



ORIGINAL ARTICLE

Integrated aquaculture approaches for Lebranche mullet juveniles (*Mugil liza* Valenciennes 1836, Mugilidae): assessing biofloc and periphyton contributions

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Abstract – This study evaluated the effects of integrating biofloc technology and periphyton grown on artificial substrates on water quality and growth performance of *Mugil liza* juveniles. Three experimental systems were tested: clear water (CW), clear water with periphyton substrates (CWS), and bioflocs with periphyton substrates (BS). Over a 30-day trial, fish in the BS treatment exhibited significantly higher final weight and improved feed conversion ratio compared with the other treatments. The BS system also exhibited lower concentrations of total ammonia and nitrate and higher levels of chlorophyll *a* in the water, indicating enhanced microbial activity and natural productivity. These results indicate that combining bioflocs and periphyton can improve water quality and reduce feed costs through natural-food supplementation. The integrated approach demonstrated the potential to enhance aquaculture sustainability in *M. liza* by minimizing water exchange and improving nutrient utilization.

Keywords: Biofilm. Microbial aggregates. Fish farming. Zero water exchange.

Abordagens integradas de aquicultura para juvenis de tainha (*Mugil liza* Valenciennes 1836, Mugilidae): avaliando as contribuições de bioflocos e perifíton

Resumo – Este estudo avaliou os efeitos da integração da tecnologia de bioflocos e do perifíton cultivado em substratos artificiais na qualidade da água e no desempenho de crescimento de juvenis de *Mugil liza*. Três sistemas experimentais foram testados: água clara (CW), água clara com substratos contendo perifíton (CWS) e bioflocos com substratos contendo perifíton (BS). Ao longo de um período de 30 dias, os peixes no tratamento BS apresentaram peso final significativamente maior e melhor taxa de conversão alimentar em comparação com os outros tratamentos. O sistema BS também apresentou menores concentrações de amônia total e nitrato, bem como maiores níveis de clorofila *a* na água, indicando maior atividade microbiana e produtividade natural. Esses resultados indicam que a combinação de bioflocos e perifíton pode melhorar a qualidade da água e reduzir os custos com ração por meio de suplementação alimentar natural. A abordagem integrada demonstrou o potencial para aumentar a sustentabilidade da aquicultura de *M. liza*, reduzindo as trocas de água e melhorando a utilização de nutrientes.

Palavras-chave: Biofilme. Agregados microbianos. Piscicultura. Troca zero de água.

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Introduction

The use of artificial substrates to promote periphyton development, also referred to as biofilm (Azim *et al.*, 2003), and the manipulation of the carbon-to-nitrogen (C:N) ratio to stimulate the formation of microbial aggregates (bioflocs) (Hargreaves, 2006; Avnimelech, 2007; Crab *et al.*, 2007), has been widely recognized as a sustainable technique in aquaculture. When applied individually or in combination (Azim *et al.*, 2003; Azim; Little; Bron, 2008; Asaduzzaman *et al.*, 2008; 2009; Cavalcante *et al.*, 2017), these approaches have demonstrated potential for enhancing the culture of herbivorous and omnivorous species while also improving water quality (Van Dam *et al.*, 2002; Jana; Garg; Patra 2004; Ballester *et al.*, 2007; Bell *et al.*, 2023).

Periphyton is a complex and nutritionally valuable natural food source composed of algae, bacteria, fungi, protozoans, and detritus that adhere to submerged surfaces (Thompson; Abreu; Wasielesky, 2002). Its role in nutrient cycling, oxygen production, and water purification has been demonstrated in various aquatic systems (Azim *et al.*, 2003), making it an effective tool for integrated aquaculture (Bell *et al.*, 2023). In addition to supplementing fish diets (Silva *et al.*, 2008), periphyton systems can reduce nitrogenous waste by facilitating nutrient uptake via microbial and algal processes (Thompson; Abreu; Wasielesky, 2002; Jana; Garg; Patra, 2004; Ballester *et al.*, 2007; Asaduzzaman *et al.*, 2009). Successful outcomes have been reported in the cultivation of tilapia (Uddin *et al.*, 2007), carp (Azim *et al.*, 2002), and mullet (Jana; Garg; Patra, 2004; Richard *et al.*, 2010; Milstein *et al.*, 2018).

