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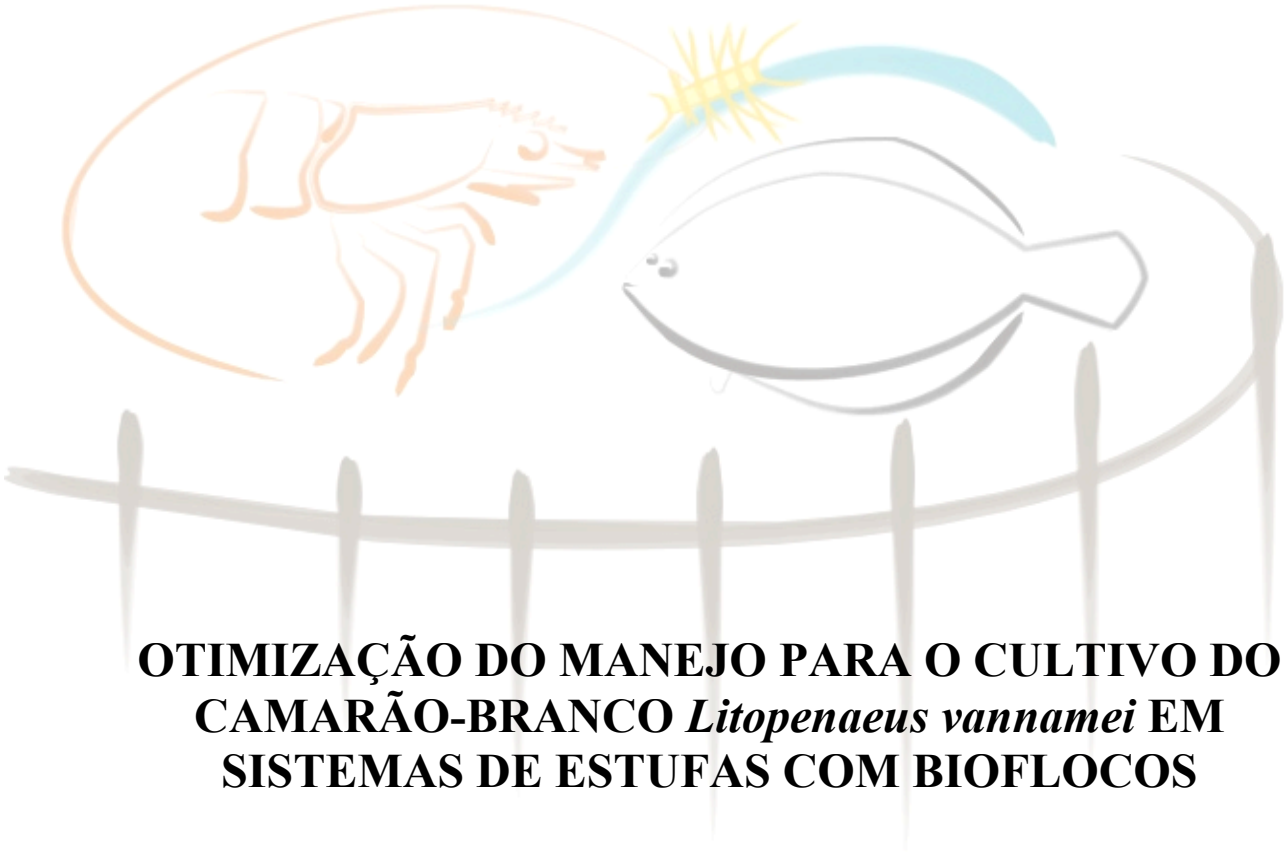

OTIMIZAÇÃO DO MANEJO PARA O CULTIVO DO CAMARÃO-BRANCO *Litopenaeus vannamei* EM SISTEMAS DE ESTUFAS COM BIOFLOCOS

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Tese de doutorado

OTIMIZAÇÃO DO MANEJO PARA O CULTIVO DO CAMARÃO-BRANCO *Litopenaeus vannamei* EM SISTEMAS DE ESTUFAS COM BIOFLOCOS.

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Tese apresentada ao Programa de Pós-graduação em Aquicultura da Universidade Federal do Rio Grande, como requisito parcial à obtenção do título de DOUTOR.

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ÍNDICE

"Filho é um ser que nos emprestaram para um curso intensivo de como amar alguém além de nós mesmos, de como mudar nossos piores defeitos para darmos os melhores exemplos e de aprendermos a ter coragem"

José Saramago

Dedico este trabalho aos meus filhos

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RESUMO GERAL

Nos últimos anos a aquacultura se direcionou para sistemas de cultivo que visam o aumento da produtividade e a redução da emissão de efluentes. Uma das estratégias que vem sendo testada é o cultivo de camarões marinhos em sistemas de bioflocos (BFT). Nesse sistema, o acúmulo dos nitrogenados é controlado através da manipulação da relação carbono/nitrogênio, que favorece o desenvolvimento de bactérias heterotróficas. Com isso, o nitrogênio amoniacal presente no cultivo é removido através da assimilação pela biomassa microbiana, gerando uma importante fonte de proteína para os camarões cultivados. Além disso, possibilita que o cultivo seja realizado sem renovação de água. Dentro deste contexto, o presente trabalho visa contribuir para o desenvolvimento do cultivo de *Litopenaeus vannamei* em sistema BFT, no sul do Brasil. Para isso, foram realizados cinco experimentos na Estação Marinha de Aquacultura (Instituto de Oceanografia, Universidade Federal do Rio Grande, RS, Brasil) e um experimento na Texas Agrilife Research (Texas A&M University, Corpus Christi, TX, EUA). Cada experimento realizado está descrito nos seguintes capítulos: (1) Efeito da reutilização de água com bioflocos com diferentes porcentagens em um novo ciclo de cultivo de camarões marinhos em sistema de bioflocos; (2) Efeito do reuso de diferentes níveis de bioflocos sobre o desempenho zootécnico e os parâmetros de qualidade da água em um sistema experimental, em alta densidade de estocagem e operado sem renovação de água; (3) Comparação do desempenho do crescimento de *L. vannamei* em sistema BFT realizado em estufas analisando o efeito de diferentes profundidades; (4) Uso de diferentes tipos de aeração em sistemas intensivos sem renovação de água: efeito na formação dos bioflocos, crescimento e sobrevivência de *L. vannamei*; (5) Análise da capacidade dos injetores de ar tipo "Taeration nozzles" no desempenho zootécnico e parâmetros de qualidade da água em um sistema super-intensivo de cultivo de *L. vannamei* e (6) Análise do uso de probiótico no cultivo de *L. vannamei* no sistema BFT contaminado com *Vibrio parahaemolyticus*. Para realização dos ensaios foram utilizadas uma estufa retangular com 9 tanques de 35.000 L (experimentos 3, 4 e 6), 15 caixas com volume útil de 800 L (experimentos 1 e 2) e uma estufa com dois tanques de 100.000 L (experimento 5). Os experimentos 1 e 2 evidenciaram as vantagens da utilização da água de despesca para um ciclo subsequente de cultivo em sistema BFT; no experimento 3, os camarões apresentaram melhor desempenho zootécnico quando foram cultivados nas profundidades 0,40 e 0,80 m; no capitulo 4, o difusor de ar (blower) apresentou o melhor resultado na formação dos agregados microbianos e no desempenho zootécnico dos camarões; no quinto experimento, os resultados comprovaram que o sistema de aeração "Taeration" pode ser utilizado como fonte de aeração nos sistemas BFT; no sexto capitulo foi possível observar a eficácia do uso de probióticos no controle de *Vibrio parahaemolyticus* presente no cultivo de *L. vannamei* em sistemas BFT. Assim, os resultados do presente estudo auxiliaram no desenvolvimento de um pacote tecnológico básico para a implementação do cultivo de *L. vannamei* em sistemas BFT, no sul do Brasil.

ABSTRACT

In the last years, the aquaculture systems have been focused in culture wich aim to increase the productivity and reduction of aquaculture wastes. Thus, the Biofloc Technology (BFT system) appears as a new production strategy. In these system, the nitrogen compounds is controlled by the manipulation of the carbon/nitrogen ratio in such a way as to promote the growth of heterotrophic bacteria. Therefore, the nitrogen is removed from the system through assimilation into microbial biomass, creating an important source of feed protein, reducing the cost of production; and also that culture can be reared without water exchange. In this way, the present study aims to development the culture of *Litopenaeus vannamei* in BFT system in Southern Brazil. Five trials were carried out at the Marine Station of Aquaculture (Institute of Oceanography, Federal University of Rio Grande – FURG, Brazil). An additional trial was carried out at the Texas Agrilife Research (Texas A&M University, Corpus Christi, TX, USA). Of these experiments are described in the following chapters: (1) Effect of different percentages of biofloc in a new cycle of culture in BFT; (2) Effect of reuse water on the Pacific white shrimp *Litopenaeus vannamei* under no water exchange; (3) Survival and growth of *Litopenaeus vannamei* reared in BFT system in different water depths; (4) Effects of different aerators on biofloc formation and its consequences on the growth and survival of *L. vannamei* raised in a BFT system; (5) Performance of the Pacific white shrimp *L. vannamei* in biofloc-dominated zero-exchange raceways using a non-venturi air injection system for aeration; and (6) The effect of probiotics in a *L. vannamei* biofloc technology culture system contaminated with *Vibrio parahaemolyticus*. For carrying out the trials were used a greenhouse with nine 35,000 L raceways (trials 2, 4 and 6), fifteen 800L tanks (trials 1 and 2) and a greenhouse with two 100,000 L raceways (trial 5). The results obtained proved the efficiency in the reuse of water from the harvest in a subsequent cycle of culture in BFT system (trials 1 and 2). In the trial 3, the results showed that higher yields are feasible when raceways are operated with low volume water. In the chapter 4, results indicate that different sources of aeration could affect the formation of bioflocs and abundance and types of microorganisms present in the water. The fifth experiment showed that the T-aeration could be used as a source of aeration in BFT system. The results from sixth trial showed that the multi-strain probiotic tested in this study controlled *V. parahaemolyticus* and improved the overall productivity in a BFT culture system. Thus, the results from the present study showed the possibility of the development of the culture of *L. vannamei* in BFT system in Southern Brazil.

Introdução Geral

A escassez da oferta de alimento de origem aquática gerou um crescimento considerável na produção mundial de camarões nas últimas quatro décadas, principalmente nos países em desenvolvimento (Subasinghe 2005; Gutierrez-Wing & Malone 2006). Entretanto, as limitações ambientais e econômicas podem dificultar esse crescimento. Efluentes aquícolas são responsabilizados pela poluição da água por um excesso de materiais orgânicos e nutrientes que são passíveis de causar efeitos tóxicos agudos aos animais aquáticos e de longo prazo ao meio ambiente. Estas descargas contem vários compostos orgânicos e inorgânicos, principalmente amônia, fósforo e carbono orgânico dissolvido (McIntosh et al. 2001; Jackson et al. 2003; Cohen et al. 2005). Outro aspecto importante é que a maioria dessas fazendas de cultivo de camarões estão intensificando a produção, o que aumenta a pressão sobre o ambiente. Os altos níveis de nutrientes gerados causam deterioração ambiental dos corpos d'água receptores. Além disso, a água drenada pode aumentar a ocorrência de microrganismos patogênicos e introduzi-los nos cultivos (Naylor et al. 2000; Piedrahita, 2003). Como resultado temos o surgimento de patógenos virais e bacterianos que geraram um efeito profundo sobre os camarões peneídeos cultivados. Pandemias devido a vírus como o da mancha branca (WSSV), síndrome de taura (TSV), vírus da cabeça amarela (YHV) e necrose hipodérmica hematopoiética e infecciosa (IHHNV) custaram bilhões de dólares em despescas e empregos perdidos, com queda nas receitas de exportação, principalmente em países nos quais a carcinocultura representa grande importância na economia (Thompson et al. 2002; Boyd, 2003; Hargreaves, 2006; Lightner, 2005; Sugiura et al. 2006). De acordo com Lotz e Lightner (2000), a principal via de acesso de agentes patógenos nos sistemas aquícolas é através da entrada de água bombeada diretamente do ambiente.

Para manter a qualidade da água dentro dos parâmetros aceitáveis para o crescimento e sobrevivência dos camarões, os sistemas tradicionais de cultivo em viveiros possuem altas taxas de renovação, variando de 10 a 15%, podendo atingir até milhares de metros cúbicos por dia (Hopkins et al. 1993; Wang, 2003; Hargreaves, 2006). Entretanto, pesquisas indicam que as trocas de água se mostram ineficazes como meio de controlar a qualidade da água dos cultivos (Sandifer & Hopkins 1996; Hopkins et al. 1993; Browdy et al. 2001). Assim, preocupações relacionadas principalmente à biossegurança e ao impacto ambiental gerado pelos efluentes, foram iniciadas pesquisas visando reduzir as taxas de renovação e tornar mais conservador e racional o uso da água, numa aquicultura ambientalmente correta. A partir desta nova visão, surgiram os cultivos com mínima ou nenhuma renovação de água em meio a Bioflocos, recentemente denominado sistema BFT (Biofloc Technology System - BFT). Nos meados dos anos 90, dois centros de pesquisas iniciaram simultaneamente estudos com essa modalidadede cultivo; nos Estados Unidos, os estudos foram liderados por Hopkins e colaboradores no Waddel Mariculture Center, na Carolina do Sul, e em Israel, por Avnimelech e colaboradores (Sandifer et al. 1991; Hopkins et. al. 1993; Avnimelech 1993, 1994). Após o sucesso nas pesquisas em pequena escala, esta tecnologia foi modificada e adaptada para produções comerciais em Belize, América Central e também nos Estados Unidos (Mcintosh 2000; Boyd & Clay 2002; Burford et al. 2003). Em 2005, na Universidade Federal do Rio Grande – FURG, Wasielesky e colaboradores, iniciaram as primeiras pesquisas em sistema BFT no Brasil (Wasielesky et al. 2006; Emerenciano et al. 2007; Krummenauer et al. 2011).

Um elemento chave da gestão da qualidade da água nos cultivos de camarões é o controle de resíduos nitrogenados: amônia, nitrito e nitrato (Kuhn et al. 2010). Grande parte da entrada de nitrogênio nos cultivos entra na forma de amônio ($NH4^{+}$) que é formado a partir do

alimento fornecido que não é convertido em tecido pelos camarões. Outra parcela da amônia entra no sistema através das excretas dos animais cultivados. Em muitos casos, os efluentes desses cultivos são lançados diretamente no meio ambiente sem a remoção prévia do excesso de nutrientes e outras partículas (Thakur & Lin 2003; Cohen et al. 2005; Maillard et al. 2005; Avnimelech 2009).

Segundo Avnimelech (2009) existem quatro maneiras de controle do acúmulo de nitrogênio orgânico nos sistemas aquícolas: (1) através das renovações de água, como ocorre principalmente em sistemas convencionais de cultivo, sendo a maneira mais simples para eliminar os nitrogenados, resultando na deterioração do ecossistema, prática condenada pelas novas regulações ambientais inviabilizando, cada vez mais, a prática da carcinocultura (Naylor et al. 1998; Burford et al. 2003); (2) através de bactérias heterotróficas e processos fotoautotróficos em que a biomassa das microalgas assimilam amônia, mecanismo limitado pela instabilidade da atividade das microalgas e também pela taxa de assimilação do carbono, abaixo das necessidades requeridas para imobilização do nitrogênio (Timmons et al. 2002); (3) através dos processos de nitrificação, estratégia em que os nitrogenados são controlados através de biorreatores que convertem nitrogênio amoniacal em nitrato através de bactérias oxidantes da amônia (AOB) e bactérias oxidantes de nitrito (NOB) (Ebeling et al. 2006; Crab et al. 2007), e (4) controle dos nitrogenados através do uso de bioflocos no sistema conhecido como BFT. Neste sistema, a acumulação da amônia é controlada através da manipulação da relação carbono/nitrogênio que promove o crescimento de bactérias heterotróficas. Estas bactérias somadas com outros microorganismos formam uma espécie de biomassa bacteriana chamada de flocos microbianos (bioflocos) que se apresentam na forma de agregados. Desta forma os

nutrientes podem ser facilmente absorvidos (Burford et al. 2004; Azim & Little 2008; Avnimelech 2009; Crab et al. 2010).

Os agregados (bioflocos) servem de suplemento alimentar aos animais, sendo uma importante fonte de proteína para os camarões, e possibilitam a utilização de ração com menores teores de proteína bruta, além de permitirem um melhor aproveitamento dos nutrientes originários da ração não consumida pelos camarões (Burford et al. 2003; Ebeling et al. 2006; Wasielesky et al. 2006; Avnimelech, 2007; Ballester et al. 2010).

Uma vantagem que o sistema BFT possui é a possibilidade da utilização da mesma água por diversos ciclos de cultivo. Segundo McIntosh (2000), a comunidade microbiana pode demorar até seis semanas para se desenvolver adequadamente em um ciclo de cultivo. Com a estratégia do reuso da água em um ciclo subsequente podem-se estabilizar as comunidades microbianas e os parâmetros de qualidade da água mais rapidamente, encurtando o período de cultivo e minimizando possíveis problemas com nitrogenados (McAbee et al, 2003; Samocha et al. 2010).

Os sistemas de cultivos convencionais requerem grandes volumes de água, sendo necessárias de 20 a 64 metros cúbicos de água para produzir 1 kg de camarões (Hopkins et al. 1993; Timmons & Losordo, 1994). Por sua vez, os cultivos em bioflocos podem ser realizados apenas com 1% deste volume de água. Por exemplo, Samocha et al. (2010) registraram a produção de 9,50 kg/m^3, ou seja, utilizando apenas 98 litros de água para produzir 1 kg de camarões.

Outro aspecto importante é a profundidade dos tanques de cultivo. Geralmente os cultivos são realizados em profundidades que variam de 0,40 a 1,20 m (Otoshi et al. 2009; Samocha et al. 2010; Krummenauer et al. 2011), a profundidade possui grande relevância em

sistemas autotróficos e heterotróficos, entretanto, existem poucos estudos e não existe um consenso entre os pesquisadores de qual a profundidade que possui a melhor relação custo beneficio para o sistema BFT.

A geração de altas concentrações de biomassa bacteriana e camarões requerem aeração suplementar para manter os níveis adequados de oxigênio dissolvido a qual também auxilia nos processos de nitrificação (Hopkins et al. 1993; Ebeling et al. 2006). Para isto, os aeradores devem funcionar continuamente, suprindo a demanda de oxigênio na água dos tanques e viveiros (Rogers, 1989; Avnimelech et al. 1992; Howerton et al. 1993). Diversos modelos de aeradores podem ser utilizados para esta suplementação de oxigênio, entre eles os aeradores de pás (paddlewheels), utilizados em viveiros revestidos com geomembrana, os quais, além da oxigenação, evitam o acumulo de matéria orgânica e quebram a estratificação da coluna do viveiro (Losordo & Piedrahita, 1991; Fast & Lannan, 1992; Avnimelech & Ritvo, 2001). Os cultivos de camarões realizados em raceways geralmente utilizam como fonte de aeração o sistema de ar difuso, injetando ar no sistema através de um soprador comumente chamado de "blower" (Browdy et al. 2001). Estudos mais recentes reportam a utilização de oxigênio líquido como fonte de oxigênio em cultivos em raceways, com isso, a produtividade pode variar de 5 a 10 kg de camarão/m^3 (McAbee et al. 2003; Otoshi et al. 2009; Samocha et al. 2010). Embora a utilização de oxigênio suplementar proporcione altas produtividades de camarões, a adoção desta estratégia no sistema de cultivo exige altos investimentos tecnológicos e ainda está distante da realidade brasileira. Dentro deste contexto, torna-se de grande importância identificar o tipo de aerador que possibilita um melhor desempenho na formação dos agregados e se reflita no desempenho zootécnico dos camarões.

Com relação às enfermidades nos cultivos de camarões, uma alternativa proposta com relativo sucesso é o uso de probióticos (Gildberg et al. 1997; Gram et al. 1999). O termo probiótico geralmente é usado para especificar bactérias que promovem a saúde de outros organismos hospedeiros (Lilley & Stillwell, 1965). Fuller (1989) define os probióticos como microrganismos vivos suplementados ao alimento que afetam beneficamente o hospedeiro melhorando seu balanço intestinal. Balcázar et al. (2006) citam cinco mecanismos de atuação dos probióticos: exclusão competitiva de bactérias patogênicas, fonte de nutrientes, contribuição enzimática para a digestão, influência sobre a qualidade da água, melhora da resposta imune e efeitos antivirais. Por outro lado, nos cultivos de camarões doenças causadas por bactérias do gênero *Vibrio* afetam o crescimento e podem causar mortalidade, resultando em uma queda significativa na produtividade (Lightner 1988; Jayasree et al. 2006). Uma alternativa no combate de vibrioses é o uso de antibióticos, entretanto, o seu uso em cultivos de camarões pode levar ao desenvolvimento de cepas resistentes (Jayasree et al. 2006). Além disso, nos cultivos BFT os antibióticos matariam toda a comunidade bacteriana presente, a qual também possui efeito benéfico no combate a vibriosoes. (Defoirdt et al. 2007; Crab et al. 2010). Assim, o uso de probioticos em sistemas BFT pode ser uma importante ferramenta no controle de possíveis patógenos, evitando-se a necessidade de antibióticos.

Estima-se que com os resultados obtidos no presente estudo, venha a ser elaborado um pacote tecnológico básico para o sistema BFT e que seja uma alternativa viável a ser aplicada por produtores das diferentes regiões do Brasil.

Objetivos

O presente trabalho visa contribuir para o desenvolvimento do cultivo do camarão marinho *Litopenaeus vannamei* em sistemas de bioflocos no Brasil.

Objetivos específicos

- Analisar o efeito da reutilização de água com bioflocos em novo ciclo de cultivo de *L. vannamei* em sistema BFT;
- Avaliar o efeito do reuso de diferentes níveis de bioflocos sobre o desempenho zootécnico e os parâmetros de qualidade da água em um sistema experimental, operado sem renovação de água;
- Comparar o desempenho de crescimento de *L. vannamei* em sistema BFT realizado em estufas, em diferentes profundidades de coluna d'água;
- Comparar diferentes tipos de aeração em sistema BFT, sem renovação de água, avaliando seus efeitos na formação dos bioflocos, no crescimento e na sobrevivência de *L. vannamei.*
- Analisar a capacidade dos injetores de ar tipo "Taeration nozzles" promoverem melhor desempenho zootécnico e influenciarem os parâmetros de qualidade da água em sistemas super-intensivos de cultivo de *L. vannamei*.
- Avaliar o efeito do uso de probiótico no cultivo de *Litopenaeus vannamei* em sistema BFT contaminado com *Vibrio parahaemolyticus.*

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CAPÍTULO I

CULTIVO DE CAMARÕES MARINHOS EM SISTEMA DE BIOFLOCOS: ANÁLISE DA REUTILIZAÇÃO DA ÁGUA

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RESUMO

A necessidade de redução dos impactos ambientais gerados pelo descarte de efluentes ricos em nutrientes e matéria orgânica é fundamental para a aquacultura. O sistema BFT (Biofloc Tecnology System) surge como uma alternativa aos sistemas convencionais, para minimizar a emissão de efluentes. Da mesma forma, fazem-se necessários estudos relativos ao reaproveitamento da água e da comunidade microbiana do cultivo em cultivos subsequentes. O presente estudo teve como objetivo analisar o uso de diferentes porcentagens de reutilização da água de cultivo do camarão branco *Litopenaeus vannamei.* O experimento teve duração de 82 dias, os camarões foram estocados em 12 tanques com volume útil de 800 L (625 camarões / m^{-2}), foram testados quatro tratamentos (água clara (0), 2,5, 10 e 100% de reutilização de água). Parâmetros de qualidade da água foram monitorados diariamente (temperatura; oxigênio dissolvido; pH; e amônia). Semanalmente foram monitorados nitrito, nitrato, fosfato, salinidade, turbidez, transparência da água e material particulado em suspensão. Possíveis variações de amônia foram controladas através da fertilização orgânica utilizando dextrose como fonte de carbono. A cada 15 dias foram realizadas biometrias, para avaliar o crescimento dos camarões. O tratamento sem reutilização de água apresentou mortalidade total no 63° dia, nos tratamentos com 2,5, 10 e 100% de reutilização a sobrevivência foi de 66,5, 81,4 e 80,7% respectivamente, o peso médio final foi 2,91, 3,25 e 3,46g nos tratamentos de 2,5, 10 e 100%. Os resultados evidenciaram a possibilidade de se utilizar inóculos de bioflocos nos cultivos BFT. Além disso, o tratamento com 100% de reutilização de água apresentou os melhores resultados para desempenho dos camarões e qualidade de água (p<0,05).

Palavras-chave: Sistemas BFT, impactos ambientais, reutilização da água, inóculos, estabilização microbiana.

ABSTRACT

The need to reduce environmental impacts generated by the discharge of wastewater rich in nutrients and organic matter is of great importance in Biofloc Culture Systems due the procedures for harvest, which may contain high concentrations of nitrogen compounds. In the same way, it is necessary to recycle the microbial community structure of the cultures. The present study aimed at using different percentages of water reuse in the culture of white shrimp *Litopenaeus vannamei* in BFT systems. The experiment lasted 82 days, shrimp were stocked in 12 tanks with a volume of 800 L (625 shrimp / m^{-2}) and was carried out with four treatments: clear water (0), 2.5, 10 and 100% of water reuse. Potential variations of ammonia concentrations were controlled by organic fertilization using dextrose as a carbon source. Water quality parameters were monitored daily (temperature, dissolved oxygen, pH and ammonia). Weekly, water samples are collected to check nitrite, nitrate, phosphate, salinity, turbidity, water transparency and suspended particulate matter. Fortnightly, shrimp samples were weigh to estimate the growth of shrimps. In the treatment without reuse of water presented total mortality at 63 days. In the treatments with 2.5, 10 and 100% of reuse the survival was 66.5, 81.4 and 80.7% respectively. The final mean weight was 2.91, 3.25 and 3.46 g in treatments of 2.5, 10 and 100%.The results demonstrated the potential use of inoculums of bioflocs in BFT cultures. Furthermore, the treatment with 100% of water reuse presented high performance of shrimps and water quality.

Key-words: BFT System, environmental impacts, water's reuse, inoculums, microbial stabilization.

INTRODUÇÃO

O desenvolvimento descontrolado do setor aquícola pode provocar alguns problemas ambientais, como destruição de mangues e de vegetações costeiras, disseminação de doenças associadas ao cultivo (na maioria das vezes por manejo inadequado), introdução de espécies exóticas e geração de efluentes com alta concentração de nutrientes e matéria orgânica (Primavera 2006). Portanto, faz-se necessária a adoção de sistemas de cultivo nos quais a renovação de água seja minimizada, reduzindo o uso dos recursos hídricos, a emissão de efluentes e a transmissão de doenças, culminando em uma atividade ambientalmente amigável. Entre as novas tecnologias de cultivo que estão sendo desenvolvidas em nível mundial destaca-se o cultivo de camarões em meio heterotrófico ou também chamado de sistema "BFT" (Biofloc Technology System ou Sistema de Bioflocos).

Os bioflocos são agregados de microorganismos (bactérias, fitoplâncton e zooplâncton), associados a partículas, colóides, polímeros orgânicos e células mortas (Forster 1976). As bactérias heterotróficas atuam na formação dos bioflocos, utilizando o nitrogênio inorgânico da água e carbono dissolvido para produção de biomassa bacteriana. Nos sistemas BFT a agregação é induzida através da manipulação da relação de carbono:nitrogênio do cultivo, mantendo essa relação entre 15 e 20:1 (Avnimelech 1999, 2009).

Além de melhorar a qualidade da água, os agregados microbianos servem como complemento na dieta dos organismos, permitindo assim uma redução na quantidade de proteína bruta utilizada na ração (Samocha et al. 2004) e até mesmo a redução da ração utilizada.

O sistema de bioflocos também possibilita uma maior biossegurança, uma vez que, com a redução de troca de água, reduz-se também a possibilidade de introdução de doenças no

sistema (Wasielesky et al. 2006) e a salinização de corpos de água (Boyd 2003). Estudos realizados com o camarão-branco *Litopenaeus vannamei* em tecnologia de bioflocos, apontam para a adoção de altas densidades de estocagem, possibilitando assim o uso de menores áreas para o emprego desta atividade. Além disso, devido às menores taxas de renovação de água, há uma menor necessidade de uso de água, comparando-se com os cultivos tradicionais (Wasielesky et al. 2006).

Porém, existe ainda, principalmente durante a despesca, a liberação de efluentes com altas taxas de nutrientes e matéria orgânica. Além disso, há um período de tempo necessário para a maturação dos bioflocos em um novo cultivo, o que ocorre entre o início do cultivo e a estabilização da comunidade microbiana na água (Suita 2009). Logo, a utilização da água de um ciclo de produção anterior para formação dos bioflocos nos cultivos subsequentes pode encurtar o tempo para o processo de estabilização da comunidade microbiana (Mcabee et al 2003). O presente estudo visa analisar a possibilidade do reaproveitamento da água em sistemas de produção superintensiva de *L. vannamei*, assim como avaliar a utilização de inóculos na formação de bioflocos, contribuindo, dessa forma, para a busca de sustentabilidade ambiental e econômica para a tecnologia de bioflocos.

Assim, o presente trabalho teve como objetivo analisar o efeito de diferentes porcentagens de reutilização da água (efluentes) para produção de camarões marinhos em sistema de bioflocos.

2 MATERIAIS E MÉTODOS

2.1 Delineamento experimental

O experimento foi realizado na Estação Marinha de Aquicultura (EMA), do Instituto de Oceanografia da Universidade Federal do Rio Grande – FURG, durante 82 dias utilizando quinze unidades experimentais com volume útil de 800 L com área de fundo de 1,4 m^2. Em cada unidade experimental foi montado um sistema de aeração abastecido por um soprador de ar (7 hp). A densidade de estocagem de camarões em cada tanque foi de 625 indivíduos/m^3, sendo que, durante o experimento, os camarões foram alimentados com ração comercial com 38% de proteína bruta.

