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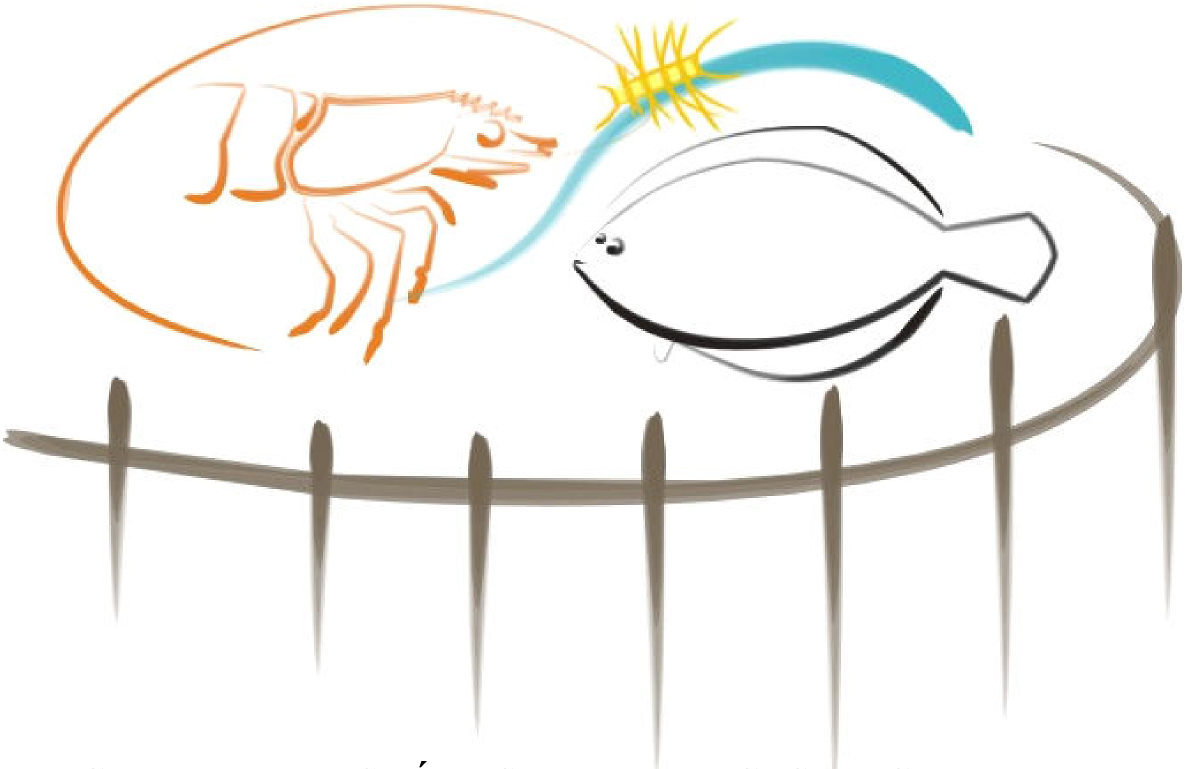

CONTROLE DOS NÍVEIS DE BIOFLOCOS NO CULTIVO DE CAMARÕES E SUAS IMPLICAÇÕES COM A QUALIDADE DE ÁGUA E O DESEMPENHO DOS ANIMAIS

CARLOS AUGUSTO PRATA GAONA

FURG
RIO GRANDE, RS
2015

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Carlos Augusto Prata Gaona

Tese apresentada ao Programa de Pós-Graduação em Aquicultura da Universidade Federal do Rio Grande, como parte dos requisitos para obtenção do título de Doutor em Aquicultura.

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Co-orientador: Prof. Dr. LuísHenrique S. Poersch

Rio Grande – RS – Brasil
Abril,2015

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DEDICATÓRIA

A toda minha incansável e maravilhosa família.

AGRADECIMENTOS

Ao meu orientador Prof. Dr. Wilson Wasielesky Jr. (Mano), pela constante orientação, consistentes contribuições a minha formação, por acreditar e por ajudar em momentos de dificuldades.

Ao meu co-orientador Prof. Dr. Luis Poersch (Mineiro) pelas sugestões durante os experimentos e pelo apoio durante o período no exterior.

Ao Dr. Dariano Krummenauer, pela amizade, companheirismo, ajuda nas horas de dificuldades e sugestões nesta tese.

Ao Prof. Dr. Rodrigo Schveitzer por aceitar o convite para participar da banca desta tese.

Ao Prof. Dr. Marcelo Tesser, por esclarecimentos e amizade consolidada a tempos.

Ao Dr. Alessandro Cardozo pela amizade e por aceitar em participar da banca desta tese.

A Fabiane Serra, por toda incondicional ajuda e apoio.

Ao Dr. Plínio Furtado, pela amizade, ajuda e sugestões.

Ao Dr. Geraldo (Leãozinho), pelo companheirismo aos longos desses anos.

Aos amigos do Projeto Camarão e da EMA por todo companheirismo.

Aos colegas da pós-graduação.

Ao Sandro, pelas análises químicas dos meus experimentos.

A toda equipe e colaboradores da EMA.

Ao Seu João, Dona Maria Alice e Miguel Dib pelo apoio em minha vida acadêmica.

Ao Seu José Fossati e Ana Maria, pelo apoio em minha realização profissional e por todos os esses bons anos.

A Ana Paula por me apoiar em meus sonhos e por ter me dado as duas maiores conquistas de minha vida.

A minha família que sempre me apoiou, incondicionalmente, nas decisões da minha vida.

RESUMO GERAL

AAquicultura tem produzido organismos aquáticos aumentando a densidade de estocagem, reduzindo a ocupação de áreas e o uso de água. A produção de camarões marinhos com a tecnologia de bioflocos (BFT) permite a prática de redução do uso de água, estimulando a produtividade natural que melhora a qualidade de água e adiciona alimento a espécie alvo. No entanto, a manutenção da água ao longo do ciclo gera aumento na quantidade de sólidos suspensos totais (SST), que podem interagir com parâmetros físicos e químicos, ocasionando mudanças na qualidade de água. Uma série de estudos foram executados buscando o aperfeiçoamento do manejo de sólidos suspensos para melhorar a qualidade de água e otimizar a produção de camarões marinhos, sendo três na Estação Marinha de Aquacultura (IO-FURG, Brasil) e um no Waddell Mariculture Center (WMC, EUA). Os capítulos da tese, referentes aos experimentos com camarões *Litopenaeus vannamei* em sistema BFT, tiveram como objetivo: (1) efeito de dois diferentes fluxos de bombeamentos de água durante o processo de remoção de sólidos suspensos por sedimentação; (2) analisar durante a formação de bioflocos, o efeito de diferentes níveis de sólidos suspensos na qualidade de água e performance dos camarões; (3) avaliar o efeito de diferentes concentrações de sólidos suspensos sobre o consumo de oxigênio e o desempenho zootécnico dos camarões; (4) avaliar o cultivo de camarões marinhos, integrando tilápias para o tratamento biológico de SST, bem como, o uso de ostras como biofiltradores e a possibilidade de integrar uma espécie de peixe carnívora ao sistema BFT. Todos os experimentos foram realizados em estufas. No Capítulo 1, um experimento de 17 semanas foi executado. Um controle sem remoção de sólidos foi comparado com dois tratamentos com diferentesfluxos de bombeamento: fluxo alto (HF) – 3945 L h^{-1}e fluxo baixo (LF) – 1750 L h^{-1}para remoção de sólidos. Melhores índices de desempenho zootécnico de *L. vannamei* foram alcançados com a remoção de sólidos. O tratamento com menor fluxo de água no clarificador facilitou a sedimentação. No Capítulo 2, três faixas de SST foram comparados durante 42 dias delineadas em três tratamentos: faixa baixa (TL) 100 – 300 mgL^{-1}, faixa média (TM)300 – 600mgL^{-1}(TM) e faixa alta (TH) 600 – 1000mgL^{-1}. Os parâmetros de qualidade de água na menor faixa (TL) resultaram em melhor performance de*L. vannamei*. No Capítulo 3, os camarões foram mantidos em cinco níveis de SST: 250, 500, 1000, 2000 e 4000 mgL^{-1}. Foi executado teste para medir o consumo específico de oxigênio (CEO) dos camarões após 24 e 42 dias do início do experimento. A performance foi semelhante nas cinco concentrações de SST. Análises de CEO e dos dados de desempenho sugerem uma adaptação de *L. vannamei* em baixas concentrações de oxigênio dissolvido. No Capítulo 4, dois sistemas multitróficos integrados foram montados com água recirculando em quatro circuitos fechados compostos por tanques separados para camarões, tilápias, tanques de sedimentação e *red drum*associados às ostras. O estudo demonstrou que a aplicação do cultivo de camarões marinhos com a tecnologia de bioflocos em sistema multitrófico integrado resulta na redução de sólidos causada por organismos consumidores de subprodutos dos camarões. Como conclusão, as pesquisas mostraram que o controle de SST pode ser realizado por clarificação, podendo aumentar a eficiência do processo de sedimentação com o ajuste do fluxo mantido no clarificador ou por tratamento biológico com utilização de organismos consumidores de resíduos da produção de camarões. O manejo das concentrações de SST durante o cultivo de *L. vannamei* em sistema BFT gera benefício na qualidade de água e melhora os índices de desempenho zootécnico.

Palavras-chave: *Litopenaeus vannamei*, bioflocos, sólidos suspensos totais, remoção de sólidos, consumo de oxigênio, sistema multitróficos.

ABSTRACT

Aquaculture has produced aquatic organisms increasing stocking density, reducing the occupied areas and the use of water. The marine shrimp production with biofloc technology (BFT) allow the practice of water reducing, stimulating the natural productivity that improves the water quality and add food to the target species. However, the retention of water over cycle generates large amounts of total suspended solids (TSS) that can interact with physical and chemical parameters, leading to changes in water quality. A series of studies were performedsearching for improving of the suspended solids management to improve water quality and optimize the marine shrimp production, being three at the Marine Station of Aquaculture (IO-FURG, Brazil) and one in Waddell Mariculture Center (WMC, USA). The chapters, relating to experiments with *L. vannamei* in BFT system, aimed to: (1) effect of two different water flow during the suspended solids removal process by settling; (2) analyzeduring biofloc formation, the effect of different suspended solids levels on water quality and *L. vannamei*performance; (3) evaluate the effect of different TSS concentrations on the oxygen consumption and performance of *Litopenaeus vannamei* and (4) evaluate the marine shrimp culture, integrating tilapia for the TSS biological treatment, as well as the use of oysters as biofilters and the possibility of integrating a carnivorous fish species in the BFT system. All experiments were conducted in greenhouses. In Chapter 1, a 17-week trial was carried out. A control with no solids removal andtwotreatments with different flows (3945L h^{-1}–HF) and (1750L h^{-1}–LF) for removalwere compared from the seventh week. Best performance indices of *L. vannamei*were achieved with the suspended solids removal. The lower water flow (LF) maintained in the clarifier facilitated the settling, allowing the flow adjustment. In Chapter 2, a 42-day trial was conducted with treatmentsofthree ranges ofTSS: 100 – 300 mgL^{-1}(TL), 300 – 600 (TM) and 600 – 1000 (TH). The water quality parameters in the lower range (TL) resulted in better performance of *L. vannamei*. In Chapter 3,*L. vannamei* was kept during 42 days infive TSS concentrations were used: 250, 500, 1000, 2000 and 4000 mg L^{-1}. It was carried out test to measure the specific oxygen consumption rate (OCR). The performance was similar in the five concentrations. OCR analysis and performance of *L. vannamei* suggest an adaptation of shrimp at low dissolved oxygen concentrations. In Chapter 4, two integrated multi-trophic systemswere mounted with recirculating water in four closed circuits composed of separate tanks for shrimp, tilapia, settling tanks and red drum associated with oysters. After 8 weeks, the study showed that application of BFT marine shrimp systemin integrated multi-trophic system, result in reduction of solids by consumers of shrimp sub-products. In conclusion, the TSS control can be performed by clarifying, and can increase the settling process efficiency with flow adjustmentin the clarifier or by biological treatment with the use of consumers of shrimp waste. The management of TSS concentrations during the *L. vannamei*culture in BFT system generates benefits in water quality and improve the growth performance indices.

Key-worlds: *Litopenaeus vannamei*, biofloc, total suspended solids, solids removal, oxygen consumption, multi-trophic system.

INTRODUÇÃO GERAL

A aquicultura pode variar de sistema desde o extensivo até o superintensivo, buscando aumento de produção em menores áreas e com redução de uso de água, possibilitando a intensificação do sistema com o aumento na densidade de estocagem e geração de resíduos (Avnimelech 2009; Martínez-Espiñera et al. 2015).

O planejamento no desenvolvimento da aquicultura não incluiu suficientes considerações no âmbito ambiental, especialmente durante o período de 1980 – 2000, quando a produção de camarão foi considerada ser altamente lucrativa e novos viveiros foram construídos, em muitos casos, com poucas considerações ambientais (Avnimelech 2009). No entanto, além das questões ambientais, a qualidade da águado cultivo deve ser considerada em sistema de produção na carcinicultura. Em viveiros escavados as interações de fatores abióticos com o solo e interface com a água são relevantes, podendo resultar em alterações de pH, consumo de oxigênio, geração de amônia e, consequentemente, interferir no crescimento dos camarões (Bratvold & Browdy2001; Avnimelech & Ritvo 2003). Mesmo com a possibilidade de maiores produtividades em viveiros com sedimento (Fóes 2011), a impermeabilização dos viveiros deve ser considerada para melhora da qualidade de água, isolando o efeito de interações entre solo e água.

Para melhor conhecimento dos cultivos em viveiros revestidos, algumas pesquisas foram desenvolvidas ainda na década de 1990 e investigaram as partículas orgânicas disponíveis na coluna de água sem interação com sedimento e associadas positivamente ao desempenho zootécnico do camarão branco em sistema intensivo de cultivo (Moss 1995; Moss & Pruder 1995). Simultaneamente, Hopkins et al. (1993) desenvolveram pesquisa para avaliar diferentes percentuais de troca de água de 25% dia^{-}

^{1}, 2,5% dia^{-1} e 0% dia^{-1}. Foi alcançando produtividade de 6.400 kg ha^{-1} ciclo^{-1}com renovação de 25 e 2,5% dia^{-1}, enquanto que, onde não houve renovação (0% dia^{-1}) foi registrado uma produtividade de 3.200 kg ha^{-1} ciclo^{-1}. Estes resultados demonstraram a possibilidade da redução de efluente de produção de camarão. Anos depois, em revisão sobre manejo de água para cultivo de camarão, Hopkins et al. (1995) destacaram algumas práticas como: (1) redução das trocas de água; (2) densidades de estocagem e taxas de arraçoamento adequadas a capacidade de produção; (3) remoção de depósitos de lodo; (4) tanque de tratamento por sedimentação para separação de resíduos sólidos; (5) transferência da água para reservatórios com o reuso em ciclo subsequente e (6) tratamento biológico da água com uso de moluscos, peixes herbívoros ou plantas halófitas para retenção e assimilação de sólidos e nutrientes. Neste sentido, Hopkins e colaboradores desenvolveram distintas pesquisas em Waddell Mariculture Center (Bluffton, Carolina do Sul, EUA) relacionadas à cultivos com mínima ou sem renovação de água, utilizando a produtividade natural para melhores resultados de desempenho zootécnico. Paralelamente, Avnimelech e colaboradores em Israel desenvolveram pesquisas com objetivos diversificados, desde controle de atividade microbiana, mínima renovação de água e utilização de fontes de carbono para degradação de nitrogênio em sistema intensivo na aquicultura (Avnimelech 2009). Todas estas pesquisas foram o marco para o desenvolvimento de um pacote tecnológico, com pesquisas voltadas para a produção de camarões em sistemas intensivo e superintensivo, visando à redução de uso de água e o aumento da produtividade.

Com estas linhas de pesquisas foi possível impulsionar o desenvolvimento de técnicas para estabelecer a transição de microrganismos fotoautotróficos no início do cultivo para predominantemente heterotrófico, que são bactérias que dependem de uma fonte de carbono orgânico e nitrogênio inorgânico de forma balanceada (Hargreaves, 2006).Para acelerar esta predominância, as bactérias heterotróficas dependem de uma demanda maior de carbono, a qual deverá provir de fertilização com fontes de carbono alóctone (Samocha et al. 2007; Serra et al. 2015). Para isso, adota-se a relação carbono:nitrogênio (C:N) inicial de 15-20:1 para estimular o surgimento de bactérias heterotróficas para a conversão de nitrogênio amoniacal em proteína bacteriana (Avnimelech 1999; Xu & Pan 2012). O sucesso do cultivo dependerá de um equilíbrio entre a produção de resíduos e a capacidade de assimilação dos nutrientes no ambiente da espécie cultivada. A partir do cálculo estequiométrico elaborado por Ebeling et al. (2006) as bactérias heterotróficas consomem 15,17 g de carbono para cada grama de nitrogênio (N) consumido do meio. Em subsequente estágio de desenvolvimento bacteriano, surgem as bactérias nitrificantes que são autotróficas e dependem de carbono inorgânico oriundo da alcalinidade ($CaCO_3$), consumindo 7,05 g $CaCO_3$ / g N (Ebeling et al. 2006). Este consumo de nitrogênio auxilia na redução das concentrações de amônia no meio de cultivo, melhorando a qualidade de água e otimizando o uso do recurso hídrico, convergindo para a prática do uso racional de água. Com isso, minimiza o descarte de água e, consequentemente, reduz a descarga de compostos nitrogenados com reflexos no aumento da produção (Wasielesky et al. 2006). Em uma denominação inicial, estes sistemas eram conhecidos também por "ZEAH" (*Zero Exchange, Aerobic, Heterotrophic Culture Systems*), mas atualmente, devido a produtividade natural ser a precursora na formação de bioflocos (agregados microbianos), o sistema leva a denominação de BFT – *Biofloc Technology* (Avnimelech 2009).

A estrutura dos bioflocos é complexa e de configuração amórfica (Figura 1), com uma matriz interna dependente de interações entre processos físicos, químicos e biológicos (De Schryver et al. 2008). Em regiões de baixa turbulência ocorrem colisões entre partículas que levam a um aumento da agregação de sólidos e formação de partículas maiores (McMillan et al. 2003).Aproximadamente, 40% da biomassa bacteriana presente na coluna da água de viveiros revestidos, está associada com partículas suspensas (Burford et al. 2003), tornando as bactérias os microrganismos precursores na agregação dos flocos microbianos. Com isso, o fornecimento de fontes de carbono, que estimulam o crescimento de bactérias heterotróficas, tem como consequência, o aumento das concentrações de sólidos suspensos (Xu & Pan 2012). Subsequentemente, o estabelecimento das comunidades bacterianas autotróficas (nitrificantes), possibilitam também a agregação de microrganismos, caracterizando a geração de SST (Ebeling et al. 2006). Analisando os resultados de dinâmica de nitrogênio de Silva et al. (2013), pode-se observar que o início das reduções de amônia e nitrito no 27° dia de cultivo de *L. vannamei* em sistema BFT, coincidiram com o aumento de sólidos suspensos totais (SST).

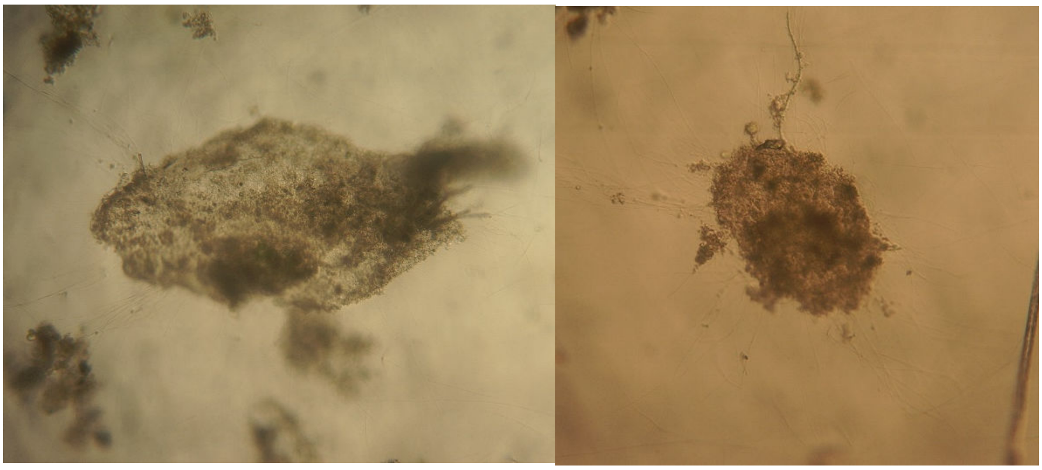

Figura 1. Imagens de bioflocos provenientes de cultivo de *L. vannamei* em sistema BFT (Foto: Carlos Gaona).

