei* under a salinity of 23 g L^{-1} (Furtado et

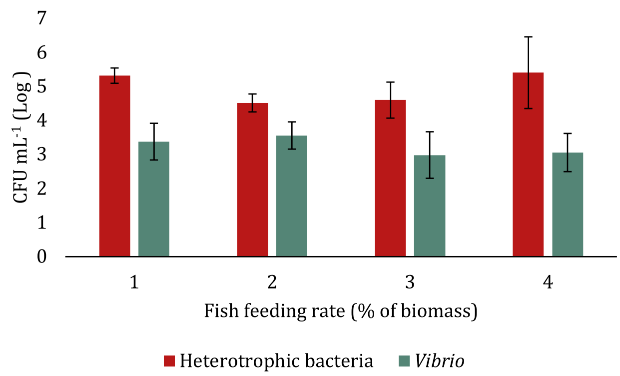

Figure 3. Water counts of total heterotrophic bacteria (THB) and *Vibrio* spp. at the end of a whiteleg shrimp (*Penaeus vannamei*) and lebranche mullet (*Mugil liza*) cultivation for 46 days using biofloc technology under different fish feeding rates. One-way analysis of variance (ANOVA) p-values: THB, 0.981; *Vibrio* spp., 0.415.

al., 2015). These two observations, the low concentrations of TAN and nitrite-N, along with the increasing nitrate-N concentration, suggest that the system was characterized as being a mature biofloc system, as expected from the management employed.

Productive performance

Regarding the shrimp, the lack of significant effects of the fish feeding rates on its performance was likely a result of the increased rates not deteriorating water quality. A similar phenomenon was also observed when *P. vannamei* was cultured in integration with Nile tilapia in a study evaluating different feeding rates for the fish (Silva et al., 2022). The authors also observed no significant effects of the fish feeding rates on the shrimp performance.

In contrast, the significant effects observed on the fish performance variables indicate that mullet growth and productivity were influenced by the feeding rates. As the feeding rate increased, there was an incremental improvement in fish final mean weight, final biomass, daily growth coefficient, and yield. This suggests that a higher feeding rate provides a greater availability of feed resources for the fish, leading to enhanced growth and productivity. The positive relationship between feeding rate and fish performance aligns with previous studies that have reported similar findings for aquatic animals reared in BFT, such as Nile tilapia (Oliveira et al., 2021; Silva et al., 2022) and whiteleg shrimp (Khanjani et al., 2016) under different feeding levels.

The observed significant effects on the overall system productive performance further support the influence of feeding rates on the integrated culture system. Specifically, there were significant effects on the final biomass and yield of the system. As the feeding rate increased, both the final biomass and yield of the system increased accordingly. This indicates that a higher feeding rate promotes a higher overall production output, highlighting the importance of feed availability for system productivity. Silva et al. (2022) also reported higher system productive performance when Nile tilapia were fed at 1% of their biomass in the integrated culture with whiteleg shrimp, when compared to a treatment in which the fish were not fed at all.

The results of this study have practical implications for the optimization of feeding strategies in the integrated culture of marine shrimp and mullets using biofloc technology. The findings suggest that increasing the fish feeding rate can positively impact fish growth and system productivity without negatively affecting shrimp performance. However, it is important to note that there may be limits to the positive effects of increasing feeding rates. Beyond a certain point, excessive feeding rates may lead to diminishing returns or potential negative impacts, such as deteriorating water quality and poor growth performance (Oliveira et al., 2021).

Water microbiology

The counts of total heterotrophic bacteria and *Vibrio* spp. in the water were measured to assess the impact of different fish feeding rates on water microbiology in the integrated culture of whiteleg shrimp and lebranche mullet using biofloc technology. The results showed no statistically significant effects of the fish feeding rate on water microbiology. This suggests that the microbial communities associated with biofloc formation and maintenance remained resilient and stable, unaffected by the feeding rates tested. This was also observed in a study evaluating the effect of fish feeding rate on the integrated culture of Nile tilapia and whiteleg shrimp, in which no statistical significance was found for *Vibrio* spp. and total heterotrophic bacteria when different fish feeding rates were used (Silva et al., 2022).

