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Tecnologia de bioflocos na larvicultura do camarão *Litopenaeus vannamei*

Tese apresentada ao Programa de Pós-Graduação em Aquicultura da Universidade Federal de Santa Catarina como requisito para obtenção de grau de Doutor em Aquicultura

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Tecnologia de bioflocos na larvicultura do camarão *Litopenaeus vannamei*

Por

MARCO ANTONIO DE LORENZO

Esta tese foi julgada adequada para a obtenção do título de

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“Quando a educação não é libertadora, o sonho do oprimido é ser o opressor”.
Paulo Freire

RESUMO

Objetivou-se com este trabalho aplicar estratégias de manejo de larvicultura de *Litopenaeus vannamei* no cultivo com bioflocos microbianos com o intuito de viabilizar a produção de pós-larvas sem renovação de água, entre as fases de misis 1 (M1) e pós-larvas 5 (PL5). Primeiramente foram avaliados melaço e dextrose como fontes de carbono para estabelecimento de uma determinada relação carbono:nitrogênio (C:N) visando o controle da amônia em comparação com o sistema de larvicultura tradicional com renovação de água na densidade de 200 larvas L^{-1}. Resultados semelhantes em termos de qualidade da água e de desempenho foram obtidos a partir do sistema convencional de produção e com a adição de fontes de carbono orgânico sendo que este consumiu apenas 12% do total de água utilizada no sistema convencional. No segundo experimento, avaliou-se o desempenho da larvicultura sob diferentes níveis de fertilização com dextrose estabelecendo relações C:N fixas de 10:1, 12,5:1 e 15:1 na densidade de 200 larvas L^{-1}. Os valores médios dos parâmetros zootécnicos e de qualidade de água avaliados foram apropriados para esta fase de produção com todas as relações avaliadas. No entanto, as relações 12,5:1 e 15:1 mantiveram níveis mais baixos de amônia. No terceiro experimento, foram avaliadas quatro densidades de estocagem (200, 250, 300 e 350 larvas por litro) com C:N fixa de 12,5:1. Os valores médios dos parâmetros de qualidade de água foram adequados para esta fase de produção em todos os tratamentos. Os dois tratamentos com maior densidade obtiveram maior produtividades, com consumo limitado a 8% da água utilizada pelo sistema convencional. Estes resultados indicam que o sistema BFT pode ser utilizado na larvicultura de camarões marinhos, aumentando a produtividade com redução do uso de água.

Palavras-chave: Aquicultura, BFT, cultivo intensivo, heterotrófico, sustentabilidade.

Abreviaturas: BFT, tecnologia de bioflocos.

ABSTRACT

The objective of this study was to apply the biofloc technology to *L. vannamei* hatchery, in order to allow the production of post-larvae in biosecure system without water exchange from misis 1 (M1) to postlarvae 5 (PL5) phases. The first experiment evaluated the dextrose and molasses as carbon sources compared to the conventional hatchery system whith water exchange, using the stock density at 200 larvae L^{-1}. Similar results in terms of water quality and performance were obtained from the conventional system and with the addition of organic carbon sources and the systems without water exchange consumed only 12% of the total water used in the conventional system. The second experiment evaluated the hatchary performance under three fixed C:N ratios (10:1, 12.5:1 and 15:1) using dextrose as carbon sourse and stocking density of 200 larvae L^{-1}. The performance parameters and water quality were appropriate for this production stage in all C: N ratios used. However, 12.5:1 and 15:1 maintained lower levels of ammonia. In the third experiment, we evaluated four stocking densities (200, 250, 300 and 350 larvae per liter) with a C:N ratio of 12.5:1. The values of water quality parameters were suitable for this production phase in all treatments. The two treatments with high density had higher productivity, with limited consumption to 8% of water used by the conventional system. These results indicate that BFT system can be used in marine shrimp hatchery increasing productivity and reducing the use of water.

Keywords: Aquaculture; BFT, intensive culture, heterotrophic, sustentability.

Abbreviations: BFT, biofloc technology.

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Nos últimos 30 anos observaram-se o crescimento e intensificação da indústria da aquicultura com relatos de níveis de crescimento três vezes mais rápido que a agricultura e com um aumento médio de 8,3% por ano desde 1970 (FAO 2015). A participação da aquicultura no fornecimento global de frutos do mar em 2012 ficou em torno de 52% (FAO 2014). Esta percentagem deverá aumentar para 62% até 2030 uma vez que capturas provenientes da pesca estão estagnadas frente a demanda de uma classe média emergente global que aumenta substancialmente (FAO 2014).

A aquicultura, de modo geral, apresenta um imenso potencial para expandir-se e intensificar-se imprescindivelmente de forma sustentável para contribuir com esta crescente demanda por alimentos até 2050, uma vez que a população humana está prevista a aumentar nos próximos 30 anos antes de se estabilizar em um mínimo de 9 bilhões de pessoas (GODFRAY *et al*., 2010).

No entanto, a aquicultura é confrontada com a competição por água, terra e outros recursos naturais gerados pela pressão do crescimento populacional e tais desafios podem ser atendidos através da intensificação de sistemas de cultivo (PIEDRAHITA, 2003). Sobre os níveis de intensificação em aquicultura, estes podem variar desde sistemas extensivos, com baixa estocagem de organismos jovens crescendo naturalmente, até sistemas muito intensivos, com altas taxas de estocagem e utilização de alimento artificial. Atualmente, a aquicultura vem passando por contínua modificação com novos métodos e tecnologias (genética, formulação de rações e gestão da água) para alcançar taxas mais elevadas de produção por unidade de área. Esta intensificação certamente continuará no futuro próximo (DIANA *et al*., 2013).

Com a expansão da produção aquícola torna-se importante o conhecimento dos impactos ambientais relacionados a ela. Adicionalmente, devem-se desenvolver estratégias que impeçam ou minimizem danos provenientes dessa atividade, como poluição de águas, grande aporte de energia e água no meio de produção, aplicação de antibióticos e químicos, além de problemas sanitários. Tal conhecimento é fundamental para o desenvolvimento futuro de sistemas produção aquícola sustentável (DIANA *et al*., 2013).

Trazendo essa abordagem para um recorte específico da carcinicultura, a importância de espécies de camarão peneídeos, com

reprodução controlada, independente de estoque selvagem, deve de ser considerada. Duas espécies de peneideos, *Litopenaeus vannamei* e *Penaeus monodon*, participam com a maior fatia da indústria mundial com 12 bilhoes de dólares ao ano. Entretanto, deste valor total estima-se uma perda de 40% (três bilhões de dólares por ano) da produção da carcinicultura tropical devido, principalmente, a patógenos virais aos quais medidas preventivas padrão (como vacinação) não são factíveis (STENTIFORD, 2012).

Na carcinicultura nacional, a produção do camarão branco do Pacífico, *Litopenaeus vannamei*, se destaca. Características como capacidade de adaptação as mais variadas condições de cultivo, altas taxas de crescimento e sobrevivência, boa produtividade e grande aceitação no mercado, transformaram o *L. vannamei* na única espécie cultivada comercialmente no país. O auge da produção nacional para a espécie se deu em 2003, atingindo 90.190 toneladas (FAO, 2012) e uma produtividade média de 6.084 kg/ha/ano, sendo a maior entre os países produtores na época (ROCHA, 2005). Porém, a partir de 2004, o setor enfrentou problemas sanitários cujo impacto negativo em temos de produtividade e nas relações comerciais prejudicou sensivelmente o seu desempenho, com destaque para o Vírus da Mancha Branca (WSSV, do inglês *White Spot Syndrome Virus*) e o Virus da Mionecrose Infeciosa (IMNV, do inglês *Infectius Myonecrosis Virus*), que reduziu a produção para atuais 65.000 toneladas (FAO, 2014; FAO-Fishstat, 2015). Diante desse quadro, há urgência no desenvolvimento de novas abordagens para aumento da produtividade através de melhorias nos reprodutores, no suprimento de larvas e no aperfeiçoamento do manejo e biosseguridade da produção (STENTIFORD, 2012).

Este duplo desafio – aumento da demanda por alimentos e perdas por questões sanitárias – pode ser atendido através de um planejamento de expansão a longo prazo. Com base na sustentabilidade futura da indústria mundial de produção de camarões, inclui-se melhorias na seleção de reprodutores, práticas rigorosas de biosseguridade e sistemas intensivos de cultivo sem renovação de água, como no caso da tecnologia de bioflocos (BFT= Biofloc Technology System). Essas melhorias podem levar a maiores índices de produtividade por unidade de área, evitando também a troca de organismos patogênicos com o ambiente (MOSS *et al*., 2012). Entretanto, torna-se importante ponderar que, se por um lado esse aumento da produção de biomassa pode causar impactos negativos no ambiente, com potencial aumento de material proveniente de ração não ingerida e produção de excretas metabólicas, por outro, pode proporcionar um sistema de tratamento de efluente mais

funcional resultando em menores níveis de descarga por unidade de biomassa produzida, quando comparado com sistemas de cultivo de menor intensificação (PIEDRAHITA, 2003).

Mais especificamente tratando-se de sistema BFT, este viabiliza a intensificação evitando a renovação de água dos sistemas de produção, minimizando o fluxo de patogenos e a descarga de efluentes ricos em nutrientes no ambiente (SAMOCHA *et al*., 2007). Em sistemas de BFT sem renovação de água, o controle da amônia (tóxica para peixes e camarões) se inicia através da manutenção de uma relação carbono:nitrogênio (C:N) que promova o crescimento da população de bactérias heterotróficas no meio através da incorporação do nitrogênio amoniacal presente na água (AVNIMELECH, 1999; EBELING *et al*., 2006). A relação C:N adequada é obtida através da adição de fontes de carbono orgânico (como melaço, farinhas, açúcar e dextrose) ao meio de cultivo. São necessários 20g de carboidrato, ou 5,7g de carbono para converter 1g de amônia em biomassa bacteriana (AVNIMELECH, 1999; EBELING *et al*., 2006). Contudo, pouco se sabe sobre a inserção do sistema de bioflocos na larvicultura de camarões marinhos.

Assim, o tema central do presente trabalho é um recorte específico com foco na larvicultura de camarões marinhos frente aos desafios do aumento da demanda alimentar mundial, cuja abordagem se estrutura sobre o tripé de sustentação – preservação de recursos e meio ambiente aquático, aumento da biosseguridade e intensificação da produção – visando aumento real da produtividade com sustentabilidade.

1.1 Larvicultura do *L. vannamei*:

Dentro do ciclo produtivo do camarão, uma das etapas que requer maior atenção é a larvicultura, que se estende desde a fase de náuplio (N), pós eclosão, até a fase de pós larva (PL) quando são transferidas para o pré berçário, o que normalmente se dá entre PL5 e PL10. Na larvicultura as unidades de produção normalmente são tanques de grande volume, entre cinco e trinta mil litros (ANDREATTA e BELTRAME, 2004) com densidades entre 100 e 250 nauplios por litro e uma sobrevivência média em torno de 60% (FAO, 2003). Nesta fase os animais se apresentam extremamente susceptíveis a estressores físicos e químicos e também à contaminação por micro-organismos de natureza bacteriana, fungica e viral, além de protozoários e parasitos, sendo estes pontos considerados um ponto crítico na produção de camarões (ANDREATTA e BELTRAME, 2004).

De modo geral as características de produção na larvicultura de camarões marinhos são a manutenção de um meio predominantemente autotrófico, através da adição e manutenção de microalgas (diatomáceas e clorofíceas) na água de cultivo. As diatomáceas, preferencialmente, pois contêm altos valores de ácidos graxos poli-insaturados como o ácido eicosapentaenóico e docosaexaenóico, importantes no desenvolvimento larval de camarões peneídeos (PRATOOMYOT *et al*., 2005). Além das microalgas as larvas recebem alimentação com rações microencapsuladas, com altos níveis de proteínas (42-55% PB), formuladas de acordo com as etapas de desenvolvimento larval, além de náuplios de artemias. A larvicultura de camarões, a partir da fase de misis 1 (M1) também se caracteriza por altos índices de renovação da água, excedendo em 100% ao dia o volume da unidade para manutenção dos parâmetros de qualidade da água de cultivo em níveis adequados no final do cultivo.

O sistema predominantemente autotrófico utilizado na produção de camarões marinhos utiliza altos níveis de renovação para controlar possível superpopulação de algas, controle da qualidade de água e manutenção dos níveis de amônia. Além disso, a utilização de rações com altos níveis de proteína, tem sido responsabilizada pela deterioração de ecossistemas costeiros e assim sofrido grandes perdas econômicas, resultado de doenças decorrentes de sua auto-poluição (SAMOCHA *et al*, 2007).

1.2 O cultivo de organismos aquáticos em sistema de bioflocos:

O cultivo em sistema de bioflocos vem ganhando força no cenário mundial seja na piscicultura como também nas fases de engorda de camarões, onde inicialmente estimula-se o estabelecimento de microbiota bacteriana heterotrófica no meio de cultivo. Outra característica deste sistema é propiciar um cultivo intensivo com trocas mínimas tendendo a regimes de troca zero de água, através do consumo do nitrogênio inorgânico produzido no meio de cultivo proveniente das excretas e restos alimentares (AVNIMELECH, 1999). Com esse procedimento reduz-se a entrada de patógenos e a descarga de efluentes ricos em nutrientes (DECAMP *et al*., 2003; CRAB, 2012), mesmo em cultivos de alta densidade.