As fontes de carbono orgânico e inorgânico, indiretamente, aumentam a quantidade de bioflocos e mantem ativo o metabolismo microbiano, fazendo com que ocorra o decréscimo nas concentrações de oxigênio dissolvido (De Schryver et al. 2008). Simultaneamente à formação de bioflocos, a produção de camarões em altas densidades de estocagem em sistema BFT resulta em elevadas quantidades de material fecal em suspensão (Wasielesky et al. 2006), fazendo com que a precipitação de sólidos e a formação de lodo sejam comuns (Hopkins et al. 1994). Reforçando este processo, dejetos sólidos são gerados tanto direta ou indiretamente pela adição de alimento no sistema e estão presentes como resíduos de alimentos, metabólitos, fezes e microrganismos mortos no sistema (Viadero & Noblett 2002). Estes sólidos decantados, mesmo na forma de partículas menores (Maillard et al. 2005) podem ser ressuspendidos em grandes quantidades na água do cultivo, podendo causar efeitos negativos no sistema de produção (McMillan et al. 2003), destacando-se a redução dos níveis de oxigênio dissolvido (Cohen et al.2005). O consumo de oxigênio está relacionado também, com o desenvolvimento de bactérias heterotróficas e nitrificantes. Para cada grama de nitrogênio consumido por estas bactérias, 4,71 g O_2 são utilizados pelas heterotróficas e 4,18 g O_2 pelas nitrificantes (Ebeling et al. 2006).

Com essa dinâmica de geração de partículas sólidas, para a manutenção dos níveis de oxigênio e de distribuição de bioflocos na coluna de água, o sistema BFT exige aeração intensa (Hargreaves 2006). Os equipamentos de aeração aplicados na aquicultura, variam tanto na capacidade de incorporação de oxigênio na água como função primária, como na mistura como um efeito secundário (Hargreaves 2006), criando características hidrodinâmicas distintas. A movimentação horizontal causada por aerador de pá na superfície da água, é reduzida com a distância do equipamento, desfavorecendo a permanência de sólidos em suspensão e, consequentemente, facilita o acúmulo de lodo no fundo dos viveiros, onde pode ocorrer troca de substâncias entre o material acumulado e água, influenciando fortemente a qualidade da água de cultivo (Avnimelech & Ritvo 2003). Como consequência, o sistema fica carregado de matéria orgânica que não entra em processo de reciclagem de nutrientes e cria uma zona anóxica, limitando a área de ocupação dos camarões, além do risco de doenças (Avnimelech & Ritvo 2003). Todos esses problemas acarretam menores índices de desempenho zootécnico, resultando em baixa produtividade. O sistema de aeração de ar difuso no fundo do tanque, resulta na verticalização do movimento da água, distribuindo o material particulado em todo o tanque, fazendo com que haja maior concentração de sólidos mantidos em suspensão.

Além da interação dos sólidos suspensos com a disponibilidade de oxigênio, o aumento de SST pode reduzir a alcalinidade em sistema BFT, devido ao consumo de carbono inorgânico na forma de alcalinidade pelas bactérias nitrificantes (Hargreaves 2013), visto que estas consomem aproximadamente o dobro de carbono inorgânico por grama de nitrogênio consumido do sistema, comparado as bactérias heterotróficas (Ebeling et al. 2006). A alcalinidade é a capacidade de tamponamento em resistir a mudanças no pH em resposta a ácidos ou bases (Hargreaves 2013). A matéria particulada pode fornecer substrato para as bactérias e outros microrganismos, sendo que quantidades elevadas de sólidos aumentam a respiração na coluna de água (Vinatea et al. 2010). A liberação de CO_2 devido aos processos respiratórios realizados pelos microrganismos, levam a redução de pH (Wasielesky et al. 2006). O dióxido de carbono dissocia-se em íons carbonatos (CO_3^{2-}) e bicarbonatos (HCO_3^-), promovendo a liberação de prótons de hidrogênio (H^+) e reduzindo o pH e a alcalinidade. O processo de nitrificação é responsável também pela acidificação, onde 1,98 mol de H^+ pode ser produzido para cada mol de NH_4^+ (amônia) oxidada (Guisasola et al. 2007)

Outro problema na qualidade da água é a rápida eutrofização decorrente do aumento das concentrações de nutrientes e matéria orgânica durante o cultivo (Thakur & Lin 2003). No caso do fósforo, este nutriente está relacionado com a constante entrada de ração durante o cultivo. Estima-se que apenas 23 % do fósforo disponível na ração seja incorporado em biomassa de camarões *L. vannamei* (Velasco et al. 1998). Assim, a decomposição do alimento não ingerido e das excretas dos animais contribui para o incremento das concentrações de fósforo do meio (Barak et al. 2003), proporcionando o acúmulo durante o cultivo, característico de sistema superintensivo, uma vez que não apresenta nenhuma rota de saída de fósforo do sistema (Silva et al. 2013). O risco do acúmulo em sistema BFT em função de eutrofização são consequentes florações de cianobactérias que podem comprometer a qualidade da água de cultivos e a sustentabilidade do sistema (Silva et al. 2013).

Dentre os esforços para melhorar a qualidade da água em cultivo superintensivo em sistemas BFT, o controle das concentrações de SST ao longo do cultivo devem ser efetivos (Hargreaves 2006). Sugestões de níveis apropriados ao cultivo de *L. vannamei* em sistema BFT variam de 200 – 600 mg L^{-1} (Samocha 2007; Avnimelech 2009; Gaona et al. 2011; Schveitzer et al. 2013). Portanto, intervenções são necessárias para o manejo de bioflocos em sistema BFT. A clarificação possui propriedades que se adequam na remoção de matéria orgânica particulada. O processo tem como característica a remoção de sólidos suspensos por ação gravitacional sobre as partículas, em massa de água com fluxo lento, para o tratamento de corpos de água. Quando a remoção de sólidosé feita por decantaçãoou sedimentação, a velocidade desedimentação de partículas é influenciada por propriedades físicas. Velocidade de sedimentação de uma partículaé uma função dadensidade de partículas, tamanho, forma, de viscosidade e densidade da água do cultivo (Merino et al. 2007). Dessa maneira, a diferença de densidade entre partícula e fluido, controlará o processo de separação (Wheaton 1977). Partículas com velocidade de sedimentação maior do que a velocidade de fluxo massa de água tendem a sedimentação (Johnson & Chen 2006). Composição, tamanho e densidade de sólidos suspensos podem variar devido à fonte de resíduos, bem como, por condições dos tanques (McMillan et al. 2003). As propriedades de sedimentação e tamanho de partículas presentes na aquacultura variam muito e estas variações têm importantes implicações no desenho e operação de sistema de remoção de sólidos (Wong & Piedrahita 2000). Especificamente em sistema BFT, se uma partícula de biofloco é altamente porosa, o fluido passará pelo agregado resultando em um transporte de nutrientes as células constituintes do floco microbiano e causará uma redução na velocidade de sedimentação da partícula no tanque (Crab et al. 2007).Em cultivo de camarões marinhos com tecnologia de bioflocos, Ray et al. (2010, 2011) testaram o uso de clarificadores para retirada de sólidos suspensos utilizando *air-lift* e bomba submersa para captação de água para o clarificador, para analisar os efeitos na produção de *L. vannamei*. Gaona et al. (2011) elaboraram um sistema de clarificação com bombeamento para captação de água para o clarificador em escala piloto, para avaliar a qualidade de água e desempenho zootécnico de *L. vannamei* em sistema BFT. No entanto, comparações entre diferentes fluxos de massa água e sedimentação das partículas durante a retirada de sólidos suspensos de cultivo superintensivo de camarão *L. vannamei*, devem ser analisados.

A otimização de processos de remoção de partículas suspensas pode auxiliar no controle de SST mesmo em fase inicial de formação de bioflocos. O processo de nitrificação tem estreita relação com a carga orgânica presente na água devido a inibição causada as bactérias nitrificantes, até mesmo pelo rápido crescimento de bactérias heterotróficas (Luo et al. 2013). Conforme observado por Ebeling et al. (2006), se tratarmos a biomassa bacteriana como SSV, pode-se perceber a diferença no crescimento entre nitrificantes (SSV_N) e heterotróficas (SSV_H), quando utilizam o nitrogênio disponível no meio. Estes autores observaram que foram produzidas 0,20g SSV_N g N^{-1}, contra 8,07 g SSV_H g N^{-1}, resultando em uma diferença de 40 vezes maior o crescimento de heterotróficas. Como destacado por Hargreaves (2006), quanto maior a quantidade de carbono orgânico menor é a taxa de nitrificação. Considerando uma concentração de SST de 500 mg L^{-1} a taxa de nitrificação pode chegar próximo de 0,025 g N g SSV^{-1} dia^{-1}, sugerindo que a nitrificação fica restringida pelo carbono orgânico acumulado no sistema (Hargreaves 2006). Dessa maneira, as concentrações de SST em cultivo de *L. vannamei* recomendadas na literatura (Samocha et al. 2007; Ray et al. 2010; Gaona et al. 2011; Schveitzer et al. 2013), podem alcançar diferentes resultados na taxa de nitrificação, resultando em tempos diferentes de estabelecimento de bactérias nitrificantes. O retardo da nitrificação leva ao acúmulo de amônia e nitrito, que dependendo das concentrações alcançadas pode interferir na taxa de sobrevivência dos camarões (Lin & Chen 2001, 2003).

Além do risco de acúmulo de compostos nitrogenados em sistema BFT, o excesso de SST totais pode causar efeitos direto aos camarões relacionados as oclusões de brânquias (Ray et al. 2010; Schveitzer et al. 2013). A concentração de oxigênio recomendada para camarões marinhos é de 5 mg L^{-1} (Van Wyk & Scarpa 1999). Os níveis letais de oxigênio dissolvido para *L. vannamei* variam de acordo com o tamanho em função das taxas metabólicas do animal (Zhang et al. 2006). Em condições de hipóxia de 3, 5 e 2,0 mg L^{-1} de oxigênio dissolvido, *L. vannamei* apresentou respostas negativas em imuno parâmetros (Jiang et al. 2005). Além de relações como peso, o consumo de oxigênio pode variar com a temperatura, salinidade e concentrações elevadas de compostos nitrogenados (Bett & Vinatea 2009; Campos et al. 2014). Estas relações de interdependência com os níveis de oxigênio disponíveis na água, tornam-se preocupantes em sistema BFT sem manejo dos SST. A interrupção do sistema de aeração por qualquer tipo de falha, reduz a disponibilidade de oxigênio dissolvido, podendo alcançar 0,65 mg L^{-1} em 30 minutos em sistema BFT (Vinatea et al. 2009). Chapman et al. (1987) observaram efeito físico na obstrução e danos nas brânquias de truta arco-íris. Para *L. vannamei* é necessário observar e entender a relação de concentrações de SST acima do recomendado com consumo de oxigênio dissolvido.

Além da mínima ou nenhuma taxa de renovação de água ao longo do período de cultivo (Hopkins et al. 1995; Wasielesky et al. 2006), outros estudos propõem técnicas para melhorar a qualidade da água dos cultivos e do efluentes, dividindo-se em diferentes estratégias, tais como: utilização de organismos filtradores como moluscos e assimiladores de nutrientes como microalgas e macroalgas (Shpigel & Neori 1996; Wong et al.1995; Pagand et al. 2000) e tratamentos integrados entre sedimentação, macroalgas e moluscos (Jones et al. 2001, Ramos et al. 2009), construção de “wetland” com macrófitas (Tilley et al. 2002), tanques de sedimentação (Teichert-Coddington et al. 1999) e clarificação em escala laboratorial e piloto (Ray et al. 2010, 2011; Gaona et al. 2011). O cultivo de camarões com tilápias tem sido demonstrado em estudos prévios com resultados que demonstram a viabilidade no controle de sólidos suspensos (Muangkeow et al. 2007; Yuan et al. 2010). Passando para uma alternativa mais abrangente, osistema multitrófico pode ser adotado como uma tecnologia para redução de resíduos de monoculturas (Martínez-Espiñeira et al. 2015). Como característica, o sistema multitrófico está baseado na integração de espécies de diferentes estratégias alimentares, combinando o cultivo de organismos alimentados com ração comercial (peixe ou camarão) com espécies extrativistas de resíduos orgânicos particulados (peixes ou invertebrados) e inorgânicos dissolvidos (plantas halófitas) (Barrington et al. 2009).

Portanto, a aplicação das técnicas de remoção de sólidos suspensos, a identificação de concentrações de SST favoráveis a formação de bioflocos e ao consumo de oxigênio dissolvido, bem como, a integração de *L. vannamei* cultivado em sistema BFT com sistema multitrófico, focando o tratamento biológico dos resíduos dos camarões, fecham uma série de estudos que podem aperfeiçoar o manejo de sólidos suspensos para melhorar a qualidade de água, buscando a otimização da produção de camarões marinhos.

OBJETIVOS

Objetivos geral

A presente tese tem como objetivo a análise das interações de sólidos suspensos na qualidade de água e performance de cultivo do camarão *Litopenaeus vannamei* em sistema BFT.

Objetivos específicos

- Efeito de dois diferentes fluxos de bombeamentos de água durante o processo de remoção de sólidos suspensos por sedimentação em clarificadores do cultivo de camarão *Litopenaeus vannamei* em sistema com bioflocos;

- Analisar durante a formação de bioflocos, o efeito de diferentes níveis de sólidos suspensos totais na qualidade de água e performance de *L. vannamei* em sistema BFT;
- Avaliar o efeito de diferentes concentrações de SST sobre o consumo de oxigênio e desempenho zootécnico de *Litopenaeus vannamei* em sistema BFT;
- Avaliar o cultivo de camarões *L. vannamei* em sistema BFT, integrando tilápias *Oreochromis niloticus* para avaliação de tratamento biológico de sólidos suspensos totais, bem como, o uso de clarificadores e de biofiltradores como as ostras *Crassostrea virginica*. Além disso, avaliar a possibilidade de integrar uma espécie de peixe carnívora *Sciaenops ocellatus* ao sistema BFT.

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CAPÍTULO I

BIOFLOC MANAGEMENT WITH DIFFERENT FLOW RATES FOR SOLIDS REMOVAL IN THE *Litopenaeus vannamei* BFT CULTURE SYSTEM

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Artigo submetido a revista Aquaculture International

RESUMO

Foi avaliado o efeito de dois fluxos de bombeamentos de água durante a remoção de sólidos suspensos por clarificação, do cultivo de camarão *Litopenaeus vannamei* em sistema com bioflocos. Foram avaliados um controle (sem remoção de sólidos) e dois tratamentos com remoção de sólidos em fluxos diferentes: alto (3945 L h^{-1} – HF) e baixo (1750 L h^{-1} – LF), sem reposição de água, avaliados por medidas de sólidos suspensos totais (SST). *L. vannamei* com peso médio de 0,18 ± 0,06 g (350 ind. m^{-2}) foram estocados em tanques de 35 m^{-3}. Durante 17 semanas, parâmetros físicos e químicos foram mantidos dentro do recomendado para a espécie. Na sétima semana, teve início a clarificação para manutenção de SST próximo a 500 – 600 mg L^{-1} até o final do estudo. Os volumes de passagem de água pelos clarificadores foram significativamente diferentes ($p < 0,05$) entre HF (205 ± 34 m^3) e LF (114 ± 24 m^3). Houve redução significativa ($p < 0,05$) de volume dos tanques no HF (28,09 ± 0,92 m^3) e LF (28,62 ± 1,38 m^3) comparados ao controle (34,43 ± 0,60 m^3). A composição proximal antes da clarificação (inicial) e no término do experimento (final) foi semelhante ($p > 0,05$) para proteína, umidade e cinza. O lipídeo em LF (final) foi significativamente menor ($p < 0,05$) comparado aos demais. A sobrevivência, produtividade e conversão alimentar aparente foram significativamente melhores ($p < 0,05$) em HF e LF, comparados ao controle. Melhores índices de desempenho foram alcançados com a remoção de sólidos. O menor fluxo de água facilitou a sedimentação no clarificador, possibilitando o ajuste de fluxo.

ABSTRACT

Different water flows for solids removal in the *Litopenaeus vannamei* BFT system were evaluated. One control (no solids removal) and two treatments using different water flows, high (3945 L h^{-1} – HF) and low (1750 L h^{-1} – LF), were used with no water replenishment after each process, and the total dry weight of the solids was measured. *L. vannamei* (0.18 ± 0.06 g; 350 ind m^{-2}), were stocked in 35 m^{-3} tanks. For 17 weeks, the physical and chemical parameters were maintained within the recommended. To keep the total suspended solids (TSS) concentrations at approximately 500 – 600 mg L^{-1}, clarifying was performed. The average water volume flowed by clarifiers was significantly different ($p < 0.05$) between HF (205 ± 34 m^3) and LF (114 ± 24 m^3). There was a significant decrease ($p < 0.05$) in the final tank volume in HF (28.09 ± 0.92 m^3) and LF (28.62 ± 1.38 m^3) due to the clarifying. Before clarifying (initial) and at the end of experiment (final) were not significantly different ($p > 0.05$) for crude protein, moisture or ash. The crude lipid of the LF in the final period were significantly lower ($p < 0.05$) compared to the others in both periods. The survival, productivity and food conversion ratio were significantly better ($p < 0.05$) in the HF and LF treatments compared to those of the control. The best shrimp performance was obtained with solids removal. The lower flow in the clarifier facilitated particle settling, allowing adjustment of the flow.

Keyword: Biofloc, *Litopenaeus vannamei*, suspended solids, clarifying, settling.

INTRODUCTION

The 1990s marked the development of research in aquatic farming systems with no or minimal water exchange toward the development of systems with bioflocs (BFT), focusing on improving techniques to stimulate bacterial growth and the formation of biofloc (Avnimelech 2009). Recently, research on the water quality and nutritional context has accompanied the development of BFT systems (Wasielesky et al. 2006a; Krummenauer et al. 2011; Schveitzer et al. 2013; Serra et al. 2015). The water quality has improved due to the recycling of nutrients, such as ammonia and nitrite, by heterotrophic and nitrifier bacteria, allowing a reduction in the amount of water that is used for marine shrimp production, given the need to use water in a sustainable manner. The nutritional composition of bioflocs contributes to a supplementary feeding with variations in the crude protein content, improving shrimp performance (Ballester et al. 2010; Wasielesky et al. 2006a; Xu and Pan et al. 2012).

In BFT systems, the contribution of food supplementation to shrimp performance is related to the consumption of suspended particles, which range in the size of the biofloc, digestibility and nutritional value (Wasielesky et al. 2006a; De Schryver et al. 2008; Kent et al. 2011; Xu and Pan 2012; Ekasari et al. 2014). The digested biofloc can replace a significant fraction of the nutrient demand by recycling residues and/or recovering some fractions of nutrients that are excreted after feeding (Burford et al. 2004; Schneider et al. 2005; Hargreaves 2006; Crab et al. 2010).

Over time, culture techniques have improved, allowing an increased stocking density and a consequently increasing feed (Wasielesky et al. 2006a; 2011 Krummenauer et al. 2011). In the BFT system, water conservation during the shrimp production cycle generates a large amount of suspended solids due to the increase in food through natural productivity in the ponds (Burford et al. 2003). In heterotrophic systems, bacterial biomass production is higher compared to that of phytoplankton biomass in photoautotrophic cultures, and there is a consequent increase in suspended solids (Ebeling et al. 2006). In closed shrimp production systems at high stocking densities, there are decreased dissolved oxygen levels (Cohen et al., 2005) and production of nitrogen compounds, such as ammonia and nitrite (Avnimelech 1999; Cohen et al. 2005), derived from organic matter in suspension.

Among the efforts to control the load of particulate matter and to improve the water quality in BFT systems, suspended solids removal by settling (clarifying) has properties that are suitable for the removal of particulate organic matter by gravitational action in a slow radial water flow (Ray et al. 2010a; Gaona et al. 2011). When the removal of solids is carried out by decantation, the particle-settling velocity is influenced by physical properties. The particle-settling velocity is a function of the particle density, size, shape, density and viscosity of the water culture (Merino et al. 2007). Particles with a higher settling velocity than the water flow tend toward sedimentation (Johnson & Chen 2006; Timmons and Ebeling 2010).

Several studies have demonstrated the use of intermediate and main settling chambers with a radial flow for suspended solids removal from fish and shrimp production systems (Johnson and Chen 2006; Azim and Little 2008; Ray et al. 2010a, 2011; Gaona et al. 2011; Schveitzer et al. 2013). However, knowledge of the capacity of particle removal by settling at different water flows for clarifying is needed.

The aim of this study was to evaluate the effect of two different water flows for the solids removal process by settling in the *Litopenaeus vannamei* BFT culture system.

MATERIALS AND METHODS

Experimental design

The study was conducted at the Marine Station of Aquaculture Prof. Marcos Alberto Marchiori (EMA) under the Oceanographic Institute of the Federal University of Rio Grande – FURG, located at Cassino Beach in Rio Grande, RS, SouthernBrazil (32°11'S; 52°10'W), for 17 weeks.

The experiment was carried out in a greenhouse with nine 35-m³ tanks of useful volume that were lined with high-density polyethylene (HDPE). One control (with no solids removal) and two solids removal treatments using a 797-L settling chamber with two different water flows, high flow (HF) 3945 L h^{-1} and low flow (LF) 1970 L h^{-1}, were usedwith three replicates.