The lack of significant effects could be related to the fact that the fish feeding represented a small percentage of the overall feed input, due to the greater shrimp biomass in the system. A support for this hypothesis is a study that evaluated the effects of different feeding rates and species configurations in the integrated culture of *Tilapia hornorum* and *P. monodon* (Tendencia et al., 2006). The system that employed shrimp and fish with both being fed resulted in statistically higher values of total bacterial count when compared with a system in which the fish were not fed. However, the proportion of biomasses differed from the ones used in the current study. For example, Tendencia et al. (2006) used 0.5 kg m^{-3} of fish and only 0.080 kg m^{-3} of shrimp, compared to the current study where the mean final biomasses were at most 0.64 kg of fish m^{-3} and 3.54 kg of shrimp m^{-3} when considering the whole volume of the system.

These findings indicate the robustness of the microbial ecosystem in biofloc-based systems and highlight the potential for adjusting feeding rates within a reasonable range without significant disruptions to the microbial balance. Future research should investigate the long-term effects of feeding rates on water microbiology and explore the functional roles of specific microbial taxa in biofloc systems for a comprehensive understanding of their contributions to system performance and sustainability.

Conclusion

This study demonstrated that different fish feeding rates in the integrated culture of marine shrimp and mullets using biofloc technology significantly influenced fish growth and system productivity, without negatively impacting shrimp performance. Water quality variables remained stable and unaffected by the feeding rates. Additionally, the counts of *Vibrio* spp. and total heterotrophic bacteria in the water were not significantly influenced by the feeding rates. These findings provide valuable insights for optimizing feeding strategies and management practices in biofloc-based integrated culture systems, enhancing efficiency, and minimizing environmental impacts. Future research should focus on comprehensive economic analyses to determine the cost-effectiveness and profitability of different feeding rates.

Ethical approval: All pertinent institutional and national guidelines for the use of the laboratory animals employed were followed.

Data availability: Data will be available on request.

Competing interests: The authors have declared that no competing interests exist.

Authors' contributions: Ana Carolina da Silveira Pereira: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Esmeralda Chamorro Legarda:** Investigation, Writing – review & editing. **Mateus Aranha Martins:** Formal analysis, Visualization, Writing – review & editing. **Claudia Machado:** Investigation, Writing – review & editing. **Caio Cesar Franca Magnotti:** Resources, Writing – review & editing. **Felipe do Nascimento Vieira:** Funding acquisition, Resources, Writing – review & editing. **Luis Alejandro Vinatea Arana:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Funding agencies/ institutions	Project / Grant
Coordination for the Improvement of Higher Education Personnel–Brazil (CAPES)	Finance code 001
European Union	Aquavitae project (Horizon 2020, No. 818173).
National Council for Scientific and Technological Development (CNPq)	Productivity grant to Felipe N. Vieira (310250/2020-0)

References

Ali M, Hayward RS, Bajer PG, Whitledge GW, 2010. Maintenance/submaximum feeding schedules for reducing solid wastes and improving feed conversion in aquaculture. J World Aquacult Soc 41: 319-331. https://doi.org/10.1111/j.1749-7345.2010.00374.x

APHA (American Public Health Association), Water Works Association, Water Environment Federation, 2005. Standard methods for the examination of water and wastewater, 21 ed. American Public Health Association, Washington, DC.

Avnimelech Y, Verdegem M, Kurup M, Keshavanath P, 2008. Sustainable land-based aquaculture: rational utilization of water, land and feed resources. Med Aquacult J 1: 45-54. https://doi.org/10.21608/maj.2008.2663

Borges BAA, Rocha JL, Pinto PHO, Zacheu T, Chede AC, Magnotti CCF, et al., 2020. Integrated culture of white shrimp *Litopenaeus vannamei* and mullet *Mugil liza* on biofloc technology: Zootechnical performance, sludge generation, and *Vibrio* spp. reduction. Aquaculture 524: 735234. https://doi.org/10.1016/j.aquaculture.2020.735234

Cavalcante DDH, Lima FRS, Rebouças VT, Sá MVC, 2017. Nile tilapia culture under feeding restriction in bioflocs and bioflocs plus periphyton tanks. Acta Sci Anim Sci 39: 223-228. https://doi.org/10.4025/actascianimsci.v39i3.33574

Chamorro Legarda E, Aranha Martins M, Moreira Pereira PK, Siqueira Carneiro RF, Pinheiro IC, Seiffert WQ, et al., 2020. Shrimp rearing in biofloc integrated with different mullet stocking densities. Aquacult Res 51: 3571-3581. https://doi.org/10.1111/are.14694