Reduzindo ou cessando a renovação, torna-se fundamental o controle da amônia resultante do catabolismo protéico para que não se acumule no ambiente de criação devido a sua toxicidade para peixes e camarões. As amônias ionizadas e não ionizada apresentam-se em

proporções variáveis sob influência de outros fatores como pH, temperatura e salinidade, sendo a não ionizada a forma mais tóxica por sua afinidade com os componentes apolares da membrana plasmática, causando uma série de danos fisiológicos (BOWER and BIDWELL, 1978; CHEN, et al., 1996; LIN and CHEN, 2001).

No sistema de bioflocos sem renovação, a estratégia de controle da amônia passa a ser o estabelecimento de uma relação carbono:nitrogênio que facilita o crescimento de microorganismos heterotróficos para incorporação do nitrogênio amoniacal do meio (MORIARTY, 1997; AVNIMELECH, 1999; EBELING *et al*., 2006; HARI, *et al*., 2006; SAMOCHA, *et al*., 2007). Esta relação é obtida utilizando-se fontes de carbono orgânico (melaço, farinhas, açúcar, dextrose, etc.) levando-se em consideração que 20 g de carboidrato ou 5,7 g de carbono são necessárias para conversão de 1g de nitrogênio amoniacal em biomassa bacteriana (AVNIMELECH, 1999; EBELING, *et al*., 2006).

Neste sistema de cultivo, bactérias heterotróficas e quimioautotróficas participam da formação do biofloco, que também consiste em uma mistura complexa compostos por bactérias, algas, fungos, protozoários, rotíferos, nematóides, detritos orgânicos e inorgânicos (BRATVOLD E BROWDY, 2001; AVNIMELECH, 2006; CRAB *et al*., 2007; DE SCHRYVER, *et a*l., 2008). Em viveiros de cultivo heterotrófico, componentes orgânicos podem estar disponíveis tanto na coluna d'água quanto no fundo do viveiro (SCHROEDER, 1978).

O cultivo em sistema de bioflocos, num segundo momento, em condições ambientais favoráveis e também de tempo, promove o estabelecimento de uma microbiota nitrificante que pode participar em maior ou menor grau no controle dos compostos nitrogenados no sistema de bioflocos. Cronologicamente o estabelecimento de bactérias heterotróficas ocorre mais rapidamente devido a uma taxa de crescimento de produção de biomassa bacteriana por unidade de substrato heterotrófico 10 vezes maior que a de bactérias nitrificantes (HARGREAVES, 2006). Segundo LEONARD *et al*. (2002), existe uma competição constante por espaço entre bactérias heterotróficas e autotróficas, entretanto, a disponibilidade dos resíduos orgânicos, conforme AVNIMELECH (2006), fornece substrato que favorece uma dominância da comunidade heterotrófica.

Uma consequência do crescimento da comunidade heterotrófica é a produção de proteína microbiana. Essa biomassa microbiana resultante da conversão de detritos orgânicos pode ser consumida regularmente

pelos camarões durante o cultivo (BARBIERI JR. e OSTRENSKY NETO, 2002; CUZON *et al*., 2004). Além de proteína, os flocos contêm quantidades importantes de macronutrientes (cálcio, fósforo, potássio e magnésio) e micronutrientes (cobre, ferro, manganês e zinco), assim como aminoácidos e ácidos graxos (MOSS, 2006, TOLEDO *et al*., 2014). Esse consumo potencialmente contribui duplamente para a dinâmica do cultivo, pois além de constituir uma fonte para a nutrição dos camarões é um eficiente instrumento de reciclagem dos nutrientes através da biomassa de animais cultivados (MCINTOSH, 2001).

1.3 Fontes de Carbono para fertilização no sistema de bioflocos:

No meio heterotrófico é imprescindível o estabelecimento e manutenção de relações Carbono:Nitrogênio (C:N), que em níveis adequados, faz com que o nitrogênio inorgânico seja incorporado pela célula bacteriana enquanto substratos orgânicos são metabolizados. A adição de hidratos de carbono é um meio prático e de baixo custo para reduzir a concentração de nitrogênio inorgânico em sistemas de aqüicultura intensiva. A quantidade de hidratos de carbono a ser inserida deve estar relacionada a entrada de ração e da mensuração dos níveis de amônia (AVNIMELECH, 2006).

Diversas fontes de carbono já vêm sendo utilizado com êxito para este fim. Destaca-se o melaço, que é subproduto do processo de fabricação do açúcar, que embora não seja constituído unicamente de carboidratos (55% de carboidratos, 3% proteínas e 42% outros componentes) vem sendo usado com êxito, bem como a dextrose, que apresenta 100% de carboidratos constituintes (SUITA, 2009). Estas fontes de carbono têm diferentes porcentagens de carbono, quanto mais pura, menos quantidade desta será preciso para reduzir a amônia (AVNIMELECH, 1999). A escolha pode estar associada a uma análise de custos, qualidade e padronização da fonte de carbono, bem como a possível diferença entre elas na dinâmica do processo de formação de bioflocos microbianos, que em um período relativamente curto como a larvicultura de camarões pode ser um fator importante.

Alguns estudos demonstram que a fonte de carbono não influencia na composição do floco microbiano (EKASARI, *et al*., 2010). No cenário produtivo é preciso conhecer o uso das diferentes fontes de carbono para usá-las segundo o custo beneficio.

2 JUSTIFICATIVA

A larvicultura do camarão marinho *Litopenaeus.vannamei*, requer um grande aporte de recursos naturais, energéticos, equipamentos e instalações além de mão de obra capacitada para que índices de sobrevivência considerados bons (acima de 60%) sejam alcançados. Alguns desses recursos estão listados abaixo frente a prováveis estratégias de manejo que lhes reduzam ou mitiguem efeitos negativos, estratégias estas das quais se trata a abordagem do presente trabalho, como se segue:

2.1 Água:

O uso e renovação de água nas unidades usualmente de 20 toneladas (t), onde renovações parciais diárias iniciam-se na fase de misis 1 (M1) tendendo a um acréscimo variável até que pode suplantar 100% do volume da unidade a partir de pós larva um (PL1), representando uma grande quantidade deste recurso e do processo envolvido na sua utilização considerando-se a sua captação, estocagem, tratamento, neutralização, aquecimento e distribuição.

A presente proposta de sistema sem renovação com fertilização para promover o desenvolvimento de bioflocos microbianos como ferramenta de controle da amônia na água de cultivo da larvicultura de camarões marinhos alteraria esse consumo a partir do momento em que o objetivo a ser alcançado seria a não renovação de água.

2.2 Energia:

Na região sul do Brasil, por suas características climáticas e de correntes, a água captada e estocada demanda de uma grande quantidade de energia para que atinja a temperatura ideal para larvicultura, podendo, em certas épocas do ano, requerer um aumento de temperatura de aproximadamente 14 °C. Este consumo energético representa um dos índices de maior custo para um laboratório nestas condições. Quando se pensa na quantidade de água que precisa ser aquecida para dar suporte ao sistema de renovação de água na larvicultura pode-se estimar a economia energética num sistema sem renovação. Também o gasto energético com o bombeamento de água para renovação seria drasticamente reduzido.

2.3 Microalgas:

O sistema sem renovação também tende a reduzir a demanda de microalgas na larvicultura, uma vez que a utilização das mesmas ficaria concentrada apenas na fase de protozoea, quando as microalgas representam a principal fonte de alimento. Após a fase de misis, as larvas passam a ser predominantemente carnívoras reduzindo a sua dependência por microalgas esperando-se que a ração adequada, náuplios de artêmia, no sistema de bioflocos de suporte as larvas. Com isso projeta-se redução nos custos de produção, distribuição e mão de obra, bem como processos e dimensões no cepário, intermediário e massivo do setor de microalgas também reduzindo em potencial os riscos de contaminações no cultivo das microalgas e aumentando assim os níveis de biossegunança das unidades de larvicultura.

2.4 Alimento:

O manejo os tanques de larvicultura com fertilização visando a formação dos bioflocos microbianos sem renovação podem, em perspectiva, trazer uma redução do aporte de ração no tanque por estes apresentarem um determinado nível de características nutricionais (não sendo objeto deste estudo) que seriam utilizadas pelas larvas como fonte complementar de alimento tendo como resultado uma redução nos custos com a alimentação. Isso se fundamenta pelo hábito alimentar dos camarões (detritívoros), e, para tanto, se faz necessário um ajuste entre quantidade de ração, níveis de fertilização do tanque, quantidade e qualidade nutricional dos sólidos suspensos frente à qualidade da água.

2.5 Biosseguridade:

A redução da taxa de renovação de água na larvicultura reduz ou elimina constantes entradas de água no sistema, o que diminuiria sensivelmente o risco de contaminações químicas, físicas ou biológicas oriundas de problemas nos processos de captação, tratamento, neutralização, aquecimento e distribuição da água até as unidades de criação. Tais problemas, podem gerar uma fonte de contaminação por micro-organismos que podem se desenvolver dentro do meio de cultivo. Com a taxa de renovação de água tendendo a zero, ambos os pontos críticos de entrada e saída de água (apresentado no próximo item) do sistema estariam controlados tornando o sistema de cultivo mais biosseguro.

2.6 Ambiental:

Sob uma perspectiva ambiental, há no sistema de cultivo proposto uma tendência a se poupar o ambiente uma vez que se evita o processo de renovações constantes que promove uma descarga de grandes volumes de água com presença de matéria orgânica e, se presentes, microorganismos patogênicos a esta e outras espécies no ambiente.

Com o sistema heterotrófico sob fertilização a qualidade da água seria mantida através do favorecimento de uma microbiota no meio de cultivo. Esses organismos seriam capazes de de incorporar a amônia resultante do metabolismo das larvas e decomposição da matéria orgânica do tanque na forma de proteína bacteriana atingindo um ponto de equilíbrio tal que a qualidade da água seria mantida em níveis controlados para o desenvolvimento da larvicultura evitando-se o grande volume de água saindo do sistema.

3 HIPÓTESE

A larvicultura do camarão-branco-do-pacífico (*Litopenaeus vannamei*) pode ser realizada com o menor uso de água mediante utilização da tecnologia de bioflocos

4 OBJETIVOS

4.1 Objetivo geral

Aplicar estratégias de manejo de larvicultura de *Litopenaeus vannamei* no cultivo com bioflocos microbianos (BFT) com o intuito de viabilizar a produção de pós-larvas sem renovação de água.

4.2 Objetivos específicos

a) Avaliar diferentes fontes de fertilização orgânica, melaço e dextrose, para formação de bioflocos microbianos na larvicultura do camarão marinho *L. vannamei* a partir do estágio de mísis.
b) Avaliar diferentes relações C:N (10:1, 12.5:1 e 15:1) sobre o desempenho de larvas de *L. vannamei* cultivados em sistemas superintensivos com bioflocos.
c) Avaliar diferentes densidades de povoamento (200, 250, 300 e 350 M1/L) sobre o desempenho de larvas de *L. vannamei* cultivados em sistemas superintensivos com bioflocos.

5 FORMATAÇÃO DOS ARTIGOS

A tese é dividida em quatro capítulos: o primeiro referente à introdução e revisão de literatura; o segundo refere-se a artigo científico original (primeiro artigo) sobre o primeiro objetivo específico estando publicado no periódico *Aquacultural Engineering* (DOI:10.1016/j.aquaeng.2015.05.007). O terceiro capítulo trata de um artigo científico (segundo artigo) que foi aceito para publicação no mesmo periódico citado acima estando no momento disponível online em PDF (não editado e não formatado) no endereço http://dx.doi.org/10.1016/j.aquaeng.2016.04.001. O quarto capítulo também descreve artigo científico original (terceiro artigo) que se encontra redigido e traduzido para o inglês para ser submetido para publicação.

6 CAPÍTULO II: INTENSIVE HATCHERY PERFORMANCE OF THE PACIFIC WHITE SHRIMP IN BIOFLOC SYSTEM

7 ABSTRACT

We assessed the hatchery performance of *Litopenaeus vannamei* between the mysis1 and postlarva5 stages, in a zero-exchange biofloc system. Two sources of organic carbon (molasses and dextrose) were evaluated and water quality, zootechnical parameters, microbiology, and water consumption during production were compared between carbon-supplemented and control groups. The mean values of the evaluated water quality parameters were appropriate for this production stage. Fertilization with molasses and dextrose efficiently controlled ammonia levels and ammonia did not reach the average concentrations that are considered toxic for the species (total ammonia <1.3 $mg \cdot L^{-1}$ and free ammonia<0.05 $mg \cdot L^{-1}$). The number of heterotrophic bacteria in the water was greater in the molasses and dextrose groups than in the control group. However, there was no difference in Vibrionaceae count between groups. There was no difference between groups in survival (>85%), length (6.15 mm), dry weight (0.17 mg) of postlarvae 5. Treatment with dextrose or molasses required approximately 12% of the water used by the control group. *L. vannamei* production rates and water quality were maintained without water exchange using a biofloc system supplemented with dextrose or molasses.

Keywords: zero-exchange; larvae; heterotrophic; BFT; intensive culture.

Abbreviations: BFT, biofloc technology;

8 INTRODUCTION

The hatchery stage is a critical stage in the production of Pacific white shrimp (*Litopenaeus vannamei*), during which strict quality criteria and constant attention are required. The hatchery stage extends from the nauplii phase to the postlarva5 (PL5) phase. At this stage, shrimp are extremely susceptible to physical, chemical, and biological stressors, such as vibriosis outbreaks (Vandenberghe et al., 1999; Aguirre-Guzman et al., 2001; Mourino et al., 2008; Martin et al., 2012). In order to minimize losses due to disease and environmental impacts, hatchery systems with biofloc technology (BFT) and reduced water exchange have been developed (Samocha et al., 2007). Intensive shrimp

production is traditionally performed in a predominantly autotrophic medium, with high rates of daily water exchange (Wang, 1990). At this stage, microalgae rich in polyunsaturated fatty acids are added every day, in addition to the water renovations (Piña et al., 2006; Ju et al., 2009; Khatoon et al., 2013). These microalgae exchanges not only contribute to the nutrition, but also enable the control of ammonia nitrogen levels in the farming tanks (Ebeling et al., 2006). Negative impacts associated with such production systems, such as the discharge of large volumes of water containing high levels of ammonia nitrogen and phosphorus (microalgae, feces, and uneaten feed) may threaten coastal ecosystems and lead to health risks due to self-pollution (Hopkins et al., 1995; Samocha et al., 2007). In this context, the sometimes-considerable economic costs of the energy required to capture, heat, and distribute large volumes of water must be considered.

