UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

UTILIZAÇÃO DE *Navicula* sp. NO CULTIVO DE CAMARÕES MARINHOS *Litopenaeus vannamei* NA FASE BERÇÁRIO EM SISTEMA DE BIOFLOCOS

Jéssika Lima de Abreu

Tese apresentada ao Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura da Universidade Federal Rural de Pernambuco como exigência para obtenção do título de Doutora.

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Tese julgada adequada para obtenção do título de doutora em Recursos Pesqueiros e Aquicultura. Defendida e aprovada em 25/09/2020 pela seguinte Banca Examinadora.

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Resumo

O uso do sistema BFT (*Biofloc Technology*) está sendo implementado nas fases de berçário e engorda comercial de camarão marinho no Brasil. Entretanto, pouco sabe-se sobre o papel da combinção de bactérias e microalgas nesse sistema. Nesse sentido, o objetivo do estudo foi avaliar o uso de sistema de BFT em berçários de camarões marinhos com diferentes frequências e densidades de inoculação da diatomácea *Navicula* sp. no crescimento, qualidade da água e composição do floco microbiano. Foram realizados dois experimentos, onde no primeiro foi avaliado a densidade de adição da *Navicula* sp. no sistema de cultivo e, no segundo, avaliou-se a melhor frequência de adição da diatomácea. Ambos os experimentos contaram com quatro tratamentos, todos em triplicata. O primeiro experimento foi formado pelos tratamentos: BFT (sem adição da *Navicula* sp.); BFT 2,5N (com adição de *Navicula* sp. a densidade de 2,5 x 10^4 células.mL); BFT 5N (com adição de *Navicula* sp. a densidade de 5 x 10^4 células.mL) e BFT 10N (com adição *Navicula* sp. a densidade de 10 x 10^4 células.mL). O cultivo teve uma duração de 42 dias e foram avaliados o desempenho zootécnico e qualidade de água. Os camarões (1 ± 0,01 mg) foram estocados a uma densidade de 3.000 pós-larvas.m³ e foram alimentados com ração comercial. A microalga foi inoculada nas unidades experimentais de acordo com a densidade descrita para cada tratamento no primeiro, décimo, vigésimo e trigésimo dias de cultivo. Os tratamentos BFT 5N e BFT 10N apresentaram valores de desempenho superiores, destacando-se os valores de produtividade (2,30 e 2,42 kg.m^3) e taxa de crescimento específico (15,92 e 16,08 %.dia), que foram superiores aos demais tratamentos. Além disso, os maiores níveis de ácidos graxos foram observados nos tratamentos com diatomáceas (BFT 5N e BFT 10N), indicando os benefícios do *Navicula* sp. no aumento do crescimento e conteúdo de ácidos graxos de pós-larvas de *L. vannamei* cultivadas em sistemas de bioflocos. No segundo experimento, quatro tratamentos foram avaliados: BFT (sem adição da *Navicula* sp.); BFT 5D (com adição de *Navicula* sp. a cada 5 dias); BFT 10D (com adição de *Navicula* sp. a cada 10 dias) e BFT 15D (com adição *Navicula* sp. a cada 15 dias), todos em triplicata. O cultivo teve uma duração de 35 dias e foram avaliados o desempenho zootécnico e qualidade de água. Os camarões (5 ± 0,01 mg) foram estocados a uma densidade de 3.000 pós-larvas.m³ e foram alimentados com ração comercial. A microalga foi inoculada nas unidades experimentais a uma densidade ceular de 10 x 10^4 cél.mL. Todos os dados foram submetidos a ANOVA ($\alpha \geq 0,05$) onde não foram observadas diferenças significativas para os dados de qualidade da água, FCA e sobrevivência, já para o peso final, produtividade e, TCE foram encontradas diferenças significativas, onde os tratamentos BFT mais adição de *Navicula* sp. foram superiores ao tratamento controle (BFT sem adição de *Navicula* sp.), destacando o tratamento BFT 10D

que apresentou maiores valores para peso final (0,50 ± 0,06 g), produtividade (1,41 ± 0,03 kg.m^3) e TCE (13,17 ± 0,03 %/dia) em relação aos demais tratamentos. Por fim, conclui-se que as concentrações de 5 x 10^4 células.mL e 10 x 10^4 células.mL a *Navicula* sp. quando inoculadas a cada 10 dias apresentaram o melhor desempenho, o que indica os benefícios de *Navicula* sp. no aumento do crescimento de *L. vannamei* pós-larvas cultivadas em sistemas bioflocos.

Palavras-chave: diatomáceas, camarão marinho, bioflocos, berçário

Abstract

The use of the BFT system (Biofloc Technology) is being implemented in the nursery and commercial grow-out phases of shrimp in Brazil. However, little is known about the role of the combination of bacteria and microalgae in this system. In this sense, the objective of the study was to evaluate the use of the BFT system in marine shrimp nurseries with different frequencies and densities of inoculation of the diatom *Navicula* sp. on growth, water quality and microbial floc composition. Two experiments were carried out, in which the density of addition of *Navicula* sp. in the culture system and the second one evaluated the best frequency of addition of the diatom. Both experiments had four treatments, all in triplicate. The first experiment: BFT (without addition of *Navicula* sp.); 2.5N BFT (with the addition of 2.5 x 10^4 cells.mL *Navicula* sp.); BFT 5N (with the addition of 5 x 10^4 cells.mL *Navicula* sp.) and BFT 10N (with the addition of 10 x 10^4 cells.mL *Navicula* sp.). The cultivation lasted 42 days and zootechnical performance and water quality were evaluated. The shrimp (1 ± 0.01 mg) were stored at a density of 3,000 post-larvae.m³ and they were fed with commercial feed. The microalgae was inoculated in the experimental units according to the density described for each treatment on the first, tenth, twentieth and thirtieth days of culture. The treatments BFT 5N and BFT 10N presented superior performance values, with emphasis on the values of productivity (2.30 and 2.42 kg.m^3) and specific growth rate (15.92 and 16.08%.day), which were superior to the other treatments. In addition, the highest levels of fatty acids were observed in treatments with diatoms (BFT 5N and BFT 10N), indicating the benefits of *Navicula* sp. in increasing the growth and fatty acid content of *L. vannamei* post-larvae grown in biofloc systems. In the second experiment, four treatments were evaluated: BFT (without the addition of *Navicula* sp.); BFT 5D (with the addition of Navicula sp. every 5 days); BFT 10D (with addition of *Navicula* sp. every 10 days) and BFT 15D (with addition of *Navicula* sp. every 15 days), all in triplicate. The cultivation lasted 35 days and zootechnical performance and water quality were evaluated. The shrimp (5 ± 0.01 mg) were stored at a density of 3,000 post-larvae.m³ and they were fed with commercial feed. The microalgae was inoculated in the experimental units at a cell density of 10 x 10^4 cells.mL. All data were submitted to ANOVA ($\alpha \geq 0.05$) where no significant differences were observed for the data of water quality. FCA, survival, final weight, productivity and SGR differed significantly, where *Navicula* BFT treatments were superior to the control treatment (BFT without the addition of *Navicula* sp.), highlighting the BFT 10D treatment that presented higher values for final weight (0.50 ± 0.06 g), productivity (1.41 ± 0.03 kg.m^3) and specific growth rate (13.17 ± 0.03% / day) in relation to the other treatments. Finally, it

is concluded that the concentrations of 5 x 10^4 cells.mL and 10 x 10^4 cells.mL of *Navicula* sp. when inoculated every 10 days they showed the best performance, which indicates the benefits of *Navicula* sp. in the growth of post-larvae *L. vannamei* grown in biofloc systems.

Key words: diatoms, marine shrimp, bioflocs, nursery

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1- Introdução

1.1- Contextualização da pesquisa

Com a redução nas capturas pesqueiras e com o aumento da população mundial, a aquicultura é uma atividade que apresenta alto potencial para suprir a demanda por alimentos de origem aquática (AHMAD & SUBASINGUE, 2010). Sorgeelos et al. (2010), relatam que a aquicultura é o setor de produção de alimentos com maior crescimento mundial e destaca que aproximadamente 50% de todo o alimento marinho consumido é advindo de cultivos.

Segundo dados da FAO (2020), entre os anos de 1986 a 1995 a atividade aquícola mundial alcançou uma produção de aproximadamente 14,9 milhões de toneladas, apresentando uma crescente nos anos seguintes. Em 2018, essa produção foi de aproximadamente 82,1 milhões de toneladas, indicando que o setor teve um incremento de mais de 150% na sua produção (FAO, 2020). Desse total, a aquicultura marinha produziu aproximadamente 30.756 mil toneladas do qual 18,64% foram advindos da produção de crustáceos (FAO, 2020).

Entre as várias atividades que compõem a aquicultura, a carcinicultura destaca-se pelo seu grande crescimento em diversas partes do mundo. No Brasil, a carcinicultura iniciou sua produção comercial na década de 1970, mas foi na década de 1990 que ocorreram as maiores transformações, a principal delas, foi a introdução do pacote tecnológico para o cultivo do *Litopenaeus vannamei*, que hoje é a principal espécie cultivada mundialmente (NATORI et al., 2011; ROCHA, 2006). Segundo dados obtidos pelo IBGE, em 2018, o Brasil produziu aproximadamente 45,8 mil toneladas apresentando um aumento de 11,4% em relação ao ano anterior, onde a região Nordeste representa mais de 99% do total da produção no país (IBGE, 2018)

Apesar de apresentar destaque dentre as atividades aquícolas, a carcinicultura é duramente criticada pelo impacto negativo que pode causar ao meio ambiente. Nos últimos anos, diversos estudos foram realizados com o objetivo de criar uma tecnologia que minimizasse esses impactos e assim surgiu o sistema de bioflocos. Esse sistema baseia-se na mínima ou zero troca de água, onde os animais são produzidos em pequenas áreas, de forma

biossegura e sustentável e com uma significativa redução no descarte de efluentes (DE SCHRYVER et al., 2008; CRAB et al., 2012). Nesses cultivos, ocorre a formação de agregados microbianos que são compostos por bactérias, microalgas, microfragelados, nematóides, zooplâncton, fungos, fezes e exoesqueleto de animais mortos. Esses agregados podem servir como fonte de suplementação alimentar, trazendo benefícios como maior taxa de crescimento, aumento no peso final e redução do fator de conversão alimentar para os camarões (ZHAO et al., 2012; PÉREZ-FUENTES et al., 2013).

Nos últimos anos, estudos vêm sendo realizados para aprimorar o pacote tecnológico do sistema de bioflocos. Alguns dos estudos são direcionados a encontrar formas de melhorar nutricionalmente o floco formado no sistema, e uma das formas estudadas é a adição de microalgas (BRITO et al., 2016; MARINHO et al., 2014, 2016; ABREU et al., 2019). Esses microorganismos podem atuar de duas formas no sistema: melhorando a qualidade da água através da absorção dos compostos nitrogenados acumulados e como uma suplementação alimentar de alta qualidade (JUAREZ et al., 2010), visto que apresentam uma rica composição nutricional (COUTTEAU, 1996). Além disso, as microalgas são uma fonte alimentar essencial nos estágios iniciais de desenvolvimento dos camarões (JUAREZ et al., 2010; ZMORA et al., 2013). Sabe-se que os camarões podem consumir as bactérias (AVNIMELECH, 1999; MOSS et al., 2000), fitoplâncton (KENT et al., 2011; OTOSHI et al., 2011) e zooplâncton (DECAMP et al., 2007) agregados aos bioflocos, e alguns autores observaram que isso têm aumentado significativamente as taxas de crescimento dos camarões (KUHN et al., 2010; AUDELO-NARANJO et al., 2012), assim como, melhora a eficiência das enzimas protease, lipase, amilase, celulase, tripsina (XU et al., 2013), e a resposta imune dos camarões da espécie *L. vannamei* (XU e PAN, 2013).

