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APLICAÇÃO DA AQUICULTURA MULTITRÓFICA PARA PRODUÇÃO DO CAMARÃO-BRANCO-DO-PACÍFICO EM SISTEMA DE BIOFLOCOS

Tese submetida ao Programa de Pós-graduação em Aquicultura da Universidade Federal de Santa Catarina para a obtenção do Grau de Doutor em Aquicultura

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Aplicação da aquicultura multitrófica para produção do camarão-branco-do-Pacífico em sistema de bioflocos

Por

MOISÉS ANGEL POLI

Esta tese foi julgada adequada para a obtenção do título de

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“É fundamental diminuir a distância entre o que se diz e o que se faz, de tal forma que, num dado momento, a tua fala seja a tua prática”.

Paulo Freire

RESUMO

O objetivo deste projeto foi aplicar os conceitos da aquicultura multitrófica integrada (AMTI) ao cultivo de camarão-branco-do-pacifico (*Litopenaeus vannamei*) em sistema de bioflocos. Para compor o sistema AMTI foram utilizadas como espécie consumidora orgânica e inorgânica a tilápia (*Oreochromis niloticus*) e sarcocórnia (*Sarcocornia ambigua*), respectivamente. Dois experimentos foram realizados e em ambos os camarões foram alimentados seguindo uma tabela de alimentação, enquanto as tilápias foram alimentadas com apenas 1% da biomassa, estimulando que buscassem comida nos bioflocos. O primeiro trabalho teve o objetivo de estabelecer a melhor relação de biomassa de tilápia em relação a biomassa camarão. Através desse trabalho conclui-se que é possível que essa relação chegue a 41 % sem prejuízo para o sistema. Além disso, a retenção de nitrogênio e fósforo nos animais aumentou 27,9 % e 223 % respectivamente com a integração da tilápia ao cultivo de camarão em sistema de bioflocos em relação ao monocultivo de camarão em sistema de biofloco. O segundo trabalho teve como objetivo avaliar o desenvolvimento do sistema AMTI no cultivo de camarão em sistema de bioflocos. Para isso, foram avaliados um sistema AMTI contendo camarões, tilápias e sarcocórnia e comparado sistema integrado de camarões e tilápias (controle), ambos em sistema de bioflocos. Nesse trabalho o sistema AMTI obteve uma produtividade em 17,2 % maior em relação ao sistema integrado. A retenção de nitrogênio e fósforo não diferiu entre os tratamentos sendo em média 33,9 ±1,2 % e 15,0 ± 1,2 % respectivamente. Apesar disso a concentração de nitrato foi menor no tratamento AMTI em relação ao tratamento controle. Como conclusão é possível dizer que a aplicação de conceitos da AMTI no cultivo de camarão em sistema de biofloco pode aumentar a produtividade e o desempenho ecológico do sistema.

Palavras chaves: Aquicultura; *Litopenaeus vannamei*; Sarcocórnia; Tilápia; Sustentabilidade.

ABSTRACT

The aim of this project was to apply the concepts of integrated multitrophic aquaculture (IMTA) to the Pacific white shrimp (*Litopenaeus vannamei*) rearing in biofloc system. In order to compose the AMTI system, tilapia (*Oreochromis niloticus*) and sarcocornia (*Sarcocornia ambigua*) were used as organic and inorganic species, respectively. Two experiments were carried out and in both shrimps were fed following a feed table, while the tilapia were fed with only 1% of the fish biomass, stimulating the tilapia to seek food in the biofloc. The first work had the objective of establishing the best ratio of tilapia biomass to biomass shrimp. Through this work it is concluded that it is possible that this ratio reaches 41% without prejudice to the system. In addition, retention of nitrogen and phosphorus in the animals increased 27.9% and 223%, respectively, with the tilapia and shrimp integration in a biofloc system relative to the shrimp monoculture in biofloc system. The second work had as objective to evaluate the IMTA system apply to the shrimp rearing in biofloc system. The IMTA system containing shrimps, tilapia and sarcocornia was evaluated and compared to the integrated system of shrimp and tilapia (control), both in a biofloc system. The result show that the IMTA system achieved a 17.2% higher productivity compared to the integrated system. Nitrogen and phosphorus retention did not differ between treatments, with a mean of 33.9 ± 1.2% and 15.0 ± 1.2%, respectively. However, the nitrate concentration was lower in the AMTI treatment in relative to the control treatment. In conclusion, it is possible to say that the IMTA concepts applied to the shrimp rearing in a biofloc system can increase the yield and the ecological performance of the system.

Keywords: Aquaculture. *Litopenaeus vannamei*. Sarcocorina. Tilapia. Sustainability.

SUMÁRIO

1 INTRODUÇÃO GERAL

A aquicultura atingiu um marco histórico em 2014 ao se tornar a principal provedora de pescados para consumo humano, ultrapassando a pesca (FAO, 2018). Dentro da aquicultura, o cultivo de camarão marinho representa um setor economicamente importante, tendo gerado em 2016 uma receita de pouco mais de 32 bilhões de dólares (FAO – FISHSTAT, 2018). No Brasil, o cultivo do camarão-branco-do-pacífico (*Litopenaeus vannamei*), representa quase a totalidade do camarão cultivado (FAO, 2018).

Apesar de dados positivos, a aquicultura é alvo de críticas, devido a fatores como a ocupação de grandes áreas, geração de efluentes com elevada carga de nutrientes no ambiente (NAYLOR, 2000; PEREZ, NIRCHIO, GOMEZ, 2000). Portanto, o uso correto de recursos hídricos, o cuidado com os ambientes adjacentes e o conflito pela terra serão grandes desafios da aquicultura num futuro próximo (NAYLOR, 2000).

Mediante a essas necessidades o setor da carcinicultura tem buscado sistemas mais produtivos, biosseguros e com baixo impacto ambiental. Assim surgiu o cultivo em sistema de bioflocos para camarões e peixes, elevando a densidade de estocagem e trabalhando com pouca ou nenhuma renovação de água (CRAB et al., 2007).

1.2 Cultivo em sistema de bioflocos:

O cultivo de organismos aquáticos está limitado por fatores físicos e químicos da água, principalmente pela concentração de oxigênio dissolvido e dos compostos nitrogenados. Em sistemas intensivos a remoção dos compostos nitrogenados geralmente é realizada por filtros biológicos acoplados ao sistema de cultivo ou, de maneira mais comum, através de renovação de água.

Paralelamente a esse modelo, existem outras formas de se reciclar os nutrientes e simultaneamente produzir alimento natural para os animais de cultivo. Neste sentido, existem modelos de cultivo baseados no uso de perifíton, mais apropriado para modelos extensivos, como também modelos de cultivo baseados em bioflocos bacterianos geralmente utilizados em sistemas intensivos (CRAB et al., 2007). Os bioflocos são conglomerados de algas, protozoários, bactérias, detritos orgânicos e inorgânicos (CRAB et al., 2007), que além de controlar os compostos nitrogenados servem de suplemento alimentar para os animais de cultivo (CRAB et al., 2007; AVNIMELECH, 2007; AZIM; LITTLE, 2008; BURFORD et al., 2004; SCHRYVER et al., 2008; ASADUZZAMAN et al.,2010).

Com base no tratamento de efluentes através dos “lodos ativados”, a tecnologia de bioflocos foi desenvolvida para controlar os compostos nitrogenados (tóxicos para os animais de cultivo), dentro do próprio ambiente de cultivo. O sistema pode ser manejado com bactérias nitrificantes que oxidam a amônia até nitrato, ou através da adição de uma fonte externa de carbono que irá favorecer o crescimento de bactérias heterotróficas que assimilam a amônia e o ortofosfato, transformando-os em biomassa celular (AVNIMELECH, 1999). Para que isso aconteça a aeração deve ser constante para o fornecimento do oxigênio e também para suspensão da matéria orgânica.

Essa forma de controle de nitrogenados permite que o cultivo seja realizado com pouca ou nenhuma troca de água. Isso lhe dá algumas vantagens como:

•Aumentar a biossegurança evitando a entrada de patógenos (SAMOCHA et al., 2007);

•Prevenir o escape de animais do cultivo (HARGREAVES, 2006);

•Evitar a contaminação de ambientes adjacentes (HARGREAVES, 2006);

• Otimizar a utilização dos recursos naturais como utilização da terra e água ao permitir o aumento significativo da densidade de estocagem e consequentemente aumento da produtividade do cultivo (AVNIMELECH, 2012).

Há também uma grande evolução em termos do uso dos recursos hídricos. No sistema de biofloco é possível produzir 1 kg de camarão em pouco mais de 100 litros de água (OTOSHI et al., 2007), enquanto sistemas tradicionais utilizam cerca de 64.000 litros para produzir 1 kg de camarão (HOPKINS et al., 1995).

Apesar de reduzir a quantidade de efluente de cultivo, podemos citar como desvantagens a constante geração de sólido. Uma vez que começamos a adicionar ração e uma fonte de carbono, há um aumento constante de sólidos suspensos totais (SST) que, quando presentes em excesso, devem ser removidos para não comprometer a saúde e o desempenho zootécnico dos animais de cultivo (AVNIMELECH, 2012; GAONA et al., 2011; SCHVEITZER et al., 2013). O processo de remoção normalmente é feito com o uso de decantadores ou sistemas de sifonamento do tanque.

O excesso de sólido ao ser removido torna-se um efluente rico em nutrientes, especialmente N e P, podendo contaminar os ambientes adjacentes. Por isso, o grande desafio para tornar esse sistema ainda mais sustentável é reaproveitar melhor os nutrientes presentes nos bioflocos e consequentemente diminuir o efluente gerado.

Nesse contexto, a aplicação dos conceitos da aquicultura multitrófica integrada (AMTI) se apresenta como uma das possibilidades. Na AMTI espécies de diferentes níveis tróficos são cultivadas visando aproveitar ao máximo os nutrientes (orgânicos e inorgânicos) presentes no cultivo da espécie principal.

1.3 Aquicultura multitrófica integrada AMTI

A integração de espécies de diferentes níveis tróficos em um mesmo ambiente de cultivo é conhecida como aquicultura multitrófica integrada (AMTI) (NEORI et al., 2004). Reconhecida por gerar avanços ambientais, econômicas e sociais (ALLSOPP et al., 2008), a AMTI é um elemento chave para uma aquicultura sustentável (CHOPIN et al., 2006; BARRINGTON et al., 2010).

Conceitualmente, a AMTI consiste em reciclar os resíduos do cultivo de uma espécie para converter-se em aportes na forma de alimento ou fertilizantes para outra (CHOPIN et al., 2001).

O conceito de IMTA é bastante flexível e pode ser aplicado a sistemas abertos em alto mar, em fazendas com tanques escavados e até sistemas fechados (FAO, 2009). O que é importante é que os organismos associados sejam escolhidos baseados na sua função ecológica e de maneira não menos importante, pelo seu valor econômico ou como um potencial econômico (FAO, 2009).