In an attempt to minimize the impact of the environmental, health, and economic problems associated with shrimp aquaculture, BFT has become increasingly common (Avnimelech, 1999, 2006; Browdy et al., 2001; Crab et al., 2007; De Schryver et al., 2008). BFT is used to intensify production and avoid the exchange of farming water, with a consequent reduction in the flow of pathogens and discharge of nutrient-rich effluents into the environment (Samocha et al., 2007).

Reducing or ceasing water exchange requires control of the ammonia that results from protein catabolism, as it is toxic to fish and shrimp. Ionized and non-ionized ammonia are present in the water of aquaculture tanks in variable proportions that are influenced by factors such as pH, temperature, and salinity. The non-ionized form of ammonia is more toxic to shrimp than the ionized form, and causes a variety of physiological damage, due to its affinity for the non-polar compounds of the plasma membrane (Bower and Bidwell, 1978; Chen et al., 1996; Lin and Chen, 2001).

In BFT systems without water exchange, the ammonia control strategy centers on the establishment of a carbon-nitrogen balance that facilitates the growth of heterotrophic bacteria, which incorporate ammonia nitrogen from the medium (Moriarty, 1997; Avnimelech, 1999; Ebeling et al., 2006; Hari et al., 2006; Samocha et al., 2007). This relationship is established by adding organic carbon sources (molasses, flours, sugar, and dextrose) to aquaculture media. It requires 20 g of carbohydrate, or about 6 g of carbon, to convert 1 g of ammonia nitrogen to bacterial biomass (Avnimelech, 1999; Ebeling et al., 2006). In BFT culture systems, chemoautotrophic and heterotrophic bacteria participate in the formation of bioflocs, which also include an aggregate

of algae, fungi, protozoa, rotifers, and nematodes (De Schryver et al., 2008). Therefore, in addition to providing ammonia control, bioflocs may represent a food source in farming tanks (Avnimelech, 1999; Cuzon et al., 2004).

The use of BFT systems in the pre-nursery and fattening stages of marine shrimp aquaculture, have been extensively studied (Emerenciano et al., 2011; 2012, 2013 Ray et al., 2011; Xu and Pan, 2012; da Silva et al., 2013; Schveitzer et al., 2013; de Souza et al., 2014; Kumar et al., 2014). However, no systematic studies have been published showing BFT without water exchange during the hatchery phase as a viable alternative to the standard production system of penaeid shrimp larvae. The hatchery stage starts at mysis1 (M1), and continues through the PL5 phase. Daily water exchanges are typically performed throughout this stage. The objective of this study was to assess the hatchery performance of *L. vannamei* that were reared between M1 and PL5 using a biofloc system supplemented with organic carbon (molasses or dextrose) and without water exchange.

9 MATERIALS AND METHODS

The experiment was conducted at the Laboratório de Camarões Marinhos (LCM), Departamento de Aquicultura of the Universidade Federal de Santa Catarina, Brazil.

9.1 Biologic material

Before the experiment, nauplii of *L. vannamei* were raised in a 20 m3 (stocking density of 100 larvae L^{-1},), semi-cylindrical hatchery tank (annex 1) in salinity of 35 ppm until they reached mysis1. The microalgae *Chaetoceros muelleri* (5×10^4 cells mL^{-1}) was added to the culture water daily. When the larvae reached the stage of M1 (average dry weight of 0.085 ± 0.004 mg and average length of 3.543 ± 0.076 mm) they were transferred to the 60 L experimental units (annex2), which were initially filled with water from the hatchery tank. This line was free of any pathogens that require notification of the International Organization of Epizootics (from Aquatec LTDA, Rio Grande do Norte, Canguaratema, Brazil).

9.2 Experimental conditions

Three groups of larvae were prepared: two experimental groups were reared in a heterotrophic system without water exchange and a control group was reared in a conventional autotrophic system with daily water exchange and the addition of microalgae. The organic source was added to the culture water of the two experimental groups. Anhydrous dextrose ($C_6H_{12}O_6$, Sigma-Aldrich®) was added to the culture water of one group and sugar cane molasses (55% carbohydrate, 3% crude protein) was added to the culture water of the second group (annex 3).

The experimental groups were randomly distributed in a unifactorial experimental design. Semi-cylindrical plastic tanks (92×68×25 cm) with a working volume of 60 L constituted the experimental units. Four tanks were prepared for each experimental condition, resulting in 12 tanks. All tanks were equipped with linear aeration supplied by a PVC pipe (90 cm long, 20 mm diameter with 36 holes of 1 mm) to keep the solids generated during cultivation in suspension and maintain the level of dissolved oxygen in the water at the recommended concentration for *L. vannamei* larval cultivation (>5 $mg \cdot L^{-1}$). The water temperature was kept constant, between 29 and 30°C, using 100-W heaters connected to a thermostat.

The experimental tanks were supplied with water from an autotrophic larval cultivation. The water supplied had the following parameters: Chaetoceros muelleri (5×10^4 cells mL^{-1}), oxygen = 5.19 $mg \cdot L^{-1}$, pH = 7.92, temperature = 31.65°C, salinity = 35.57 $mg \cdot L^{-1}$, total ammonia = 0.92 $mg \cdot L^{-1}$, free ammonia = 0.05 $mg \cdot L^{-1}$, nitrite = 0.01 $mg \cdot L^{-1}$, nitrate = 1.56 $mg \cdot L^{-1}$, phosphate = 0.187 $mg \cdot L^{-1}$, total suspended solid (TSS) = 170.1 $mg \cdot L^{-1}$, volatile suspended solid (VSS) = 42.7 $mg \cdot L^{-1}$, and alkalinity = 132 $mg \cdot L^{-1}$.

Each experimental unit was stocked with 12,000 larvae in M1, which represents a stocking density of 200 larvae L^{-1}. The experiment was conducted until the larvae reach the post larvae stage 5 (seven days after stoking). The water in the biofloc experimental units was not exchanged during the experimental period, but evaporated water was replaced with fresh water in order to maintain salinity. No suspended solids were removed from the water during the experiment. To keep total ammonia nitrogen (TAN) below the established maximum of 1 $mg \cdot L^{-1}$, the water of the control units was exchanged at rates that ranged from 50% per day at the start of the experiment to 200% in the final stage of cultivation. Water samples were taken from the control units

after each water exchange. Chaetoceros muelleri was counted in the water samples. In order to provide food for larvae and to maintain water quality, *C. muelleri* was added as needed to maintain a concentration of 5×10^4 cells mL^{-1}.

The larval and postlarval shrimp were fed microencapsulated commercial diets (INVE). The Lansy ZM diet (minimum protein 48%, minimum ether extract 13%, maximum fibrous matter 2.5%, maximum mineral matter 13%, maximum humidity 8.0%, minimum calcium 0.25%, maximum calcium 1.5%, and minimum phosphorus 1.0%) was fed from M1 to mysis3/postlarva 1. After this period, and until harvesting, postlarvae were fed the Lansy MPL diet (minimum protein 48%, minimum ether extract 9.0%, maximum fibrous matter 2.5%, maximum mineral matter 13%, maximum humidity 9.0%, minimum calcium 1.0%, maximum calcium 2.2%, and minimum phosphorus 1.0%). Larvae were fed nine times a day (0800, 1000, 1200, 1400, 1600, 1800, 2100, 2300, and 0300) and were provided INVE quantities according to the manufacturer's recommendation for each larval stage. Artemia nauplii were also provided to the larvae at a rate of six nauplii for each mysis or postlarva, five times each day (0900, 1100, 1500, 1700, and 0000).

9.3 Addition of carbohydrates

Sources of organic carbon were added (divided in four times per day) to the biofloc treatment tanks (dextrose or sugar cane molasses) to maintain ammonia levels of <1 $mg \cdot L^{-1}$. The percentage of carbohydrate was assumed to be 100% for dextrose and 55% for molasses.

Controlling fertilization with organic carbon to regulate ammonia was done in two ways: 1) The amount of additional carbohydrate required to neutralize the ammonium excreted by shrimp was estimated assuming that shrimp assimilate about 25% of the nitrogen added in the feed and 75% of added nitrogen is transformed into ammonia dissolved in water. Sources of carbon were added to each tank at a ratio of 20 g carbohydrate for each gram of TAN created (Avnimelech, 1999). 2) When TAN surpassed 1 $mg \cdot L^{-1}$, additional carbohydrate (molasses or dextrose) was added to the system at a carbohydrate:TAN ratio.

9.4 Chemical and Physical variables of the water

Dissolved oxygen, temperature (YSI 55, YSI Incorporated, Yellow Springs, OH, USA) and pH (YSI 100, YSI Incorporated, Yellow

Springs, OH, USA) were measured twice a day. Salinity (YSI 30, YSI Incorporated, Yellow Springs, OH, USA), alkalinity (APHA 2005-2320 B), nitrite, and total ammonia were analyzed daily (APHA 2005). Total suspended solids (TSS) and volatile suspended solids (VSS) were assessed every other day (APHA 2005-2040 D and 2005- 2540 E) using 0.6-µm glass fiber micro-filters (GF-6, Macherey-Nagel, Düren, Germany). Two hundred milliliters of water samples were collected from each tank three times each week. Samples were frozen until nitrate (HACH method 8039, cadmium reduction) and orthophosphate analysis. The TAN, nitrite, nitrate, and orthophosphate analyses were carried out using a spectrophotometer and analyzed according to Strickland and Parsons (1984), and following the guidelines contained in APHA (2005).

9.5 Larval quality and zootechnical performance

Each day, 20 larvae from each tank were analyzed at the macro and microscopic level to assess larval quality. We observed the following parameters: swimming activity, lipid reserves, and color of the hepatopancreas, intestinal contents, deformities, presence of epibionts, adhered particles, necrosis, and muscular opacity (FAO, 2003).

Zootechnical parameters used to evaluate treatments included ultimate survival (%), final dry weight (mg), and final larval length (mm). We also examined survival (%) during a salinity stress test, which is related to larval quality (Samocha et al., 1998; Racotta et al., 2003). In order to perform this test, 100 larvae from each replicate were placed in cylinders containing 15 L of water with a salinity of 19 $g \cdot L^{-1}$. The test water was the same temperature as the culture water and the shrimp were kept in the test water for 60 min. After that time, the larvae were transferred to similar cylinders containing water with 35 $g \cdot L^{-1}$ salinity (the same of the culture) where they remained for an additional 60 min. Larval survival was estimated at the end of the procedure.

9.6 Microbiological analysis of water

Before harvest, 0.25 mL water samples were collected from each tank for microbiological analysis. Samples were homogenized and serially diluted (1/10) in sterile saline solution (3%) and seeded in duplicate on marine agar culture medium (Difco) to count viable and total heterotrophic bacteria. Diluted samples were also seeded in

duplicate on thiosulfate-bile-sucrose-agar medium (TCBS, Difco) to count Vibrionaceae bacteria. Seeded media were incubated in a microbiological oven at 30°C. After 24 h, the colony forming units (CFU) were counted.

Water consumption

The final amount of water used by the experimental group was expressed in liters per thousand of PL5 produced and includes the initial water used to fill the experimental units and the water for daily water exchange in the control group or to fresh water used to replenish evaporation losses in the experimental groups (biofloc).

9.7 Statistical analysis

One-factor ANOVA followed by Tukey's test (Zar, 1984), was used to compare treatments at a significance level of 0.05. Normality and homoscedasticity were assessed by the Shapiro–Wilk and Levene tests, respectively (Zar, 1984). Data expressed as a percentage underwent angular transformation before analysis. Microbiological analysis data showed no homoscedasticity and were Log10 transformed. The analysis of changes in ammonia over time was performed by repeated-measures ANOVA. Treatments were considered to be the main factors, and duration of culture was the additional factor. Significant differences were analyzed by Tukey's test (Zar, 1984) with a significance level of 0.05.

10 RESULTS AND DISCUSSION

All water quality parameters (Table 1, Figure 1) remained within the appropriate range for the hatchery stage of *L. vannamei*. These parameters were similar in the conventional production system with high rates of daily exchange (control), and the BFT production systems with both sources of organic carbon (dextrose and molasses).

Table 1: Water quality parameters and final water microbiology in three Pacific white shrimp (*Litopenaeus vannamei*) hatchery systems between the mysis 1 and postlarva 5 phase+s (200 larvae L^{-1}): conventional water-exchange system (control), biofloc system supplemented with dextrose (dextrose), and biofloc system supplemented with molasses (molasses).