Dentro do grupo de microalgas presentes nesses sistemas, as diatomáceas se destacam por apresentarem um alto valor nutricional, ricas em ácidos graxos altamente insaturados e aminoácidos essenciais, e por serem facilmente digeríveis pelos camarões (JU et al. 2008, 2009; JAIME-CEBALLOS et al., 2006; PHILLIPS, 1984). Alguns autores já relataram que a inoculação de algumas espécies de diatomáceas no sistema de cultivo trás benefícios e

melhora o desempenho zootécnico dos camarões (BRITO et al., 2016; MARINHO et al., 2014, 2016; ABREU et al., 2019). Dentre as diatomáceas utilizadas nos trabalhos citados anteriormente, destaca-se a *Navicula* sp. que segundo KHATOO et al. (2009) ao avaliar a sua composição nutricional encontrou níveis em peso seco de proteína de 430-490 g / kg, de lípidos entre 230 e 260 g / kg, de EPA entre 30 e 150 g de lípidos / kg e, de DHA, entre 20 e 30 g de lípido / kg.

Diante do exposto, objetivou-se com o desenvolvimento do presente estudo avaliar qual a melhor densidade e a melhor frequência de adição da diatomácea *Navicula* sp. no cultivo do *Litopenaeus vannamei* em sistema de bioflocos.

Hipótese

A adição da diatomácea *Navicula* sp. em uma densidade e frequência específica contribui para o melhor desempenho zootécnico de pós larvas de *L. vannamei* cultivadas em sistema de bioflocos na fase de berçário.

1.3- Objetivos do trabalho (geral e específicos)

- ✓ Geral

Aprimorar a tecnologia do cultivo de *Litopenaeus vannamei* na fase berçário em sistema BFT com adição de diatomácea.

- ✓ Específico

1. Avaliar o cultivo berçário de *Litopenaeus vannamei* em sistema de bioflocos com adição de *Navicula* sp. em diferentes densidades e frequências;
2. Avaliar a dinâmica dos compostos fosfatados e nitrogenados na água ao longo do cultivo na fase berçário em sistema de bioflocos com adição de *Navicula* sp. em diferentes densidades e frequências;
3. Avaliar o crescimento e sobrevivência dos camarões na fase berçário em sistema de bioflocos com adição de *Navicula* sp. em diferentes densidades e frequências;
4. Analisar a composição centesimal dos camarões na fase berçário em sistema de bioflocos com adição de *Navicula* sp. em diferentes densidades.

2- Effects of addition of *Navicula* sp. (diatom) in different densities to postlarvae of shrimp *Litopenaeus vannamei* reared in a BFT system: Growth, survival, productivity and fatty acid profile.

Artigo científico publicado na revista Aquaculture Research (ISSN: ISSN: 1365-2109), conforme Anexo I.

Effects of addition of *Navicula* sp. (diatom) in different densities to postlarvae of shrimp *Litopenaeus vannamei* reared in a BFT system: Growth, survival, productivity and fatty acid profile

Abreu et al., 2019

Abstract

The objective of this study was to evaluate the effect of the addition of *Navicula* sp. on the growth and fatty acids profile of *Litopenaeus vannamei* postlarvae in a biofloc system (BFT). Four treatments were used: BFT; BFT 2.5N (addition of 2.5×10^4 cells/ ml of Navicula sp.); BFT 5N (addition of 5×10^4 cells/ml of *Navicula* sp.) and BFT 10N (addition of 10×10^4 cells/ml of *Navicula* sp.), all in triplicate. The shrimp (1 ± 0.01 mg) were stocked at a density of 3,000 postlarvae/m^3 and fed with commercial feed. The diatom was added every 10 days, and at the end of 42 days, shrimp performance, water quality and proximal composition were evaluated. The BFT 5N and BFT 10N treatments had higher performance values, highlighting the values of productivity (2.30 and 2.42 kg/m^3) and specific growth rate (15.92 and 16.08%/day), which were higher than the other treatments. In addition, the highest levels of fatty acids were observed in treatments with diatom (BFT 5N and BFT 10N), indicating the benefits of *Navicula* sp. on growth enhancement and fatty acid content of *L. vannamei* postlarvae grown in biofloc systems.

KEYWORDS diatoms, presumptive diagnosis, proximal composition, stress response, water quality

1. Introduction

Shrimp farming in Brazil is almost entirely based on the cultivation of *Litopenaeus vannamei* and in 2017 reached production of approximately 41 thousand tonnes (IBGE [Instituto Brasileiro de Geografia e Estatística], 2018). Much of this production is in semi-intensive farming systems, with regular water exchanges and less biosecurity. However, in recent years, problems of outbreaks of viruses such as infectious myonecrosis virus (IMNV) and white spot syndrome virus (WSSV) have resulted in reduced productivity. Therefore, it is necessary to use systems with minimum water changes and emission of effluents (Krummenauer & Seifert, 2012), such as the biofloc system (Crab, Defoirdt, Bossier, & Verstraete, 2012; Pérez-Fuentes, PérezRostro, & Hernández-Vergara, 2013).

The key to biofloc systems is their manipulation of the carbon–nitrogen relationship in the ponds or raceways, stimulating the growth of the microbial community formed by bacteria, phytoplankton, zooplankton, uneaten feed and faeces remains, exoskeletons and other invertebrates (Avnimelech, 2009; De Schryver, Crab, Defoirdt, Boon, & Verstraete, 2008; Samocha et al., 2017), providing an increase in final weight, a higher growth rate and a reduction in feed conversion ratio (FCR) (Avnimelech, 2009; PérezFuentes et al., 2013; Zhao et al., 2012). However, some studies indicate a deficiency in essential amino acids on bioflocs, such as methionine and lysine (Gamboa-Delgado et al., 2017; Valle et al., 2015), and polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Ekasari, Crab, & Verstraete, 2010).

The presence of microalgae may be beneficial because of its contribution as a food source for the farmed shrimp. Ray, Shuler, Leffler, and Browdy (2009) reported that the biomass in an autotrophic– heterotrophic shrimp system was 17% higher and the FCR was 18% lower than those in a completely heterotrophic system. The planktonic community could play an important role in shrimp nutrition in biofloc systems because of its good nutritional quality, considering plankton protein, lipid and carbohydrate levels ranging, respectively,

from 12% to 35%, 7% to 23% and 4% to 23% of their dry weight, making them a significant food source in the early stages of cultivation (Brito et al., 2016; Hemaiswarya, Raja, Ravikumar, Ganesa, & Anbazhagan, 2011; Marinho, Brito, Silva, Santos, & Gàlvez, 2014; Marinho et al., 2016; Muller-Feuga, Robert, Cahu, Robin, & Divanach, 2003; Richmond, 2004). In terms of microalgal communities, diatoms are distinguished by their high nutritional content and can contribute with essential amino acids and highly unsaturated fatty acids (HUFAs) (Ju et al., 2008; Ju, Forster, & Dominy, 2009).

Navicula sp. is a benthic diatom that presents approximate dry weight levels of 430–490 g/kg of protein, 230–260 g/kg of lipids, which are 30–150 g of EPA g/kg and 20–30 g of DHA g/kg (Khatoon, Banerjee, Yusoff, & Shariff, 2009). Other studies have shown that the lipid content ranges from 4.3% to 31.7% and protein from 12.7% to 13.31% (Courtois, Viera, Huchetteb, & Izquierdo, 2012; Pacheco-Vega, Cadena-Roa, Ascencio, Rangel-Dávalos, & Rojas-contreras, 2015). In this context, some studies using Navicula sp. (5 × 104 cells/ ml addition every 5 days) in biofloc systems in the nursery phase presented good results regarding the nutritional contribution of this diatom to the growth of L. vannamei postlarvae (Brito et al., 2016; Marinho et al., 2014, 2016).

According to Underwood, Boulcott, Raines, and Waldron (2004) bethic diatoms produce fucose, xylose, mannose, galactose, glucose and glucans as intracellular storage compounds. These extracellular polymeric substances are important as adhesive components for the attachment of symbiotic bacteria (Watanabe et al., 2005). Another beneficial effect of microalgal–bacterial interactions is the excretion into the environment of carbon sources or other products by microalgae that have a stimulatory effect on bacteria, including bacterial DNA synthesis (Murray, Cooksey, & Priscu, 1986), and increased bacterial biofilm formation (Espeland, Francoeur, & Wetzel, 2001). This beneficial effect depends on microalgae and bacterial density and species (Natrah, Bossier, Sorgeloos, Yusoff, & Defoirdt, 2014).

Therefore, the objective of this study was to evaluate the effect of the addition of different densities of the diatom *Navicula* sp. on the shrimp performance and fatty acids profile of *L. vannamei* in the nursery phase reared in a biofloc system.

2. Materials and Methods

2.1 Experimental conditions

An indoor trial was conducted for 42 days at the Sustainable Mariculture Laboratory (LAMARSU) of the Fisheries and Aquaculture Department (DEPAq) of the Rural Federal University at Pernambuco (UFRPE), Recife, Brazil. The experimental design was completely randomized with four treatments: BFT (biofloc system without addition of *Navicula* sp); BFT with the addition of 2.5×10^4 cells/ml *Navicula* sp. (BFT 2.5N); BFT with the addition of 5×10^4 cells/ml *Navicula* sp. (BFT 5N); and BFT with the addition of 10×10^4 cells/ml *Navicula* sp. (BFT 10N), all in triplicate.

Five days prior to stocking, water from a matrix tank (TAN 0.05 mg/L, N-NO2 0.25 mg/L, N-NO3 0.5 mg/L, alkalinity 150 mg CaCO3/L, pH 8.8, salinity 30 g/L, P-orthophosphate 0.9 mg/L, TSS 100 mg/L and settleable solids 4.16 ml/L) was mixed and distributed equally to fill 12 black plastic tanks (50 L, 50 × 35 × 23 cm). The experimental units were constantly aerated by three airstones per tank. No water exchange was carried out during the experimental period, except for the addition of dechlorinated freshwater to compensate for evaporation losses. Light intensity was kept at 27 µmol m^{-2} s^{-1} using a fluorescent lamp with a natural photoperiod.

Molasses (30% organic carbon) was added once a day as a carbohydrates source to maintain the CHO:N ratio at 12:1, and was calculated based on Ebeling, Timmons, and Bisogni (2006). Calcium hydroxide ($Ca(OH)_2$) with high neutralizing power (132%), reactivity of 62% and relative total neutralization power of 81% was added at 10% (by weight) of the daily feed lot throughout the study.

2.2 Shrimp stocking, feeding and monitoring

The postlarvae (1 ± 0.01 mg) of *L. vannamei* were obtained from a commercial laboratory (Aquasul, RN, Brazil) and stocked at a density of 3,000 postlarvae/m^3 (150 shrimp by experimental units) until 42 days. The postlarvae were fed four times a day (at 08:00 a.m., 11:00 a.m., 02:00 p.m. and 04:00 p.m.), with a commercial shrimp feed (0.4–1 mm in diameter) with 40% crude protein and 8% lipids (In vivo Animal Nutrition and Health). The daily feeding rate of 35% of body weight used at the start of the culture was gradually reduced to 10% of body weight after 42 days based on the Van Wyk (1999) table and adjusted daily according to estimated shrimp consumption and mortality rate.