Chopin T, Robinson SMC, Troell M, Neori A, Buschmann AH, Fang J, 2008. Multitrophic integration for sustainable marine aquaculture. In: Encyclopedia of Ecology. Elsevier, pp. 2463-2475. https://doi.org/10.1016/B978-008045405-4.00065-3

Ebeling JM, Timmons MB, Bisogni JJ, 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture 257: 346-358. https://doi.org/10.1016/j.aquaculture.2006.03.019

Emerenciano MGC, Rombenso AN, Vieira FN, Martins MA, Coman GJ, Truong HH, et al., 2022. Intensification of penaeid shrimp culture: An applied review of advances in production systems, nutrition and breeding. Animals 12: 236. https://doi.org/10.3390/ani12030236

FAO, 2022. The State of World Fisheries and Aquaculture 2022. Food and Agriculture Organization of the United Nations, Rome. 236 pp.

Furtado PS, Poersch LH, Wasielesky W, 2011. Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical performance of shrimp *Litopenaeus vannamei* reared in bio-flocs technology (BFT) systems. Aquaculture 321: 130-135. https://doi.org/10.1016/j.aquaculture.2011.08.034

Furtado PS, Campos BR, Serra FP, Klosterhoff M, Romano LA, Wasielesky W, 2015. Effects of nitrate toxicity in the Pacific white shrimp, *Litopenaeus vannamei*, reared with biofloc technology (BFT). Aquacult Int 23: 315-327. https://doi.org/10.1007/s10499-014-9817-z

Glencross BD, Booth M, Allan GL, 2007. A feed is only as good as its ingredients? A review of ingredient evaluation strategies for aquaculture feeds. Aquacult Nutr 13: 17-34. https://doi.org/10.1111/j.1365-2095.2007.00450.x

Grasshoff K, Ehrhardt M, Kremling K, 1983. Methods of seawater analysis, 2nd Ed. Verlag Chemie Weinhein, New York.

Holanda M, Santana G, Furtado P, Rodrigues RV, Cerqueira VR, Sampaio LA, et al., 2020. Evidence of total suspended solids control by Mugil liza reared in an integrated system with pacific white shrimp *Litopenaeus vannamei* using biofloc technology. Aquacult Rep 18: 100479. https://doi.org/10.1016/j.aqrep.2020.100479

Holanda M, Wasielesky W, de Lara GR, Poersch LH, 2022. Production of marine shrimp integrated with tilapia at high densities and in a biofloc system: Choosing the best spatial configuration. Fishes 7: 283. https://doi.org/10.3390/fishes7050283

Khanjani MH, Sajjadi MM, Alizadeh M, Sourinejad I, 2016. Study on nursery growth performance of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) under different feeding levels in zero water exchange system. Iran J Fish Sci 15(4): 1465-1484.

Khanjani MH, Zahedi S, Mohammadi A, 2022. Integrated multitrophic aquaculture (IMTA) as an environmentally friendly system for sustainable aquaculture: functionality, species, and application of biofloc technology (BFT). Environ Sci Pollut Res 29: 67513-67531. https://doi.org/10.1007/s11356-022-22371-8

Khanjani MH, Mozanzadeh MT, Sharifinia M, Emerenciano MGC, 2023. Broodstock and seed production in biofloc technology (BFT): an updated review focused on fish and penaeid shrimp. Aquaculture, 740278. https://doi.org/10.1016/j.aquaculture.2023.740278

Lacerda LD, Ward RD, Godoy MDP, de Andrade Meireles AJ, Borges R, Ferreira AC, 2021. 20-Years cumulative impact from shrimp farming on mangroves of northeast Brazil. Front For Glob Change 4: 653096. https://doi.org/10.3389/ffgc.2021.653096

Legarda EC, Poli MA, Martins MA, Pereira SA, Martins ML, Machado C, et al., 2019. Integrated recirculating aquaculture system for mullet and shrimp using biofloc technology. Aquaculture 512: 734308. https://doi.org/10.1016/j.aquaculture.2019.734308

Legarda EC, da Silva D, Miranda CS, Pereira PKM, Martins MA, Machado C, et al., 2021. Sea lettuce integrated with Pacific white shrimp and mullet cultivation in biofloc impact system performance and the sea lettuce nutritional composition. Aquaculture 534: 736265. https://doi.org/10.1016/j.aquaculture.2020.736265