Ao total foram utilizados cinco tratamentos com três repetições. O tratamento controle consistiu de tanques com 0% de reutilização de água e os demais tratamentos compostos por diferentes percentuais (2,5%, 10%, e 100%) de reutilização de água de um cultivo prévio do *L. vannamei* produzido em sistema de bioflocos.

Para estimular a formação dos agregados microbianos, no tratamento controle, foram inoculadas diatomáceas *Thalassiosira weissflogii*. Após a inoculação de microalga foi acrescentado uma fonte de carbono (dextrose) seguindo a metodologia proposta por Avnimelech (1999) e Ebeling et al. (2006), onde se mantinha uma relação C/N entre 15 e 20:1, juntamente com aplicações de farelo de trigo como substrato para fixação dos microorganismos. Além disso, aplicações de probiótico comercial (INVE®) foram realizadas semanalmente a fim de auxiliar na manutenção da qualidade da água em todos os tratamentos. Os parâmetros físicos e químicos monitorados diariamente foram: temperatura e oxigênio dissolvido (medidos com um oxímetro YSI® 55), pH (utilizando um pHmetro YSI® 100) e concentração de amônia (UNESCO 1983). Semanalmente foram analisadas as concentrações de nitrito, nitrato e fosfato,

utilizando a metodologia de Strickland & Parsons (1972), salinidade (utilizando um refratômetro), turbidez (através de um turbidímetro Hach® 2100P), transparência da água (mensurada com a utilização de um disco de Secchi) e material particulado em suspensão (Strickland & Parsons 1972).

2.2 Desempenho zootécnico dos camarões

Para avaliar o ganho de peso dos camarões, foram realizadas biometrias em intervalos de quinze dias ao longo do período experimental, quando 60 camarões eram amostrados, pesados e repostos às respectivas unidades experimentais. Para análise da sobrevivência e produção de biomassa, foi realizada a contagem total dos indivíduos bem como uma biometria total ao término do estudo. A taxa de conversão alimentar aparente (TCA) foi determinada de acordo com a fórmula:

$$\mathbf{TCA = RF/(B_f - B_i)}$$

Onde: RF=quantidade de ração fornecida ao longo de todo o período experimental, B_f=Biomassa final, B_i=Biomassa inicial.

2.3 Análise Estatística

Foram realizados testes estatísticos para comparar a sobrevivência e crescimento dos camarões nos diferentes tratamentos. Depois de verificadas a homocedasticidade das variâncias e a normalidade da distribuição dos dados foi aplicada ANOVA de uma via ($\alpha = 0,05$). Quando detectadas diferenças significativas foi aplicado teste de Tukey HSD (Sokal & Rohlf 1969).

3 RESULTADOS

3.1 Parâmetros físicos e químicos

Os resultados dos parâmetros físicos e químicos de qualidade de água observados nos tratamentos ao longo do estudo estão apresentados na tabela 1. É importante ressaltar que o tratamento sem reutilização de água teve duração de 63 dias quando ocorreu mortalidade total dos animais.

Tabela 1 – Médias e desvios padrões dos parâmetros físicos e químicos ao longo do período experimental nos diferentes tratamentos.

Tratamentos (Porcentagem de reutilização)	**0%**	**2,5%**	**10%**	**100%**
Temperatura (°C)	24,38±1,91 [a]	25,18±2,09 [a]	24,96±2,06 [a]	25,05±2,04 [a]
Oxigênio dissolvido (mg/L)	5,49±0,98 [a]	5,28±1,02 [a]	5,18±0,97 [a]	5,47±0,92 [a]
pH	7,66±0,42 [a]	7,59±0,33 [a]	7,64±0,31 [a]	7,62±0,33 [a]
Amônia (mg/L)	0,63±0,71 [a]	0,25±0,38 [a]	0,29±0,41 [a]	0,09±0,18 [a]
NO_2 (mg/L)	11,43±15,46 [a]	0,87±2,02 [b]	0,60±2,21 [b]	0,09±0,14 [b]
NO_3 (mg/L)	8,29±10,96 [a]	29,78±28,66 [b]	29,45±28,43 [b]	36,70±24,60 [b]
PO_4 (mg/L)	1,07±0,60 [a]	0,87±0,97 [a]	0,69±0,53 [a]	0,66±0,59 [a]
Alcalinidade (mg/L)	149,79±46,78 [a]	145,83±53,37 [a]	158,33±44,09 [a]	132,5±40,13 [a]
Salinidade	28,45±2,48 [a]	29,07±2,53 [a]	29,87±2,47 [a]	29,78±2,60 [a]
Transparência da água (cm)	29,82±18,24 [a]	23,19±19,19 [a]	22±17,33 [a]	9,77±4,11 [b]
Turbidez (NTU)	135±129 [a]	335±236 [b]	348±197 [b]	371±170 [b]
Sólidos em suspensão (mg/L)	535,71±453,53 [a]	1100,37±988,52 [b]	1152,22±965,10 [b]	1153,78±787,51 [b]

A temperatura média foi de 24,92°C e não foram encontradas variações entre os tratamentos (P>0,05). Para o oxigênio dissolvido foi observada uma tendência de queda ao longo do período experimental para todos os tratamentos variando as médias entre 5,5 e 7,5 mg/L no início do experimento e 3,0 e 5,5 mg/L ao final do período experimental. As quedas

mais significativas foram observadas no tratamento sem reutilização de água ao final do experimento.

O pH não diferiu estatisticamente ($p>0,05$) entre os tratamentos com reutilização de água ao longo do período experimental, apresentando valores entre aproximadamente 7 e 8,5. Exceção foi observada no tratamento com 0% de reutilização de água, que apresentou uma queda significativa (6,7) ($P<0,05$) precedendo o 63º dia experimental (Figura 1).

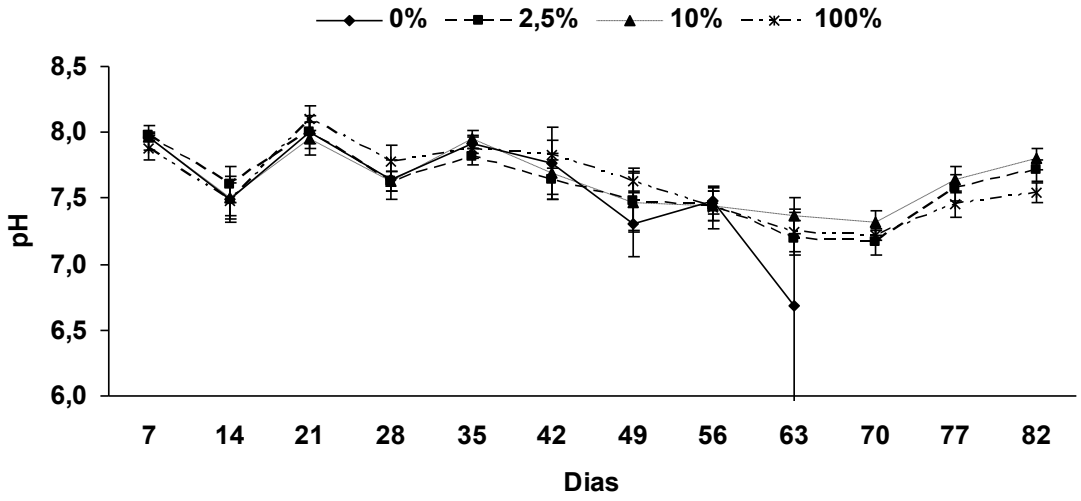

Figura 1 – Variação do pH ao longo do período experimental nos diferentes tratamentos no cultivo de *Litopenaeus vannamei* em sistema BFT com reutilização de água.

Variações significativas foram observadas entre os tratamentos com relação à amônia (Figura 2). Observa-se que o tratamento com 0% de reutilização de água apresentou maiores médias, chegando a pouco mais de 2 mg/L. Por outro lado, o tratamento com 100% de reutilização de água apresentou concentrações menores que 0,5 mg/L em quase todo o período experimental.

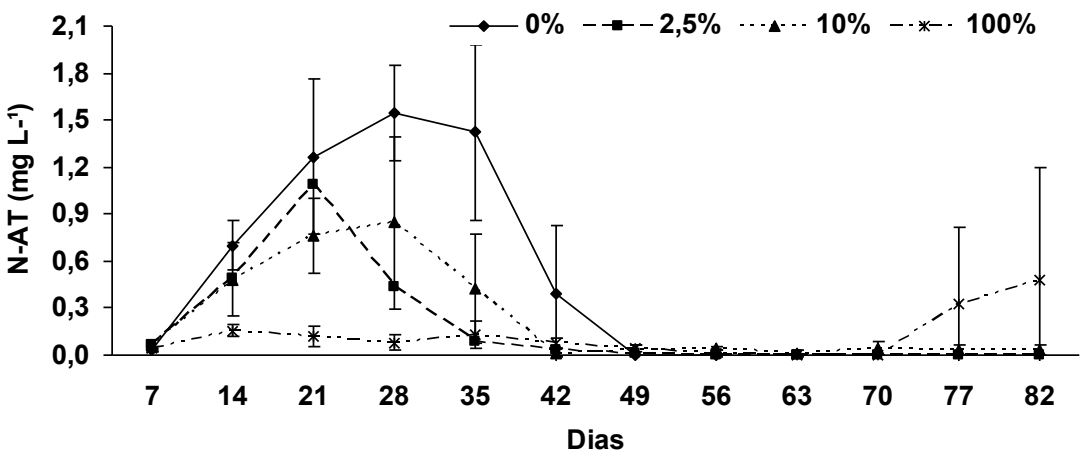

Figura 2 – Variação na concentração de nitrogênio amoniacal ao longo do período experimental nos diferentes tratamentos no cultivo de *Litopenaeus vannamei* em sistema BFT com reutilização de água.

Na figura 3, pode-se observar uma diferença expressiva (P<0,05) entre as concentrações de nitrito no tratamento com 0% de reutilização de água e os restantes tratamentos. Sem a reutilização de água as concentrações alcançaram 35 mg/L, a partir dos 30 dias de experimento, enquanto que nos demais tratamentos as concentrações mantiveram-se inferiores a 8 mg/L.

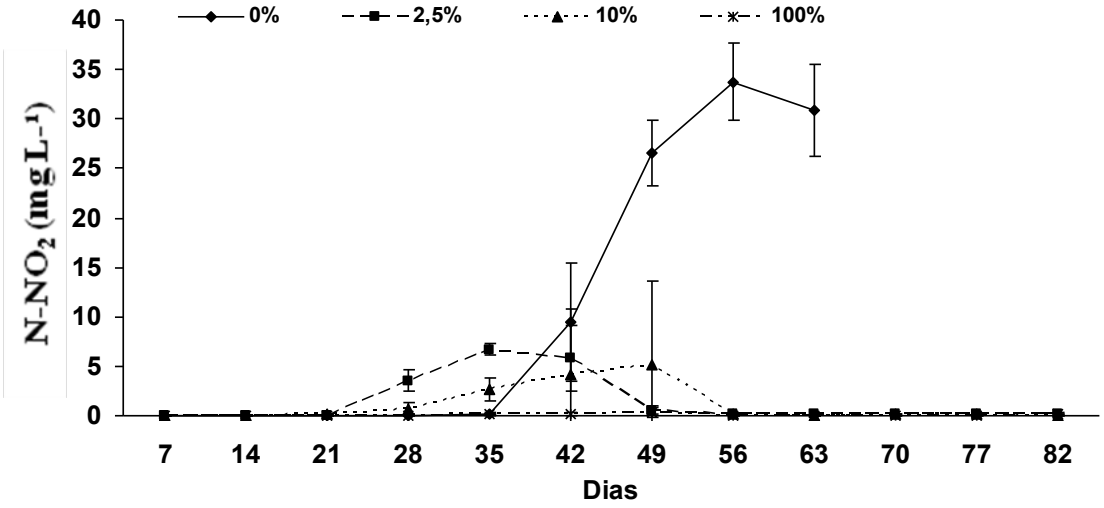

Figura 3 – Variação na concentração de nitrito ao longo do período experimental nos diferentes tratamentos no cultivo de *Litopenaeus vannamei* em sistema BFT com reutilização de água.

As concentrações de nitrato apresentaram elevação ao longo do experimento, sem que diferenças significativas fossem observadas (P>0,05). Ao final do período experimental se observaram concentrações médias oscilando próximas a 80 mg/L para os diferentes tratamentos testados (figura 4).

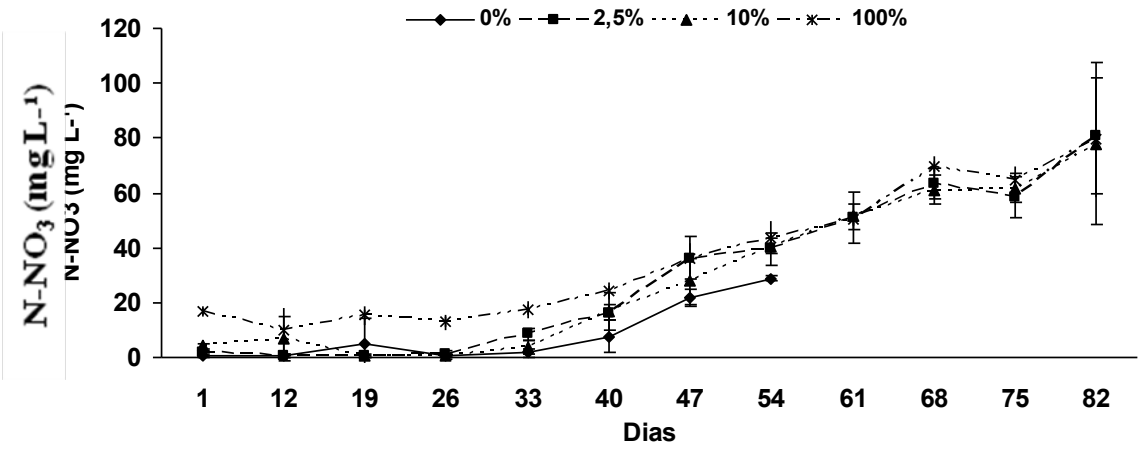

Figura 4 – Variação na concentração de nitrato ao longo do período experimental nos diferentes tratamentos no cultivo de *Litopenaeus vannamei* em sistema BFT com reutilização de água.

A alcalinidade apresentou tendências uniformes nos diferentes tratamentos (*P*>0,05), sendo que os valores mínimos registrados apareceram no tratamento com 100% de reutilização de água, com valores próximos de 75 mg/L. Os valores máximos foram registrados ao final do experimento para o tratamento com 2,5% de reutilização de água com concentração próxima a 240 mg/L.

Os valores de turbidez foram maiores (*P*<0,05) no tratamento com 100% de reutilização de água no início do experimento (figura 5).

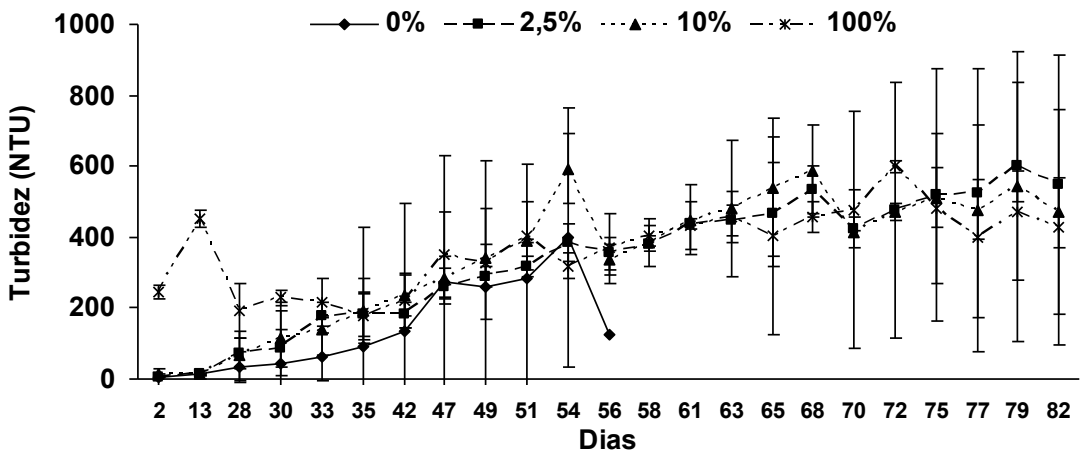

Figura 5 – Variação na turbidez ao longo do período experimental nos diferentes tratamentos no cultivo de *Litopenaeus vannamei* em sistema BFT com reutilização de água.

Os sólidos em suspensos totais (SST) (figura 6) percebem-se uma tendência de aumento nas concentrações semelhantemente em todos os tratamentos.

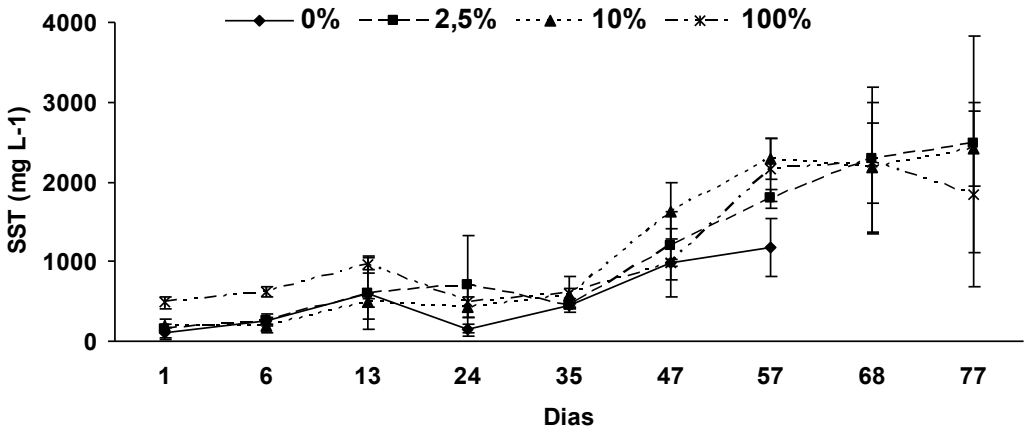

Figura 6 – Variação na concentração de sólidos totais em suspensão ao longo do período experimental nos diferentes tratamentos no cultivo de *Litopenaeus vannamei* em sistema BFT com reutilização de água.

3.2 Desempenho dos camarões

Os índices de desempenho zootécnico dos camarões cultivados estão apresentados na tabela 2.

Tabela 2 – Média do desempenho zootécnico dos camarões ao longo do período experimental nos diferentes tratamentos.

Tratamentos (Porcentagem de reutilização)	**0%**	**2,5%**	**10%**	**100%**
Peso inicial (g)	0,008±0,003	0,008±0,003	0,008±0,003	0,008±0,003
Peso final (g)		2,91±1,09[a]	3,25±1,40[b]	3,46±1,26[b]
Sobrevivência (%)	0	66,5±11,1[a]	81,4±10,3[a]	80,73±13,6[a]
Biomassa final (g)	0	969,24±99,2[a]	1325,19±160.1[b]	1396,63±143,2[b]
TCA	-	2,89±0,23[a]	2,09±0.54[b]	2,13±0,49[b]
Crescimento semanal (g)	-	0,25±0,02[a]	0,28±0,07[a]	0,29±0,05[a]

*O tratamento sem reutilização de água apresentou mortalidade total após 63 dias de experimento.

As sobrevivências dos camarões no experimento não apresentaram diferenças estatísticas entre os tratamentos com 2,5%, 10% e 100% de reutilização de água. No tratamento com 0% de reutilização de água, não foi verificada sobrevivência, uma vez que no dia 63 do período experimental se observou a mortalidade total dos camarões deste tratamento.

Na figura 7, constata-se, para todos os tratamentos, que os camarões tiveram um crescimento maior a partir 28º dia do período experimental. Os maiores pesos estiveram sempre relacionados com o tratamento com 100% de reutilização da água, chegando a um peso médio de 3,46 g, uma vez que a partir do 15º dia já foram observadas diferenças estatísticas significativas em relação aos outros tratamentos.

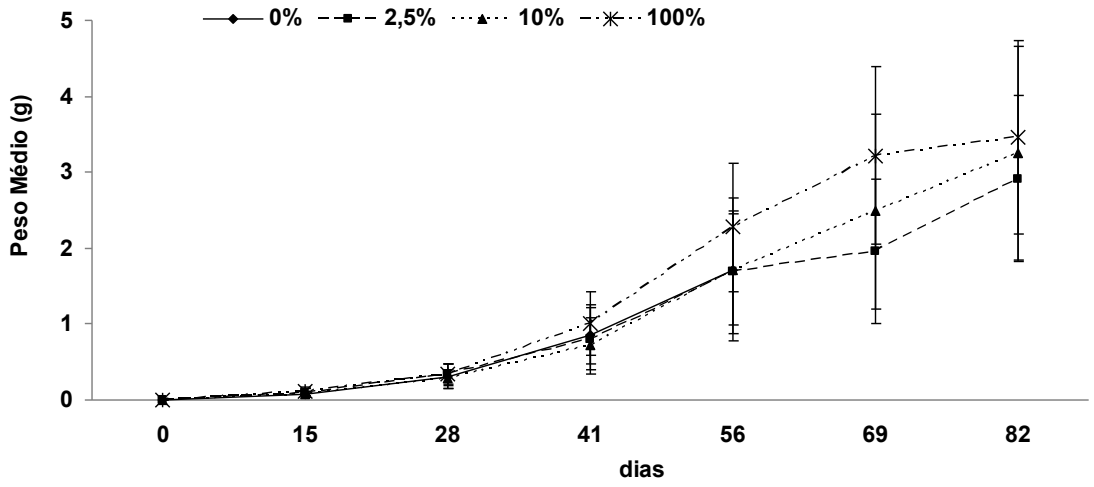

Figura 7 – Peso médio dos camarões ao longo do período experimental nos diferentes tratamentos no cultivo de *Litopenaeus vannamei* em sistema BFT com reutilização de água.

4 DISCUSSÃO

O reutilização de água na aquicultura pode resultar no aumento da concentração de alguns compostos, como nitrogenados e fosfatados, no sistema de produção (Colt 2006). No presente estudo observa-se exatamente este efeito, com aumento inicial das concentrações de amônia seguido de aumento de nitrito e de nitrato. Da mesma forma pode-se observar aumento nas concentrações de sólidos em suspensão, turbidez e alcalinidade, porém sem efeitos de mortalidade dos camarões cultivados ao longo do tempo, mantendo-se dentro do intervalo ideal para crescimento da espécie em todos os tratamentos (Van Wyk & Scarpa 1999).

Lin & Chen (2001) indicam que o nível de segurança da amônia para juvenis de *L. vannamei* em salinidade 25 é de 3,55 mg/L N-AT. No presente estudo, nenhum dos tratamentos atingiu valores de concentração de amônia que pudessem estar relacionados a uma menor sobrevivência dos camarões. As concentrações de nitrito registradas no presente estudo foram inferiores a 10 mg/L para os tratamentos com reutilização de água. No tratamento com 0% de reutilização, os valores médios observados foram acima de 25,7 mg/l que são os valores

máximos recomendados por Lin & Chen (2003). Deste modo, a mortalidade observada no tratamento sem reutilização de água provavelmente foi decorrente das altas concentrações de nitrito neste tratamento.

Van Rijn et al. (2006) afirmam que ao contrário da amônia e nitrito, o nitrato é pouco tóxico aos organismos aquáticos. Segundo Kuhn et al. (2010), concentrações inferiores a 220 mg/L de nitrato não afetam a sobrevivência, o crescimento e a biomassa de *L. vannamei*. No presente estudo, as concentrações médias de nitrato dissolvido na água foram inferiores aos reportados como letais pelos autores acima citados.

Segundo Godoy (2008), a matéria particulada na coluna d'água consiste de organismos vivos, partículas inorgânicas e detritos. Sua agregação é um processo complexo e envolve interações físicas, químicas e biológicas entre as partículas. A partir dessa informação, pode-se concluir que deve ocorrer um aumento da concentração de sólidos em suspensão, turbidez e diminuição da transparência da água devido a uma maior concentração da comunidade microbiana no cultivo.

Cohen et al. (2005), afirma que as bactérias heterotróficas assimilam os produtos nitrogenados do sistema e convertem em proteína microbiana, sendo consumidas pelos camarões, reduzindo a conversão alimentar aparente. Este fato não foi observado no presente trabalho, onde foram registradas taxas de conversão alimentar relativamente altas quando comparadas com outros trabalhos em sistema BFT (Krummenauer et al. 2011).

Com relação à temperatura, ao oxigênio dissolvido e ao pH, estes podem ter afetado o crescimento e sobrevivência dos camarões. Segundo Ponce-Palafox et al. (1997) em temperaturas abaixo de 25ºC os camarões são relativamente inativos diminuindo o consumo alimentar e consequentemente reduzindo seu crescimento. Portanto, as baixas temperaturas

podem ter causado um menor crescimento dos camarões no início do período experimental. Segundo Van Wyk & Scarpa (1999), as concentrações ótimas de oxigênio dissolvido, para *L. vannamei* são iguais ou maiores que 5 mg/L. Pode-se observar que em todos os tratamentos, no presente estudo, as concentrações médias de oxigênio dissolvido mostraram-se próximas a este valor. Entretanto, no 57º dia experimental, no tratamento com 0%, se percebeu uma queda acentuada nas concentrações de oxigênio dissolvido, que pode ser associada à mortalidade dos camarões nessa data. Alem disso, no período final do experimento ocorreu um aumento da colonização microbiana, o que pode estar associado à diminuição das concentrações de oxigênio dissolvido observadas no presente estudo (De Shryver et al. 2008).

Com exceção do tratamento 0% a variação de pH durante o período experimental esteve dentro da faixa de tolerância da espécie reportada por Van Wyk & Scarpa (1999). No tratamento 0% a queda do pH ocorrida no 57º dia experimental, apresentou valores abaixo do limite aceitável para os camarões, mostrando ter relação com o evento de mortalidade registrado no período.

CONCLUSÃO

Os resultados evidenciam que a utilização de um inóculo mínimo (2,5%) acelera a formação dos agregados microbianos em sistemas BFT. Além disso, o tratamento com 100% de reutilização de água apresentou os melhores índices de sobrevivência e crescimento, demonstrando ser a melhor estratégia a ser adotada. Dessa maneira, pode-se garantir um sistema de cultivo sustentável e ambientalmente correto, minimizando assim, impactos ambientais resultantes da liberação de efluentes ricos em nutrientes e matéria orgânica, bem como,

minimizando o uso de água necessário para o cultivo e também possibilitando o cultivo em áreas onde a água salgada é restrita.

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CAPÍTULO II

EFFECT OF WATER REUSE ON THE CULTURE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* WITHOUT WATER EXCHANGE

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Resumo

O uso da mesma água em mais de um ciclo de cultivo pode trazer muitos benefícios no cultivos em meio a flocos bacterianos. O estudo avaliou o efeito de diferentes níveis de reaproveitamento de bioflocos no desenvolvimento da comunidade microbiana e no desempenho zootécnico de juvenis de *Litopenaeus vannamei.* O experimento foi realizado na Estação Marinha de Aquacultura, Instituto de Oceanografia da Universidade Federal do Rio Grande, durante 30 dias. Foram utilizados 15 tanques de 800 L, povoados com camarões com peso médio de 3,5 g em uma densidade de 312 camarões/m^3. Foram testadas 5 diferentes níveis de reaproveitamento de água com bioflocos (0%, 25%, 50%, 75% e 100%) provenientes de um cultivo anterior. Para os compostos nitrogenados (amônia, nitrito e nitrato), foram detectadas diferenças significativas entre o tratamento controle (0%) e os demais tratamentos. No tratamento controle, o peso médio final foi significativamente menor (7,37 g vs 8,25 g), com maior TCA (1,52 vs 1,08). O estudo indica que o reuso de água com biofloco melhora a qualidade da água e o desempenho zootécnico dos camarões cultivados de forma super-intensiva.

Abstract

The use of the same water over a many culture cicles of biofloc technology system can provide many benefits. The study evaluated the effect of different levels of biofloc rich water on selected water quality indicators and productive performance of *Litopenaeus vannamei* juveniles (3.5 g) stocked at 312 juveniles/m^3 and cultured under no water exchange conditions. The study was carried out by 30 days and was conducted in 800 L tank-system. A total of five biofloc enrichment levels (0%, 25%, 50%, 75% and 100%) were tested with three replicates each. Significant differences were found among treatments in nitrogen species between the biofloc-enriched water and the zero enrichment treatment. No statistically significant differences were found among the biofloc-enriched treatments in survival, final weight (8.25 g) and FCR (1.08). Shrimp raised in clear seawater (e.g., 0% biofloc enrichment) were significantly smaller (7.37 g vs. 8.25 g) with higher FCR (1.52 vs. 1.08) than shrimp cultured in the biofloc-rich water. Nevertheless, no differences were found in yields between treatments. The results from this study suggest improvement in water quality and shrimp performance when cultured in biofloc-enriched water compared to natural seawater.

Keywords: BFT system, *L. vannamei*, Biofloc-rich, High density.

1. Introduction

In recent years, new management practices have been studied for production of shrimp with emphasis on reduced water exchange. These practices focused on optimization of culture conditions and improvement in biosecurity. Several studies showed that shrimp can be produced successfully under limited or no water exchange systems (Browdy and Moss, 2005; McIntosh, 2000; Mishra et al., 2008; Wasielesky et al., 2006). These systems are characterized by rich microbial communities that form flocs in the water column. Growing shrimp under these conditions have been found to have several advantages compared to conventional aquaculture practices. These systems are characterized by high yields, small footprint, and reduced environmental impact (Browdy et al., 2001; Neal et al., 2010). The use of limited water exchange minimize the introduction of pathogens with the incoming water, while increasing awareness to biosecurity which leads to reduce crop losses due to disease outbreaks. Furthermore, as under these growing conditions assimilation of nitrogen compounds is enhanced, the same water have been used for several production cycles with no negative impact on yields Another advantage stemming from this technology was the ability to construct and operate these facilities inland far from the high price coastal areas (Ray et al., 2009; Vinatea et al., 2010; Samocha et al., 2012).