Periphyton-based systems, which enhance productivity and resource-use efficiency in aquaculture (Van Dam *et al.*, 2002), are a promising approach for improving the sustainability and profitability of small-

scale production units (David *et al.*, 2022). Likewise, biofloc technology (BFT) has emerged as an eco-friendly system that enhances aquaculture sustainability by promoting microbial floc formation through the addition of external carbon and intense aeration. This microbial community, comprising heterotrophic and autotrophic/nitrifying bacteria, algae, protozoa, and detritus, assimilates nitrogen compounds and improves water quality while producing protein-rich flocs that serve as feed (Avnimelech, 2007; Alkhamis *et al.*, 2023). Meta-analyses have shown that BFT significantly reduces nitrite concentrations and increases total suspended solids, positively influencing growth and feed efficiency in aquaculture systems (Alkhamis *et al.*, 2023). Widely adopted in shrimp and freshwater fish farming, its application in marine fish culture has shown great potential.

Cavalcante *et al.* (2017) demonstrated that the integration of bioflocs and periphyton in tilapia culture effectively reduced ammonia concentrations compared to control systems. Furthermore, with regard to both systems, it is possible that they could contribute to providing low-cost protein, minimize environmental impacts, and reinforce their value as sustainable alternatives in aquaculture.

These findings reinforce the potential of integrated systems, especially for species that can substantially benefit from the availability of natural food sources such as mullet (Van Dam *et al.*, 2002). *Mugil liza*, due to its feeding habit, can benefit from this combination.

The Lebranche mullet (*Mugil liza*) is a euryhaline species of high ecological and socioeconomic relevance, especially in southern and southeastern Brazil, Uruguay, and Argentina, where it supports artisanal fisheries in estuarine and coastal lagoon systems (Vieira; Scalabrin, 1991). With delayed reproductive maturity





(six years of age, ~40 cm length) and migratory behavior during colder months (Garbin; Castello; Kinas, 2014; Lemos *et al.*, 2016), this species supports thousands of families and plays an important role in fish processing industries (Steenbock, 2019). Despite the reported production of 7,209 tons in Brazil in 2018 (Brasil, 2018), *M. liza* has been classified as overexploited, with a risk of fishery collapse (Brasil, 2004; 2015).

Although only a few mullet species are cultivated globally, primarily in the Mediterranean, Taiwan, and China (Saleh, 2008), *M. liza* was among the first native Brazilian species to be studied for aquaculture. Advances have been made in captive reproduction (Godinho, 1993), hatchery development (Galvão *et al.*, 1997), optimal temperature and salinity (Okamoto; Sampaio; Maçada, 2006; Sampaio; Bianchini, 2002; Lisboa *et al.*, 2015), ammonia and nitrite tolerance (Sampaio; Wasielesky; Miranda-Filho, 2002; Poersch *et al.*, 2007), nutrition (Ramos *et al.*, 2015; Zamora-Sillero *et al.*, 2013; Carvalho *et al.*, 2010), and stocking densities (Sampaio; Ferreira; Tesser, 2001). The feeding ecology of *M. liza* includes initial consumption of periphyton, followed by detritus, microalgae, detritus, sediments, metazoa (Oliveira; Soares, 1996), and organisms associated with fine sediments (Thompson *et al.*, 2015), suggesting suitability for systems that utilize natural foods such as biofilms and bioflocs. Biofloc consumption by mullet species, such as *M. liza* (Holanda *et al.*, 2020), *Mugil cephalus* (Larson; Shanks, 1996; Vinatea *et al.*, 2018; Garcés; Lara, 2023), and *Mugil curema* (Larson; Shanks, 1996; Legarda *et al.*, 2019), has been reported, and its maintenance in mullet tanks was demonstrated in trials with *Mugil cf hospes* (Rocha *et al.*, 2012) and *M. liza* (Rocha *et al.*, 2022). The combined use of periphyton and biofloc systems may optimize nutrient utilization and reduce

environmental impacts, particularly in low- or zero-exchange systems (Guo *et al.*, 2022; Bell *et al.*, 2023).

Considering the ecological adaptability and iliophagous-periphyton feeding behavior of *M. liza*, this study aimed to evaluate the effects of three culture systems (clear water, clear water with periphyton substrates, and bioflocs with periphyton substrates) on juvenile growth performance and water quality. These findings aim to support the development of sustainable and efficient aquaculture strategies for this important native species.

Material and Methods

Experimental design

This study aimed to evaluate the effects of different culture systems on the growth performance of *M. liza* juveniles and their impact on water quality, focusing on the contribution of natural food sources. A completely randomized design was adopted, consisting of three treatments with three replicates each (90 fish per treatment): Clear Water (CW): The control group was maintained in clear water without the addition of bioflocs or periphyton substrates. Clear Water with Periphyton Substrate (CWS): Tanks containing artificial substrates pre-colonized with periphyton without biofloc inoculation. Biofloc and Periphyton Substrate (BS): Tanks containing artificial substrates with pre-formed periphyton and an initial biofloc inoculum (20 mL L⁻¹).