Shrimp weight was monitored weekly after 15 days of culture to determine shrimp growth and adjust the amount of feed offered. At the end of the experiment, biomass gain, mean final weight, specific growth rate (SGR), FCR, survival and productivity were determined based on the following equations:

- ✓ Biomass gain (g) = final biomass (g) − initial biomass (g);
- ✓ Final weight (g) = final biomass (g)/number of individuals at the end of evaluation period; SGR (%/day) = 100 × [ln final weight (g) − ln initial weight (g)]/time (days);
- ✓ FCR = feed supplied/biomass gain; Survival (%) = (number of individuals at the end of evaluation period/ initial number of individuals) × 100;
- ✓ Productivity (kg/m3) = final biomass (kg)/volume of experimental unit (m^3).

2.3 *Navicula* addition

The benthic diatom *Navicula* sp. was obtained from the Live Food Production Laboratory (LAPAVI) – DEPAq – UFRPE, and cultured in a Conway medium (Walne, 1966) with $FeCl_3 \cdot 6H_2O$ 1.30; $MnCl_2 \cdot 4H_2O$ 0.36; H_3BO_3 33.6; EDTA 45.0; $NaH_2PO_4 \cdot 2H_2O$ 20.0; $NaNO_3$ 100.0; $ZnCl_2$ 1.1; $CoCl_2 \cdot 6H2O$ 1.0; $(NH_4)6MO_7O_{24} \cdot 4H_2O$ 0.45; $CuSO_4 \cdot 5H_2O$ 1.0;

$Na_2SiO_3 \cdot 5H_2O$ 2.0; B_{12} 0.1; B_1 1.0 which was used in a 1.0 ml/L solution, maintained in water with 30 g/L salinity, pH 8.0, temperature 25 ± 1°C, and light intensity kept at 37 µmol m^{-2} s^{-1} using a fluorescent lamp with a 12-hr light/dark photoperiod. Diatoms were added on the 1st, 10th, 20th and 30th days of experimental time in each experimental tank, regardless of the wastes of unconsumed diatoms, based on Marinho et al. (2014), Marinho et al. (2016) and Brito et al. (2016).

2.4 Water quality

Dissolved oxygen, temperature, salinity and pH (YSI model 100, Yellow Springs, Ohio, USA) were monitored twice a day (at 08:00 a.m. and 04:00 p.m.). Settleable solids (SS) (Imhoff cone) (Avnimelech, 2009) were monitored three times a week. Total ammonia nitrogen (TAN), nitrogen-nitrite ($N\text{-}NO_2$), nitrogen-nitrate ($N\text{-}NO_3$), total suspended solids (TSS), phosphorus - orthophosphate ($P\text{-}PO_{43}$) and alkalinity (mg/L $CaCO_3$) were monitored once a week, following the methods described by Koroleff (1976), Golterman, Clymo, and Ohnstad (1978), Mackereth, Heron, and Talling (1978), APHA (2005) and Felföldy, Szabo, and Tothl (1987) respectively. Alkalinity and dissolved nutrients were analysed from effluent samples filtered with 0.45 µm pore size HAWP Millipore membranes, while samples for analysing suspended solids were filtered through prehydrated 0.7 µm pore size AP40 Millipore glass fibre filters. For TSS determination, membranes were weighed after being dried at 105°C.

2.5 Proximate composition and fatty acids profile

Biofloc samples were collected for proximal composition at the end of the experiment from each tank using a 100-µm mesh, and oven-dried at 60°C. Shrimp samples were also collected from each tank at the end of the experiment, washed with distilled water to remove epiphytes and encrusting material, and oven-dried at 60°C. The dried samples, in triplicate, were ground and processed for biochemical analysis following AOAC (2000) at the Physical

Analysis and Food Chemistry Laboratory, UFRPE, Recife, Brazil. For moisture content, the quantities of samples were oven-dried at 105°C until constant weight (315 SE model, Fanem). The difference in weight before and after sample drying was taken and expressed in percentage. Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method (TE 0363 model, Tecnal), and ash by oven incineration at 550°C (Q318 D24 model, Quimis).

The crude lipids were extracted for analyses of their fatty acid profile by a transesterification procedure of extracted fatty acids or quantification of the lipid content as methyl ester derivatives (FAME) (Comeau, Hall, & Oldham, 1988; Wychen & Laurens, 2013).

2.6 Salinity stress test

At the end of the experiment, a salinity stress test was carried out. This test consisted of transferring the juvenile shrimps from each treatment to three replicated units (10 shrimp per unit) containing freshwater, gently aerated by one air stone per recipient for 30 min. The shrimp were then transferred to a salinity of 35 g/L. After an additional exposure of 30 min, all shrimp not responding to mechanical stimulus were considered dead. Experimental conditions were 29.0 ± 0.5°C and pH 7.8 ± 0.1, using twelve 2-L plastic bottles as experimental units (Burbano-Gallardo et al., 2015).

2.7 Presumptive diagnosis and total haemocyte count

The measurement of presumptive analysis (Morales-Covarrubias, 2010) and total haemocyte count (THC) (Guertler et al., 2013) were performed for 40 shrimp at the end of the rearing period (day 42). To measure presumptive analysis, the following scores were considered: degree of intestinal repletion—(0) empty; (1) slightly full; (2) moderately full; and (3) full; hepatopancreas–Tubules: (0) shows no signs of infection or deformation; (1) low

presence of tubular deformation (1–5/field/organism); (2) moderate presence of tubular deformation (6–10/field/organism), atrophy, melanization and tubular necrosis; and (3) high presence of tubular deformation (11–16/field/ organism), with moderate to severe lesions, with melanization, necrosis and tubular atrophy; epicomensals in the gills lamellae—(0) does not present lesions caused by epicomensals; (1) low presence of protozoa (1–5/lamella/organism) and lesions caused by epicomensals; (2) moderate presence of protozoa (6–10/lamella/organism) and increased epicomensal lesions (melanization and formation of haemocytic nodules); and (3) high presence of protozoa (10–15/lamella/organism), moderate to severe lesions caused by epicomensals (multifocal melanized areas and formation of haemocytic nodules).

The haemolymph sample was taken with a ^{1}-ml syringe containing 200 µl of precooled anticoagulant solution (modified Alsever solution (MAS) (336 mmol/L NaCl, 115 mmol/L glucose, 27 mmol/L sodium citrate, 9 mmol/L EDTA, pH 7.2) in the proportion of 1:2 (v:v). For total haemocyte counting, duplicates of 0.8 ml of diluted haemolymph were counted for the number of haemocytes using a haemocytometer under a light microscope.

2.8 Statistical analysis

A parametric one-way ANOVA was used to analyse production parameters and stress salinity analysis, after confirming homocedasticity (Cochran $p < 0.05$) and normality (Shapiro–Wilk $p < 0.05$). Tukey's test ($p < 0.05$) was performed to compare and rank means from the three treatments and the control. Water quality parameters were analysed by performing repeated ANOVA measures. For nonparametric statistics data (temperature, pH, TAN, N-nitrite, N-nitrate and P-orthophosphate), the Kruskal–Wallis ($\alpha < 0.05$) and Dunn tests ($\alpha < 0.05$) were used to compare and rank medians from the three treatments and the control. For the THC (date log transformation), the t test was used. Data analyses were performed using ASSISTAT Version 7.7 (Assistat Analytical Software).

2.9 Results

The water quality parameters of the culture treatments are presented in Table 1. Water temperature was maintained at 28°C, dissolved oxygen above 5 mg/L, pH at 8.3, alkalinity between 140.5 and 158.9 mg $CaCO_3$/L, P-orthophosphate around 1 mg/L, and TSS between 147 and 182 mg/L, with no significant difference ($\alpha \geq 0.05$) between treatments. The results for dissolved inorganic nitrogen compounds were TAN < 0.2 mg/L, N-NO2 < 0.4 mg/L and N-NO3 < 1 mg/L with no significant difference ($\alpha \geq 0.05$) between treatments. There was no difference in salinity among the treatments with *Navicul*a (31.7–32.0 g/L), but all were slightly higher than the control (31.1 g/L).

The proximal compositions of the bioflocs are presented in Table 2. Moisture results varied from 87.6 to 88.9 g/kg, crude protein from 172.0 to 225.5 g/kg, crude lipids from 63 to 150 g/kg and ash from 553.9 to 587.2 g/kg, with a significant difference ($\alpha \geq 0.05$) between treatments for crude protein, lipids and ash.

The fatty acids profile in the biofloc is presented in Table 2. In general, an increase in microalgae led to an increase in linoleic acid, EPA and DHA. The BFT 10N treatment presented high amounts of EPA and DHA of 3.140 and 5.163 g/kg respectively. It is also noteworthy that the BFT 5N and BFT 2.5N treatments also presented high values of EPA (1.281 and 2.529 g/kg) and DHA (1.088 and 3.690 g/kg). The highest values for linolenic acid and arachidonic acid were found in treatment BFT 2.5N at 1.156 and 1.035 g/kg respectively. In addition, the ratio (n-3)/(n-6) was higher in BFT 5N and BFT 10N. (Table 2).

Table 3 summarizes the shrimp performance during the 42-day experimental period. Shrimp survival rates were all above 93% and FCR above 0.8 with no significant difference ($\alpha \geq 0.05$) between treatments. In terms of final weight, the different treatments with *Navicula* sp. had similar results, but the BFT 2.5N and BFT 5N treatments were also similar to the control BFT and only the BFT 10N treatment (0.86 ± 0.03 g) was higher to the control BFT (0.69 ± 0.03 g). However, for biomass gain, productivity and SGR, the treatments with

Navicula sp. had similar results, and while the BFT 2.5N did not differ from the BFT control, the results of BFT 5N and BFT 10N treatments were higher than the control BFT ($\alpha \leq 0.05$).Shrimp survival after the salinity stress test was above 90% without a significant difference ($\alpha \geq 0.05$) between the treatments.

The results of the presumptive diagnosis determined that 80% of all the animals in the treatments with the addition of *Navicula* sp. (BFT 2.5N, BFT 5N and BFT 10N) had a high degree of gut tissue repletion (grade 3), as opposed to 57% of the animals in the control group. In relation to the hepatopancreas, 95% of the animals showed no or very-low tubular deformity (degrees of severity between 0 and 1) in all of the treatments, and 71–91% of the animals submitted to the treatments with an addition of diatoms had high rates of lipid droplets (grade 3). In contrast, 28% of the control group (BFT) had a low to moderate presence of protozoa and melanization in the gill lamellae, with a maximum of 15% in the tested groups (BFT 2.5N, BFT 5N and BFT 10N) (Table 4). The THC was significantly higher ($\alpha < 0.05$) in the animals submitted to treatments with the addition of diatoms (41.25×10^6 cells/ml) than in the control group (22.84×10^6 cells/ml) (Figure 1).