Atualmente os sistemas de IMTA mais avançados e conhecidos são os cultivos em tanque-redes no mar os quais tem a seguinte composição: Peixe (geralmente salmão); espécies consumidoras de material orgânico em suspenção como os moluscos; macroalgas como consumidores de material inorgânico dissolvido (CHOPIN et al., 2001). Porém, esses cultivos podem ser ainda mais elaborados ganhando outros componentes como: consumidores de matéria orgânica do fundo como pepinos-do-mar; poliquetas no fundo do tanque-rede; peixes bentônicos (FAO, 2009).

A integração de espécies de diferentes níveis tróficos não é um conceito novo na aquicultura. Países asiáticos, responsáveis por mais da metade da produção aquícola, praticam a aquicultura integrada há séculos (Edwards, 1992, Quian et al., 1996). Fazendas integradas são uma pratica antiga na China, porém a partir de 1949, um novo conceito de fazendas com alta produtividade transformou essa realidade para fazendas de monocultura (CHOPIN et al., 2001). Apesar de produtividades altas, fazendas de monocultivo muito próximas umas das outras passaram a gerar problemas ambientais e por profiláticos, consequentemente econômicos. Por isso há nesses países uma retomada nos conceitos de fazendas integradas a fim de viabilizar a atividade de maneira sustentável.

Porém, a grande transformação deverá acontecer quando os consumidores exigirem produtos produzidos de maneira sustentável (CHOPIN et al., 2001).

Pouco se sabe sobre a Aquicultura Multitrófica Integrada aplicada ao sistema de bioflocos. Apenas um trabalho cientifico foi encontrado descrevendo a integração de uma espécie de planta conhecida como espinafre-da-água (*Ipomoea aquatic*) e uma espécie de peixe ornamental *Scatophagus argus* integrada ao cultivo de camarão em sistema de bioflocos. Os autores encontraram o dobro de biomassa produzida no tratamento com sistema integrado em relação ao tratamento com monocultivo. A salinidade usada no trabalho foi de 1 g L^{-1} e a espécie principal *L. vannamei,* cultivado em uma densidade de 90 cam. m^{-2} em sistema de bioflocos. As outras duas espécies complementares foram selecionadas por terem também um viés comercial na China. Os talos recém brotados do espinafre-da-água tem valor culinário, enquanto que *S. argus* pode custar até 12 dólares a unidade no mercado ornamental (Liu et al. 2014).

É importante ressaltar que a AMTI aplicada ao sistema de bioflocos deve-se focar em espécies que aproveitem a grande quantidade de material orgânico em suspenção e outras espécies que aproveitem o acumulo de elementos inorgânicos como nitrato. Por isso abaixo estão descritas algumas espécies que tem potencial para integrar inicialmente a AMTI aplicada ao cultivo de camarão em sistema de bioflocos.

1.4 Espécies promissórias para o sistema AMTI aplicada ao cultivo de camarão em sistema de bioflocos

1.4.1 Camarão (*Litopenaeus vannamei*)

Espécie principal do sistema, o camarão-branco-do-pacífico (*Litopenaeus vannamei*) é uma espécie amplamente cultivada no mundo e representa pouco mais de 80 % do camarão produzido no mundo (Fao-Fishstat 2018).

Isso se deve ao fato de ter um desempenho zootécnico que se destaca perante as outras espécies de cultivo. Por exemplo, tem um custo de produção relativamente mais baixo que *Penaeus monodon* o qual exige maior quantidade de proteína na ração (VAN-WYK, 1999). Chega a um tamanho comercial em três meses e conta com inúmeros programas de melhoramento genético. É passível de ser cultivada em sistemas que vão do extensivo (5 camarões m^{-3}) a superintensivos (500 camarões m^{-3}) (BALOI et al., 2013). Pode ser cultivada em policultivos (COSTA et al., 2013; SIMAO et al., 2013) e em cultivos integrados (NEORI et al., 2004; LIU et al., 2014).

1.4.2 Tilápia (*Oreochromis niloticus*)

Uma das espécies de peixe mais cultivada no mundo, a tilápia se destaca pela rusticidade, desempenho zootécnico e filé com alto valor de mercado. Tolera salinidades de até 20 g L^{-1} e já foi cultivada em sistema de policultivo com camarões obtendo bons resultados (BESSA et al., 2012; HERNANDEZ-BARRAZA et al., 2013; SIMAO et al., 2013).

Além disso é uma espécie já cultivada em sistema de bioflocos e vários estudos relataram o seu bom aproveitamento do biofloco como fonte de alimento (AZIM; LITTLE, 2008; AVNIMELECH; KOCHBA, 2009; CRAB et al., 2009). Isso demonstra seu potencial como espécie extratora orgânica dos bioflocos presentes no cultivo de camarão em sistema de bioflocos.

1.4.3 Halófitas

Um potencial como espécie extratora inorgânica são as plantas adaptadas a solos salinizados e as halófitas são plantas que toleram solos ou lugares onde a salinidade é alta e que a maioria das plantas não toleraria (FLOWERS; COLMER, 2008). No Brasil a espécie *Sarcocornia ambigua* está amplamente difundida em regiões de manguezais e marismas (COSTA et al., 2006).

As plantas do gênero *Sarcocornia* e *Salicornia* já são reportadas como fonte de alimento e matéria prima para outros produtos desde o século XVIII (CHEVALIER, 1922). Além disso, já é utilizada como forragens para animais, produção de fármacos, e extração de óleo (DÍAZ; BENES; GRATTAN, 2013; TIKHOMIROVA et al., 2008; VENTURA; SAGI, 2013).

Atualmente, os mercados Europeus e Nortes Americanos têm consumido como vegetal semelhante ao aspargo verde por seu alto valor nutricional, sendo consumida na forma de salada e também como tempero devido ao seu sabor salgado (BERTIN et al., 2014). No Brasil já é possível encontrar produtos em conserva, sal verde e até cerveja a base de salicornia a um preço bastante elevado.

Essas plantas têm ganhado espaço no tratamento de efluente da aquicultura marinha por serem tolerantes a salinidades altas, apresentarem boa absorção de nutrientes inorgânicos e consequentemente boa produtividade (BUHMANN et al., 2015; GLENN et al., 2013; ROZEMA; SCHAT, 2013; SHPIGEL et al., 2013; WEBB et al., 2012).

Isso demonstra que espécie pode ser a componente extratora inorgânica dos bioflocos oriundos do cultivo de camarão.

A espécie *Sarcocornia ambigua* foi recentemente utilizada em cultivo aquapônico com camarões cultivados em sistema de biofloco (PINHEIRO et al., 2017). Nesse trabalho os autores descrevem que foi possível produzir 2 kg de sarcocorina para cada kg de camarão além de aumentar a retenção de nitrogênio em 10,4% em relação ao tratamento sem a presença da planta.

Levando em consideração o que foi descrito acima, o presente projeto propõe o estudo da combinação do sistema de cultivo de camarões em bioflocos com tilápias (*Oreochromis niloticus*) e sarcocornia (*Sarcornia ambigua)*.

Caracterizado pelo aumento da produtividade, por reutilizar a água e os nutrientes oriundos da ração, esse modelo produtivo irá contribuir para a diminuição do impacto gerado pela carcinicultura nos ambientes adjacentes e melhorar a eficiência econômica do cultivo.

Sendo assim, este projeto tem como objetivo avaliar o sistema multitrófico integrado (AMTI) aplicado ao cultivo de camarão em sistema de bioflocos como potencial sistema de produção aquícola sustentável.

1.5 OBJETIVOS

1.5.1 Objetivo geral

Desenvolver um cultivo multitrófico integrado de peixes, plantas e camarões em sistema de bioflocos.

1.5.2 Objetivos específicos

a. Estabelecer a melhor relação entre a biomassa de tilápia (*Oreochromis niloticus*) em relação à biomassa de camarão-branco-do-pacífico (*Litopenaeus vannamei*) cultivado em sistema de bioflocos.
b. Avaliar o desempenho produtivo do cultivo multitrófico integrado (AMTI) composto por cultivo de camarão (*L. vannamei*) em sistema de bioflocos, tilápia (*O. niloticus*) e salicórnia (*Sarcocornia ambigua*).

1.6 Estrutura do trabalho

A tese é composta por dois artigos. O primeiro artigo científico original intitulado "*Pacific white shrimp and Nile tilapia integration in* *biofloc system under different fish-stocking densities*” publicado no periódico Aquaculture e que foi agraciado com o prêmio de melhor trabalho da América Latina na competição *Alltech Young Scientist* 2018 e posteriormente ao prêmio global *Impact Award* durante a fase final da competição

O segundo artigo científico original intitulado “*Integrated Multi-Trophic Aquaculture apply to the shrimp rearing in a biofloc system*” formatado para submissão para a revista Aquaculture.

2 DESENVOLVIMENTO – ARTIGOS CIENTIFICOS

2.1 Artigo 1- Pacific white shrimp and Nile tilapia integration in biofloc system under different fish-stocking densities

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Abstract

This study aimed to evaluate the effect of different stocking densities of Nile tilapia, *Oreochromis niloticus*, integrated with Pacific white shrimp, *Litopenaeus vannamei,* reared in a biofloc system for 57 days. The performance of both species and the ecological efficiency of the system were evaluated. Four levels of tilapia stocking density were evaluated: 0, 8, 16 and 24 fish per tank (90 L useful volume). The initial weight was 4.8 ± 0.1 g and 9.6 ± 0.1 g for shrimp and fish, respectively. The shrimp were fed according to the feed table, and the fish were fed with 1% of fish biomass, stimulating the tilapia to seek food in the biofloc. Results show no difference between average shrimp weight (14.9 ± 0.6 g) and survival (93.0% ± 1.0%). Similarly, fish obtained a final mean weight of 61.9 ± 3.8 g and survival of 91.1 ± 7.9%. Total yield was higher based on the increase in fish density. The sludge produced per animal biomass (sludge:biomass ratio) decreased as fish density increased ($y = -0.0083x + 0.5995$ $r^2 = 0.87$). Nitrogen recovery rose linearly with the increase in fish density ($y = 0.036x + 0.2725$, $r^2 = 0.84$), as did phosphorus recovery ($y = 0.00711x + 0.1395$, $r^2 = 0.91$). These results demonstrate the feasibility of increasing yield up to 31.2% by integrating *L. vannamei* and *O. niloticus* in a biofloc system. Also, the decrease in sludge:biomass ratio and the higher nitrogen and phosphorus recovery increased the sustainability of *L. vannamei* rearing in a biofloc system

2.1.1 Introduction

Aquaculture is the fastest growing animal food sector, producing 73.8 million tons and a turnover of 160.2 billion dollars in 2014 and surpassing capture fisheries production (FAO-FIGIS, 2017). Shrimp farming has grown constantly in the past years, reaching a production of 4.8 million tons in 2015 (FAO-FIGIS, 2017). FAO projects that aquaculture will continue to grow rapidly, owing to intensification of production systems, diversification of species and introduction of innovative new technologies to make production more efficient (FAO, 2016). Biofloc technology (BFT) is now the premier technology that contributes to the intensification of Pacific white shrimp production, and it uses high stocking densities and minimum, or zero, water exchange, which considerably reduces the area used for rearing and also the water resources, in comparison with semi-intensive systems (Samocha et al., 2012). It is possible, for example, to produce 1.0 kg of shrimp in just over 100 liters of water in a biofloc system, while traditional systems use about 64,000 liters (Otoshi et al., 2007).