Experimental facilities and conditions

A 30-day experiment was conducted at the Marine Aquaculture Station of the Institute of Oceanography, Federal University of Rio Grande (FURG), Brazil. Juvenile *M. liza* (0.66 ± 0.21 g initial weight) were collected from Cassino Beach (Rio Grande do Sul, Brazil) and stocked in 50 L rectangular





polyethylene tanks at a density of 0.6 fish L⁻¹.

The study was conducted during the summer, with an average air temperature of 25.8 °C and approximately 250 mm of accumulated rainfall (Instituto Nacional de Meteorologia, 2010).

The tanks were placed outdoors under natural light and photoperiod. Partial shading was achieved using reflective screens. Water was filtered through a 5 µm Cuno filter and adjusted to 10 ppt salinity. Aeration and water circulation were ensured using two 10 cm porous stones per tank connected to a blower system.

Fish were fed a commercial diet containing 38 % crude protein, 7.5 % ether extract, and 13 % ash (on a dry matter basis) at a rate of 5 % of biomass per day, divided into three meals. The diet supplied to the fish consisted of a commercial feed formulated for omnivorous species, containing 38 % crude protein, which closely aligned with the estimated dietary protein requirement of 34 % for juvenile mullet (*Mugil platanus*), as reported by Carvalho *et al.* (2010).

Biofloc management

The biofloc inoculum for the BS treatment originated from a *Litopenaeus vannamei* grow-out pond at the Marine Aquaculture Station's Marine Shrimp Culture Laboratory. This inoculum was prepared as described by Rocha *et al.* (2012). Dextrose was added when the total ammonia nitrogen (TAN) exceeded 1 mg L⁻¹, maintaining a C:N ratio of 6:1 (Ebeling; Timmons; Bisogni, 2006; Hargreaves, 2006).

Biofloc volume was monitored weekly using 1 L Imhoff cone samples that were settled for 15 min (Avnimelech, 2007). When the volume dropped below 5 mL L⁻¹, additional inoculum (20 mL L⁻¹) was added. No water exchange was conducted in the BS treatment, except to compensate for the evaporation. The CW and

CWS treatments received 90 % water exchange daily.

Water quality analyses

Daily monitoring included temperature, dissolved oxygen (YSI 550A), salinity (Atago refractometer ± 1), and pH (YSI PH100 ± 0.01). Weekly measurements included alkalinity (as CaCO₃, APHA, 2005), turbidity (Hach 2100P), and nitrate (N-NO₃⁻, Aminot; Chaussepied, 1983). Total suspended solids (TSS) were measured by filtering 25 mL of water through pre-weighed Whatman GF/A filters, determined by drying and reweighing. Nitrite (N-NO₂⁻) and orthophosphate (P-PO₄⁺) levels were analyzed twice weekly (Aminot; Chaussepied, 1983). Total ammonia nitrogen (TAN) was measured five times a week (IOC; UNESCO, 1983).

Chlorophyll *a* was analyzed weekly by filtering 20 mL of water through Whatman GF/A filters, which were frozen at -12 °C. Pigments were extracted with 90 % acetone and quantified using a Turner TD-700 fluorometer (Welschmeyer, 1994).

Periphyton analyses

In the BS and CWS tanks, two polyethylene mesh screens (20 × 15 cm) were installed per tank, covering 82 % of the vertical surface area, and pre-colonized with periphyton. Nine smaller mesh pieces (3 × 3 cm) were used for sampling.

Biomass was estimated by drying the samples at 60 °C for 24 h and recording the dry weight. For chlorophyll *a*, samples were frozen and extracted with 90 % acetone, and concentrations were determined using the Turner TD-700 fluorometer (Welschmeyer, 1994).

Growth performance and survival

Fish were sampled at the beginning, day 15 (10





fish per replicate), and end of the trial. The fish were anesthetized with 50 ppm benzocaine and weighed (precision: 0.1 g). The performance indicators were calculated as follows: Survival Rate (%) = (Number of surviving animals at the end of the study / Number of animals at the beginning of the study) $\times$ 100. Feed Conversion Ratio - FCR = Total feed offered / Weight gain. Specific Growth Rate - SGR (% day⁻¹) = 100 $\times$ [Ln*(Final biomass) – Ln*(Initial biomass)] / Days *Ln = natural logarithm

Ethical Approval

All relevant institutional and national guidelines for the use of laboratory animals at the time were followed.