By adding of carbon source into the culture medium in limited discharge systems (e.g., changing C/N ratio) there is a significant enhancement of bacterial growth and in fixation of toxic nitrogen metabolite species (Avnimelech, 2009; Chamberlain et al., 2001; Ebeling et al., 2006; Hari et al., 2006; Crab et al., 2010). Beside the improvement in water quality, the increase in bacterial biomass, which provided supplemental feed, was also associated with improved shrimp survival and growth while reducing nutrient-rich water releases into receiving streams

(Avnimelech, 2009; De Schryver et al., 2008; Krummenauer et al., 2011; Timmons et al., 2002; Wasielesky et al., 2006).

Several authors mentioned the high volume of water needed (20–64 m^3) to produce 1 kg of shrimp when traditional production practices are used (Hopkins et al., 1993; Moss et al., 2001; Timmons and Losordo, 1994). On the other hand, the water usage by biofloc-rich systems is greatly reduced. For example, Otoshi et al. (2009) documented the use 163 L to produce 1 kg of the Pacific white shrimp *Litopenaeus vannamei* while working with a super-intensive production system. Similar low water usage (169 L/1 kg shrimp produced) was documented by Krummenauer et al., (2011) in 35 m^{-3} raceways. Samocha et al. (2010), in an experimental zero exchange super-intensive system, reported use of only 98 L to produce 1 kg of food shrimp.

It should be noted that the development of the microbial bioflocs in the culture medium is a lengthy process that can take few weeks. Usually, in outdoor ponds the development of these heterotrophic conditions takes seven to eight weeks (McIntosh, 2000) from filling the ponds. Samocha et al. (2012), in their work with super-intensive raceway systems under zero exchange, showed that application of molasses to the culture medium can help reduce the time needed for the development of the bioflocs from seven down to five weeks. Other researchers (McAbee et al., 2003) suggested the use of biofloc-rich water from a prior production cycle to accelerate the formation of bioflocs in newly started systems.

This study was designed to evaluate the effect of different enrichment levels of biofloc-rich water on shrimp performance and selected water quality indicators in an experimental tank system stocked at high density and operated under no water exchange

2. Material and Methods

2.1. Culture Conditions

The experiment was carried out at the Marine Station of Aquaculture, Federal University of Rio Grande, Southern Brazil (32°12´16S 52°10´38W) in fifteen rectangular tanks (bottom area 1.2 m²) with working volume of 800 L, which were positioned in a greenhouse. Each tank was equipped with 2 air stones (12 x 1.8 x 2.0 cm) powered by a 4 hp air blower. Juveniles (3.5 g ± 0.93) of *L. vannamei* raised at the laboratory from ten-day-old postlarvae (PL_{10}). Postlarvae were received from Aquatec® hatchery, Canguaretama, Rio Grande do Norte State, Brazil and were kept under no water exchange in a 70 m^3 tank stocked at a density of 1,000 PL/m^3. Throughout the 72-day nursery period, shrimp were fed by a commercial 40% crude protein feed (0.8-1.2 mm, Guabi®, Campinas, SP, Brazil).

The experiment evaluated five inoculation levels of biofloc-rich water: 0% (control, natural seawater - 31 ppt), 25%, 50%, 75% and 100% biofloc-rich water (salinity 34 ppt). The water used in this study was filtered (sand filter) natural seawater treated with a chlorine solution (10 ppm measured immediately after chlorination) and dechlorinated using ascorbic acid powder (1 g/1,000 L). Tanks were kept with no water exchange through the duration of the study. Dechlorinated municipal freshwater was used to compensate for evaporative losses and to maintain salinity.

The biofloc-rich water was obtained from a 35 m^3 tank stocked with PL_5 at a density of 300/m^3 and operated during 120 days with no water exchange. Shrimps fed the same diet offered to the shrimp used in this study. The biofloc-rich water used on the preparation of the test-water had concentrations of 0.67 mg L^{-1} (TA-N), 0.06 mg L^{-1} (NO^-_2-N), 22.0 mg L^{-1} (NO^-_3-N), 1.85 mg L^{-1} (PO_4^{+3}-P), and alkalinity of 140 mg L^{-1} as $CaCO_3$. In order to accelerate the

development of the bioflocs, molasses was added when the total ammonia levels reached 0.5 mg L^{-1}. Supplementation of carbon was based on addition of 6 g of carbon for each 1 g of total ammonium nitrogen (TA-N) in the water. This procedure followed the method described by Avnimelech (2009), Ebeling et al. (2006) and Samocha et al. (2007).

2.2. Water Quality Monitoring

Water temperature, salinity, dissolved oxygen (DO) and pH were recorded twice daily (0800 and 1700) using a YSI 556 MPS meter (YSI Inc., Yellow Springs, Ohio, United States). Secchi measurements were done daily. Water samples were tested daily for TA-N (UNESCO, 1983). Monitoring of NO_2-N, NO_3-N, and PO_4^{+3}-P was done every five days following methods adapted from Strickland and Parsons (1972). Alkalinity was measured once a week (APHA, 1989). Adjustments of water pH were made anytime its levels dropped below 7.2 using 0.05 g of $Ca(OH)_2$ for each liter of water (added directly into water). Water turbidity was measured once a week using a Turbidimeter (Hach 2100P, Hack Company, Loveland, Colorado, United States). Settleable solids (ml L^{-1}) was measured three times per week using an Imhoff cone with readings recorded after 15–20 min, following Eaton et al. (1995) method. Water collected for testing of total suspended solids (TSS) following the method described by Strickland and Parsons (1972).

2.3. Shrimp Stocking, Feeding and Monitoring

All tanks were randomly assigned and stocked at a density of 312 juveniles m^{-3}. Shrimp were fed three times day^{-1} using a commercial diet (38% CP, 1.6 mm, Guabi®, Campinas, SP, Brazil), offered on a feed tray (10 cm diameter, 5 mm mesh size, one per tank). At study initiation shrimp were fed 10% their total biomass following the recommendation by Jory et al. (2001). Daily rations after the first week were adjusted based on shrimp consumption and

growth performance. Every week, 60 shrimps were randomly sampled from each tank and individually weighed; using a digital scale with readability of 0.01 g (Marte® científica AS2000, Santa Rita do Sapucaí, MG, Brazil). At the end of the trial, total shrimp biomass along with individual weights of 200 randomly selected shrimp from each tank were recorded. The weekly growth rate (WGR) was determined by the net increase in final weight over the four week study duration. The feed conversion ratio (FCR) was calculated as offered feed / net biomass increase. Survival was calculated as [(final biomass / average final individual weight) / number of individuals stocked]*100, where (final biomass / average final individual weight) is the total of shrimps at the end of the experiment. Yield was calculated as total biomass / volume of tank.

2.4. Statistical Analysis

Water quality parameters were compared by two-way repeated measures ANOVA with treatment (system type) as main factor and sampling date as repeated measures factor. Significant differences of $P<0.05$ was used in all zootechnical performance and shrimp performance, Tukey's multiple-range test was applied when significant differences were detected. All tests were performed after the confirmation of homogeneity of variance (Levene's test) and normality of data distribution (Kolmogorov-Smirnov test). To satisfy the ANOVA assumptions, survival data were arcsine-square root transformed using a constant exponent (arcsine x 0.5) (Zar, 1996).

3. Results

No significant differences in concentrations of DO, pH and temperature were found between the treatments (Table 1). Figures 1–3 show temporal variation in TAN, NO^-_2-N and NO^-_3-N, respectively, for the five treatments. Although still low, TAN levels fluctuated substantially (between 0.001 and 1.5 mg L^{-1}) only in the 0% treatment. The concentrations of TAN in the biofloc-rich water treatments remained low and were significantly lower than the levels found for the 0% treatment. Similarly, the levels of NO^-_2-N in the 0% biofloc-rich were significantly higher than all other treatments (10.11 vs. 0.54 to 1.85 mg L^{-1}). As expected, a positive relationship was found between the amount of reused water and the dissolved nitrate concentrations in the culture media.

TABLE 1. Water quality parameters for culturing *L. vannamei* in a 30-day study under no water exchange using biofloc rich water.

	Percent enrichment with biofloc-rich water				
	0	25	50	75	100
TA-N (mg L^{-1})	0.52±0.082[a1]	0.09±0.21[b]	0.08±0.010[b]	0.11±0.24[b]	0.04±0.006[b]
NO^-_2-N (mg L^{-1})	10.11±2.32[a]	1.26±0.29[b]	1.85±1.16[b]	1.56±0.56[b]	0.54±0.05[b]
NO^-_3-N (mg L^{-1})	6.72±6.75[a]	23.98±12.24[b]	28.18±15.22[b]	34.49±14.00[bc]	42.94±10.44[c]
PO_4^3-P (mg L^{-1})	1.80±0.91[a]	2.79±1.61[ab]	3.04±1.55[b]	4.66±2.15[b]	5.18±2.51[b]
Alkalinity (mg L^{-1} $CaCO_3$)	140.66±37.5[a]	128.33±20.6[a]	127.00±28.3[a]	123.33±21.3[a]	127.33±40.1[a]
DO (mg L^{-1})	7.88±0.09 [a]	7.91±0.07[a]	8.17±0.07[a]	7.99±0.08[a]	8.11±0.07[a]
pH	7.58±0.02[a]	7.67±0.03[a]	7.65±0.02[a]	7.61±0.02[a]	7.58±0.02[a]
Salinity (ppm)	31.5±0.23[a]	32.5±0.28[ab]	33.2±0.27[bc]	33.9±0.25[cd]	34.6±0.26[d]
Temperature (^oC)	26.7±0.14[a]	26.6±0.15[a]	26.7±0.15[a]	26.5±0.13[a]	26.8±0.16[a]

[1]Values are means of replicates ± standard deviation. Different superscripts in the same row indicate significant differences ($P<0.05$).

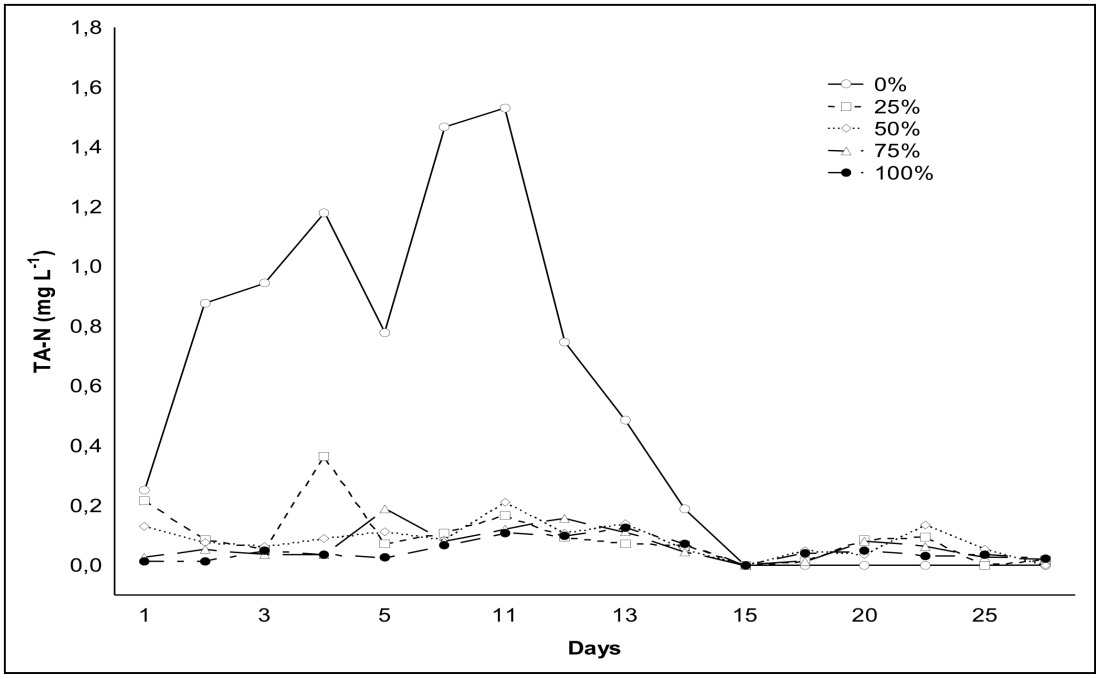

Fig. 1. Total ammonia nitrogen (TAN) concentration in tanks with white shrimps under no water exchange using biofloc rich water.

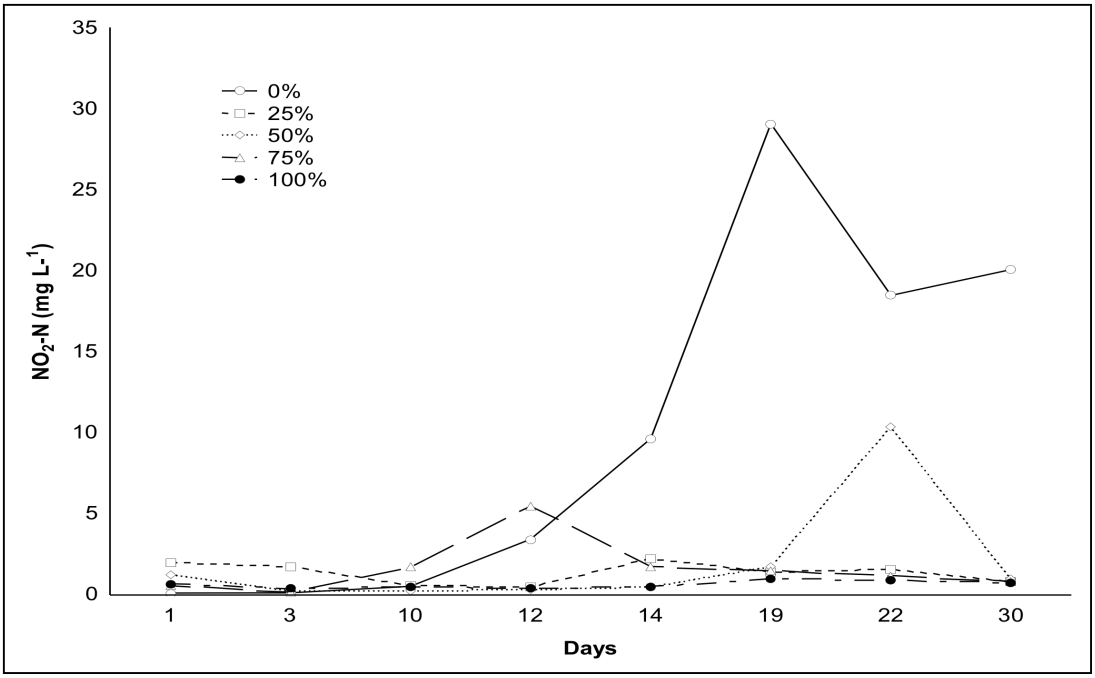

Fig. 2. Nitrite (NO_2-N) concentration in tanks with white shrimps under no water exchange.

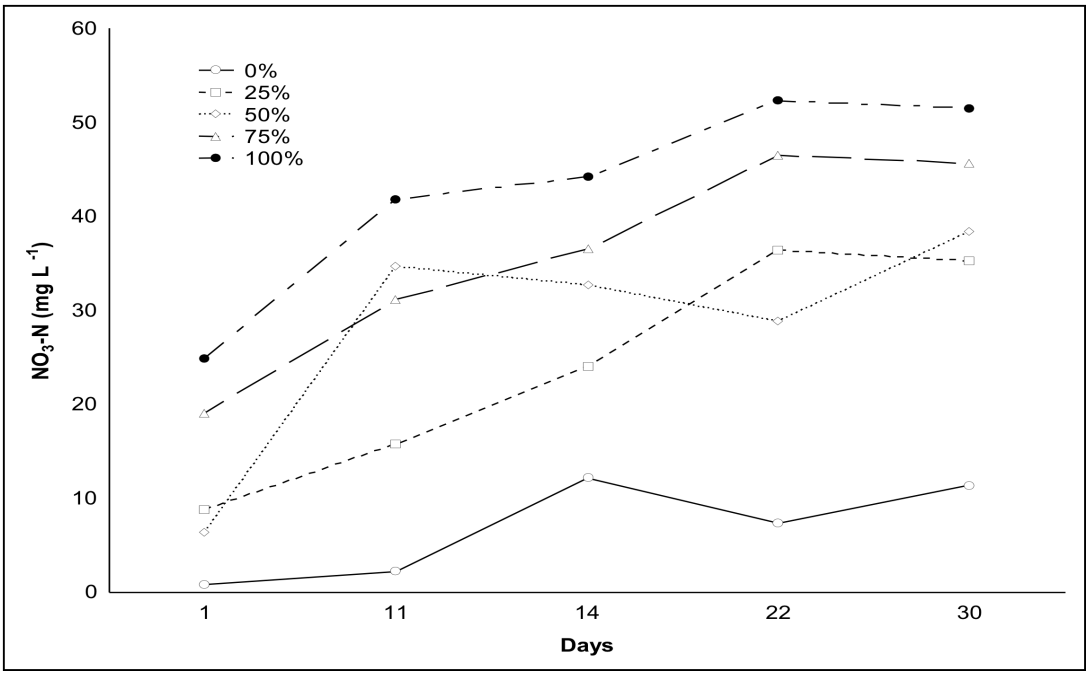

Fig. 3. Nitrate (NO_3-N) concentration in tanks with white shrimps under no water exchange using biofloc rich water.

Table 2 summarizes the TSS, SS, Turbidity and Transparency. No significant difference in TSS and turbidity were found between treatments. Settleable solids concentrations were significantly higher in the biofloc-rich treatments while water transparency was significantly higher in the treatment without water reuse. Figures 4-6 show the changes over time in TSS, SS, and Turbidity for the five treatments.

TABLE 2. Settleable solids (SS), Total suspended solids (TSS), Turbidity and Transparency recorded in *L. vannamei* cultured in a 30-day study under no water exchange.

	Percent enrichment with biofloc-rich water				
	0	25	50	75	100
SS (ml L^{-1})	8.48±1.14^{a}	14.18±1.53^{b}	13.16±0.82^{b}	12.51±0.98^{b}	16.20±1.98^{b}
TSS (mg L^{-1})	383.5±293.3^{a}	466.3±277.2^{a}	525.6±353.4^{a}	636.6±312.5^{a}	714.0±358.4^{b}
Turbidity (NTU)	79.12±36.37^{a}	104.18±64.06^{a}	112.86±50.62^{a}	105.20±54.95^{a}	131.94±50.66^{a}
Transparency (cm)	22.19±5.77^{a}	19.75±4.13^{ab}	17.70±5.77^{bc}	18.33±7.17^{bc}	15.08±4.55^{c}

Values are means of replicates ± standard deviation. Different superscripts in the same row indicate significant differences ($P<0.05$).

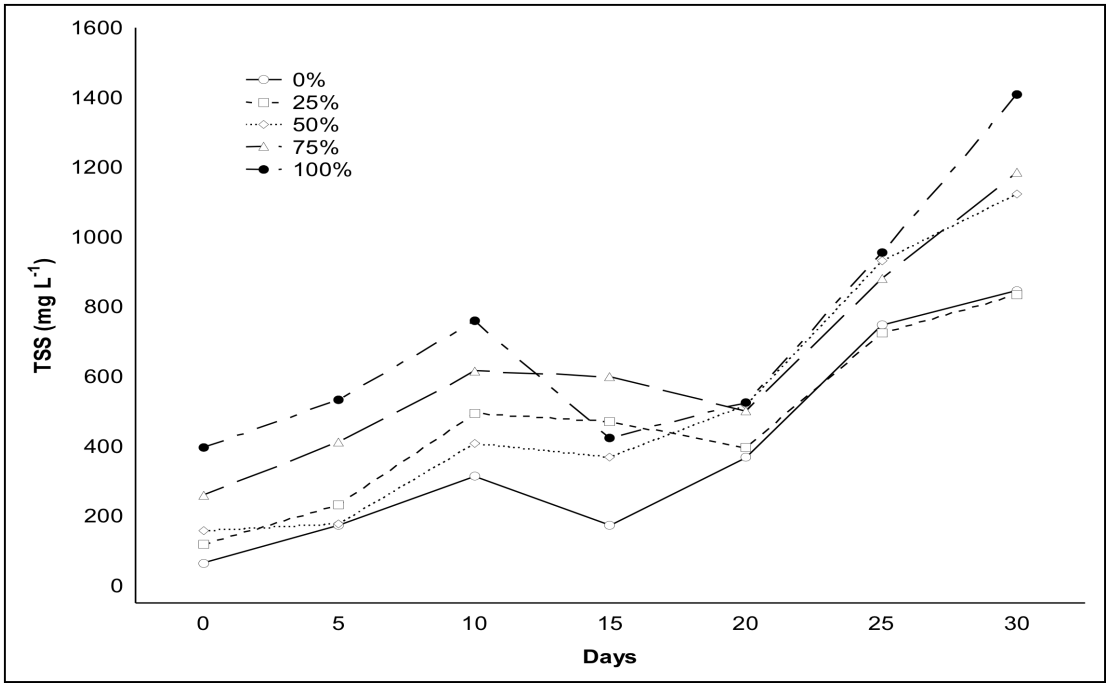

Fig. 4. Total suspended solids (TSS) over the 30-day study with different levels of biofloc-rich water with no water exchange.

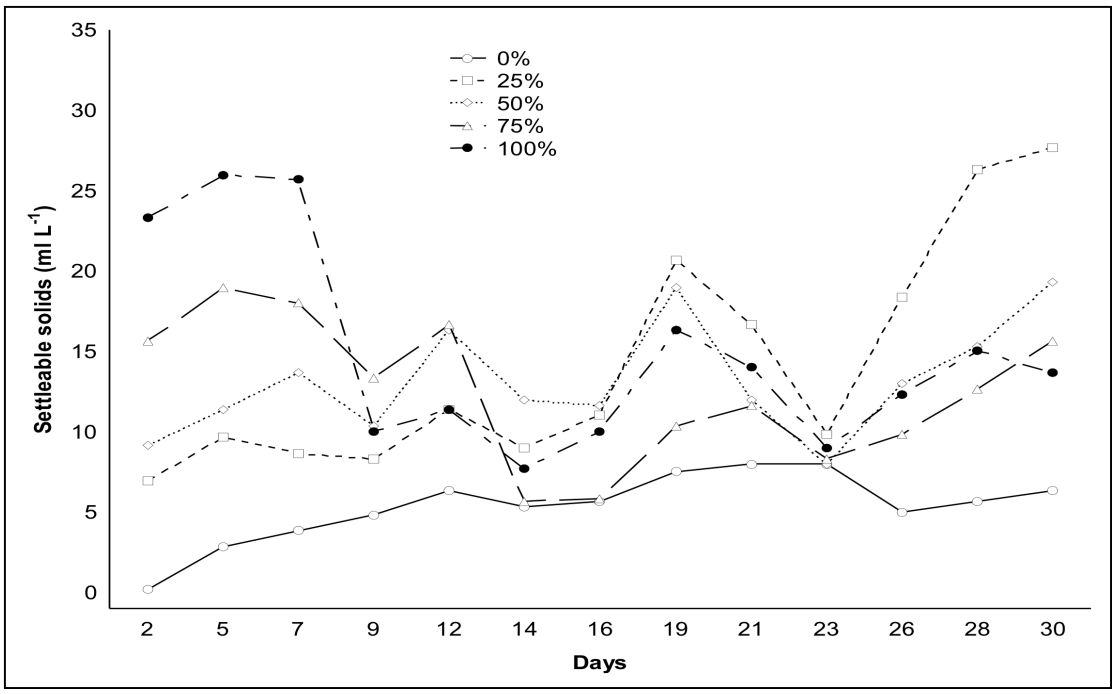

Fig. 5. Settleable solids changes over the 30-day study with different levels of biofloc-rich water in BFT, *L. vannamei* culture under no water exchange.

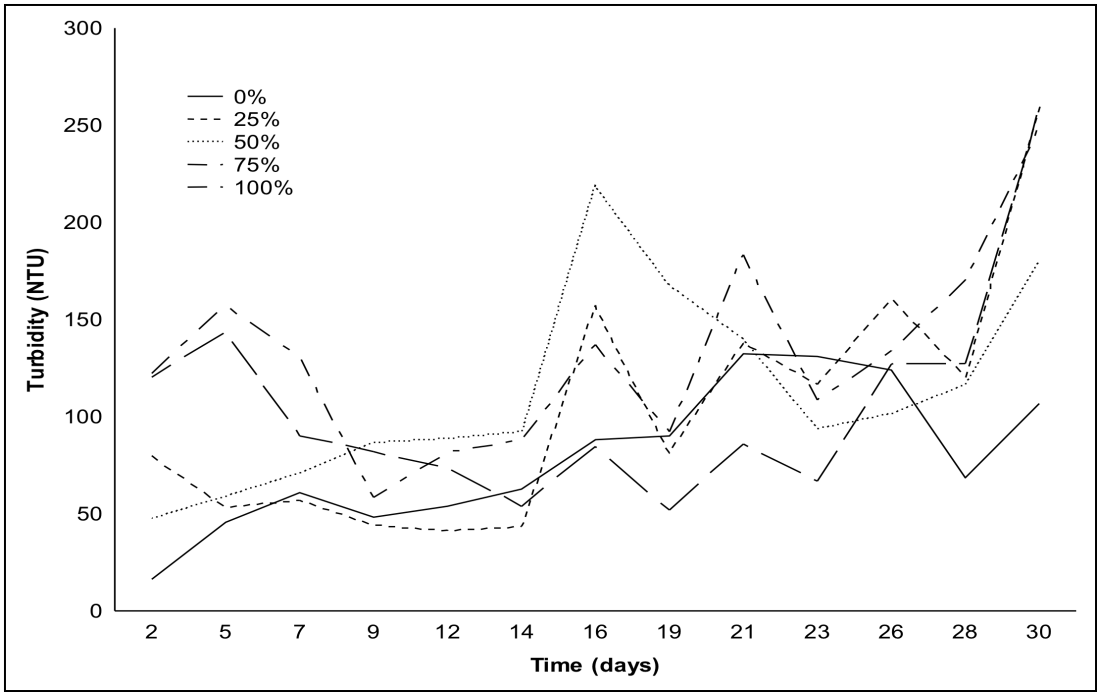

Fig. 6. Turbidity changes over the 30-day study with different levels of biofloc-rich water in BFT, *L. vannamei* culture under no water exchange.

Mean weight of the shrimp at the study termination was 7.37, 8.30, 8.28, 8.42 and 8.01 g for the 0, 25, 50, 75 and 100% treatment, respectively (Table 3). Shrimp final weight was significantly lower in the 0% floc-rich water treatment (Table 3). No significant difference was found in shrimp survival between treatments (90.9 to 99.06%). The FCR was significantly lower in the biofloc-rich water treatments than the control (Table 3).

TABLE 3. Performance parameters of *L. vannamei* in a 30-day study under no water exchange using biofloc rich water

	Percent enrichment with biofloc-rich water				
	0	25	50	75	100
Final weight (g)	7.37 ± 0.11^{a}	8.30 ± 0.11^{b}	8.28 ± 0.09^{b}	8.42 ± 0.13^{b}	8.01 ± 0.10^{b}
Survival (%)	92.13 ± 6.5^{a}	91.0 ± 4.2^{a}	90.93 ± 4.1^{a}	91.60 ± 6.24^{a}	99.06 ± 4.49^{a}
WGR (g)	0.90 ± 0.01^{a}	1.12 ± 0.02^{b}	1.11 ± 0.02^{b}	1.14 ± 0.03^{b}	1.05 ± 0.02^{b}
FCR	1.52 ± 0.12^{a}	1.23 ± 0.18^{b}	1.19 ± 0.17^{b}	0.84 ± 0.19^{b}	1.09 ± 0.21^{b}
Yield /kg/m^{-3}	2.12 ± 0.31^{a}	2.35 ± 0.40^{a}	2.35 ± 0.59^{a}	2.41 ± 0.55^{a}	2.48 ± 0.67^{a}

Values are means of replicates ± standard deviation. Different superscripts in the same row indicate significant differences ($P<0.05$).

4. Discussion

The mean values of the water quality indicators monitored in this study remained within the recommended range for optimal growth and survival for *L. vannamei* (Ponce-Palafox et al., 1997; Van Wyk and Scarpa, 1999). Salinity was significantly higher ($P < 0.05$) in all of the biofloc-rich treatments, due to the mix between the water from the matrix tank, where the biofloc inoculum was removed (34.0 ppt) and the water from the laboratory (31.0 ppt). Despite differences in salinity among treatments, this parameter was within acceptable ranges for survival and growth for *L. vannamei* (Van Wyk and Scarpa, 1999). Furthermore, since this species is known to tolerate wide salinity range varying between 5 and 40 ppt, these changes are

not considered. The TAN concentrations in all treatments remained far below the 3.95 mg L^{-1} limite reported by Lin and Chen (2001) to be toxic to juvenile shrimp. According Avnimelech (2009) there is four routes to control inorganic nitrogen accumulation: (1) water exchange; (2) algae control; (3) nitrification, and (4) nitrogen control by bacterial biofloc (Avnimelech, 1999). Several authors (Avnimelech, 2009; Ebeling et al., 2006; Samocha et al., 2007) documented successful removal of ammonia, in limited exchange culture systems, via adjustment of the carbon and nitrogen to reach a ratio of 6:1 that favor assimilation of the ammonia and production of microbial biomass. Cohen et al. (2005) reported gradual increase in ammonia levels which followed by similar increase in nitrite while working in limited water exchange system. These authors mentioned that it took 5 to 7 weeks for the nitrifying bacteria to develop in the culture media before significant reduction in the ammonia took place. The level of TAN in the 0% biofloc-rich treatment showed the normal route usually observed in BFT system, with a reduction of the levels after two weeks of culture, that fluctuation were controlled by addition of molasses (Avnimelech, 1999; Avnimelech, 2009). On the other hand, the levels of TAN in the treatments with biofloc-rich water did not show any fluctuation, probably, due to the fact that the bacteria uptakes were higher than the clear water treatment.