The heart of the BFT system is the formation of microbial aggregates which are initiated by heterotrophic bacterial colonization stimulated by the addition of an external carbon source (Avnimelech, 2015). Heterotrophic bacteria incorporate the inorganic nitrogen and orthophosphate by assimilation, turning them into cell biomass and, consequently, reducing water exchange and improving biosecurity (McIntosh, 2000). In addition, microbial aggregates can be used as a food source by the cultured animals (Xu et al., 2012).

However, implementing a BFT system can encounter some obstacles, especially the high concentration of solids generated. The excessive accumulation of suspended solids in the water can militate against shrimp growth and, therefore, must be removed from the system (Schveitzer et al., 2013). At the same time, however, the solids removed are an effluent rich in nitrogen and phosphorus.

Faced with these obstacles, the utilization of an integrated multi-trophic aquaculture (IMTA) system could result in a better development of Pacific white shrimp reared in a BFT system. IMTA is an aquaculture model which integrates different trophic levels in the same environmental system, resulting in a conversion of the culture residues of the main species into food, or fertilization, for the other species (Chopin et al., 2001). The application of IMTA could contribute to productivity growth based on trophic level diversity. In addition, the utilization of different trophic level species would allow for maximum utilization of nutrients present in the solids generated in a BFT system. Thus, tilapia would seem to be a good species to integrate with shrimp in a biofloc system, owing to its capacity to consume biofloc, as well as its rusticity and salt-tolerance (El-Sayed, 2006, Avnimelech, 2006).

Some studies have reported on the integration of shrimp and tilapia, however, these have generally focused on traditional rearing systems (Cruz et al., 2008; Martínez-Porchas et al., 2010; Yuan et al., 2010; Simão et al., 2013), or the improvement of shrimp health (Tendencia et al., 2004; Tendencia et al., 2006). Therefore, the present work has focused on co-culture of shrimp and fish as a first step toward a future IMTA for shrimp rearing in biofloc technology.

As an IMTA case study, this work aimed to evaluate the yield, as well as the ecological performance of the system relative to different stocking densities of *Oreochromis niloticus* integrated with *Litopenaeus vannamei* reared in a biofloc system.

2.1.2 Material and methods

2.1.2.1 Biological material

The experiment was conducted for 57 days between May and June of 2017 at the Laboratório de Camarões Marinhos (LCM), which is part of the Aquaculture Department of the Universidade Federal de Santa Catrina (UFSC). Shrimp juveniles of *Litopenaeus vannamei* (HB12-Aquatec LTDA) were obtained from an LCM biofloc rearing tank. Nile tilapia juveniles (*Oreochromis niloticus*) were provided from the Empresa de Pesquisa e Extensão Rural de Santa Catarina (EPAGRI). This work was approved by the Ethics Committee on Animal Use of the UFSC (Protocol 1023030417).

2.1.2.2 Experimental design, experimental units and system management

The experiment was performed in a completely randomized design with four replicates, integrating tilapia with shrimp reared in a biofloc system.

The experimental units consisted of a 1000 L (800 L of useful volume) tank for the rearing of shrimp and another 100 L tank (90 L of useful volume) for rearing tilapias, both allocated inside a greenhouse. Water was recirculated via a submerged pump (Sarlo-Better 650 L $hour^{-1}$) into the tilapia tanks and returned by gravity to the shrimp tanks (Figure 1).

The shrimp rearing unit had an 800 W heater, a thermostat to maintain the temperature at 28±1 ºC, a microperforated hose and a blower aeration system to keep the bioflocs in suspension and maintain oxygen above 5 mg L^{-1}. It was also equipped with artificial substrates (high-density polyester, Nedlona®), which corresponded to 80% of the surface area of the tank. The tilapia tank had a 100 W heater, a thermostat to maintain the temperature at 28±1 ºC, and four air stones attached to the same blower that fed the aeration of the shrimp tank. There was no water exchange during this time, but to compensate evaporation pipe freshwater was used. The control of ammonia was performed with the daily addition of sugar cane as an organic carbon source, in accordance with Avnimelech (1999).

At the beginning of the experiment, shrimp and fish weighed an average of 4.78 ± 0.02 g and 9.64 ± 0.14 g, respectively. The shrimp were fed with a 35% crude protein commercial feed four times a day according the Van Wyk (1999) feed table, and the fish were fed with 38% crude protein commercial feed only once a day with 1% of the fish biomass, stimulating the tilapia to seek food in the bioflocs.

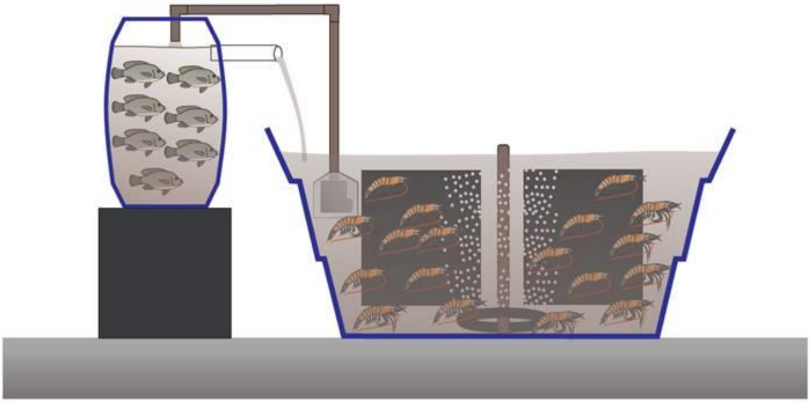


Figure 1: Flow diagram of the integrated experimental unit of shrimp in bioflocs with tilapia.

Shrimp were reared at a density of 281 shrimp m^{-3} (225 animals $tank^{-1}$) in all experimental units. The experiment consisted of four treatments corresponding to four levels of tilapia stocking density, one of which stocked no fish at all (zero point). The tilapia densities were determined based on the estimated shrimp and fish final biomass ratio. To make this estimation, it was assumed that a survival of 90% of the 225 shrimp stocked per tank would be obtained at the end of the rearing and that the average final weight would be 15 g. Based on these predictions, the shrimp estimated final biomass (SEFB) was calculated as follows:

$$SEFB = (\#SS * EFS) * EFW,$$

where *SEFB* is the *Shrimp Estimated Final Biomass* (3037 g),

#SS is the *Number of Shrimp Stocked* (225),

EFS is the *Estimated Final Survival* (90 %), and

EFW is the *Estimated Final Weight* (15 g).

Based on the calculated SEFB and the assumption that tilapia would reach the estimated average final weight of 40 g, the following densities were determined and evaluated:

T0 – No tilapia were stocked in this control tank; however, recirculation with the shrimp tank was maintained.

T8 - In this treatment, the final biomass of tilapia was assumed to correspond to 10% of the shrimp estimated final biomass (SEFB), resulting in an estimated 303 g of tilapia per tank at the end of the experiment. Thus, the initial density of tilapia per tank was calculated using the following formula:

$$TSD = \frac{TEFB}{TEFW},$$

where *TSD* is the *Tilapia Stocking Density*,

TEFB is the *Tilapia Estimated Final Biomass* (303 g), and

TEFW is the *Tilapia Estimated Final Weight* (40 g).

Therefore, the initial density was 8 fish per tank.

T16 - In this treatment, the final biomass of tilapia was assumed to correspond to 20% of the estimated final biomass of shrimp. Following the prior calculation method presented for the T8 treatment, the density in this treatment was 16 fish per tank.

T24 - In this treatment, the final biomass of tilapia was assumed to correspond to 30% of the estimated final biomass of shrimp, resulting in 24 fish per tank based on the detailed calculations in the T8 treatment.

The methodology to define tilapia density relative to the shrimp is summarized in Table 1.

Table 1: Methodology applied to establish the fish stock density based on the shrimp final biomass for *Litopenaeus vannamei* reared in a biofloc system integrated with different fish-stocking densities of *Oreochromis niloticus* for 57 days.

Treatments		**T0**	**T8**	**T16**	**T24**
Parameter	**Shrimp**	**Nile Tilapia**			
Estimated survival (%)	90	95	95	95	95
Fish:Shrimp final biomass rate	-	0%	10%	20%	30%
Estimated final biomass (g)	3037	0	303	607	911
Final mean weigh (g)	15	40	40	40	40
Stock Density (animal tank $^{-1}$)	225	0	8	16	24

The initial water was prepared with 491 liters of water from the shrimp biofloc tank with salinity of 33 g L^{-1} in order to provide biofloc inoculation and salt water. The remaining volume was filled with fresh water provided by Santa Catarina Water and Sanitation Authority, with salinity reaching 18 g L^{-1}. The initial physical and chemical characteristics of the water were as follows: total ammoniacal nitrogen (TAN) 1.6 ± 0.3 mg L^{-1}; nitrite (N-NO_2^-) 1.1 ± 0.1 mg L^{-1}; nitrate (N-NO_3) 9.0 ± 1.8 mg L^{-1}; total suspended solids (TSS) 213 ± 20 mg L^{-1}; pH 7.5 ± 0.2, and alkalinity 120 ± 10 mg L^{-1}.

2.1.2.3 Water quality

Dissolved oxygen and temperature were measured twice a day with a digital oximeter (YSI Pro20). Alkalinity (APHA, 2005), pH (pH-metro Tecnal®) and salinity (Eco-Sense YSI EC3) were measured twice a week.

Total Suspended Solids (TSS), volatile suspended solids (VSS) and fixed suspended solids (FSS) were analyzed twice a week (APHA, 2005). Fiberglass filters with a porosity of 0.6 μm (GF6 Macherey-Nagel) were used for analysis of TSS. TSS were maintained between 400 and 600 mg L^{-1}, a level considered adequate for *L. vannamei* (Schveitzer et al., 2013), and the excess was removed through the use of conical settling tanks. The removed solids were quantified in terms of volume and TSS concentration. In order to quantify the volume removed, graduated buckets were used, and to quantify the concentration of TSS, the methodology proposed by APHA (2005) was used. Thus, at the end of the crop, the total values of sludge produced in each experimental unit were measured by the following equation:

$Sludge\ produced\ (kg\ tank^{-1}) = \frac{(final\ TSS\ (mg\ L^{-1})\ *v(L)) - (initial\ TSS\ (mg\ L^{-1})\ *v\ (L)}{1{,}000{,}000} + (RS\ (kg))$, where v corresponds to the volume of the tank in liters, and RS corresponds to solids removed by settling tanks. To measure the RS, a graduated bucket was used to collect all removed sludge.Sludge was then homogenated, and a sample was collected to measure measure the TSS (APHA, 2005). The TSS result was multiplied by the sludge volume measured in the graduated bucket.