2.10 Discussion

The temperature, dissolved oxygen concentration and pH of the water remained within the ideal range for the cultivation of the species during the whole experimental period (Ponce-Palafox,

Martinez-Palacios, & Ross, 1997; Van Wyk & Scarpa, 1999). In biofloc systems, the increase in the microbial biomass and respective consumption of alkalinity causes a drop in pH levels (Silva, Wasielesky, & Abreu, 2013). To avoid pH fluctuations, alkalinity shouldbe kept above 100 mg $CaCO_3$/L, since it acts on the system as a buffer solution (Van Wyk & Scarpa, 1999). In this study, due to daily alkalinity correction, no decrease in pH levels was observed during the experimental period. The low TAN and N-NO2 values observed during the experiment indicate the transformation of the nitrogen compounds (Ebeling et al., 2006),

which were within the ideal range for cultivation of the species (Van Wyk & Scarpa, 1999). In relation to P-orthophosphate, the addition of the diatoms did not reduce this nutrient, similar to what was observed with *Navicula* sp., *Amphora coffeaeformis*, *Cylindrotheca closterium* and *Conticribra weissflogii* addition in BFT systems (Brito et al., 2016; Martins, Odebrecht, Jensen, D'Oca, & Wasielesky, 2016).

In biofloc systems, the reduction in water exchange, high organic matter loading and the rapid growth of heterotrophic bacteria provide an increase in the amount of TSS in the system (Van Wyk, 2006). During cultivation, TSS remained close to the values recommended for the development of the species. Recent studies report that the concentration of TSS should be maintained between 250 and 350 mg/L (Samocha et al., 2017).

Regarding the proximal composition of bioflocs, it was observed that the addition of diatoms did not contribute to an increase in protein. The lowest protein level was observed in the treatment with the highest diatom concentration, and conversely the highest protein level was observed in the treatment without an addition of diatoms. Therefore, it is necessary to evaluate the amino acids profile of the bioflocs, mainly due to the better shrimp performance obtained in treatments with the addition of diatoms. According to Ekasari et al. (2014), the biofloc size class >100 μm contains the highest protein (27.8%) and lipid (7.5%) levels but the lowest essential amino acids content.

The highest ash content percentage in the biofloc was found in the treatments with a higher addition of diatoms than in the BFT. Ash content is a measure of the total amount of minerals present in a food. Seven minerals (calcium, copper, magnesium, phosphorus, potassium, selenium and zinc) have been recommended for inclusion in penaeid shrimp's diets (Shiau, 1998), since dietary sources of these minerals is necessary for growth because of the repeated losses of certain minerals during moulting. Despite the benefit of using diatoms to increase ash, it is necessary to determine the concentrations of the minerals present in the biofloc and their bioavailability for the cultivated animals.

The lipid content was higher in the treatment without an addition of diatoms. However, the EPA and DHA levels in the BFT 5N and BFT 10N treatments were at ideal levels for the

shrimp's diet. Four fatty acids are particularly important dietary ingredients for crustaceans: linoleic acid (18:2n-6), linolenic acid (18:3n-3), EPA (20:5n-3) and DHA (22:6n-3) (Wickins & Lee, 2002). The latter two n-3 HUFAs are the most indispensable, and it is generally safe to assume that they are better utilized by crustaceans and have better nutritional value than other lipids (Lim, Ako, Brown, & Hahn, 1997). They also increase resistance to environmental stress and disease (Wickins & Lee, 2002), and promote higher growth, better survival and higher feed efficiency of L. vannamei (Lim et al., 1997). Diatoms are rich in fatty acids, especially EPA (20:5n-3) and DHA (22:6 n-3), with a variation of 5%-35% of total polyunsaturated fatty acids (Hemaiswarya et al., 2011; Patil et al., 2007), and promote increased concentrations of fatty acids in the bioflocs and probably improve shrimp performance in the nursery phase.

It was observed that the BFT 10N treatment presented a higher shrimp performance than the control. Some studies have shown that the inoculation of *Navicula* in *L. vannamei* postlarvae culture improves growth performance (Brito et al., 2016; Marinho et al., 2014, 2016), corroborating the observations of this study. The results for biomass gain (F = 7.4926), productivity (F = 7.6790) and SGR (F = 7.5064) were also significantly higher in the BFT 5N and BFT 10N treatments, highlighting the higher productivity values (2.30 and 2.42 kg/m3) than the other treatments. Brito et al. (2016) reported productivity of 1.76 ± 0.51 kg/m3, using a *Navicula* sp. concentration of 5 × 104 cells/ml in an *L. vannamei* culture in a biofloc system for 35 days. Such result is lower than that observed in this study, indicating that higher density inoculation of diatoms increases productivity. Regarding the SGR, the BFT 5N and BFT 10N treatments differed significantly from the other treatments. The results were similar to those observed with the addition of microalage in bioflocs by Marinho et al. (2014), who found a rate of 14.87 ± 0.61%/ day, and higher than that attained by Brito et al. (2016), who found a rate of 9.41 ± 0.41%/day. For FCR and survival, no significant differences were observed between treatments, indicating that the density of *Navicula* sp. did not influence these parameters. The better shrimp performance in the BFT 10N treatment may be associated with the fatty acids in the bioflocs. Courtois et al. (2012) evaluated the nutritional value of four

diatom species for shrimp, one of them being *Navicula incerta*, and observed 4.35 ± 0.17% of lipids in dry weight, of which 21.8% was EPA and 0.07% DHA, indicating that diatoms can contribute nutritionally to the performance of *L. vannamei*.

The hepatopancreas is an important indicator of shrimp health because it is extremely sensitive to different diets and water pollutants, and can be observed directly under a microscope (Manan, Zhong, Othman, & Ikhwanuddin, 2015). In this study, by means of fresh examination, it was possible to determine that the animals in the treatments with the addition of microalgae (BFT 2.5N, BFT 5N and BFT 10N) had hepatopancreas with tubules full of lipid droplets and without significant lesions, as well as a filled intestine, revealing the positive effect of the inclusion of *Navicula* sp. on the health of the shrimp.

As for the presence of epicomensals on the gill lamellae, 28% of the animals in the control group had 1–10 protozoa per lamella, while the animals in the treatments BFT 2.5N, BFT 5N and BFT 10N an occurrence of at most 14%–18% was observed at the same counting interval. In general, high concentrations of organic material (low water quality) increases the epicomensal counts in the gill lamellae (Cuéllar-Anjel, 2008), and the number of unhealthy shrimp (Guzmán & Valle, 2000). In this study, no significant differences were found in the water quality parameters evaluated, suggesting that the differences in the epicomensal counts could be linked to the better health conditions recorded in the treatments inoculated with diatoms.

Regarding the THC, higher mean values were found in the animals of the treatments with the addition of Navicula sp. in relation to BFT. According to Krupesha, Seema, Philipose, and Radhkrishnan (2009), higher THC provides higher immunological status, since its increase may provide the crustaceans greater protection against infections since haemocytes are the main immunocompetent cell in crustaceans (Jiravanichpaisal, Lee, & Soderhall, 2006).

2.11 Conclusion

It is concluded that the diatom *Navicula* sp. inoculated at a density of 5×10^4 cells/ml

(BFT 5N) and 10 × 10^4 cells/ml (BFT 10N) provides benefits for the development of *L. vannamei* postlarvae, since it presented higher values in the performance variables for final mean weight, productivity, biomass gain, SGR and shrimp health status. In addition, the inoculation with diatoms improved the nutritional quality of bioflocs by increasing the amount of fatty acids.

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2.14 References

AOAC (2000). Official methods of analysis of AOAC. International (17th ed.). Gaithersburg, MD: USA Association of Analytical Communities. APHA (2005). Standard methods for the examination of water and wastewater. Washington, DC: American Public Health Association.

Avnimelech, Y. (2009). Biofloc technology – A practical guide book. Baton Rouge, LA: The World Aquaculture Society.

Brito, L. O., Santos, I. G. S., Abreu, J. L., Araújo, M. T., Severi, W., & Gálvez, A. O. (2016). Effect of addition of diatoms (*Navicula* spp.) and rotifers (*Brachionus plicatilis*) on growth and water quality of the *Litopenaeus vannamei* postlarvae reared in biofloc system. Aquaculture Research, 47, 3990–3997. https ://doi.org/10.1111/ are.12849

Burbano-Gallardo, E., Imués-Figueroa, M. A., Gonzalez-Legarda, E. A., Brito, L. O., Galvez, A. O., & Arana, L. A. V. (2015). Supervivencia de poslarvas de *Litopenaeus vannamei* sometidas a la prueba de estrés osmótico y su relación con el estado de muda. Revista de Biología Marina y Oceanografía, 50, 323–329. https ://doi.org/10.4067/S071819572 01500 0300010

Comeau, Y., Hall, K. J., & Oldham, W. K. (1988). Determination of poly-βhydroxybutyrate and poly-β-hydroxyvalerate in activated sludge by gas liquid chromatography. Applied and Environmental Microbiology, 54, 2325–2327. 0099-2240/88/092325-03$02.00/0

Courtois, V. G., Viera, M. P., Huchetteb, S., & Izquierdo, M. S. (2012). Improving nursery performances of Haliotis tuberculata coccinea: Nutritional value of four species of benthic diatoms and green macroalgae germlings. Aquaculture, 334–337, 124–131. https ://doi.

org/10.1016/j.aquac ulture.2011.12.040

Crab, R., Defoirdt, T., Bossier, P., & Verstraete, W. (2012). Biofloc technology in aquaculture: Beneficial effects and future challenges. Aquaculture, 356–357, 351–356. https ://doi.org/10.1016/j.aquaculture.2012.04.046

Cuéllar-Anjel, J. (2008). Enfermedade por parasitos. In: V. Morales, & Y. J. Cuéllar (Eds.), Guía Técnica – Patología e Inmunología de Camarones Penaeidos. Programa CYTED-Red II-D: *Vannamei* (pp. 137–157). Panamá, Rep Panamá.

De Schryver, P., Crab, R., Defoirdt, T., Boon, N., & Verstraete, W. (2008). The basics of bioflocs technology: The added value for aquaculture. Aquaculture, 277, 125–137. https ://doi.org/10.1016/j.aquaculture.2008.02.019

Ebeling, J. M., Timmons, M. B., & Bisogni, J. J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture, 257, 346–358. https ://doi.org/10.1016/j.aquac ulture.2006.03.019

Ekasari, J., Angela, D., Waluyo, S. H., Bachtiar, T., Surawidjaja, E. H., Bossier, P., & De Schryver, P. (2014). The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. Aquaculture, 426–427, 105–111. https ://doi.org/10.1016/j. aquac ulture.2014.01.023

Ekasari, J., Crab, R., & Verstraete, W. (2010). Primary nutritional content of bio-flocs cultured with different organic carbon sources and salinity. HAYATI Journal of Biosciences, 17, 125–130. https ://doi. org/10.4308/hjb.17.3.125

Espeland, E. M., Francoeur, S. N., & Wetzel, R. G. (2001). Influence of algal photosynthesis on biofilm bacterial production and associated glucosidase and xylosidase activities. Microbial Ecology, 42, 524–530. https ://doi.org/10.1007/s00248-001-1022-8

Felföldy, L., Szabo, E., & Tothl, L. (1987). A biológiai vizminösités. Budapest, Hungary: Vizügyi Hodrobiológia Vizdok.

Gamboa-Delgado, J., Oca, G. A. R. M., Reyes, J. C. R., Villarreal-Cavazos, D. A., Nieto-Lopez, M., & Cruz-Suarez, L. E. (2017). Assessment of the relative contribution of dietary nitrogen from fish meal and biofloc meal to the growth of Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture Research, 48, 2963–2972. https ://doi.org/10.1111/ are.13129

Golterman, H. J., Clymo, R. S., & Ohnstad, M. A. (1978). Methods for physical and chemical analysis of freshwaters. Oxford/London, UK: Blackwell Scientific Publications.