Vertical substrates were used for the natural formation of periphyton, providing an additional source of food for the shrimp (Ballester et al. 2007). Seawater with a salinity of 35 was used and was filtered and treated to 10 ppm chlorine and neutralized with ascorbic acid 24 h later. Organic fertilization was performed by manipulating the C:N ratio (6:1)-based methodologies of Avnimelech (1999) and Ebeling et al. (2006) for nitrogen conversion in bacterial biomass. The carbon source was molasses from sugar cane, containing 37.46% carbon. The pH corrections were made to maintain the values above 7 by adding lime hydrate - $Ca(OH)_2$ according to the dosage that was suggested by Furtado et al. (2011). A 7.5-hp blower was used to keep a diffused aeration trough hose with micropores (Aero-Tube™, Swan®, Marion, OH, USA) at the bottom of the tanks.

L. vannamei, with an average weight of 0.18 ± 0.06 g, were stocked in nine experimental units at a stocking density of 350 individuals m^{-2}. The shrimp were fed three times a day with commercial feed (Potimar Active 38 – Centro Oeste Rações SA, Campinas, SP, Brazil) specific for the species, containing 38% crude protein and 8% lipid. The feed was offered in feeding trays at an initial rate of 10% of shrimp biomass and was adjusted according to the consumption that was observed within the interval between each feeding.

Biofloc management

For the solids removal process, clarifiers were used (Figure 1) corresponding to 2.28% of the initial volume of the culture tank following the procedure that was proposed by Gaona et al. (2011) with a duration of 6 continuous hours for each application to maintain the TSS concentrations at approximately 500 – 600 mg L^{-1} (Samocha et al. 2007; Schveitzer et al. 2013). For each treatment, submerged pumps flowed at 4500 L h^{-1}, whereas for the HF treatment, the inflow into the clarifier was 3945 L h^{-1} and that in the LF treatment was adjusted to 1970 L h^{-1} by a valve (Figure 1) in the water intake pipe. The flow was measured using a stopwatch to measure the time to fill a 1-L flask. After each application, the water that was retained in the clarifier was homogenized, and the sample was collected to analyze the total suspended solids concentration (Strickland and Parsons, 1972) for solids quantification in a 797-L clarifier volume to assess the removal capacity in two flows. After this step, the volume of each clarifier was disposed without replacing this volume in the culture tanks. The following were recorded: (1) Cumulative time (h) whole clarifying process (6 h × number of processes); (2) Water volume flowed (m^3) by clarifiers [(6 h × flow of each treatment) × number of processes]; (3) Solids removed as total dry weight (kg) of solids kept in the clarifier (TSS removed accumulated × 797 L); and (4) Tank final volume (m^3) as measured at the end of the experiment.

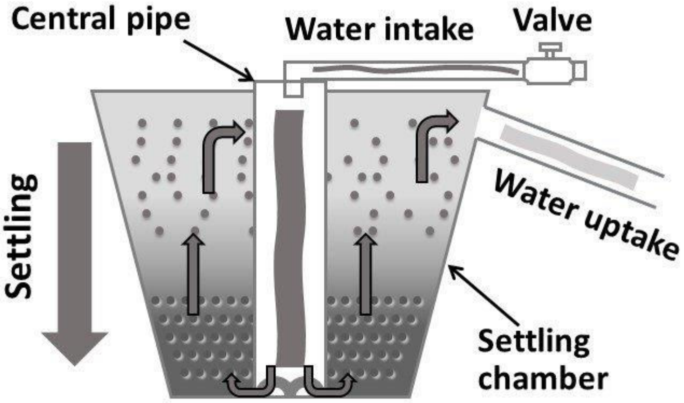

Figure 1. Clarifier: mounted in a 1000-L plastic water box (settling chamber) with a central pipe with a 300-mm diameter and a 700-mm height at the center of the box to reduce turbulence. The operation volume was maintained at 797 L.

Physical and chemical parameters

Daily was monitored temperature, pH, salinity and dissolved oxygen using multiparameter YSI® model 556 (YSI Incorporated, Yellow Springs, OH, USA). Analyses based on the total ammonia nitrogen (TAN) and nitrite ($N–NO_2^-$) concentrations were performed daily, following methods described in UNESCO (1983) and Bendschneider and Robinson (1952), respectively. Every seven days were monitored nitrate ($N–NO_3^-$) and phosphate ($P–PO_4^{-3}$) both according to Aminot and Chaussepied (1983), total suspended solids (Strickland and Parsons 1972) and alkalinity (APHA 1998). The settleable solids volume was quantified by Imhoff cone, as described in Avnimelech (2009).

Proximate composition analysis

Proximate analysis to evaluate the content of protein, lipid, moisture and ash of bioflocs in the culture tanks was performed. The samples were collected in the seventh week before the first clarifying, considered as initial period and at the end of the experiment considered the final period. For the analysis, we used specific methodology for each component according to AOAC (2000).

Shrimp performance

The shrimp growth was accompanied by biometric every seven days, using digital scale with 0.01 g precision (Marte® cientifica AS2000, Santa Rita do Sapucai, Minas Gerais, Brazil), followed by feeding adjustment suggested by Jory et al. (2001). The weekly weight gain (WWG) was determined from the week that the shrimp reached 1 g (Furtado et al. 2015), by the following calculation: WWG (g w^{-1}) = [(Final weight – initial weight) / nº weeks of culture]. The feed conversion rate (FCR) was calculated by the following formula: FCR = Offered food / biomass increment. Survival was calculated by: S% = [(final biomass / average individual weight) / nº stocked individuals] x 100. The productivity (per volume and area) was obtained according to calculation: Prod (kg m^{-3}) = (final biomass / tank volume) and Prod (kg m^{-2}) = (final biomass / tank area).

Statistical analysis

The water quality data, proximal composition and growth performance were analyzed by ANOVA (one way). The homoscedasticity of variances and the normality of the data were verified by the Levene and Kolmogorov-Smirnov tests, respectively, followed by the Tukey test to detect possible differences ($p < 0.05$) between the experimental groups (Sokal and Rohlf, 1969). For the cumulative time of the entire clarifying process, the water volume that flowed by clarifiers, and the total dry weight of the solids that were kept in the clarifier between the two treatments (HF and LF), a *t*-test was used. The survival data were transformed (arcsine $x^{0.5}$) before analysis (Zar 1996).

RESULTS

Water quality

Throughout the experiment, the average temperature, salinity and dissolved oxygen values were greater than 29 °C, 5 mg L^{-1} and 34, respectively (Table 1).

The nitrogen compound mean concentrations (Table 1) showed no significant differences ($p > 0.05$) and followed the natural nitrification process, with decreasing ammonia in the second week in the control and HF and LF treatments. The nitrite concentrations decreased in the fifth week in the LF treatment and in sixth week in the HF treatment and control. The nitrate buildup began in the eighth week and lasted until the end of the experiment.

The TSS concentrations and SS varied compared to the control according to the management that was applied during the study. The clarifying process began in the seventh week, and decreased TSS concentrations of 500 – 600 mg L^{-1}were observed from the twelfth week in both treatments with solids removal (Figure 2a). In the control, the suspended particulate matter accumulation remained until the end of the experiment. Similarly, SS volumes (Figure 2b) were maintained at less than 50 ml L^{-1} from the tenth week in the HF and LF treatments.

Table 1. Mean values ± standard deviation (minimum-maximum) of the physical and chemical parameters that were monitored during the study period for the control and treatments with high (HF) and low (LF) flows for total suspended solids (TSS) removal in the *L. vannamei* BFT culture system.

PARAMETER	**Controle**	**HF**	**LF**
Temperature (°C)	29.06 ± 0.91	29.20 ± 1.04	29.19 ± 0.99
	(26.8-31.5)	(26.7-31.7)	(26.8-31.6)
Dissolved oxygen (mg L^{-1})	5.47 ± 0.67	5.62 ± 0.54	5.58 ± 0.58
	(3.76-6.84)	(4.10-7.11)	(3.22-7.23)
Salinity	35.43 ± 2.80	34.50 ± 2.16	34.91 ± 2.09
	(30.0-39.7)	(30.3-38.3)	(31.7-39.0)
Alkalinity (mg $CaCO_3$ L^{-1})	113.98 ± 36.68	117.68 ± 29.88	120.00 ± 33.44
	(66.7-170.0)	(75.0-176.7)	(75-186.7)
Ammonia (mg L^{-1})	0.31 ± 0.19	0.25 ± 0.17	0.27 ± 0.17
	(0-5.21)	(0-4.03)	(0-4.73)
Nitrite (mg L^{-1})	4.18 ± 0.85	3.81 ± 0.87	4.14 ± 0.86
	(0-22.23)	(0-22.33)	(0-24.33)
Nitrate (mg L^{-1})	54.72 ± 12.48	44.63 ± 14.12	43.58 ± 9.78
	(0-209)	(0.17-166.33)	(0-136.67)
Phosphate (mg L^{-1})	5.34 ± 1.86	4.83 ± 1.76	5.03 ± 1.94
	(2.40-7.60)	(2.20-7.84)	(2.09-7.79)

The pH values decreased during the experiment, and significant differences ($p < 0.05$) were recorded from the thirteenth week (Figure 2c). The pH values below 7 were frequent in the control from the twelfth week. The corrections by the application of $Ca(OH)_2$ were carried out in the three experimental groups did not mask the clarifying effect.

Biofloc management

There were significant differences ($p < 0.05$) between the water volumes flowed by clarifiers during the solids removal process in the HF and LF treatments (Table 2). The HF treatment had a water volume flowed through the clarifier of 205 ± 34 m^3, and for the LF treatment, the value was 114 ± 24 m^3. However, the amounts of solids that were removed in the HF and LF treatments (Table 2) were not significantly different ($p > 0.5$). The application of clarifying from the seventh week resulted in the significant decrease ($p < 0.05$) in the tank final volumes in the HF and LF treatments because of the water that was discharged at the end of each solids removal process compared to the control (Table 2).

The alkalinity had a similar pattern among the experimental groups throughout the trial, and no significant differences ($p > 0.05$) were observed between the groups (Table 1).

The phosphate concentrations accumulated until the end of the experiment, with no significant differences ($p > 0.05$) among the control or HF or LF treatments (Table 1).

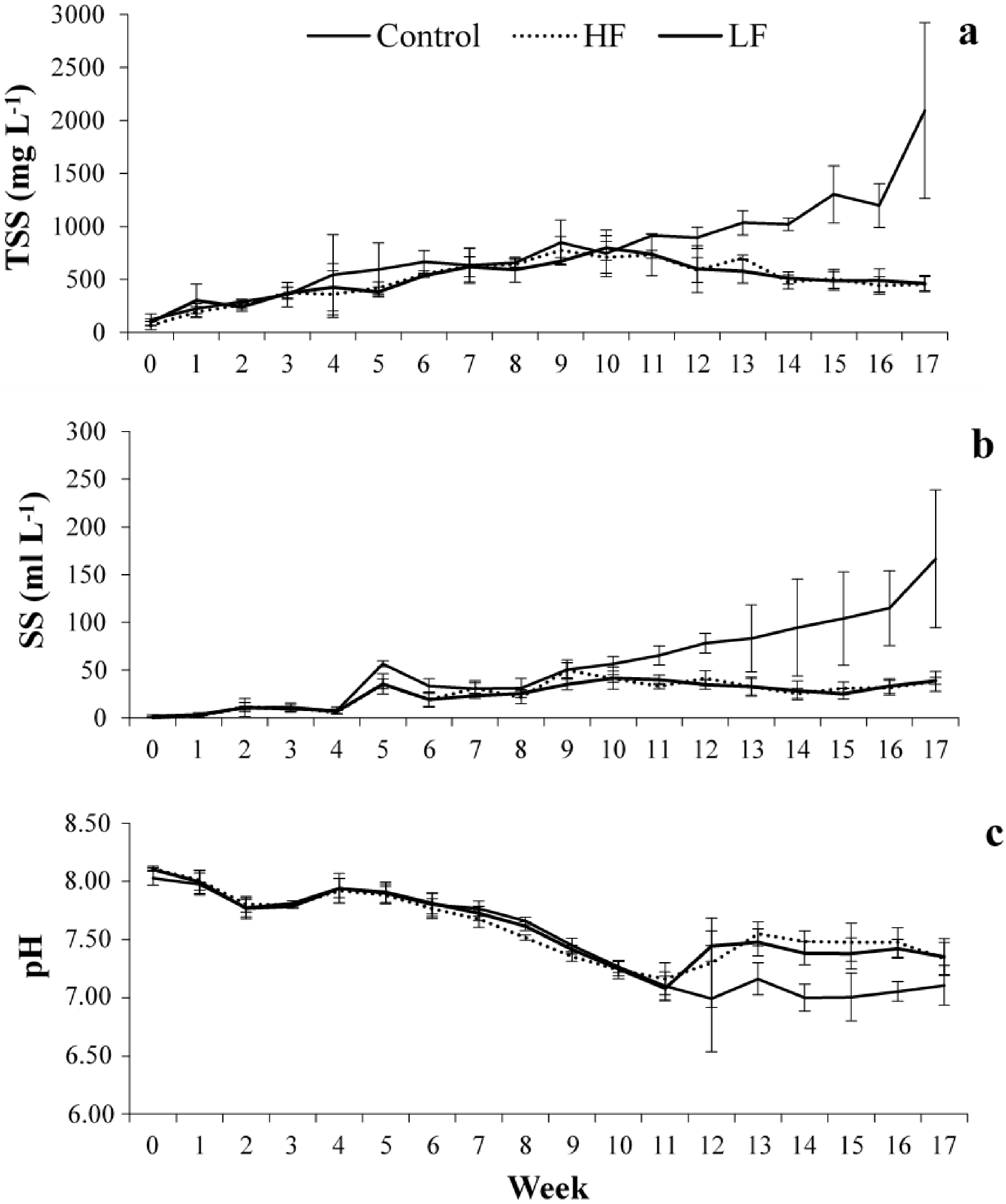

Figure 2. Variation in the tanks throughout the experiment of (a) total suspended solids (TSS) concentrations, (b) settleable solids volumes, and (c) pH in the control and HF and LF treatments in the *L. vannamei* BFT culture system. Vertical bars indicate standard deviation.

Table 2. Clarifying process values that were recorded during the study period, including mean ± standard deviation of the control and TSS removal treatments with high (HF) and low (LF) flows in the *L. vannamei* BFT culture system. Different letters in the same row indicate significant differences ($p < 0.05$).

PARAMETER	**Control**	**HF**	**LF**
Cumulative time (h)	–	52±8.72	58±12.17
Water volume flowed (m^3)	–	205±34^a	114±24^b
Solids removed (kg)*	–	27.87±4.01	26.51±4.98
Tank final volume (m^3)	3.43±0.60	28.09±0.92	28.62±1.38

*Total dry weight (kg) of solid kept in the clarifier

Proximate composition

The proximal composition values related to the crude protein, crude lipid, moisture and ash are presented in Table 3. No significant differences were observed ($p > 0.05$) in the crude protein and ash between the experimental groups in both of the analyzed periods. The moisture of the samples was not significantly different ($p > 0.05$) among the control and treatments (HF and LF) in the initial and final periods. The crude lipid of the LF treatment in the final period was significantly lower ($p < 0.05$) compared to that of the other experimental groups in both periods.

Table 3. Proximate composition of bioflocs that were collected before (initial period) clarifying and at the termination of the experiment (final period) after using the clarifiers in the control and TSS-removal treatments with high (HF) and low (LF) flows in the *L. vannamei* BFT culture system. Different letters in the same row indicate significant differences ($p < 0.05$).

	INITIAL PERIOD			**FINAL PERIOD**		
	Control	**HF**	**LF**	**Control**	**HF**	**LF**
CP* (%)	24.28±0.92	25.06±0.87	26.03±0.39	23.05±0.16	25.44±0.46	25.58±0.46
CL (%)**	$0.58±0.04^{a}$	$0.73±0.15^{a}$	$0.77±0.13^{a}$	$0.62±0.08^{a}$	$0.62±0.07^{a}$	$0.33±0.12^{b}$
Moisture (%)	7.17±0.35	7.01±0.56	7.30±0.69	6.99±0.08	6.60±0.44	6.77±0.12
Ash (%)	40.65±1.63	39.60±5.66	39.15±3.32	40.15±4.03	41.50±5.52	45.40±5.52

*Crude protein; **Crude lipid

Shrimp performance

Throughout the experiment, there were no significant differences ($p > 0.05$) in the growth of *L. vannamei* among the control and treatments with the removal of TSS (HF and LF). At the end of the experiment, both the HF and LB treatments showed significant differences ($p < 0.05$) in survival, productivity and FCR (Table 4) compared to those of the control.

Table 4. Shrimp performance of *L. vannamei* between the control and TSS-removal treatments with high (HF) and low (LF) flows in the BFT system. Different letters in the same row indicate significant differences ($p < 0.05$).

PARAMETER	**Control**	**HF**	**LF**
Initial weight (g)	0.18 ± 0.06	0.18 ± 0.06	0.18 ± 0.06
Final weight (g)	10.58 ± 1.11	11.65 ± 1.84	11.51 ± 1.23
Survival (%)	93.84 ± 1.74^{a}	98.15 ± 0.26^{b}	98.13 ± 0.71^{b}
WWG* (g w^{-1})	0.62 ± 0.06	0.70 ± 0.13	0.69 ± 0.07
Productivity (kg m^{-2})	3.03 ± 0.22^{a}	3.76 ± 0.30^{b}	3.69 ± 0.08^{b}
Productivity (kg m^{-3})	3.08 ± 0.27^{a}	4.56 ± 0.42^{b}	4.52 ± 0.16^{b}
FCR**	1.49 ± 0.14^{a}	1.25 ± 0.07^{b}	1.23 ± 0.01^{b}

*Weekly weight gain; **Feed conversion rate

DISCUSSION

The parameters of temperature, dissolved oxygen, salinity, pH and alkalinity were within tolerable values for *L. vannamei* (Van Wyk and Scarpa 1999; Ebeling et al. 2006; Wasielesky et al. 2006b). The pH differences that were observed among the control and solids removal treatments coincided with lower TSS concentrations between the thirteenth and sixteenth weeks, resulting in an inverse relationship between the CO_2 and TSS concentrations in the BFT system due to the respiration processes of microorganisms in the water column (Wasielesky et al. 2006a; Vinatea et al. 2010; Furtado et al. 2011).

The nitrification process occurred due to the natural culture conditions and not due to solids removal. The initial management with carbon source application reduced the ammonia concentrations (Avnimelech 2009; Ebeling et al. 2006; Serra et al. 2015). Later, the natural nitrification process occurred, which is characteristic of the BFT system (Avnimelch 2009; Silva et al. 2013), maintaining ammonia and nitrite at tolerable levels (Lin and Chen 2001, 2003) and consequent buildup of nitrate at the end of the experiment (Silva et al. 2013) at acceptable concentrations for the species (Kuhn et al. 2010).

Most of the phosphorus in the BFT system is in the dissolved and particulate form, and its buildup is common throughout the cycle (Silva et al. 2013). Previous studies have demonstrated phosphorus reduction by removing suspended solids by settling in a radial flow system (Ray et al. 2010a; Schveitzer et al. 2013). In the present study, no phosphorous reduction was observed in the solids removal treatments.

From the seventh week, the solids removal from culture tanks was approximately within the recommended values of 500 – 600 mg L^{-1} (Samocha et al. 2007; Gaona et al. 2011; Schveitzer et al. 2013). The TSS and SS differences in the clarified treatments (HF and LF) compared to the control indicate the efficiency in the suspended solids removal by clarifying (Ray et al. 2010a; Gaona et al. 2011). In the control, both of the parameters followed a natural aggregation process of microorganisms and the consequent formation of particles in the water column (Avnimelech 2009), accumulating until the end of the experiment (Furtado et al. 2011; Xu and Pan 2012; Schveitzer et al. 2013). In the HF and LF treatments, the operating time of the clarifying process and the dry weight of the solids that were removed were similar in both flows, indicating that the LF treatment was more efficient due to the smaller water volume flowed that was required for TSS removal. In this way, the lower flow provided a lower radial flow in the settling chamber, making the settling velocity higher than the flow and leading to the deposition of suspended solids (Johnson and Chen 2006; Merino et al. 2007; Timmons and Ebeling 2010). Ray et al. (2011) tested two flows in two settling chamber volumes: 3.4% (1200 L h^{-1}) and 1.5% (600 L h^{-1}) of the size of the culture tank. These authors observed higher suspended solids removal in the treatment with a greater flow using a larger clarifier. In contrast, in the present study, two different flows were tested in clarifiers of the same size, corresponding to 2.28% of the culture tank volume (Gaona et al. 2011). Thus, it was possible to observe the need for less water circulating in the clarifier for better particle retention by sedimentation. Ray et al. (2011) replaced water in the culture tanks as a function of water disposal after the clarifying process. These authors found no effect of the replacement on the water quality parameters throughout the study. In the present study, the water disposal after each solids removal process decreased the tank volume in the HF and LF treatments. However, the tank volume decreased from the beginning of clarifying and continued slowly until the end of the present study, not interfering with the water quality parameters compared to those of the control. Meantime, the wide variation in the volume of culture tanks as used in previous studies demonstrates the feasibility of shrimp production in the BFT system (Samocha et al. 2007; Ray et al. 2010a, 2011; Krummenauer et al. 2011; Gaona et al. 2011).

The nutritional composition of bioflocs promotes supplemental food and results in better shrimp growth in the BFT system (Wasielesky et al. 2006a; Xu and Pan 2012). The crude protein content of the bioflocs coming from the culture tanks in the present study was similar between the control and the HF and LF treatments, ranging from 23-26%, and was similar to the variation that was found in previous publications (Wasielesky et al. 2006a; Ballester et al. 2010; Xu and Pan 2012; Ekasari et al. 2014).