Sludge:biomass ratio, represents the sludge produced per total final biomass, and it was measured as:

$$Sludge: biomass\ ratio = \frac{Sludge\ produced\ (kg)}{Total\ biomass\ (kg)}.$$

TAN (Grasshoff; Ehrhardt and KremLing, 1983) and nitrite ($N\text{-}NO_2$) (Strickland and Parson, 1970) concentrations were measured twice a week. Nitrate ($N\text{-}NO_3$) and orthophosphate (PO_4^{3-}) (APHA, 2005) were analyzed once a week.

2.1.2.4 Shrimp and tilapia performance

Shrimp performance was evaluated by weekly biometry. At the end of rearing, survival (%), final mean weight (g), apparent feed conversion ratio (FCR), yield (kg m^{-3}) and weekly growth (g) were measured.

The fish were subjected to biometrics analysis after 30 days of rearing and also at the end of experimentation. In order to evaluate the performance of the fish, survival (%), final mean weight (g), apparent feed conversion ratio (FCR), yield (kg m^{-3}) and specific growth rate (% day^{-1}) were measured.

2.1.2.5 Nitrogen and phosphorus recovery

The recovery of nitrogen and phosphorus was calculated according to the respective equations provided by Hossain, Pandey and Satoh (2007), as

Recovery (%P or N) = (Final content - Initial content) / input*100.

To determine the content of total N and P Kjeldahl (NTK), we used the methodology described by AOAC (2005), using the animal's total body.

2.1.2.6 Statistical analysis

One-way analysis of variance (ANOVA), followed by the Tukey's test (Zar, 2010) when necessary, was applied, using a significance level of 0.05. Normality and homoscedasticity were evaluated by the Shapiro-Wilk and Levene tests (Zar, 2010), respectively. Percentage data analysis (survival, SSV and SSF) were subject to angular transformation. The final survival and FCR data of the tilapias did not meet the prerequisite for ANOVA and consequently were analyzed by the Kruskal-Wallis test.

Total sludge:biomass ratio and nitrogen and phosphorus recovery were analyzed by linear regression, and their coefficients were evaluated for significance by the Student's *t* test ($\alpha = 0.05$).

2.1.3 Results

2.1.3.1 Water quality

During the experiment, the oxygen remained above 6 mg L^{-1}, temperature close to 28 °C and salinity close to 19 g L^{-1}. The pH remained stable near 8.0 and alkalinity near 150 mg L^{-1}. No significant difference was observed among treatments for dissolved nitrogen compounds (Table 2). The amount of cane sugar for ammonia control and the final C:N ratio were shown in Table 3.

2.1.3.2 Total suspended solids, sludge produced and sludge:biomass ratio

No significant difference was detected among treatments relative to TSS, VSS or FSS. TSS remained around 450 mg L^{-1}, while VSS corresponded to approximately 60% of this value, and FSS corresponded to 40%. The sludge produced was similar among treatments (Table 4). The amount of sludge produced by animal biomass (sludge:biomass ratio) decreased as fish density increased (Figure 2).

Table 2: Physical and chemical variables of water, total heterotrophic bacteria and total *Vibrio* count for *Litopenaeus vannamei* reared in a biofloc system integrated with different stocking densities of *Oreochromis niloticus* for 57 days.

	T0	**T8**	**T16**	**T24**	***p* ANOVA**
Dissolved oxygen (Shrimp)(mg L^{-1})	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.0	6.3 ± 0.1	0.5385
Temperature (Shrimp) (°C)	28.6 ± 0.3	28.5 ± 0.2	28.7 ± 0.4	29.0 ± 0.6	0.8444
Dissolved oxygen (Fish) (mg L^{-1})	6.4 ± 0.1	6.4 ± 0.2	6.3 ± 0.2	6.1 ± 0.1	0.4778
Temperature (Fish) (°C)	28.2 ± 0.5	28.4 ± 0.4	28.3 ± 0.6	28.6 ± 0.9	0.3836
Salinity (g L^{-1})	19.2 ± 0.4	18.8 ± 0.4	18.9 ± 0.2	18.8 ± 0.1	0.2995
pH	8.04 ± 0.02	8.08 ±0.04	8.07 ± 0.02	8.06 ± 0.03	0.4800
Alkalinity (mg L^{-1})	142.0 ± 6.0	144.0 ± 7.0	149.0 ± 5.0	147.0 ± 8.0	0.5179
TAN – N* (mg L^{-1})	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.1	0.8173
Nitrite N - NO_2 (mg L^{-1})	0.6 ± 0.2	0.7 ± 1.9	0.6 ± 0.1	0.8 ± 0.2	0.3654
Nitrate N – NO_3^{2-} (mg L^{-1})	2.3 ± 0.1	2.4 ± 0.2	2.4 ± 0.5	3.1 ± 0.8	0.1090

Data presented in mean ± standard deviation. Different letters on the same line indicate statistical differences by the Tukey test ($p < 0.05$). * Total Ammonia Nitrogen

Table 3: Total shrimp feed and fish feed input, total cane sugar input and final Carbon:Nitrogen (C:N) ratio for *Litopenaeus vannamei* reared in a biofloc system integrated with different fish-stocking densities of *Oreochromis niloticus* for 57 days.

	T0	**T8**	**T16**	**T24**	***p* ANOVA**
Shrimp total feed (kg)	3.75 ± 0.06	3.75 ± 0.1'	3.69 ± 0.15	3.73 ± 0.11	0.90
Fish total feed (kg)	-	0.08 ± 0.0[a]	0.16 ± 0.01[b]	0.27 ± 0.02[c]	0.00
Total cane sugar (kg)	3.15 ± 0.05[a]	3.23 ± 0.0[a]	3.25 ± 1.46 [ab]	3.38 ± 0.11[b]	0.03
C:N ratio [1]	14.93 ± 0.00[a]	14.91 ± 0.0	14.90 ± 0.00[c]	14.88 ± 0.00[d]	0.00

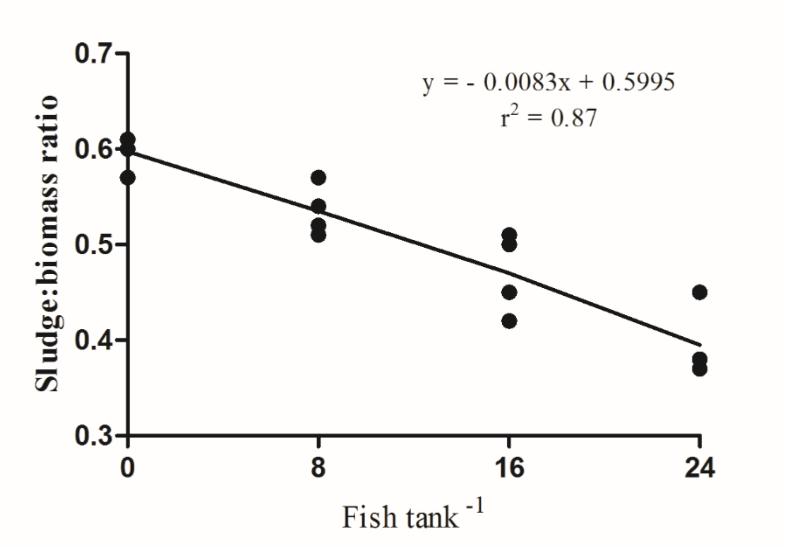
Data presented in mean ± standard deviation. Different letters on the same line indicate statistical differences by the Tukey test (p <0.05). [1] Assuming that: shrimp feed 35% crude protein and 50 % of carbon; fish feed 38% crude protein and 50% of carbon; cane sugar 99.9 % carbohydrate.

Table 4: Total sludge produced, total suspendend solids (TSS), volatile suspended solids (VSS) and fixed suspended solids (FSS) for *Litopenaeus vannamei* reared in a biofloc system integrated with different fish stocking densities of *Oreochromis. niloticus* for 57 days.

Parameter	**T0**	**T8**	**T16**	**T24**	***p***
Total sludge production (kg tank $^{-1}$)	1.85 ± 0.06	1.91 ± 0.07	1.85 ± 0.14	1.82 ± 0.16	0.65
TSS (mg L^{-1})	443.0 ± 24.0	445.0 ± 12.0	457.0 ± 25.0	421.0 ± 15.0	0.11
VSS (%)	59.0 ± 1.0	60.0 ± 1.0	60.7 ± 1.0	59.9 ± 1.0	0.08
FSS (%)	41.2 ± 0.8	39.93 ± 1.0	39.7 ± 0.7	40.9 ± 1.3	0.08

Data presented in mean ± standard deviation. Different letters on the same line indicate statistical differences by the Tukey test (p <0.05).

Figure 2: Linear regression of the total biomass and total sludge



produced (sludge:biomass ratio) in a *Litopenaeus vannamei* and *Orechromis niloticus* cultivated in an integrated biofloc system for 57 days.

2.1.3.3 Shrimp and tilapia performance

Shrimp performance was similar among treatments. The final mean weight among treatments was 14.9 ± 0.6 g, and mean survival was 93.0% ± 1.0%. The mean weekly growth was 1.2 ± 0.01 g week^{-1}, and the feed conversion ratio (FCR) was 1.8 ± 0.1. The final biomass was close to 3.1 ± 0.1 kg tank $^{-1}$, and the average yield was 3.9 ± 0.1 kg m^{-3} (Table 5).

Tilapia performance presented differences for fish biomass and final yield. Treatments with higher fish density reached higher biomass and yield. The specific growth rate (SGR) did not present differences among treatments and was, on average, 3.3 ± 0.3%. Feed conversion ratio (FCR) also presented no differences and was 0.2 ± 0.01 on average (Table 5).

The total biomass of the integrated system, i.e., shrimp biomass plus tilapia biomass (total biomass), was also higher in treatments with higher fish density (Table 5) with resultant higher yield.

Table 5: *Litopenaeus vannamei* and *Oreochromis niloticus* performance in an integrated biofloc culture system for 57 days.