The ammonium can be oxidized by Ammonia-Oxidizing Bacteria (AOB) to form nitrite, which is an intermediate product of the nitrification process (Avnimelech, 2009; Maillard et al., 2005). Lin and Chen (2003) estimated the "safe level" for *L. vannamei* juveniles (pH 8.02, 18 °C and salinity 35) to be 25.7 mg L^{-1} of NO_2-N. In the present study, the highest nitrite concentration (30.0 mg L^{-1} NO_2-N) was found in the control treatment. However, nitrite-related mortalities were not observed at any time during the trial and shrimp continue to grow. According Browdy and Moss (2005), in limited/no exchange systems the shrimps can tolerate

high TAN and NO_2–N concentrations. Furthermore, Kuhn et al. (2010) and Schuler and Boardman (2010) reported an increase in shrimp tolerance to nitrite with the increase in salinity, i.e., higher salinities can dampen the toxic effects of nitrogenous wastes. Unlike the high levels found in the control treatment, the NO_2–N concentrations in the biofloc-rich treatments were below 1.85 mg L^{-1}. These low concentrations can be explained by the uptake of the *Nitrobacter* like bacteria that were introduced into the culture water with the biofloc. According to Otoshi et al. (2009), the bacterial dominated systems promote better water quality stability than mixed algal dominated systems because they do not experience the bloom and crash cycles typical of algal dominated systems. This stabilization process was also observed by other researchers working with *L. vannamei* in limited or no water exchange systems; for example, McAbee et al. (2003) in their intensive shrimp production raceway study with reused water showed this water helped to maintain low ammonia and nitrite levels throughout the trial. A similar reduction in ammonia and nitrite levels observed in our biofloc-rich water treatments was also mentioned by Gaona et al. (2012), which used only 10% of biofloc-rich water as inoculum to accelerate the formation of the nitrifying bacteria in the culture medium, a study conducted with no water exchange. Other studies mentioned that in the absence of denitrification, nitrate would often accumulate in systems operated with no water exchange (Arnold et al., 2009; Cohen et al., 2005; Ebeling and Timmons, 2007; Kuhn et al., 2010). A gradual increase in nitrate over time in such systems was mentioned by several researchers and documented levels over 400 mg L^{-1} NO_3-N in these system over production cycle (Krummenauer et al., 2011; Samocha et al., 2010, 2012). Kuhn et al. (2010), while working with water salinity of 11 ppt, reported negative effect of nitrate on the *L. vannamei* growth and survival at concentration of 220 mg L^{-1} of NO_3-N. In our study the maximum level of NO_3-N for all treatments (including control), nitrate

concentrations toward the end of the trial varied between 13.25 and 55.64 mg L^{-1} and increased according to the amount of biofloc-rich water reused in each treatment. Probably the increase of nitrate concentrations in higher rich-biofloc treatments did not inhibited survival rates and growth.

In limited/zero exchange systems TSS tends to increase over time primarily due to the increase in bacterial biomass. In the current study, the maximum TSS concentrations reported was 1,510 mg L^{-1} (treatment 100%). This concentration was above the 400-500 mg L^{-1} targeted for optimal range mentioned by Gaona et al. (2012) and Samocha et al. (2007). Avnimelech (2009) warns that TSS concentration above 500 mg L^{-1} may interfere with water quality parameters and zootechnical performance of the shrimps, and suggests keeping the levels between 200-500 mg L^{-1}. Different methods have been listed as potential tools to control the levels of particulate matter in limited/zero exchange systems including clarification, filtration, water exchange (Ebeling et al., 2006; Gaona et al., 2012; Ray et al., 2010, 2011). In the present trial, no attempts were made to control the culture TSS levels. An increase in turbidity was observed over the study, a similar increase trend was noticed by other BFT studies (Ebeling et al., 2006; Vinatea et al., 2010). Apparently high turbidity values did not affect the shrimp zootechnical parameters (Van Wyk and Scarpa, 1999). According to results of this study, the development of the bacterial community in the biofloc-rich treatments was more effective than the 0% treatment. Similar results were reported by McAbee et al. (2003), in a study that used this strategy performed with a comparison with clear water. In addition, other studies in closed culture systems that used biofloc-rich to accelerate the development of the biofloc community have been observed quickly bacterial stabilization (Krummenauer et al., 2011; Gaona et al., 2012; Samocha et al., 2012).

Survival was higher in all treatments, high survival rates have been observed in studies under higher stocking densities: Krummenauer et al. (2011) evaluated the effect of stocking density on survival of *L. vannamei*, with survival ranging from 75.0% to 92.0%, at stocking densities ranging from 150 to 450 shrimps.m^{-2}; Otoshi et al. (2009) operating 75 m^{-2} RAS stocked at densities ranging from 301 to 408 shrimps m^{-2} reported survival of 82.3 to 91.8%.

The data suggest significant improvement in mean final weight of the shrimp raised in biofloc-rich water. Probably, the better growth in those treatments with biofloc-rich is associated to natural productivity and better water quality parameters in these treatments. Wasielesky et al. (2006) and Ballester et al. (2010) also observed the benefit of the microbial community on the growth of *L. vannamei* and *Farfantepenaeus paulensis* respectively. The average weekly growth rates in our study were a little over 1.0 g $week^{-1}$ in the biofloc-rich treatments. This growth was significantly better than the rate observed for the clear water treatment. McAbee et al. (2003) working with water reuse and clear water at stocking density of 200 shrimp/m^2 reported high survival (91%) and average growth rate of 1.44 g wk^{-1} in that treatment with biofloc-rich. This result are in agreement with those reported by Krummenauer et al. (2011) for *L. vannamei*, which were stocked at densities that varied between 150 and 450 shrimps m^{-2} in a zero water exchange super-intensive system (0.85–0.92) g wk^{-1}. On the other hand, while working with aged water at stocking density of 450 shrimp m^{-3} Samocha et al. (2010) reported a weekly growth between 1.35 and 1.39 g wk^{-1} with 95.54% survival and FCR of 1.56. The low FCR values (0.8 to 1.2) observed in those treatments using biofloc-rich are similar to reported by other studies with limited discharge systems (Browdy and Moss, 2005; Krummenauer et al., 2011; Samocha et al., 2007; Wasielesky et al., 2006). These values confirm the importance of natural productivity provided by rich-biofloc enhanced treatments.

5. Conclusion

The use of biofloc-rich water in different fractions (25-100%) suggest accelerated rate of the biofloc allowed the rapid establishment of nitrifying microbial community resulting in fast removal of ammonia and nitrite from the culture water. The results suggest that supplementation of virgin seawater with biofloc-rich water at level as low as 25% also showed that even reusing small amount of biofloc-rich water provides better nutritional conditions for shrimp growth. Thus, to generate a biosecurity and environmentally friendly system, minimizing even more the use of water.

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CAPÍTULO III

SURVIVAL AND GROWTH OF *Litopenaeus vannamei* REARED IN BFT SYSTEM UNDER DIFFERENT WATER DEPTHS

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Resumo

Atualmente, os cultivos de camarão estão se voltando para sistemas que minimizam a utilização de água. O sistema de criação que utiliza a tecnologia de bioflocos (BFT) contribui para a qualidade da água, através da remoção de compostos nitrogenados, além disso, é realizado com mínima ou sem renovação de água e também possibilita a utilização de densidades elevadas. O presente trabalho teve como objetivo o cultivo de *L. vannamei* em sistema BFT analisando três diferentes profundidades (0,40; 0,80 e 1,20 m). O experimento foi realizado na Estação Marinha de Aquacultura, Instituto de Oceanografia da Universidade Federal do Rio Grande, RS, Brasil, em uma estufa retangular com nove tanques com volume útil de 52.500 L cada. Cada unidade experimental foi estocada com 14.000 camarões (400 ind/m^2). Os camarões foram alimentados três vezes por dia com ração comercial contendo 38% de proteína bruta. Ao longo de 120 dias foram avaliados os parâmetros de qualidade da água, ganho de peso, conversão alimentar e sobrevivência. Os resultados foram submetidos a análise de variância (ANOVA) e, quando observadas diferenças significativas, as médias foram comparadas pelo teste de Tukey (α = 0,05). Não foram detectadas diferenças significativas nos parâmetros de qualidade da água ($P>0,05$), exceto para alcalinidade, que foi maior no tratamento com 1,20 m de profundidade ($P<0,05$) . Peso final e sobrevivência não apresentaram diferenças significativas ($P> 0,05$), porém a biomassa final (kg/m^3) foi significativamente maior no tratamento com profundidade de 0,40 m ($P <0,05$). Neste tratamento, a produtividade foi de 8,45 kg/m^3 e foram utilizados 119 L de água para produzir 1 kg de camarão. De acordo com os resultados, *L. vannamei* pode ser cultivado em sistemas BFT com profundidades de água entre 0,4 e 0,8 m.

Abstract

Currently, the shrimp culture has been directed to systems that minimize the use of water. The biofloc technology culture system (BFT) contributes to the water quality, through the removal of nitrogen compounds, supplements the shrimp diet and allows the use of high stocking densities. In addition, this system enables the culture with minimal water exchange. This study aims to rear *Litopenaeus vannamei* in BFT system under different water depths. The experiment was carried out at the Marine Station of Aquaculture (Federal University of Rio Grande -, RS, Brazil). Three treatments were compared with 0.40, 0.80 and 1.20 m of deep. Treatments were randomly assigned to nine 52,500 L lined tanks in a greenhouse. Each tank was stocked with 14,000 animals (400 shrimp m^{-2}). Feed (38 % crude protein - CP) was offered three times per day, through feeding trays. The study lasted 120 days. Water quality parameters, weight gain, FCR and survival were evaluated. Results were analyzed by one-way ANOVA and, when sugnificative differences were observed, the means were compared by Tukey Test (α=0.05). No significant differences were detected for water quality parameters ($P>0.05$), except for alkalinity, that was higher in the 1.20 m deep treatment ($P<0.05$). Final weight and survival did not show significant differences ($P>0.05$). Final biomass ($kg.m^{-3}$) was significantly higher in the treatment 0.40 m ($P<0.05$). The productivity was 8.45 $kg.m^{-3}$ in 17 weeks and used 119 L of water to produce 1.0 kg of shrimp. According to the results, *L. vannamei* can be reared in BFT systems with water depths from 0.4 to 0.8 m.

Keywords: *L. vannamei*, BFT system, different depths.

1. INTRODUCTION

In order for aquaculture to be wholly successful, the industry will need to develop technologies that will increase economic and environmental sustainability (Kuhn et al. 2010). The conventional shrimp ponds usually need daily water exchange rates around 10–15 % of total volume to maintain optimum water quality. However, this strategy is an important factor contributing to development of several diseases in shrimp growing areas (Lightner 2005; Hargreaves 2006). In addition, the effluent from aquaculture rich in organic materials and nutrients, can cause environmental pollution as well as expenses with pumping water (De Schryver et al. 2008). Thus, the shrimp culture has been directed to biosecurity systems that minimize the use of water.

Biofloc Technology Culture Systems (BFT) is characterized by zero water exchange and super-intensive culture of shrimp in enclosed raceways. This system is considered environmentally friendly and avoids nutrient rich waste from polluting coastal waters (Avnimelech, 2009). The nutrients transformation in bioflocs (microorganisms) contribute to the water quality, through the removal of nitrogen compounds, supplements the shrimp diet and allows the use of high stocking densities (Krummenauer et al. 2011; Wasielesky et al. 2006)

Earthen ponds are used to produce the overwhelming majority of finfish and crustacean production in aquaculture (Hargreaves 2006). The water volume needed for even small to medium aquaculture systems can reach up to several hundreds of cubic meters per day (De Schryver et al. 2008). In general, shrimp farming is commonly practiced in ponds of about 1.0 m deep, but there is a wide range of depths in use. Depth is usually determined by reasons related to construction costs and habitat preference of the primary cultured species (Abdel-Aal 2008).

Depth as a factor in pond ecosystem management has been given little attention, despite its theoretical importance in autotrophic and heterotrophic systems (Szyper et al. 1991). Usually the greenhouse-enclosed raceways are carried out with depths ranging from 0.4 to 1.2 m (Gaona et al. 2012; Krummenauer et al. 2011; Otoshi et al. 2009; Samocha et al. 2010). Furthermore, with the scarcity of water resources and possible environment pollution, studies are needed to determine the ideal amount of water necessary to be used in shrimp culture. Besides of this, the shrimp behavior and water quality parameters could be completely changed according to the amount of water available in the culture system.

The objective of this study was to compare the growth performance of *Litopenaeus vannamei* in greenhouse-enclosed raceways with different depths, in BFT culture system.

2. MATERIAL AND METHODS

2.1.Culture Conditions:

The growout study was carried out in a 120-day at Marine Station of Aquaculture, Federal University of Rio Grande, Southern Brazil (32°12´16 S 52°10´38 W) in a greenhouse with nine 52,500 L (total volume) rectangular tanks (35 m^{-2}, each). The aeration system consisted in 7-HP blower with one air stone per m^{-2} (10 cm each). *L. vannamei* post-larvae were obtained from commercial hatchery (Aquatec™, Canguaretama, Rio Grande do Norte State, Brazil). Each tank was stocked with 14,000 animals in at a stocking density of 400 shrimps m^{-2} (initial weight 0.08 g). The experiment had three treatments: 0.40, 0.80 and 1.20 m of water depth (with three replicates each). Before the experiment tanks were filled with filtered seawater (salinity 30) and treated with a chlorine solution (10 ppm measured immediately after chlorine) dechlorinated with ascorbic acid powder (1 gram per 1000 L). Losses of water due to evaporation were replaced with dechlorinated freshwater. Structures with vertical substrates

(Needlona™, Cachoeirinha, Rio Grande do Sul State, Brazil) were used to increase the surface area (150% front and back) available for nitrifying bacteria colonization. The greenhouse was covered by a 50% light attenuation shade cloth to avoid high temperatures during the culture period. The organic fertilization began with the addition of 6.0 g of carbon (molasses) for each 1.0 g of total ammonium nitrogen (TA-N) in the water. This procedure followed the methodology described by Ebeling et al. (2006) and Avnimelech (2009). Adjustments of culture water pH in all test-tanks were made anytime the levels dropped below 7.2 using 0.05 g of $Ca(OH)_2$ for each liter of water (added directly into water).

2.2.Water Quality Monitoring:

The values of temperature, salinity, dissolved oxygen (DO) and pH were recorded twice daily (0800 and 1700) using a multiparameter apparatus (YSI 556, YSI Inc., Yellow Springs, Ohio, United States). Water samples were collected daily to quantify the concentration of total ammonia nitrogen (TA-N) (UNESCO 1983). The analysis of nitrite (NO_2-N), nitrate (NO_3-N) and phosphate (PO_4^3-P) concentrations was performed every five days following the methodology adapted from Strickland and Parsons (1972). Alkalinity was determined following the methodology described in APHA (1989). The water transparency was measured using a Secchi disc. The turbidity was measured weekly using a turbidimeter (Hach 2100P Hack Company, Loveland, CO, United States). The volume of settling solids was measured using an Imhoff cone registering the volume taken in by the bioflocs in 1.0 L of tank water after 15 min sedimentation, following Eaton et al. (1995). Water collected for total suspended solids (TSS) analysis (particles larger than 45 μm) according to the method of Strickland and Parsons (1972).

2.3.Shrimp Stocking, Feeding and Monitoring:

Shrimp were fed three times per day with a commercial diet (38% CP and 1.6 mm, Guabi™, Campinas, SP, Brazil) using feed trays (10 cm diameter, one per tank), according to Jory et al. (2001). Feed was placed at an initial rate of 10% of shrimp biomass and was adjusted based on consumption. Every week, 60 shrimps were randomly sampled from each tank and individually weighed, using a digital scale accurate to 0.01 g. (Marte® científica AS2000, Santa Rita do Sapucaí, MG, Brazil). At the end of the study, 200 shrimps were individually weighed from each experimental unit, and survival was estimated based on total harvest weight. The weekly growth rate (WGR) was determined by the net increase in final weight over the study period. The feed conversion ratio (FCR) was calculated as offered feed / net biomass increase. Survival was calculated as [(final biomass / final average individual weight) / number of individuals stocked]*100. Yield was calculated as total biomass / tank volume.

2.4.Statistical Analysis:

Significant differences ($P<0.05$) in zootechnical performance and water quality were analyzed using one-way ANOVA. Tukey's multiple-range test was applied when significant differences were detected. All tests were performed after the confirmation of homogeneity of variance (Levene's test) and normality of data distribution (Kolmogorov-Smirnov test). To satisfy the ANOVA assumptions, survival data were arcsine-square root transformed using a constant exponent (arcsine x0.5) (Zar, 1996).

3. RESULTS

The results of water quality parameters over the 120-day study are shown in Table 1. Temperature and dissolved oxygen did not differ between treatments. Dissolved oxygen decreased throughout the experimental period in all treatments (Fig. 1).

Table 1. Water quality parameters of rearing *L. vannamei* in a bioflocs technology system under different water depths over 120 days[1].

	Depth (m)		
	0.40	**0.80**	**1.20**
TA-N (mg L^{-1})	0.22±0.57 [a]	0.55±0.58[a]	0.56±0.57[a]
NO_2-N (mg L^{-1})	10.92±12.4[a]	5.40±8.44[b]	9.52±8.88[a]
NO_3-N (mg L^{-1})	0.54±0.68[a]	0.57±0.59[a]	0.54±0.58[a]
PO_4^3-P (mg L^{-1})	4.88±4.43[a]	4.68±4.51[a]	4.74±4.53[a]
DO (mg L^{-1})	4.25±0.74[a]	4.50±0.74[a]	3.89±0.89[a]
Alkalinity (mg L^{-1})	139.93±45.3[a]	129.97±28.8[a]	150.20±21.63[b]
TSS (ml L^{-1})	628.00±347.3[a]	577.12±372.7[a]	468.48±241.6[a]
Settleable solids (ml L^{-1})	27.04±17.2[a]	17.95±13.7[a]	15.71±11.1[a]
Transparency (cm)	10.25±6.45[a]	17.65±13.5[a]	17.63±12.82[a]
Turbidity (NTU)	277.05±161.2[a]	170.50±119.6[b]	152.10±105.3[b]
pH	7.35±0.35[a]	7.35±0.36[a]	7.34±0.33[a]
Temperature (^oC)	27.98±1.45[a]	28.10±1.47[a]	28.64±1.34[a]

Values are means of replicates ± standard deviation. Different superscripts in the same row indicate significant differences (P>0.05).

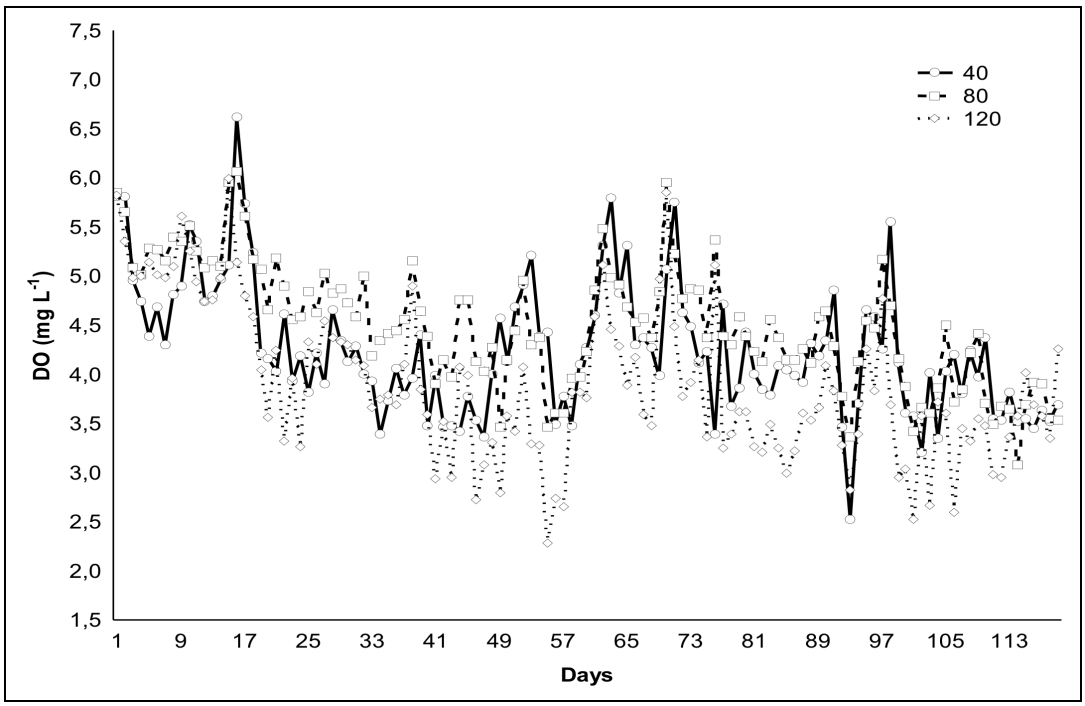

Fig. 1. Dissolved Oxygen (DO) concentration of *L. vannamei* in a bioflocs technology system under different water depths over 120 days.

Total suspended solids (TSS), settleable solids (SS), transparency and turbidity did not differ significantly in the treatments ($P>0.05$) (table 1). Figs. 2 and 3 summarize the changes in total suspended solids (TSS) and settleable solids (SS) over the 120-day period in the three treatments.

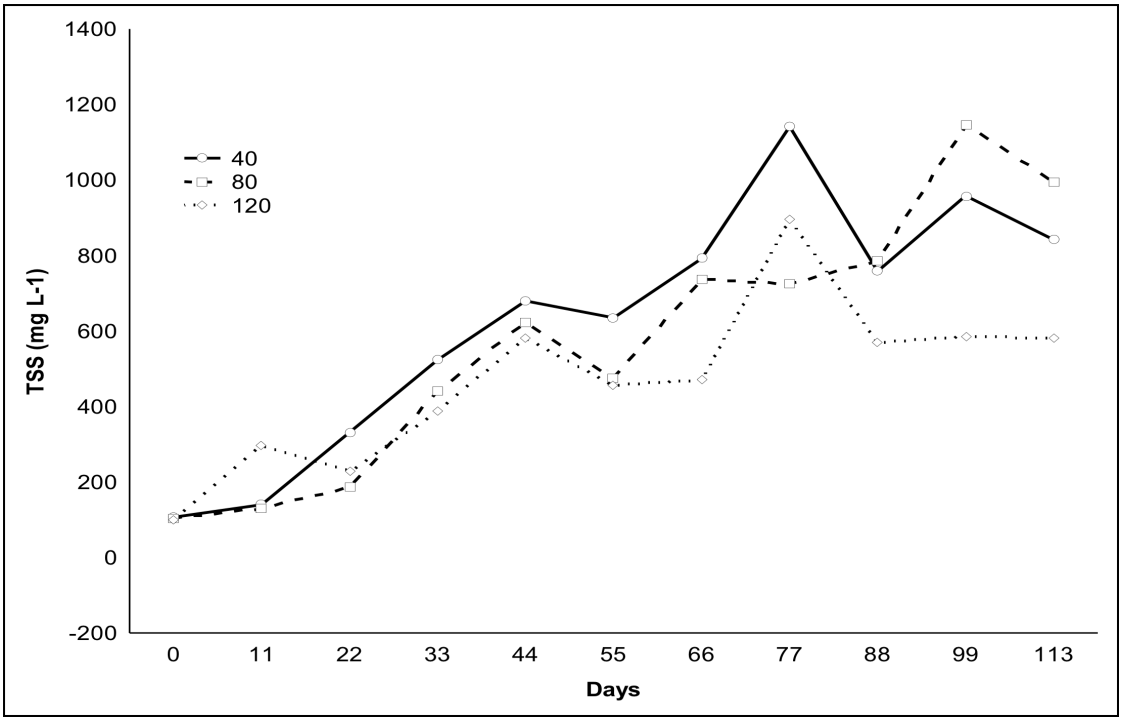

Fig. 2.Total suspended solids (TSS) concentration of *L. vannamei* in a bioflocs technology system under different water depths over 120 days.

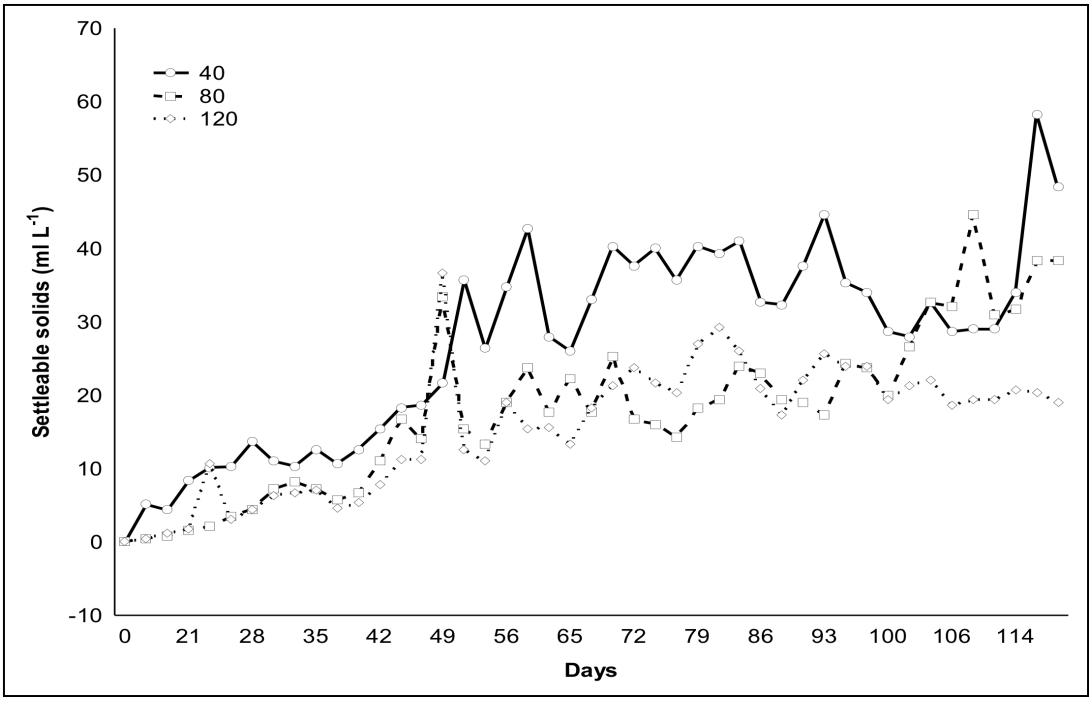

Fig. 3. Settleable solids (SS) of *L. vannamei* in a bioflocs technology system under different water depths over 120 days.

No significant differences (P>0.05) in nitrogenous compounds between treatments were showed over the culture period, except for nitrite, which differed significantly in the 0.8 treatment. Figs. 3 and 5 show temporal variation in total ammonia (TAN), nitrite (NO_2-N) and nitrate (NO_3-N), respectively. Nitrite concentrations fluctuated substantially in all treatments, in particular in the 0.4 treatment. Nitrate concentrations did not differ between treatments and the concentrations remained low during the study.

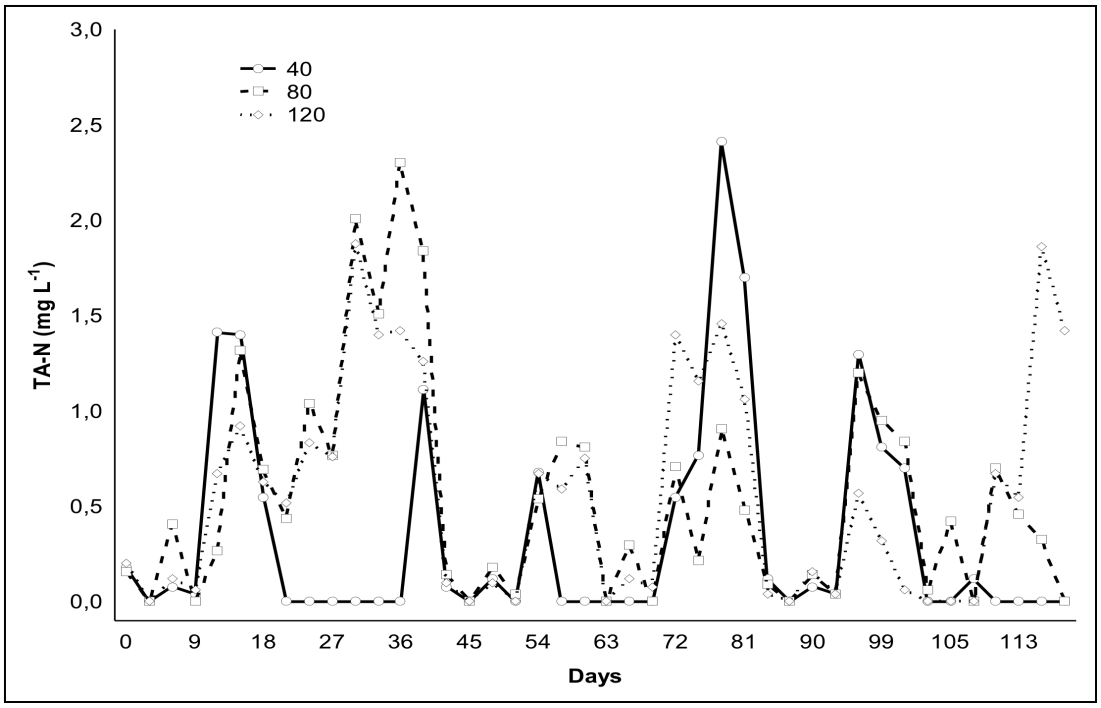

Fig. 4. Total ammonia nitrogen (TAN) concentration of *L. vannamei* in a bioflocs technology system under different water depths over 120 days.