Schveitzer et al. (2013) observed a higher percentage of protein in the treatment with a TSS concentration close to 200 mg L^{-1}, but this percentage was associated with the use of organic fertilization, increasing the C:N ratio to reduce the ammonia concentrations and increasing the amount of protein coming from heterotrophic bacteria. Ray et al. (2010b) observed at the end of their study that solids removal reduced the average final fatty acid concentration from bacteria in 60.3% compared to that of a system without biofloc management. It is likely that the lower flow in the LF treatment permitted the lipid removal from the microorganisms that aggregated in the bioflocs that were removed by clarifying. The high ash content that was observed in this study, ranging from 39-45%, may be related to the large amount of fecal material in suspension, a salinity above 30 and a wide range of minerals that are present in bioflocs, similar to previous publications (Wasielesky et al. 2006a; Avnimelech 2009; Ballester et al. 2010; Maica et al. 2014).

Solids removal has been shown in previous research to improve the water quality and growth performance of the shrimp *L. vannamei* (Ray et al. 2010a, 2011; Gaona et al. 2011). However, varying results regarding shrimp performance can be found in the literature for *L. vannamei* when cultured at different stocking densities, initial weight and time of culture, as well as varying TSS concentrations (Samocha et al. 2007; Krummenauer et al. 2011, 2014; Schveitzer et al. 2013; Furtado et al. 2015; Serra et al. 2015). The WWG and final weight at the end of cycles between 12 to 16 weeks have been reported in previous studies on *L. vannamei* in the BFT culture system, ranging from 0.30 to 1.7 g w^{-1} and 7.19 to 22.1 g, respectively (Samocha et al. 2007; Ray et al. 2010a, 2011; Gaona et al. 2011; Schveitzer et al. 2013; Serra et al. 2015). In the present study, these indices were within these intervals, with the WWG from 0.62 to

0.70 g w^{-1} and the final weight from 10.58 to 11.65 g. The stocking density that was used in this experiment was within the range of 150 to 390 m^{-2} individuals, as previously used in a superintensive system (Krummenauer et al. 2011; Schveitzer et al. 2013). However, in both of the suspended solids removal treatments, there was a greater survival (98%) and productivity (3.69 – 3.76 kg m^{-2}; 4.52 – 4.56 m^{-3}) and a better FCR (1.23 – 1.25). These parameters were within the range that was found in previous publications related to research on SST (Ray et al. 2010a, 2011; Gaona et al. 2011; Schveitzer et al. 2013), carbon sources (Samocha et al. 2007, Xu and Pan 2012, Serra et al. 2015), different stocking densities (Krummenauer et al. 2011) and water quality (Krummenauer et al. 2014; Furtado et al. 2015).

CONCLUSION

The smallest amount of water flowed through the clarifier showed a greater solids removal efficiency due to the greater retention of particles in the settling chamber. Furthermore, not water in the culture tanks following water withdrawal in the clarifying process improves the use of hydric resources, optimizing the marine shrimp production system.

ACKNOWLEDGEMENTS

The authors are grateful for the financial support provided by the National Council for Scientific and Technological Development (CNPq), Ministry of Fishery and Aquaculture (MPA) and Coordination for the Improvement of Higher Level Personnel (CAPES). Special thanks to Centro Oeste Rações S.A. (GUABI) for donating the experimental diets. W. Wasielesky, and L.H. Poersch are research fellows of CNPq.

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CAPÍTULO II

EFFECT OF DIFFERENT TOTAL SUSPENDED SOLIDS LEVELS ON A *LITOPENAEUS VANNAMEI* (BOONE, 1931) BFT CULTURE SYSTEM DURING BIOFLOC FORMATION

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RUNNING TITLE

Suspended solids levels in BFT shrimp system

KEY-WORDS

Suspended solids, biofloc, *Litopenaeus vannamei*, clarifier

Artigo submetido a revista Aquaculture Research

RESUMO

Em um sistema com tecnologia biofloc (BFT), há constante formação de agregados microbianos e acúmulo de sólidos em suspensão. Este acúmulo pode alterar os parâmetros de qualidade da água que podem afetar o desempenho de crescimento de camarão cultivado. O presente estudo teve como objetivo, analisar durante a formação dos bioflocos, o efeito de diferentes níveis de sólidos suspensos totais (SST) sobre a qualidade da água e sobre o desempenho do crescimento de *Litopenaeus vannamei* em sistema BFT. Um experimento foi realizado durante 42 dias com tratamentos distintos em três faixas de SST: 100-300 mgL^{-1} como baixo (TL), 200-600 mgL^{-1} como médio (TM) e 500-1000 mgL^{-1} como alto (TH). As concentrações iniciais de 100 (TL), 200 (TM) e 500 mgL^{-1} (TH) foram alcançadas por fertilização previamente ao início do experimento. Juvenis de *L. vannamei* com um peso médio de 4,54 ± 1,19 g foram estocados a uma densidade de estocagem de 372 camarões m^{-3}. Foram analisados os parâmetros físicos e químicos da água e desempenho zootécnico dos camarões. Depois de seis semanas, as concentrações médias de SST foram: 306,37; 532,43 e 745,2 mg L^{-1}, respectivamente, para os tratamentos TL, TM e TH. Diferenças significativas ($p<0,05$) foram observadas em SST, sólidos sedimentáveis, pH, alcalinidade e nitrito, especialmente entre os tratamentos TL e TH. Similarmente, as diferenças significativas ($p<0,05$) foram observadas nos parâmetros de desempenho zootécnico, especificamente, para peso final, sobrevivência, conversão alimentar e produtividade. Os parâmetros de qualidade da água na menor faixa (TL) de concentração de SST, resultou em um melhor desempenho zootécnico de *L. vannamei* em sistema BFT. A manutenção na faixa de 100 – 300 mgL^{-1} SST é, portanto, importante para o sucesso do cultivo de camarão.

ABSTRACT

In a Biofloc Technology System (BFT), there is constant biofloc formation and suspended solids accumulation, leading to effects on water quality parameters that may affect the growth performance of cultured shrimp. The present study aimed to analyze during biofloc formation the effect of different total suspended solids (TSS) levels on water quality and the growth performance of *Litopenaeus vannamei* shrimp in a BFT system. A 42-day trial was conducted with treatments of three ranges of TSS: 100 – 300 mgL^{-1} as low (TL), 300 – 600 as medium (TM) and 600 – 1000 as high (TH). The initial concentrations of 100 (TL), 300 (TM) and 600 mgL^{-1} (TH) were achieved by fertilization before starting the experiment. *L. vannamei* juveniles with an average weight of 4.54 ± 1.19 g were stocked at a density of 372 shrimp m^{-3}. Physical and chemical water parameters and shrimp growth performance were analyzed. After six weeks, TSS mean concentrations were 306.37, 532.43 and 745.2 mg/L^{-1} for, respectively, TL, TM and TH treatments. Significant differences ($p<0.05$) were observed in TSS, settleable solids, pH, alkalinity and nitrite, especially between the TL and TH treatments. Similarly, differences ($p<0.05$) were observed in the growth performance parameters, specifically final weight, survival, feed conversion and productivity. The water quality parameters at lower range of total suspended solids concentration (TL) treatment resulted in a better performance of *L. vannamei* in the BFT system. The maintenance at range of 100 – 300 mgL^{-1} TSS is thus important to the success of shrimp culture.

1. INTRODUCTION

A super-intensive marine shrimp culture system (BFT) works by stimulating natural productivity, consisting in the formation of bioflocs, which are microbial aggregates (Samocha, Patnaik, Speed, Ali, Burger, Almeida, Ayub, Harisanto, Horowitz & Brook 2007; Avnimelech 2009). The suspended bioflocs constitute the suspended solids, which remain distributed throughout the water column according to the dynamics caused by aeration. These aggregate particles distinguish intensive culture systems from natural environments by the large amount of particulate organic carbon distributed among different taxa of microorganisms (Pruder & Moss 1995; Burford, Thompson, McIntosh, Bauman & Pearson 2004; Ray, Seaborn, Leffler, Wilde, Lawson & Browdy 2010a).

In a BFT system, where stocking densities can reach 450 shrimp m^{-3} with higher feeding rates (Krummenauer, Cavalli, Poersch & Wasielesky 2011), the aeration system that diffuses air through the water column promotes vertical motion, distributing particulate matter throughout the tank via intensive mixing and keeping the biofloc suspended (Hargreaves 2006; De Schryver, Crab, Defoirdt, Boon & Verstraete 2008). This mixing becomes more important in impermeable structures where there is minimal or no water culture renewal, providing access to the solids and organic matter accumulated from offered food and the natural productivity in the suspended aggregates (Ray, Lewis, Browdy & Leffler 2010b; Gaona, Poersch, Krummenauer, Foes & Wasielesky 2011; Schveitzer, Arantes, Costódio, Santo, Vinatea, Seiffert & Andreatta 2013). The natural biota maintained in suspension acts in the recycling of nitrogen compounds (ammonia and nitrite) and supplements the feeding of penaeid shrimp (Wasielesky, Atwood, Stokes & Browdy 2006a; Arnold, Coman, Jackson &

Groves2009; Ballester, Abreu, Cavalli, Emerenciano, Abreu & Wasielesky 2010; Megahed 2010).

Simultaneously, there is an increase in nutrient concentrations, which can result in rapid eutrophication in closed systems (Thakur & Lin 2003). Silva, Wasielesky & Abreu (2013) analyzed the nitrogen and phosphorus dynamics in BFT systems and found that 39% of the nitrogen input into the system is in dissolved form and 7.7% of that fraction is in inorganic form. Of the phosphorus that remained in the system, 34.1% was in dissolved form, with 50.7% of that portion being inorganic. However, the bacterial community in the water culture assimilates and converts inorganic nitrogen species, improving water quality (De Schryver & Verstraete 2009; Avnimelech 2009; Luo, Avnimelech, Pan & Tan 2013).

The capacity of an intensive biofiltration system is dependent on a continuous oxygen supply (Hargreaves 2006). Nitrifying bacteria, which maintain water quality by reducing nitrite concentrations, depend on an environment with high dissolved oxygen (Avnimelech 2009; Ebeling, Timmons & Bisogni 2006). Organic carbon is the energetic substrate for many microorganisms, and its consumption contributes to the use of dissolved oxygen, posing a risk of inadequate oxygen supply for the animals reared in the system if facilities are inadequate (Avnimelech 2009; Mook, Chakrabarti, Aroua, Khan, Ali, Islam & Abu Hassan 2012). In addition, the interactions between the biofloc and water quality may interfere with the stability of the system, potentially causing changes in pH, alkalinity and carbon dioxide concentration (Ebeling *et al.* 2006; Furtado, Poersch & Wasielesky 2011; Furtado, Gaona, Poersch & Wasielesky 2014).

As the density of suspended solids increases, a culture's success will depend on a balance between waste production and the capacity of the environment of the cultured species to assimilate nutrients. One of the strategies for formation of biofloc is to stimulate heterotrophic bacteria growth and metabolism by adding organic carbon (C) sources, balancing this substrate with the total ammonia nitrogen (N) through the C:N ratio (Avnimelech 1999; Ebeling *et al.* 2006). Nitrifying bacteria require an inorganic carbon substrate for nitrite assimilation to reduce the concentrations of this compound (Ebeling *et al*. 2006). Due to concerns about the establishment of ammonium-oxidizing and nitrite-oxidizing bacteria in cultures, some studies have focused on the analysis of suspended solids concentrations and zootechnical performance using biofloc inoculum from previous crops (Ray, Dillon & Lotz 2011; Gaona *et al.* 2011; Schveitzer *et al.* 2013).

Aside from the nutritional benefits of bioflocs and their ability to maintain water quality, monitoring of biofloc formation must be conducted through a specific analysis of the progression of the concentration of suspended solids and water quality parameters. However, none study started with different concentration of total suspended solids on biofloc system without inoculum. In this sense, the objective of this study was to analyze during biofloc formation, the effect of different suspended solids levels on water quality and the growth performance of the penaeid shrimp *Litopenaeus vannamei* in a BFT system.

2. MATERIALS AND METHODS

2.1. Location and period of the study

A 42-day trial was conducted at the Marine Station of Aquaculture (EMA), Institute of Oceanography, Federal University of Rio Grande, located at Cassino Beach in Rio Grande, RS, Southern Brazil (32°11'S; 52°10’W).

2.2. Experimental design

The experimental design was completely randomized, consisting of three treatments with three replicates each. Nine tanks (1.0 m^3) with a useful capacity of 0.86 m^3were installed in a greenhouse for shrimp growth via a BFT system. The aeration system consisted of an Aero-Tube™ (a hose with micropores distributed evenly across its length) to optimize oxygen transfer and aeration efficiency. Forty-centimeter lengths of this hose were cut and installed in pairs in each experimental unit, coupled to a PVC pipe (20 mm diameter) and supplied by mechanical aeration from a 2 hp blower.

Three treatments were defined by ranges of TSS: 100 – 300 mgL^{-1} as low (TL), 300 – 600 as medium (TM) and 600 – 1000 as high (TH). The water used in the experiment was obtained by pumping directly from Cassino Beach, which has a salinity of 33 gL^{-1}. Before the beginning of the experiment, the water was treated with 10 mgL^{-1} of chlorine and neutralized with ascorbic acid 24 h later and then fertilized with sugar cane molasses in a 20:1 ratio (C:N) to achieve the initial suspended solids concentrations: 100, 200 and 500 mgL^{-1}. A first adjustment was made to the total suspended solids concentrations before the start of the experiment. Further adjustments to the suspended solids levels were made (third and fifth week) by suspended solids removal (six hour each removing) in all treatments, based on sedimentation methods (clarification) used in previous studies (Ray *et al.* 2010b, 2011; Gaona *et al.* 2011). Clarifiers were assembled intoa conical-cylindrical glass fiber chamber with a diameter of 0.48 m, height of 0.50 m and useful volume of 48 liters, representing 5.5% of the total volume of the experimental unit. A PVC pipe (100 mm diameter) was placed inside this chamber to reduce the turbulence of the water pumped from the culture tank by a submerged pump with a flow of 1500 Lh^{-1}.

2.3. Biological material and feeding

The juveniles of *L. vannamei* used in the present study were first kept for 60 days in the nursery and grow-out BFT system inside the greenhouse at the Marine Station of Aquaculture (EMA). Each experimental unit was stocked at a density of 372 shrimp m^{-3} (265 individuals m^{-2}) with an average weight per shrimp of 4.54 ± 1.19 g. Shrimp were fed twice a day (at 9:00 and 16:00) with commercial feed (Potimar Active 38 – Centro Oeste Rações SA, Campinas, SP, Brazil) containing 38% crude protein and 8% lipid. The food was offered on feeding trays (Wasielesky *et al.* 2006a) at an initial rate of 10% of shrimp biomass and was adjusted according to consumption observed in a period of 24 h. This value was adjusted posteriorly according to the consumption observed in the trays within each interval between feedings.

2.4. Physical and chemical water parameters

Monitoring of dissolved oxygen, pH, temperature and salinity was performed daily. Samples were collected daily for total ammonia nitrogen (TAN) and nitrite (NO_2^-–N) analysis and once a week for nitrate (NO_3^-–N), orthophosphate (PO_4–P) and alkalinity. Total suspended solids (TSS) and settleable solids (SS) were monitored twice a week. Dissolved oxygen, pH, temperature and salinity were measured using multiparameter YSI® mod. 556 (YSI Incorporated,Yellow Springs, OH, USA). The concentrations of ammonia (TAN) and nitrite (NO_2^-–N)were measured according to UNESCO (1983) and Bendschneider& Robinson (1952), respectively. The method used for nitrate (NO_3^-–N) and orthophosphate (PO_4–P) was Aminot & Chaussepied (1983), and alkalinity was measured according to APHA (1998). TSS was determined through gravimetry by filtering aliquots of 20 mL of water through GF 50-A glass fiber filters, according to Strickland and Parsons (1972) and AOAC (2000). SS was analyzed using Imhoff cone and the volume of floc on the bottom of the cone was measured after 15 minutes of sedimentation (Avnimelech 2009).

2.5. Growth performance

Shrimp growth was monitored every 15 days, and used to adjust the amount of supplied feed according to Jory, Cabreras, Durwood, Fegan, Lee, Lawrence, Jackson, Mcintosh& Castañeda (2001).For that purpose, 30 shrimps were collected randomly from each experimental unit, individually weighed and returned to their respective tank. After weighing, the mean individual weight (g) was calculated.

At the end of the experiment the following variables were calculated:

- Survival (%) = (final shrimp number / initial shrimp number) x 100.
- Feed conversion ratio (FCR) = offered feed (g) / (final biomass (g) – initial biomass (g));
- Productivity (kgm^{-3}) = (final biomass – initial biomass) / volume (m^3)

2.6. Statistical analysis

The water quality parameters and growth performances in the different treatments were submitted to a one-way analysis of variance (ANOVA), taking into account the assumptions (Levene and Kolmogorov-Smirnov tests) necessary for its implementation. Tukey's test was applied when significant differences were detected ($p<0.05$). The survival data were transformed (arcsine $x^{0.5}$) before analysis (Zar 1996).

3. RESULTS

3.1. Water quality

Temperature and dissolved oxygen were maintained above 26 °C and 5 mgL^{-1}, respectively, throughout the experiment and did not exhibit significant differences ($p>0.05$) between treatments. Salinity increased at the end of the study; however, the mean salinity values were similar ($p>0.05$) between groups.

Means and standard deviations of the parameters monitored throughout the experiment are presented in Table 1.

The concentrations of total suspended solids (TSS) outside of setting ranges of the treatments were recorded during the culture. Significant differences ($p<0.05$) in total suspended solids concentrations were found between the TL and TH treatments throughout the study, whereas the TM group exhibited intermediate values (Fig. 1a). However, after six weeks of the experiment, the TSS concentrations did not present a significant difference ($p>0.05$) between the TM and TL treatments (Fig. 1a).

From de second week onwards, the settleable solids (SS) presented significant differences ($p<0.05$) among all treatments (Fig. 1b).

The pH was significantly higher ($p<0.05$) in TL compared to TH tanks from the third week onwards (Fig. 1c).

The variations in alkalinity observed in the study (Fig. 1d) were marked by significant differences ($p<0.05$) between treatments in the last two weeks, when the concentrations were significantly higher ($p<0.05$) in TL with respect to TH.

Ammonia and nitrate concentrations were not differences ($p>0.05$) between treatments during the experiment. However, the nitrite concentration increased throughout the experiment for the TM and TH treatments while it remained low for the

TL group (Fig. 1e). Concentrations were significantly lower (p<0.05) in the low TSS treatment (TL) relative to the other two groups during the final two weeks.

Table 1. Physical and chemical parameters data for *L. vannamei* in a BFT system with three suspended solids levels (low=TL, medium=TM, and high=TH), with mean values ± standard deviation.

	TREATMENT		
	TL	**TM**	**TH**
Temperature (°C)	27.86±1.37	28.07±1.42	28.17±1.45
Dissolved oxygen (mgL^{-1})	5.85±0.64	5.76±0.69	5.68±0.71
pH	7.93±0.23	7.86±0.25	7.74±0.31
Alkalinity (mg $CaCO_3$ L^{-1})	159.29±19.96^{a}	136.43±20.88^{ab}	116.71±43.75^{bc}
Salinity (gL^{-1})	37.23±3.13	37.69±2.92	38.49±2.95
TSS (mgL^{-1})	306.37±119.71^{a}	532.43±321.00^{ab}	745.20±283.85^{bc}
SS (mgL^{-1})	11.48±3.79^{a}	57.00±41.22^{ab}	210.83±161.47^{c}
Ammonia (mgL^{-1})	3.42±3.01	3.33±3.03	2.54±3.25
Nitrite (mgL^{-1})	0.73±1.08	3.66±8.00	10.28±14.25
Nitrate (mgL^{-1})	0.38±0.63	0.46±0.72	1.73±2.11
Orthophosphate (mgL^{-1})	0.51±0.27^{a}	1.39±0.84^{ab}	4.82±1.99^{bc}

Different letters in the same row indicate significant difference (p<0.05) between treatments.

