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Production of Marine Shrimp Integrated with Tilapia at High Densities and in a Biofloc System: Choosing the Best Spatial Configuration

Mariana Holanda [1], Wilson Wasielesky, Jr. [1], Gabriele Rodrigues de Lara [2,*] and Luís H. Poersch [1]

[1] Marine Aquaculture Station, Institute of Oceanography, Federal University of Rio Grande—FURG, Rua do Hotel, n°2, Cassino, Rio Grande 96210030, Brazil
[2] Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Avenida Universidad 330, Valparaíso 2340000, Chile
* Correspondence: gabriele.rodrigues@pucv.cl

Abstract: Integrating marine shrimp and tilapia has been shown to be a viable alternative in a system based on biofloc, but there is no consensus on the spatial arrangement of farmed animals. The present study aims to (1) compare the performance of shrimp and fish in high density when subjected to polyculture (species in the same tank) and multitrophic (species in different tanks) arrangements, and (2) effects on water quality, especially on organic matter (biofloc). The experiment was carried out for 30 days, and three treatments with bioflocs were evaluated in triplicate: control: shrimp monoculture, polyculture: shrimp and tilapia in the same tank, and multitrophic: shrimp and tilapia in separate tanks. The results show that the best product configuration is the multitrophic system, where the biological control of bioflocs took place, and the best performance of the Pacific white shrimp *L. vannamei* and Nile tilapia *O. niloticus* was obtained. Tilapia, as an organic consumer, was effective in controlling bioflocs.

Keywords: organic consumer; IMTA; pacific white shrimp; tilapia; multitrophic aquaculture; suspended solids

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1. Introduction

Shrimp production is steadily increasing, and Pacific white shrimp *Litopenaeus vannamei* continues to be one of the most produced species in the world [1]. This activity tends to intensify using technologies that allow production in high stocking densities, such as the biofloc system. Biofloc technology (or biofloc system or BFT system—Biofloc Technology system) is characterized by super intensive production of aquatic organisms, minimal or no water renewal, and cycling of the nutrients in the tank itself by the stimulation of a microbiota that forms microbial aggregates [2]. Besides maintaining water quality in the system, as the ammonia is removed by heterotrophic bacteria [3], these aggregates also serve as food for the reared animals [4]. The removal of toxic nitrogen compounds in the biofloc system is the key to the success of this technology in aquaculture.

In closed aquaculture systems, ammonia is excreted by aquatic animals and, in the case of using biofloc technology, is assimilated by heterotrophic bacteria after the addition of an organic carbon source [5]. For each gram of ammonia metabolized by heterotrophic bacteria, nearly 8 g of total suspended solids (the bioflocs) are produced [3], producing an accumulation of suspended solids in the system, which may negatively affect the reared organisms. Samocha and Prangnell [6] recommend that for shrimp production, the total suspended solids (TSS) concentrations should not exceed 350 mg L^{-1}. For this purpose, mechanical clarifiers, which function as particle decanters for the removal of particulate organic matter by gravitational action in a slow radial water flow, are generally used [7].

This equipment is relatively easy to operate but requires extra space in the facility and depends directly on the type of biofloc [8], as the size and nature of the biofloc will directly influence the speed and efficiency of sedimentation [9], and clarifiers may not be as efficient in some specific cases. Furthermore, recent studies have shown that clarifiers can modify the structure of the microbial community present in bioflocs [10] and may open doors for opportunistic microorganisms in the culture system [11].

Another way to remove the excess of TSS is by integrating other species of interest in aquaculture, which consumes these particles, reducing the TSS accumulation. This technique, designated Integrated Multitrophic Aquaculture (IMTA), has been gaining importance in recent decades due to the need to boost environmentally friendly technologies while maintaining productivity in aquaculture systems. IMTA is based on the concept that residues, such as unconsumed feed, feces, and metabolic excretion of one species, are useful to feed species of different trophic levels in the same culture environment [12,13]. Therefore, the coupling of biofloc and IMTA systems could be an effective way to deal with the accumulation of TSS generated by the rapid formation of biofloc and diversify the production, taking advantage of the nutritional benefits of the aggregates.