Parameter	Control	Dextrose	Molasses	p
Temperature (°C)	30.20 ± 0.47[a] *	30.45 ± 0.26[a]	30.28 ± 0.21[a]	0.58
Oxygen (mg·L-1)	5.18 ± 0.05[a]	4.88 ± 0.10 [b]	4.84 ± 0.13 [b]	0.0015
pH	8.00 ± 0.02[a]	7.80 ± 0.04 [b]	7.90 ± 0.03 [c]	0.0001
Salinity (g·L-1)	35.50 ± 0.03[a]	35.43 ± 0.09[a]	35.41 ± 0.21[a]	0.64
Total Ammonia Nitrogen (mg·L-1)	1.02 ± 0.29[a]	1.21 ± 0.66 [a]	0.44 ± 0.29[b]	0.0186
NH3-N (mg·L-1)	0.05 ± 0.09[a]	0.02 ± 0.11[a]	0.01 ± 0.05 [b]	0.0276
NO2--N (mg·L-1)	0.02 ± 0.012[a]	0.01 ± 0.01[a]	0.01 ± 0.01[a]	0.0742
NO3-N (mg·L-1)	1.74 ± 0.63[a]	1.58 ± 0.69[a]	3.18 ± 1.30[a]	0.1434
PO43-(mg·L-1)	0.11 ± 0.72[a]	0.15 ± 0.31[a]	1.28 ± 0.33[b]	0.0463
Alkalinity (mg·L-1)	129.3 ± 10.12[a]	134.3 ± 12.77[a]	156.70 ± 39.31[a]	0.4049
Total Suspended Solid (mg·L-1)	259.8 ± 8.88[a]	281.3 ± 5.29[a]	278.30 ± 11.39[a]	0.051
Volatile Suspended Solid (mg·L-1)	78.69 ± 11.51[a]	91.44 ± 7.49 [a]	94.94 ± 17.38 [a]	0.2217
Total heterotrophic bacteria (Log CFU mL-1)	4.446 ± 0.3034[a]	6.859 ± 1.254 [b]	5.828 ± 0.5863[b]	0.0077
Total Vibrio spp. (Log CFU mL-1)	1.500 ± 1.000[a]	3.406 ± 1.851[a]	2.771 ± 2.074[a]	0.3205

*Values are expressed as means ± standard deviation. Values in the same row with different letters are significantly different ($p < 0.05$), as indicated by Tukey's test of mean separation.

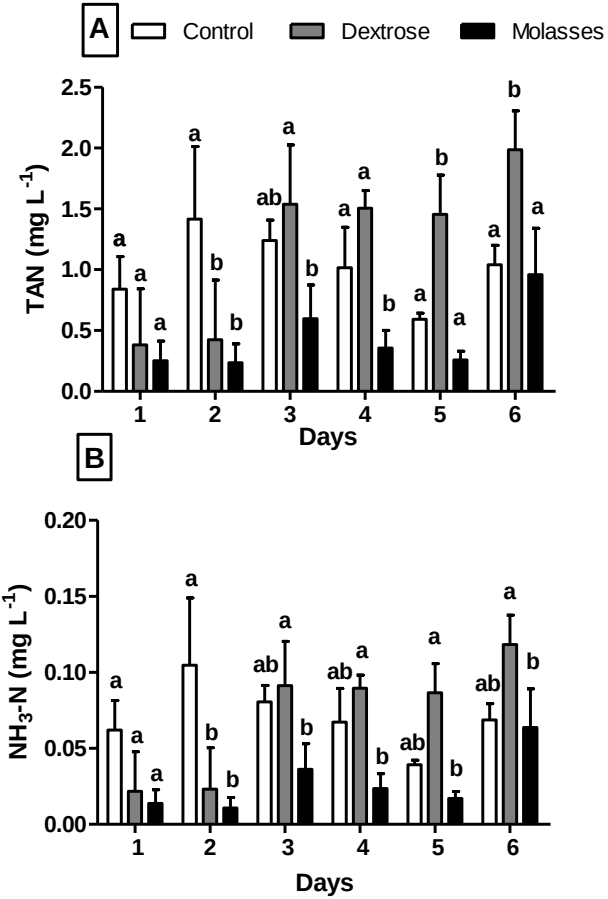

Figure 1: Daily mean total (A) and free ammonia (B) in Pacific white shrimp (*Litopenaeus vannamei*) hatcheries between the mysis 1 and postlarva 5 phases. White bars represent controls maintained using a conventional water exchange technique, gray bars represent biofloc systems supplemented with dextrose, and black bars represent biofloc systems supplemented with sugar cane molasses. Water was not exchanged in either of the biofloc systems. Different letters on the same day indicate significant differences, as indicated by Tukey's test of mean separation ($p < 0.05$).

Dissolved oxygen and pH (Table 1) were statistically different between groups; however, these differences were not large enough to affect cultivation. When heterotrophic bacteria use ammonia for growth, the addition of carbon results in an increase in the respiration rate of the growing community. This reduces the amount of dissolved oxygen in the water of bioflocs systems (Moriarty, 1997). A decrease in pH, relative with control, was also observed by Emerenciano et al. (2012) in the culture water of Farfantepenaeus paulensis as postlarvae. As bacterial respiration increased, so did CO2 concentration due to bacterial respiration. This resulted in a corresponding decrease in pH. In bioflocs systems, alkalinity is also reduced by a small amount of inorganic carbon use (Ebeling et al., 2006; Furtado et al., 2011).This reduction in alkalinity might promote a decrease in pH (Xu and Pan, 2012). However, we did not observe any alkalinity reduction in the culture water of the bioflocs groups in the present study (Table 1).

As in previous studies, nitrite and nitrate (Table 1) did not change significantly during the experiment (Emerenciano et al., 2012). Ebeling et al. (2006) concluded that in entirely heterotrophic systems there is no production of nitrite or nitrate from ammonia, suggesting that nitrification was not established during the course of our experiment. In our study, the activities of the heterotrophic bacterial community successfully controlled ammonia in BFT groups. The control of ammonia may have inhibited or delayed the emergence of nitrifying bacteria, as communities of nitrifying bacteria grew at a much slower rate than communities of heterotrophic bacteria (Ebeling et al., 2006).

Table 2: Inputs of feed, artemia nauplii, dextrose, molasses and C:N ratio in three Pacific white shrimp (Litopenaeus vannamei) hatchery systems between the mysis 1 and postlarva 5 phases (200 larvae L-1): conventional water-exchange system (control), biofloc system supplemented with dextrose (dextrose), and biofloc system supplemented with molasses (molasses).

Treatment	Input	Day						
		1	2	3	4	5	6	7
Control	Diet[1] (g m^{-3})	9.36	12.78	13.50	16.50	19.50	19.50	21.00
	Artemia[2] (g m^{-3})	0.00	14.52	17.42	20.33	17.42	17.42	17.42
	C:N	6.5:1	6.5:1	6.5:1	6.5:1	6.5:1	6.5:1	6.5:1
Dextrose	Diet (g m^{-3})	9.36	12.78	13.50	16.50	19.50	19.50	21.00
	Artemia (g m^{-3})	0.00	14.52	17.42	20.33	17.42	17.42	17.42
	Dextrose[3] (g m^{-3})	10.78	31.45	35.62	42.43	42.53	42.53	44.27
	*Extra dextrose (g m^{-3})	19.93				23.60	24.4	21.60
	C:N	23.6:1	12.5:1	12.5:1	12.5:1	15.8:1	16:1	15.4:1
Molasses	Diet (g m^{-3})	9.36	12.78	13.50	16.50	19.50	19.50	21.00
	Artemia (g m^{-3})	0.00	14.52	17.42	20.33	17.42	17.42	17.42
	Molasses[4] (g m^{-3})	26.67	78.33	88.33	105.00	106.67	106.67	110.00
	*Extra molasses (g m^{-3})	36.25						
	C:N	18.1:1	12.5:1	12.5:1	12.5:1	12.5:1	12.5:1	12.5:1

* Ammonia was maintained around 1,0 mg·L^{-1} by adding organic carbon sources when this limit was exceeded. Assuming that: [1]48% of crude protein and 50% of carbon; [2]48% of crude protein and 50% of carbon, [3]100% of carbohydrate, [4]55% of carbohydrate and 3% of crude protein.

We observed an increase in the concentration of reactive phosphorus in the molasses group (Table 1). This difference may be associated with the low assimilation of reactive phosphorous into predominantly heterotrophic environments, unlike systems dominated by phytoplankton, into which reactive phosphorous is readily assimilated (Hargreaves, 2006). Furthermore, phosphorous was present in the molasses that was added daily to the molasses group for ammonia control. The phosphorous content of molasses total dry matter varies between 0.07 and 0.74% (OECD, 2011).

There was no difference between groups in the mean values of total suspended solids or volatile solids (Table 1). We believe that this result is associated with the high levels of solids in the initial water used in all experimental units. Similar results have been observed in cultures of postlarval shrimp (Mishra et al., 2008; Emerenciano et al., 2012; Xu and Pan, 2012). Although an acceptable range of total solids in shrimp hatcheries has not yet been established, very high levels of total solids can have negative impacts on developing larvae. In the early stages of postlarval development, shrimp are small and can be harmed by high levels of solids (Schveitzer et al., 2013).

The mean values of total ammonia and non-ionized ammonia (free) remained below toxic levels throughout the experimental period (Cobo et al., 2012). This indicates that ammonia was effectively controlled in all treatments, with molasses treatment resulting in the lowest values (Table 1). The concentration profile of total and free ammonia remained close to 1 $mg \cdot L^{-1}$ in all treatments during the experimental period (Figure 1). The addition of organic carbon, as either dextrose or molasses, effectively stimulated the production of bacterial biomass from ammonia in the BFT systems (Avnimelech, 1999). The C:N ratio that results from the input of organic matter (artemia + feed + source of carbon) was greater than 12,5:1, and was close to the ratios reported to be optimal for bacterial growth (Schneider et al., 2007) and assimilation of ammonia into microbial proteins (Avnimlech, 1999; Ebeling *et. al.*, 2006).

Mean levels of total and free ammonia were significantly lower in the molasses group than in the other two groups (Table 1, Figure 1). Total ammonia increased somewhat in the dextrose group on the last two days of the experiment (Figure 1A). However, the average levels of toxic ammonia in the BFT groups did not significantly differ from that of the control group on any day of the experiment (Figure 1B). Neither toxic nor total ammonia exceeded the levels recommended for juvenile *Penaeus monodon* (Chen and Lin, 1992) or the hatchery stage of *L.*

vannamei (Cobo et al., 2012) in any of the treatments throughout the experiment.

In the present experiment, the addition of molasses to BFT systems resulted in the most stable ammonia control. This may have been due to the presence of non-carbohydrate compounds in molasses, such as minerals and amino acids (aspartic acid, glutamic acid, isoleucine, valine, glycine, and alanine), that favor the establishment of heterotrophic bacterial communities (Curtin, 1983). However, it is should be noted that the composition of molasses can be highly variable, as it depends on the processing technology used to produce it and the composition of sugarcane (OECD, 2011).

During the experiment, we did not observe any differences between treatments in larval quality parameters. All larvae were active (high swimming activity), and had lipid reserves, normal hepatopancreas color, and full intestines. We found no deformities, epibionts, adhered particles necrosis, or muscular opacity.

Final survival, length, dry weight, and survival of the salinity stress test did not differ between groups (Table 3). Ultimate survival in all groups surpassed the rate appropriate for the species (70%, FAO, 2003) and that appropriate for experimental hatcheries (Aranguren et al., 2006; D'Abramo et al., 2006). Similarly, no difference was observed between groups in larval quality, including development, feeding, and signs of diseases (FAO, 2003).

Table 3: Final survival, salinity stress survival, final length, final dry weight, larval quality, and water consumption, in three Pacific white shrimp (*Litopenaeus vannamei*) hatchery systems between the mysis 1 and postlarva 5 phases (200 larvae L^{-1}): conventional water-exchange system (control), biofloc system supplemented with dextrose (dextrose), and biofloc system supplemented with molasses (molasses).

Parameter	Control	Dextrose	Molasses	p
Survival (%)	90.58 ± 5.40[a*]	90.23 ± 10.51[a]	85.13 ± 11.15[a]	0.7058
Stress survival (%)	97.45 ± 2.01[a]	95.39 ± 3.25[a]	93.67 ± 6.11[a]	0.4731
Final length (mm)	6.14 ± 0.21[a]	6.11 ± 0.19[a]	6.20 ± 0.23[a]	0.5093
Final weight (mg)	0.155 ± 0.02[a]	0.197 ± 0.06[a]	0.178 ± 0.01[a]	0.3206
Water consumption (L per thousand post-larvae 5)	56.22 ± 3.31[a]	6.49 ± 0.79[b]	6.89 ± 0.95[b]	<0.0001

* Values are expressed as means ± standard deviation. Values in the same row with different letters are significantly different, as indicated by one-way ANOVA followed by Tukey's test of mean separation ($p < 0.05$)

At the end of cultivation, water from the BFT groups contained significantly more heterotrophic bacteria than water from the control group. The relatively high number of heterotrophic bacteria in BFT groups was expected due to organic fertilization. However, the quantity of potentially pathogenic Vibrionaceae bacteria did not differ significantly between groups (Table 1).

Treatment with dextrose or molasses required approximately 12% of the water used by the control group because water was not exchanged in these groups (Table 2). Such reduction in the amount of water required for the hatchery phase of shrimp aquaculture would proportionally reduce the costs associated with the capture, disinfection, neutralization, heating, and pumping of water for hatcheries. At a commercial scale, converting conventional hatchery systems to BFT systems supplemented with organic carbon could result in substantial reduction in production costs. Reducing the amount of water required for the hatchery phase of shrimp aquaculture would also decrease environmental impacts and improve biosecurity.

11 CONCLUSION

The use of biofloc systems without water exchange that are supplemented with molasses or dextrose as a carbon source results in adequate production indexes and water quality during the hatchery phase *L. vannamei*. Because water is not exchanged in these biofloc systems, it requires approximately 12% of the water used in the conventional autotrophic system.