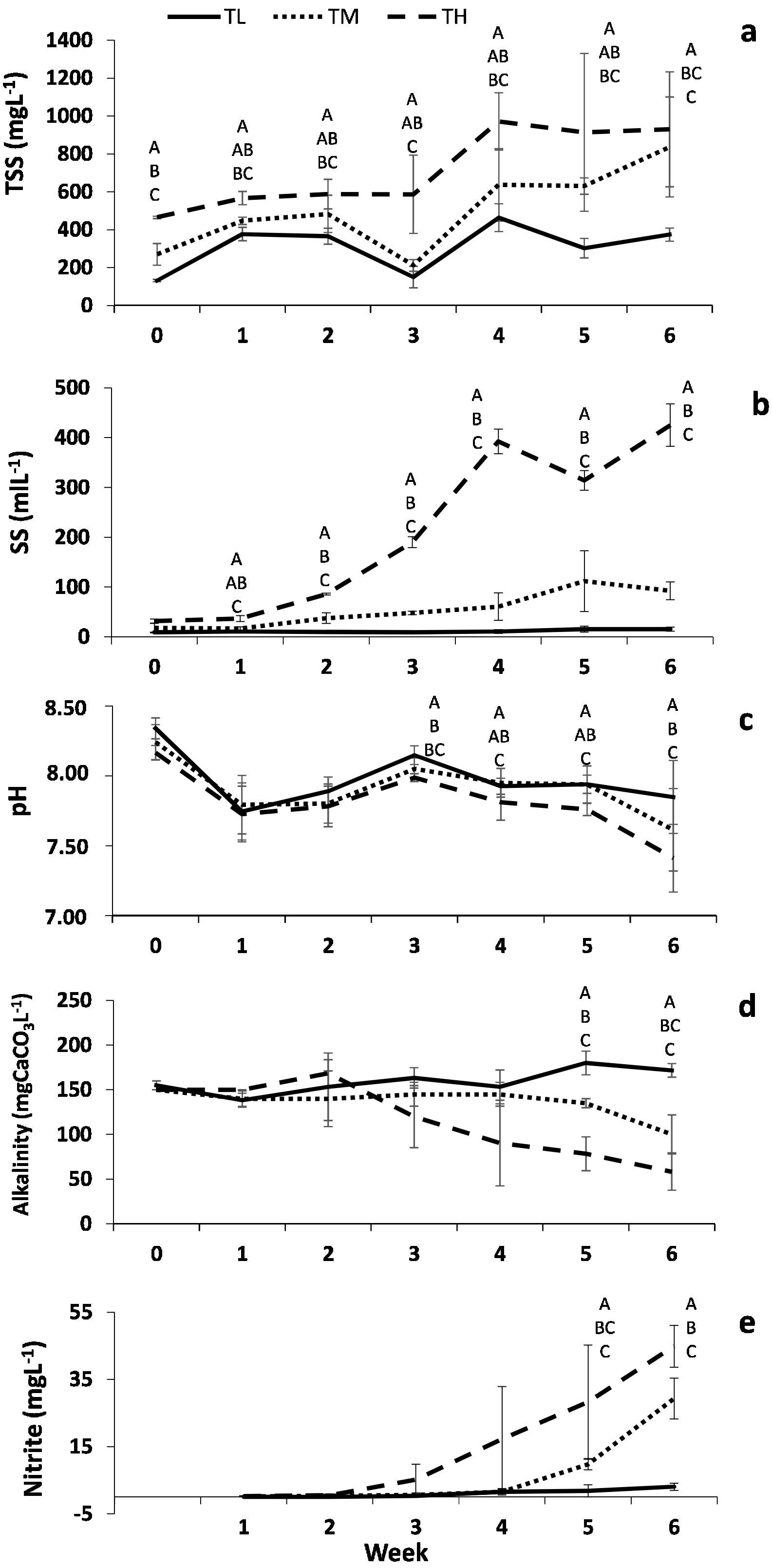

Figure 1. Variations in total suspended solids – TSS (a), settleable solids – SS (b), pH (c), alkalinity (d) and nitrite (e) throughout the experiment. Significant differences

(p<0.05) are indicated by the letters A, B and C, for Low, Medium and High treatments, respectively. Different letters indicate significant differences (p<0.05) between treatments for each sample period.

The highest orthophosphate concentration was recorded for the TH treatment during the last week, being significantly different (p<0.05) from the TL group.

3.2. Growth performance

Shrimp kept at low (TL) and medium (TM) TSS concentrations exhibited similar (p>0.05) growth during the experiment, reaching the highest (p<0.05) weights relative to the TH treatment after 6 semanas~~42 days~~ (Fig. 2).

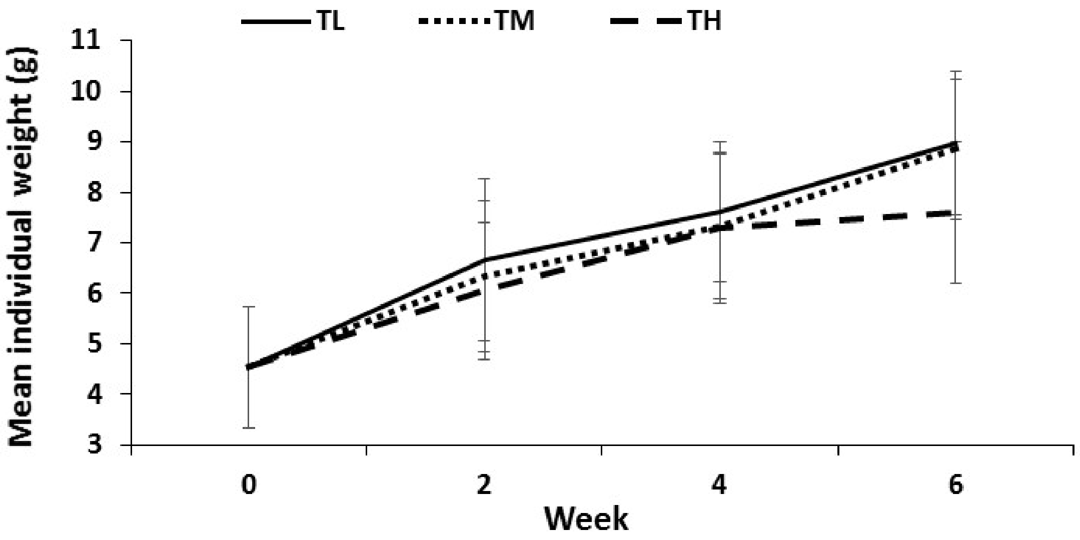

Figure 2. Shrimp growth during the 42-day trial in the presence of low (TL), medium (TM) and high (TH) suspended solids levels.

The growth performance results in terms of final mean weight, survival, feed conversion ratio (FCR) and productivity presented significant differences between treatments, as indicated in Table 2.

Table 2. Growth performance data for *L. vannamei* in a BFT system with three suspended solids levels (low=TL, medium=TM, and high=TH), with mean values ± standard deviation.

	TREATMENT		
	TL	**TM**	**TH**
Mean initial weight (g)	4.54±1.19	4.54±1.19	4.54±1.19
Mean final weight (g)	8.98 ± 1.42^a	8.85 ± 1.39^a	7.60 ± 1.41^b
Survival (%)	94.79 ± 1.41^a	84.17 ± 7.73^a	20.73 ± 8.58^b
FCR*	1.08 ± 0.04^a	1.21 ± 0.08^b	5.28 ± 1.92^c
Productivity (kgm^{-3})	3.40 ± 0.08^a	2.98 ± 0.21^b	0.65 ± 0.37^c

*FCR – Feed conversion ratio. Different letters in the same row indicate significant differences (p<0.05) between treatments.

Significant differences (p<0.05) were observed in the last week, distinguishing the final weight of TH treatment shrimp from the other two treatments. The survival for the TL and TM treatments were similar (p>0.05), both being significantly higher (p<0.05) than that for the TH treatment. The FCR and productivity exhibited significance differences (p<0.05) among all treatments at the end of the trial, with the best feed conversion ratio (FCR) and productivity obtained for the TL group in relation to the other groups (Table 2).

4. DISCUSSION

This study observed the effects of TSS of the three ranges along the experiment into BFT water quality and its consequences on *L. vannamei* growth performance. The changes on water quality occurred together with the evolution of every range of TSS concentrations.

Parameters such as temperature and oxygen availability directly affect the growth performance of *L. vannamei* by regulating metabolic functions, determining the growth of the animals. In this study, temperature and oxygen availability were maintained at levels favorable to the growth of the species (Van Wyk & Scarpa 1999; Zhang, Zhang, Li & Huang 2006). Salinity varied over the course of the experiment within the tolerance range of the species (Van Wyk & Scarpa 1999). A previous study of the combined effect of temperature and salinity on the culture of the shrimp *L. vannamei* observed the great osmoregulatory ability of this species, which quickly adapted to variations in salinity between 10 and 40 gL^{-1} (Re, Díaz, Ponce-Rivas, Giffard, Muñoz-Marquez & Sigala-Andrade 2012).

It is possible to estimate the dry matter present in settleable solids, estimating the proportion of TSS as dry weight (i.e., 1.4% TSS) present in biofloc, but this may not be the rule (Avnimelech 2007). According Schveitzer et al. (2013) the degree of correlation between TSS and SS may be affected by independent variations of each parameter. These same authors observed that even in stable levels of TSS, changes occurred in the SS measures. In this study, the SS had similar behavior to TSS with increasing throughout the experiment, as noted by Furtado et al. (2011). According to Avnimelech (2009) typical floc volumes for shrimp culture are between 2 – 40 mlL^{-1}, whereas in the present study the values of TL treatment were within these ranges.

L. vannamei has the capacity to feed on the natural biota and also contributes to the generation of a significant amount of biofloc in a BFT system (Burford *et al.* 2004). This was observed by Ferreira (2008) studying microbial floc formation in two cultures of marine shrimp species, who reported higher TSS concentrations in an *L. vannamei* culture than a *Farfantepenaeus paulensis* culture. The most notable variations in the average concentrations of total suspended solids were observed throughout the study in the TH and TL treatments, which, respectively, varied below and above 500 mgL^{-1}, a value recommended by Samocha *et al.* (2007). Suspended solids are involved in the organic matter decomposition processes and reflect changes in water quality (Vinatea, Galvez, Browdy, Stokes, Venero, Haveman, Lewis, Lawson, Shuler& Leffler 2010). Changes in pH and alkalinity during the study correlated inversely with TSS levels; a similar pattern was noted by Furtado *et al.* (2011) for TSS levels above 850 mgL^{-1}. The respiration in the water column by microorganisms present in the biofloc results in the excretion of carbon dioxide, which drives a reduction in pH (Wasielesky *et al.* 2006a; Vinatea *et al.* 2,010; Ray *et al.* 2010a). Simultaneously, the dissociation of carbonate and bicarbonate ions reduces the alkalinity of the water culture (Ebeling *et al.* 2006). The alkalinity and pH remained above 100 mgCaCO^{3}L^{-1} and 7, respectively, and within the recommended values for *L. vannamei* in a BFT system (Ebeling *et al.* 2006; Wasielesky, Atwood, Kegl, Bruce, Stokes & Browdy 2006b).

Nitrogen cycling by heterotrophic and nitrifying bacteria resulted, respectively, in similar reductions in ammonia concentrations and increases in nitrite with increasing organic matter at the higher TSS levels. Due to the addition of molasses, heterotrophic bacteria had a substrate for obtaining carbon and subsequently metabolizing ammonia (Avnimelech 1999; Samocha *et al.* 2007), reducing the ammonia concentrations to levels tolerable to *L. vannamei* (Lin & Chen 2001). Luo *et al.* (2013) observed that the effect of the organic substrate in the inhibition of nitrifying bacteria was not toxic, but stimulated rapid heterotrophic growth and competition for dissolved oxygen, space, total ammonia and micronutrients. Inhibition of nitrifying bacteria may have led to slowed growth, and the reduction of alkalinity in the TH treatment may have limited the availability of inorganic carbon (Ebeling *et al.* 2006). In the present study, there were increased concentrations of nitrite in treatments TM and TH, in which the oxidation necessary to decrease and stack nitrate did not occur. Nitrite concentrations in the TL treatment also increased, but it was a very gentle rise. It was clear that the length of the experiment was most likely not sufficient for the establishment of characteristic nitrification in BFT systems. According to Silva *et al.* (2013), the route of nitrification occurs with successive conversions of ammonia to nitrite and nitrate prevalence at the end of the crop cycle.

Phosphorus concentrations remained at concentrations that accompanying TSS levels, remaining accumulated in the water culture. Barak, Cytryn, Gelfand, Krom & Van Rijn (2003) reported that the source of phosphorus in the culture is mainly due to unconsumed feed and nutrients excreted by cultured organisms. In cultures with biofloc technology, *L. vannamei* can incorporate up to 35% of the total phosphorus that enters the system, whereas the majority remains in the dissolved and particulate forms, with accumulation expected in a BFT system (Silva *et al.* 2013).

The shrimp's performance was determined by the water quality during the experiment. The lower total suspended solids concentration (at range of 100 – 300 mgL^{-1}) used in the study provided the best conditions for the shrimp, most likely due to the low levels of nitrites maintained throughout the 42-day trial. Lin & Chen (2003) verified a nitrite (NO_2^-–N) safe level of 25.7 mgL^{-1} for *L. vannamei* juveniles at a salinity of 35 gL^{-1} indicating the high tolerance of the species, as the concentration indicated by these authors was only achieved in the sixth week for the TM treatment in higher salinity. The TH treatment exceeded this concentration from the fifth week, exposing the animals for longer toxicity of nitrite. At low salinity to 48 h LC50 (median lethal concentration) studies, Shuler et al. (2010) in salinity of 10 gL^{-1} observed 154 $mgL^{-1}NO_2^{-}$–N, while Lin & Chen (2003) at 15 gL^{-1} recorded 143 $mgL^{-1}NO_2^{-}$–N. However, the nitrite concentration was determinant for the growth rate of the animals, and the two variables may have an inverse relationship (Vinatea *et al*. 2010). These interactions were reflected in the better growth and survival in treatment TL, which was observed to have the lowest nitrite concentration. In an *L. vannamei* culture with a TSS concentration of 465 mgL^{-1}, Ray *et al.* (2010b) recorded a maximum nitrite concentration of 5.4 mgL^{-1}, obtaining a final mean weight of 11.6 g and mean survival of 71% at a stocking density of 460 shrimp m^{-3} after 12 weeks. Gaona *et al*. (2011) observed, after 16 weeks of culture, that shrimp stocked at a density of 250 individuals m^{-2} reached 10.76 g with a survival of 81%, maintaining the TSS level at 500 mgL^{-1}. Schveitzer *et al*. (2013) observed 83% survival in *L. vannamei* reared under suspended solids conditions kept within the range of 400-600 mgL^{-1}. Concomitantly, there was a better conversion of feed consumed to shrimp growth in the present study. The best FCR was observed in the treatment with low TSS, with a ratio of 1.08. In accordance with this result, Samocha *et al*. (2007) obtained the best FCR in a culture with a total suspended solids concentration near 100 mgL^{-1} but with a lower stocking density than in the current study. Ray *et al*. (2011), testing two TSS levels (197 and 313 mgL^{-1}), obtained a FCR of 2.5 in the treatment with lower TSS. However, some studies have observed variations in productivity between 2.15 and 4.10 kgm^{-3} at different stocking densities (Ray *et al.* 2011; Gaona *et al.* 2011; Krummenauer *et al.* 2011; Baloi, Arantes, Schveitzer, Magnotti & Vinatea 2013; Schveitzer *et al.* 2013). In the present study, an intermediate value of productivity (3.40 kgm^{-3}) was observed at the lowest concentration of TSS with range of 100 – 300 mgL^{-1}. To keep TSS in such range becomes a strategy to await the establishment of nitrite oxidizers which occurs around 4 to 6 weeks (Avnimelech 2009) and to start a cicle without inoculum containing nitrifiers.

5. CONCLUSION

TSS concentrations about 100 – 300 mgL^{-1} during biofloc formation are important for maintaining water quality, particularly when the nitrification process is not well established. In addition, negative impacts on water quality parameters can be reduced by using low TSS concentrations, starting the culture at approximately 100 mgL^{-1}, reducing variations over culture. Obviously, a culture environment with better physical and chemical conditions favors a better growth performance of cultured organisms. As a recommendation, preventive actions to maintain a TSS concentration by means of clarification are indicated for water quality improvement in a BFT system.

6. ACKNOWLEDGEMENTS

The authors are grateful for the financial support provided by the National Council for Scientific and Technological Development (CNPq), Ministry of Fishery and Aquaculture (MPA) and Coordination for the Improvement of Higher Level Personnel (CAPES). Special thanks to Centro Oeste Rações S.A. (GUABI) for donating the experimental diets. W. Wasielesky, and L.H. Poersch are research fellows of CNPq.

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CAPÍTULO III

EFFECT OF DIFFERENT TOTAL SUSPENDED SOLIDS CONCENTRATIONS ON THE OXYGEN CONSUMPTION AND PERFORMANCE OF *Litopenaeus vannamei* IN A BFT SYSTEM

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Artigo submetido a revista Aquacultural Engineering

RESUMO

Reduções de desempenho zootécnico por excesso de sólidos suspensos totais (SST) são destacadas em estudos prévios. O objetivo deste estudo foi avaliar o efeito de diferentes concentrações de SST sobre o consumo de oxigênio e performance de *Litopenaeus vannamei* em sistema BFT, durante 42 dias. Foram utilizadas cinco concentrações de SST: 250, 500, 1000, 2000 e 4000 mg L^{-1} com três repetições cada, respectivamente, T250, T500, T1000, T2000 e T4000, em tanques com volume útil de 200 L cada. As concentrações de oxigênio dissolvido (OD) foram mantidas acima de 5 mg L^{-1}. Foram utilizados camarões com peso médio inicial de 4,57 ± 1,07 g (277 camarões m^{-2}). Foram monitorados osparâmetros físicos e químicos da água**.** Foi analisado o consumo específico de oxigênio (CEO) em dois períodos de exposição (CEO_I – 24 dias e CEO_F – 42 dias). Em cada teste de cada tratamento (três réplicas) e foram medidas as concentrações inicias de OD e interrompida a aeração com posteriores medições das concentrações finais de OD após uma hora. Os parâmetros de qualidade de água e desempenho zootécnico foram submetidos à análise de variância (ANOVA – uma via). Para o CEO foi utilizado regressão linear simples ($y = \beta_0 + \beta_1 x$) e teste de identidade dos modelos: coeficientes angulares (H_0: $\beta_{11} = \beta_{12}$) e interceptos (H_0: $\beta_{01} = \beta_{02}$). Os parâmetros físicos e químicos ficaram dentro do recomendado para a espécie. As regressões (CEO_1: $R^2 = 0,68$, $p<0,05$; CEO_2: $R^2 = 0,42$, $p<0,05$) apresentaram redução de CEO com aumento de SST e foram semelhantes nos dois testes. Dados de performance não apresentaram diferenças significativas ($p>0,05$). Análises de CEO e performance sugerem uma adaptação de *L. vannamei* em baixas concentrações de OD. Elevadas concentrações de SST parecem não interferir no desempenho zootécnico desta espécie, desde que as concentrações de OD sejam mantidas acima de 5 mg L^{-1}.

ABSTRACT

Decreased *Litopenaeus vannamei* performance resulting from excess total suspended solids (TSS) has been highlighted in previous studies. The aim of this study was to evaluate the effect of different TSS levels on the oxygen consumption and shrimp performance in a BFT system for 42 days. Five TSS concentrations were used —250, 500, 1000, 2000, and 4000 mg L^{-1} — in three replicates identified as T250, T500, T1000, T2000, and T4000, respectively, in 200 L-tanks each. Dissolved oxygen concentration (DO) was maintained above 5 mg L^{-1}. Shrimp with an initial average weight of 4.57 ± 1.07 g (277 shrimp m^{-2}). Physical and chemical parameters were monitored. The specific oxygen consumption rate (OCR) was analyzed for two periods of exposure (OCR_I – 24 days and OCR_F – 42 days) with three replicates of each treatment. The initial DO concentrations and the concentrations after 1 h without aeration were measured. Water quality parameters and animal performance were subjected to analysis of variance (ANOVA – one way). For the OCR, a simple linear regression ($y = \beta_0 + \beta_1 x$) with angular coefficients (H_0: $\beta_{11} = \beta_{12}$) and intercepts (H_0: $\beta_{01} = \beta_{02}$) was used. The physical and chemical parameters were within the recommended range for *L. vannamei*. The regressions (OCR_1: $R^2 = 0.68$, $p<0.05$; OCR_2: $R^2 = 0.42$, $p<0.05$) showed that as TSS increased, the OCR decreased, and similar results were found for both tests. Performance data showed no significant differences ($p>0.05$). OCR and performance analysis suggested that there was an adaptation of *L. vannamei* at low dissolved oxygen concentrations. The high TSS levels did not seem to affect the performance of this species when DO concentrations were maintained above 5 mg L^{-1}.

Keywords: Total suspended solids, biofloc, *Litopenaeus vannamei*, oxygen consumption

1. INTRODUCTION

Several of the current production systems of penaeid shrimp utilize parallel microorganism cultures that supplement the food of the target species (Wasielesky et al., 2006a; Ballester et al., 2007). The BFT system contributes directly by means of microorganisms that use ammonia as nutrients for the formation of bacterial proteins (Wasielesky et al., 2006a). Simultaneously, the water quality benefits from the recycling of nutrients that are present throughout the culture as bioflocs (Ebeling et al., 2006).

An increase in stocking density implies an increase in feeding because of biomass increases (Krummenauer et al., 2011) and consequent rises in particulate organic matter in cultured water that is involved in the formation and aggregation of microorganisms. The impermeabilization of tanks and water confinement without or minimal renewal expose organisms to direct contact with particulate matter and also determine the importance of aeration and water movement in BFT systems (De Schryver et al., 2008). The dynamics caused by mechanical aeration promotes the mixing and distribution of particles (Hargreaves, 2006) that vary in size and composition. These variations can be expressed by microbial composition that reflects in the structure of bioflocs. The microbial composition may determine the size and density of bioflocs in water and also reflect in the consumption by cultured organisms (Ekasari et al., 2014).