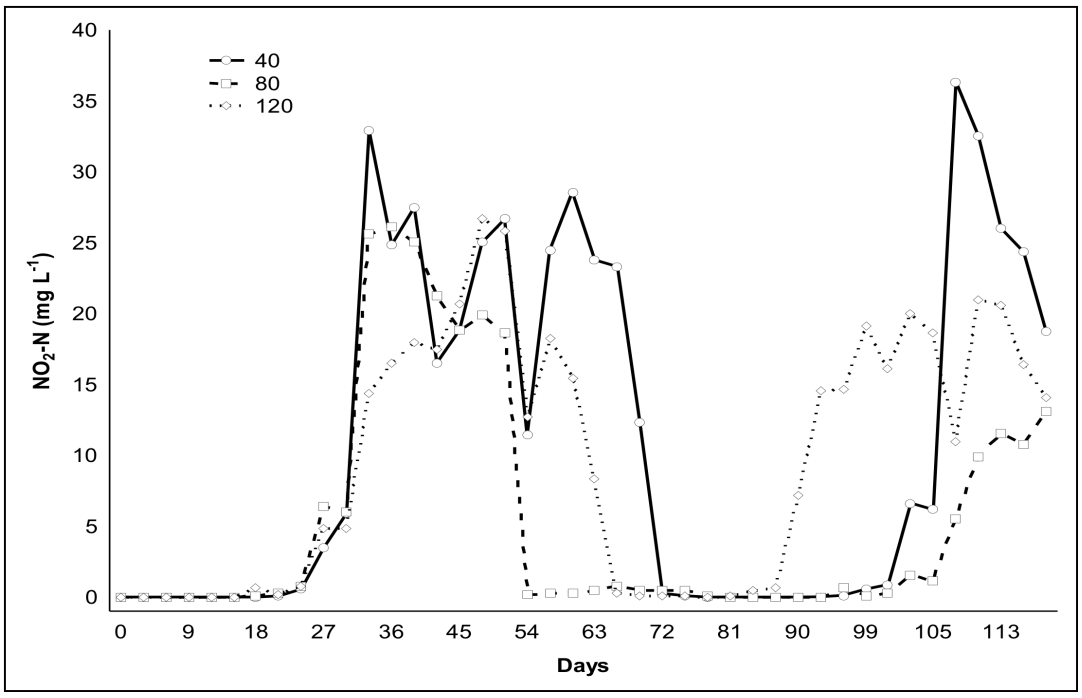

Fig. 5. Nitrite (NO_2-N) concentration of *L. vannamei* in a bioflocs technology system under different water depths over 120 days.

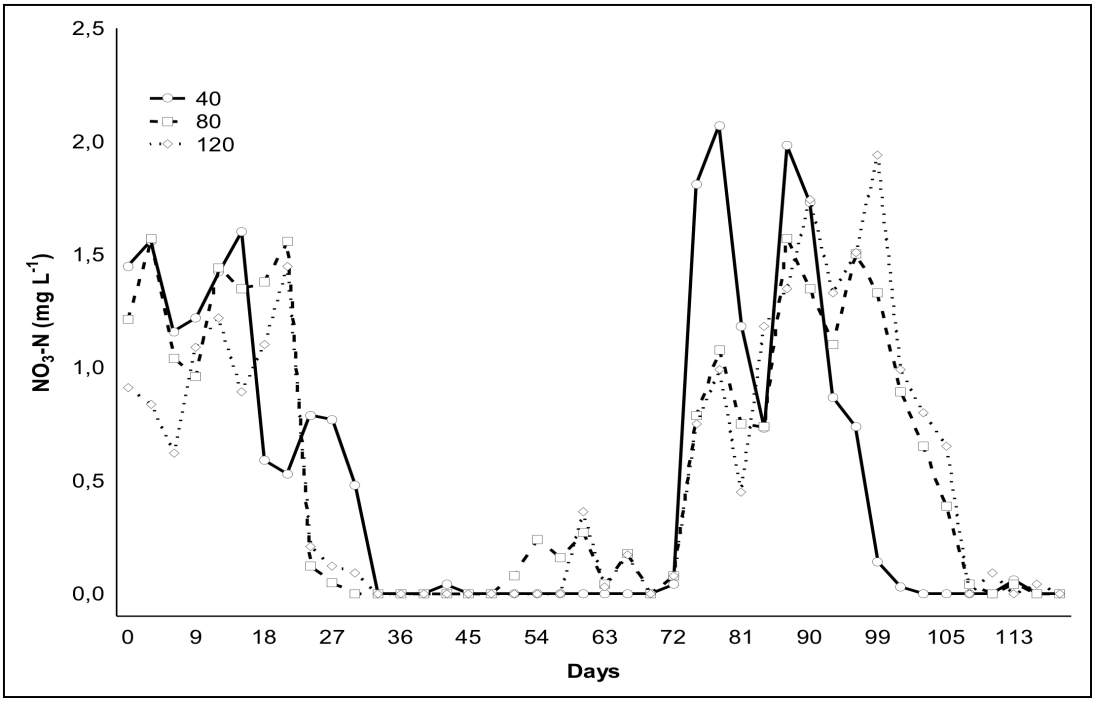

Fig. 6. Nitrate (NO_3-N) concentration of *L. vannamei* in a bioflocs technology system under different water depths over 120 days.

No significant differences (P>0.05) were observed for final weight, survival, WGR, FCR, final biomass and productivity (table 2). However, productivity (kg m^{-3}) (table 2) was highest in treatment 0.4 (P>0,05). The water used to produce 1.0 kg of shrimp over 120-day study was 118, 207 and 347 liters of water for the 0.4, 0.8 and 1.2 m respectively.

Table 2. Growth parameters of *L. vannamei* in a bioflocs technology system under different water depths over 120 days[1].

	Depth (m)		
	0.40	**0.80**	**1.20**
Initial weight (g)	0.08±0.02[a]	0.08±0.02[a]	0.08±0.02[a]
Final weight (g)	11.36±0.59[a]	11.43±1.12[a]	11.30±2.58[a]
Survival (%)	74.0±7.0[a]	84.6±13.6[a]	78.0±15.7[a]
WGR	0.66±0.03[a]	0.66±0.06[a]	0.65±0.15[a]
FCR	1.60±0.18[a]	1.53±0.15[a]	1.49±0.06[a]
Final biomass (kg)	118.32±14.6[a]	135.13±17.2[a]	121.12±20.2[a]
Prod. (kg m^{-2})	3.38±0.41[a]	3.86±0.49[a]	3.46±0.57[a]
Prod. (kg m^{-3})	8.45±1.04[a]	4.83±0.61[b]	2.88±0.47[c]
Water Use (L kg^{-1} shrimp)	119.6±15.7[a]	209.6±28.7[b]	352.7±53.9[c]

Values are means of replicates ± standard deviation. Different superscripts in the same row indicate significant differences (P<0.05).

4. DISCUSSION

The nitrogen compounds in systems with limited discharge are controlled by a combination of natural (nitrification and phytoplankton uptake, and incorporation into cell biomass by heterotrophic bacteria) and mechanical processes (oxygenation devices, aerators, destratifiers) (Hargreaves 2006). In the present study, the levels of ammonia increase throughout the 120 days of the trial (2.1 mg L^{-1}) however were within safe levels for *L. vannamei* (Frias-Espericueta et al. 1999; Lin and Chen 2001). In BFT system, the increase in ammonia values has been linked to the slow development of nitrifying bacteria or the increase of decomposition of organic matter (Cohen et al. 2005). According to Burford et al. (2003) variation in ammonia concentrations in systems without water exchange is usually observed and has limited impact on growth and survival of shrimp. Lin and Chen (2003) reported a 96h LC50 for exposure of *L. vannamei* juveniles to NO_2-N of 321.7 mg L^{-1} and recommended a ‘‘safe level” of exposure of less than 25.7 mg L^{-1} at 35 of salinity. In this study, high nitrite levels after the fourth week of the trial may have negatively impacted growth in all treatments, especially in the 0.4 treatment were it may have caused some mortality. According to Chen et al. (2006), the accumulation of nitrite has a negative influence on the growth and survival of aquaculture species. This accumulation has been observed in studies reared in BFT system and has been of great concern today (Cohen et al. 2005; Furtado et al. 2011; Maicá et al. 2012; Vinatea et al. 2010). An alternative to minimize the effect of nitrite is through the use of water from the harvest and reused for the subsequent cycle, this strategy has been used by several researchers with relative success (Krummenauer et al. 2011; Otoshi et al. 2009; Samocha et al. 2010). The low levels of nitrate could indicate delayed or limited oxidation of nitrite to nitrate by autotrophic bacteria over the 120 days of study. Probably this no nitrification is related to the

low levels (<3.0 mg L^{-1}) of oxygen that were recorded in this work (Fig. 1). According to Hargreaves (2006), the mineralization of organic matter and autotrophic nitrification require the maintenance of oxygen concentration above an optimal level for de organisms. The autotrophic bacteria that perform nitrification are obligate aerobes, which require oxygen to grow (Hagopian and Riley, 1998), therefore, if oxygen concentrations are low, the growth of bacteria can be restricted.

Water quality parameters were within desired ranges for shrimp health in terms of dissolved oxygen, pH, temperature and salinity (Van Wyk and Scarpa 1999). Moreover DO levels dropped with the increase of shrimp and bacterial biomasses. Samocha et al. (2010) suggest that a high shrimp biomass (>7.5 kg m^{-3}) can be maintained with only occasional oxygen supplementation. The same authors emphasize that the real-time DO data provided can be a valuable tool for the management of this system.

According to Avnimelech (2009), the total suspended solids and settling solids should not exceed 500 mg L^{-1} and 40 ml L^{-1} respectively in BFT systems. In the present work, the values of settling solids remained within the recommended range, except during the last week culture in the treatment 0.4, due to accumulation of particulate organic matter in low volume of water. Avnimelech (2009) warns that TSS concentration above 500 mg L^{-1} may interfere with adequate mixing of the water and can reduce the DO levels. TSS levels in all treatments were above for recommended for *L. vannamei* and did not appear to influence the DO concentrations in the water.

The survival rates of shrimps were similar among the treatments showing that different deeps of the tanks did not have a significant effect in this parameter. However, the shrimp survival was lower (74 %) in 0.40 treatment. The low survival can be attributed to the

high concentration of nitrite present in all treatments (>35.0 mg L^{-1} NO_2-N). Krummenauer et al. (2011) observed mortality in nitrite concentrations similar to the current study. The FCR values were similar to those observed by Otoshi et al. (2009) and Samocha et al. (2010, 2012) in studies with minimal water exchange in intensive raceways. Weekly growth rates (0.65–0.66 g) were lower to those reported for *L. vannamei* cultured in the same structure (0.85–0.92 g at the densities of 150–300 shrimp m^{-2}), however, with a similar density (450 shrimp m^{-2}) of the present study it was observed 0.47 g / week (Krummenauer et al. 2011). Also, working in the same experimental units, Gaona et al. (2012) in 120-day growout trial reported a WGR between 0.46 and 0.51 g / week while operating an investigation with a clarification process at stocking density of 250 shrimp m^{-2}. On the other hand, in study conducted at Texas Agrilife Research (Texas A&M university, USA), Samocha et al. (2010) has reported 1.36 g in the same time. Browdy and Moss (2005) at Waddell Mariculture Center and Oceanic Institute (USA), recorded 1.44 g and 1.47g / week respectively. Probably, the best results reported by these authors are associated with the quality of the feed used and strains of fast growth shrimp, which are developed in that country (Wyban 2009). The yield registered in 0.4 treatment (8.45 kg m^{-3}) is very closed to reported by Samocha et al. (2012) operating two raceways with 390 shrimps/m^{-2}, where after 106 days of culture the authors recorded 8.4 kg m^{-3}.

Traditional production practices require large volumes of water. In general, these system use from 20 to 64 m^3 of water to produce 1.0 kg of shrimp (Hopkins et al., 1993; Moss et al., 2001; Timmons and Losordo, 1994). On the other hand, BFT system can be performed with lower amount of water. In this work, the amount of water to produce 1.0 kg of shrimp ranged from 119 to 352 L kg^{-1}. These values are similar to those reported by other researchers that work with BFT system. For example, Samocha et al. (2010) in a 108-day growout study

conducted in 40 m^3 (68.5 m^2) raceways with 450 shrimp/m^3 used only 98 L of water to produce 1.0 kg of shrimps. In the same raceways, Samocha et al. (2012) at density of 500 shrimp/m^3 reported the use of 157 L of water to produce 1.0 kg of shrimp. At Oceanic Institute in Hawaii, in a 75x m^{-2}, obtained production of 7 kg/m^2 with an average growth rate of 1.8 g/week and FCR of 1.42. Otoshi et al. (2009) estimated a total use of 163 L water/kg shrimp produced. Venero et al. (2009) working at Waddell Mariculture Center, in a 271 m^2 raceway reported production of 6.92 kg/m^2 using only 60 L of 35 ppt saltwater to produce 1.0 kg of shrimp. These results confirm the possibility to use smaller amounts of water in BFT systems. Then, in this culture system it is likely to reduce pumping costs and the quantity of effluent generated. Also, These amounts of water could be reduced, particularly if the same salt water could be stored and used later to fill another raceway for a new production cycle.

5. CONCLUSION

Higher yields are feasible when raceways are operated with low volume of water. Also, it is possible to produce 8.45 kg of Brazilian market-sized shrimp (>11.0 g) per m^{-3} on BFT system in 17 weeks, using 119 L of water per kg of shrimp.

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CAPÍTULO IV

EFFECTS OF DIFFERENT AERATORS ON BIOFLOC FORMATION AND ITS CONSEQUENCES ON THE GROWTH AND SURVIVAL OF *Litopenaeus vannamei* REARED IN A BIOFLOC TECHNOLOGY SYSTEM

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Resumo

O objetivo deste estudo foi avaliar o efeito de diferentes tipos de aeração sobre as comunidades microbianas, a qualidade da água, a sobrevivência e crescimento de juvenis de *Litopenaeus vannamei* em um sistema super-intensivo sem renovação de água. O experimento foi realizado na Estação Marinha de Aquacultura, FURG, Rio Grande, RS. Foram testados três modelos de aeração: (1) Aerador propulsor (PR), (2) Aerador tipo chafariz (VP) e (3) Difusor de ar (Blower) (BL), com três repetições. Os tratamentos foram distribuídos aleatoriamente em nove tanques de 35.000 L cada, localizados em uma estufa retangular. A densidade de estocagem utilizada foi de 140 camarões/m^2. Os camarões foram alimentados três vezes ao dia com ração de 38% de proteína bruta, durante 35 dias. Durante o período experimental foram registrados ganho de peso, sobrevivência e taxa de conversão alimentar (TCA). Sólidos suspensos (SS), sólidos suspensos totais (SST) e os níveis de clorofila α foram medidos três vezes por semana. Além disso, foram coletadas amostras para quantificar os principais microrganismos presentes nos tanques, utilizando microscopia de epifluorescência. Os resultados foram submetidos à análise de variância (ANOVA) e, se as diferenças foram significativas, as médias foram comparadas pelo teste de Tukey (α=0,05). O peso médio final foi maior no tratamento com aerador propulsor ($P < 0,05$), porém a sobrevivência foi significativamente menor neste tratamento do que nos outros. Entre os parâmetros de qualidade da água, a concentração da amônia foi maior apenas no tratamento aerador propulsor. No tratamento blower, a formação de flocos foi significativamente maior ($P<0,05$). A biomassa final foi significativamente maior no tratamento blower e no tratamento chafariz. Os resultados sugerem que sistemas de ar difuso (blower) em tanques de cultivo intensivo pode melhorar a formação dos bioflocos e, consequentemente, obter melhor crescimento e produtividade dos camarões.

Abstract

The aim of this study was to evaluate the effects of different aerators on biofloc formation, microbial communities and water quality as well as their influence on the growth parameters of *Litopenaeus vannamei* juveniles raised in a biofloc technology system. The study was conducted with three treatments using three types of aerators: (1) PR, a ½ HP propeller-aspirator pump aerator; (2) VP, a ½ HP vertical pump aerator; and (3) BL, a 4 HP diffused-air-blower. The study was carried out at the Marine Station of Aquaculture, Federal University of Rio Grande (FURG), in a greenhouse with nine 35,000 L rectangular tanks. The temperature, salinity, dissolved oxygen (DO) and pH was recorded twice daily. Water samples were collected daily to quantify the concentration of total ammonia nitrogen. Analyses of nitrite, nitrate and phosphate concentrations were performed for water samples collected every five days. The settling solids (SS), total suspended solids (TSS) and chlorophyll α levels were measured three times a week. Moreover, samples were taken to quantify the main microorganisms present in the tanks, using epifluorescence microscopy. Throughout the experiment, the temperature, pH, salinity and alkalinity were kept within the recommended levels for the growth and survival of *L. vannamei*. The propeller treatment showed a concentration of total ammonia above the recommended level and lower densities of ciliates and flagellates, probably because of inadequate biofloc formation in this treatment that hampered the establishment of ammonium-oxidizing bacteria. Rotifers were recorded only in the blower treatment. The shrimp final weight was higher in the blower and in the propeller treatments. However, survival was lower in the propeller treatment than in the other treatments. The results suggest that of diffused air systems (blower) can improve the formation of the bioflocos

Introduction

The aquaculture technique of biofloc technology systems (BFT) uses minimal or no water exchange and can improve water quality through the formation of a microbial community. Therefore, BFT represents a new alternative for shrimp cultures, in accord with the concepts of an environmentally friendly aquaculture.

The BFT system reduces the use of water, minimizing the emission of effluents and environmental impacts, and enhances production, with the possibility of increasing stocking densities (Hopkins et al., 1995; Browdy et al., 2001). In this system, biofloc formation is stimulated by the addition of a carbon source to maintain a C:N ratio ranging from 12 to 20:1. The microbial flocs consist of a conglomerate of different microorganisms, such as bacteria, microalgae, protozoa and zooplankton (Avnimelech, 2009). In addition, the bioflocs serve as a complement in the diet of animals that use the natural productivity stimulated in ponds as additionally feed (McIntosh et al., 2000; Moss et al., 2001). Wasielesky et al. (2006) and Otoshi et al. (2011) analyzed the effects of natural productivity on juveniles of *Litopenaeus vannamei* and confirmed the positive effects of culture environment on the growth, feed consumption, weight gain, feed conversion and survival of shrimps. Thus, mixing intensity parameters, dissolved oxygen, pH, temperature and organic carbon sources may require adjustments to achieve good aggregation and high quality of bioflocs with optimal conditions for the growth of the organisms (De Schryver et al., 2008). One requirement for the use of bioflocs is the high oxygenation of the culture tanks, which is provided by aeration devices. Thus, the choice of such a device may be crucial to obtaim high productivity in the system. According to Avnimelech (2009), aerators are inserted into cultures with several goals: (a) to supply oxygen for livestock beyond their limitations and thus maintain elevated stocking densities and

productivity; (b) to distribute the oxygen into the culture tanks horizontally and vertically; (c) to mix the water column; and (d) and to oxygenate the sediment coverage.

In addition to affect the growth and performance of cultured animals, low oxygen concentrations in BFT systems may influence the microbial communities forming bioflocs (Ray et al., 2010). Furthermore, the processes of nitrogen assimilation and nitrification, carried out by bacteria, consume oxygen and reduce the alkalinity of the system, such that it is sometimes necessary to supplement the cultures with oxygen and carbonate (Ebeling et al., 2006; Hargreaves, 2006).

The formation of microbial aggregates (bioflocs) is dependent upon physical, chemical and biological interactions. In early stages of a culture, there is an increase in the abundance of bacteria that use the dissolved organic matter present in the environment for their growth (Biddanda, 1985). Thus, because of the adherent mucus production by these bacteria, there is an increase in the size of bioflocs caused by aggregation of the particles. Then, the abundance of other microorganisms may increase, such as flagellates, ciliates and amoeboid forms, because most of these graze upon the bacteria (Biddanda and Pomeroy, 1988). Even the cultured organisms, such as crustaceans and fish, can affect the dynamics of aggregation, independently of particle consumption (Dilling and Alldredge, 2000). Moreover, the addition of feed generates products that will be metabolized, increasing the number of bacteria if enough oxygen is available in the water. Therefore, the activity of microbial communities is defined by the rates of mixing and oxygenation generated by aeration processes (Avnimelech, 2009).

In this context, it is of great significance to evaluate the performance of the aerators on the oxygenation, water-column turbulence and formation of microbial aggregates to achieve higher productivity and better performance in BFT systems. The aim of this study was to

evaluate the effects of different aeration types available in the market on biofloc formation, microbial community and water quality and the influence of these factors on the growth parameters of *Litopenaeus vannamei* juveniles raised in a biofloc technology culture system.

Methods

Culture Conditions

The 33-day growout study was performed at the Marine Station of Aquaculture, Federal University of Rio Grande (FURG), Southern Brazil (32°12´16 S, 52°10´38 W) in a greenhouse with nine 35,000 L rectangular tanks (35 m², each) covered with a liner (EPDM, 1.5 mm). *Litopenaeus vannamei* post larvae (PL) were obtained from Aquatec®, Canguaretama, Rio Grande do Norte State, Brazil, and were raised for 60 days until each individual reached 4.3 ± 0.93 g in a 70 m^3 tank operated with no water exchange. The nursery was stocked (2,000 individuals per m^2) with approximately 140,000 (10-day-old) PL. The shrimp were fed with a commercial 40%-protein feed (0.8-1.2 mm, Guabi®, Campinas, SP, Brazil).

The study consisted of three treatments with three replicates each, in which three types of aerators were tested: (1) PR, a ½ HP propeller-aspirator pump aerator; (2) VP, a ½ HP vertical pump aerator; and (3) BL, a 4 HP diffusion-air blower. The tanks of the BL treatment were equipped with 35 air stones (10 cm length each, 1 stone per m², placed at the bottom of the tank), connected with 20 mm PVC pipes and powered using a 4 HP air blower. The stocking density was 140 shrimps m^{-2} (approximately 4900 shrimps per tank). The tanks were filled with filtered seawater (salinity 30), treated with a chlorine solution (10 ppm, measured immediately after the chlorine addition) and de-chlorinated with ascorbic acid powder (1 gram per 1000 L). Structures with vertical substrates (Needlona™, Cachoeirinha, Rio Grande do Sul State, Brazil) were used to increase the surface area (150% front and back) available for nitrifying bacteria

colonization. The greenhouse was covered with a 50% light attenuation shade cloth to avoid high temperatures during the culturing period. The organic fertilization began with the addition of 6.0 g of carbon (molasses) for each 1.0 g of total ammonium nitrogen (TA-N) in the water. This procedure followed the method described by Ebeling et al. (2006) and Avnimelech (2009). When necessary, corrections of pH were made by adding 700 g of hydrated lime ($Ca(OH)_2$) to maintain pH values above seven.

Physical and Chemical factors

The water temperature, salinity, dissolved oxygen (DO) and pH were recorded twice a day (0800 and 1700 h) using a YSI 556 multiparameter probe (YSI Inc., Yellow Springs, Ohio, United States). The water transparency was determined using a Secchi disc. Water samples were collected daily to quantify the concentration of total ammonia nitrogen (TA-N) (UNESCO 1983). Analyses of the nitrite (NO_2-N) nitrate (NO_3-N) and phosphate (PO_4^{-3}-P) concentrations were performed every five days, following the methods described by Strickland and Parsons (1972). The alkalinity was determined according to the method described in APHA (1989). The turbidity was measured once a week using a turbidimeter (Hach 2100P Hack Company, Loveland, CO, United States). The volume of settling solids was measured using an Imhoff cone and was based on the volume of the flocs in 1 L after 15–20 min of sedimentation (Avnimelech 2009). The levels of total suspended solids (TSS) (particles larger than 45 μm) were determined according to Strickland and Parsons (1972).

Chlorophyll a and the microbial community

An analysis of the chlorophyll *a* concentration was carried out weekly in 20 ml samples collected from each tank. This volume was concentrated on fiberglass filters (Whatman GF/F 45 μm) in a dark room, and the filters were stored with 90% acetone in dark glass bottles at –12

°C. After 24 hours, the concentration of chlorophyll *a* was determined using a calibrated Turner TD700 fluorometer (Welschmeyer 1994).

Water samples (50 ml) from the treatments were collected every 3 days and preserved with borate buffered formalin (4% v/v) for further analysis of the microbial floc composition (Thompson et al. 2002). The abundances of ciliates and rotifers were determined in 2.1 mL samples poured into sedimentation chambers using an Olympus inverted light microscope equipped with phase contrast (Utermöhl 1958). For bacterial and flagellate counts, 0.1 mL sub-samples were concentrated on darkened polycarbonate membrane filters (Nuclepore, 0.2 μm), stained with the fluorochrome Acridine Orange (5 μg mL^{-1}) and counted using a Zeiss Axiolplan epifluorescence microscope (Thornwood, NY) equipped with a 487 701 light filter set (BP365/11; FT 395; LP 397) at 1000x final magnification (Porter and Feig, 1980).

Shrimp Stocking, Feeding and Monitoring

The shrimp were fed three times a day with a commercial diet (Active 38% CP, 2 mm, Guabi®, Campinas, SP, Brazil), using feed trays (10 cm diameter, one tray per tank). The feeding rate followed the method proposed by Jory et al. (2001). Initially, the feeding rate was 10% of the total shrimp biomass and was later adjusted according to the consumption.

Every week, 60 shrimps were randomly sampled from each tank and individually weighed; the wet weight was individually measured using a digital scale accurate to 0.01 g (Marte®científica AS2000, Santa Rita do Sapucaí, MG, Brazil). At the end of the culture cycle, 200 shrimp were individually weighed, and the survival was estimated from the total harvest weight. The weekly growth rate (WGR) was determined as follows: WGR = (final weight/number of weeks of culture). The feed conversion ratio (FCR) was calculated as FCR = offered feed/biomass increment. The survival was calculated as S (%) = [(final biomass/average

individual weight)/number of individuals stocked)] x 100. The productivity was calculated as Prod = (final biomass/tank volume).

Statistical Analysis

Significant differences ($P < 0.05$) in the zootechnical performance and water parameters were analyzed using a one-way ANOVA. Tukey's multiple-range test was applied when significant differences were detected. All of the tests were performed after confirmation of the homogeneity of variance (Levene's test) and normality of the data distribution (Kolmogorov-Smirnov test). To satisfy the ANOVA assumptions, the survival data were arcsine/square-root transformed using a constant exponent (arcsine x 0.5) (Zar, 1996).

Results

The mean values (± SD) of the physical and chemical parameters during the experiment with the blower (BL), vertical pump (VP) and propeller (PR) are shown in Table 1.

Table 1. Mean ± SD of the physical, chemical and nitrogen compound characteristics of the water from the treatments with the blower, vertical pump and propeller over the culture of *Litopenaeus vannamei* in systems without water exchange.

Treatment	**Blower**	**Vertical pump**	**Propeller**
Temperature (°C)	29.46±2.65[a]	28.38±2.1[a]	30.79±2.95[a]
DO (mg L^{-1})	4.91±0.45[a]	4.49±0.46[a]	4.50±0.48[a]
pH	7.36±0.25[a]	7.46±0.31[a]	7.46±0.28[a]
Salinity	36±1.69[a]	35.74±2.57[a]	35.30±2.75[a]
Alkalinity	133.4±28.7[a]	185.90±53.1[b]	229.70±68.8[c]
Phosphate	3.67± 3.19[a]	2.70±1.58[a]	2.94±1.66[a]
Total ammonia (N-TAN) (mg L^{-1})	1.00±1.20[a]	2.13±2.22[a]	5.91±6.08[b]
Nitrite (mg L^{-1})	8.97±9.59[a]	4.54±4.61[a]	1.97±2.24[b]
Nitrate (mg L^{-1})	14.88±9.60[a]	4.53±3.59[b]	3.97±2.38[b]

The values are the means of replicates ± standard deviation. Different superscripts in the same row indicate significant differences ($P<0.05$).

The temperature throughout the experimental period did not differ significantly among the three treatments ($P>0.05$). The maximum and minimum temperatures in the BL treatment were 26.3 and 31.6 ºC, respectively. In the VP treatment, the minimum was 26.55 °C, and the maximum was 30.75 °C. For the PR treatment, the minimum and maximum temperatures recorded were 28.5 and 34.4 ºC, respectively.

Similarly, no significant differences were detected for the values of dissolved oxygen in the water of all of the tanks. For the BL treatment, the minimum concentration was 3.07 mg L^{-1}, and the maximum concentration was 6.4 mg L^{-1}. In the VP treatment, the minimum value recorded was 2.13 mg L^{-1}, and the maximum concentration was 6.7 mg L^{-1}. The propeller treatment showed the lowest minimum concentration of all of the treatments (0.82 mg L^{-1}), and the highest concentration recorded was 6.4 mg L^{-1}.

The total ammonia concentrations did not differ significantly between the BL and VP treatments; however, both of those treatments differed statistically ($P<0.05$) from the PR treatment, which presented the highest values during the culturing period (Table 1; Fig. 1). The nitrite levels were significantly higher ($P<0.05$) in the BL treatment than in the PR treatment (Fig. 2). Similarly, for nitrate, the highest values were recorded in the BL treatment (Fig. 3). This treatment differed statistically ($p<0.05$) from the VP and PR treatments, which did not significantly differ.

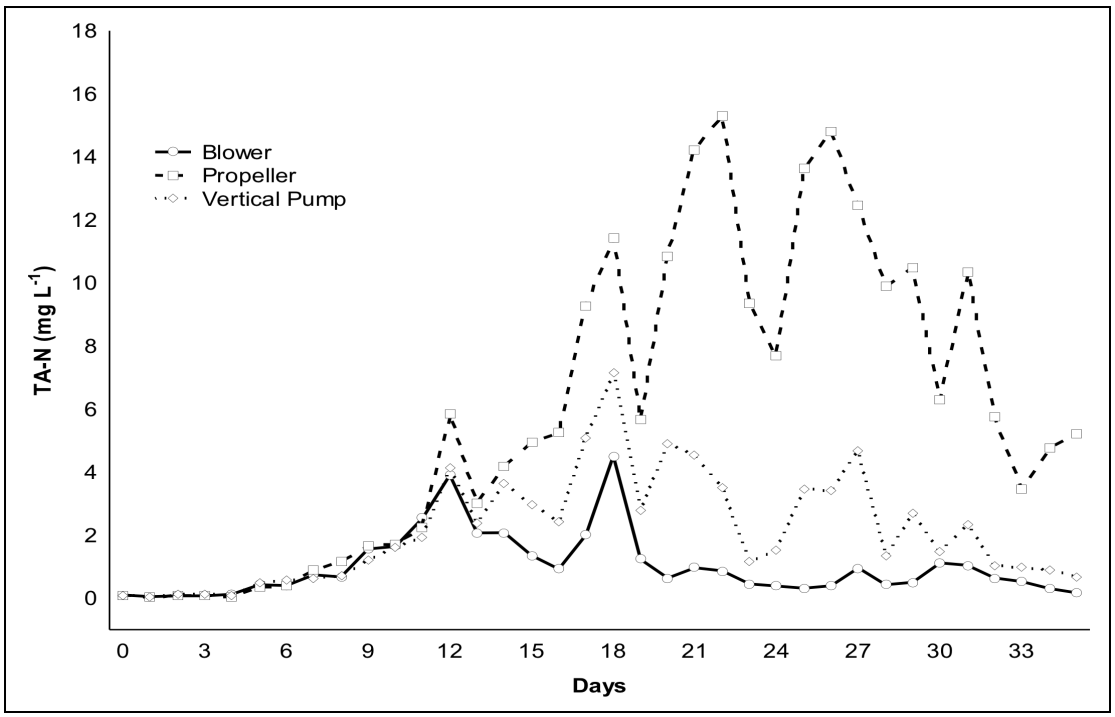

Figure 1. Average levels of total ammonia recorded during the 33 experimental days in *Litopenaeus vannamei* BFT cultures with different types of aerators.