Guertler, C., Rieg, T., Mejía-Ruíz, C. H., Lehmann, M., Barracco, M. A., & Perazzolo, L. M. (2013). Hemograma e sobrevivência de camarões marinhos após silenciamento do WSSV por RNA de interferência. Pesquisa Agropecuária Brasileira, 48, 983–990. https ://doi. org/10.1590/S0100-204X2 01300 0800025

Guzmán, G. A., & Valle, F. A. (2000). Infectious disease in shrimp species with aquaculture potential. Recent Research Developments in Microbiology, 4, 333–348.

Hemaiswarya, S., Raja, R., Ravikumar, R., Ganesa, N. V., & Anbazhagan, C. (2011). Microalgae: A sustainable feed source for aquaculture. World Journal Microbiology and Biotechnology, 27, 1737–1746. https ://doi. org/10.1007/s11274-010-0632-z

IBGE (Instituto Brasileiro de Geografia e Estatística). (2018). Produção da Pecuária

Municipal. 45, 1–8, Rio de Janeiro, Brazil.

Jiravanichpaisal, P., Lee, L. B., & Soderhall, K. (2006). Cell- mediated immunity in arthropods: Hematopoiesis, coagulation, melanization and opsonization. Immunobiolog, 211, 213–236. https ://doi. org/10.1016/j.imbio.2005.10.015

Ju, Z. Y., Forster, I., Conquest, L., Dominy, W., Kuo, W. C., & Horgen, F. D. (2008). Determination of microbial community structures of shrimp floc culture by biomarkers and analysis of floc amino acid profiles. Aquaculture Research, 39, 118–133. https ://doi. org/10.1111/j.1365-2109.2007.01856.x

Ju, Z. Y., Forster, I. P., & Dominy, W. G. (2009). Effects of supplementing two species of marine algae or their fractions to a formulated diet on growth, survival and composition of shrimp *(Litopenaeus vannamei*). Aquaculture, 292, 237–243. https ://doi.org/10.1016/j. aquac ulture.2009.04.040

Khatoon, H., Banerjee, S., Yusoff, F. M., & Shariff, M. (2009). Evaluation of indigenous marine periphytic *Amphora*, *Navicula* and *Cymbella* grown on substrate as feed supplement in *Penaeus monodon* postlarvae hatchery systems. Aquaculture Nutrition, 15, 186–193. https :// doi.org/10.1111/j.1365-2095.2008.00582.x

Koroleff. (1976). Determination of nutrients. In K. Grasshoff (Ed.), Methods of seawater analysis (pp. 97–115). New York, NY: Verlag Chemie Weinhein.

Krummenauer, D., Seifert, C. A. Jr, Poersch, L. H., Foes, G. K., Lara, G. R., & Wasielesky, W. Jr (2012). Cultivo de camarões marinhos em sistema de bioflocos: análise de reutilização da água. Atlântica, Rio Grande do Norte, 34, 103–111. https ://doi.org/10.5088/atlântica. v34i2.3118

Krupesha, S. R., Seema, J., Philipose, K. K., & Radhkrishnan, E. V. (2009). Effect of salinity an on pH selected immune functions of the Indian white shrimp, *Fenneropenaeus indicus* (H. Milne Edwards, 1937). Indian Journal of Fisheries, 56, 183–187.

Lim, C., Ako, H., Brown, C. L., & Hahn, K. (1997). Growth response and fatty acid composition of juvenile *Penaeus vannamei* fed different sources of dietary lipid. Aquaculture, 151, 143–153. https ://doi. org/10.1016/S0044-8486(96)01500-1

Mackereth, F. J. H., Heron, J., & Talling, J. F. (1978). Water analysis: Some revised methods for limnologists. Oxford/London, UK: Blackwell Scientific Publications.

Manan, H., Zhong, J. M. H., Othman, F., & Ikhwanuddin, M. H. D. (2015). Histopathology of the hepatopancreas of pacific white shrimp, *Penaeus vannamei* from none early mortality syndrome (EMS) Shrimp Ponds. Journal of Fisheries and Aquatic Science, 10, 562–568. https :// doi.org/10.3923/jfas.2015.562.568

Marinho, Y. F., Brito, L. O., Campos, C. V. F. S., Severi, W., Andrade, H. A., & Galvez, A. O. (2016). Effect of the addition of *Chaetoceros calcitrans*, *Navicula* sp. and *Phaeodactylum tricornutum* (diatoms) on phytoplankton composition and growth of *Litopenaeus vannamei* (Boone) postlarvae reared in a biofloc system. Aquaculture Research, 48, 4155–4164. https ://doi.org/10.1111/are.13235

Marinho, Y. F., Brito, L. O., Silva, C. V. F., … Gàlvez, A. O. (2014). Effect of addition of *Navicula* sp. on plankton composition and postlarvae growth of *Litopenaeus vannamei* reared in culture tanks with zero water exchange. Latin American Journal of Aquatic Research, 42, 427– 437. 10385 6/vol42-issue3-fullt ext-4

Martins, T. G., Odebrecht, C., Jensen, L. V., D'Oca, M. G. M., & Wasielesky, W. Jr (2016). The contribution of diatoms to bioflocs lipid content and the performance of juvenile *Litopenaeus vannamei* (Boone, 1931) in a BFT culture system. Aquaculture Research, 47, 1315–1326. https ://doi.org/10.1111/are.12592

Morales-Covarrubias, M. S. (2010). Enfermidades del camarón: detectión mediante análisis en fresco e histopatología (Diseases shrimp detection by means of gross analysis and histopathology) (2ndª ed.). Mexico City: Trillas.

Muller-Feuga, A., Robert, R., Cahu, C., Robin, J., & Divanach, P. (2003). Uses of microalgae in aquaculture In J. G. Støttrup, & L. A. McEvoy (Eds.), Live feeds in marine aquaculture (pp. 253–299). Berlin, Germany: Blackwell Publishing Company.

Murray, R. E., Cooksey, K. E., & Priscu, J. C. (1986). Simulation of bacterial DNA synthesis by algal exudates in attached algal-bacterial consortia. Applied and Environmental Microbiology, 52, 1177–1182. 0099-2240/86/111177-06$02.00/0

Natrah, F. M. I., Bossier, P., Sorgeloos, P., Yusoff, F. M., & Defoirdt, T. (2014). Significance of microalgal–bacterial interactions for Aquaculture. Reviews in Aquaculture, 6, 48–61. https ://doi.org/10.1111/raq.12024

Pacheco-Vega, J. M., Cadena-Roa, M. A., Ascencio, F., Rangel-Dávalos, C., & Rojas-contreras, M. (2015). Assessment of endemic microalgae as potential food for *Artemia franciscana* culture. Latin American Journal of Aquatic Research., 43, 23–32. https ://doi.org/10.3856/ vol43-issue1-fullt ext-3

Patil, V., Kallqvist, T., Olsen, E., Vogt, G., & Gislerod, H. R. (2007). Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquaculture International, 15, 1–9. https

://doi.org/10.1007/ s10499-006-9060-3

Pérez-Fuentes, J. A., Pérez-Rostro, C. I., & Hernández-Vergara, M. P. (2013). Pond-reared Malaysian prawn *Macrobrachium rosenbergii* with biofloc system. Aquaculture, 400–401, 105–110. https ://doi. org/10.1016/j.aquaculture.2013.02.028

Ponce-Palafox, J., Martinez-Palacios, C. A., & Ross, L. G. (1997). The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone, 1931. Aquaculture, 157, 107–115. https ://doi.org/10.1016/S0044-8486(97)00148-8

Ray, A. J., Shuler, A. J., Leffler, J. W., & Browdy, C. L. (2009). Microbial ecology and management of biofloc systems. In B. L. Craig, & D. Jory (Eds.), The rising tide, proceedings of the special session on sustainable shrimp farming, world aquaculture 2009 (pp. 255–266). Baton Rouge, LA: The World Aquaculture Society.

Richmond, A. (2004). Handbook of microalgal culture: Biotechnology and applied phycology. Ames, IA: Blackwell Science Ltd a Blackwell Publishing Company.

Samocha, T. M., Prangnell, D. I., Hanson, T. R., Treece, G. D., Morris, T. C., Castro, L. F., & Staresinic, N. (2017). Design and operation of super intensive, biofloc-dominated systems for indoor production of the Pacific White Shrimp, *Litopenaeus vannamei* – The Texas A&M AgriLife Research Experience. Baton Rouge, LA: The World Aquaculture Society.

Shiau, S. Y. (1998). Nutrient requirements of penaeid shrimps. Aquaculture, 164, 77–93. https ://doi.org/10.1016/S0044-8486(98)00178-1.

Silva, K. R., Wasielesky, J. R., & Abreu, P. C. (2013). Nitrogen and phosphorus dynamics in the biofloc production of the Pacific White Shrimp, *Litopenaeus vannamei*. Journal of the

World Aquaculture Society, 44, 30–41. https ://doi.org/10.1111/jwas.12009

Underwood, G. J. C., Boulcott, M., Raines, C. A., & Waldron, K. (2004). Environmental effects on exopolymer production by marine benthic diatoms: Dynamics, changes in composition and pathways of production. Journal of Phycology, 40, 293–304.

Valle, B. C. S., Dantas, E. M. Jr, Silva, J. F. X., Bezerra, R. S., Correia, E. S., Peixoto, S. R. M., & Soares, R. B. (2015). Replacement of fishmeal by fish protein hydrolysate and biofloc in the diets of *Litopenaeus vannamei* postlarvae. Aquaculture Nutrition, 21, 105–112. https ://doi. org/10.1111/anu.12149

Van Wyk, P. (2006). Production of *L. vannamei* in recirculating aquaculture systems: Management and design considerations. In: T. T. Rakestraw, L. S. Douglas, L. Marsh, L. Granata, A. Correa, & G. J. Flick (Eds.), Proceedings of the 6th international conference on recirculation aquaculture (pp. 38–47). Roanoke, VA. Van Wyk, P., Scarpa, J., et al. (1999). Water quality and management. In P. Van Wyk (Ed.), Farming marine shrimp in recirculating freshwater systems (pp. 128–138). Tallahassee, FL: Department of Agriculture and Consumer Services.

Walne, P. R. (1966). Experiments in the large scale culture of the larvae of Ostrea edulis. Fish Investigation, 25, 1–53.

Watanabe, K., Takihana, N., Aoyagi, H., Hanada, S., Watanabe, Y., Ohmura, N., et al. (2005). Symbiotic association in *Chlorella* culture. FEMS Microbiology Ecology, 51, 187–196.

Wickins, J. F., & Lee, D. O'C. (2002). Crustacean farming – Ranching and culture (2nd ed.) Oxford, UK: Blackwell Science. Wychen, V. S., & Laurens, L. M. L. (2013). Determination of total lipids as fatty acid methyl esters (FAME) by in situ transesterification. NREL.

Van Wyk. (1999). Nutrition and feeding of *Litopenaeus vannamei* in intensive culture systems. In: P. Van Wyk, M. Davis-Hodgkins, R. Laramore, K. L. Main, J. Mountain, & J. Scarpa (Eds.), Farming marine shrimp in recirculating freshwater systems (pp. 125–140). Fort Pierce, FL: Florida Department of Agriculture and Consumer Services - Harbor Branch Oceanic Institute.