The Nile tilapia *Oreochromis niloticus* (hereafter referred to as tilapia) is a potential candidate to compose the multitrophic system with shrimps. Tilapia has been considered a species of great interest [14,15] for its rusticity, tolerance to a wide range of salinity, and because it presents better growth rates when cultivated in environments rich in natural productivity. Furthermore, as an omnivorous species, tilapia can consume the bioflocs of the system, as already observed in the integrated system with Pacific white shrimp [16–18].

The major ecological advantage of the IMTA system is the conversion of waste into protein, such as excess TSS from shrimp production in the biofloc system. This ecological advantage can improve the production system. Some studies have already successfully reported the integration of tilapia to *L. vannamei* culture in the biofloc system, even highlighting its role as an organic extractivist in the system, consuming the biofloc present in the system and transforming this excess into fish biomass [18]. However, there is no study that identifies which is the best spatial arrangement to integrate tilapia into the system, which can combine the biological advantage and the economic advantage of this integration. Therefore, the present work investigates the use of two different spatial conformations: tilapia in the same tank as the shrimp and tilapia in a separate tank, to optimize the integrated culture of white shrimp and tilapia in order to obtain a better zootechnical performance of the shrimp and higher consumption of TSS by the tilapia.

2. Materials & Methods

2.1. Study Location and Origin of the Animals

The study was conducted at the Marine Aquaculture Station (EMA), Institute of Oceanography of the Federal University of Rio Grande (FURG), located in Cassino beach, Rio Grande, RS, southern Brazil. The study lasted 30 days. Juvenile tilapias (*O. niloticus*) were provided from a commercial fish farm (Piscicultura Amorim, Rio Grande do Sul, Brazil). Before the experiment, the tilapia juveniles were acclimatized in a biofloc system for two weeks. Shrimp juveniles of *L. vannamei* were obtained from a commercial hatchery, and they were acclimated in a biofloc system for 2 weeks. The experiment was approved by the Ethics and Animal Welfare Committee of FURG (Case number 23116.005895/2016-42).

2.2. Experimental Design

The experiment consisted of three treatments in triplicate, randomly distributed: MONO—monoculture of shrimp; IMTA ST—integrated tilapia and shrimp in the same tank; IMTA DT—integrated culture tilapia and shrimp in different tanks. In all treatments, the shrimp density was 204 shrimp m^{-3} (45 shrimp $tank^{-1}$), and the fish density was 100 fish m^{-3} (18 fish $tank^{-1}$). At the beginning of the experiment, shrimp and fish had initial weights of 2.67 $\pm$ 0.17 g and 7.44 $\pm$ 1.18 g, respectively. Shrimps and fish were individually weighed before being placed in the experimental units. A biofloc inoculum

was used in the experimental units stocked with mature bioflocs, corresponding to 20% of the volume of the experimental tank (44 L of inoculum + 176 L of seawater), according to Krummenauer et al. [19]. The inoculum was taken from a 60-day culture of *L. vannamei* with a density of 400 m^{-3} in 35 m^3 tanks from *L. vannamei* cultivation in a greenhouse. Molasses was used as a carbon source in the initial phases of cultivation for ammonia control. The inoculum's initial concentration was TSS ± 350 mg L^{-1} and ±70 mg L^{-1} of nitrate, indicating that the nitrification process was taking place in this matrix tank.

2.3. Recirculation System

A blend of seawater (previously chlorinated and neutralized with ascorbic acid, according to Roselet et al. [20]) and fresh water from the local supply company was prepared to achieve salinity 15. In IMTA DT treatment, water from each fish tank was constantly circulated to tanks containing shrimp with a submerged pump (Sarlo Better®, SB 1000C, São Caetano do Sul, São Paulo, Brazil, 13 W and 720 L h^{-1} flow rate) and returned to the fish tanks by gravity. Twelve tanks with 220 L useful volume (bottom area of 0.36 m^2) were used. Water was constantly aired through air stones (two air stones per tank with 3.4 cm^2 and 13 cm in length) to maintain the suspension of the biofloc and adequate dissolved oxygen concentration. The stability of water temperature was obtained using heaters with thermostats (Stealth, ETP250, Ohio, USA, 250 W).