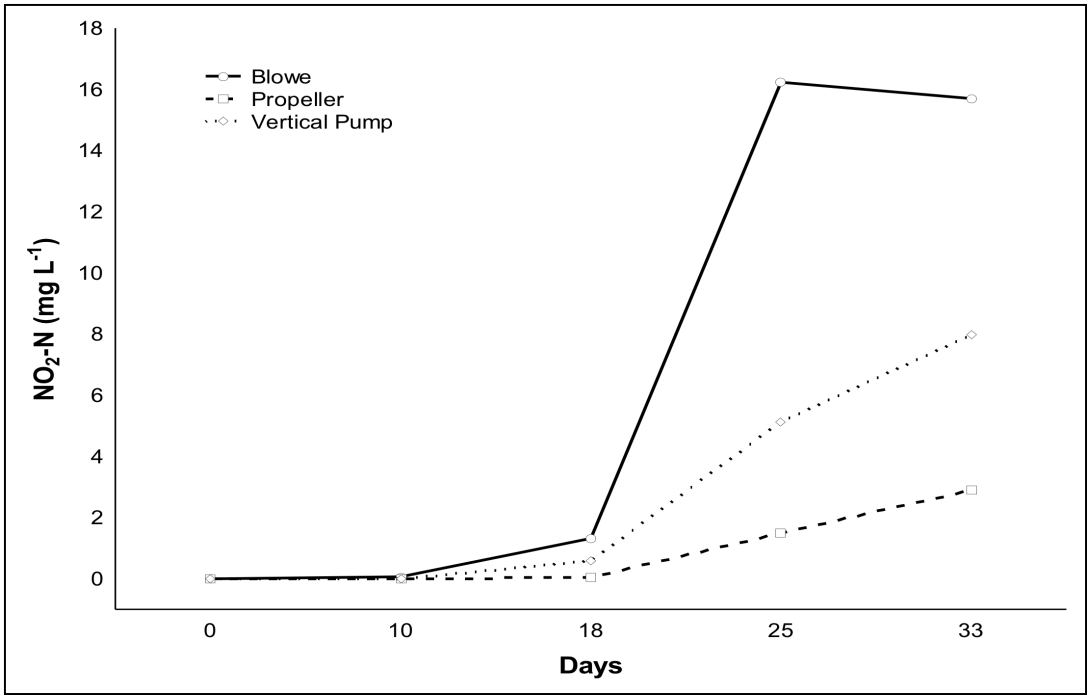

Figure 2. Average levels of nitrite recorded during 33 experimental days in *Litopenaeus vannamei* BFT cultures with different types of aerators.

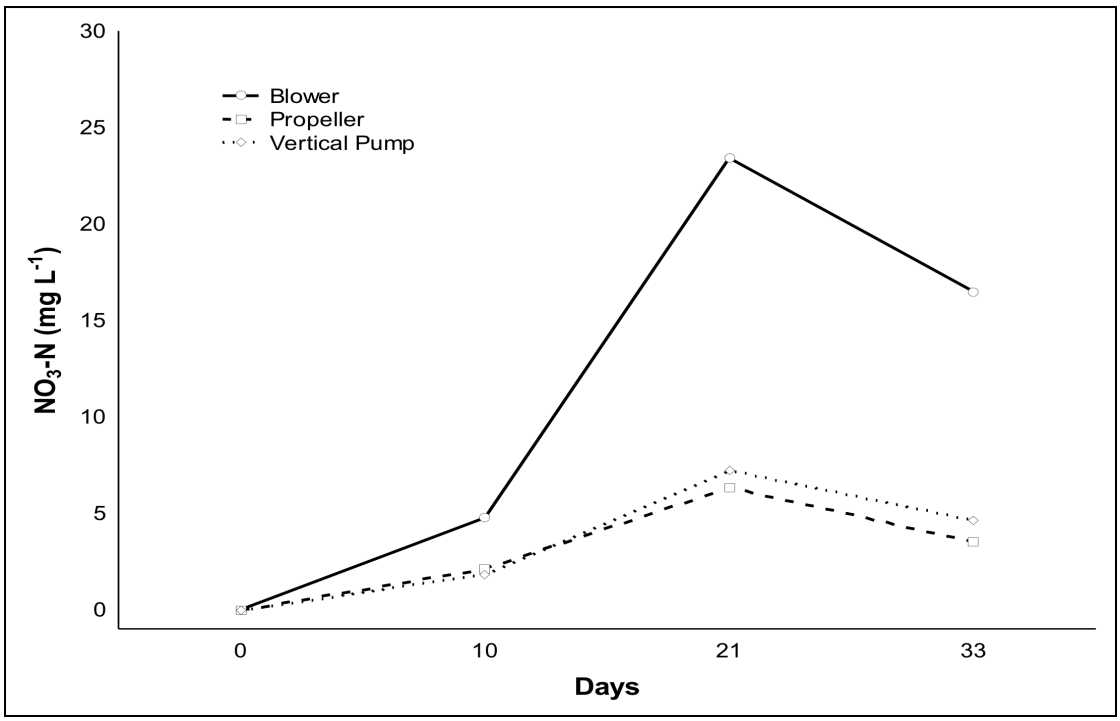

Figure 3. Average levels of nitrate recorded during 33 experimental days in *Litopenaeus vannamei* BFT cultures with different types of aerators.

The results from the settling solids showed statistically higher values in the BL treatment (20.5 ± 28.94 ml L^{-1}) than the other treatments, with 3.65 (± 5.79) ml L^{-1} and 3.03 (± 4.49) ml L^{-1} in the VP and PR treatments, respectively (Fig. 4). Similarly, for the total suspended solids, the BL treatment also had significantly higher values (460.50 ± 174.31 mg L^{-1}) than the VP and PR treatments, which did not significantly differ (fig. 5).

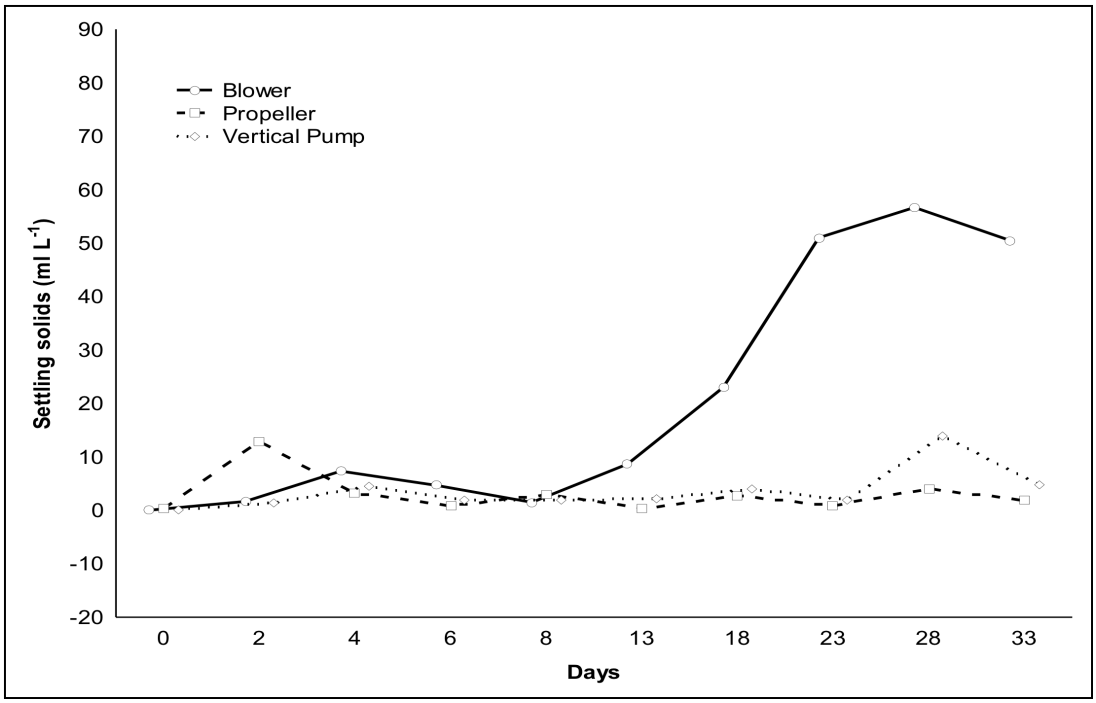

Figure 4. Settling solids recorded during 33 experimental days in *Litopenaeus vannamei* BFT cultures with different types of aerators.

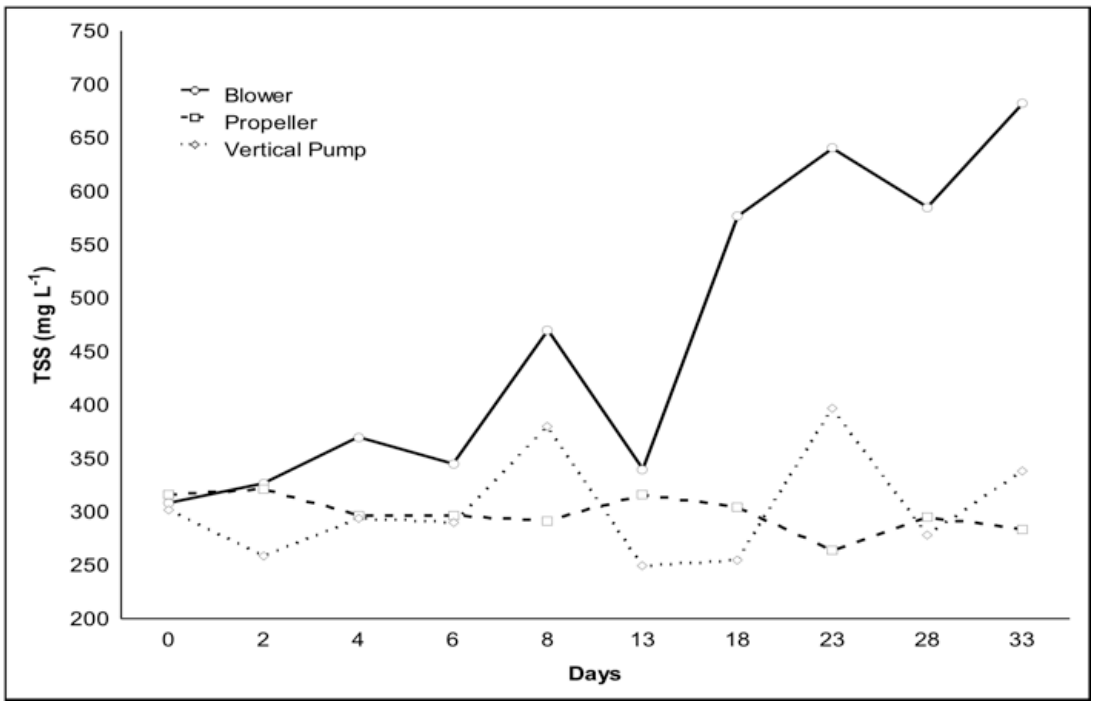

Figure 5. Total suspended solid levels recorded during 33 experimental days in *Litopenaeus vannamei* BFT cultures with different types of aerators.

The values of chlorophyll *a* showed no significant differences among the treatments, although the BL treatment had slightly higher concentrations (93.67 ± 34.21 mg L^{-1}) than the VP and PR treatments, which had 78.75 (±38.92) mg L^{-1} and 70.08 (±26.28) mg L^{-1}, respectively (fig. 6). In the BL treatment, a significant increase in the chlorophyll *a* concentrations occurred on the 6th experimental day, and on the 13th day, the highest concentration of chlorophyll *a* throughout the experiment was recorded. The chlorophyll *a* levels were statistically different ($p<0.05$) from the values of the initial day until the 23rd day of the experiment.

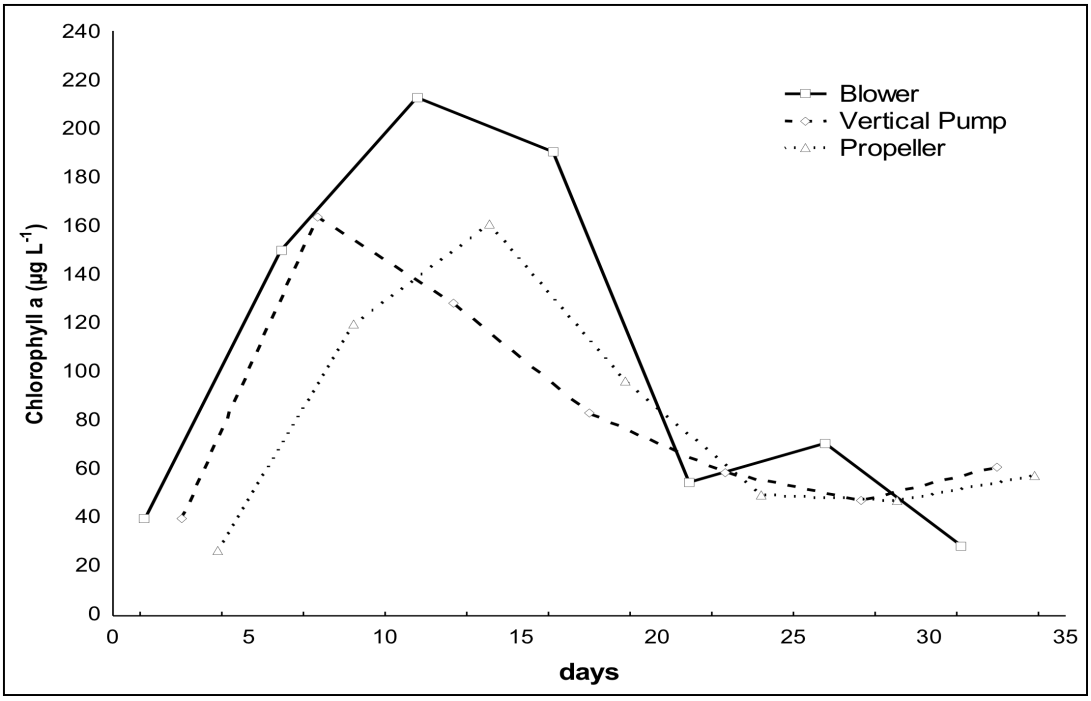

Figure 6. Average chlorophyll *a* concentrations recorded during 33 experimental days in *Litopenaeus vannamei* BFT cultures with different types of aeration.

Table 2 summarizes the ciliate, flagellate and diatom abundances observed in the different treatments and days. The densities of ciliates and flagellates were constant throughout the culture in the BL and PR treatments; however, the density was lower in the PR treatment. In the VP treatment, the densities of ciliates and flagellates had a temporal variation, with a strong decrease after the 8th day. However, from the 18th day, there was an increase, with the abundances reaching tenfold greater than those in the other treatments at the 23rd day. The rotifers were observed only in the BL treatment in the last few days of the culture (8.80 ± 8.23 organisms x10^{3} mL^{-1}).

Table 2. Abundances of ciliates, flagellates and diatoms (mean ± SD) in *Litopenaeus vannamei* BFT culture water aerated with blower, vertical pump and propeller.

Day	Treatment	**Ciliates (org. x10^5 mL^{-1})**	**Flagellates (org. x10^3 mL^{-1})**	**Diatom (org. x10^3 mL^{-1})**
8	Blower	5.51 ± 3.17^a	1.61 ± 0.55^a	0.34 ± 0.25^a
	Vertical Pump	8.28 ± 2.49^a	0.32 ± 0.20^b	11.46 ± 5.46^b
	Propeller	2.30 ± 1.69^b	0.35 ± 0.21^b	0.21 ± 0.17^a
13	Blower	3.72 ± 2.75^a	0.14 ± 0.12^a	0.25 ± 0.17^a
	Vertical Pump	0.32 ± 0.20^b	0.02 ± 0.01^b	0.32 ± 0.15^a
	Propeller	0.43 ± 0.17^b	0.02 ± 0.02^b	0.04 ± 0.01^b
18	Blower	5.29 ± 2.66^a	0.78 ± 0.31^a	0.44 ± 0.26^a
	Vertical Pump	3.42 ± 2.71^a	5.24 ± 2.78^b	0.11 ± 0.12^b
	Propeller	0.19 ± 0.13^b	0.04 ± 0.02^c	0.07 ± 0.02^b
23	Blower	1.62 ± 1.56^a	1.58 ± 0.80^a	0.30 ± 0.21^a
	Vertical Pump	12.72 ± 5.47^b	1.90 ± 0.57^a	0.94 ± 0.36^b
	Propeller	1.25 ± 0.59^a	0.42 ± 0.13^b	0.45 ± 0.18^a

Different superscripts in the same collum indicate significant differences ($P<0.05$).

The abundance of coccoid bacteria did not differ significantly among the treatments until day 23. On the 28th day, the PR treatment had significantly higher densities than the BL and VP treatments. For the bacilli, no significant differences were observed until the 23rd day, when the BL treatment became significantly different than PR treatment; however, the VP treatment showed no significant difference to the other two treatments. There were high numbers of amoeboid organisms in the treatments with the blower and vertical pump. For filamentous cyanobacteria, there were no significant differences among the treatments until the 28th day of the experiment (table 3).

Table 3. Densities of coccoid morphotypes of bacteria, bacilli, filamentous cyanobacteria and amoeboid organisms C present in *Litopenaeus vannamei* BFT culture water aerated with blower, vertical pump and propeller.

Day	Treatment	**Coccoid (org. x10^{7} mL^{-1})**	**Bacillus (org. x10^{6} mL^{-1})**	**Filamentous cyanobacteria (org. x10^{6} mL^{-1})**	**Amoebae (org. x10^{5} mL^{-1})**
13	Blower	0.09±0.05 [a]	0.69±0.11 [a]	2.81±2.33 [a]	0.24±0.22 [a]
	Vertical Pump	0.35±0.06 [b]	0.36±0.11 [b]	4.32±1.16 [a]	1.52±2.20 [b]
	Propeller	0.30±0.26 [b]	6.11±0.14 [c]	3.04±1.73 [a]	ND
18	Blower	0.33±0.27 [a]	1.24±1.26 [a]	3.75±4.38 [a]	0.37±0.16 [a]
	Vertical Pump	0.47±0.29 [a]	1.04±0.83 [a]	5.33±1.66 [a]	4.27±6.24 [b]
	Propeller	0.57±0.43 [a]	1.54±1.31 [a]	3.45±1.15 [a]	ND
23	Blower	0.54±0.18 [a]	0.58±0.25 [a]	5.48±3.27 [a]	0.04±0.06 [a]
	Vertical Pump	0.41±0.17 [a]	1.36±0.71 [a]	4.99±1.52 [a]	2.65±1.06 [a]
	Propeller	0.93±0.69 [a]	2.49±1.44 [a]	7.68±413 [a]	ND
28	Blower	0.54±0.15 [a]	4.84±6.73 [a]	4.66±1.69 [a]	0.36±0.41 [a]
	Vertical Pump	1.00±0.27 [b]	4.51±1.46 [a]	10.01±8.12 [a]	4.26±6.03 [b]
	Propeller	2.08±0.66 [c]	4.08±1.65 [a]	7.22±3.40 [a]	ND
33	Blower	0.79±0.23 [a]	2.59±1.39 [a]	5.30±5.13 [a]	0.67±0.62 [a]
	Vertical Pump	1.03±0.33 [b]	4.55±1.08 [b]	9.40±2.78 [b]	2.16±2.69 [b]
	Propeller	5.33±4.96 [c]	6.03±4.20 [b]	11.39±4.66 [b]	ND

[1]Values are means of replicates ± standard deviation. Different superscripts in the same column, for the same day, indicate significant differences ($P<0.05$).

The main results regarding the growth performance of the shrimp are presented in Table 4. The final weights of the shrimp did not differ statistically differ ($p>0.05$) between the treatments with the blower or propeller; however, the weights in both of those treatments were significantly higher than the final weight observed in the vertical pump treatment. The survival and final biomass were significantly lower ($p<0.05$) in the propeller treatment.

Table 4. Results (Mean±SD) of the growth performance of shrimp *Litopenaeus vannamei* in treatments with a blower, propeller and vertical pump in systems without water exchange.

	Aerator type		
	Blower	Propeller	Vertical Pump
Days of culture	35	35	35
Initial weight (g)	4.30±0.93[a]	4.30±0.93[a]	4.30±0.93[a]
Survival (%)	86.0±3.0[a]	55.0±18.3[b]	92.3±5.68[a]
WGR (g .week^{-1})	1.73±0.1[a]	1.75±0.07[a]	1.29±0.32[a]
Final weight (g)	12.96±2.63[a]	12.81±2.21[a]	10.93±2.66[b]
FCR	1.71±0.15[ab]	1.99±0.21[b]	1.56± 0.17[a]
Productivity (kg m^{-2})	1.55±0.02[a]	0.98±0.33[a]	1.41±0.23[a]

Different superscripts in the same row indicate significant differences (P<0.05).

Discussion

In different culturing systems, the management of water quality parameters is extremely important. In BFT cultures with minimal or no water exchange, such management is greater because the water quality is strongly influenced by the rates of respiration and excretion of the cultivated livestock in high stocking densities as well by the respiratory processes of the microbial community present in the water (Vinatea et al., 2010). The microbial community may have a positive effect, such as improving the water quality and enhancing the productivity in the culture system by serving as a complementary food source (Avnimelech et al., 1995; Wasielesky et al. 2006).

In this study, the temperature was maintained between 28 and 30 °C in all treatments, which probably did not affect the survival of shrimp since temperature remained within the optimal ranges for the growth of the *L. vannamei*. According Ponce-Palafox et al. (1997), the highest growth rates for *L. vannamei* are obtained when the water temperature varies from 25 to 35 °C. Usually, the comfort zone for dissolved oxygen concentrations in shrimp farms is higher

than 5 mg L^{-1}. Prolonged periods of exposure to concentrations below 1.5 mg L^{-1} of DO can be lethal for the shrimp; however, when exposed to these low concentrations for short periods, shrimp can still survive (Van Wyk and Scarpa, 1999). In this study, the mean oxygen concentrations recorded in all treatments remained below those recommended by the same author and might have affected the growth of the shrimp, especially in the propeller treatment, where low DO (0.82 mg L^{-1}) coupled with high temperatures (34.4 ºC) and probably negatively affect the shrimp survival.

Ammonia is the major excretion product of aquatic organisms. Accumulation of ammonia in the water of a culture can deteriorate the water quality, reduce growth and increase the oxygen uptake by the cultured organisms or even to cause high mortality (Wickins, 1976). According to Lin and Chen (2001), the safe level of ammonia for juveniles of *L. vannamei* in the range of salinity used in this study is 3.55 to 3.95 mg L^{-1}, and the toxicity of this compound increases with exposure time. In this trial, the ammonia concentrations for the treatments with the blower and vertical pump remained within this range, while the mean concentrations reported for the propeller treatment (5.91 ± 6.08 mg L^{-1}) exceeded these values, indicating that the growth and survival of the shrimp were affected by high concentrations of this compound. Nitrite is an intermediate product of nitrification. According Lin and Chen (2003), the safe level for *L. vannamei* in salinities similar to those in this study is in the range of 15.2 to 25.7 mg L^{-1}. The values reported in this study are within the range recommended by these authors. Nitrate is the least toxic nitrogen compound, and according to Kuhn et al. (2010), the performance of *L. vannamei* begins to be affected at nitrate concentrations over 220 mg L^{-1}, in lower salinity. The values recorded in this study were below the values cited by the authors, in higher salinity.

It is clear from the data presented in this study that the ammonia-oxidizing bacteria were affected by the smaller amount and probably the smaller size of particles in the propeller treatment. It is well known that ammonium and nitrite-oxidizing bacteria are normally present on aggregates, forming colonies (Delatolla et al., 2009; Vlaeminck et al., 2010), and the establishment of these colonies may be influenced by the particle size, which is determined by the water shear rate. Larsen et al. (2008) showed that increased shear rates in activated sludge systems formed particles smaller than the medium size of a colony (13–22.5 μm) of the ammonia-oxidizing bacteria *Nitrosomonas oligotropha*, leading to a low rate of ammonia oxidation, similar to the rate observed in our study.

The smaller values of nitrate in the propeller and vertical pump treatments indicate that the nitrite-oxidizing bacteria were also affected by the reduction of particle size and quantity. Problably, the water turbulence and propeller action may have contributed to the decrease in particle size in the biofloc system, hampering the establishment of nitrifying bacteria.

The settling solids (SS) in an ImHoff cone are the volume occupied by 1.0 g of flocculated material in 1.0 L of water (De Schriver et al., 2008). The mixing intensity imposed by different aerators can affect the rate of aggregation and disaggregation of flocs (Chaignon et al., 2002; Spiecer and Pratsinis, 1996). In the present study, high values from settling solids were recorded only in the blower treatment. The total suspended solid levels (TSS) recommended for BFT systems is in the range of 200-500 $mg.L^{-1}$ (Avnimelech, 2009; Samocha et al., 2007). De Schriver et al. (2008) mention that TSS are determined by the amount of particulate matter present in a water sample from a culture. The amounts recorded in this study are close to the recommended range, and the highest rates of particulate matter were observed in the treatment using diffused air (the blower), presenting the same pattern as that found for the

settling solids. These results indicate that there was a higher aggregation rate in the blower treatment, while the propeller and vertical pump disrupted flocs.

The lower abundances of ciliate and flagellates found in the PR treatment showed a possible inhibition of the development of microorganisms that form bioflocs by this type of aerator. Furthermore, the oscillations in the abundance of ciliates observed in the other treatments suggested possible shrimp predation on protozoa. As demonstrated by Thompson et al. (1999), ciliates have a significant role in the diet of shrimp larvae and potentially control pathogenic organisms through grazing. In the other treatments, the densities of ciliates and flagellates had an inverse relationship throughout the experiment, with trophic interactions typical of the "microbial loop" reported by Azam et al. (1982). According to Decamp and Nagano (2001), ciliates and flagellates are sources of highly unsaturated fatty acids (HUFAs) and steroids and have a high intracellular concentration of free amino acids; thus, these microorganisms may have stimulated the growth rates of the shrimp.

Amoebas appeared in the VP treatment in higher numbers than in the BL treatment and were not recorded in the PR treatment. These microorganisms are heterotrophic and feed on small organisms, such as bacteria, flagellates, diatoms, ciliates and small metazoan, like rotifers. Amoebas are typically associated with surfaces and, when active, can ingest bacteria at high rates in places where ciliates and metazoans are not present (Decamp et al., 2007). Therefore, these microorganisms may have influenced the microbial food chain, potentially affecting the growth performance of the cultured shrimp.

Rotifers were recorded only in the final stage of the study and only in the BL treatment. These organisms are efficient predators of ciliates (Decamp et al., 2007). Silva (2009), evaluating the stomach contents of sub-adult *L. vannamei* in biofloc systems with different

stocking densities, noted a probable preference of shrimps for rotifers, demonstrating the importance of rotifers in the diet of *L. vannamei*. Furthermore, the presence of rotifers using the blower may indicate a mature state of a stable microbial community resulting from the use of this aeration device.

The presence of cyanobacteria (filamentous bacteria) was probably due to the availability of phosphorus in all of the treatments because phosphorus is a source of nutrients for cyanobacteria (Burford et al., 2003). Another factor that may have influenced the appearance of cyanobacteria in all of the treatments is the low dissolved oxygen concentrations that were observed in some periods of the culture (Ray et al., 2010). The presence of these organisms in culture may have affected the shrimp performance because some cyanobacteria produce substances that may be toxic or contribute to 'off' flavors of cultured animals (Alonso-Rodriguez and Paez-Osuna, 2003; Zimba et al., 2006).

The final weights did not differ significantly among the BL and PR treatments; nevertheless, the lower survival rate reported in the PR treatment might have influenced this result. The decrease of the density at which the animals were stored might have promoted the growth of the shrimp; several authors have reported an inverse relationship between stocking densities and the performance of shrimp (Krummenauer et al., 2011; Moss and Moss, 2004; Otoshi et al., 2007; Williams et al., 1996).

L. vannamei uses bioflocs as a source of supplementary food, improving feed utilization and reducing the rate of feed conversion (FCR) (Burford et al., 2003; Wasielesky et al., 2006). The best feed-conversion rates were registered in the BL and VP treatments, the same treatments for which more diversity was recorded in the dominant microbes, potentially indicating greater use of such organisms as a dietary supplement for shrimp. Thus, the blower

treatment had better zootechnical indexes than the other aerators, probably because of a higher formation of microbial aggregates, which was reflected in the improved water quality of this treatment.

Conclusion

Different sources of aeration can affect the formation of bioflocs and the abundance and types of microorganisms present in shrimp cultures. Furthermore, because of the better formation of aggregates, water quality was improved in the treatment with the blower, and this improvement might have contributed to a higher final shrimp biomass.