Zhao, P., Huang, J., Wang, X. H., Song, X. Z., Yang, C. H., Zhang, X. G., & Wang, G. C. (2012). The application of bioflocs technology in highintensive, zero exchange farming systems of *Marsupenaeus japonicus*. Aquaculture, 354–355, 97–106. https ://doi.org/10.1016/j.aquac ulture.2012.03.034

Table 1. Water quality parameters in the culture of *Litopenaeus vannamei* under nursery biofloc system with and without the addition of diatoms

Parameters	Treatments				
	BFT	**BFT 2.5N**	**BFT 5N**	**BFT10N**	
Salinity (g L^{-1})	31.07 ± 0.57^{b}	31.72 ± 0.39^{ab}	$31.94 \pm 0,39^{ab}$	32.05 ± 0.32^{a}	p = 0.016
Temperature (ºC)	28.23 ± 0.12^{a}	28.42 ± 0.10^{a}	$28.39 \pm 0,37^{a}$	28.38 ± 0.03^{a}	h = 1.3204
Dissolved oxygen (mg L^{-1})	5.12 ± 0.02^{a}	5.09 ± 0.04^{a}	$5.08 \pm 0,03^{a}$	5.01 ± 0.03^{a}	p = 0.196
pH	8.30 ± 0.03^{a}	8.31 ± 0.05^{a}	8.31 ± 0.02^{a}	8.30 ± 0.02^{a}	h = 0.2156
TAN (mg L)	0.19 ± 0.03^{a}	0.20 ± 0.06^{a}	$0.08 \pm 0,06^{a}$	0.08 ± 0.02^{a}	h = 6.3385
N- Nitrite (mg L)	0.40 ± 0.01^{a}	0.40 ± 0.01^{a}	$0.38 \pm 0,01^{a}$	$0.38 \pm 0,01^{a}$	h = 1.9183
N-Nitrate (mg L)	0.97 ± 0.02^{a}	0.95 ± 0.01^{a}	0.97 ± 0.01^{a}	0.98 ± 0.01^{a}	h = 0.5906
Alkalinity (mg $CaCO_3$ L)	158.93 ± 11.48^{a}	140.53 ± 11.60^{a}	$156,53 \pm 19,61^{a}$	$157,60 \pm 9,47^{a}$	p = 0.406
Orthophosphate (mg L)	1.18 ± 0.14^{a}	1.11 ± 0.12^{a}	$1,19 \pm 0,06^{a}$	$1,17 \pm 0,09^{a}$	h = 0.7978
TSS (mg L)	170 ± 44^{a}	147 ± 65^{a}	159 ± 65^{a}	182 ± 76^{a}	p = 0.449

Results from ANOVA, Tukey's test (*P*) and the Kruskall-Wallis, Dunn (*H*). Values in the same column with different superscripts differ significantly (α <0.05); BFT (biofloc system without addition of *Navicula* sp.); BFT 2.5N (addition of 2.5 x 10^4 cells mL^{-1} of *Navicula* sp.); BFT 5.0N (addition of 5 x 10^4 cells mL^{-1} of *Navicula* sp.) and 10.0N BFT (addition of 10 x 10^4 cells mL^{-1} of *Navicula* sp.) TAN- total ammonia nitrogen; TSS - total suspended solids.

Table 2. Proximate composition of microbial floc samples and fatty acids profiles at the end of the experimental period of *Litopenaeus vannamei* postlarvae reared in biofloc system without addition of diatoms and with different densities of diatom addition.

	Treatments				Recommended levels
	BFT	BFT 2.5N	BFT5N	BFT10N	
Proximal composition					
Moisture	89.57 ± 0.66[a]	88.87 ± 0.35[a]	88.68 ± 2.83[a]	87.98 ± 0.84[a]	
Crude protein	225.5 ± 1.66[a]	206.7 ± 0.81[a]	220.7 ± 0.09[a]	172.0 ± 1.28[b]	45%-50%[2]
Crude lipids	150 ± 0.03[a]	98 ± 0.03[b]	97 ± 0.01[b]	63 ± 0.00[c]	9%-15%[2]
Ash	553.9 ± 4.2[c]	567.0 ± 3.4[bc]	579.9 ± 8.9[ab]	587.2 ± 1.8[a]	
Fatty acids					
C18:2n-6 (LA)	4.301	6.390	8.484	12.466	0.4%[2]
C18:3n-3 (ALA)	0.767	1.156	0.613	0.699	0.3%[2]
C20:4n-6 (ARA)	0.775	1.035	0.538	0.884	
C20:5n-3 (EPA)	0.835	1.281	2.529	3.140	0,4%[2]
C22:6n-3 (DHA)	0.709	1.088	3.690	5.163	0,4%[2]
Σ Saturated	15.268	20.911	23.717	30.034	
Σ Monounsaturated	7.328	9.346	6.066	7.177	
Σ n-3	2.312	3.526	6.833	9.003	
Σ n-6	5.076	7.425	9.023	13.350	

*Except for moisture (%). The data correspond to the mean of three replicates ± standard deviation. Results from one-way ANOVA and Tukey test. Mean values in the same row with different superscripts differ significantly ($\alpha \leq 0.05$). BFT (biofloc system without addition of *Navicula* sp.); BFT 2.5N (addition of 2.5 x 10^4 cells mL^{-1} of *Navicula* sp.); BFT 5.0N (addition of 5 x 10^4 cells mL^{-1} of *Navicula* sp.) and 10.0N BFT (addition of 10 x 10^4 cells mL^{-1} of *Navicula* sp.)

Table 3. Shrimp performance in the culture (42 days) of *Litopenaeus vannamei* under nursery biofloc system with and without the addition of diatoms.

Parameters	Treatment					
	BFT	BFT 2,5N	BFT5N	BFT10N	Probability	F ratio
Final weight (g)	0.69 ± 0.03 ^{b}	0.79 ± 0.03 ^{ab}	0.80 ± 0.08 ^{ab}	0.86 ± 0.03 ^{a}	P=0.0149	F=6.5730*
Biomass Gain (g)	97.25 ± 5.94 ^{b}	109.51 ± 5.69 ^{ab}	114.59 ± 8.37 ^{a}	120.97 ± 4.90 ^{a}	P= 0.0103	F=7.4926*
Productivity (Kg m^{-3})	1.95 ± 0.11 ^{b}	2.19 ± 0.12 ^{ab}	2.30 ± 0.17 ^{a}	2.42 ± 0.10 ^{a}	P=0.0096	F=7.6790**
Survival (%)	93.6 ± 2.52 ^{a}	95.6 ± 2.52 ^{a}	95.3 ± 3.21 ^{a}	93.6 ± 5.77 ^{a}	P=0.8646	F=0.2426^{ns}
SGR (% dia^{-1})	15.56 ± 0.10 ^{b}	15.88 ± 0.08 ^{ab}	15.92 ± 0.23 ^{a}	16.08 ± 0.08 ^{a}	P=0.0103	F=7.5064*
FCR	0.84 ± 0.66 ^{a}	0.77 ± 0.03 ^{a}	0.82 ± 0.01 ^{a}	0.80 ± 0.04 ^{a}	P=0.7847	F=0.3585^{ns}
Salinity stress test	100 ± 0^{a}	90 ± 0^{a}	100 ± 0^{a}	95.5 ± 5.77^{a}	P= 0.3289	F= 1.0526^{ns}

Results from Tukey's test. Values in the same row with different superscripts differ significantly BFT (biofloc system without addition of *Navicula* sp.); BFT 2.5N (addition of 2.5 x 10^4 cells/ml of *Navicula* sp.); BFT 5N (addition of 5 x 10^4 cells/ml of *Navicula* sp.) and BFT 10N (addition of 10 x 10^4 cells/ml of *Navicula* sp.).

Abbreviations: SGR, specific growth rate; FCR, feed conversion ratio; ns, not significant.

*$\alpha \leq 0.05$.

* $\alpha \leq 0.01$.

Table 4. Presumptive diagnosis of nursery culture juvenile *Litopenaeus vannamei* in a biofoc system with and without the addition of diatoms

Treatments	N	Gut repletion degree				Hepatopancreas Tubules				Epicomensals on gill lamellae			
		0	1	2	3	0	1	2	3	0	1	2	3
BFT	14	-	1	5	8	10	4	-	-	10	4	-	-
BFT-2.5N	11	-	-	1	10	9	1	1	-	9	1	1	-
BFT-5.0N	7	-	1	1	5	4	2	-	-	6	-	1	-
BFT-10.0N	8	-	-	2	6	5	3	-	-	7	1	-	-

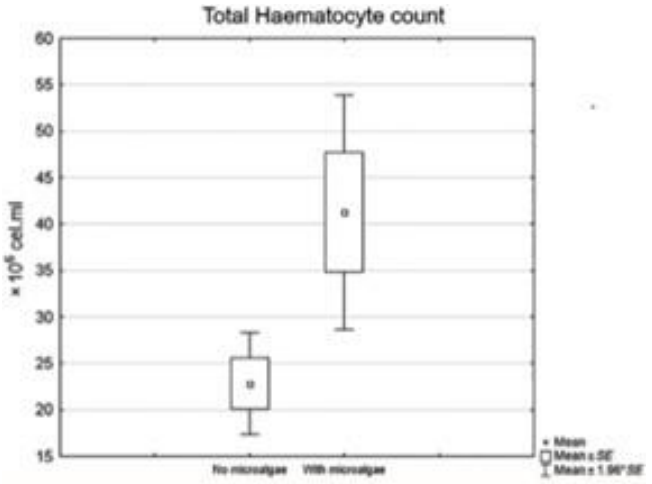

Figure 1. Total hematocyte count (THC) (p=0.0194) of juvenile *Litopenaeus vannamei* under osmotic stress after nursery culture in a biofloco system with an without the addition of diatoms. Significant differences according to the t test

3- Efeito da frequência de adição da microalga *Navicula* sp. sobre o desempenho zootécnico de pós-larvas do *Litopenaeus vannamei* em sistema de bioflocos

Artigo científico II

Aquaculture Research

Efeito da frequência de adição da microalga *Navicula* sp. sobre o desempenho zootécnico de pós-larvas do *Litopenaeus vannamei* em sistema de bioflocos

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Keywords: diatoms, biofloc, microalgae, nursery

Resumo

O objetivo do estudo foi avaliar o efeito da frequência de inoculação da diatomácea *Navicula* sp. no crescimento de pós-larvas de *Litopenaeus vannamei* em um sistema de bioflocos. Foram utilizados quatro tratamentos: BFT (sem adição da *Navicula* sp.); BFT 5D (com adição de *Navicula* sp. a cada 5 dias); BFT 10D (com adição de *Navicula* sp. a cada 10 dias) e BFT 15D (com adição *Navicula* sp. a cada 15 dias), todos em triplicata. O cultivo teve uma duração de 35 dias e foram avaliados o desempenho zootécnico e qualidade de água. Os camarões (5 ± 0,03 mg) foram estocados a uma densidade de 3.000 pós-larvas.m³ e foram alimentados com ração comercial. A microalga foi inoculada nas unidades experimentais a uma densidade ceular de 10 x 10^4 cél.mL. Todos os dados foram submetidos a ANOVA ($\alpha \geq 0,05$) e Tukey ($\alpha \geq 0,05$) onde não foram observadas diferenças significativas para os dados de qualidade da água, FCA e sobrevivência, já para o peso final, produtividade e TCE foram encontradas diferenças significativas, onde os tratamentos BFT mais adição de *Navicula* sp. foram superiores ao tratamento controle (BFT sem adição de *Navicula* sp.), destacando o tratamento BFT 10D que apresentou maiores valores para peso final (0,50 ± 0,06 g), produtividade (1,41 ± 0,03 kg.m^3) e TCE (13,17 ± 0,03 %.dia) em relação aos demais tratamentos. Por fim, sugere-se também que o tratamento BFT 10D com inoculação das microalgas a cada 10 dias tenha o melhor desempenho, o que indica os benefícios de *Navicula* sp. no aumento do crescimento de *L. vannamei* pós-larvas cultivadas em sistemas bioflocos.