	T0	T8	T16	T24	p ANOVA
Shrimp performance					
Mean final weight (g)	15.0 ± 0.2	14.9 ± 0.4	14.8 ± 1.1	14.9 ± 0.6	0.8354
Survival (%)	92.4 ± 1.7	93.6 ± 1.6	96.1 ± 8.8	92.7 ± 5.9	0.9313
Feed Conversion Ratio	1.8 ± 0.0	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	0.0680
Specific Growth rate (g week $^{-1}$)	1.2 ± 0.1	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.1	0.8730
Final biomass (kg)	3.1 ± 0.0	3.1 ± 0.1	3.2 ± 0.1	3.1 ± 0.1	0.2895
Tilapia performance					
Mean final weight (g)	-	66.4 ± 11.7	59.7 ± 9.7	59.6 ± 6.7	0.5432
Survival (%)	-	84.5 ± 18.5	89.0 ± 3.1	100 ± 0.0	0.0826*
Feed Conversion Ratio	-	0.21 ± 0.1	0.24 ± 0.0	0.22 ± 0.0	0.4724*
Specific Growth Rate (% day $^{-1}$)	-	3.5 ± 0.4	3.2 ± 0.1	3.2 ± 0.2	0.3288
Final biomass (kg)	-	0.44 ± 0.1^{a}	0.67 ± 0.1^{b}	1.4 ± 0.1^{c}	0.0000
Shrimp plus tilapia					
Total final biomass (kg)	3.1 ± 0.0^{a}	3.6 ± 0.2^{b}	4.0 ± 0.1^{c}	4.5 ± 0.1^{d}	0.0000
Total yield (kg m^{-3})	3.5 ± 0.0^{a}	4.1 ± 0.2^{b}	4.6 ± 0.1^{c}	5.1 ± 0.2^{d}	0.0000

Data presented as mean ± standard deviation. Different letters on the same line indicate statistical differences by the Tukey test (p <0.05).*P value for the Kruskal-Wallis test.

2.1.3.4 Nitrogen and phosphorus recovery

Nitrogen recovery rose linearly as stocking densities increased (Figure 3A). Nitrogen recovery in treatments with higher fish density was 27.9% higher relative to control. In the treatments with higher fish density, nitrogen recovery reached 37.6 %, out of which 32.3% accounted for recovery by fish, and 67.7 % accounted for recovery by shrimp (Figure 3C).

Phosphorus recovery also rose linearly as stocking densities increased (Figure 3B). In the treatment with the highest stocking density phosphorus recovery reached 31.3 %, out of which fish accounted for 59.4% of the total phosphorus recovery (Figure 3D), despite representing 41% of the total biomass of the system.

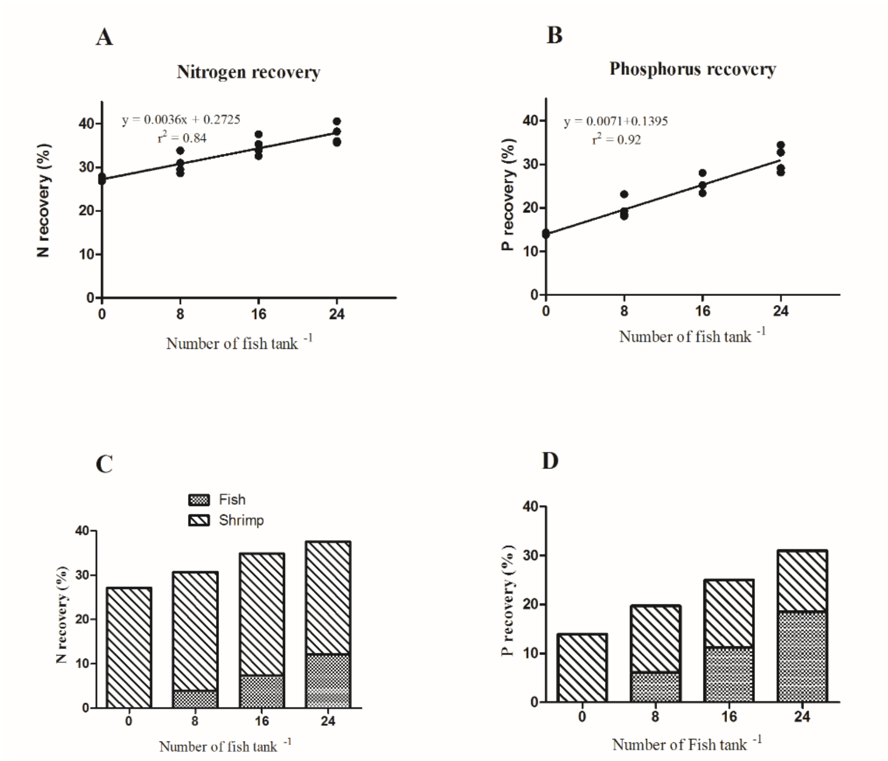


Figure 3: Nitrogen and phosphorus recovery in a *Litopenaeus vannamei* and *Oreochromis niloticus* cultivated in an integrated biofloc system for 57 days: A) linear regression for nitrogen recovery in the system; B) linear regression for phosphorous recovery in the system; C) Nitrogen recovery (%) in shrimp and fish; D) Phosphorus recovery (%) in shrimp and fish.

2.1.4 Discussion

2.1.4.1 Water quality

Oxygen was not a limiting factor for the growth of animals or the development of bioflocs. The temperature, pH and salinity of the water varied within the limits considered appropriate for *L. vannamei* and *O. niloticus* (Van Wyk and Scarpa; 1999; McGinty and Rakocy, 2003). Dissolved nitrogenous compounds were also within the limits considered suitable for rearing both species (Lin and Chen, 2001; Lin and Chen, 2003; El-Shafai et al., 2004; Cobo et al., 2014). The stable pH and alkalinity, the high percentage of VSS, the low nitrite and nitrate values and the constant addition of organic carbon (cane sugar) suggested that ammonia control was mostly driven by the heterotrophic pathway (Cohen et al., 2005; Ebeling et al., 2006; Schveitzer et al. 2013, Poli et al., 2015).

2.1.4.2 Total suspended solids, sludge produced and sludge:biomass ratio

Total suspended solids were kept within the levels indicated for both species (Schveitzer et al., 2013; Avnimelech, 2015). The constant input of organic carbon correspondingly increased TSS since heterotrophic bacteria produce approximately 40 times more solids than chemoautotrophic bacteria when stimulated by the high C:N ratio (Ebeling et al., 2006).

The amount of sludge produced was equal among the treatments, even with the largest biomass in the treatments that contained fish. Therefore, the relationship between kilogram of sludge produced per kilogram of animal biomass produced, the sludge:biomass ratio, decreased as fish stocking densities increased, suggesting consumption of solids by the fish, which represents a significant ecological gain.

2.1.4.3 Shrimp and tilapia performance

The results of this study show that the presence of tilapia did not affect shrimp yield and that the final biomass of shrimp reached the amount initially estimated according to shrimp and fish final biomass ratio.

The shrimp mean growth rate was 1.2 ± 0.01 g week^{-1}, with mean growth below 1 g only in the first week after starting (mean growth of 0.9 g) and in the last week (mean growth of 0.8 g), at which time the carrying capacity of the tank had possibly been reached. Despite this, weekly growth was higher than that reported by other authors (Krummenauer et al., 2011; Baloi et al., 2013; Schveitzer et al., 2013).

The feed conversion ratio (FCR) of the shrimp was lower than that of other studies with shrimp and diets of the same origin (Baloi et al., 2013; Schveitzer et al., 2013), but it is still high when compared to work done outside Brazil (Samocha et al., 2012). Feed consumption was monitored throughout the crop with the aid of a feed tray, and small leftovers were observed only during moulting.

The average yield of the shrimp was similar to that found by other authors using stocking densities close to those used in the present study (Krummenauer et al., 2011). Final yield of tilapia reached values close to those reported by other studies that used the biofloc system (Pérez-

Fuentes et al., 2016; Azim and Little, 2008; Crab et al., 2007). The highest mean reached in the treatment with higher stocking density is relevant because the animals were fed with only 1.0% of the biomass during the whole experimental period.

Despite the large difference between the means of the treatments, the final survival of the tilapia did not present significant differences by the Kruskal-Wallis test. In the treatment with greater density, a survival of 100% was observed in all experimental units, indicating no standard deviation. In the treatment with 8 fish per tank (84.5 ± 18.5%), two experimental units presented survival of 100%, and the other two units showed survival of 75 and 63%. This high standard deviation in one treatment and the absence of standard deviation in another justify the absence of statistical difference.

SGR did not show differences among the treatments and it was a smaller SGR than that previously described for tilapia fingerlings cultivated in biofloc (Cavalcante et al., 2017). These authors submitted tilapia (1.22 ± 0.08 g) to treatments with 15 and 30% of feed restriction and a treatment without food restriction and obtained SGRs of 5.5, 5.0 and 5.6% day $^{-1}$, respectively. In the present study, the food restriction was, on average, 81%, considering the feed table proposed by Ostrensky and Boeger (1998).

Tilapia feed rate fixed at 1% of fish biomass resulted in a low FCR, which did not differ statistically among treatments and was, on average, 0.2 ± 0.01. This fact corroborates the idea expressed above that tilapias would consume biofloc.

The integration of tilapia with a Pacific white shrimp culture in the biofloc system is an example of diversified production and resulted in a 31.2% increase in yield in the treatment with the higher fish density relative to the treatment without fish. In relation to shrimp and fish final biomass ratio foreseen in the planning of the experiment, only small differences in final tilapia biomass were observed, essentially because tilapia reached a final weight above expected. The percentage of tilapia biomass relative to shrimp biomass was 14%, 21% and 46% in treatments T8, T16 and T24, respectively. It is important to emphasize that it was possible to increase the stocking density of tilapia without compromising the specific growth rate and feed conversion ratio in both species.

2.1.4.4 Nitrogen and phosphorus recovery

Low nitrogen uptake is one of the major technical, economic and environmental problems of aquaculture. On average, only 25% of nitrogen is absorbed by aquatic animals (Crab et al., 2007; Avnimelech, 2015). The remainder of this nitrogen is excreted in the water in the form of ammonia, which, in turn, becomes toxic to the aquatic animals. In addition, the feed accounts for more than 50% of aquaculture production costs, and nitrogen is the most expensive feed ingredient.

Therefore, the recovery of nitrogen, which, in the present study, increased to 27.9% in the treatment with higher density of fish relative to control, represents an economic and environmental advancement for shrimp reared in a biofloc system. Similarly, in a study that integrates shrimp reared in a biofloc system with the halophyte *Sarcocornia ambigua*, reported a 20% increase in nitrogen recovery relative to the treatment without plants (Pinheiro et al., 2017), supporting the idea that integration is a successful ways to achieve a better use of nitrogen from feed.

Phosphorus, along with nitrogen, is also a main source of pollutants to aquatic environments. Its recovery in shrimp is approximately 11% (Avnimelech and Ritvo, 2003), while the remainder is excreted and deposited in adjacent environments. In the present experiment, the treatment with the highest density of fish retained 223% more phosphorus in relation to control. This could be explained by the amount of bone structure present in the fish.