12 ACKNOWLEDGMENTS

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14 CAPÍTULO III: INTENSIVE HATCHERY PERFORMANCE OF PACIFIC WHITE SHRIMP IN THE BIOFLOC SYSTEM UNDER THREE DIFFERENT FERTILIZATION LEVELS

15 ABSTRACT

Bacterial inorganic nitrogen control using carbon:nitrogen ratio (C:N) manipulation is a tool for aquaculture systems. The present study assessed the hatchery performance of *Litopenaeus vannamei* between the mysis 1 and postlarvae 5 stages in a zero-exchange biofloc system under different C:N fertilization levels (10:1, 12.5:1 and 15:1) with dextrose. Water quality, performance parameters and water microbiology were compared among treatments. The mean values of the evaluated water quality parameters were appropriate for this production stage. Fertilization with dextrose efficiently controlled ammonia levels, which did not reach the average concentrations considered toxic for the species. In the 10:1 C:N ratio treatment, the levels of ammonia started to increase early and showed significantly higher levels from the third to the last experimental day. There was no difference among groups in means of survival (>76%) and dry weight (0.26 mg) of *L. vannamei* production parameters and water quality were maintained without water exchange using a biofloc system supplemented with dextrose. Therefore, the use of biofloc systems without water exchange with dextrose as a carbon source in C:N ratios of 10:1, 12,5:1 and 15:1 results in both adequate production indexes and water quality during the misis 1 to post-larvae 5 hatchery phases of *L. vannamei*. However, the ratios of 12,5:1 and 15:1 keep lower levels of ammonia.
Keywords: *Litopenaeus vannamei*, zero-exchange, hatchery, heterotrophic system, BFT, intensive culture.
Abbreviations: BFT, biofloc technology;

16 INTRODUCTION

The challenge in intensive aquaculture systems is to offer a favorable environment for shrimp and fish production in a high-density culture with little or no water exchange (Ray et al., 2010). Biofloc technology (BFT) has become increasingly common to meet this challenge (Avnimelech, 1999, 2006; Browdy et al., 2001; Crab et al., 2007; De Schryver et al., 2008). BFT is used to intensify production and avoid the water exchange of farming, with a consequent reduction in the flow of pathogens and the discharge of nutrient-rich effluents into the

environment (Samocha et al., 2007). Biofloc is composed of a variety of microorganisms, uneaten feed, feces and detritus in particles that are kept in suspension with vigorous aeration (De Schryver et al., 2008).

In BFT systems, reduction or ceasing of water exchange requires control of the ammonia from protein catabolism, which is toxic for fish and shrimp. In BFT systems, ammonia control starts with a carbon:nitrogen balance that promotes the growth of heterotrophic bacteria, which incorporate ammonia nitrogen from the water (Moriarty, 1997; Avnimelech, 1999; Ebeling et al., 2006; Hari et al., 2006; Samocha et al., 2007). This relationship is established by adding organic carbon sources (such as molasses, flour, sugar and dextrose) to aquaculture media. It requires 20 g of carbohydrate, or about 6 g of carbon, to convert 1 g of ammonia nitrogen into a bacterial biomass (Avnimelech, 1999; Ebeling et al., 2006).

The first systematic approach with BFT in the hatchery phase demonstrated that the performance of *L. vannamei* between the mysis 1 (M1) and postlarva 5 (PL5) stages in a zero-exchange biofloc system using molasses and dextrose as organic carbon sources has the same results as the standard production system (Lorenzo et al., 2015). Water quality, performance and microbiology during this previous study were similar between carbon-supplemented and control groups. Water exchange (control) and the addition of organic carbon sources (BFT) maintained total ammonia nitrogen (TAN) levels bellow 1 mg L-1. The efficient mean C:N ratio that results from the input of organic matter (artemia nauplii + feed + source of carbon) to control ammonia was around 12.5:1.

The objective of the present study was to assess the hatchery performance of *L. vannamei* using a biofloc system between the mysis1 and postlarva5 stage in a zero-exchange biofloc system under three defined C:N ratios (10:1, 12.5:1 and 15:1) using dextrose as a carbon source.

17 MATERIALS AND METHODS

The experiment was conducted at the Laboratório de Camarões Marinhos (LCM), Departamento de Aquicultura of the Universidade Federal de Santa Catarina, Brazil.

17.1 Biologic material

The utilized larval line was free of any pathogens that require notification of the International Organization of Epizootics (from Aquatec LTDA, Rio Grande do Norte, Canguaratema, Brazil). Before the experiment, nauplii of *L. vannamei* were raised in a 20 m3 (stocking density of 100 larvae L^{-1}) semi-cylindrical hatchery tank in salinity of 35 ppm until they reached mysis1. The microalgae Chaetoceros muelleri (5×10^4 cells mL^{-1}) were added to the culture water daily. When the larvae reached the stage of misis1 (average dry weight of 0.087 ± 0.006 mg and average length of 3.50 ± 0.17 mm), they were transferred to the experimental units, which were initially filled with water from the hatchery tank.

17.2 Experimental conditions

The larvae were reared under three treatments with different C:N ratios: 10:1, 12.5:1 and 15:1. The organic source used was anhydrous dextrose ($C_6H_{12}O_6$, Sigma-Aldrich) added four times per day. The carbohydrate percentage of dextrose was assumed to be 100% (annex 4).

Controlling fertilization with organic carbon to regulate ammonia was done based on the amount of additional carbohydrate required to neutralize the ammonium excreted by shrimp assuming that shrimp assimilate 25% of the nitrogen added in the feed and 75% of added nitrogen is transformed into ammonia dissolved in water. Source of carbon was added to each tank at a ratio of 20 g carbohydrate for each gram of TAN created (Avnimelech, 1999).

The experimental groups were randomly distributed in a unifactorial experimental design. Semi-cylindrical plastic tanks (92 × 68 × 25 cm) with a working volume of 60 L constituted the experimental units. Four tanks were prepared for each experimental treatment, resulting in 12 tanks. All tanks were equipped with linear aeration supplied by a PVC pipe (90 cm long, 20 mm diameter with 36 holes of 1 mm) to keep the solids generated during cultivation in suspension and to maintain the level of dissolved oxygen in the water at the recommended concentration for *L. vannamei* larval cultivation (> 5 mg L^{-1}). The water temperature was kept constant, between 29 and 30 °C using 100 W heaters connected to a thermostat.

The experimental tanks were supplied with water from an autotrophic larval cultivation. The supplied water had the following parameters: Chaetoceros muelleri (5×10^4 cells mL^{-1}), oxygen = 6.0 mg

L^{-1}, pH = 7.83, temperature = 30 °C, salinity = 35.0 mg L^{-1}, total ammonia = 0.2 mg L^{-1}, free ammonia = 0.02 mg L^{-1}, nitrite = 0.01 mg L^{-1}, nitrate = 0.26 mg L^{-1}, phosphate = 0.001 mg L^{-1}, total suspended solid (TSS) = 278.3 mg L^{-1}, volatile suspended solid (VSS) = 60.0 mg L^{-1} and alkalinity = 125.33 mg L^{-1}.

Each experimental unit was stocked with 12,000 larvae in the M1 stage, which represents a stocking density of 200 larvae L^{-1}. The experiment was conducted until the larvae reach post larvae stage 5 (seven days after stoking). The water in the biofloc experimental units was not exchanged during the experimental period, but evaporated water was replaced with fresh water in order to maintain salinity. No suspended solids were removed from the water during the experiment.

The larval and postlarval shrimp were fed microencapsulated commercial diets (INVE) according to Lorenzo et al. (2015) and based on the manufacturer's recommendation for each larval stage. The Lansy ZM diet (minimum protein 48%, minimum ether extract 13%, maximum fibrous matter 2.5%, maximum mineral matter 13%, maximum humidity 8.0%, minimum calcium 0.25%, maximum calcium 1.5% and minimum phosphorus 1.0%) was fed from mysis 1 to mysis 3. After this period and until harvesting, were fed with the Lansy MPL diet (minimum protein 48%, minimum ether extract 9.0%, maximum fibrous matter 2.5%, maximum mineral matter 13%, maximum humidity 9.0%, minimum calcium 1.0%, maximum calcium 2.2% and minimum phosphorus 1.0%).

Larvae were fed nine times a day (0800, 1000, 1200, 1400, 1600, 1800, 2100, 2300, and 0300), and Artemia nauplii were also provided to the larvae at a rate of six nauplii for each mysis or postlarva five times each day (0900, 1100, 1500, 1700, and 0000).

17.3 Chemical and physical variables of the water

Dissolved oxygen, temperature (YSI 55, YSI Incorporated, Yellow Springs, OH, USA) and pH (YSI 100, YSI Incorporated, Yellow Springs, OH, USA) were measured twice a day. Salinity (YSI 30, YSI Incorporated, Yellow Springs, OH, USA), alkalinity (APHA 2005-2320 B), nitrite and total ammonia were analyzed daily (APHA, 2005). Total suspended solids (TSS) and volatile suspended solids (VSS) were assessed every other day (APHA 2005-2040 D and 2005-2540 E) using 0.6-µm glass fiber micro-filters (GF-6, Macherey-Nagel, Düren, Germany).

Two hundred milliliters of water samples were collected from each tank. Samples were frozen until nitrate (HACH method 8039, cadmium reduction) and orthophosphate analysis. The TAN, nitrite, nitrate and orthophosphate analyses were carried out using a spectrophotometer and analyzed according to Strickland and Parsons (1984) and following the guidelines contained in APHA (2005).

17.4 Larval quality and performance

Each day, 20 larvae/postlarvae from each tank were analyzed at the macro and microscopic level to assess larval quality. We observed the following parameters: swimming activity, lipid reserves and color of the hepatopancreas, intestinal contents, deformities, the presence of epibionts, adhered particles, necrosis and muscular opacity (FAO, 2003). Performance parameters used to evaluate treatments included ultimate survival (%), final dry weight in mg (oven at 60 ° C during 180 minutes) and final larval length in mm (from the tip of the rostrum to the telson border using a labeled magnifying glass with accuracy of 0.01 mm).

17.5 Microbiological analysis of water

Before harvest, 1 mL water samples were collected from each tank for microbiological analysis. Samples were homogenized and serially diluted (1/10) in a sterile saline solution (3% NaCl) and seeded in duplicate on a Marine agar culture medium (Difco) to count viable and total cultivable heterotrophic bacteria. Diluted samples were also seeded in duplicate on a thiosulfate-bile-sucrose-agar medium (TCBS, Difco) to count Vibrionaceae bacteria. Seeded media were incubated in a microbiological oven at 30°C. After 24 h, the colony forming units (CFU) were counted.

17.6 Statistical analysis

A one-factor ANOVA followed by a Tukey's test (Zar, 1984), was used to compare treatments at a significance level of 0.05. Shapiro-Wilk and Levene tests assessed normality and homoscedasticity respectively (Zar, 1984). Data expressed as a percentage underwent angular transformation before analysis. Microbiological analysis data showed no homoscedasticity and were Log10 transformed.
The analysis of changes in ammonia over time was performed by a repeated-measures ANOVA. Treatments were considered to be the main

factors, and the duration of the culture was the additional factor. Significant differences were analyzed by a Tukey's test (Zar, 1984) with a significance level of 0.05.

18 RESULTS AND DISCUSSION

The water quality parameters were similar among treatments (Table 4, Figure 2) and remained appropriate for the hatchery stage of L. vannamei. These parameters were similar to those found in a comparative test between a conventional hatchery system with high rates of daily exchange and BFT hatchery systems with sources of organic carbon (Lorenzo et al, 2015).

The treatments were able to maintain the TAN mean level near to 1 mg L-1 until day six when this parameter started to increase, except for the 10:1 C:N TAN levels that increased prior to those of the other groups starting on day three (Figure 2). However, mean values of total and free ammonia remained below toxic levels throughout the experimental period (Cobo et al., 2012). This finding corroborate with Lorenzo et al. (2015) where the efficient C:N ratio was higher than 12,5:1. Similar C:N ratios have been reported to achieve optimal bacterial growth (Schneider et al., 2007) and the assimilation of ammonia into microbial proteins (Avnimlech, 1999; Ebeling et. al., 2006).

These results demonstrated that a fixed C:N ratio from 10 to 15:1 in BFT hatchery systems is able to control TAN and free ammonia during M1 to PL5 stages. However, the lowest C:N ratio starts to increase TAN significantly from the third day to the end of the experiment (Figure 2), so, if a harvest were, for any reason, to be delayed for some days, the ammonia could quickly reach toxic levels. In the same way, the 15:1 C:N ratio was able to control the ammonia levels in the tanks without affecting the water or productive parameters. However, if carbon were added to the system, the level of solids could be a threat. In another experiment (data not published), we used C:N ratios from 15:1 to 30:1, and the limiting factor in this case was not ammonia but the solid levels that reached a toxic level for the larvae, with high larval mortality, mainly in the M3 to PL1 metamorphosis.

The low concentration of nitrite (Table 4) suggests that nitrification was not established during the course of our experiment, and that the activity of the heterotrophic bacterial community was responsible for controlling ammonia in BFT groups.

During the experiment, we did not observe differences among treatments in larval quality parameters. All larvae were active (high swimming activity) and had lipid reserves, a normal hepatopancreas color and full intestines. We found no deformities, epibionts, adhered particles necrosis or muscular opacity. Final survival and dry weight did not differ between groups, and only the 15:1 C:N ratio length parameter showed statistical difference from other groups (Table 5). Final survival in all groups surpassed the rate appropriate for the species (70%, FAO, 2003) and that appropriate for experimental hatcheries (Aranguren et al., 2006; D'Abramo et al., 2006, Lorenzo et al., 2015).

Table 4: Water quality parameters and final water microbiology in Pacific white shrimp (*Litopenaeus vannamei*) hatchery systems, whit dextrose at C:N ratios fixed at 10:1, 12,5:1 and 15:1 C:N, between the mysis 1 and postlarva 5 phases (200 larvae L^{-1}).