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CAPÍTULO V

PERFORMANCE OF THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei* IN BIOFLOC-DOMINATED ZERO-EXCHANGE RACEWAYS USING A NON-VENTURI AIR INJECTION SYSTEM FOR AERATION, MIXING, AND FOAM FRACTIONATION

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Resumo

Perdas por doenças virais e o impacto negativo dos efluentes emitidos pela aquicultura são dois grandes desafios para o desenvolvimento das práticas aquícolas sustentáveis. A utilização de estufas fechadas utilizando cultivos super intensivos dominados por bioflocos vem sendo testada para amenizar estes problemas. O presente trabalho foi realizado na Texas Agrilife Research da Texas A&M University, Corpus Christi, TX, EUA. Foram utilizados dois raceways de 100 m^3 cada, revestidos com EPDM (Firestone Specialty Products, Indianapolis, IN). Foram abastecidos com uma mistura de água do mar (55 m^3), água doce clorada (10 m^3), e água com biofloco (35 m^3) oriunda de um estudo anterior. Os raceways foram estocados em uma densidade de 390 camarões/m^3 com juvenis de *L. vannamei* (3,4 g), linhagem: "Taura resistente" (from Shrimp Improvement System, Islamorada, FL). Para aeração e mistura da água foram instalados 14 injetores (Taeration) paralelamente à direção do fluxo, ao longo da parte inferior das paredes dos tanques. Além disso, a remoção dos sólidos foi realizada através de um fracionador de espuma conectado a cada tanque. Os níveis de sólidos em suspensão foram mantidos em 400-500 mg/L. Os camarões foram alimentados com uma dieta contendo 35% de proteína bruta (Hyper-Intensive-35, Zeigler Bros. Gardners, PA). O experimento foi conduzido sem renovação de água. Cada raceway foi equipado com um sistema de monitoramento de oxigênio dissolvido (YSI 5200, YSI Inc., Yellow Springs, OH). Temperatura da água, salinidade, oxigênio dissolvido (OD) e pH foram monitorados duas vezes por dia. Amonia total (TAN), Nitrito (NO_2-N), Nitrato (NO_3-N), alcalinidade, Volume de Floco (SS), turbidez, Solidos suspensos totaos (SST), Solidos suspensos voláteis (VSS) e BOD_5 foram monitorados pelo menos uma vez por semana. TAN e NO_2-N se mantiveram muito baixos (<0,5 mg / L), o peso médio final dos camarões foi 25,26 g, crescendo 1,46 g / semana, com uma biomassa de 8,36 kg/m^3 e uma taxa de conversão alimentar de 1,77.

Abstract

In the current study two 100 m^3 EPDM (Firestone Specialty Products, Indianapolis, IN) lined RWs were each filled with a mixture of seawater (55 m^3), municipal chlorinated freshwater (10 m^3), and biofloc-rich water (35 m^3) from a previous nursery study. RWs were stocked (390 shrimp/m^3) with juvenile (3.14 g) Taura resistant *L. vannamei* from Shrimp Improvement System, Islamorada, FL. To provide aeration, mixing, and circulation, a total of 14 non-Venturi injectors were positioned parallel to the direction of flow along the bottom of each RW wall. In addition, one nozzle was used to power a home-made foam fractionator to enable the removal of particulate and dissolved organic matter, the target TSS levels were 400-500 mg/L in hopes of lowering the FCRs. Shrimp were fed a 35% CP diet (Hyper-Intensive-35, Zeigler Bros. Gardners, PA). Chlorinated freshwater was added weekly to maintain salinity, and RWs were maintained with no water exchange. Alkalinity was adjusted to 150-200 mg/L (as $CaCO_3$) using sodium bicarbonate and agricultural lime. Each RW was equipped with a DO monitoring system (YSI 5200, YSI Inc., Yellow Springs, OH). Water temperature, salinity, DO, and pH were monitored twice/d. TAN, NO2-N, NO3-N, alkalinity, SS, turbidity, TSS, VSS, and cBOD5 were monitored at least once a week. Based on sampling data collected on Day-60 TAN and NO_2-N remained very low (<0.5 mg/L), shrimp averaged 25.26 g in weight, grew 1.46 g/wk, with a biomass of 8.36 kg/m^3 and an estimated FCR of 1.7:1. Thus far each RW has achieved an estimated total biomass of 635 kg (6.35 kg/m^3) supported until this point by one 2 HP pump without supplemental oxygen. A second 2 HP pump was available to provide additional aeration if DO levels drop below 4.3 mg/L for an extended period. RWs will be harvested when mean weights reach 25 g.

1. Introduction

In the last few decades, production of the Pacific White Shrimp, *Litopenaeus vannamei*, has been negatively affected by disease epizootics and environmental concerns over effluent impact on receiving streams (Lotz and Lightner, 2000; Lightner 2005; Neal et al. 2010). Traditional shrimp grow-out methods use outdoor ponds and exchange high volumes of water on a regular basis (Hopkins et al. 1993; Timmons and Losordo 1994). The possible introduction of harmful pathogens with the incoming water and the release of nutrient-rich effluent into receiving streams are two major challenges for the development of sustainable, biosecurity, and cost-effective shrimp farming practices, These systems offer improved biosecurity with reduced risk of crop losses to viral disease outbreaks (Burford et al., 2004; Cohen et al. 2005; De Schryver et al. 2008; Mishra et al. 2008). Use of greenhouse-enclosed, super-intensive, biofloc-dominated, zero-exchange systems may alleviate these problems. Previous research has indicated that good shrimp production can be achieved under low water exchange. Furthermore, operating these systems with no water exchange minimizes the negative effluent impact on receiving waters (McAbee et al. 2003; Browdy & Moss 2005; Wasielesky et al. 2006; Avnimelech 2009; Krummenauer et al. 2011). Some reports suggest better shrimp growth and survival in biofloc water when compared to clear water where a more complete and expensive diet is needed (Wasielesky et al. 2006; Emerenciano et al. 2007). However, operating biofloc systems at high production levels (>8 kg/m^3) requires substantial energy to satisfy the high oxygen demand of the shrimp and the microbial communities (Samocha et al. 2010). Recent advances in super-intensive, limited-discharge, biofloc-dominated systems for raising *L. vannamei*, suggest that these systems can be profitable when used to produce live or fresh (never frozen) shrimp for niche markets (Samocha et al.2012).

Previous studies by AgriLife research have utilized a pump driven Venturi to mix water and to oxygenate the water. Additional circulation and mixing was provided by airlifts and air diffusers. This system has worked well for numerous studies in the past however, in an effort to reduce operation costs, an investigation was initiated to explore ways to reduce energy use, and to eliminate the need to use pure oxygen in operating these systems when dealing with yields above 8 kg/m^3. Super-intensive biofloc systems require large amounts of oxygen input that increases production costs. It can also limit feeding and thus potential growth of the shrimp. The Taeration® (Advanced Industrial Aeration, Tampa, FL) device is currently used in several wastewater treatment facilities requiring only little maintenance compared to other aeration alternatives. This technology may be successfully transferred to biofloc systems and other types of aquaculture systems. According to the manufacturer's specs the Taeration® nozzle provides 3:1 air to water ratio. In contrast, our current Venturi system provides < 1:1 and requires use of supplemental oxygen at high biomass loading (>7-8 kg/m^3) to maintain the desired DO levels.

Researchers, supported by the United States Marine Shrimp Farming Program have been working to improve the economic viability of super-intensive zero exchange systems for production of food shrimp. Members of the USMSFP use economic modeling and other metrics to evaluate advances in management practices and culture systems used to produce market size shrimp. The AgriLife Research Mariculture Lab at Flour Bluff has been developing cost-effective, sustainable and biosecure super-intensive systems for the production of food-size Pacific White Shrimp with an emphasis on optimization of the growing conditions while reducing operating costs.

The objectives of this study were:

1. To evaluate the ability of the Taeration injectors to maintain adequate DO levels and mixing in

a zero exchange super-intensive RW system without the use of supplemental oxygen;

2. To evaluate if the current foam fractionator would be adequate to maintain solids levels within the desired range, and

3.To evaluate the effect of the injectors on shrimp performance during the culture period**.**

2. Material and Methods

2.1. System Descriptions

The system consists of two 100 m^3 RWs (33 m x 3 m) inside a 40 x 9.5 m double-layered polyfilm greenhouse. To reduce operational costs by reducing energy use and eliminating the need for supplemental oxygen, a newly patented non-Venturi injector was installed to provide aeration, mixing, and circulation. These injectors are currently used in several wastewater treatment facilities and require little maintenance compared to other aeration methods. A total of 14 nozzles were positioned parallel to the direction of flow along the bottom of each RW wall (Figure 1). In addition, one nozzle was used to power a homemade foam fractionator (Figure 2) to remove particulate and dissolved organic matter. Two 2 HP pumps are available to power the 15 nozzles in each RW; however, the entire RW can be operated with just one pump when loading is low.

The raceway systems were equipped with DO monitoring and alarm systems (Figure 3) that uploaded data to a computer in the lab, which could also be accessed from remote locations. Real-time monitoring is a valuable management tool, helping to minimize stress, conserve resources, and often diverting what would otherwise become catastrophic events.

Figure 1. Raceways (100 m^{-3}) showing airlifts.

Figure 2. Home-made foam fractionator and DO monitors.

2.2. Culture Conditions

The two RWs were each filled to 100 m^3 with a mixture of seawater (55 m^3), municipal chlorinated freshwater (10 m^3), and biofloc-rich water (35 m^3) from a previous nursery study. As was the case in 2010, water mixing, circulation, and aeration were generated by 14 non-Venturi injectors positioned parallel to the direction of flow along the bottom of each RW wall, however, the 2011 study was initiated using only one of the available 2 HP pumps. RWs were stocked (390 shrimp m^{-3}) with Taura resistant *L. vannamei* juvenile (1.90 g) from Shrimp Improvement System, Islamorada, FL. Shrimp were fed a 35% crude protein diet (Hyper-Intensive-35 Extra Short- cut, Zeigler Bros. Gardners, PA). Half of the daily ration was offered in four equal portions during the day (8:30, 11:30, 14:30, and 16:30), and the remainder was fed by belt feeders during the night. Initial daily rations were based on an assumed FCR of 1:1.4, growth of 1.2 g/wk and mortality rate of 0.5%/wk. Rations were later adjusted based on results of twice-weekly shrimp sampling. Chlorinated freshwater was added weekly (equivalent to 0.3 m^3/d) to maintain salinity and compensate for water losses from operating the foam fractionator. Alkalinity was adjusted to 150-200 mg/L (as $CaCO_3$) using sodium bicarbonate and agricultural lime. Water temperature, salinity, DO, and pH were monitored twice a day. TAN, NO_2-N, NO_3-N alkalinity, SS, turbidity, TSS, VSS, and cBOD5 were monitored at least once a week (Table 1). Each RW was equipped with a DO monitoring system (YSI 5200, YSI Inc., Yellow Springs, OH).

Table 1. Water quality parameters determined during the experiment and methods used for the analyses.

Parameter (units)	Method
Water temperature Temperature (^{0}C)	Model MDS 650, YSI Inc., Yellow Springs, OH, USA
Salinity	Model MDS 650, YSI Inc., Yellow Springs, OH, USA
Dissolved oxygen (mg O_2 L^{-1})	Model MDS 650, YSI Inc., Yellow Springs, OH, USA
pH	Model MDS 650, YSI Inc., Yellow Springs, OH, USA
Alkalinity (mg $CaCO_3$ L^{-1})	Titration method (HCl 0.1 N)
Total ammonium nitrogen-TAN (mg L^{-1})	UNESCO 1983
Nitrite nitrogen (mg N-NO_2 L^{-1})	Strickland and Parsons 1972
Nitrate nitrogen (mg N-NO_3 L^{-1})	Strickland and Parsons 1972
Volume of settling solids (SS ml L^{-1})	Eaton et al. (1995)
Turbidity	Hach 2100P Hack Company, Loveland, CO, USA
Total suspended solids: (TSS mg L^{-1})	EPA method 340.2
Volatile suspended solids: (VSS mg L^{-1})	EPA method 340.2
cBOD5	Yellow Spring Instruments, Yellow Springs, OH, USA

3. Results

A summary of the mean DO, pH, temp and other selective water quality indicators are presented in Table 2. Mean water temperature, salinity, pH, and DO were: 29.8 C, 28.5 ppt, 7.1, and 5.8 mg L^{-1}, respectively. TAN and NO_2-N remained very low (<0.5 mg L^{-1}) throughout the study however, NO_3-N levels increased from 10 mg/L at stocking and averaged 562.7 mg L^{-1} at harvest. Figures 3 through 10 provide graphic representation of the changes in selected water quality indicators throughout the study. TSS and SS values reached >1,000 mg L^{-1} and 39 ml L^{-1}, respectively.

The system was operated with one 2 HP pump until Day-62 (6.5 kg shrimp / m^{-3}) when DOs began to drop below 4.5 mg L^{-1}, and the second 2 HP pump was started to provide additional aeration.

Table 2. A summary of mean water quality values during the 106-d grow-out study with juvenile *Litopenaeus vannamei* in two 100 m^2 raceways stocked at a density of 390 m^{-3} using Taeration® system for mixing and aeration.

RW	DO (mg/L)	pH	Temp. (C)	Sal. (ppt)	TAN (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	SS (mL/L)	TSS (mg/L)	VSS (mg/L)	$cBOD_5$ (mg/L)	Alk (mg/L)	CO_2 (mg/L)
1	5.8	7.1	30.6	28.4	0.4	0.33	154.6	17.6	417	250	34	204.3	35.6
2	5.8	7.1	30.2	28.5	0.5	0.21	158.8	14.4	480	281	46	214.5	37.3
Av.	5.8	7.1	29.8	28.5	0.5	0.27	156.7	16.0	449	266	40	209.4	36.5
SD	0.01	0.0	0.26	0.1	0.1	0.08	3.0	2.3	43	21	9	7.3	1.2

Shrimp were harvested from the harvest basin using a commercial harvest pump. Survival was good (83% on average) with average shrimp growth of 1.46 g/wk and mean final weights of 25.2 g. FCRs averaged 1.77 and the average yield obtained in this trial was 8.4 kg shrimp/m^3 (Table 3).

Table 3. Summary of a 106-d grow-out study with juvenile *Litopenaeus vannamei* stocked at 390 shrimp/m^3 in two 100 m^2 raceways using the Taeration® system for mixing and aeration.

RW	Stocking (Juv/m^3)	Stocking (g)	Harvest (g)	Growth (g/wk)	SGR (g/d)	Sur. (%)	Yield (kg/m^3)	FCR	Water Use (L/1 kg)
1	390	3.14	25.14	1.45	0.21	79.7%	8.04	1.83	166.6
2	390	3.14	25.39	1.47	0.21	86.3%	8.69	1.70	149.7
Av.			25.26	1.46	0.20	83.0%	8.36	1.77	158.1
SD			0.18	0.01	0.001	3.3%	0.32	0.06	8.5

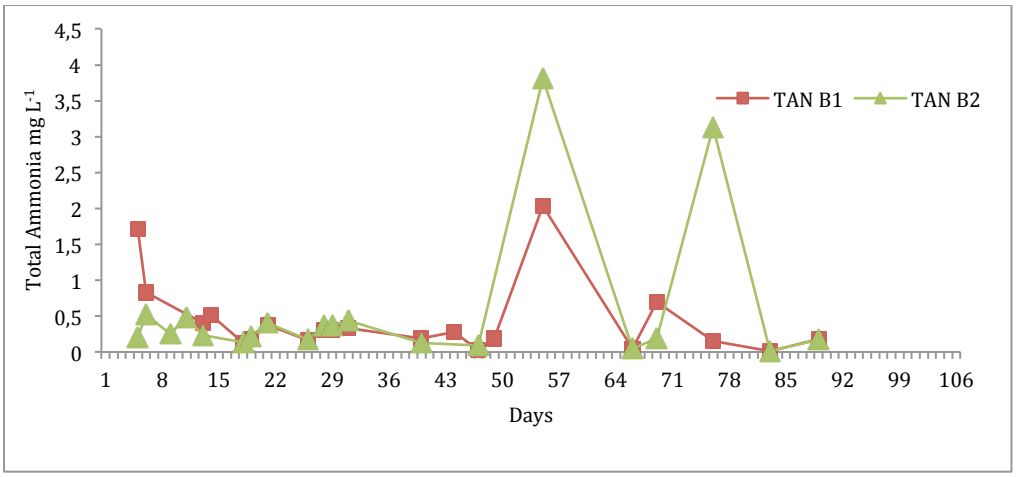

Figure 3: Total ammonia, daily changes in water in raceways operated in a 106-day grow out study.

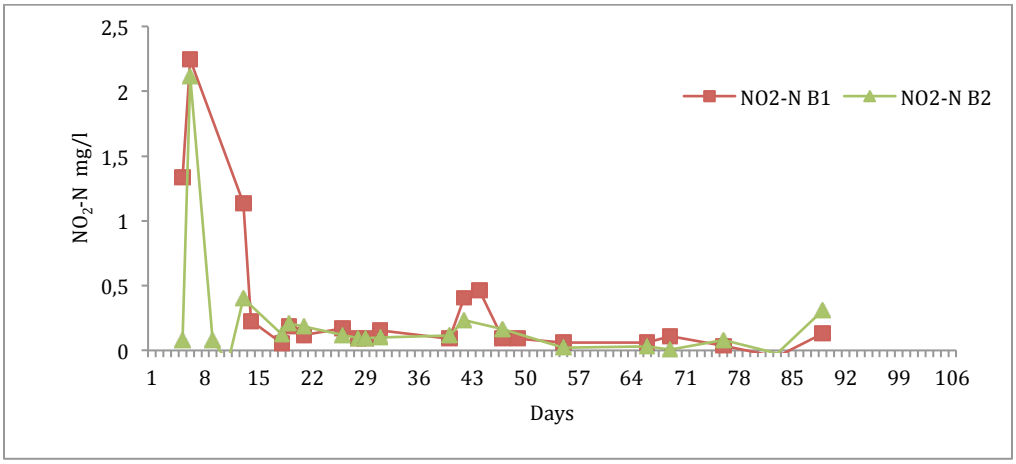

Figure 4: NO_2-N daily changes in water in raceways operated in a 106-day grow out study.

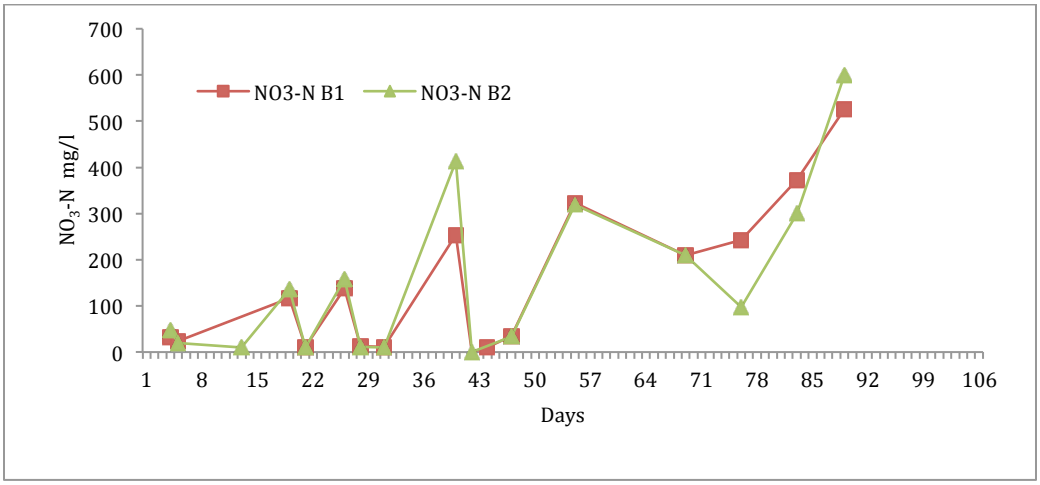

Figure 5: NO_3-N daily changes in water in raceways operated in a 106-day grow out study.

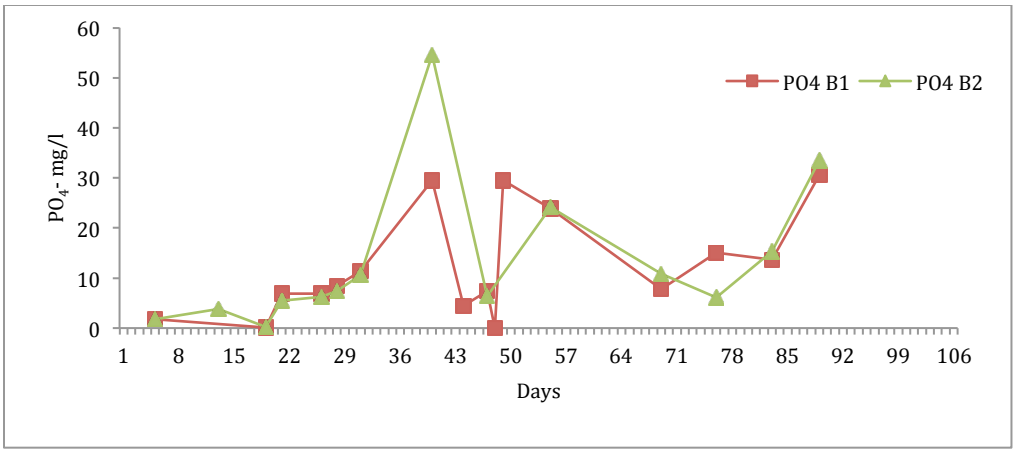

Figure 6: PO_4^- daily changes in water in raceways operated in a 106-day grow out study.

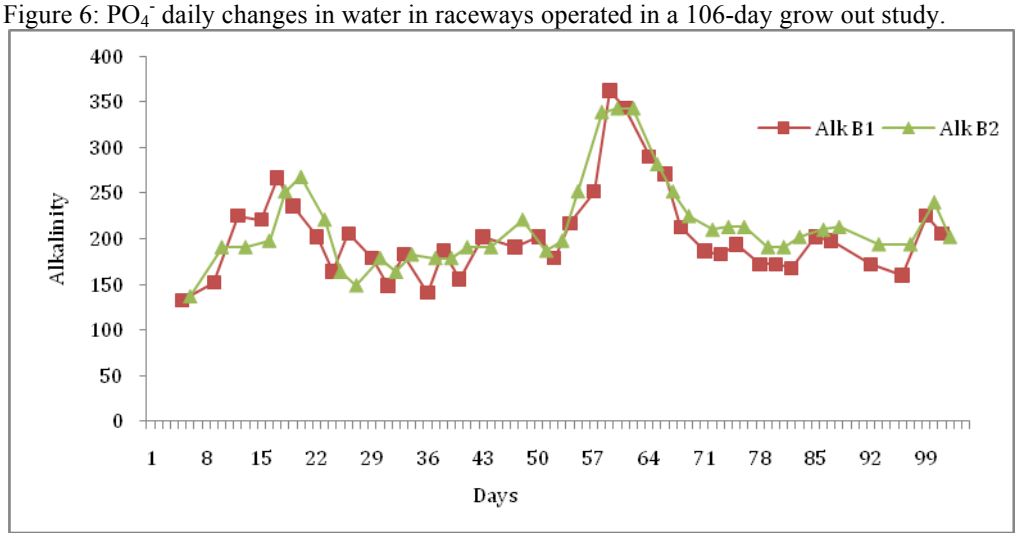

Figure 7: Alkalinity daily changes in water in raceways operated in a 106-day grow out study.

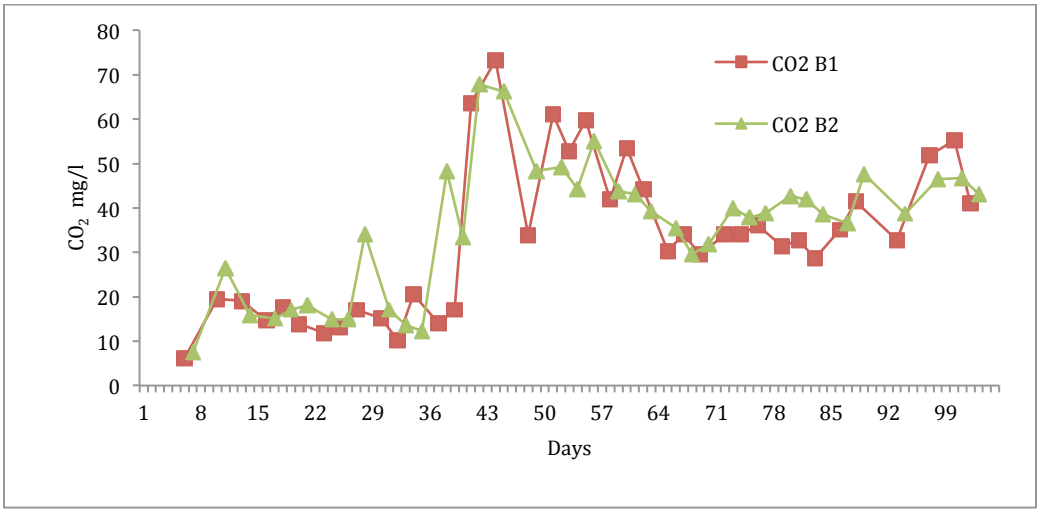

Figure 8: CO_2 daily changes in water in raceways operated in a 106-day grow out study.

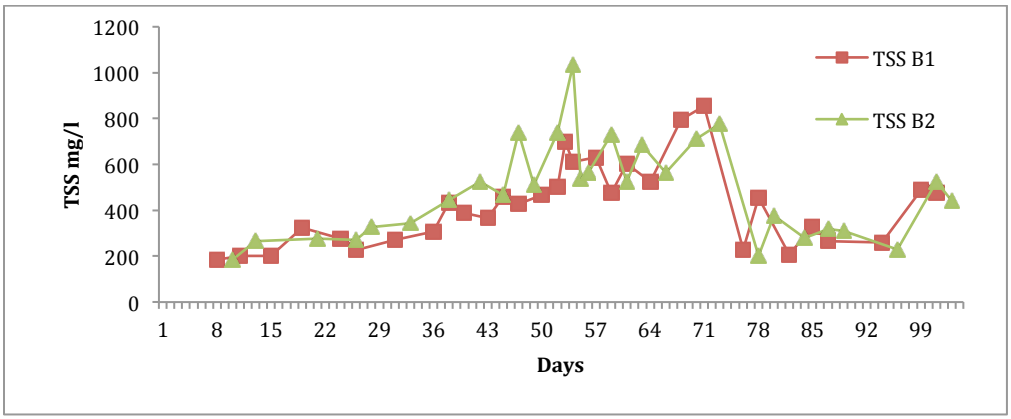

Figure 9: TSS daily changes in water in raceways operated in a 106-day grow out study.

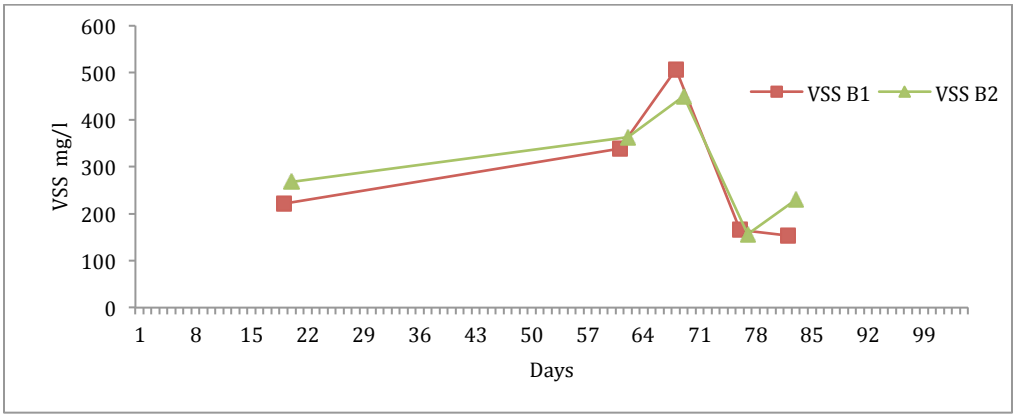

Figure 10: VSS daily changes in water in raceways operated in a 106-day grow out study.

4. Discussion

The mean values of the water quality indicators monitored in this study remained within the recommended range for optimal growth and survival for *L. vannamei,* no significant differences were found both raceways ($P > 0.05$) (Ponce-Palafox et al., 1997; Van Wyk and Scarpa, 1999).

The production figures from this year have been very encouraging (over 3,588 Kg produced). The foam fractionators currently employed in both RW systems were undersized and some mortality was observed during periods of high total suspended solids (>1,000 mg/L), combined with high temperatures and moderate DO levels. This results contrast with others studies that reported large removal of fine suspended solids when Foam Fractionators were used (Mishra et al., 2008; Samocha et al., 2007, 2010). Apparently, with the increase of biomass (over 5.0 kg m^{-3}) the foam fractionators losing their efficiency. In this study, simple but effective settling tanks were installed to control solids and supplemental oxygen was provided until mortality tapered off.

The results of this study suggest that the Taeration® injectors were able to maintain adequate DO levels and mixing for the production of marketable size shrimp in a biofloc system. Dissolved oxygen levels in the two RWs were in most cases above 5 mg/L (70-85% saturation) even after feeding. During the initial 62 days of the production cycle the aeration method used in the New 100 m^3 RW system was able to support 6.5 kg shrimp per m^3, using one 2 HP without any blowers. Samocha et al. 2010, operating six 40-m^3 raceway with a 7HP blower plus a centrifugal pump and a venturi injector capable of mixing the culture water with atmospheric air, suggested that a shrimp biomass load of about 7.5 kg/m^3 can be maintained with only oxygen supplementation.

Shrimp growth in 2011 was 1.4 g/week. The result was the same observed by Samocha et al. (2010) in the same raceways, however, the density used in this year was higher than that study (270 m^{-3}). It should be noted that neither study utilized any of the fast growth lines that are presently available. If future funding becomes available we will operate this system with a fast growth line shrimp which will reduce the time required to get the same or better results. FCRs were significantly lower in 2011 compared to study of 2010, 1.77 and 2.4, respectively. A preliminary economic analysis indicates that this system is more efficient in terms of energy and labor requirements than our previous Venturi based system (Hanson et al. 2012).

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CAPÍTULO VI

THE EFFECT OF PROBIOTICS IN A *Litopenaeus vannamei* BIOFLOC TECHNOLOGY CULTURE SYSTEM CONTAMINATED WITH *Vibrio parahaemolyticus*

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Resumo

O objetivo do presente trabalho foi analisar o efeito do uso de probiótico em um cultivo de *Litopenaeus vannamei,* contaminado com *Vibrio parahaemolyticus,* mantido em sistema de bioflocos. Foram comparados dois tratamentos, um controle sem adição de probiótico e outro com adição de probiótico. No tratamento probiótico, foi utilizado um produto comercial empregado em duas vias de aplicação, conforme recomendação do fabricante. A primeira, aplicação foi na água e a segunda, na ração. O probiótico aplicado na água continha *Bacillus sp*., *Enterococcus sp*., *Lactobacillus sp* e *Paracoccus Thiobacillus* (AquaAtar Pond), o probiótico aplicado na ração continha *Bacillus spp*., *Enterococcus spp*. e *Lactobacillus spp*. (AquaStar Growout). Foram utilizados seis tanques de 35.000 L, revestidos com geomembrana e cobertos por uma estufa. Cada tanque foi estocado com 10.500 camarões (peso inicial 1,15 g), densidade de 300 camarões/m², provenientes de um berçário fortemente infectado por *Vibrio parahaemolyticus*. A ração (38% PB) foi fornecida três vezes ao dia e o experimento teve duração de 60 dias. O peso final, taxa de crescimento e sobrevivência foram significativamente maiores no tratamento probiótico ($P<0,05$), assim como a biomassa final ($P<0,05$). Resultando em uma produção de biomassa significativamente maior ($P<0,05$) em comparação com o tratamento controle (70%). Shrimp in the probiotic treatment group also had a lower ($P<0.05$) FCR compared to shrimp in the control group (1.4 vs. 2.7). Não foram detectadas diferenças significativas entre os parâmetros de qualidade de água ($P>0,05$). Os resultados mostraram que o uso de probiótico pode controlar a infecção por *Vibrio parahaemolyticus* em sistema de cultivo em bioflocos, bem como, melhorar os parâmetros de crescimento.