2.4. Feed Management

The animals were fed twice a day (9:00 a.m. and 5:00 p.m.). Shrimp feeding was carried out following the methodology described by [21] with commercial feed containing 38% crude protein (Poty Active 38, 1.6 mm, Guabi Aqua, Guabi Nutrition and Animal Health S.A., Campinas, São Paulo, Brazil). Tilapias were fed with a 36% crude protein commercial feed (Guabitech Mirim QS 2–3 mm, Guabi Aqua, Guabi Nutrition and Animal Health S.A.). The amount of food provided to tilapias was restricted to 1% of the tank's biomass in order to stimulate the consumption of biofloc by the fish. The total feed used (shrimp + fish) during the experiment was calculated using the average of the sum of feed that went into each replicate of each treatment. The nitrogen input was calculated according to [3], taking into account 38% crude protein in the shrimp feed and 36% in the fish feed.

2.5. Shrimp and Fish Performance

The growth of the shrimp (15 shrimp/tank) and fish (10 fish/tank) was assessed by performing weekly and biweekly biometrics, respectively, using a digital scale with 0.01 g precision (Marte®científica AS2000; Santa Rita do Sapucaí, MG, Brazil). In these instances, the amount of food provided to the animals was adjusted according to the increase in biomass in the tanks. To perform the biometrics, the tilapias were anesthetized with 50 mg L^{-1} benzocaine hydrochloride in an aqueous solution, and after complete recovery, they were returned to the experimental units. At the end of the experimental period, the remaining animals were counted for the determination of survival and growth rates. The parameters analyzed were: Survival (%) = (final shrimp or fish number/initial shrimp or fish number) ×100; Final mean weight (g): ∑ final weight of live animals (g)/total number of animals; total biomass (g): ∑ final weight of all live animals (g); feed conversion ratio (FCR) = offered feed (g)/(final biomass (g)—initial biomass (g)); productivity (kg m^{-3}): [(final biomass (kg) − initial biomass (kg)) × 1000]/useful tank volume (L); specific growth rate (g $week^{-1}$): weight gain (g)/number of weeks.

For IMTA DT and IMTA ST treatments, some parameters were calculated considering the system integrated by shrimp and fish. The parameters analyzed were:

1. System productivity (Kg m^{-3}): (Fsb + Ffb) − (Isb + Ifb)/Total useful volume (m^3), where: Fsb is final shrimp biomass; Ffb is final fish biomass; Isb is initial shrimp biomass; Ifb is initial fish biomass; The total volume considered in the IMTA DT

treatment was 0.44 m^3 and the total volume considered in the IMTA ST treatment was 0.22 m^3;

2. Feed conversion rate (FCR) = (Fos+Fof)/(Fsb + Ffb) − (Isb − Ifb), where: Fos = feed offered to the shrimp; Fof = Feed offered to the fish
3. Total final biomass (g): Σ final biomass of shrimps and biomass of the fish.

2.6. Water Quality

Dissolved oxygen, temperature, and pH were checked twice a day after feeding the organisms with a multiparameter probe (YSI, model Pro-20, Ohio, USA) and a benchtop pH meter (Mettler Toledo, FEP20, Brazil), respectively. The salinity was verified once a week with an optical refractometer (ATC, RTP-20ATC, São Paulo, Brazil).

Turbidity and total suspended solids (TSS) were analyzed twice a week, turbidity with a portable turbidimeter (Hach 2100P, Hach Company; Loveland, CO, United States) and total suspended solids (TSS) were measured according to the methodology described by [22]. Alkalinity was verified twice a week following the methodology proposed by APHA (2017). Total ammoniacal nitrogen (TAN) and nitrite ($N–NO_2$) were measured daily according to the methodology described by [23] and [22], respectively. Organic fertilization with molasses occurred whenever concentrations of total ammoniacal nitrogen (TAN) exceeded 1mg L^{-1}, according to [3,5]. Nitrate ($N–NO_3$) and orthophosphate ($P–PO_4{}^3$) were measured once a week, following the methodology proposed by [22]. When pH and alkalinity values dropped below 7.2 and 120 mg CaCO3 L^{-1}, respectively, hydrated lime [$Ca(OH)_2$] was used according to [24] for correction. Fresh water was added weekly to compensate for the evaporation in the tanks. The water quality parameters and addition of hydrated lime in the IMTA DT treatment tanks were measured in both tanks and the data presented are an average of these measurements.