Parameter	10:1	12,5:1	15:1	p
Temperature (°C)	29.91 ± 0.18[a]	29.86 ± 0.25[a]	29.92 ± 0.20[a]	0.92
Oxygen ($mg \cdot L^{-1}$)	6.05 ± 0.09[a]	6.05 ± 0.06[a]	5.97 ± 0.09[a]	0.35
pH	8.01 ± 0.06[a]	8.00 ± 0.06[a]	7.98 ± 0.06[a]	0.19
Salinity ($g \cdot L^{-1}$)	35.41 ± 0.20[a]	35.47 ± 0.07[a]	35.53 ± 0.09[a]	0.45
TAN ($mg \cdot L^{-1}$)	1.14 ± 0.67[a]	0.66 ± 0.44[b]	0.44 ± 0.46[b]	0.0001
NH_3-N ($mg \cdot L^{-1}$)	0.09 ± 0.05[a]	0.05 ± 0.03 [b]	0,03 ± 0,03 [b]	0.0001
NO_2^--N ($mg \cdot L^{-1}$)	0.01 ± 0.03[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0,38
NO_3^--N ($mg \cdot L^{-1}$)	0.24 ± 0.11[a]	0.24 ± 0.11[a]	0.18 ± 0.10[a]	0.50
PO_4^{3-}($mg \cdot L^{-1}$)	0.70 ± 0.61[a]	0.41 ± 0.52[a]	0.29 ± 0.34[a]	0.27
Alkalinit ($mg \cdot L^{-1}$)	132.5 ± 4.50[a]	133.0 ± 6.32[a]	127.5 ± 3.34[a]	0.07
TSS ($mg \cdot L^{-1}$)	293.5 ± 16.90[a]	292.0 ± 14.60[a]	304.7 ± 21.25[a]	0.46
VSS ($mg \cdot L^{-1}$)	97.43 ± 21.19 [a]	90.61 ± 29.50 [a]	96.14 ± 25.00 [a]	0.71
Total heterotrophic bacteria (Log CFU mL^{-1})	5.37 ± 0.54[a]	5.65 ± 0. 84[a]	5.29 ± 1.11[a]	0. 82
Total *Vibrio* spp. (Log CFU mL^{-1})	3.98 ± 0.28[a]	3.69 ± 0.33[a]	3.76 ± 0.16[a]	0.32

* Values are expressed as means ± standard deviation. Values in the same row with different letters are significantly different ($p < 0.05$), as indicated by Tukey's test of mean separation.

Table 5: Final survival, length and dry weight in Pacific white shrimp (*Litopenaeus vannamei*) hatchery systems, whit dextrose at C:N ratios fixed at 10:1, 12,5:1 and 15:1 C:N, between the mysis 1 and postlarva 5 phases (200 larvae L^{-1}).

Parameter	10:1	12,5:1	15:1	p
Survival (%)	76.44 ± 12.06[a]	81.55 ± 15.29[a]	78.67 ± 22.64[a]	0.93
Final length (mm)	6.68± 0.21[a]	6.55 ± 0.26[a]	6.93 ± 0.20[b]	0.0002
Final dry weight (mg)	0.31 ± 0.07[a]	0.28 ± 0.08[a]	0.27 ± 0.02[a]	0.60

* Values are expressed as means ± standard deviation. Values in the same row with different letters are significantly different, as indicated by one-way ANOVA followed by Tukey's test of mean separation ($p < 0.05$).

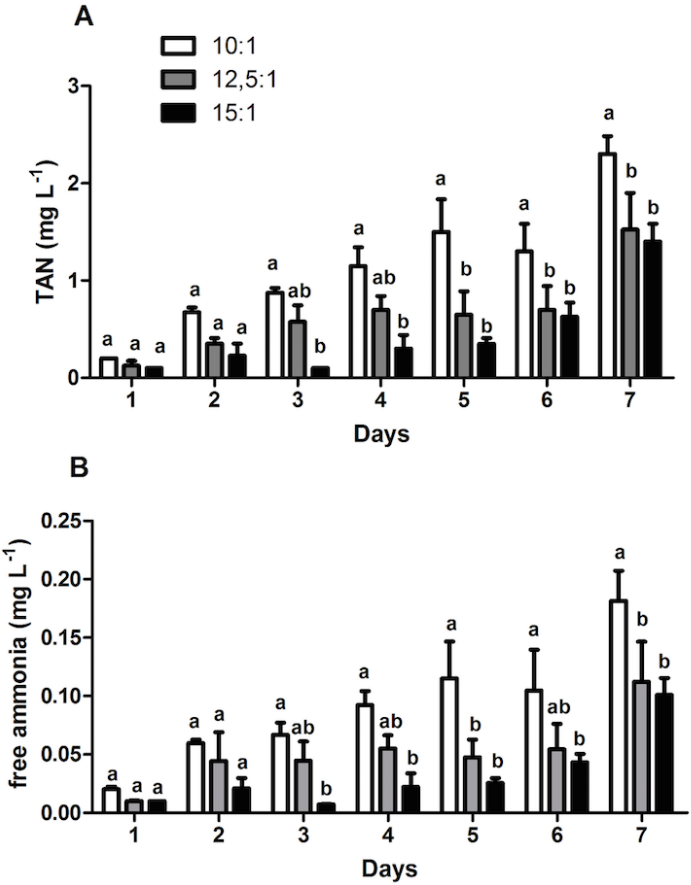

Figure 2: Total ammonia (A) and free ammonia (B) in diferent treatments for Pacific white shrimp (*Litopenaeus vannamei*) hatcheries between the mysis 1 and postlarva 5 phases. White, grey and black bars represent respectively 10:1, 12,5:1 and 15:1 C:N ratios treatments. Water was not exchanged in either of the biofloc systems. Different letters on the same day indicate significant differences, as indicated by Tukey's test of mean separation ($p < 0.05$).

19 CONCLUSION

The use of biofloc systems without water exchange with dextrose as a carbon source in C:N ratios of 10:1, 12.5:1 and 15:1 results in both adequate production indexes and water quality during the misis1 to post-

larvae 5 hatchery phase of *L. vannamei*. However, the ratios of 12.5:1 and 15:1 keep lower levels of ammonia.

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22 CAPÍTULO IV: INTENSIVE HATCHERY PERFORMANCE OF THE PACIFIC WHITE SHRIMP IN BIOFLOC SYSTEM USING DIFFERENT STOCKING DENSITIES

23 ABSTRACT

Due to an increase in the world population, there is an increasing demand for food. To meet this demand through aquaculture with higher rates of production per unit area, avoiding or minimizing significant environmental damage, is a challenge. The present study assessed the hatchery performance of the Pacific white shrimp *Litopenaeus vannamei* between the mysis 1 (M1) and postlarval 5 (PL5) stages, in a zero-exchange biofloc system under a 12,5:1 fixed C:N ratio (previously experimentally selected) with dextrose as the carbon source, in four stocking densities: 200, 250, 300, and 350 larvae per liter (D200, D250, D300, and D350, respectively). Water quality and performance parameters were compared among treatments. The mean values of the water quality parameters were appropriate for this production stage in all treatments. Fertilization with dextrose efficiently controlled ammonia levels below the average concentrations considered toxic for the species. Lower values of pH and higher values of volatile solids were found in D350. There was no difference between groups for survival and dry weight of PL5. Therefore, the use of biofloc systems without water exchange with dextrose as a carbon source in a 12,5:1 C:N ratio resulted in adequate production indexes and water quality during the M1 to PL 5 hatchery phase of *L. vannamei*. These results indicated that the density limit for a viable biofloc technology (BFT) hatchery was not reached. Our findings have potential ecofriendly applications to increase productivity of several intensive culture systems.

Keywords: Shrimp; *Litopenaeus vannamei*; hatchery; heterotrophic; biofloc technology; intensive culture.

Abbreviations: BFT, biofloc technology.

24 INTRODUCTION

Aquaculture has immense potential to expand and intensify sustainably to contribute to the growing demand for food in 2050 since the human population is predicted to rise for the next 40 years before stabilizing at a minimum of 9 billion people (Godfray et al., 2010). However, aquaculture is faced with competition for water, land, and other natural resources created by the pressure from population growth.

In general, these challenges are met by intensifying culture operations (Piedrahita, 2003). The intensity levels of aquaculture systems vary from extensive systems stocking young organisms growing naturally, to very intensive systems with high stocking rates and commercial feed. Presently, aquaculture undergoes continual modification with new methods and technologies (genetic, feed formulation, and water management) to achieve higher rates of production per unit area. This intensification certainly will continue in the near future (Diana et al., 2013).

Furthermore, the importance of penaeid shrimp species with controlled reproduction, independent from wild stock, must to be considered. The two cultured penaeid species, *Litopenaeus vannamei* and *Penaeus monodon*, contribute to the majority of the $12bn/year global industry. However, an approximately 40% loss has been estimated for tropical shrimp production (> $3bn/year), mainly due to viral pathogens for which standard preventive measures (e.g., vaccination) are not feasible. Regarding this problem, new approaches are urgently required to enhance yield by improving broodstock, larval supply, and promoting the best management practices (Stentiford, 2012).

This double challenge (food increase demand and sanitation) could be met for future expansion and long-term sustainability of the global shrimp industry, including improvements in selective breeding, strict on-farm biosecurity practices, and intensive shrimp culture systems with zero water exchange like Biofloc technology (BFT). These improvements would drive higher rates of production per unit area avoiding pathogen exchange with environment (Moss et al., 2012). However, if this enhancement in biomass caused negative environmental impacts with potential increases of waste materials from uneaten feed and metabolic waste products, it could lead to more functional effluent treatment systems, resulting in lower discharges per unit of biomass production when compared with lower intensity systems (Piedrahita, 2003).

BFT allows production intensification and avoids the water exchange of other farming systems, thereby minimizing the flow of pathogens and discharge of nutrient-rich effluents into the environment (Samocha et al., 2007). In BFT systems without water exchange, ammonia (toxic for fish and shrimp) control starts with a carbon/nitrogen balance that promotes the growth of heterotrophic bacteria, which incorporate ammonia nitrogen from the water under intensive aeration (Avnimelech, 1999; Ebeling et al., 2006). The suitable

ratio for this control is obtained by adding organic carbon sources (such as molasses, flours, sugar, and dextrose) to ponds. It requires 20 g of carbohydrate, or about 6 g of carbon, to convert 1 g of ammonia nitrogen to bacterial biomass (Avnimelech, 1999; Ebeling et al., 2006).

This approach could be applied specifically to shrimp hatcheries considering the same general advantages and disadvantages, besides the potential production intensification and sustainability. Previously, two important experimental steps have been taken in a BFT hatchery and strongly contributed to this knowledge. Firstly, a first systematic approach with BFT in the hatchery phase, evaluated the performance of *L. vannamei* between the mysis 1 (M1) and postlarva 5 (PL5) stages in zero-exchange BFT systems using molasses and dextrose as carbon sources compared to conventional hatchery systems with a stocking density of 200 larvae L^{-1} (Lorenzo et al., 2015). Similar results in terms of water quality and performance were obtained from the standard production system with water exchange (control group) and with the addition of organic carbon sources (BFT group) to maintain the total ammonia nitrogen (TAN) levels near 1 $mg \cdot L^{-1}$. Secondly efficient average C/N ratio that resulted from the input of organic matter (artemia + feed + source of carbon) to control ammonia was over 12,5:1 according to our second hatchery approach usig different fertilization leves (Lorenzo, 2016 accepted for publication). In addition, the BFT zero-exchange hatchery consumed only approximately 12% of the total water used in the conventional autotrophic system. Second, an experimental study assessed the hatchery performance of *L. vannamei* between the M1 and PL5 stages in a zero-exchange biofloc system under different fixed C:N fertilization levels (10:1, 12,5:1, and 15:1) with dextrose at a stocking density of 200 larvae L^{-1} (unpublished data). The elected C:N range ratio was established from above previous study of the efficient C:N ratio (over 12,5:1) and the central focus was to determine an optimal C:N ratio to control ammonia without the extra punctual carbohydrate addition based on frequent laboratory ammonia analysis. The mean values of the evaluated water quality parameters were appropriate for this production stage. Fertilization with dextrose efficiently controlled ammonia levels, which did not reach the average concentrations considered toxic for the species. Results in both adequate production indexes and water quality during M1 to PL5 hatchery phases of *L. vannamei* were in accordance with these findings. However, the ratios of 12,5:1 and 15:1 maintained lower levels of ammonia.

Both previous studies showed, firstly that the BFT hatchery is a feasible strategy with secure indexes in water quality and performance,

and secondly, we can use a standardized protocol with secure fixed C:N ratios for this BFT hatchery. Based on these findings, the objective of the present study was to assess the hatchery performance of *L. vannamei* using a zero-exchange BFT system, with a 12,5:1 C:N ratio using dextrose as a carbon source from M1 to PL5 stages under three stocking densities: 200, 250, 300 and 350 larvae per liter (D200, D250, D300, D350, respectively) to determine the optimal BFT hatchery intensification potential.

25 MATERIALS AND METHODS

The experiment was conducted at the Laboratório de Camarões Marinhos (LCM), Departamento de Aquicultura of the Universidade Federal de Santa Catarina, Brazil.

25.1 Biological material

The utilized larval line was free of any pathogens that require notification at the International Organization of Epizootics (from Aquatec LTDA, Rio Grande do Norte, Canguaratema, Brazil). Before the experiment, nauplii of *L. vannamei* were raised in a 4 m3 (stocking density of 100 larvae L^{-1}), semi-cylindrical hatchery tank in a salinity of 35 ppm until they reached M1. The microalgal species *Chaetoceros muelleri* (5×10^4 cells mL^{-1}) was added to the culture daily. When the larvae reached the stage of M1 (average dry weight of 0,031 ± 0,004 mg and average length of 3,57 ± 0,029 mm) they were transferred to the experimental units, which were initially filled with 50% water from the hatchery tank, and 50% filtered and disinfected water.