Statistical analysis

Differences in survival, weight gain, FCR, SGR, and TSS were tested using One-way ANOVA after confirming normality (Kolmogorov–Smirnov test) and homoscedasticity (Levene's test). Tukey's post-hoc test was applied when differences were significant. The Kruskal–Wallis test was used when the assumptions were not met. Percentage data were transformed using the arcsine square-root method (Sokal; Rohlf, 1995). Analyses were performed in Statistica 7.0 (StatSoft, Tulsa, OK, USA, free trial version), with significance set at $P < 0.05$.

Results and Discussion

Water quality

The experiment was conducted during the summer under outdoor conditions, resulting in only slight fluctuations in water temperature (26.7 ± 1.7 °C), dissolved oxygen (7.36 ± 0.25 mg L⁻¹), salinity (9.16 ± 3.37), and pH (7.74 ± 0.28).

Average alkalinity and turbidity values were

significantly higher in the treatments involving biofloc and periphyton substrates than in the other treatments. Similarly, total suspended solids (TSS) concentrations were elevated in the BS treatment, significantly differing from the CW treatment and showing no statistical difference from the CWS treatment (Table 1).

Notably, the BS treatment exhibited lower mean concentrations of nitrate (N-NO₃⁻) and total ammonia nitrogen (TAN) in the water than the other treatments (Table 1). Additionally, this treatment resulted in higher chlorophyll concentrations in the water column (Fig. 1). No significant differences were found among the treatments regarding nitrite (N-NO₂⁻) and orthophosphate (P-PO₄⁺) concentrations (Table 1).

Periphyton growth

After 30 d, periphyton biomass (dry matter) reached 0.0103 mg cm⁻² in the BS treatment and 0.0092 mg cm⁻² in the CWS treatment, with no statistically significant difference between them (Fig. 2A). Similarly, chlorophyll *a* concentrations in the periphyton did not differ significantly, averaging 0.32 ± 0.06 µg cm⁻² and 0.64 ± 0.41 µg cm⁻² for the BS and CWS treatments, respectively (Fig. 2B).

Growth performance and survival

The survival rates exceeded 90 % across all treatments, with no significant differences observed. Juvenile mullets from all treatments exhibited approximately a four-fold increase in weight over the experimental period. However, fish reared under BS treatment achieved significantly higher final weights than those under CW and CWS treatments. In addition, fish in the BS treatment showed lower feed conversion ratios (FCR) than those in the CWS treatment, although this difference was not statistically significant when compared to the CW control. The specific growth rate





(SGR) did not differ significantly among the treatments (Table 2).

Despite no significant differences in temperature, dissolved oxygen, salinity, or pH among the treatments, these parameters consistently remained within the optimal ranges for the development of eurythermal and

euryhaline species, such as *M. liza* (Miranda Filho *et al.*, 2005). In this study, mullet survival was greater than 90 % in all treatments. Similar results were observed for mullet raised in biofloc systems in trials by Holanda *et al.* (2020) with *M. liza*, Legarda *et al.* (2019) with *M. curema*, and Vinatea *et al.* (2018) with *M. cephalus*.

Table 1. Water quality parameters during the experimental period for the treatments: bioflocs with periphyton substrate (BS), clear water (CW), and clear water with periphyton substrate (CWS).

Parameter	BS	CW	CWS
Alkalinity (mg L ⁻¹)	105.9 ± 26.5 ^a	73.3 ± 19.4 ^b	67.8 ± 15.6 ^b
Turbidity (ntu)	94.0 ± 78.7 ^a	16.5 ± 6.2 ^b	7.22 ± 3.43 ^c
Total Suspended Solids (mg L ⁻¹)	206.6 ± 88.9 ^a	128.8 ± 107.8 ^b	138.6 ± 90.3 ^{ab}
Total Ammonia (mg L ⁻¹)	0.73 ± 0.78 ^a	1.19 ± 0.48 ^b	1.27 ± 0.63 ^b
Nitrite (mg L ⁻¹)	0.08 ± 0.17 ^a	0.04 ± 0.08 ^a	0.18 ± 0.44 ^a
Nitrate (mg L ⁻¹)	0.52 ± 0.53 ^a	1.36 ± 0.80 ^b	1.32 ± 1.01 ^b
Orthophosphate (mg L ⁻¹)	0.35 ± 0.42 ^a	0.40 ± 0.41 ^a	0.41 ± 0.31 ^a

Values are presented as mean ± standard deviation. Different letters in the same row indicate significant differences (*P* < 0.05) among treatments after the Kruskal–Wallis test (by multiple comparisons of mean ranks for all groups).