2.7. Statistical Analysis

All data were analyzed by one-way analysis of variance (ANOVA), except the zootechnical performance data of the tilapia that were analyzed by the t-test. Homoscedasticity and normality were analyzed by the Levene test and the Kolmogorov–Smirnov test, respectively. The Tukey test was applied when significant differences were detected ($p < 0.05$). Survival results were arcsine transformed prior to the analysis [25].

3. Results

3.1. Water Quality

The mean temperature (>28 °C), dissolved oxygen (>6 mg L^{-1}), and pH (>7.9) values were similar in all treatments. In addition, the mean values of ammonia, nitrite, nitrate, and orthophosphate did not present significant differences among the treatments (Table 1). The mean alkalinity, on the other hand, was significantly higher in the IMTA DT than in the IMTA ST and MONO treatments (Table 1). The average TSS and turbidity values were higher in the MONO treatment and lowered in the IMTA DT. The IMTA ST treatment did not differ statistically from the last two (Table 1).

subsectionShrimp and Tilapia Performances

Survival, final average weight, weekly weight gain, final biomass, and shrimp productivity were higher in MONO and IMTA DT treatments, with no significant difference between them, and lower in IMTA ST treatment. Shrimp FCR was lower in MONO and IMTA DT treatments and did not present significant differences between these two treatments. The FCR was significantly higher in IMTA ST treatment.

The opposite was observed for tilapia. Final average weight, weekly weight gain, final biomass, and productivity were higher in IMTA ST treatment and lower in IMTA DT, while FCR was lower in IMTA ST and higher in IMTA DT treatment.

For the system as a whole (shrimp and tilapia together), the total final biomass was alike in IMTA DT and IMTA ST, with significantly lower values in MONO treatment. The system productivity was significantly higher in IMTA ST than in Mono and IMTA DT

treatments. The system FCR, on the other hand, was higher in MONO than in IMTA DT and IMTA ST treatments, which did not differ significantly between them (Table 2).

Table 1. Water quality parameters (mean±standard deviation) of *L. vannamei* and *O. niloticus* during the experiment in the different treatments: MONO, IMTA DT, and IMTA ST.

	MONO	**IMTA DT**	**IMTA ST**
Temperature °C	29 ± 2 (25.7–31.2)	29 ± 2 (25.7–31.3)	28 ± 2 (25–31.1)
DO (mg L^{-1})	6 ± 0 (5.2–7.3)	7 ± 0 (5.5–7.3)	6 ± 0 (5.2–7.3)
pH	8 ± 0 (7.5–8.0)	8 ± 0 (7.7–8.2)	8 ± 0 (7.5–8.1)
Alkalinity (mg $CaCO_3$ L^{-1})	125 ± 45 ^{ab} (70–255)	150 ± 49 ^{a} (60–270)	115 ± 43 ^{b} (50–250)
TSS (mg L^{-1})	294 ± 146 ^{a} (115–540)	211 ± 79 ^{b} (95–340)	247 ± 106 ^{ab} (140–455)
Turbity (NTU)	127 ± 70 (44–244)	110 ± 57 (29–188)	101 ± 50 (34–217)
TAN (mg L^{-1})	0.2 ± 0.3 (0–1.0)	0.1 ± 0.2 (0–0.84)	0.3 ± 0.8 (0–3.57)
NO_2-N (mg L^{-1})	0.9 ± 0.5 (0.21–1.89)	1.2 ± 0.7 (0.24–2.73)	1.5 ± 1.6 (0.15–5.25)
NO_3-N (mg L^{-1})	31.3 ± 27.6 (6–66)	32.3 ± 33.9 (6–66)	31.5 ± 29.7 (6–75)
PO_4^3 (mg L^{-1})	2.2 ± 2.1 (0.2–4.2)	2 ± 1.9 (0.2–4.2)	2.1 ± 2.1 (0.2–4.6)

Different letters in the same row represent significant differences ($p < 0.05$) between treatments after one-way ANOVA followed by Tukey's test. OD (dissolved oxygen), TSS (total suspended solids), TAN (total ammonia). In parentheses, the minimum and maximum values are represented.