These results show that is possible improve the system's eco-efficiency by increasing nitrogen and phosphorus recovery with a significant gain over shrimp monoculture systems.

2.1.5 Conclusion

The integration of *O. niloticus* in the cultivation of *L. vannamei* in a biofloc system did not affect the performance of either species. The system yield increased with the integration of *L. vannamei* and *O. niloticus* species in a biofloc system. The relationship between sludge produced and animal biomass produced decreased as fish densities increased. Therefore, the integration of these species in the biofloc system represents an overall ecological gain by the increased recovery of nitrogen and phosphorus.

2.1.6 Acknowledgments

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2.2 Artigo 2 – Integrated multitrophic aquaculture applied to shrimp rearing in a biofloc system

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Abstract

This study aimed to evaluate the performance of an integrated multitrophic aquaculture (IMTA) system applied to shrimp rearing in biofloc technology (BFT). The IMTA system consisted of shrimp (*Litopenaeus vannamei*) in a rearing tank (800 L), a tilapia (*Oreochromis niloticus*) rearing tank (90 L), and hydroponic bench with 0.33 m^2 of planting for the sarcocornia plant (*Sarcocornia ambigua*) culture. A submerged pump constantly pumped water from the shrimp tank to the tilapia tank. Then, by gravity, water flowed through the sarcocornia hydroponic bench and returned to the shrimp tank. The hydroponic bench had enough capacity for 32 plants. The shrimp, tilapia and sarcocornia stock densities were 312 shrimp m^{-3} (250 shrimps per 800 L tank), 445 tilapia m^{-3} (40 tilapias per 90 L tank), and 97 plants m^{-2} (32 plant per system), respectively. The same experimental units were used in the control system which only differed by the absence of sarcocornia. The initial weight was 4.09 ± 0.05 g, 1.16 ± 0.04 g and 1.17 ± 0.35 g for shrimp, fish and sarcocornia, respectively. Shrimps were fed according to the feed table, and the fishes were fed with 1% of fish biomass, stimulating tilapia to use biofloc as a food source. Results show no difference between shrimp and tilapia performance in both treatments. Only IMTA total yield (4.83 ± 0.38 kg m^{-3}) was significantly higher than that in the control system (3.99 ± 0.09 kg m^{-3}). Nitrate was higher in the control system (12.28 ± 2.54 mg L -1) compared to the IMTA system (9.38 ± 2.18 mg L -1).These results demonstrate a yield increase of up to 21.5 % by integrating *L. vannamei*, *O. niloticus* and *S. ambigua* in a biofloc system.

2.2.1 Introduction

Shrimp farming has grown constantly in past years, reaching a production of 5.1 million tons in 2016 (FAO-FIGIS, 2018). FAO projects that aquaculture will continue to grow rapidly, owing to intensification of production systems, diversification of species and introduction of innovative new technologies to make production more efficient (FAO, 2018). Biofloc technology (BFT) is now the premier technology that contributes to the intensification of Pacific white shrimp production, and it uses high stocking densities and minimum, or zero, water exchange, which considerably reduces the area used for rearing and also water resources, in comparison with semi-intensive systems (Samocha et al., 2012).

In biofloc technology, ammonia is controlled inside the system by two pathways. The heterotrophic pathway is stimulated by the addition of an external carbon source (Avnimelech, 2015). Heterotrophic bacteria assimilate the inorganic nitrogen and orthophosphate, turning them into cell biomass. The chemoautotrophic pathway oxidizes ammonia and, as a result, produce nitrate by nitrifying bacteria (Ebeling et al., 2006). These two ammonia control pathways permit the biofloc system to operate without water exchange, consequently improving biosecurity (McIntosh, 2000). In addition, microbial aggregates can be used as a food source by the cultured animals (Xu et al., 2012).

However, in the biofloc system, the amount of solids increases every day, and shrimp are intolerant to the accumulation of solids (Gaona et al., 2013; Schveitzer et al., 2013). Therefore, the excess of solids must be removed from the system, thus becoming a salinized effluent rich in nitrogen and phosphorus.

In this context, one alternative that could improve the development of Pacific white shrimp reared in biofloc would consist of an integrated multitrophic aquaculture (IMTA) system. IMTA is an aquaculture model which integrates different trophic levels in the same environmental system, resulting in a conversion of the culture residues of the main species into food, or fertilization, for the other species (Chopin et al., 2001). This concept can improve aquaculture sustainability by reducing the effluent and bringing economic diversity by producing other value-added species (Chopin et al., 2001).

The composition of an IMTA system that meets the requirements of shrimp rearing in biofloc technology involves different trophic levels of species that are salt-tolerant and able to take advantage of biofloc nutrients. Tilapia is a particularly good species to integrate with shrimp in a biofloc system owing to its capacity to consume biofloc, as well as its rusticity and salt-tolerance (El-Sayed, 2006; Avnimelech, 2015). Furthermore, tilapia has shown good performance when integrated with shrimp rearing in biofloc technology using only 1% of fish biomass for feeding, thus improving nitrogen and phosphorus recovery (Poli et al., 2018).

Halophytes are plants from another trophic level. Being salt-tolerant, they can be included in the composition of IMTA applied to shrimp rearing in a biofloc system. This type of plant was reported as a food for its high salt contents and medicinal uses (Davy et al., 2001; Lieth, 2000). Halophytes of the genera *Salicornia* and *Sarcocornia* are marketed as 'Sea asparagus' (Ventura and Sagi, 2013). In a recent study, *Sarcocornia ambigua* was reported to have good performance when integrated with Pacific white shrimp in BFT in addition to improving nitrogen recovery (Pinheiro et al., 2017). Thus, for a completely integrated shrimp rearing IMTA system, we include Nile tilapia (*Oreochromis niloticus*) and sarcocornia (*Sarcocornia ambigua*).

As an IMTA case study, this work aimed to evaluate the yield, as well as the ecological performance, of the system relative to the integration of Nile tilapia and sarcocornia along with Pacific white shrimp (*Litopenaeus vannamei*) reared in a biofloc system.

2.2.2 Material and methods

2.2.2.1 Biological Material

The experiment was conducted for 57 days from December of 2017 through February of 2018 at the Laboratório de Camarões Marinhos (LCM), a facility of the Aquaculture Department of the Universidade Federal de Santa Catrina (UFSC). Shrimp juveniles of *Litopenaeus vannamei* (HB16-Aquatec LTDA) were obtained from an LCM biofloc rearing tank. Nile tilapia juveniles (*Oreochromis niloticus*) were provided by the Empresa de Pesquisa e Extensão Rural de Santa Catarina (EPAGRI), while sarcocornia (*Sarcocornia ambigua*) were obtained from an LCM plant bank. The UFSC Ethics Committee on Animal Use approved this work (Protocol 1023030417).

2.2.2.2 Experimental design, experimental units and system management

Two treatments were performed in a biofloc environment. One was an integrated multitrophic aquaculture treatment (IMTA), consisting of shrimp, tilapia and sarcocornia rearing, and another was our control consisting of shrimp and tilapia rearing. The control treatment consisted of the same system and operating mode, but without plants. Each treatment had four replicates, totaling eight experimental units that were randomized in a 243 m^2 greenhouse.

The experimental units consisted of a round 1000 L tank (800 L of useful volume) for rearing shrimp, another round 100 L tank (90 L of useful volume) for rearing tilapias, and a NFT (Nutrient Film Technique) hydroponic bench for the plants. Water was recirculated via a submerged pump (Sarlo-Better 650 L $hour^{-1}$) from the shrimp tank into the tilapia tank. Then, by gravity, water flowed through the sarcocornia NFT hydroponic bench and returned to the shrimp tank (Figure 1).

The shrimp rearing unit had an 800 W heater, a thermostat to maintain the temperature at 28±1°C, a microperforated hose and a blower aeration system to keep the bioflocs in suspension and maintain oxygen above 5 mg L^{-1}. The shrimp tank was also equipped with four artificial substrates (high-density 100 % polyester, Needlona®), having a vertically oriented and rectangular design (0.40 x 0.55 m), which corresponded to 80% of the surface area of the tank.

The tilapia tank had a 100 W heater, a thermostat to maintain the temperature at 28 ± 1°C, and four air stones attached to the same blower that fed the aeration of the shrimp tank.

The structures for growing the plants followed the design of Pinheiro et al. (2017), with some adaptation. They were built 0.5 m above the water level of shrimp tank. This structure was based on the nutrient film technique (NFT) system whereby plant roots are partially submerged in a film of water passing through the irrigation channel (Lennard and Leonard, 2006). These channels were composed of four PVC pipes 75 mm in diameter and 1.10 m in length, arranged side by side. The pipes were painted with aluminum enamel to reflect the light and avoid heating the water film (Rodrigues, 2002) and placed on wooden supports with a 4% slope. Each channel contained eight plants, spaced 12 cm apart (Izeppi, 2011). Each bench had 0.33 m^2 of planting area with capacity for 32 *S. ambigua* seedlings, which is equivalent to a density of 97 plants m^{-2}. Plants were placed in supports made of 50 mm diameter PVC pipe and nylon screening with perlite added as a substrate (Ventura et al., 2011).

No water exchange occurred during this time; instead, only fresh water was used to replace water lost by evaporation. The initial water was provided from a LCM biofloc shrimp tank with a complete predominance of nitrifying bacteria. No organic carbon was used because the ammonia did not reach levels above 1 mg L^{-1}. Inorganic carbon ($Ca(OH)_2$) was used to keep the alkalinity above 150 mg L^{-1}.

The initial physicochemical characteristics of the water were as follows: total ammoniacal nitrogen (TAN), 0.32 ± 0.02 mg L^{-1}; nitrite ($N\text{-}NO_2^-$), 0.03 ± 0.001 mg L^{-1}; nitrate ($N\text{-}NO_3$), 6.49 ± 0.19 mg L^{-1}; total suspended solids (TSS), 447.63 ± 2.65 mg L^{-1}; pH 8.09 ± 0.02, salinity 20.38 ± 0.05 and alkalinity 132.5 ± 2.12 mg L^{-1}.

At the beginning of the experiment, shrimp, fish and sarcocornia weighed an average of 4.09 ± 0.05 g, 1.16 ± 0.04 g, and 1.17 ± 0.35 g, respectively. The shrimp were fed with a 35% crude protein commercial feed (Guabi-Potimirim 1.6 mm) four times a day according the Van Wyk (1999) feed table, and fish were fed with 38% crude protein commercial feed (Guabi-Onívoros QS 1.4 mm) only once a day with 1% of fish biomass, stimulating the tilapia to seek natural food in the bioflocs.