Abstract

The aim of this study was to analyze the effect of bacterial probiotics on a *Litopenaeus vannamei* biofloc technology culture system contaminated with *Vibrio parahaemolyticus*. The work was carried out at the Marine Station of Aquaculture (Federal University of Rio Grande – Brazil). In this experiment, two treatments were compared: control and commercial probiotics. For the probiotic treatment, a multi-strain probiotic containing *Bacillus spp*., *Enterococcus spp*., *Thiobacillus spp.* and *Paracoccus spp*. (AquaStar Pond) was applied to the water, and a multi-strain probiotic containing *Bacillus spp*., *Enterococcus spp*. and *Lactobacillus spp*. (AquaStar Growout) was added to the feed to improve water and gut health, respectively. The treatments were randomly assigned in triplicate to six 35,000 L enclosed and lined raceway greenhouses. Each tank was stocked with 10,500 nursed *V. parahaemolyticus*-infected shrimp to a final stocking density of 300/m^2. The experiment lasted for 60 days, during which time water quality, biological parameters, microorganism communities, growth, weight gain, FCR and survival were analyzed. The results showed that the final weight, weight gain and survival rate were significantly higher in the probiotic treatment group ($P<0.05$), resulting in a significantly higher ($P<0.05$) biomass production (70%) compared to the control. Shrimp in the probiotic treatment group also had a lower ($P<0.05$) FCR compared to shrimp in the control group (1.4 vs. 2.7). There were no significant differences between water quality parameters ($P>0.05$) in each treatment. The results showed that the tested probiotics can control *V. parahaemolyticus* shrimp infection in a biofloc culture system while improving growth parameters.

Key Words: Biofloc Technology System, *Litopenaeus vannamei,* Probiotic, *Vibrio parahaemolyticus,* Biosecurity

Introduction

In shrimp cultures around the world, diseases caused by different species of the genus Vibrio affect growth and mortality, resulting in a significant drop in productivity (Lightner 1988; Chen et al. 2000; Molina-Aja, et al. 2002; Jayasree et al. 2006). *Vibrio parahaemolyticus* is a gram-negative bacterium that occurs naturally in estuarine and marine environments (Khuntia et al. 2008; DePaola et al. 2003). *Vibrio spp*. usually make up the majority of the micro flora associated with penaeid shrimp. However, if the natural defense mechanisms of shrimp are suppressed and the interactions of the microorganisms with the animals and the environment are not well understood, then the Vibrios may become pathogenic (Lightner 1993; Moriarty 1998). The use of antibiotics to combat bacterial infections can be an effective treatment, however, their use in shrimp culture systems may lead to the development of resistance (Jayasree et al. 2006). Additionally, the inappropriate use of antibiotics has led to the development and dissemination of antibiotic-resistant strains (Tendencia and De la Pena 2001; Defoirdt et al. 2007). Defoirdt et al. (2007) recommend the use of probiotics as alternatives to the use of antibiotics in culture systems, claiming that new strategies to control infections are urgently needed. As a result, there is currently a search for alternatives to antibiotics as a more sustainable control technique (Crab et al. 2010). Several studies are analyzing the use of bacteria that promote the health of other organisms. These beneficial bacteria are known as probiotic bacteria (Gildberg et al. 1997; Moriarty 1998; Gram et al. 1999; Verschuere et al. 2000; Balcázar et al. 2006; Souza et al. 2011). Fuller (1989) defines probiotics as food supplements with live microorganisms that benefit the host by improving the balance of gut flora. Probiotics have many mechanisms of action: competitive exclusion of pathogenic bacteria, being a nutrient source, contribution to enzymatic digestion, beneficial effects on water

quality and improvement of the animal's immune response (Balcázar et al. 2006). Thus, probiotics may have substantial effects on the microorganisms present in the aquatic environment, offering higher productivities, increased weight gain, high FCR and improved aquaculture production performance. These responses may occur through various modes of action including the improvement of food digestion and water quality (Planas and Cunha 1999; Verschuere et al. 2000).

Bioflocs Technology (BFT) system culture could also be an alternative to antibiotics for the control of pathogens. In a recent study, Crab et al. (2010) suggest that bioflocs may assist in the control of disease caused by *Vibrio spp*. In this culture system, microbial growth is stimulated through the absorption of nitrogen derived from microbial protein production (Wasielesky et al. 2006; Avnimelech 2009). This stimulation is carried out by the addition of a carbohydrate to the water, thus manipulating the C:N ratio (Avnimelech 1999; Ebeling et al. 2006). Microbial protein is produced in the form of aggregates that are consumed by the shrimp and become an additional source of protein (Schneider et al. 2006; Schryver et al. 2008; Kuhn et al. 2009). The heterotrophic bacteria assimilate the inorganic nitrogen available in the system and convert it into microbial protein (Avnimelech 1999; Burford et al. 2004; Hari et al. 2006). However, manipulation of the C:N ratio may result in significant changes to the microbial community and the development of pathogenic bacteria. Thus, the use of probiotic bacteria in BFT systems could be an important tool in avoiding possible pathogenic infections.

The aim of this study was to analyze the effect of bacterial probiotics in *Litopenaeus vannamei* BFT culture systems contaminated with *Vibrio parahaemolyticus*.

Materials and Methods

Culture Conditions

The experiment was carried out at the Marine Station of Aquaculture, Federal University of Rio Grande, Southern Brazil (32°12´16S 52°10´38W), in a greenhouse with 35 m² tanks lined with high density polyethylene (HDPE) and water depth of 1.0 m. Aeration was performed using one 10 cm airstone for each square meter attached to a seven hp blower. Tanks were filled with filtered seawater (salinity 30) and treated with a chlorine solution (10 ppm measured immediately after chlorine) dechlorinated with ascorbic acid powder (1.0 g per 1,000 L). Structures with vertical substrates were used to increase the surface area (150%) available for nitrifying bacteria colonization. The greenhouse was covered by a 50% light attenuation shade cloth to avoid high temperatures during the culture period.

L. vannamei post-larvae were obtained from Aquatec®, Canguaretama, Rio Grande do Norte State, Brazil. Post-larvae with an average weight of 0.0123 g (±0.007) were maintained in a nursery during 35 days in a stocking density of 3000 m^{-2} until they achieved an average weight of 1.15 g (± 0.89). It was detected that those nursed juveniles were entirely affected by *V. parahaemolyticus*. Then shrimps were transferred to the growout greenhouse tanks in a stocking density of 300 shrimp m^{-2}. In the experiment, three replicates of two experimental treatments were conducted: a control system and a system with application of AquaStar® (Biomin GmbH) commercial probiotics. For the probiotic treatment, a multi-strain probiotic containing *Bacillus sp., Enterococcus sp., Thiobacillus sp.* and *Paracoccus sp.* (AquaStar®Pond) was applied to the water, while a multi-strain probiotic containing *Bacillus sp., Enterococcus sp.* and *Lactobacillus sp*. (AquaStar®Growout) was added to the feed to

improve water and gut health, respectively. The experiment lasted for 60 days. For the formation of microbial aggregates, tanks were initially inoculated with diatoms (microalgae - *Thalassiosira weissflogii*). After three days, organic fertilization was started with the addition of 6.0 g of carbon (molasses) for each 1.0 g of total ammonium nitrogen (TA-N) in the water. This procedure followed the methodology described by Ebeling et al. (2006), Samocha et al. (2007) and Avnimelech (2009).

Vibrio identification

Shrimp samples collected from the nursery raceways were screened for the presence of bacterial diseases. The shrimp samples were fixed in Davidson solution (Humason 1979) and embedded with Paraplast® Plus embedding media (Microsystems Inc., Bannockburn, IL, USA). Serial longitudinal sections of 5 μm were stained with Gram, hematoxylin and eosin and then examined with an Olympus BX41 microscope.

Immunohistochemistry

Tissues were incubated for 2 hours at room temperature with monoclonal antibody conjugated with rhodamine"8 in combination with fluorescein to *V. parahaemolyticus* (Chemetron Dako - Argentina) (1:200 in PBS). Immunofluorescence was examined with a Leitz Orthoplan microscope equipped with a Ploem-type vertical illuminator for selective observation of green fluorescein.

Water Quality Monitoring

The values for temperature, salinity, dissolved oxygen (DO) and pH were recorded twice daily (0800 and 1700 h) using a multiparameter apparatus (YSI 556, YSI Inc., Yellow Springs, Ohio, United States). The water clarity was measured using a Secchi disc. Water

samples were collected daily to quantify the concentration of total ammonia nitrogen (TA-N) (UNESCO 1983). The analysis of nitrite (NO_2^--N), nitrate (NO_3^--N) and phosphate (PO_4^{-3}-P) concentrations was performed every five days following the methodology adapted from Strickland and Parsons (1972). Alkalinity was determined following the methodology described in APHA (1998). The turbidity was measured once a week using turbidimeter (Hach 2100P Hack Company, Loveland, CO, United States). The volume of settling flocs was quantified using an Imhoff cone according to the methodology of Eaton et al. (1995) as adapted by Avnimelech (2009). The volume of the floc on the bottom of the cone was measured after 15 minutes of sedimentation. Water was collected for the analysis of suspended matter (particles larger than 45 μm) according to the method of Strickland and Parsons (1972). The weight of the suspended solids was determined gravimetrically from the filtration rates of up to 20 mL of culture water through glass fiber Whatman GF/F filters. The filters were dried for approximately 24 hours at 60 ºC and then weighed on an analytical balance (Sartorius MC1, analytic AC 210 S, ± 0.0001 g) to determine the final weight (AOAC 2000).

Shrimp Stocking, Feeding and Monitoring

The shrimp were fed three times per day with a commercial diet (Active 38% CP, 2 mm, Guabi®, Campinas, SP, Brazil). The quantities used were those proposed by Jory et al. (2001). The diet was offered in feeding trays at an initial rate of 10% of shrimp biomass and was adjusted according to consumption. Every week, 60 shrimp were randomly sampled from each tank and individually weighed; wet weight was individually measured using a digital scale accurate to 0.01 g (Marte® científica AS2000, Santa Rita do Sapucaí, MG, Brazil). At the end of the culture cycle, 200 shrimps were weighed, and survival was estimated based on total harvest weight. The weekly growth rate (WGR) was determined by the following calculation: WGR =

weight gain / number of weeks of culture. The feed conversion ratio (FCR) was calculated as FCR = offered feed / biomass increment. Survival was calculated as S% = [(final biomass / average individual final weight) / number of individuals stocked)] x 100. Productivity was calculated as Prod = final biomass / tank area.

Statistical Analysis

Significant differences ($P<0.05$) in zootechnical performance and water quality were analyzed using one-way ANOVA. Tukey's multiple-range test was applied when significant differences were detected. All tests were performed after the confirmation of homogeneity of variance (Levene's test) and normality of data distribution (Kolmogorov-Smirnov test). To satisfy the ANOVA assumptions, survival data were arcsine-square root transformed using a constant exponent (arcsine x 0.5) (Zar 1996)

Results

In the end of nursery time, macroscopically and fluorescent lesions were observed in the body especially in the caudal region (Fig. 1). The nerve cord also showed blue fluorescent Vibrio colonies (Fig. 3). In the muscle tissue, there was necrosis, abundant colonies of Gram-negative bacteria and fluorescence indicating *V. parahaemolyticus* (Fig. 3a and 3b). Conversely, in the end of growout no lesions were detected.

The mean values for water quality parameters are shown in Table 1. There were no significant differences ($P>0.05$) in water quality parameters among the different treatments. Dissolved oxygen decreased throughout the experimental period. The lowest values of dissolved oxygen recorded for the control and probiotic treatment groups were 2.02 and 1.90 mg L^{-1}, respectively. The pH and temperature remained stable throughout the experimental period. Ammonia increased at the beginning of the experiment and then stabilized as the bioflocs formed (Fig 4a). High concentrations of nitrite were recorded during the experimental period, with maximum values of 68.0 and 52.3 mg/L in the control and probiotic treatments, respectively (Fig 4b). Imhoff cones and TSS were increase similar to that while the 60 days of trial (Fig 5a and 5b).

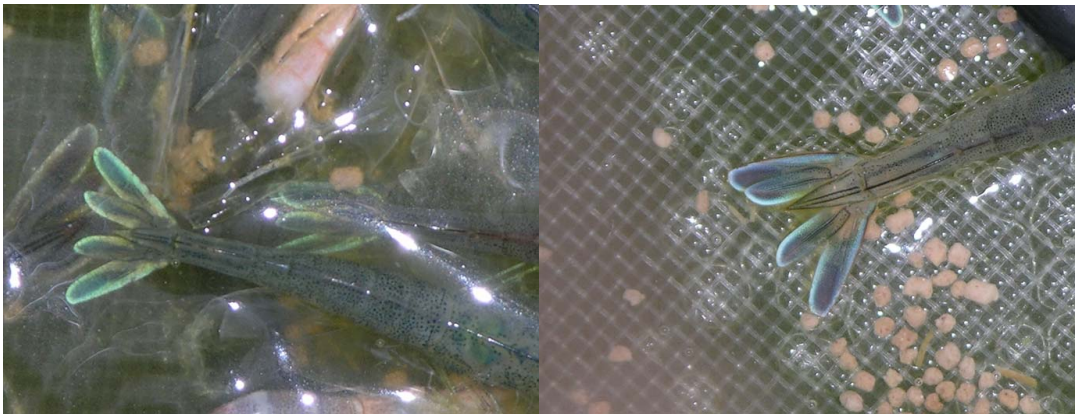

Figure 1: *L. vannamei* with a greenish fluorescence in the caudal region.

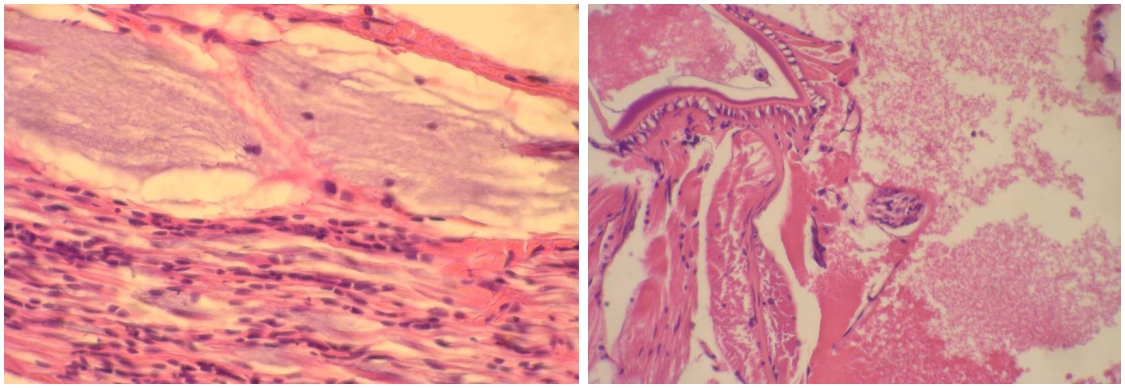

Figure 2: a) Nervous cords with blue bacterial colonies belonging to Vibrios; H-E 20 X. b) Caudal region rich in bacteria colonies; H-E 10 X.

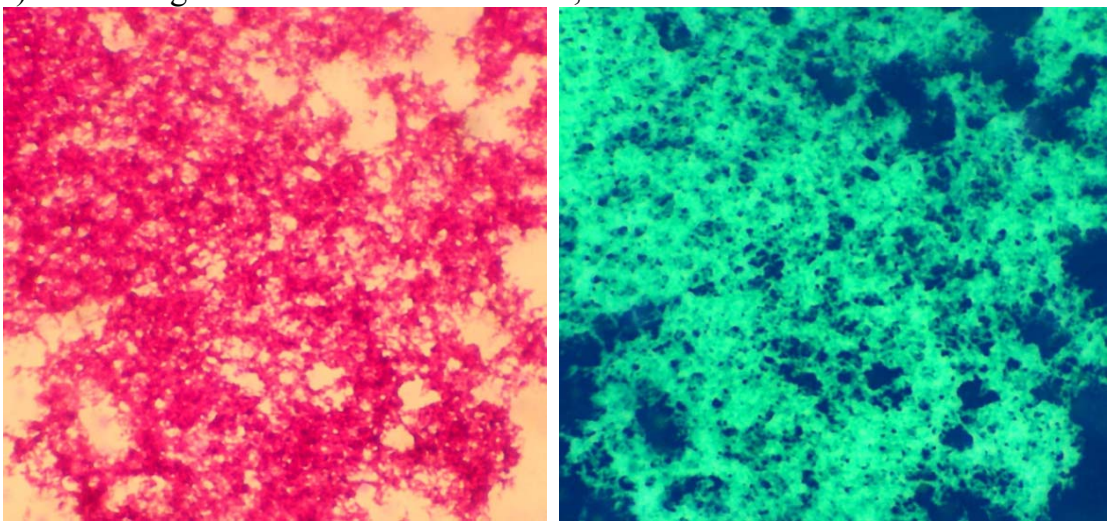

Figure 3: a) Colonies of Gram-negative bacteria (red) 40 X. b) Colonies of green fluorescence *V. parahaemolyticus* 100 X.

TABLE 1. Water quality parameters for *L. vannamei* culture in a BFT system over 70 days[1].

	Control	**Probiotic**
TA-N (mg L^{-1})	0.48 ± 0.63[a]	0.45 ±0.63[a]
NO_2-N (mg L^{-1})	20.91 ± 19.5[a]	14.68 ± 17.7[a]
NO_3-N (mg L^{-1})	8.68 ± 14.5[a]	7.42 ± 11.5[a]
PO_4^3-P (mg L^{-1})	4.27 ±3.01[a]	4.57 ±3.40[a]
Alkalinity	202.00 ± 65.4[a]	185.66 ±68.1[a]
DO (mg L^{-1})	4.02 ±0.77[a]	4.10 ± 0.72[a]
pH	7.45 ± 0.22[a]	7.46 ± 0.21[a]
Salinity	35.35 ± 1.15[a]	34.78 ± 1.63[a]
Temperature (^oC)	29.10 ±1.77[a]	29.02 ±1.62[a]
Settleable solids (ml L^{-1})	15.71 ±12.43[a]	15.13 ±11.27[a]
TSS (mg L^{-1})	513.78 ± 229.85[a]	526.02 ± 207.7[a]
Turbidity (NTU)	192.52 ± 116.0[a]	175.53 ± 112.7[a]
Transparency (cm)	21.74 ±22.6[a]	21.915 ± 20.0[a]

[1]Values are the means of replicates ± standard deviation. Different superscripts in the same row indicate significant differences ($P<0.05$).

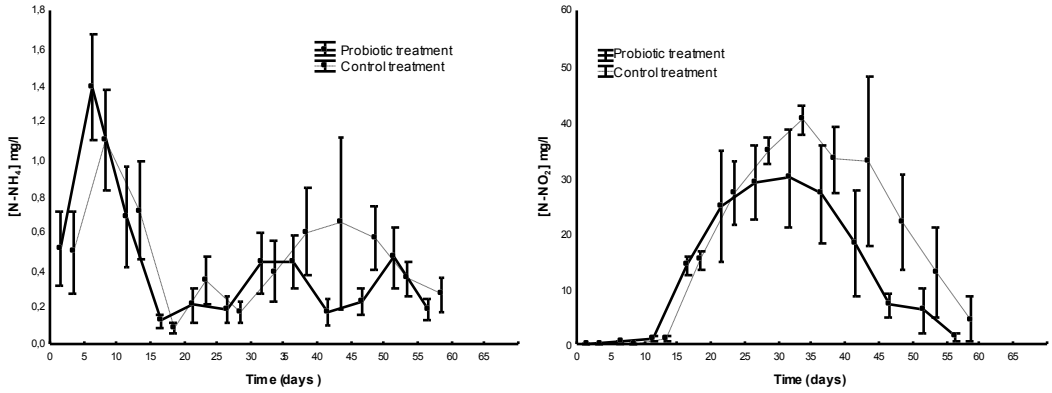

Figure 4. Mean Ammonia (a) and Nitrite (b) concentrations in Pacific white shrimp *L. vannamei* culture during the experimental period.

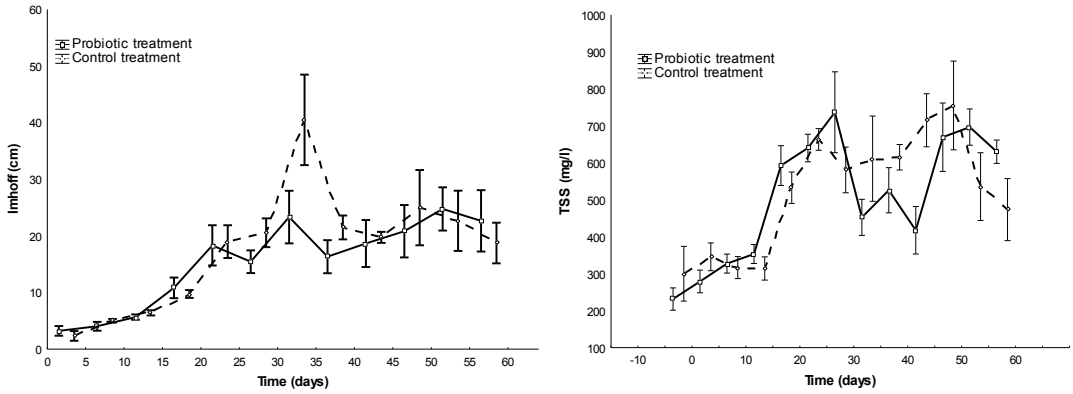

Figure 5. Performance of biofloc during the experimental period, (a) Imhoff Cones and (b) Total Suspended Solids).

The results for zootechnical performance showed significant differences ($P<0.05$) between the treatments (Table 2). The final weight and survival rate were significantly lower for the shrimp cultured in the treatment without probiotic (control). The FCR ranged from 2.70 in the control to 1.40 in the probiotic treatment group, with significant differences ($P<0.05$) between treatments. Productivity (kg m^{-2}) was significantly higher ($P<0.05$) in the probiotic treatment group (2.25 kg m^{-2}) compared to the control (1.31 kg m^{-2})

TABLE 2. Mean survival, growth rate, final weight, final biomass and FCR

	Treatment	
	Control	Probiotic
Survival (%)	$52.0^{a} \pm 12.3$	$83.0^{b} \pm 6.5$
Growth rate (g/week)	$0.85^{a} \pm 0.2$	$0.92^{b} \pm 0.3$
Final weight (g)	$8.42^{a} \pm 2.32$	$9.05^{b} \pm 2.54$
Final biomass (kg/tank)	$45.97^{a} \pm 6.4$	$78.87^{b} \pm 10.9$
FCR	$2.70^{a} \pm 0.41$	$1.40^{b} \pm 0.25$
Productivity (kg/m²)	$1.31^{a} \pm 0.2$	$2.25^{a} \pm 0.5$

Different superscripts in the same row indicate significant differences ($P<0.05$).

Discussion

Along experiment dissolved oxygen, pH and temperature remained within recommended ranges for the growth of *L. vannamei* during the study (Van Wyk and Scarpa 1999). The DO reduction during the culture period was probably due to the increases in shrimp biomass and the bacterial population in the cultured water. According to Avnimelech (2009), the addition of carbohydrates is a potential means for reducing the levels of inorganic nitrogen in BFT systems by increasing the C:N ratio and converting the TA-N to bacterial proteins (Avnimelech 1999; Samocha et al. 2007). In this study, the process of ammonia oxidation occurred relatively quickly (fig 04a). Nitrite is an intermediate product of ammonia, either by bacterial nitrification of ammonia or denitrification of nitrate (Avnimelech 2009). The accumulation of nitrite is a significant negative factor in the growth of aquaculture species. In addition, low DO concentrations can also cause nitrite accumulation in the nitrification biofilters (Chen et al. 2006). In BFT systems, high concentrations of nitrite have been reported in several studies (Otoshi et al. 2009; Vinatea et al. 2010; Krummenauer et al. 2011; Furtado et al. 2011; Maicá et al. 2011). In this study, high nitrite levels during the fourth and fifth weeks of the trial may have negatively impacted growth in both treatments.

TSS, Imhoff cone, turbidity and water clarity are commonly used to quantify the volume of biofloc present in the culture system. According to Samocha et al. (2007), the target TSS level lies in the ranges of 400-500 mg L^{-1}. During the study period, the mean value was within that range; however, recorded values near the end of the study were close to 800 mg / L^{-1}, but they did not differ significantly between treatments.

The identification of *Vibrio spp.* bacteria has been described by a number of researchers (West et al. 1984; Bryant et al. 1986). These identifications have played important roles, but

they usually involve many techniques that require extensive microbiological and immunological skills of the laboratory staff. However, during the 1980s, monoclonal antibodies were produced against a variety of bacteria and used for the rapid identification of bacteria (Hanna et al. 1992). Several authors suggest that in aquaculture, the inoculation of probiotic bacteria into the culture system is a viable alternative for avoidance of *Vibrio spp*.-based diseases (Rengpipat et al. 1998; Moriarty 1998; Balcazar et al. 2006; Kesarcodi-Watson et al. 2008). According to Kesarcodi-Watson et al. (2008), diseases caused by *Vibrio* spp. are commonly implicated in episodes of increased mortality. The Gram-negative bacterium Vibrio is reputed to have caused mass mortalities in penaeid shrimp culture in many regions, and they can also affect luminescence in the cultured animals (Jayasree et al. 2006; Defoirdt et al. 2007; Phuoc et al. 2009). In this study, the experimental animals that received a probiotic showed significantly higher survival rates than the control treatment (survival 83% and 52%, respectively). This indicates that the strains present in the probiotic were effective in protecting the shrimp against *V. parahaemolyticus*. Similarly, Swain et al. (2008) reported that *E. faecium* was able to protect *P. monodon* post-larvae when challenged with *V. parahaemolyticus*. In addition, several studies (Rengpipat et al. 1998; Decamp et al. 2008) have also shown that B*acillus* sp. strains can increase shrimp survival when exposed to pathogenic Vibrio.

The mean weight of individual shrimp at the end of the experiment was also slightly higher in the probiotic treated group (9.05 g) compared to the control (8.42 g). This fact is even more remarkable when taking into account that the higher survival rates resulted in higher shrimp density in the probiotic tanks. Nevertheless, the shrimp in the probiotic treatment achieved a higher growth rate than the shrimp grown at lower density conditions in the control group. These results could be associated with the positive effects from probiotic bacteria, which

provided a better culture condition for the shrimp compared to the treatment without probiotics (control). Similar results were observed by Widanarni et al. (2010), who showed that nursery culture with probiotic addition resulted in significantly better growth performance in Pacific white shrimp (*L. vannamei*).

From a production point of view, it is clear that improved growth performance along with increased survival rate in the group that received probiotics resulted in a significant ($P<0.05$) increase in the final biomass: a 70% increase in production (79 kg versus 46 kg) for the probiotic treatment compared to the control. The lower observed FCR is similar to the result obtained by Krummenauer et al. (2011) in a study with the same stocking densities used in this research.

On the other hand, when shrimp stocking density decreased from 3000 m^{-2} in the nursery to 300 m^{-2} in growout, even contaminated, survival was higher than 50% in the control. The results also showed the capacity of BFT systems to help in the health of the shrimp in the rearing. According to Balcázar et al. (2006), the composition of microbial communities can be changed by production practices and environmental conditions, which stimulate the proliferation of selected bacterial species. Several authors emphasize the benefit of the ingestion of other microorganisms, as a tool for eliminate or reduce the incidence of opportunistic pathogens (Gatesoupe 1999; Thompson et al. 1999; Balcázar 2006; Li et al. 2008; Crab et al. 2010; Souza et al. 2011). Overall, the results show that the multi-strain probiotic tested in this study controlled *V. parahaemolyticus* and improved the overall productivity in a BFT culture system.

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CONCLUSÃO E RECOMENDAÇÕES

Os resultados do presente estudo auxiliaram no desenvolvimento de um pacote tecnológico dentro da realidade econômica do Brasil, para a implementação do cultivo de *L. vannamei* em sistemas de bioflocos (BFT system). Com os experimentos realizados podemos concluir que:

1) A utilização de um inóculo mínimo (2,5%) de biofloco formado acelera a formação dos agregados microbianos em sistemas BFT. Além disso, com a reutilização de água os índices de sobrevivência e crescimento foram melhores que o tratamento com água clara, demonstrando ser a melhor estratégia a ser adotada;

2) Os cultivos em sistemas BFT podem ser realizados com baixo volume de água (0,40m), reduzindo a quantidade de água utilizada. Foram produzidos 8,45 kg de camarões com apenas 119 litros de água;

3) Diferentes equipamentos de aeração podem afetar a formação dos bioflocos e a abundância e tipos de microrganismos presentes nos cultivos. Além disso, o difusro de ar (Blower) proporcionou melhor formação dos agregados microbianos que resultaram em melhores parâmetros de qualidade da água e desempenho zootécnico dos camarões;

4) O sistema de aeração "Taeration" também pode ser utilizado como ferramenta na formação dos bioflocos e no aporte de oxigênio na agua de cutivo;

5) O uso de probióticos pode ser utilizado na prevenção e controle de *V. Parahaemolyticus* além de proporcionar aumento na produtividade dos camarões cultivados em sistema BFT.

Com estes resultados torna-se possível o repasse desta tecnologia de cultivo para pequenos e médios produtores aquícolas do Brasil.