Table 2. Zootechnical performance (mean ± standard deviation) of *L. vannamei* and *O. niloticus* during the experiment in the different treatments: MONO (shrimp monoculture), IMTA DT (integrated tilapia and shrimp in the same tank), and IMTA DT (integrated culture tilapia and shrimp in different tanks).

	MONO	**IMTA DT**	**IMTA ST**
Shrimp			
Survival (%)	98 ± 2 ^{a}	96 ± 7 ^{a}	79 ± 16 ^{b}
Final mean weight (g)	8.1 ± 0.5 ^{a}	8.0 ± 0.3 ^{a}	5.1 ± 0.8 ^{b}
FCR	1.4 ± 0.1 ^{a}	1.4 ± 0.1 ^{a}	3.1 ± 1.3 ^{b}
WWG (g week $^{-1}$)	1.3 ± 0.1 ^{a}	1.3 ± 0.1 ^{a}	0.6 ± 0.2 ^{b}
Final biomass (g)	356 ± 23 ^{a}	353 ± 15 ^{a}	226 ± 38 ^{b}
Yield (kg m^{-3})	1.1 ± 0.2 ^{a}	1.1 ± 0.0 ^{a}	0.5 ± 0.2 ^{b}
Tilapia			
Survival (%)	-	92 ± 7	88 ± 0
Final mean weight (g)	-	15.2 ± 1.2 ^{b}	27.2 ± 2.4 ^{a}
FCR	-	0.6 ± 0.0 ^{b}	0.3 ± 0.0 ^{a}
WWG (g week $^{-1}$)	-	2.1 ± 0.3 ^{b}	4.7 ± 0.5 ^{a}
Final biomass (g)	-	274 ± 22 ^{b}	490 ± 44 ^{a}
Yield (kg m^{-3})	-	0.7 ± 0.1 ^{b}	1.6 ± 0.2 ^{a}
Shrimp + Tilapia			
Total feed use (g)	174.4 ± 12.6 ^{b}	214.3 ± 7.7 ^{a}	200.6 ± 11.5 ^{ab}
Total nitrogen input (g)	10.6 ± 0.7 ^{b}	12.9 ± 0.4 ^{a}	12 ± 0.7 ^{ab}
Total nitrogen input (g m^{-3})	48.2 ± 3.5 ^{b}	29.27 ± 1.0 ^{a}	54.7 ± 3.1 ^{b}
Total final biomass (g)	356.4 ± 22.6 ^{b}	627.1 ± 37.9 ^{a}	716.1 ± 46.6 ^{a}
Total Yield (kg m^{-3})	1.1 ± 0.2 ^{b}	1.0 ± 0.0 ^{b}	2.0 ± 0.0 ^{a}
FCR	1.4 ± 0.1 ^{a}	1.0 ± 0.1 ^{b}	0.8 ± 0.01 ^{b}

Different letters after mean values ± standard deviation in the same row represent significant differences ($p < 0.05$) among treatments after one-way ANOVA followed by Tukey's test. FCR = Feed conversion rate, WWG: weekly weight gain. For system calculations, a useful volume of 0.22 m^3 was considered for the Mono and IMTA ST treatments and 0.44 m^3 for the IMTA DT treatment.

In terms of feed use, there was a higher input of feed in the treatments where tilapia was present and a lower input in the Mono treatment. The same pattern was observed for the total nitrogen (N) input in the ponds. When the N-input m^{-3} was calculated, IMTA DT showed the lowest nitrogen input m^{-3} and Mono and IMTA ST showed the highest N-input m^{-3}, with no significant difference between the latter two (Table 2).

4. Discussion

4.1. Water Quality

Integrating tilapia with *L. vannamei* culture either in the same pond or in separate ponds did not affect water quality parameters for either cultured species [18,26–28].

Nitrogen compounds also did not undergo any significant variation during the experiment as a result of the use of biofloc inoculum [19]. These values did not reach toxic levels for shrimp or tilapia [29–33]. Despite the high nitrate values at the end of the experimental period, the pH values were above 7.9, without acidification of the medium, because of the successive pH and alkalinity corrections during the experiment, as described by Furtado et al. [24]. Despite the decrease in the mean value of TSS, the presence of tilapia did not affect the microbial community in the biofloc system since there was no significant difference in the mean value of nitrogen compounds with the presence of fish.