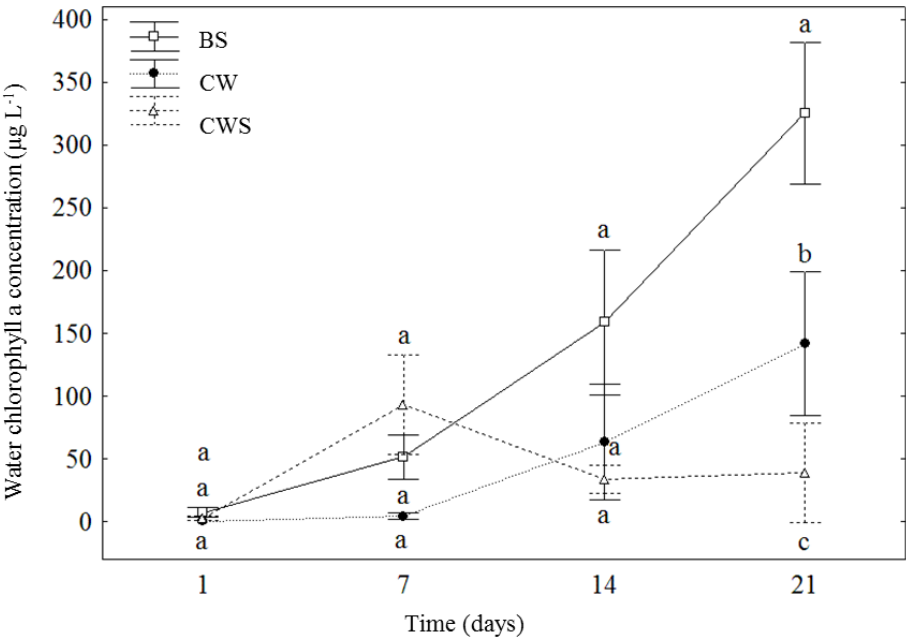


Figure 1. Mean concentrations of water chlorophyll *a* (mean ± standard deviation), over the 21-day experimental period, in the different treatments: BS (bioflocs and periphyton substrate); CWS (clear water with periphyton substrate); CW (clear water). Different letters indicate statistical difference (*P* < 0.05) between treatments after the Kruskal-Wallis test (by multiple comparisons of mean ranks for all groups) in each sampling period.