Microorganisms consume dissolved oxygen (DO) to maintain metabolic activities during the decomposition of organic matter (Avnimelech, 2009). In this sense, the aeration system must be sufficient to supply dissolved oxygen to the target species and microorganisms (Van Wyk and Scarpa, 1999; De Schryver et al., 2008). However, in some studies, the DO concentrations were not maintained until the end of the cycle, especially in conditions of high concentrations of suspended solids (Ray et al., 2010a, Gaona et al., 2011; Krummenauer et al., 2011). Furthermore, the absence of aeration in BFT systems can lead to a reduction of dissolved oxygen to lethal levels after approximately 30 minutes (Vinatea et al., 2009).

The increase of suspended solids in BFT systems is constant and could pose hazards to production. The recommended maximum concentration of total suspended solids is 500 – 600 mg L^{-1} (Samocha et al., 2007; Gaona et al., 2011; Schveitzer et al., 2013). As mentioned in previous publications, the suspended particulate matter excess beyond interactions with water quality parameters (Furtado et al., 2011; Gaona et al., 2011), which can cause stress to cultured organisms (Ray et al., 2010a). Chapman et al. (1987) reported direct physical effects on the gills of rainbow trout caused by the impregnation of the gill arches by solid particles, which caused mortalities because of the destruction of the gill epithelium and/or the blockage of water passage over the gills. In a study assessing the degree of gill occlusion in *L. vannamei*, Schveitzer et al. (2013) reported that higher suspended solids concentrations may have clogged the gills and committed to respiration of the shrimp.

The oxygen consumption is a physiological response that can be correlated with changes in environmental factors, because the respiration rate is related to the metabolic work and the energy flow that the organisms canalize by homeostatic control mechanisms (Salvato et al., 2001). Published studies with penaeid shrimp describe the importance interactions of oxygen consumption and metabolism with factors such as temperature, salinity and weight (Villarreal et al., 1994; Spanopoulos-Hernández et al., 2005; Salvato et al., 2001; Li et al., 2007; Bett & Vinatea, 2009; Vinatea et al., 2009;

Palafox-Ponce et al., 2013), however, none study of interaction with suspended solids was conducted.

The aim of this study was to evaluate the effect of different concentrations of TSS on the oxygen consumption and performance of *Litopenaeus vannamei* in BFT systems to evaluate the effects of suspended solids in shrimp cultured in BFT systems.

2. MATERIALS AND METHODS

2.1. Experimental design

The study was conducted at the Marine Station of Aquaculture Prof. Marcos Alberto Marchiori (EMA) under the Oceanographic Institute of the Federal University of Rio Grande - FURG, located at Cassino Beach in Rio Grande, RS, Southern Brazil (32°11'S; 52°10’W). The experiment lasted 42 days.

The treatments were defined according to the concentrations of total suspended solids (TSS): 250, 500, 1000, 2000, and 4000 mg L^{-1} in three replicates, which were identified as T250, T500, T1000, T2000, and T4000, respectively. Fifteen tanks, each with a volume of 200 L each, were installed in a greenhouse for culture studies of marine shrimp in BFT systems. Aeration was supplied by a 2 hp blower for air diffusion in the water column via hose with micropores (Aero-Tube™, Swan®, Marion, OH, USA) in order to maintain DO concentrations at high levels.

To begin the study, the proposed TSS concentrations were used with concentrated biofloc inoculum from clarifiers (Gaona et al., 2011) with approximately 5000 mg L^{-1}. For each treatment, appropriate dilutions were performed for each concentration, maintaining a salinity of 33. Fresh and salt water were used and were chlorinated with 10 ppm and dechlorinated with 1 ppm of ascorbic acid, even when replacements were needed by evaporation. To keep the TSS levels constant in each treatment throughout the experimental period, 50 µm mesh filters were used.

2.2. Biological material and feeding

The animals for the experiment were kept in a nursery tank inside a greenhouse in a Marine Aquaculture Station (EMA). The shrimp were stocked with an initial average weight of 4.57 ± 1.07 g at a stocking density of 277 shrimp m^{-2} (500 individuals m^{-3}).

The shrimp were fed twice a day (at 9:00 and 16:00) with commercial feed (Potimar Active 38 – Centro Oeste Rações SA, Campinas, SP, Brazil) containing 38% crude protein and 8% lipids. The food was offered on feeding trays (Wasielesky et al., 2006a) at an initial rate of 5% of shrimp biomass and was adjusted according to consumption observed in a 24-h period. This value was subsequently adjusted according to the consumption observed in the trays within each interval between feedings.

2.3. Physical and chemical water parameters

Physical and chemical parameters were monitored daily (dissolved oxygen, pH, temperature and salinity) using multiparameter equipment YSI® mod. 556 (YSI Incorporated, Yellow Springs, OH, USA). Water quality was monitored by measuring total ammonia nitrogen levels (TAN), nitrite (NO_2^-–N), nitrate (NO_3^-–N), orthophosphate (PO_4–P), total suspended solids (TSS), and alkalinity. Daily samples were collected to analyze ammonia and nitrite (UNESCO 1983 and Bendschneider and Robinson, 1952, respectively), and samples were collected once a week for nitrate (Aminot and Chaussepied 1983) and alkalinity (APHA 1998). TSS was monitoredevery two days to the adjustment of levels according to the treatments (Strickland and Parsons 1972, AOAC 2000). When necessary, corrections were made to maintain pH values above 7.2 using calcium hydroxide ($Ca(OH)_2$) according to the dosages suggested by Furtado et al. (2011).

2.4. Specific oxygen consumption rate (OCR)

The specific consumption of dissolved oxygen by the shrimp was evaluated for each treatment to assess the effects of different TSS levels for two different time periods that were considered chronic (Gross et al., 2004): the initial period (OCR_I), which included the first 24 days after the beginning of study, and the final period (OCR_F), which ended when the study was completed (42 days). The intervals between OCR_I and OCR_Fwere used to assess whether the exposure time of the shrimp to different TSS concentrations during the experimental period changed the dissolved oxygen consumption. The analyses were carried out using three replicates for each treatment. According to a procedure adapted from González et al. (2010), each replicate was made using a set of two flasks (with and without shrimp) with a lid and a volume of 1 L with no water flow or any aeration for one hour. To maintain the temperature, the flasks were kept in a water table with a constant temperature of 29 °C. Prior to the beginning of the test, the shrimp were kept for two hours in each flask with aeration to eliminate the stress caused by their adaptation to the experimental container (Bett and Vinatea, 2009). Table 1 shows the average weights of the shrimp, which showed no significant differences between the treatments ($p>0.05$) for the OCR_I and OCR_F measurements. The container with no shrimp was included to exclude oxygen consumption by microorganisms. At the beginning of the test, using a multiparameter YSI$^{®}$ mod. 556, the initial dissolved oxygen values of all experimental containers were measured, aeration was immediately stopped, and all flasks were closed. After one hour, the final concentrations of dissolved oxygen were measured. For the OCR (mgO_2 g^{-1} h^{-1}) calculation, the initial and final dissolved oxygen concentrations were determined using the following equation:

$\text{OCR} = [(DOs - DOn) \times V] \div t \times W$, where:

DOs = Final – initial dissolved oxygen concentration (mg L^{-1}), measured in the flask with shrimp;

DOn = Final – initial dissolved oxygen concentration (mg L^{-1}), measured in the flask with no shrimp;

V = useful volume (L) of flask;

t = experiment time (h);

W = wet weight (g) of shrimp

Table 1. Weight ± standard deviation (g) of shrimp used in each test of the specific oxygen consumption rate (OCR). In each test weights were similar ($p>0.05$) between treatments.

	TREATMENT				
Weight $^{(Test)}$	**T250**	**T500**	**T1000**	**T2000**	**T4000**
Weight$^{(OCR_I)}$	8.19 ± 0.22	8.05 ± 0.41	7.93 ± 0.50	7.81 ± 0.48	8.11 ± 0.13
Weight$^{(OCR_F)}$	9.97 ± 0.21	10.04 ± 0.34	9.84 ± 0.24	9.94 ± 0.35	10.33 ± 0.10

2.5. Shrimp performance

The shrimp growth performance was monitored weekly using biometrics. Thirty shrimp were collected at random from each experimental unit. The animals were individually weighed and then returned to their respective tanks. The amount of supplied feed was adjusted according to Jory et al. (2001). At the end of the experiment, all animals were counted in order to assess survival and final biomass.

To evaluate the *L. vannamei* performance, the following parameters were used:

- Survival (%) = (final nº of shrimp ÷ initial nº of shrimp) × 100;
- Weekly Weight Gain (WWG) (g week^{-1}) = (final mean weight – initial mean weight) ÷ week of culture
- Feed Conversion Rate (FCR) = offered feed / (final biomass – initial biomass);
- Productivity (kg m^{-3}) = (final biomass – initial biomass) ÷ volume

2.6. Statistical analysis

Water quality parameters and growth performance in the different treatments were subjected to analysis of variance (ANOVA – one way), taking into account homocedasticity and normality (Levene and Kolmogorov-Smirnov tests, respectively), followed by the Tukey test for possible differences ($p<0.05$) between treatment means. For the OCR, a simple linear regression ($y = \beta_0 + \beta_1 x$) was used, as well as an identity test of the regression models to verify the similarity of the interactions between OCR and TSS in both tests (OCR_I and OCR_F) by using the *F* test of the angular coefficients (H_0: $\beta_{11} = \beta_{12}$) and intercepts (H_0: $\beta_{01} = \beta_{02}$) (Graybill, 1976). Survival data were transformed (arcsine of the square root) before being analyzed (Zar, 1996).

3. RESULTS

3.1. Water quality

The TSS levels showed significant differences ($p<0.05$) under different treatments throughout the experiment. The mean (± standard deviation) values of the monitored parameters are presented in Table 2. Parameters such as temperature, dissolved oxygen, and pH exhibited no significant differences ($p>0.05$) between treatments, and the average values of these parameters were above 28 °C, 5 mg L^{-1} and 7, respectively. The average salinity was above 33 during the study and exhibited similar values ($p>0.05$) between treatments.

Table 2. Physical and chemical parameters monitored during the trial period with different TSS concentrations in the culture of *L. vannamei* in a BFT system. Different letters in the same row indicate significant differences ($p<0.05$).

	TREATMENT				
PARAMETER	**T250**	**T500**	**T1000**	**T2000**	**T4000**
TSS (mg L^{-1})	276.56±44.75 ^{a}	563.00±88.88 ^{b}	1106.78±133.53 ^{c}	2054.22±160.38 ^{d}	3829.33±283.25 ^{e}
Temperature (°C)	28.83±2.13	29.47±2.16	29.36±2.12	28.98±2.06	29.16±2.34
DO (mg L^{-1})*	6.03±0.41	5.88±0.41	6.01±0.42	6.03±0.40	5.96±039
Salinity	34.30±0.56	34.40±0.60	34.10±0.35	33.90±0.60	34.50±0.40
pH	7.92±0.24	7.71±0.28	7.53±0.30	7.53±0.25	7.51±0.27
Alkalinity (mg $CaCO_3$ L^{-1})	111.25±13,78^{a}	98.33±6.66^{b}	92.08±10.78^{b}	90.42±7.89^{b}	91.25±5.58^{b}
Ammonia (mg L^{-1})	0.33±0.14	0.18±0.06	0.15±0.06	0.18±0.13	0.12±0.05
Nitrite (mg L^{-1})	3.55±1.48^{a}	0.22±0.15^{b}	0.07±0.02^{b}	0.05±0.01^{b}	0.04±0.01^{b}
Nitrate (mg L^{-1})	30.03±4.08^{a}	55.37±9.10^{b}	169.28±47.94^{c}	223.91±22.08^{c}	216.72±22.54^{c}

DO = Dissolved Oxygen

Significant differences were observed in alkalinity, where only the T250 treatment was significantly higher ($p<0.05$) than the other.

The nitrogen compounds showed variations during the experimental period, with some differences observed in different periods between treatments. The T250 treatment had a significantly higher mean ammonia concentration ($p<0.05$) than the other treatments in the second week. In the first three weeks, nitrite concentrations in the T250 treatment were significantly lower ($p<0.05$) compared to those in the T500, T1000, T2000, and T4000 treatments, which all exhibited similar ($p>0.05$) values. The highest concentration of this compound was detected in treatment T250 in the second week, where it reached an average of 9.61 ± 2.81 mg L^{-1}. Treatments T1000, T2000, and T4000 were similar and showed higher nitrate concentrations with significant differences ($p<0.05$) compared to T250 and T500. Moreover, significant differences ($p<0.05$) were observed between T250 and T500.

3.2. Specific Oxygen Consumption Rate (OCR)

At the end of the OCR tests, no mortality was observed in any replicate. The different TSS concentrations explained 68 and 42% of the variability in specific oxygen consumption in the OCR_I and OCR_F tests with average values of 0.30 ± 0.08 and 0.30 ± 0.10 (mg O_2 g^{-1} h^{-1}), respectively, and were inversely correlated (Fig. 1). However, the angular coefficients and intercepts were not significantly different ($p>0.05$) (Table 3).

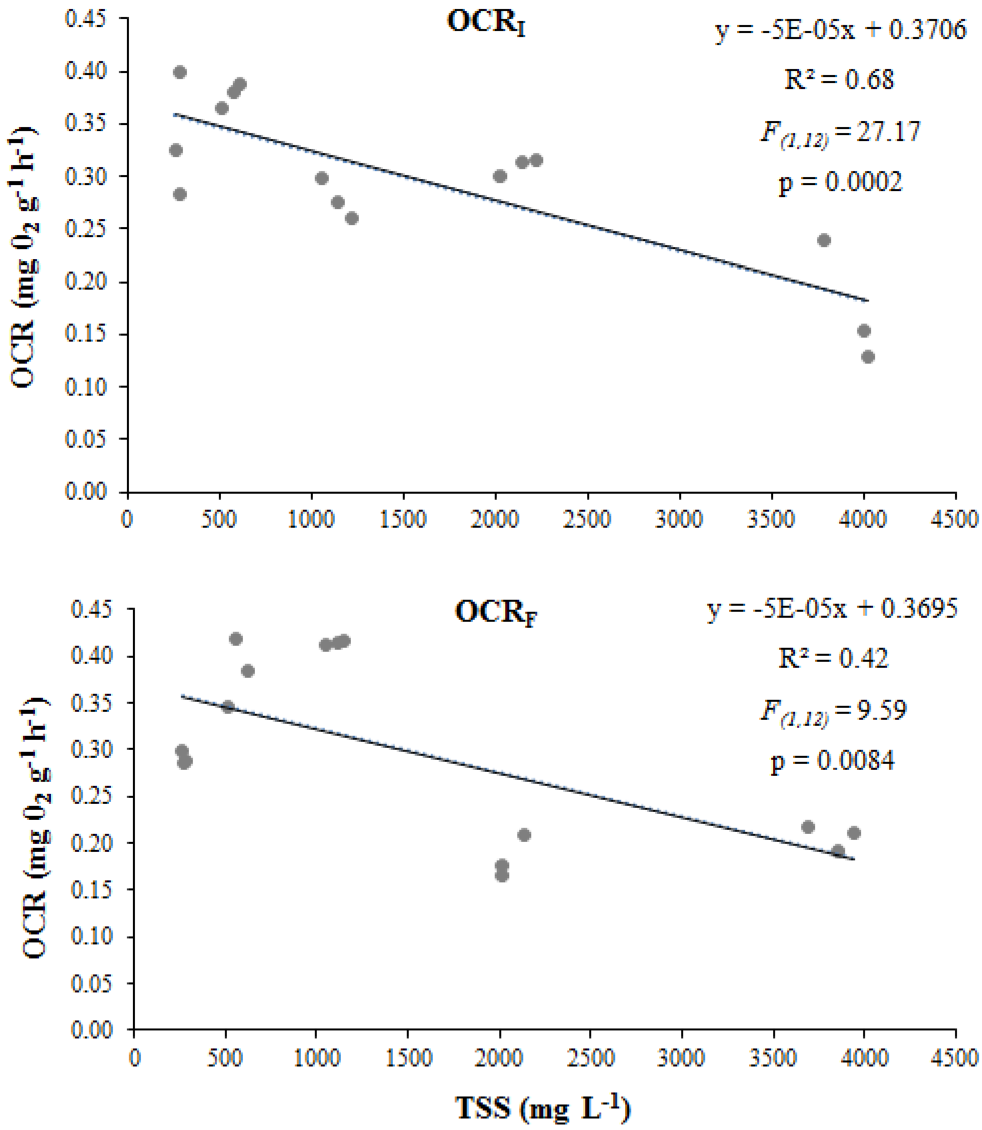

Fig. 1. Linear regressions of OCR_I and OCR_F tests for different TSS levels in a BFT system with *L. vannamei* using two exposure times.

Table 3. Comparison of angular coefficients and intercepts using an *F* test to verify the identity between the two linear regressions ($\mathrm{OCR_I}$ and $\mathrm{OCR_F}$).

Constant	**GL**	***F***	***p***
Angular (β_{01}; β_{02})	1, 26	0.0002	0.9856
Intercept (β_{11}; β_{12})	1, 27	0.0001	0.9902

GL = Graus de Liberdade

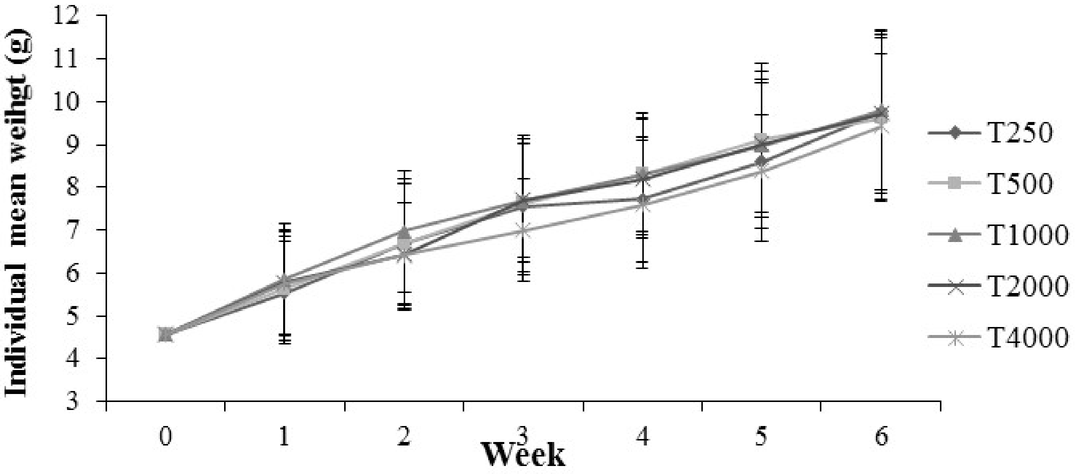

Fig. 2. Individual mean weight of *L. vannamei* in different TSS concentrations in a BFT system. Vertical bars indicate one standard deviation.

3.3. Shrimp performance

During the experiment, there was no significant difference (p>0.05) in individual mean weight among treatments (Fig. 2). At the end of the experiment, the final mean weight and the calculated parameters of weekly weight gain (WWG), feed conversion rate (FCR), survival, and productivity (Table 4) were similar (p>0.05).

Table 4. Shrimp performance indices of *L. vannamei* with different TSS concentrations in a BFT system.

	TREATMENT				
PARAMETER	**T250**	**T500**	**T1000**	**T2000**	**T4000**
Initial mean weight (g)	4.57±1.07	4.57±1.07	4.57±1.07	4.57±1.07	4.57±1.07
Final mean weight (g)	9.77±1.88	9.61±1.94	9.81±1.87	9.72±1.77	9.42±1.69
WWG (g/sem)	0.87±0.13	0.84±0.15	0.87±0.03	0.86±0.12	0.81±0.08
FCR	1.40±0.15	1.47±0.21	1.46±0.08	1.46±0.07	1.55±0.02
Survival (%)	94.00±5.00	91.67±3.51	90.00±5.20	90.00±3.61	86.00±3.62
Productivity (kg/m^3)	4.60±0.52	4.41±0.55	4.41±0.18	4.37±0.29	4.05±0.05

4. DISCUSSION

In the present study, reused water rich in biofloc was used to facilitate efficient establishment of nitrifying bacteria (Krummenauer et al., 2014) to maintain the ammonia and nitrite concentrations at levels below those recommended for *L. vannamei* (Lin and Chen, 2001, 2003). In the T2000 and T4000 treatments, the average concentrations of nitrate exceeded 200 ppm in salinity 11 suggested by Kuhn et al. (2010) for the study period; however, the authors kept limited animals during the entire experimental period. The alkalinity and pH were maintained above 7 and approximately 100 mg $CaCO_3$ L^{-1}, as recommended by Wasielesky et al. (2006b) and Ebeling et al. (2006), respectively. The temperature, dissolved oxygen, and salinity were maintained in the ranges recommended for this species (Zhang et al., 1995; Palafox-Ponce et al., 1997; Van Wyk and Scarpa 1999).