Total suspended solids (TSS) and turbidity values were kept at acceptable levels for both species [34,35]. Despite the higher input of feed and, consequently, nitrogen in the treatments where tilapia was present, the average TSS values were significantly higher where tilapia was not present, even though the biomass produced with shrimp + fish integration was higher in these treatments. That is, the higher nitrogen input did not reflect in bacterial biomass accumulation as expected in a shrimp monoculture biofloc system [3,36], suggesting the consumption of solids by tilapia, which demonstrates an ecological benefit.

Despite higher feed input in IMTA DT and IMTA ST treatments when compared to the MONO treatment, orthophosphate levels were not affected by the presence of the fish. According to Poli et al. [37], tilapia can retain up to 223% more phosphorus in an integrated system with shrimp than in a shrimp monoculture. Therefore, it can be shown that the presence of tilapia in integration with shrimp is a viable alternative for greater recycling of nutrients in the culture system.

4.2. Shrimp and Tilapia Performances

The presence of tilapia in the integrated system with shrimp in the same pond negatively affected the husbandry performance of the shrimp. There are some theories for the favored husbandry performance of tilapia: (1) due to the high biomass (shrimp + fish) in the same pond. Shrimp growth is density dependent [27,36], i.e., the higher the stocking density, the lower the weight of the individual. The density of shrimp combined with the density of fish in a small area (bottom area of 0.36 m^2), together with a total initial biomass 2.2 times higher than the shrimp that were grown in the Mono and IMTA DT treatments, contributed to the worse zootechnical performance of the shrimp in the tilapia in treatment; (2) the tilapia may have ingested the shrimp feed or ingested the shrimp themselves: This explains the better performance of the tilapia when grown in the same pond as the shrimp.

The total suspended solids were a possibly significant source of energy for the tilapia, as the FCR was 0.6 in IMTA DT and 0.3 in IMTA ST treatments. Poli et al. [37] observed low feed conversion rates for tilapia (from 0.21 to 0.24) when fed with 1% of the biomass and shrimp FCR values close to those found in this study.

In IMTA ST treatment, half of the FCR was found, with double the growth and productivity of tilapia when compared to IMTA DT. This result shows that if we increase the feeding rate of the tilapia when growing in separate tanks from the shrimp, tilapia will possibly still exert its ecological role in the integrated system as a consumer of bioflocs and show higher growth rates, as seen in the IMTA ST treatment. Even though undernourished, the tilapia, consuming the flake, showed weekly growth expected for culture in the biofloc

system. Similar results of biofloc consumption by tilapia when growing in integrated shrimp culture have been observed in other studies [15,38–40].

According to Troell et al. [13], IMTA is the only practical remediation approach with the prospect of generating revenue by diversifying production, whereas all other biomonitoring approaches usually only represent additional costs to the producer. The goal is to increase long-term sustainability and profitability per culture unit (and not by species, as practiced in monocultures) by recovering some of the nutrients and energy lost in monocultures, turning them into additional products with commercial value. The integrated culture of white shrimp and tilapia is a way to diversify production and improve the profitability of aquaculture. The final biomass of IMTA DT and IMTA ST treatments doubles the final biomass of shrimp monoculture, justifying the use of IMTA and proving it to be an ecologically and economically viable alternative. It is worth noting that it was possible to increase the total biomass produced in the IMTA DT treatment by 175% without compromising the specific growth rate and feed conversion of the shrimp.

5. Conclusions

Tilapia proved to be efficient in consuming and maintaining TSS levels in the integrated system with shrimps and did not affect water quality. The zootechnical performance of shrimp *L. vannamei* was impaired when growing in the same tank as the tilapia. Therefore, the best integrated super-intensive system for *L. vannamei* and *O. niloticus* in the tilapia-to-shrimp ratio of 0.49 is in separate tanks, as observed in the IMTA DT treatment. It is recommended that under the experimental conditions tested, shrimp and fish be grown in separate tanks so that the producer can have two final products for marketing at the end of the production cycle without negatively affecting shrimp production.

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