25.2 Experimental conditions

The larvae were reared under four treatments with different densities ratios: 200, 250, 300, and 350 postlarvae L^{-1} (D200, D250, D300, and D350, respectively). The organic source, anhydrous dextrose ($C_6H_{12}O_6$, Sigma-Aldrich), and was added four times per day (annex 5).

The experimental groups were randomly distributed in a unifactorial experimental design. Semi-cylindrical plastic tanks (92 × 68 × 25 cm) with a working volume of 60 L constituted the experimental units. Four tanks were prepared for each treatment, resulting in 16 tanks. All tanks were equipped with linear aeration supplied by a PVC pipe (90 cm long, 20 mm diameter with 36 holes of 1 mm) to keep the solids generated during cultivation in suspension and maintain the level of

dissolved oxygen in the water at the recommended concentration for *L. vannamei* larval cultivation (> 5 $mg \cdot L^{-1}$). The water temperature was kept constant, between 29 and 30°C using 100-W heaters connected to a thermostat.

The water supplied had the following parameters: Chaetoceros muelleri (5×10^4 cells mL^{-1}), oxygen = 6.0 $mg \cdot L^{-1}$, pH = 8,1, temperature = 31°C, salinity = 35.0 $mg \cdot L^{-1}$, total ammonia = 0.6 $mg \cdot L^{-1}$, free ammonia = 0.06 $mg \cdot L^{-1}$, nitrite = 0.01 $mg \cdot L^{-1}$, nitrate = 2.8 $mg \cdot L^{-1}$, phosphate = 0.001 $mg \cdot L^{-1}$, total suspended solids (TSS) = 243.5 $mg \cdot L^{-1}$, volatile suspended solids (VSS) = 44.0 $mg \cdot L^{-1}$, and alkalinity = 120 $mg \cdot L^{-1}$.

Each experimental unit was stocked with 12,000, 15,000, 18,000, and 21,000 larvae at the M1 stage according to density treatments D200, D250, D300, and D350, respectively. The experiment was conducted until the larvae reach the PL5 (7 d after stocking). The water in the BFT experimental units was not exchanged during the experimental period, but evaporated water was replaced with fresh water to maintain salinity. No suspended solids were removed from the water during the experiment.

Larval and postlarval shrimp were fed microencapsulated commercial diets (INVE) according to Lorenzo et al. (2015) based on the manufacturer's recommendation for each larval stage. The Lansy ZM diet (minimum protein 48%, minimum ether extract 13%, maximum fibrous matter 2.5%, maximum mineral matter 13%, maximum humidity 8.0%, minimum calcium 0.25%, maximum calcium 1.5%, and minimum phosphorus 1.0%) was fed from M1 to M3/PL 1. After this period, and until harvesting, postlarvae were fed with a Lansy MPL diet (minimum protein 48%, minimum ether extract 9.0%, maximum fibrous matter 2.5%, maximum mineral matter 13%, maximum humidity 9.0%, minimum calcium 1.0%, maximum calcium 2.2%, and minimum phosphorus 1.0%).

Larvae were fed nine times a day (0800, 1000, 1200, 1400, 1600, 1800, 2100, and 2300, and 0300), and Artemia nauplii were also provided to the larvae at a rate of six nauplii for each mysis or postlarva, five times each day (0900, 1100, 1500, 1700, and 0000).

25.3 Chemical and physical variables of the water

Dissolved oxygen, temperature (YSI 55, YSI Incorporated, Yellow Springs, OH, USA), and pH (YSI 100, YSI Incorporated, Yellow Springs, OH, USA) were measured twice a day. Salinity (YSI

30, YSI Incorporated, Yellow Springs, OH, USA), alkalinity (APHA 2005-2320 B), TSS, and total ammonia were analyzed daily (APHA 2005). VSS, nitrite, and nitrate were assessed every other day (APHA 2005-2040 D and 2005-2540 E). SST and SSV were filtered using 0.6-µm glass fiber micro-filters (GF-6, Macherey-Nagel, Düren, Germany).

Two hundred milliliters of water samples was collected from each tank. Samples were frozen until nitrate (HACH method 8039, cadmium reduction) and orthophosphate analysis. The TAN, nitrite, nitrate, and orthophosphate analyses were carried out using a spectrophotometer and analyzed according to Strickland and Parsons (1984), and following the guidelines contained in APHA (2005).

25.4 Larval quality and performance

Each day, 20 larvae from each tank were analyzed at the macro and microscopic level to assess larval quality. We observed the following parameters: swimming activity, lipid reserves, and color of the hepatopancreas, intestinal contents, deformities, presence of epibionts, adhered particles, necrosis, and muscular opacity (FAO, 2003). Performance parameters used to evaluate treatments included ultimate survival (%), final dry weight (mg), and final larval length (mm). At the end of experiment, a survival (%) salinity stress test, which is related to larval quality, was performed (Samocha et al., 1998; Racotta et al., 2003). To perform this test, 100 larvae from each replicate were placed in cylinders containing 15 L of water with a salinity of 19 $g \cdot L^{-1}$. The test water was the same temperature as the culture water and the shrimp were kept in the test water for 60 min. After that time, the larvae were transferred to similar cylinders containing water with 35 $g \cdot L^{-1}$ salinity (the same as that of the culture) where they remained for an additional 60 min. Larval survival was estimated at the end of the procedure.

25.5 Water consumption

The final amount of water used by the experimental group was expressed in liters per thousand of PL5 produced and includes the initial water used to fill the experimental units and the water for fresh water used to replenish evaporation losses in the experimental groups.

2.6. Statistical analysis

One-factor ANOVA followed by Tukey's test (Zar, 1984), was used to compare treatments at a significance level of 0.05. The Shapiro–

Wilk and Levene tests, assessed normality and homoscedasticity, respectively (Zar, 1984). Data expressed as a percentage underwent angular transformation before analysis. Microbiological analysis data showed no homoscedasticity and were Log10 transformed.

The analysis of changes in ammonia over time was performed by repeated-measures ANOVA. Treatments were considered to be the main factors, and duration of culture was the additional factor. Significant differences were analyzed by Tukey's test (Zar, 1984) with a significance level of 0.05.

26 RESULTS AND DISCUSSION

The water quality parameters among treatments were similar (Table 6, Figure 3) and although analysis of variance indicated significant differences between groups in some parameters (pH and volatile solids), the water quality remained appropriate for the observed hatchery stage of *L. vannamei*. These parameters were similar to those found in a comparative test between the conventional hatchery system with high rates of daily exchange and the BFT hatchery systems with sources of organic carbon (Lorenzo et al, 2015).

A significant decrease in pH relative to the higher density was observed (Table 6). The bacterial population should increase similar to the stocking densities due to higher food inputs and consequently higher availability of nutrients and inorganic nitrogen compounds. Therefore, the bacterial respiration rate possibly increased the CO2 concentration resulting in a corresponding decrease in pH. This decrease in pH, even in the higher density group, did not significantly affect the buffering capacity without affecting alkalinity levels among groups and larvae survival (Table 6).

The 12,5:1 fixed C:N ratio by dextrose input in all experimental groups maintained the TAN mean level near to 1 $mg \cdot L^{-1}$ until d 5 when this parameter steadily increased in the two higher densities groups D300 and D350 (Figure 3A). Free ammonia increased considerably on the last two days of the experiment following the same profile of TAN (Figure 3B). However, mean values of total and free ammonia remained below toxic levels throughout the experimental period (Cobo et al., 2012).

The low measurements of nitrite (Table 6) suggest that nitrification was not established during the course of our experiment and

the activity of the heterotrophic bacterial community was responsible for controlling ammonia in the BFT groups.

No difference was observed in the mean values between groups of total suspended solids (Table 6). Similar results have been observed in cultures of postlarval shrimp (Mishra et al., 2008; Emerenciano et al., 2012; Xu and Pan, 2012). Although an acceptable range of total solids in shrimp hatcheries has not yet been established, very high levels of total solids can have negative impacts on developing larvae. In the early stages of postlarval development, shrimp are small and can be harmed by high levels of solids (Schveitzer et al., 2013).

However, in the present study, more volatile solids were present in the two higher density BFT groups with statistically significant differences from D350 (representing 42% SST) to D200 (35.9% SST), and D250 (36.1% SST), and did not differ statistically from D300 (39.6% SST) density groups (Table 6). This suggests that there was a large quantity of organic matter of bacterial origin with high densities probably due to the higher input of nutrients and nitrogen compound formation and the increased fertilization (Moriarty, 1997; Avnimelech, 1999; Ebeling et al., 2006).

Table 6: Water quality parameters in four BFT Pacific white shrimp (*Litopenaeus vannamei*) hatchery systems between the mysis 1 and postlarva 5 phases: Biofloc systems supplemented whit dextrose at 12,5:1 C:N ratio and four densities were compared: 200 larvae L^{-1}, 250 larvae L^{-1}, 300 larvae L^{-1} and 350 larvae L^{-1}.

Parameter	D 200	D 250	D 300	D 350	p
Temperature (°C)	29.78 ± 0.32[a] *	29.88 ± 0.35[a]	29.98 ± 0.37[a]	29.83 ± 0.29[a]	0.46
Oxygen (mg·L-1)	5.74 ± 0.28[a]	5.85 ± 0.23[a]	5.75 ± 0.26[a]	5.80 ± 0.28[a]	0.72
pH	8.24 ± 0.04[a]	8.23 ± 0.02[ab]	8.19 ± 0.03[ab]	8.17 ± 0.03[b]	0.01
Salinity (g·L-1)	35.32 ± 0.32[a]	35.30 ± 0.26[a]	35.29 ± 0.24[a]	35.32 ± 0.28[a]	0.99
TAN (mg·L-1)	0.80 ± 0.53 [a]	0.62 ± 0.50 [a]	1.20 ± 0.91[a]	1.26 ± 1.18[a]	0.42
NH3-N (mg·L-1)	0.09 ± 0.06 [a]	0.07 ± 0.05[a]	0.13 ± 0.09 [a]	0,13 ± 0,12 [a]	0,51
NO2--N (mg·L-1)	0.02 ± 0.07[a]	0.01 ± 0.03[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.54
NO3--N (mg·L-1)	2.24 ± 0.33[a]	2.39 ± 0.64[a]	2.35 ± 0.55[a]	2.30 ± 0.40[a]	0.94
PO43-(mg·L-1)	0.70 ± 0.40[a]	0.82 ± 0.10[a]	1.07 ± 0.30[a]	1.22 ± 0.15[a]	0.06
Alkalinity (mg·L-1)	128.5 ± 10.35[a]	128.5 ± 10.99[a]	137.5 ± 10.01[a]	134.0 ± 9.07[a]	0.23
TSS (mg·L-1)	292.1 ± 44.49[a]	278.0 ± 40.59[a]	310.6 ± 45.18[a]	313.6 ± 33.98[a]	0.35
VSS (mg·L-1)	104.9 ± 9.28[a]	100. 3 ± 4.53 [a]	123.0 ± 18.21 [ab]	131.0 ± 30.97 [b]	0.001

* Values are expressed as means ± standard deviation. Values in the same row with different letters are significantly different ($p < 0.05$), as indicated by Tukey's test of mean separation.

Furthermore, we did not observe differences between treatments in larval quality parameters. All larvae were active (high swimming activity), and had lipid reserves, normal hepatopancreas color, and full intestines. We found no deformities, epibionts, adhered particles, necrosis, or muscular opacity. Final survival, dry weight, and length did not differ between groups, and only D200 and D250 lengths were statistically different (Table 7). Final survival in all groups surpassed the rate appropriate for the species (70%, FAO, 2003) and that appropriate for experimental hatcheries (Aranguren et al., 2006; D'Abramo et al., 2006, Lorenzo et al., 2015), and the salinity stress test did not differ between groups (Table 7).

In a previous study, the water consumed in BFT treatments, without water exchange, and with dextrose or molasses as a carbon source (6.49 ± 0.79 and 6.89 ± 0.95, respectively L per thousand PL5) required approximately 12% of the water used by the conventional system (56,22 ± 3,31 L per thousand PL5) (Lorenzo et al., 2015). In the present study, the water consumption (Table 7) in the two higher stocking densities of BFT was reduced by about 40% from the previous BFT system, with consumption limited to only 8% of the water used by the conventional system. The two lower stocking density water consumption results were similar (11,44% and 11, 51% for D200 and D 250, respectively) from BFT groups of the prior experiment. The water consumption per thousand postlarvae, associated with hatchery performance, demonstrated that the intensification in shrimp BFT hatchery culture has the potential to contribute to the food demand challenge, once the system achieves high productivity and low water use. The use of BFT sistem decreases the environmental impacts and costs associated with pumping, disinfection, neutralization, and heating of water for hatcheries, and improve the system biosecurity.

Table 7: Final survival, final length, final dry weight, in four Pacific white shrimp (Litopenaeus vannamei) hatchery systems between the mysis 1 and postlarva 5 phases (200 larvae L-1).

Parameter	D 200	D 250	D 300	D 350	p
Survival (%)	87.04 ± 7.50[a]*	70.03 ± 4.80[a]	80.57 ± 8.31[a]	76.55 ± 7.80[a]	0.08
Final length (mm)	6.44 ± 0,26[a]	6.11 ± 0.30[b]	6.31 ± 0.32[ab]	6.32 ± 0.25[ab]	0.02
Final dry weight (mg)	0.22 ± 0.07[a]	0.14 ± 0.05[a]	0.15 ± 0.03[a]	0.19 ± 0.01[a]	0.13

* Values are expressed as means ± standard deviation. Values in the same row with different letters are significantly different, as indicated by one-way ANOVA followed by Tukey's test of mean separation ($p < 0.05$).