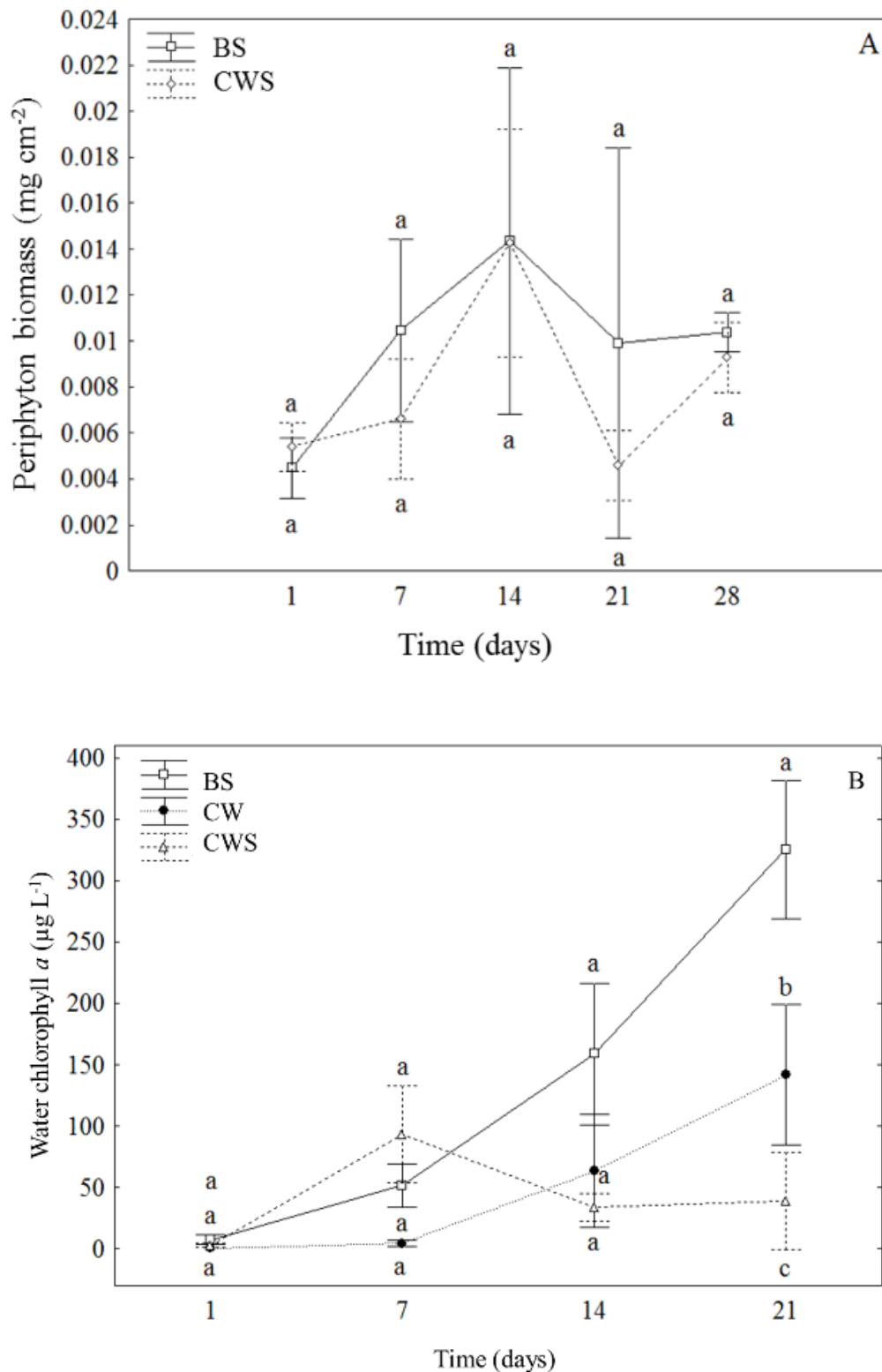


Figure 2. Mean concentrations of periphyton biomass (A) and water chlorophyll *a* concentration (B) (mean $\pm$ standard deviation), over the 21-day experimental period, in the different treatments: BS (bioflocs and periphyton substrate); CWS (clear water with periphyton substrate); CW (clear water). Different letters indicate statistical difference ($P < 0.05$) between treatments after the Kruskal-Wallis test (by multiple comparisons of mean ranks for all groups) in each sampling period.



Table 2. Final weight, feed conversion ratio (FCR), specific growth rate (SGR), and survival of *Mugil liza* juveniles after 30 days under the treatments: bioflocs with periphyton substrate (BS); clear water (CW); and clear water with periphyton substrate (CWS).

Treatments tested	Final weight (g)	FCR	SGR (% day ⁻¹)	Survival (%)
Biofloc + Substrate – BS	2.87 ± 0.7 ^a	1.82 ± 0.1 ^a	4.80 ± 0.2 ^a	100 ^a
Clear Water – CW	2.49 ± 0.6 ^b	2.21 ± 0.1 ^{ab}	4.38 ± 0.03 ^a	97.78 ± 3.9 ^a
Clear Water + Substrate – CWS	2.39 ± 0.6 ^b	2.30 ± 0.2 ^b	4.25 ± 0.3 ^a	93.33 ± 5.8 ^a

Values are presented as mean ± standard deviation. Different letters in the same row indicate significant differences (*P* < 0.05) after One-way ANOVA followed Tukey's test.

In biofloc systems, a decrease in alkalinity is typically associated with the assimilation of ammonia by heterotrophic bacteria and phytoplankton, as well as respiratory activity of aquatic organisms (Ebeling; Timmons; Bisogni, 2006). Notably, the highest alkalinity values were found in the biofloc and substrate treatments. This observation can be attributed to the uptake of nitrate by marine phytoplankton, which leads to increased alkalinity via algal biosynthesis (Ebeling; Timmons; Bisogni, 2006). This hypothesis is supported by the lower average nitrate concentrations and higher chlorophyll *a* levels observed in this treatment.

Recent studies have shown that integrating periphyton and bioflocs enhances nitrogen and carbon assimilation, thereby improving water quality and fish performance in polyculture systems (Guo *et al.*, 2022; Bell *et al.*, 2023). The combination of ecological substrates and carbon addition has been shown to improve nutrient utilization and overall system stability, which aligns with our observation of more efficient nitrogen assimilation in the presence of periphyton and bioflocs. Bell *et al.* (2023) reported that periphyton removed 32 % of nitrogen and 61 % of carbon from uneaten feed in marine recirculating systems, highlighting its effectiveness as a natural biofilter.

Additionally, periphyton plays a significant role

in oxygen dynamics within culture systems. According to Bell *et al.* (2023), the average oxygen production by periphyton was estimated to be 1.31 ± 0.20 mg O₂ / (L m² day). This level of production was sufficient to meet the respiratory needs of both microorganisms and fish under specific conditions. This finding may help clarify the adequate levels of dissolved oxygen noted in biofloc and substrate treatments, even with infrequent water exchanges.