Abstract

The objective of the study was to evaluate the effect of the frequency of inoculation of the diatom *Navicula* sp. on the growth of *Litopenaeus vannamei* post-larvae in a biofloc system. Four treatments were used: BFT (without the addition of *Navicula* sp.); BFT 5D (with the addition of *Navicula* sp. every 5 days); BFT 10D (with addition of *Navicula* sp. every 10 days) and BFT 15D (with addition of *Navicula* sp. every 15 days), all in triplicate. The culture lasted 35 days and zootechnical performance and water quality were evaluated. The shrimp (5 ± 0.01 mg) were stored at a density of 3,000 post-larvae.m³ and they were fed with commercial feed. The microalgae was inoculated in the experimental units at a cell density of 10 x 10^4 cells.mL. All data were submitted to ANOVA ($\alpha \geq 0.05$) and Tukey ($\alpha \geq 0.05$) where no significant differences were observed for the data of water quality, FCR and survival, as for the final weight, productivity and SGR, significant differences were found, where the treatments BFT higher addition of *Navicula* sp. were superior to the control treatment (BFT without the addition of *Navicula* sp.), highlighting the BFT 10D treatment that presented higher values for final weight (0.50 ± 0.06 g), productivity (1.41 ± 0.03 kg.m^3) and SGR (13.17 ± 0.03 %.day) as compared than to the other treatments. Finally, it is also suggested that the BFT 10D treatment with inoculation of microalgae every 10 days has the best performance, which indicates the benefits of *Navicula* sp. in the growth of post-larvae *L. vannamei* grown in biofloc systems.

Introdução

A aquicultura é uma atividade que vêm apresentando elevado crescimento ao longo dos anos, alcançando em 2018 uma produção mundial de aproximadamente 82,1 milhões de toneladas, desse total, aproximadamente 31 mil toneladas foram produzidas pela aquicultura marinha sendo 18,64% advindos da produção de crustáceos (FAO, 2020). Sendo o camarão branco do pacífico (*Litopenaeus vannamei*) a espécie mais produzida.

Apesar do crescimento nos últimos anos o cultivo de crustáceo tem vários desafios relacionados a diferentes patógenos principalmente, Mionecrose infecciosa e a mancha branca (MADRID, 2005; ROCHA, 2007, 2008). Desta forma, novas tecnologias são necessárias para convivência com os patogenos.

O BFT (*Biofloc Technology*) aumenta a biossegurança nos cultivos decorrente do maior controle ambiental, visto que, reduz ou elimina as trocas de água, além de proporcionar maiores produtividades e diminuir a necessidade de grandes áreas para a produção, pelas maiores densidades de estocagem praticadas (AVNIMELECH, 2015; SAMOCHA et al., 2017). O biofloco é composto por bactérias, protozoários, microalgas, nematóides, zooplâncton, além de restos de alimentos e fezes dos animais cultivados (HARGREAVES, 2013; SAMOCHA et al., 2017). Segundo Cardona et al. (2015), os bioflocos formados no sistema podem servir como suplemento alimentar, contribuindo com aproximadamente 37 a 40% da oferta de alimento (fonte alimentar), além de estimular as atividades de enzimas digestivas, podendo melhorar o crescimento dos animais cultivados.

Estudos relatam que em sistemas de bioflocos mistos, onde bactérias e diatomáceas são predominantes, o crescimento do camarão é superior quando comparado a sistemas onde existem apenas a dominância de bactérias heterotróficas (CESAR et al., 2012; MARINHO et al., 2014; BRITO et al., 2016; MARINHO et al., 2016; XU et al., 2016; ABREU et al., 2019). Nutricionalmente, as microalgas apresentam um teor lipídico que pode variar de 20 a 70% em peso seco, enquanto que os ácidos graxos poliinsaturados de cadeia longa (DHA e EPA) podem variar de 80 a 45% para as cepas que apresentam alto rendimento (DRAAISMA et al., 2013; CUELLAR-BERMUDEZ et al., 2015; ADARME-VEJA et al., 2012).

Dentre os grupos de microalgas, as diatomáceas se destacam por possuirem uma alta produção de lipídeos quando comparado a outros grupos (ALEXANDER et al., 2015). A microalga *Navicula* sp. é uma diatomácea bentônica bastante utilizada na aquicultura, ficando entre as microalgas mais utilizadas como alimento vivo (LOURENÇO, 2006). Khatoon et al. (2009) notaram que, quando cultivadas em meio Conway, a *Navicula* apresentou altas taxas de proteína bruta, lipídeos e carboidratos. Essa microalga quando adicionada em BFT melhora a composição de ácidos graxos poliinsaturados no floco microbiano (ABREU et al., 2019).

Além disso, existem estudos que relatam a presença de imunoestimulantes naturais específicos e compostos antimicrobianos em algumas microalgas que podem aumentar a resistência dos animais à patógenos aquáticos (CHAROONNART et al., 2018).

Diante do exposto objetivou-se com o presente estudo avaliar qual a melhor frequência de adição da diatomácea *Navicula* sp. no cultivo do *Litopenaeus vannamei*.

Material e Métodos

Um experimento foi conduzido durante 35 dias no Laboratório de Maricultura Sustentável (LAMARSU) do Departamento de Pesca e Aquicultura (DEPAq) da Universidade Federal Rural de Pernambuco (UFRPE), Recife, Brasil. O delineamento experimental foi inteiramente casualizado com quatro tratamentos: Bioflocos sem adição de *Navicula* sp. (BFT) e bioflocos com adição de *Navicula* sp. nas frequências de inoculação a cada 5, 10 e 15 dias (BFT 5D, BFT 10D e BFT 15D, respectivamente), todos em triplicata. A microalga *Navicula* sp. foi adicionada na concentração de $10x10^4$ células.mL^{-1} nos tratamentos BFT 5D, BFT 10D e BFT 15D de acordo com a metodologia proposta por Abreu et al. (2019).

A maturação do biofloco utilizado como inóculo inicial foi realizada 40 dias antes do início do experimento. O tanque matriz (capacidade de 1,4 m^3) foi abastecido com água do mar a uma salinidade de 35 $g.L^{-1}$ previamente clorada (65% de cloro ativo) na proporção de 13 $mg.L^{-1}$ de cloro ativo. Após três dias sob aeração constante, foram iniciadas as fertilizações inorgânicas com uréia, superfosfato triplo e metassilicato de sódio, nas concentrações de 2 $g.m^3$ N, 0,1 $g.m^3$ P e 3 $g.m^3$ Si, respectivamente, e posteriormente as orgânicas, com melaço

de cana e ração pulverizada com 40% de proteína bruta, para formação da biomassa bacteriana.

Cinco dias antes do início do experimento, as unidades experimentais foram abastecidas com um inóculo de 25 L da água maturada no tanque matriz (NAT 0,24 $mg.L^{-1}$, N -NO_2 0,83 $mg.L^{-1}$, N-NO_3 49,7 $mg.L^{-1}$, alcalinidade 165 $mg.L^{-1}$ $CaCO_3$, pH 8,14, salinidade 32 $g.L^{-1}$, ortofosfato 9,2 $mg.L^{-1}$ e sólidos sedimentáveis 4,63 $ml.L^{-1}$) e 25 L de água marinha (salinidade de 35 $g.L^{-1}$) previamente tratada. Foram utilizados 12 tanques de polietileno preto (50 L, 50 × 35 × 23 cm) como unidades de cultivo, cada um deles foram aeradas constantemente por três *airstones*.

Durante o período experimental não foi realizada nenhuma troca de água, exceto a adição de água doce desclorada para compensar as perdas por evaporação. A intensidade da luz foi mantida em 27 µmol m^{-2} s^{-1}. O melaço (30% de carbono orgânico), foi adicionado uma vez ao dia como fonte de carboidrato para manter a razão CHO:N em 12:1, e foi calculado com base em Ebeling, Timmons e Bisogni (2006). Hidróxido de cálcio ($Ca(OH)_2$) com alto poder neutralizante (132%), reatividade de 62% e poder real de neutralização total de 81% foi adicionado a 10% (em peso) do lote de alimentação diária ao longo do estudo.

A diatomácea bentônica *Navicula* sp. foi obtida no Laboratório de Produção de Alimento Vivo (LAPAVI) - DEPAq - UFRPE, a mesma foi cultivada em meio Conway (WALNE, 1966) com $FeCl_3 \cdot 6H_2O$ 1,30; $MnCl_2 \cdot 4H_2O$ 0,36; H_3BO_3 33,6; EDTA 45,0; $NaH_2PO_4 \cdot 2H_2O$ 20,0; $NaNO_3$ 100,0; $ZnCl_2$ 1.1; $CoCl_2 \cdot 6H_2O$ 1.0; (NH_4) $6MO_7O_{24} \cdot 4H_2O$ 0,45; $CuSO_4 \cdot 5H_2O$ 1,0; $Na_2SiO_3 \cdot 5H_2O$ 2,0; B_{12} 0,1; B_1 1.0 que foi usado em uma solução de 1,0 $ml.L^{-1}$, mantida em água com 30 $g.L^{-1}$ de salinidade, pH 8,0, temperatura 25 ± 1 ° C e intensidade de luz mantida a 37 µmol m^{-2} s^{-1} usando uma lâmpada fluorescente com fotoperíodo claro/escuro de 12 horas. As diatomáceas foram adicionadas de acordo com a frequência sugerida para cada tratamento, a densidade de algas foi baseada no estudo de Abreu et al. (2019).

Os parâmetros de qualidade de água, temperatura (°C), salinidade ($g.L^{-1}$), oxigênio dissolvido ($mg.L^{-1}$) e pH, foram mensurados duas vezes ao dia com auxílio de multiparâmetro

(YSI model 556, Yellow Springs, Ohio, EUA). Os sólidos sedimentáveis foram analisados com o auxílio de cone Imhoff (AVNIMELECH, 2009), na frequência de duas vezes por semana. Para a manutenção dos valores de sólidos sedimentáveis (<14 $mL.L^{-1}$), foi utilizado sedimentador, quando necessário. As análises de nitrogênio amoniacal (NAT) (APHA, 2012), nitrito ($N\text{-}NO_2$) (FRIES, 1971) e alcalinidade (APHA, 2012), foram mensurados uma vez por semana, já o N-nitrato ($N\text{-}NO_3$) (APHA, 2012) e o ortofosfato (APHA, 2012) foram mensurados a cada quinze dias.