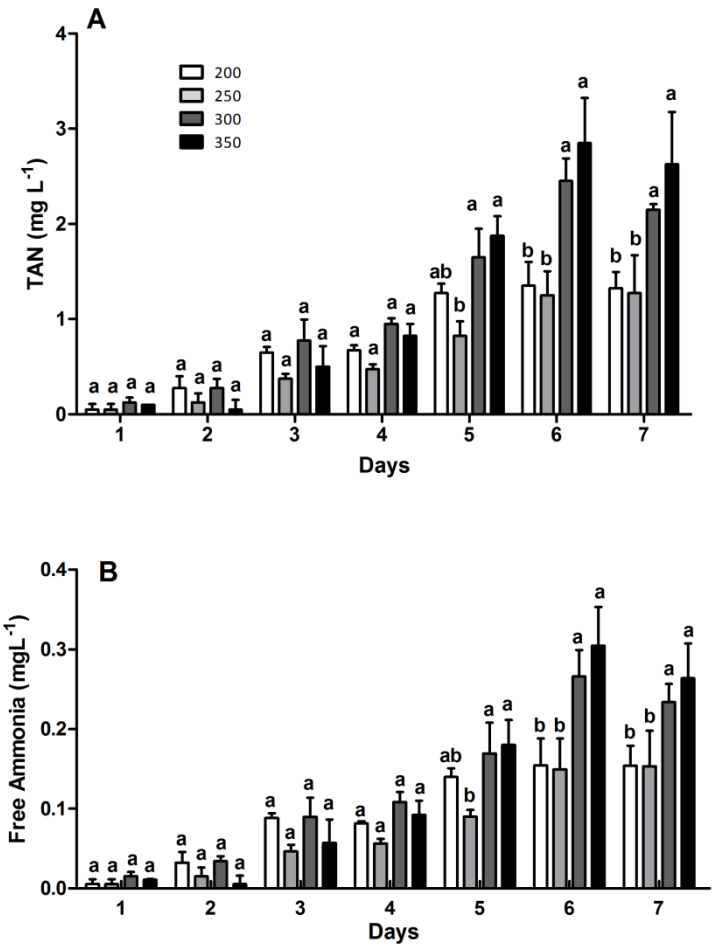

Figure 3: Daily mean total (A) and free ammonia (B) in Pacific white shrimp (Litopenaeus vannamei) hatcheries between the mysis 1 and postlarva 5 phases. Bars represent Biofloc treatments supplemented whit dextrose at 12,5:1 C:N ratio at four stocking densities: 200 larvae L-1, 250 larvae L-1, 300 larvae L-1 and 350 larvae L-1 treatments, according to legend . Water was not exchanged in either of the biofloc systems. Different letters on the same day indicate significant differences, as indicated by Tukey's test of mean separation ($p < 0.05$).

27 CONCLUSION

The intensification in BFT hatchery culture from 200 to 350 larvae per liter of water with dextrose as a carbon source in fixed C:N ratio of 12,5:1 results in adequate production indexes and water quality during the M1 to PL5 hatchery phase of *L. vannamei*, resulting in higher production with lower water consumption.

28 ACKNOWLEDGMENTS

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30 CONCLUSOES GERAIS

a) A larvicultura do *Litopenaeus vannamei* pode ser realizada em sistema de bioflocos sem a renovação de água entre as fases de misis 1 e pós-larva 5 sem prejuízos quantitativos ou qualitativos na produção de pós larvas em relação ao sistema convencional.
b) As relações C:N fixas de 12,5:1 e 15:1 são mais eficientes no controle da amônia.
c) No sistema sem renovação, foi possível incrementar a taxa de estocagem até 350 larvas L^{-1}, sem prejudicar os parâmetros zootécnicos da larvicultura e incrementando a produtividade.
d) O uso de água por milheiro de pós-larvas produzida no sistema de larvicultura em bioflocos com alta densidade (300 e 350 larvas L^{-1}) foi apenas 8% em realção ao sistema convencional se comparado ao resultado do grupo controle do primeiro experimento..

31 CONSIDERAÇÕES FINAIS

O desenvolvimento deste trabalho de tese teve o apoio financeiro proveniente do Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq); Financiadora de Estudos e Projetos (FINEP); e do Ministério da Pesca e Aquicultura (MPA).

O objetivo proposto neste trabalho partiu da premissa de se realizar a larvicultura do camarão *Litopenaeus vannamei* utilizando o sistema de bioflocos justamente em substituição ao período onde, usualmente, iniciam-se as renovações de água com reposição de microalgas (entre M1 e PL5), buscando preservação de recursos naturais, preservação do meio ambiente aquático, aumento da biosseguridade e intensificação da produção com perspectiva de um aumento real da produtividade por área visando a sustentabilidade.

Para a prospecção de dados experimentais que atingissem esse objetivo fornecendo respostas confiávies dentro do conjunto de sua aplicabilidade para gerar potencial de extrapolação para um ambiente de produção futuro, três abordagens sequenciais e complementares foram utilizadas:

1) Avaliar se a utilização da tecnologia de bioflocos durante o período supracitado da larvicultura seria viável. A aferição da qualidade da água em termos físicos e químicos, bem como microbiológicos além da performance produtiva com dados zootécnicos aqui apresentados em resposta a essa primeira abordagem, onde testamos duas fontes de carbono orgânico (melaço e dextrose) em comparação ao sistema convencional nos mostrou um bom potencial desta tecnologia de produção em termos experimentais.

Ainda nesta primeira etapa, confrontamos os dados quantitativos de uso de água utilizada por unidade de produção e percebemos uma redução extremamente significativa neste parâmetro. Um aumento previsto de bactérias heterotróficas totais sem aumento da vibrionáceas também foi um dado de grande importância. Concluímos ainda que o sistema de fertilização padrão diário com base na entrada de alimento utilizado, mais os acréscimos de carbono realizados pontualmente para correção dos níveis de amônia total para 1 mg L^{-1}, ao final do experimento nos apresentou uma relação C:N média acima de 12,5:1.

Desse modo, o nosso primeiro objetivo específico nos respondeu que é possivel empregar bioflocos ainda na fase larvicultura mantendo a qualidade da água de cultivo em condições ideiais de produção e sem nenhum comprometimento quantitativo ou qualitativo das pós larvas e ainda com uma grande economia de água.

2) Com os dados obtidos na etapa anterior que confirmaram a possibilidade de se controlar os níveis de amônia de maneira segura através de uma estrégia de fertilização com base no monitoramento deste parâmetro, pensamos em utilizar o valor médio de adição de carbono durante o experimento como ponto de partida para estabelecermos uma fertilização fixa efetiva sem a necessidade de monitoramento diário dos níveis de amônia.

Partimos de uma relação C:N de 12,5:1 com base no experimento anterior, uma relação abaixo deste valor (10:1) e uma de valor eqüidistante acima do valor central (15:1). O objetivo era, embora as análises de amônia e demais parâmetros de qualidade de água fossem realizadas durante todo experimento, nenhum acréscimo pontual de carbono seria feito para que pudéssemos acompanhar o comportamento dos parâmetros de qualidade de água e performance da larvicultura.

Observamos ao final do experimento uma performance semelhante das pós larvas, o que sugere que a qualidade de água não foi um fator de prejuízo para nenhum dos tratamentos, embora a menor relação C:N (10:1) tenha apresentado um aumento mais acentuado nos níveis de amônia do meio para o término do experimento finalizando com níveis de amônia significativamente maiores que os demais tratamentos.

Assim nossa abordagem referente ao segundo objetivo específico mostrou que a relação C:N pré estabelecida de 12,5:1 mantida mediante fertilização com dextrose durante o experimento foi capaz de manter os níveis de amônia e demais parâmetros de qualidade de água dentro de limites viáveis de produção, o que também ocorreu com a relação C:N de 15:1.

3) Finalmente, de posse de uma relação carbono nitrogênio fixa e comprovadamente eficiente, passamos para os testes do nosso terceiro objetivo específico, que seria aumentar a densidade de estocagem da larvicultura, intensificando ainda mais o sistema de produção experimental, que nas etapas anteriores contou com uma densidade de povoamento de 200 M1 L^{-1} e que agora alem desta, seriam testadas no total, quatro densidades crescentes a partir de 200, 250, 300 até 350 M1 L^{-1}. Vale lembrar que nas etapas anteriores a densidade de estocagem já pode ser considerada alta, uma vez que na introdução geral deste trabalho foi mostrado que a densidade considerada padrão para larvicultura esta no povoamento com nauplios (N) de 100 a 250 N L^{-1}, pois as maiores perdas normalmente se dão nas fases de nauplio e protozoea chegando na fase de misis 1 com uma densidade real inferior a de povoamento com os náuplios.

Neste experimento a fertilização com dextrose mantendo uma relação C:N de 12,5:1 resultou em valores médios de qualidade de água e performance apropriados para todos os grupos experimentais, e, embora os dois grupos com maiores densidades de povoamento (300 e 350 M1 L^{-1}) tenham apresentado maiores níveis de amônia do quinto dia até o término do experimento, níveis tóxicos de amônia que pudessem causar prejuízos as pos larvas não foram atingidos.

Com relação ao consumo de água utilizada por quantitativo de produção, os dois grupos com maiores densidades reduziram em 40% os valores desse parâmetro referentes aos sistemas de bioflocos realizados na abordagem do nosso primeiro objetivo específico. Comparando com o grupo controle daquele experimento, uma redução de 12% de consumo de água para os grupos BFT naquela ocasião e, nesta abordagem, para aproximadamente 8% em resultado ao aumento da densidade de estocagem.

Finalizando a abordagem deste terceiro objetivo específico, concluímos pelos resultados encontrados que a intensificação da larvicultura do *L. vannamei* em sistemas de BFT pode ser ainda aumentada por não termos encontrado uma densidade que fosse limitante a produção de acordo com os parâmetros utilizados.

Desse modo, concluímos, a partir dos resultados do presente trabalho que o tipo de cultivo aqui abordado e o desenvolvimento de sua técnica pode ser aplicado a sistemas de produção com potencial para atender ao desafio do aumento da demanda por alimentos sob a perspectiva da aquicultura buscando altos níveis de produtividade, baixo consumo de recursos naturais (água e energia, principalmente), diminuição do impacto ambiental e aumento da biosseguridade no processo de produção acreditando que os resultados apresentados neste trabalho de tese possam contribuir para este fim.

Ainda sob o ponto de vista deste trabalho, entre outras possíveis abordagens, destacamos a possibilidade de aumentar ainda mais a densidade de estocagem, além de uma análise de custos comparada ao sistema convencional para uma maior precisão em termos do potencial econômico real, alem da utilização de ferramentas para análises de impacto ambiental como perspectiva futura.

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ANEXOS

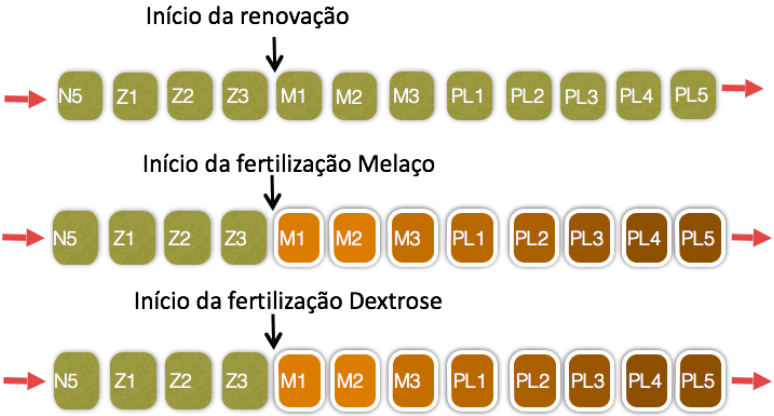
Anexo 1: Unidade de produção de 20.000 L.

* Foto de Felipe do Nascimento Vieira.

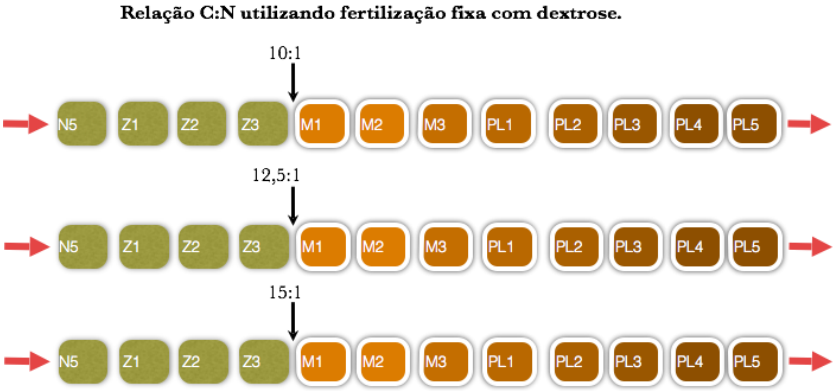
Anexo 2: Unidades experimentais de 60 L.

* Foto de Marco Antonio de Lorenzo

Anexo 3: Esquema do comparativo da larvicultura experimental de *Litopenaeus vannamei* convencional e de bioflocos:



Anexo 4: Esquema da larvicultura experimental de *Litopenaeus vannamei* em bioflocos com relações fixas de carbono:nitrogênio.



Anexo 5: Esquema da larvicultura experimental *de Litopenaeus vannamei* em bioflocos com diferentes densidades de estocagem.