The effectiveness of biofloc technology in aquaculture depends on the proper development and stabilization of microbial communities. Total suspended solids (TSS) are crucial indicators of microbial productivity, increasing due to the input of organic matter and the growth of microbes (Schneider *et al.*, 2006; Samocha *et al.*, 2007; Schryver *et al.*, 2008). However, TSS concentrations in the current study remained below the upper safety threshold of 400 mg L⁻¹ recommended by Avnimelech (2009), likely due to the relatively low feed inputs and the short duration of the experiment. Nevertheless, previous studies have demonstrated that *Mugil* species can be reared across a wide range of average TSS concentrations, from 89.79 ± 18.32 mg L⁻¹ (Holanda *et al.*, 2020) to 932 ± 309 mg L⁻¹ (Rocha *et al.*, 2022), including intermediate values (Rocha *et al.*, 2012;





Legarda *et al.*, 2019; Borges *et al.*, 2020; Pereira *et al.*, 2024). These findings highlight the substantial variability in the TSS levels tolerated within mullet culture systems.

However, previous studies have shown that it is possible to maintain *Mugil* species under average TSS concentrations ranging from $89.79 \pm 18.32 \text{ mg L}^{-1}$ (Holanda *et al.*, 2020) to $932 \pm 309 \text{ mg L}^{-1}$ (Rocha *et al.*, 2022), including intermediate values (Legarda *et al.*, 2019; Borges *et al.*, 2020; Pereira *et al.*, 2024). These results demonstrate a broad variability in total suspended solids (TSS) concentrations associated with mullet culture systems.

Lower total ammonia concentrations in the BS treatment were due to the presence of autotrophic and heterotrophic microorganisms in the bioflocs and biofilms on the artificial substrates. These microbial communities, particularly in systems with minimal water renewal, play crucial roles in nitrogen assimilation (Hargreaves, 2006; Sanli *et al.*, 2015). The efficiency of ammonia removal in heterotrophic-dominated systems is typically higher than that in autotrophic systems because of the faster establishment of heterotrophic bacteria (Hargreaves, 2006). Nevertheless, efficient ammonia removal has been reported in systems dominated by both nitrifying bacteria and periphyton (Thompson; Abreu; Wasielesky, 2002; Silva; Wasielesky Junior; Abreu, 2013). Moreover, microbial aggregates formed in biofloc systems act as highly assimilable protein sources when consumed by fish, recycling nutrients and improving feed conversion ratios, as reported by Alkhamis *et al.* (2023). This observation supports the significantly lower feed conversion ratio recorded in the BS treatment in the present study.

According to Alkhamis *et al.* (2023), biofloc systems significantly reduce nitrite concentrations and

increase microbial protein availability, thereby supporting improved feed conversion ratios and overall growth performance. Morais *et al.* (2020) also reported lower ammonia and nitrite concentrations in *Penaeus vannamei* culture systems with artificial substrates colonized with periphyton, indicating enhanced removal of nitrogen compound compared to biofloc-only systems.

In the present study, BS treatment was initiated with water from a mature biofloc system, which likely accelerated microbial community establishment, further supported by the continuous addition of dextrose as a carbon source. Conversely, the systems without biofloc (CW) required daily water exchange (~90 %) to maintain water quality, particularly for controlling elevated ammonia concentrations. Although CWS treatment included an extensive substrate area for periphyton growth, it was insufficient to establish an effective microbial community within the experimental timeframe, potentially due to water exchange disrupting periphyton stability and microbial colonization. The high-water exchange rate limits the accumulation and stability of periphyton, reducing its efficiency as a food source and biofilter. Intense exchanges tend to remove dissolved nutrients and particulate matter necessary for microbial colonization of substrates, reduce nutrient retention, and destabilize the periphyton matrix (Bondar-Kunze; Kasper; Hein, 2021), which is essential for periphyton colonization.

Furthermore, it is important to emphasize that without active control of the C:N ratio, heterotrophic nitrogen assimilation and the formation of microbial biomass are restricted. In BFT systems, supplemental carbon favors nitrogen retention in the culture medium, stimulating microbial communities that simultaneously increase periphyton productivity and provide natural food for fish (Ebeling; Timmons; Bisogni, 2006). This





microbial feedback loop helps explain the better performance observed in BS compared to that in CWS.