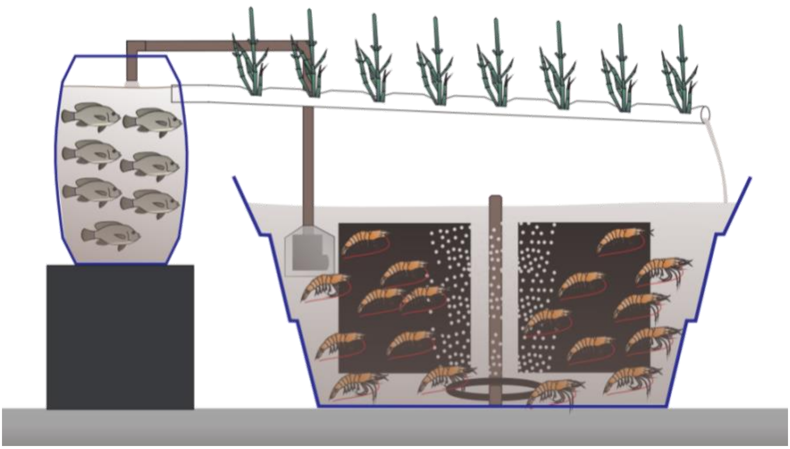

Figure 4: Flow diagram of the integrated experimental unit of shrimp in bioflocs with tilapia.

Shrimp were reared at a density of 312 shrimp m^{-3} (250 animals $tank^{-1}$) in all experimental units. Tilapia densities were determined according to the methodology proposed by Poli et al. (2018), assuming tilapia final biomass at 30 % of shrimp final biomass and tilapia final mean weight of 20 g, resulting in 40 animals $tank^{-1}$. The total plants $tank^{-1}$ was 32, using the methodology proposed per Pinheiro et al. (2017).

2.2.2.3 Water quality

Dissolved oxygen and temperature were measured twice a day with a digital oximeter (YSI Pro20). Alkalinity (APHA, 2005), pH (pH-metro Tecnal®) and salinity (Eco-Sense YSI EC3) were measured twice a week.

Total Suspended Solids (TSS), volatile suspended solids (VSS) and fixed suspended solids (FSS) were analyzed twice a week (APHA, 2005). Fiberglass filters with a porosity of 0.6 µm (GF6 Macherey-Nagel) were used for analysis of TSS. TSS were maintained between 400 and 600 mg L^{-1}, a level considered adequate for *L. vannamei* (Schveitzer et al., 2013), and the excess was removed through the use of a conical settling chamber. The removed solids were quantified in terms of volume and TSS concentration. In order to quantify the volume removed, graduated buckets were used, and to quantify the concentration of TSS, the methodology proposed by APHA (2005) was used. Thus, at the end of the crop, the total values of sludge produced in each experimental unit were measured by the following equation:

$$Sludge\ produced\ (kg) = \frac{(final\ TSS * v) - (initial\ TSS * v)}{1000000} + (RS),$$

where *v* corresponds to the volume of the tank in liters, and *RS* corresponds to solids removed by settling tanks. To measure final TSS, the sarcocornia roots, along with the entire hydroponic bench structure, were washed with water of each respective tank to include the solids retained in the roots.

TAN (Grasshoff; Ehrhardt and KremLing, 1983) and nitrite (N-NO_2) (Strickland and Parson, 1970) concentrations were measured twice a week. Nitrate (N-NO_3) and orthophosphate (PO_4^{3-}) (APHA, 2005) were analyzed once a week.

2.2.2.4 Shrimp, tilapia and sarcocornia performance

Shrimp performance was evaluated by weekly biometry. At the end of rearing, survival (%), final mean weight (g), apparent feed conversion ratio (FCR), yield (kg m^{-3}) and weekly growth (g) were measured.

Fish were subjected to biometrics analysis after 30 days of rearing and also at the end of experimentation. In order to evaluate the performance of the fish, survival (%), final mean weight (g), apparent feed conversion ratio (FCR), yield (kg m^{-3}) and specific growth rate (% day^{-1}) were measured.

At the end of the 57-day experiment, all plants were weighed individually. The average final weight (g), final biomass (kg) and production (kg m^{-2}) were then calculated.

2.2.2.5 Nitrogen and phosphorus recovery

The recovery of nitrogen and phosphorus was calculated according to the respective equations provided by Hossain, Pandey and Satoh (2007), as

Recovery (%P or N) = [(Final content - Initial content) / Input]*100.

To determine the content of total N and P Kjeldahl (NTK), we used the methodology described by AOAC (2005) using the total body of both animals and plants.

2.2.2.6 Statistical analysis

The Student's *t*-test was applied using a significance level of 0.05. Normality and homoscedasticity were evaluated by the Shapiro-Wilk and Levene tests (Zar, 2010), respectively. Percentage data were subjected to angular transformation for analysis.

2.2.3 Results and discussion

2.2.3.1 Water quality

Oxygen remained above 5.0 mg L^{-1} throughout the crop and was not a limiting factor for growth of the animals or development of bioflocs. Temperature, pH and salinity of the water varied within the limits considered appropriate for *L. vannamei* and *O. niloticus* (Van Wyk and Scarpa; 1999; McGinty and Rakocy, 2003) (Table 1).

Dissolved nitrogenous compounds were also within the limits considered suitable for rearing both species (Lin and Chen, 2001; Lin and Chen, 2003; El-Shafai et al., 2004; Cobo et al., 2014). However, a significant difference was noted for nitrate concentration, which was lower in the IMTA treatment, compared to control, most likely because of the sarcocornia presence. This result was different from that found by Pinheiro et al. (2017) who did not observe significant difference between the treatments with and without sarcocornia in an aquaponic system with shrimp in biofloc technology. Concentrations of ammonia and nitrite were significantly different between the treatments, and they were slightly higher in the IMTA treatment. However, these parameters were very stable.

Throughout the experiment, it was necessary to add calcium hydroxide to maintain pH and alkalinity, the levels of which tended to fall, most likely by nitrification. The low ammonia and nitrite values and the high nitrate values corroborate this hypothesis (Cohen et al., 2005; Ebeling et al., 2006; Schveitzer et al. 2013).

No significant difference was noted in orthophosphate and chlorophyll *a* between treatments, and similar results were found in another study with sarcocornia and shrimp reared in a biofloc system (Pinheiro et al., 2017).

Table 2: Physicochemical variables of water for *Litopenaeus vannamei*, *Oreochromis niloticus* and *Sarcocornia ambigua* performance in an integrated biofloc culture system for 57 days.

	IMTA	**Control**	***p* t-test**
Dissolved oxygen (Shrimp)(mg L^{-1})	6.16 ± 0.06	6.19 ± 0.08	0.571
Temperature (Shrimp) (°C)	28.6 ± 0.17	28.5 ± 0.23	0.569
Dissolved oxygen (Fish) (mg L^{-1})	5.92 ± 0.04	5.88 ± 0.05	0.267
Temperature (Fish) (°C)	28.7 ± 0.2	28.6 ± 0.2	0.671
Salinity (g L^{-1})	20.25 ± 0.24	20.42 ± 0.24	0.258
pH	7.98 ± 0.04	7.95 ± 0.03	0.198
Alkalinity (mg L^{-1})	149.00 ± 7.57[a]	129.0 ± 3.83[b]	0.026
NAT - N (mg L^{-1})	0.17 ± 0.05 [a]	0.14 ± 0.03 [b]	0.014
Nitrite N - NO_2 (mg L^{-1})	0.45 ± 0.07 [a]	0.36 ±0.09 [b]	0.007
Nitrate N – NO_3^{2-} (mg L^{-1})	9.38± 2.18 [a]	12.28 ± 2.54 [b]	0.003
Orthophosphate PO_4^{3-} (mg L^{-1})	5.38 ± 0.21	5.40 ± 0.14	0.3592
Chlorophyll -a (µg L^{-1})	38.89 ± 15.4	44.10 ± 14.1	0.6361

Data presented in mean ± standard deviation. Different letters on the same line indicate statistical differences by the t-test ($p < 0.05$).

2.2.3.2 Total suspended solids and sludge production

A significant difference was detected in treatments relative to TSS which was slightly low in the IMTA treatment (Table 2); however, levels were kept within those indicated for shrimp and tilapia species (Schveitzer et al., 2013; Avnimelech, 2015). No significant difference was observed in the level of VSS between the treatments, which was around 51% of TSS and FSS that corresponded to 49 % of TSS (Table 2).

The amount of sludge produced was 48.5 % lower in the treatments with sarcocornia (Table 2), even with the largest biomass in the IMTA treatment that contained *Salicornia*, which represents a significant ecological gain. In another study with tilapia and shrimps reared in a biofloc system with heterotrophic condition, sludge production was around 1.8 kg m^{-3} (Poli et al., 2019), or ten-fold more sludge than the results observed in the present study.

Table 3 Total sludge produced, total suspended solids (TSS), volatile suspended solids (VSS) and fixed suspended solids (FSS) for *Litopenaeus vannamei*, *Oreochromis niloticus* and *Sarcocornia ambigua* performance in an integrated biofloc culture system for 57 days.

Parameter	IMTA	Control	*p* t-test
Total sludge production (kg tank $^{-1}$)	0.18 ± 0.04 ^{a}	0.35 ± 0.11^{b}	0.0395
TSS (mg L^{-1})	437.9 ± 11.4 ^{a}	484.4 ± 14.7 ^{b}	0.0024
VSS (%)	50.9 ± 1.0	51.1 ± 1.0	0.2270
FSS (%)	49.1 ± 0.9	48.9 ± 1.4	0.6573

Data presented in mean ± standard deviation. Different letters on the same line indicate statistical differences by the t-test ($p < 0.05$).

2.2.3.3 Shrimp, tilapia and sarcocornia performance

Shrimp performance was similar between treatments (Table 3). The results of this study show that the presence of Nile tilapia and sarcocornia did not affect shrimp performance. Shrimp mean growth rate was 1.4 ± 0.01 g $week^{-1}$ and was higher than that reported by other authors

(Krummenauer et al., 2011; Baloi et al., 2013; Schveitzer et al., 2013; Poli et al., 2019).

The feed conversion ratio (FCR) of the shrimp was 1.7 ± 0.1, which is lower than other studies with shrimp and diets of the same origin (Baloi et al., 2013; Schveitzer et al., 2013; Poli et al., 2019), but it is still high when compared to work done outside Brazil (Samocha et al., 2012). The average shrimp yield (3.9 ± 0.1 kg m^{-3}) was similar to that found by other authors using stocking densities close to those used in the present study (Krummenauer et al., 2011; Poli et al., 2019).

Tilapia performance was also similar between treatments (Table 3). Tilapia final yield reached values close to those reported by other studies that used the biofloc system (Pérez-Fuentes et al., 2016; Azim and Little, 2008; Crab et al., 2007).