The TSS concentrations recommended for *L. vannamei* in a BFT culture system vary between 200 and 600 mg L^{-1} (Samocha et al., 2007; Avnimelech, 2009; Ray et al., 2010a; Ray et al., 2011; Gaona et al., 2011; Schveitzer et al., 2013). These ranges produce directly proportional changes in the dissolved oxygen concentrations in the water because of the presence of aerobic microorganisms that aggregate to bioflocs (Avnimelech, 2009; Ray et al., 2010b). Decreasing the dissolved oxygen levels below the recommended value (5 mg L^{-1}) for *L. vannamei* may occur during the production cycle because of the increased suspended solids and shrimp biomass. Dissolved oxygen concentrations below 3 mg L^{-1}were recorded by Ray et al. (2010a), Ray et al. (2011), Gaona et al. (2011), and Krummenauer et al. (2011). Because decreased dissolved oxygen can be a problem in BFT systems, previous studies used aeration devices increase the supply of dissolved oxygen. In addition to mechanical aeration, Vinatea et al. (2010) used pure oxygen injections to maintain the dissolved oxygen level above 3 mg L^{-1}. Ray et al. (2011) used airlifts and venturi systems to inject atmospheric air in combination with pure oxygen injection to stabilize the concentrations of dissolved oxygen. These concentrations were below the recommended value (5 mg L^{-1}) for *L. vannamei* (Van Wyk and Scarpa, 1999) and were close to the hypoxic levels (Mugnier and Soyez, 2005) recorded in some times in prior studies, combined with TSS concentrations exceeding the recommended range, which could have compromised the performance indices. However, in the present study, the shrimp growth was not determined by the different TSS concentrations, where the aeration was sufficient to keep the dissolved oxygen levels above the recommended value.

Bett and Vinatea (2009) observed an interaction between temperature, salinity, and the weight of animals with regard to specific oxygen consumption in a semi-open respirometer system. In a growth study with different salinities and ammonia concentrations, Li et al. (2007) observed interactions between abiotic factors and OCR in a closed system without aeration. These observed interactions indicate that when there is an interruption of aeration in a BFT system, dissolved oxygen can decrease to lethal levels after approximately 30 minutes and cause a decrease in oxygen consumption per individual (Vinatea et al., 2009), especially when the oxygen reduction rate may be higher in culture with higher TSS concentrations. However, the coincidence (angular coefficients and intercepts equals) observed between the regressions showed that the exposure time to different TSS concentrations did not change the interactions between specific oxygen consumption and TSS. Notably, the tolerance may be vary between species and the impact of high TSS concentrations is not known (Hargreaves, 2006). However, the decrease in OCR coinciding with the increase in TSS levels appears to be related to an adaptive response of the animals rather than a reduction in respiratory capacity caused by the reduced dissolved oxygen as a function of suspended solids. This finding supports the hypothesis that insufficient aeration systems affect the water quality and, consequently, the performance of cultured organisms. The species *L. vannamei* has the ability to regulate oxygen consumption according to the size of the animals up to a certain critical point; below this point, they become dependent on the oxygen tension (Villarreal et al., 1994; Ponce-Palafox et al., 2013).

The similarities in the performance data recorded in this study support the findings of non-interference from different TSS levels in the development of animals. In a study of stocking density, which involved 390 shrimp m^{-2} and TSS concentrations between 400-600 mg L^{-1}, Schveitzer et al. (2013) recorded GPS, CAA, survival, and productivity values of 0.64 g week^{-1}, 3.2, 83%, and 4.01 kg m^{-3}, respectively, after 44 days of evaluating the effect of different biofloc levels on microbial activity, water quality, and performance of *L. vannamei*.To analyze the reuse of water from a BFT system, Krummenauer et al. (2014) stocked 312 *L. vannamei* juveniles per m² and obtained a GPS of 1.05 g week^{-1}, CAA of 1.09, survival of 99.06%, and productivity of 2.48 kg m^{-3} after 30 days of experiment, where the TSS mean concentration was 714 mg L^{-1} using water from a previous crop without dilution. The indices in the present study were also close to the results found by Xu and Pan (2012), who stocked 224 ind. m^{-3} with TSS concentrations of 320 mg L^{-1}. These authors evaluated the effect of bioflocsin *L. vannamei* performance over 30 days and showed GPS, CAA and survival values of approximately 1 g week^{-1}, 1.45, and 91%, respectively. Furtado et al. (2014) evaluated the effect of different alkalinity levels on the same penaeid species and observed that for an alkalinity of 300 mg CaCO3 L^{-1} (TSS = 290 mg L^{-1}), the shrimp had GPS of 1.05 g week^{-1}, CAA of 1.08, and survival approximately 91%.

These previous publications showed varying performance of *L. vannamei* in terms of growth performance data of cultured animals at different stocking densities and varied TSS concentrations. Some of these studies controlled the TSS levels, and others increased the levels across experiment or did not expose the animals to higher concentrations throughout the trial period.

5. CONCLUSION

The different TSS concentrations tested did not affect the shrimp *L. vannamei* growth over the study period. Dissolved oxygen concentrations were above 5 mg L^{-1} in this study and met the recommended level for this species. In shrimp production, the TSS concentrations gradually increased with the culture time, slowly exposing the cultured organisms increasing values. TSS interactions with other parameters, such as nitrogen compounds, should be taken into account, especially when water is not reused from a previous BFT culture system because this can reach undesirable levels considering that bacterial communities are already not established. Finally, the TSS concentrations can be varied in BFT systems, exceeding the reported ranges; however, the aeration system must produce the DO concentration indicated above for the target species.

6. ACKNOWLEDGMENTS

The authors are grateful for the financial support provided by the National Council for Scientific and Technological Development (CNPq), Ministry of Fishery and Aquaculture (MPA) and Coordination for the Improvement of Higher Level Personnel (CAPES). Special thanks to Centro Oeste Rações S.A. (GUABI) for donating the experimental diets. W.J. Wasielesky and L.H. Poersch are research fellows of CNPq.

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CAPÍTULO IV

REMOÇÃO DE SÓLIDOS SUSPENSOS DO CULTIVO DE CAMARÃO EM SISTEMA BFT INTEGRADO COM PEIXES E OSTRAS

Estudo realizado em Waddell Mariculture Center (WMC), EUA, como parte do estágio no exterior (Doutorado sanduiche).

RESUMO

Sistema de cultivo com tecnologia de bioflocos (BFT) tem grande potencial para produção integrada de peixes, moluscos e crustáceos (sistema multitrófico). O objetivo deste estudo foi cultivar *L. vannamei* em sistema BFT, integrando tilápias para avaliação de tratamento biológico de sólidos suspensos totais, bem como, o uso de tanques de sedimentação e de biofiltradores como as ostras. Além disso, avaliar a possibilidade de integrar uma espécie de peixe carnívora ao sistema BFT, deve ser considerado para a otimização da produção aquícola. Neste estudo foram utilizadas quatro espécies: camarão branco do Pacífico *Litopenaeus vannamei*, tilápia do Nilo *Oreochromis niloticus*, *red drum Sciaenops ocellatus* e ostra americana *Crassostrea virginica*. Foram montados quatro sistemas integrados com água recirculando em circuitos fechados compostos por tanques separados para cada espécie. O delineamento experimental foi definido em controle (sem tilápias) e tratamento biológico TB (com tilápias), em duas réplicas. Os pesos iniciais e as densidades de estocagem foram: *L. vannamei*(2 g – 250 ind m^{-3}); *O. niloticus* (85 g – 5,12 kg m^{-3}); *S. ocellatus* (71 g – 1,04 kg m^{-3}). Foram estocadas 100 ostars *C. virginica* (51 mm) por tanque. Ao longo das 8 semanas do experimento foram avaliados temperatura, oxigênio dissolvido, pH, alcalinidade, salinidade, sólidos suspensos totais e amônia. Todos os parâmetros foram mantidos dentro do recomendado para camarões, tilápias e *red drum*. As concentrações de nitrito estiveram dentro do sugerido, exceto, para os camarões. A salinidade e temperatura podem ter interferido no crescimento ostras. No final do experimento, a medida de sólidos sedimentáveis foi significativamente menor ($p < 0,05$) no tratamento TB. Considerando o desempenho zootécnico não verificou-se diferenças significativa ($p > 0,05$) entre o controle e TB, porém o consumo de sólidos nos sistemas com tilápias apresentaram redução de material particulado no efluente, comparado ao controle. O estudo demonstrou que a aplicação de cultivo de camarões marinhos com tecnologia de bioflocos em sistema multitrófico integrado, resulta na redução de sólidos causada por organismos consumidores de subprodutos dos camarões.

ABSTRACT

Biofloc Technology Culture system (BFT) has great potential for integrated production of fish and shellfish (multi-trophic system). The objective of this study was grow*L. vannamei* in BFT system, integrating tilapia for evaluation biological treatment of suspended solids, as well as the use of biofilters as oysters. In addition, to evaluate the possibility to integrate a carnivorous fish in BFT system should be considered for optimization of aquaculture production. In this study, four species were used: Pacific white shrimp *Litopenaeus vannamei*, Nile tilapia *Oreochromis niloticus*, red drum *Sciaenops ocellatus* and American oyster *Crassostrea virginica*. It was structured four integrated systems with recirculating water in closed circuits composed of separate tanks for each species. The experiment was set in control (with no tilapia) and biological treatment BT (with tilapia) in two replicates. The initial weights and stocking densities were: shrimp (2 g – 250 ind m^3); tilapia (85 g – 5.12 kg m^{-3}); red drum (71 g – 1.04 kg m^{-3}), and one hundred oysters *C. virginica* (51 mm) per tank. During the 8 weeks of the experiment were evaluated temperature, dissolved oxygen, pH, alkalinity, salinity, total suspended solids and ammonia. All parameters were kept within the recommended for shrimp, tilapia and red drum. The nitrite concentrations were within the suggested except for the shrimp. The salinity and temperature may have interfered in oysters growth. At the end of the experiment, the measurement of settleable solids was significantly lower ($p < 0.05$) in TB treatment. Considering the growth performance not there was a significant difference ($p > 0.05$) between the control and TB, but the consumption of solids in systems with tilapia resulted in the particulate matter decreased in the effluent, compared to the control. The study showed that the application of marine shrimp culture with biofloc technology in integrated multi-trophic system, result in reduction of solid by consumers of shrimp waste.

INTRODUÇÃO

Um dos aspectos considerados no desenvolvimento da aquicultura sustentável é a otimização do uso de água. Neste sentido, a produção de uma espécie em sistema com bioflocos (BFT) é de alta sustentabilidade, já que é realizada sem nenhuma ou mínima renovação de água (Otoshi et al. 2009) e permite a reutilização da agua ao longo dos ciclos de produção (Krummenauer et al. 2014). No sistema BFT os nutrientes gerados pelas excretas dos animais são reciclados por bactérias que promovem a agregação de outros microrganismos, reduzindo os teores de compostos tóxicos, melhorando a qualidade de água e disponibilizando alimento natural suplementar para a alimentação das espécies produzidas (Wasielesky et al. 2006; Avnimelech 2009).

Com a produtividade natural no sistema BFT, há um acúmulo de partículas mantidas em suspensão pela aeração mecanizada (Hargreaves, 2006), especialmente quando há reuso de água de ciclo anterior (Krummenauer et al. 2014).Para controle das quantidades de sólidos durante o ciclo de produção, pode ser utilizado tanque de sedimentação para a remoção de sólidos suspensos (Ray et al. 2010; Gaona et al. 2011).

Sistemas multitróficos, produzem espécies alimentadas com ração comercial e simultaneamente, os seus resíduos orgânicos e inorgânicos são aproveitados para suprir a alimentação de outros organismos aquáticos (Barrington et al. 2009). A produção integrada de diferentes espécies, pode reduzir a concentração de sólidos suspensos pelo consumo de partículas em suspensão, aproveitar os dejetos e nutrientes provenientes da produção de uma espécie alvo como alimento para outra espécie e promover a mitigação dos impactos ambientais (Muangkeow et al. 2007; Ramos et al. 2009; Yuan et al. 2010; Abreu et al. 2011; Lander et al. 2013).

A integração de camarões, peixes e ostras em sistema BFT, possibilita a associação de duas alternativas na otimização da produção. A produção de *Litopenaeus vannamei* com tecnologia de bioflocos tem alcançado excelentes resultados em função da melhora de qualidade de água e desempenho zootécnico, resultando em maiores produtividades (Wasielesky et al. 2006, Samocha et al. 2007; Krummenauer et al. 2011). As tilápias, por serem peixes omnívoros que podem utilizar material particulado em sistemas intensivos, também tem sido produzidas de forma efetiva em sistema BFT (Azim & Little, 2008; Luo et al. 2014). *Red drum* é uma espécie procurada na pesca e com boa aceitação para consumo, sendo que já foi avaliada a sua produção em sistemas de engorda (Sandifer et al. 1993). As ostras podem auxiliar no controle de sólidos presentes na água do cultivo pela capacidade de filtração de partículas de tamanhos variados (Dupuy et al. 2000).

No entanto, a produção de organismos aquáticos em sistema multitrófico integrado, com as espécies confinadas em tanques mantidos com água recirculante, não é bem conhecida. Portanto, o objetivo deste estudo foi cultivar camarões *L. vannamei* em sistema BFT, integrando tilápias *Oreochromis niloticus* para avaliação de tratamento biológico de sólidos suspensos totais, bem como, o uso de ostras *Crassostera virginica* como biofiltradores. Ainda, avaliar a possibilidade de integrar uma espécie de peixe carnívora (*Sciaenops ocellatus*) ao sistema BFT, buscando a otimização da produção aquícola.

MATERIAL E MÉTODOS

Delineamento experimental

O experimento foi realizado durante oito semanas em duas estufas em Waddell Mariculture Center (WMC) localizado em Bluffton, SC, USA. A água utilizada no experimento foi captada do Colleton River, adjacente ao WMC e misturada a água doce para reduzir a salinidade 32 para 13. O sistema multitrófico foi constituído pelas espécies: camarão branco do Pacífico *Litopenaeus vannamei*, tilápia do Nilo *Oreochromis niloticus*, *red drumSciaenops ocellatus* e ostra americana *Crassostrea virginica*. As quatro espécies foram aclimatadas previamente ao início do experimento, por um período de 48 h para redução da salinidade. As concentrações de sólidos suspensos totais (SST) foram avaliadas de forma integrada ao longo do experimento e uma medida de sólidos sedimentáveis (SS) foi tomada no encerramento. Foram delineados dois tratamentos, sendo um sistema controle (sem tilápias) e um sistema com tratamento biológico – TB (com tilápias), ambos com duas réplicas. Tanto no controle como no TB, a água foi mantida recirculando em circuito fechado em uma taxa de 1500 L h^{-1}, sendo bombeada dos tanques com camarões por bomba submersa de 2000 L h^{-1} aos tanques sem e com tilápias. Destes, a água seguiu por gravidade aos tanques de sedimentação e na sequência, passou aos tanques com *red drum* associados as ostras, retornando aos tanques com camarões (Figura 1). A capacidade dos tanques com camarões, tilápias, *red drum* + ostras e de sedimentação, foram de 22 m^3, 1 m^3, 6.7 m^3 e 1m^3, respectivamente.

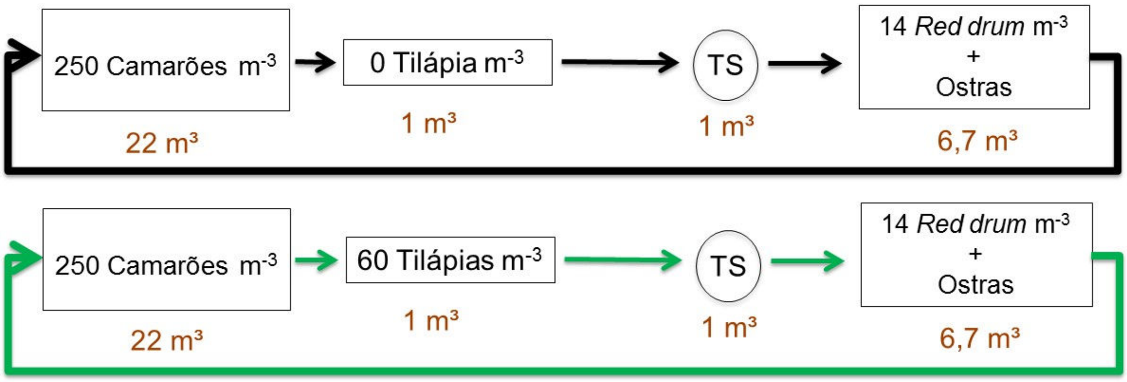

Figura 1. Desenho esquemático do sistema multitrófico desenvolvido em experimento de tratamento biológico de sólidos suspensos totais, utilizando camarões, tilápias, *red drum* e ostras. TS refere-se aos tanques de sedimentação. A setas pretas indicam o sistema mantido no tratamento TB e as setas verdes indicam o controle.

Previamente ao início do experimento, os tanques preparados para estocagem dos camarões foram inoculados com 4 m³ de água com bioflocos do berçário de *L. vannamei*, com subsequente fertilização orgânica manipulando a relação C:N (6:1) baseada em metodologias de Avnimelech (1999) e Ebeling et al. (2006) para a conversão de nitrogênio em biomassa bacteriana, considerando uma concentração de 1 mg L^{-1} de amônia. Como fonte de carbono foi utilizada dextrose com teor de 40% de carbono.

A aeração de todo sistema foi suprida por um soprador de 7 hp para difusão de ar por pedras porosas no fundo dos tanques.

Material biológico e arraçoamento

Camarão

Pós-larvas de *L. vannamei* (PL 10) foram adquiridas do *Shrimp Improvement Systems* (SIS), Islamorada, Florida, EUA e foram mantidas em berçário até peso médio de 2,0 ± 0,62 g. Para o início do experimento os camarões foram transferidos aos tanques a uma densidade de estocagem de 250 indivíduos m^{-3}. Os animais foram alimentados três vezes ao dia com ração comercial com 35% de proteína bruta (Zeigler HI 35®, Zeigler Brothers, Inc., Gardners, PA, EUA). A ração foi ofertada a lanço e em bandejas de alimentação, a uma taxa de 10% de biomassa inicial dos camarões e foi ajustado de acordo com o consumo observado nas bandejas entre cada alimentação.

Tilápia

Tilápias (*O. niloticus*) provenientes do próprio laboratório, com peso médio de 85,39 ± 38,63 g foram estocadas a uma densidade de estocagem de 60 indivíduos m^{-3} (5,12 kg m^{-3}) no tratamento TB. Foi utilizada ração comercial com 40% de proteína bruta (Zeigler Finfish Silver 40-10®, Zeigler Brothers, Inc., Gardners, PA, EUA) ofertada três vezes ao dia, com taxa média de arraçoamento de 1,75% da biomassa durante todo o experimento.

Red drum

Foram utilizados *Red drum* (*S. ocellatus*) mantidos no próprio laboratório, com peso médio de 70,94 ± 25,47 e 71,39 ± 25,88 g, respectivamente, no controle e tratamento TB, foram transferidos para as unidades experimentais a uma densidade de estocagem de 14,6 peixes m^{-3} (1,04 kg m^{-3}). Foi ofertada ração comercial com 40% de proteína bruta (Zeigler Finfish Silver 40-10®, Zeigler Brothers, Inc., Gardners, PA, EUA) três vezes ao dia, com taxa de arraçoamento de 2% da biomassa durante todo o experimento.

Ostra

As ostras (*C. virginica*) foram adquiridas de um produtor local, com comprimento médio de 52,00 ± 7,02 e 51,31 ± 8,06 mm, respectivamente no controle e tratamento TB. Dois cestos flutuantes com 50 animais cada, foram mantidos em cada tanque com *red drum*.

Parâmetros físicos e químicos

Dados de parâmetros físicos e químicos, tanto por equipamento eletrônico como por análise laboratorial, foram coletados em cada tanque. Diariamente foram monitorados temperatura, oxigênio dissolvido, pH e salinidade utilizando multiparâmetro YSI® model *Professional Plus* (YSI Incorporated, Yellow Springs, OH, EUA). Análises de amônia total (N–AT) e nitrito ($N–NO_2^-$) foram realizadas diariamente e de nitrato ($N–NO_3^-$) semanalmente, de acordo com os métodos da HACH 8155, 8507 e 8039, respectivamente, (HACH Company, 2003). As concentrações de fosfato foram medidas uma vez por semana, conforme método da HACH 8048 (HACH Company, 2003). A alcalinidade foi medida semanalmente através do método de titulação conforme descrito em APHA (1989). Sólidos suspensos totais (SST) e sólidos suspensos voláteis (SSV) foram medidos a cada semana com diluição de 90% com água deionizada segundo o método ESS 340.2 (ESS, 1993). Com o uso de cone Imhoff (Avnimelech, 2009), a medida final de sólidos sedimentáveis foi realizada a partir de amostras de água dos sistemas controle e dos sistemas com tilápias (TB), após a retirada dos animais.

Desempenho zootécnico

Camarão e peixes

O crescimento foi acompanhado por meio de biometrias quinzenais para os camarões e cada 30 dias para as duas espécies de peixes, utilizando balança digital com precisão de 0,01 g. O ajuste do arraçoamento para os camarões foi de acordo com Jory et al. (2001). O ganho de peso semanal (GPS) foi determinado pelo seguinte cálculo: GPS (g/sem) = (GP/ nº semanas de cultivo). A conversão alimentar aparente (CAA) foi obtida pela seguinte fórmula: CAA = alimento oferecido / incremento de biomassa. A sobrevivência dos camarões foi calculada através de: S% = [(biomassa final / peso médio individual) / nº indivíduos estocados)] x 100. Para as espécies de peixes a sobrevivência foi calculada por: S% = [(nº inicial de indivíduos / nº final de indivíduos) x 100]. A produtividade foi calculada para camarões e peixes, pelo seguinte cálculo: Prod (kg/m^3) = (biomassa final / volume do tanque).