Lin YC, Chen JC, 2001. Acute toxicity of ammonia on *Litopenaeus vannamei* Boone juveniles at different salinity levels. J Exp Mar Biol Ecol 259(1): 109-119. https://doi.org/10.1016/S0022-0981(01)00227-1

Oliveira LK, Pilz L, Furtado PS, Ballester ELC, Bicudo ÁJA, 2021. Growth, nutritional efficiency, and profitability of juvenile GIFT strain of Nile tilapia (*Oreochromis niloticus*) reared in biofloc system on graded feeding rates. Aquaculture 541: 736830. https://doi.org/10.1016/j.aquaculture.2021.736830

Pellegrin L, Nitz LF, Pinto DSB, Copatti CE, Wasielesky W, Garcia L, 2022. Effects of suspended solids in the survival and haematological parameters of pacu juveniles (*Piaractus mesopotamicus*) in a biofloc technology culture system. Aquacult Res 53: 276-284. https://doi.org/10.1111/are.15575

Poli MA, Schveitzer R, de Oliveira Nuñer AP, 2015. The use of biofloc technology in a South American catfish (*Rhamdia quelen*) hatchery: Effect of suspended solids in the performance of larvae. Aquacult Eng 66: 17-21. https://doi.org/10.1016/j.aquaeng.2015.01.004

Poli MA, Legarda EC, de Lorenzo MA, Martins MA, do Nascimento Vieira F, 2019. Pacific white shrimp and Nile tilapia integrated in a biofloc system under different fish-stocking densities. Aquaculture 498: 83-89. https://doi.org/10.1016/j.aquaculture.2018.08.045

Samocha TM, 2019. Sustainable biofloc systems for marine shrimp. Academic Press: Elsevier, London; San Diego, CA. 431 pp. https://doi.org/10.1016/B978-0-12-818040-2.00003-4

Schveitzer R, Arantes R, Costódio PFS, do Espírito Santo CM, Arana LV, Seiffert WQ, et al., 2013. Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. Aquacult Eng 56: 59-70. https://doi.org/10.1016/j.aquaeng.2013.04.006

Silva KR, Wasielesky Jr W, Abreu PC, 2013. Nitrogen and phosphorus dynamics in the biofloc production of the pacific white shrimp, *Litopenaeus vannamei*. J World Aquacult Soc 44: 30-41. https://doi.org/10.1111/jwas.12009

Silva VF, Pereira PKM, Martins MA, Lorenzo MA, Cella H, Lopes RG, et al., 2022. Effects of microalgae addition and fish feed supplementation in the integrated rearing of Pacific white shrimp and Nile tilapia using biofloc technology. Animals 12: 1527. https://doi.org/10.3390/ani12121527

Strickland JDH, Parsons TR, 1972. A practical handbook of seawater analysis, 2nd ed. Fisheries Research Board of Canada, Ottawa.

Sun Y, Hou H, Dong D, Zhang J, Yang X, Li X, et al., 2023. Comparative life cycle assessment of whiteleg shrimp (*Penaeus vannamei*) cultured in recirculating aquaculture systems (RAS), biofloc technology (BFT) and higher-place ponds (HPP) farming systems in China. Aquaculture 574: 739625. https://doi.org/10.1016/j.aquaculture.2023.739625

Tendencia EA, dela Peña MR, Choresca Jr. CH, 2006. Effect of shrimp biomass and feeding on the anti-Vibrio harveyi activity of Tilapia sp. in a simulated shrimp-tilapia polyculture system, 2006. Aquaculture 253: 154-162. https://doi.org/10.1016/j.aquaculture.2005.08.004

The jamovi project, 2022. jamovi. (version 2.3) [Computer Software]. https://www.jamovi.org Van Wyk P, 1999. Nutrition and feeding of *Litopenaeus vannamei* in intensive culture systems. In: Farming marine shrimp in recirculating freshwater systems;

Van Wyk P et al. (Eds.). Department of Agriculture and Consumer Services, Tallahassee, pp. 125-139.

Van Wyk P, Scarpa J, 1999. Water quality management. In: Farming marine shrimp in recirculating freshwater systems; Van Wyk P et al. (Eds.). Department of Agriculture and Consumer Services, Tallahassee, pp. 141-161.