Although some trials have reported limited additive effects of integrating biofloc and periphyton (Cavalcante *et al.*, 2017), the benefits may vary depending on the species and system conditions. Ferreira *et al.* (2016) reported that the addition of artificial substrates for biofilm fixation did not enhance nitrogen metabolism in biofloc systems, even with supplementary feeding, suggesting that substrate effects may be context-dependent. In the present study, juvenile *M. liza* exhibited significantly higher weight gain and lower apparent feed conversion ratios in the BS. Although periphyton alone (CWS) did not result in improved mullet performance compared to the control (CW), both substrate treatments showed visible grazing of periphyton, and an integrated effect between the two, periphyton + bioflocs, provided significant benefits.

These findings align with those of Richard *et al.* (2010), who observed no significant growth improvements in *Liza aurata* reared with periphyton, even when periphyton biomass and chlorophyll *a* concentration were substantially higher than those in the present study. Azim *et al.* (2003) estimated that a daily periphyton biomass of 0.176 mg cm⁻² (dry matter) would be required to sustain tilapia growth exclusively, suggesting that periphyton alone may be insufficient when offered in lower amounts or in combination with formulated feeds.

The limited impact of periphyton in the CWS treatment may also be attributed to factors such as the inadequate nutritional value of the feed, low palatability or digestibility of the periphyton, and insufficient periphyton regeneration. Similar issues were highlighted by Silva *et al.* (2008), who observed selective feeding behavior in *Penaeus paulensis* shrimp, which potentially reduces the nutritional value of biofilms over

time. These limitations of periphyton-only systems have also been documented by David *et al.* (2022), who reported that Nile tilapia subjected to feed restriction in a periphyton-based system exhibited significantly lower growth performance when the periphyton biomass was insufficient.

Additionally, the high-water exchange rate in the CWS treatment may have impaired nutrient uptake and biomass accumulation by periphyton. These operational limitations explain why the presence of substrates alone (CWS) does not always translate into short-term productivity gains, as observed in the present experiment, with BS and CWS exhibiting low periphyton biomass compared to expectations for periphyton-based systems.

Milstein *et al.* (2018) emphasized the importance of managing water flow to optimize periphyton function, as excessive flow can remove nutrient-metabolizing microorganisms and suspended particles essential for nitrogen removal. In addition, the accumulation of organic matter in the BS treatment may have created anaerobic zones, which enhanced the denitrification process and contributed to the observed reductions in nitrite, nitrate, and phosphate (Neori; Krom; van Rijn, 2007).

Overall, the use of natural food sources, such as bioflocs and/or periphyton, in *M. liza* culture systems appears to enhance growth and feed efficiency. These findings support earlier studies (Cohen *et al.*, 2005; Avnimelech, 2007; Legarda *et al.*, 2019) that reported enhanced nutrient utilization and reduced feed conversion ratios when biofloc-based diets were used. A broader comparison with other mullet species, including *M. cephalus* (Haridas *et al.*, 2021; Garcés; Lara, 2023) and *M. curema*, indicates variability in feeding plasticity and efficiency in the utilization of microbial aggregates. Some species exhibit stronger responses to microbe-





based diets, potentially due to differences in digestive morphology or trophic adaptability. The present findings suggest that *M. liza* may require higher periphyton availability or a specific community composition (e.g., diatom-rich assemblages) to convert natural productivity into measurable growth.

Together, these observations reinforce the importance of integrating substrate availability, water exchange management, and C:N control when designing periphyton-based or hybrid BFT–periphyton systems. Future studies should consider reduced water exchange rates, longer rearing periods, and detailed characterization of periphyton communities to better understand species-specific responses and optimize natural productivity pathways for *M. liza*.

Conclusion

The results of this study demonstrate that integrating biofloc technology with artificial substrates for periphyton growth can significantly enhance water quality and growth performance in juvenile Lebranche mullet culture systems with minimal water exchange. Additionally, the biofloc with periphyton substrate treatment improved feed conversion efficiency among the tested systems, indicating better nutrient utilization and natural food availability. Although periphyton alone, tested in clean water with periphyton substrate (CWS) treatment, did not result in improved mullet performance compared to the control, the integration of periphyton with bioflocs provided significant benefits, demonstrating the importance of the system's ecological interactions for improving productivity.

These findings highlight the potential of using integrated culture systems to support sustainable and environmentally friendly aquaculture practices, particularly with low water exchange techniques. Future research should investigate the long-term stability of

these systems in *M. liza* aquaculture, as well as the optimal substrate types and economic viability of integrated approaches under commercial-scale conditions.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any potential conflicts of interest.

Ethical Statements

The authors confirm that the ethical guidelines adopted by the journal were followed by this work, and all authors agree with the submission, content and transfer of the publication rights of the article to the journal. They also declare that the work has not been previously published nor is it being considered for publication in another journal.

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All procedures involving animals followed ethical and animal welfare principles according to the Brazilian legislation.





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