Ostra

O crescimento das ostras foi monitorado após 30 e 60 dias do início do experimento, através de medidas do comprimento da concha com o uso de paquímetro digital. A sobrevivência das ostras foi calculada por: S% = [(nº inicial de indivíduos / nº final de indivíduos) x 100].

Análise estatística

Os dados de parâmetros físicos e químicos foram coletados em cada tanque e, posteriormente, foram unificados em cada sistema (controle e TB) para a análise estatística. Os dados de desempenho zootécnico de cada espécie foram comparados entre o controle e tratamento TB. Para as duas análises foi utilizado teste *t*. Dados de sobrevivência foram transformados (arcoseno $x^{0.5}$) antes das análises (Zar 1996).

RESULTADOS

O sistema integrado não apresentou diferenças significativas ($p > 0{,}05$) entre as médias de temperatura, oxigênio dissolvido, pH, salinidade, alcalinidade e fosfato (Tabela 1).

As concentrações de SST a partir da primeira semana decresceram até a quarta e quinta semanas e permaneceram sem grandes variações até o final do experimento (Figura 2a). As variações de SSV foram semelhantes às de SST, porém, com diferenças significativas entre as semanas 4 e 6. As medidas de SS (Figura 3) realizadas no final do experimento nos tanques retangulares sem e com tilápias, foram significativamente diferentes ($p < 0{,}05$) entre o controle (940 ± 56,57 ml L^{-1}) e tratamento TB (7 ± 4,24 ml L^{-1}).

Os picos de amônia no controle e tratamento TB ocorreram em tempos diferentes. Na Figura 2c pode ser observado que a concentração de amônia no tratamento TB foi significativamente maior ($p < 0{,}05$) comparado ao controle antes da primeira semana de experimento. Ao contrário, diferenças significativas ($p < 0{,}05$) foram observadas na terceira e quarta semanas, com maiores concentrações no tratamento TB. O nitrito acumulou até o final do experimento e diferenças significativas ($p < 0{,}05$) entre o controle e o tratamento TB, foram detectadas nas duas últimas semanas (Figura 2d). Em um dos sistemas com tratamento biológico (TB), a concentração de nitrito alcançou 11,7 ± 0,2 mg L^{-1}, sendo observadas mortalidades de camarões e ostras.

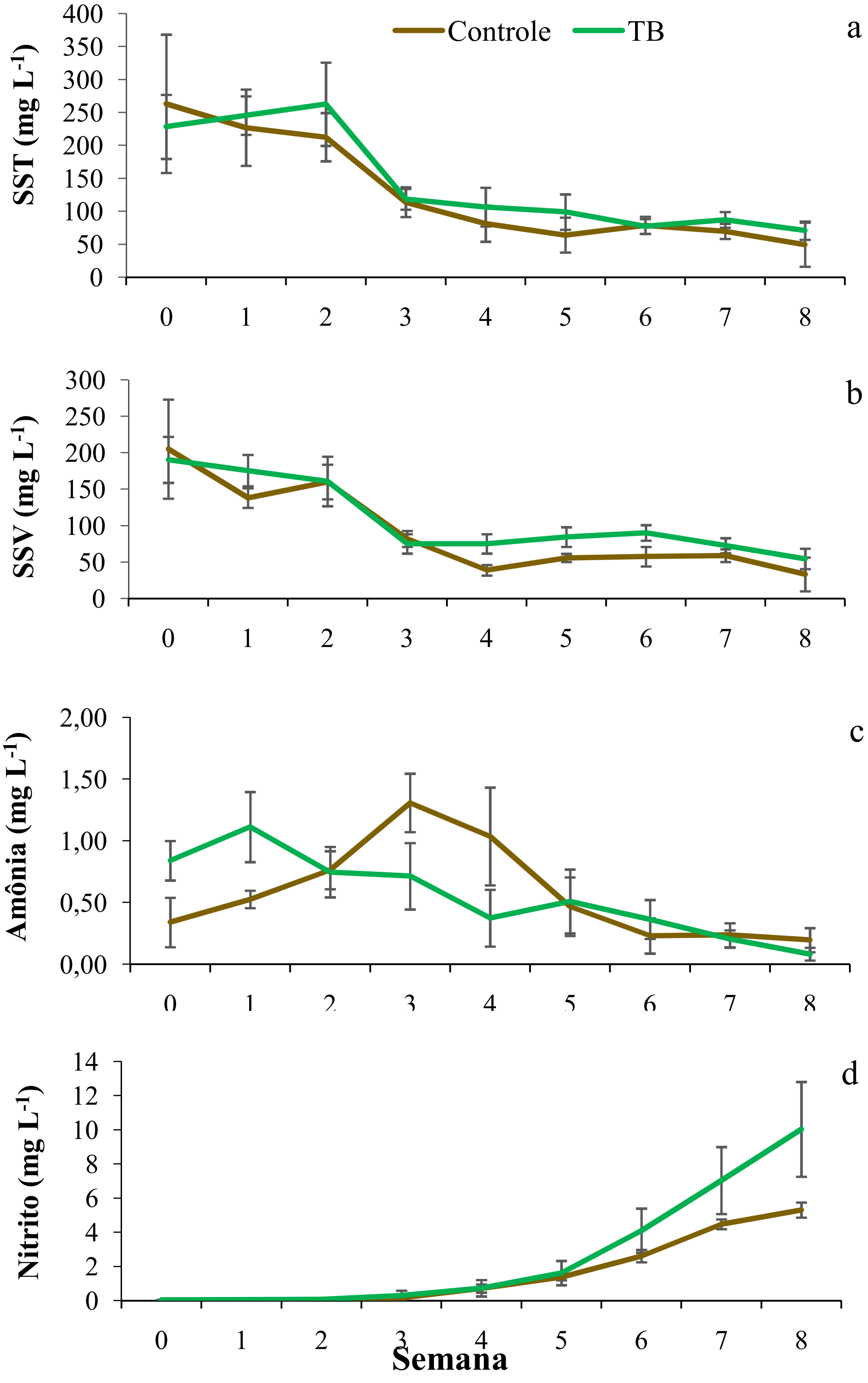

Figura 2. Variações de: (a) sólidos suspensos totais (SST); (b) sólidos suspensos voláteis (SSV); (c) amônia; e (d) nitrito, em cultivo de camarões em sistema BFT integrado com peixes e ostras.

Tabela 1. Valores médios± desvio padrão dos parâmetros físicos e químicos monitorados durante o período de estudo no controle e tratamento TB em cultivo de camarões em sistema BFT integrado com tilápias, *red drum* e ostras.

PARÂMETRO	**Controle**	**TB**
Temperatura (°C)	28,04±1,02	27,78±0,75
Oxigênio dissolvido (mg L^{-1})	6,58±1,15	6,24±0,41
pH	7,17±0,38	7,18±0,35
Salinidade	13,40±0,23	13,17±0,34
Alcalinidade (mg $CaCO_3$ L^{-1})	131,38±4,80	130,15±6,04
Fosfato (mg L^{-1})	3,74±1,63	3,14±1,85

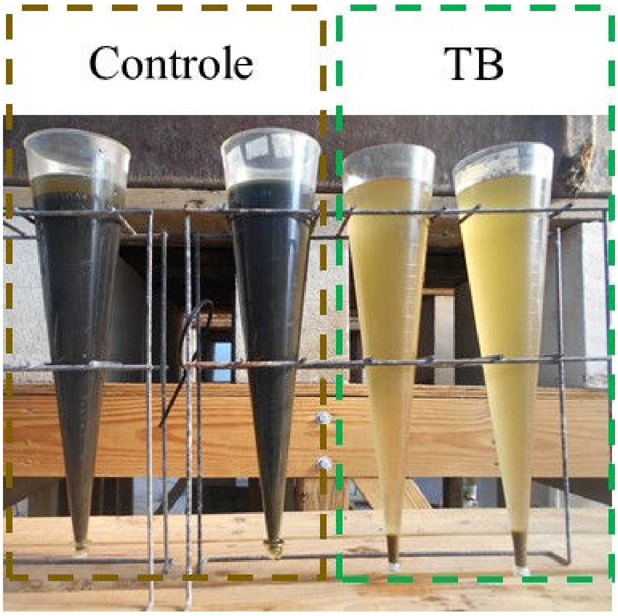

Figura 3. Medidas de sólidos sedimentáveis em cone Imhoff no final do experimento de cultivo de camarões em sistema BFT integrado com tilápias, *red drum* e ostras. As amostras foram tomadas nos tanques retangulares sem tilápias (controle) e com tilápias (TB).

Não foram detectadas diferenças significativas ($p > 0,05$) nos índices de desempenho zootécnico para camarões, tilápias e *red drum*, conforme apresentado na Tabela 2. As ostras tiveram crescimento semanal pequeno no controle (0,40 ± 0,51 cm) e tratamento TB (0,12 ± 0,06 cm), ambos sem diferenças significativas ($p > 0,05$). As taxas de sobrevivência das ostras não foram significativamente diferentes ($p > 0,05$), alcançando 100% no controle e 91,50 ± 8,18% no tratamento TB.

Tabela 2. Índices de desempenho zootécnico (média±desvio padrão) com relação a peso inicial (PI), peso final (PF), ganho de peso semanal (GPS), conversão alimentar aparente (CAA), sobrevivência (S) e produtividade (P) dos camarões, tilápias e *red drum* em sistema BFT.

Espécie	**PI (g)**	**PF (g)**	**GPS (g sem^{-1})**	**CAA**	**S (%)**	**P (kg m^{-3})**
			Camarão			
Controle	2,00±0,62	12,39±2,35	1,30±0,01	1,05±0,07	94,04±2,83	2,79±0,19
TB	2,00±0,62	12,66±1,93	1,33±0,07	1,34±0,48	72,18±25,56	2,06±0,76
			Tilápia			
TB	85,39±38,63	203,41±12,04	14,77±1,30	0,85±0,04	100	12,20±0,72
			Red drum			
Controle	70,94 ± 25,47	167,48±52,93	12,07±0,33	0,98±0,05	98,00±1,41	2,40±0,01
TB	71,39 ± 25,88	164,48±60,41	11,66±1,80	1,00±0,14	99,00±0,14	2,38±0,20

DISCUSSÃO

Os parâmetros temperatura, oxigênio dissolvido, pH e salinidade, foram controlados em função *L. vannamei* e *O. niloticus* e estiveram dentro do recomendado para estas espécies (Van Wyk & Scarpa, 1999; Azim & Little, 2008; Yuan et al. 2010). Estes parâmetros permaneceram próximos a valores monitorados por Lunger et al. (2006) para *S. ocellatus*. Em ambiente natural, *C. virginica* pode estar sujeita a variações de temperatura e salinidade entre 12 – 35 °C e 5 – 39, respectivamente (Surge et al. 2001). No entanto, exposição prolongada a temperatura acima de 28 °C e salinidade de 15 pode resultar em cessação de crescimento e mortalidades em ostras (Surge et al. 2001; Dickinson et al. 2012; Mattoo et al. 2013). No presente estudo a elevada temperatura e baixa salinidade impostas as ostras pode ter reduzido o crescimento, porém não ficou evidente o efeito na redução da sobrevivência registrada apenas no tratamento TB. As ostras *C. virginica* não tiveram crescimento notável e foi inferior ao encontrado por Kuhn et al. (2013) e não foi possível detectar a viabilidade de cultivo de ostras integradas em sistema BFT.

As concentrações de SST não ultrapassaram o intervalo de 400 – 600 mg L^{-1} sugerido por Azim & Little (2008) e Schveitzer et al. (2013), que trabalharam com *O. niloticus* e *L. vannamei*, respectivamente. A fertilização inicial com dextrose em todos os compartimentos estimulou a formação de bioflocos previamente a estocagem dos animais. Após a primeira semana de experimento, as concentrações de SST tiveram uma redução até a quarta semana. Esta redução pode estar associada a capacidade de camarões e tilápias em consumir bioflocos em diferentes tamanhos de partículas (Ekasari et al. 2014). Também pode estar relacionado a decantação de sólidos nos tanques de sedimentação, nos tanques retangulares sem tilápias (Merino et al. 2007; Ray et al. 2010; Gaona et al. 2011) e nos tanques circulares com *red drum* e ostras, devido ao fluxo radial que facilitou o acúmulo de material particulado na porção central do fundo do tanque (Timmons & Ebeling, 2010). Integrando tilápias em sistema de produção de camarões, as concentrações de SST são menores comparados a monocultura de camarões (Yuan et al. 2010). Avnimelech (2007) observou que, quando mantidas sem alimento inerte durante seis dias, as tilápias consumiram bioflocos que era a única fonte de alimento, resultando em redução de SST neste período. Neste sentido, o menor volume de sólidos sedimentáveis observado nos tanques com tilápias (TB) reforça a capacidade de *O. niloticus* em consumir grandes quantidades de material particulado. Esta característica denota a funcionalidade de tilápias no tratamento biológico de efluentes de carcinicultura.

O acúmulo de nutrientes como nitrogênio e fósforo em produção de peixes e/ou camarões, pode variar em função da quantidade excretada ou assimilada por cada espécie (Muangkeow et al. 2007; Ferreira et al. 2014). O comportamento dos compostos nitrogenados típicas de sistema BFT, com pico de amônia próximo as duas primeiras semanas de cultivo, seguido de pico de nitrito entre a quarta e sexta semana e início do acúmulo de nitrato até o final do ciclo (Avnimelech, 2009). Muangkeow et al. (2007) registraram maiores concentrações de amônia e nitrito no tanque estocado apenas com camarões comparado aos tanques integrados com camarão-tilápia. No presente estudo, provavelmente a amônia proveniente dos tanques com camarões interligados aos tanques com tilápias no tratamento TB provocou o incremento nas concentrações de amônia até a primeira semana, seguido de processo de nitrificação para a redução deste composto (Avnimelech, 2009). Ao contrário, a maior concentração média de amônia no controle, ocorreu duas semanas mais tarde, seguido do mesmo processo de nitrificação.

Estas diferenças de tempo no comportamento de amônia pode explicar as concentrações maiores de nitrito no tratamento TB. No entanto, tanto no controle como no tratamento TB o processo de nitrificação foi incompleto, o mesmo observado por Muangkeow et al. (2007) em tanques de cultivo de camarões. No presente estudo, o lento crescimento de bactérias nitrito-oxidantes pode ter ocorrido pela aplicação de dextrose para estimular crescimento de bactérias heterotróficas, as quais podem competir por oxigênio dissolvido, espaço, amônia total e micronutrientes (Luo et al. 2013).

As concentrações de amônia foram mantidas dentro do recomendado para *L. vannamei* (Lin & Chen, 2001). O nitrito excedeu o nível de segurança de 6,1 mg L^{-1} para *L. vannamei*, estimado por Lin & Chen (2003) no tratamento TB. Este fato pode ter influenciado a redução da sobrevivência dos camarões no presente estudo. Para *O. niloticus* as concentrações de amônia e nitrito ficaram abaixo dos níveis encontrados por Luo et al. (2014), os quais não observaram efeitos negativos no crescimento e sobrevivência.

O fosfato teve comportamento semelhante no controle e tratamento TB, não sendo possível detectar efeito das tilápias no tratamento TB, uma vez que pode haver maior retenção de fósforo por parte das tilápias (Tian et al. 2001).

Com respeito aos índices zootécnicos de cada espécie utilizada no presente estudo, não foi comprovada a influência do tratamento biológico por parte das tilápias sobre o desenvolvimento dos camarões, ostras e *red drum*. No entanto, os resultados sugerem a possibilidade de integrar tilápias ao sistema de produção, viabilizando a integração das espécies de forma compartimentada nos tanques de produção, evitando a necessidade de ajuste de densidade de estocagem entre as espécies, sendo esta relação observada em estudos anteriores (Muangkeow et al. 2007; Yuan et al. 2010). Em cultivo de *L. vannamei* em sistema BFT pode haver variabilidade em densidades de estocagem de camarões, parâmetros de qualidade de água e tempo de cultivo, resultando em variabilidades no peso final (8,4 – 15,6 g), GPS (0,5 – 0,9 g), CAA (1,3 – 4,8) sobrevivência (66 – 92%) e produtividade (2,1 – 4,1 kg m^{-3}) (Ray et al. 2010; Krummenauer et al. 2011; Schveitzer et al. 2013). No presente estudo, os resultados obtidos para peso final, GPS, CAA, sobrevivência e produtividade, foram 12,39 – 12,66 g, 1,33 g sem^{-1}, 1,05 – 1,34, 72 – 94% e 2,06 – 2,79 kg m^{-3}, respectivamente. Azim & Little (2008), em estudo de tilápias *O. niloticus* em sistema BFT, registrou peso final, GPS, CAA, sobrevivência e produtividade, variando de 138,58 – 140,72 g, 3,34 g sem^{-1}, 3,44 – 3,51, 100% e 4,80 – 4,90, respectivamente. Luo et al. (2014), em experimento com tilápias em sistema com tecnologia de biofloco, alcançaram peso final de 168,58 g, CAA de 1,20, sobrevivência de 100% e produtividade de 36,95 kg m^{-3}. Os resultados de desempenho zootécnico das tilápias *O. niloticus* do presente estudo, foram melhores que os estudos prévios com relação ao peso final = 203,41; GPS = 14,77 g sem^{-1}; sobrevivência = 100%, com destaque para a CAA (0,85), uma vez que o percentual de alimentação das tilápias não foi ajustado ao longo do experimento e provavelmente, isso levou a um maior consumo de biofloco. A produtividade ficou com valor intermediário de 12,20 kg m^{-3} comparado aos estudos anteriores. *Red drum* tem registros na fase de engorda durante 18 meses em viveiros revestidos de 0,1 ha, com valores de peso final, CAA, sobrevivência e produtividade variando de 0,89 – 1,37 kg, 2,15 – 2,60, 55 – 95% e 7251 – 24083 kg, respectivamente (Sandifer et al. 1993). Recentemente, Rossi et al. (2015) estocando *red drum* com peso inicial de 68 g durante 15 semanas, reportaram peso final = 237,8 – 247,9 g; CAA = 0,53 – 0,57; sobrevivência = 95 – 99% e GPS aproximado de 12 g sem^{-1}. No presente estudo, após 8 semanas em sistema BFT integrado, foram alcançados peso final, CAA, sobrevivência, GPS variando de, 164 – 167 g; 1,00; 98 – 99% e 12 g sem^{-1}, respectivamente, peso final, CAA, sobrevivência, GPS.

CONCLUSÃO

Os resultados indicam a possibilidade de diversificação de produtos da aquicultura integrados ao sistema com bioflocos. O consumo de material particulado pelas tilápias foi equivalente a um tratamento biológico, reduzindo os resíduos de efluente da aquicultura. A produção observada, especialmente a taxa de crescimento e CAA, confirmam a importância dos bioflocos como suplemento alimentar para camarões e tilápias, e sugere a possibilidade de cultivo de *red drum* em sistema BFT, porém deve ser melhor investigada.

AGRADECIMENTOS

Ao apoio financeiro concedido pelo Conselho Nacional deDesenvolvimento Científico e Tecnológico (CNPq), Ministério da Pesca e Aquicultura(MPA) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Projeto Camarão (IO/FURG). Wilson Wasielesky Jr. e Luis Henrique Poersch são bolsistas de produtividade do CNPq. Waddell Mariculture Center (EUA).

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CONCLUSÃO GERAL

O conhecimento das interações de SST com parâmetros de qualidade de água e desempenho zootécnico dos camarões reforçam a necessidade de intervenções para manutenção das concentrações de SST dentro da faixa recomendada. A série de estudos executados nesta tese, geraram informações para melhor manejo de bioflocos em cultivo de *L. vannamei* em sistemas BFT.

1. O menor volume de água que circulou pelo clarificador, demonstrou maior eficiência na remoção de partículas. O menor bombeamento de água mantido no clarificador manteve a velocidade de fluxo de água menor que a de sedimentação das partículas, facilitando a decantação das mesmas. A não reposição de água retirada a cada processo de clarificação, melhorou a utilização do recurso hídrico e otimizou a produção de camarões em sistema BFT.
2. A carga orgânica presente na água dos cultivos de camarões em sistema BFT durante a formação de bioflocos interfere nos processos de nitrificação. Estes processos podem ser mais efetivos se a faixa de SST na fase inicial do cultivo, for mantido entre 100 – 300 mg L^{-1}.
3. Diferentes concentrações de SST testadas, não afetaramo desempenho zootécnico de *L. vannamei*, em condições de oxigênio dissolvido acima de 5 mg L^{-1}. No entanto, o consumo de oxigênio é reduzido em baixas concentrações de oxigênio dissolvido e sugere uma adaptação de *L. vannamei* nestas condições.
4. Os níveis de SST em cultivo de camarões marinhos podem ser controlados com a utilização de espécies consumidoras de resíduos como tilápias, de forma integrada em sistema multitrófico, caracterizando um tratamento biológico do efluente.