The specific growth rate (SGR) did not show differences between the treatments and was, on average, 4.4 ± 0.18 % day^{-1}, which is a slightly lower SGR than that described for tilapia fingerlings cultivated in biofloc (Cavalcante et al., 2017). These authors submitted tilapia of similar weight (1.22 ± 0.08 g) to feed restriction of 15 and 30% and a treatment without food restriction and obtained SGR of 5.5, 5.0 and 5.6% day $^{-1}$, respectively. In the present study, food restriction was, on average, 90%, considering the feed table proposed by Ostrensky and Boeger (1998).

Tilapia feed rate fixed at 1% of fish biomass resulted in a low FCR, which did not differ statistically between treatments and was, on average, 0.15 ± 0.01. This fact supports the hypothesis of consumption of biofloc by tilapias.

However, the tilapia final mean weight was lower than initially estimated. The tilapia final mean weight was estimated to be 20 g, and the tilapia reached around 11.4 ± 1.1 g; consequently, the final shrimp and tilapia biomass ratio was around 12.5%, while it was initially estimated to be 20 %. This result was different from that reported in another study that integrated tilapia and shrimp in a biofloc system where the authors initially estimated 30% of shrimp and tilapia final biomass ratio, but reported 41%. In this study, the authors reported the initial weight of tilapia to be 9.7 ± 0.1 g, and tilapia reached around 61.9 ± 9.3 g in a predominantly heterotrophic condition in a biofloc system in which tilapia were fed with 1% of fish biomass (Poli et al., 2019). In the present study, the initial weight of tilapia was 1.17 g. Fish were fed with the same 1% of fish biomass and grown in a predominantly chemoautotrophic condition in a biofloc system.

Halophyte production can vary according to species and salt concentration (Ventura et al., 2011; Ventura and Sagi, 2013). In the present study, production performance of *S. ambigua* (Table 3) was lower than that reported in a marine aquaponic study (Pinheiro et al., 2017) and marine hydroponic study (Ventura et al., 2011). Nevertheless, the present results were similar to those reported by Izeppi et al. (2011), who irrigated plants with shrimp farm effluent during 150 days. However, the important point in the present study was the fact that the presence of sarcocornia in the IMTA system decreased the production of nitrate (Table 2) and solids relative to control (Table 3). This represents a considerable ecological gain because it is possible to produce more biomass and reduce the rearing effluent.

In the integrated system, shrimp biomass plus tilapia biomass plus sarcocornia biomass, or total biomass, was higher than the control treatment (Table 3). Other studies reported a production increase with the integration of Pacific white shrimp reared in biofloc technology with different species. Pinheiro et al. (2018) integrated *S. ambigua* with Pacific white shrimp in biofloc technology and reported two-fold more production than the monoculture system. Poli et al. (2019) reported a 31.2 % yield increase with the integration of tilapia and Pacific white shrimp reared in biofloc technology. In the present study, it was possible increase 21.05 % the yield in the IMTA treatment relative to the control.

Table 3: *Litopenaeus vannamei*, *Oreochromis niloticus* and *Sarcocornia ambigua* performance in an integrated biofloc culture system for 57 days.

	IMTA	**Control**	*p* t-test
Shrimp performance			
Mean final weight (g)	14.6 ± 0.4	14.1 ± 0.2	0.0906
Survival (%)	88.0 ± 4.0	89.3 ± 2.4	0.6083
FCR	1.7 ± 0.1	1.7 ± 0.1	0.6535
Growth rate (g week $^{-1}$)	1.5 ± 0.1	1.4 ± 0.0	0.1313
Final biomass (kg)	3.2 ± 1.5	3.1 ± 0.9	0.5886
Tilapia performance			
Mean final weight (g)	11.4 ± 1,2	11.5 ± 1.1	0.8589
Survival (%)	91.3 ± 3.2	87.5 ± 7.4	0.4453
FCR	0.15 ± 0.02	0.16 ± 0.01	0.6535
SGR (% day $^{-1}$)	4.41 ± 0.17	4.39 ± 0.20	0.8943
Final biomass (kg)	0.42 ± 0.06	0.40 ± 0.03	0.5737
Salicornia performance			
Mean final weight (g)	23.0 ± 6.26		
Survival (%)	92.19 ± 4.03		
Final biomass (kg)	0.68 ± 0.20		
Shrimp plus tilapia plus salicornia			
Total final biomass (kg)	4.30 ± 0.34^{a}	3.56 ± 0.08^{b}	0.0051
Total yield (kg m^{-3})	4.83 ± 0.38^{a}	3.99 ± 0.09^{b}	0.0051

Data presented in mean ± standard deviation. Different letters on the same line indicate statistical differences by the T-test ($p < 0.05$).

2.2.3.4 Nitrogen and phosphorus recovery

Low nitrogen uptake is one of the major technical, economic and environmental problems of aquaculture. On average, only 25% of nitrogen is absorbed by aquatic animals (Crab et al., 2007; Avnimelech, 2015). The remainder of this nitrogen is excreted in the water in the form of ammonia, which, in turn, becomes toxic to aquatic animals. In addition, feed accounts for more than 50% of aquaculture production costs, and nitrogen is the most expensive feed ingredient.

Phosphorus, along with nitrogen, is also a main source of pollutants to aquatic environments. Its recovery in shrimp is approximately 11% (Avnimelech and Ritvo, 2003), while the remainder is excreted and deposited in adjacent environments. In a recent study, Poli et al. (2019) reported an increase of 223% in phosphorus recovery in an integrated system with shrimp and tilapias in the biofloc system in relation to shrimp monoculture.

No difference in nitrogen and phosphorus recovery was seen between the treatments (Table 4). However, the value reached in the present study was higher than the values reported in a biofloc shrimp monoculture (Poli et al, 2019). Similarly, in a study that integrated shrimp reared in a biofloc system with the halophyte *Sarcocornia ambigua*, Pinheiro et al. (2017) reported a 20% increase in nitrogen recovery relative to monoculture treatment, supporting the idea that integration can achieve a better use of nitrogen from feed.

Table 4: Nitrogen and phosphorus recovery for *Litopenaeus vannamei*, *Oreochromis niloticus* and *Sarcocornia ambigua* performance in an integrated biofloc culture system for 57 days.

Parameter	**AMTI**	**Control**	**P value**
Nitrogen recovery (%)	34.8 ± 1.3	33.0 ± 1.2	0.185
Phosphorus recovery (%)	15.2± 1.4	14.8 ± 1.0	0.193

Data presented in mean ± standard deviation. Different letters on the same line indicate statistical differences by the T-test ($p < 0.05$).

2.2.4 Conclusion

Yield in the IMTA system increased with the integration of *L. vannamei, S. ambigua* and *O. niloticus* species in a biofloc system. However, the presence of sarcocornia did not affect nitrogen and phosphorus recovery, despite reducing the amount of nitrate.

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3 CONCLUSÃO GERAL

a. Foi possível cultivar a tilápia com uma relação de até 40 % de biomassa de peixe em relação à biomassa de camarão.
b. Houve um aumento da produtividade total do sistema ao aplicar a AMTI ao cultivo de *Litopenaeus vannamei* em sistema de bioflocos.
c. A maior retenção de nitrogênio e fósforo no sistema AMTI proposto representa um ganho ambiental para aquicultura.

4 CONSIDERAÇÕES FINAIS

O objetivo desse trabalho nasceu com a necessidade de dar um melhor destino aos sólidos salinizados oriundos do cultivo de camarão em sistema de biofloco. Apesar de ser um sistema com troca mínima ou zero de água, o excesso de sólido salinizado rico em nitrogênio e fosforo deve ser removido do sistema.

O camarão da espécie *Litopenaeus vannamei* tolera níveis de sólido de no máximo 600 mg L^{-1} e quando a quantidade de sólido atinge esse valor, deve ser removido do sistema por meio de decantadores. Esse sólido removido torna-se um efluente carregado de nutrientes que podem causar eutrofização.

Além do dano ambiental também há uma perda econômica pouco visível sem que haja um grau de abstração. Para isso devemos analisar de onde vem os nutrientes presente no sólido removido do sistema. A maior fonte desses nutrientes é sem dúvida a ração, item que representa mais de 60% dos custos de produção. O ingrediente mais oneroso para produzir a ração é a proteína. Do nitrogênio contido na proteína ingerida pelo camarão, apenas 25% em média fica retido no animal, o restante vai para água em forma de compostos nitrogenados. Do fósforo contido na ração apenas 11% em média fica retido no camarão. Portanto podemos concluir que estamos jogando dinheiro fora.

A aquicultura deve estar atenta a esses detalhes para se tornar eficiente e competitiva. É nessa direção que o presente trabalho concentrou esforços.

Para tanto foram aplicados os conceitos de aquicultura multitrófica integrada (AMTI) para mitigar essas perdas. As espécies escolhidas foram a tilápia e a sarcocórnia pelos motivos já descritos no decorrer do trabalho. Primeiramente estabelecemos a densidade de tilápia e no segundo experimento aplicamos a AMTI completa já com a presença da planta.

No primeiro trabalho não tínhamos biofloco maduro, ou seja, um biofloco com nitrificação estabelecida. Por tanto foi iniciado o biofloco para o experimento numa condição predominantemente heterotrófica. Essa condição se manteve durante os 57 dias de cultivo, produzindo muito sólido. Porém a tilápia teve um crescimento surpreendente nesse período, crescendo 60 g em média com uma conversão alimentar de 0,2. Observamos um comportamento mais agressivo nas densidades mais baixas e um 100% de sobrevivência na densidade mais alta.

No segundo trabalho, já com a sarcocórnia presente, o experimento começou com um biofloco já maduro e não foi necessário usar uma fonte de carbono orgânico para controlar a amônia. Nesse trabalho a produção de sólido foi menor que no primeiro trabalho. Também observamos que a quantidade de nitrato foi menor no tratamento com a sarcocórnia, porém, a tilápias cresceu muito menos do que esperávamos tanto no tratamento AMTI quanto no controle.

Por isso pensamos em um terceiro experimento que recentemente foi executado, porém não faz parte dessa tese. Nesse experimento testamos os dois ambientes, heterotrófico e quimioautotrófico, no cultivo integrado de camarões e tilápias. Os resultados prévios indicam uma grande diferença no crescimento das tilápias, tendo crescido mais no sistema com predominância da via heterotrófica. Mas existe uma outra grande diferença que é a quantidade de sólido gerado, que foi maior no sistema com predominância heterotrófica também.

No decorrer do processo outras perguntas foram levantadas e que precisam ser respondidas por outros trabalhos:

1. É possível não alimentar ou diminuir a frequência de alimentação para as tilápias?
2. É possível aumentar a densidade de tilápias e sarcocórnia?

Vale ressaltar que não foi atingido um teto de densidade de estocagem de tilápias no primeiro experimento. Já no segundo experimento houve ainda sobra dos nutrientes dissolvidos na água que podem ser aproveitados por mais biomassa vegetal.

Para finalizar, ainda há espaço para realizar outros trabalhos com análises de ciclo de vida e de eficiência ecológica do sistema proposto nesse trabalho.

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