As pós-larvas de *L. vannamei* (5 ± 0,01 mg) foram obtidas em um laboratório comercial (Aquatec LTDA, RN, Brasil) e estocadas na densidade de 3.000 pós-larvas.m^3 (150 camarões por unidades experimentais) e cultivadas por 35 dias. As pós-larvas foram alimentadas quatro vezes ao dia (às 8, 11, 14 e 16 horas), com ração comercial para camarões (0,4-1 mm de diâmetro) com 45% de proteína bruta e 8% de lipídios (Nutrição e Saúde Animal In vivo). A taxa de alimentação diária de 45% do peso corporal usada no início da cultura foi gradualmente reduzida para 15% do peso corporal após 35 dias com base na tabela de Van Wyk (1999) e ajustada diariamente de acordo com o consumo estimado de camarão e a taxa de mortalidade. O peso do camarão foi monitorado semanalmente após 15 dias de cultivo para determinar o crescimento dos camarões e ajustar a quantidade de ração oferecida. No final do experimento, o ganho de biomassa, peso final médio, taxa de crescimento específico (TCE), fator de conversão alimentar (FCR), sobrevivência e produtividade foram determinados com base nas seguintes equações:

- Ganho de biomassa (g) = biomassa final (g) – biomassa inicial (g);
- Peso final (g) = biomassa final (g)/número de indivíduos ao final do cultivo
- TCE (%/dia) = 100 × [ln peso final (g) – ln peso inicial (g)]/ tempo (dias);
- FCR = alimentação ofertada/ganho de biomassa;
- Sobrevivência (%) = (número de indivíduos ao final do cultivo/ número inicial de indivíduos) × 100;
- Produtividade (kg/m^3) = biomassa final (kg)/volume da unidade experimental

(m^3).

Uma ANOVA paramétrica unilateral foi usada para analisar os parâmetros de desempenho zootécnico, após confirmar a homocedasticidade (Cochran $p < 0,05$) e normalidade (Shapiro-Wilk $p < 0,05$) e, quando observada diferenças significativas, foi realizado o teste de Tukey ($p < 0,05$) para comparar e classificar as médias dos três tratamentos e do controle. Os parâmetros de qualidade da água foram analisados através da realização de ANOVA de medidas repetidas. Para dados estatísticos não paramétricos (temperatura, pH, NAT, N-nitrito, N-nitrato e Ortofosfato), os testes de Kruskall-Wallis ($\alpha < 0,05$) e Dunn ($\alpha < 0,05$) foram usados para comparar e classificar as medianas dos três tratamentos e o controle.

Resultados

Os dados de qualidade de água: oxigênio dissolvido, pH, temperatura, salinidade, alcalinidade e ortofosfato não diferiram significativamente entre os tratamentos ($\alpha \geq 0,05$), apresentando uma variação média de 4,07 a 4,54 mg L^{-1}, 7,81 a 8,18, 26,80 a 31,05 ºC, 29,57 a 36,12 g L^{-1}, 125 a 200 mg $CaCO_3$ L^{-1} e 0,22 a 4,10 mg L^{-1}, respectivamente. Os compostos nitrogenados: nitrogênio da amônia total, N- nitrito e o N-nitrato também não diferiram significativamente entre os tratamentos ($\alpha \geq 0,05$), apresentando média de $0,55 \pm 0,03$ mg.L^{-1}, $0,97 \pm 0,06$ mg.L^{-1} e $125,08 \pm 76,51$ mg.L^{-1}, respectivamente.

Os sólidos sedimentáveis diferiram significativamente entre os tratamentos, apresentando uma variação média de e 0,1 a 12,50 mg L^{-1}, respectivamente. Onde o tratamento BFT 10D apresentou maior volume de sólidos sedimentáveis quando comparado aos demais tratamentos. Todos os dados acima encontram-se sumarizados na Tabela 1.

Quanto ao desempenho zootécnico dos animais cultivados, observou-se que os dados de sobrevivência e fator de conversão alimentar (FCA) não apresentaram diferenças significativas entre os tratamentos ($\alpha \geq 0,05$), enquanto que os dados de peso final, produtividade e taxa de crescimento específico apresentaram diferenças significativas entre os tratamentos ($\alpha \geq 0,05$) (Figura 1). Os dados de produtividade nos tratamentos BFT 5D ($1,22 \pm 0,1$ Kg.m^{-3}) e BFT 10D ($1,41 \pm 0,03$ Kg.m^{-3}) foram significativamente superiores quando

comparados ao tratamento controle (sem adição de alga) (1,00 ± 0,2 $Kg.m^{-3}$) (Figura 1). O FCA foi semelhante entre os tratamentos, enquanto que o peso médio final foi superior para o tratamento BFT 10D (0,51 ± 0,06 g) em compração ao tratamento BFT (sem adição de alga) (0,36 ± 0,05 g) (Figura 2). A taxa de crescimento específico foi superior nos tratamentos BFT 5D (12,82 ± 0,18 % dia^{-1}) e BFT 10D (13,17 ± 0,03 % dia^{-1}) em comparação ao tratamento BFT (12,14 ± 0,46 % dia^{-1}) (Figura 3).

Discussão

A salinidade, temperatura, oxigênio dissolvido e pH da água permaneceram dentro da faixa ideal para *Litopenaeus vannamei* durante todo o cultivo (VAN WYK e SCARPA, 1999; PONCE-PALAFOX et al, 2013). No sistema de bioflocos, um dos principais problemas de qualidade de água é o acúmulo de substâncias tóxicas inorgânicas, como amônia e nitrito (TIMMONS e EBELING, 2010; BARBIERE et al., 2014). O acúmulo da amônia pode ocasionar a deteriorização da qualidade de água, diminuindo o crescimento dos animais, aumentando o consumo do oxigênio e, se alcançar valores muito elevados, pode ocasionar a morte dos animais cultivados (CHEN e LIN, 1992). Enquanto que o nitrito, é o produto intermediário no processo de nitrificação ou desnitrificação do nitrato no ciclo de nitrogênio e, seu acúmulo, pode acarretar prejuízo ao desempenho dos animais cultivados (JENSEN, 2003; KROUPOVA et al., 2005).

Durante o experimento os valores de NAT e $N\text{-}NO_2$ permaneceram dentro da faixa ideal para o cultivo da espécie, 0,90 e 25,7 $mg.L^{-1}$, respectivamente (LIN e CHEN, 2001; 2003; BUFORD et al., 2003; MELO et al., 2016). A manutenção dos níveis de NAT e $N\text{-}NO_2$ em valores recomendados em sistema com altas relações de carbono:nitrogênio indica que a comunidade microbiana (heterotróficas e autotróficas) estiveram presentes contribuindo no controle dos níveis desses compostos na água (AVNIMELECH, 1999; BROWDY et al., 2001; DE SCHRYVER et al., 2008). Além disso, a adição de *Navicula* sp. em diferentes frequências não ocasionaram modificações significativas nessa comunidade em relação ao controle dos nitrogenados, resultados semelhantes aos observados por Marinho et al., (2016); Brito et al.,

(2016) e Abreu et al. (2019). Segundo Avnimelech (2012), as bactérias presentes no floco possuem uma maior capacidade de absorver nitrogenados, com o aumento de carbono no sistema, quando comparado as algas.

O nitrato é o produto final do processo de oxidação da amônia, porém, diferente da amônia e do nitrito, aparentemente sua toxicidade não acarreta grandes problemas aos organismos aquáticos. Por ser menos tóxico, poucos são os estudos relacionados aos efeitos agudos e crônicos que esse composto pode trazer para o desempenho zootécnico do *L. vannamei*. Porém, como no sistema BFT não ocorre renovação de água, o processo de nitrificação pode causar o acúmulo de nitrato no sistema, principalmente se a água for reutilizada por vários ciclos (KUHN et al. 2010; SAMOCHA et al. 2010). No presente estudo foram observados alguns valores acima de 200 mg L^{-1} na terceira semana de cultivo. Esses altos níveis de nitrato no sistema em comparação ao NAT e N-NO_2 indicam um bom processo de nitrificação no ambiente de cultivo (EBELING et al., 2006), além disso, Furtado et al., (2014) descreve que apenas valores acima de (> 300 mg/L em salinidade 23 $g.L^{-1}$) são considerados prejudiciais ao cultivo do camarões em sistema de bioflocos. Segundo Ji et al. (2014), o nitrato é a fonte de nitrogênio mais utilizada na fisiologia das microalgas, entretanto, no presente estudo, não foram observadas diferenças signficativas com a adição das microalgas em diferentes frequências. Tal fato pode estar atrelado ao aumento dos níveis de sólidos ao longo do ciclo de cultivo, que culminaram com uma baixa luminosidade, inviabilizando o processo de fotossíntese realizado pelas microalgas e, com isso, a não utilização dos compostos nitrogenados presentes no sistema.

Quanto ao ortofosfato, os valores encontrados durante o cultivo ficaram próximos a 4,10 mg L^{-1}. Em BFT, ocorre um aumento substancial desse nutriente ao longo dos ciclos de cultivo, ocasionando altas concentrações no sistema (HAKANSON et al., 1998), diferente dos sistemas semi-intensivos, que o fósforo é controlado através da troca de água, e principalmente, pela adsorção e acumulação deste nutriente no sedimento (CASILAS-HERNANDES et al., 2006). No presente estudo, não foram observados valores elevados de ortofosfato e também não houve diferenças significativas entre os tratamentos.

Para contribuir com os processos de transformação do NAT em biomassa microbiana e auxiliar na oxidação da amônia em nitrito e nitrato, a alcalinidade foi mantida acima de 100 mg de $CaCO^3$ L^{-1}, sendo realizadas reposições de carbono inorgânico, quando necessárias. É importante manter os valores adequados para os sistemas BFT, visto que, o carbono inorgânico é consumido pelas bactérias heterotróficas e nitrificantes, que formam o floco, para os processos microbianos (EBELING et al., 2006). Além disso, quando a alcalinidade é mantida, dentro dos valores recomendados a mesma atua no sistema como uma solução tampão evitando assim a oscilação do pH (VAN WYK & SCARPA, 1999), assim sendo, não foram observadas reduções nos níveis de pH durante o período experimental mesmo com maiores frequências de adição da microalga.

Os sólidos sedimentáveis (SS) também são uma variável importante e podem afetar o desenvolvimento dos animais cultivados. Os níveis de SS devem ser mantidos entre 5 e 15 $mL.L^{-1}$, para que os microrganismos presentes no floco atuem de forma positiva na manutenção da qualidade de água do sistema e não ocorra uma alta demanda biológica do oxigênio (EMERENCIANO et al., 2017). Além disso, com a adição da fonte de carbono orgânico, é normal que ocorra um acúmulo dos sólidos ao longo do cultivo, visto que o mesmo constitui-se por altas quantidades de bactérias heterotróficas e autotróficas (LUO et al., 2013). No presente estudo, foi observada diferença significativa entre os tratamentos, onde o tratamento sem inoculação da microalga apresentou um nível mais elevado de SS, porém todos os valores ficaram dentro da faixa recomendada pelo autor supracitado, para *L. vannamei*.

Nos dados de desempenho zootécnico, a sobrevivência e o fator de conversão alimentar não diferiram entre os tratamentos, corroborando com os resultados encontrados por Abreu et al. (2019). Já nos estudos realizados por Marinho et al. (2016) e Brito et al. (2016), estes não observaram diferenças significativas para a sobrevivência, mas encontraram diferenças significativas no FCA, onde os tratamentos com microalgas mostraram-se superior. Os resultados diferentes podem ter sido devido ao tipo de ração ofertada, uma vez que, no presente estudo foi usada uma ração microextrusada, enquanto que nos estudos citados esta,

foi triturada, o que pode ter influenciado no FCA.

A produtividade, peso médio final e taxa de crescimento específico diferiram significativamente entre os tratamentos, onde os maiores valores foram obtidos nos tratamentos com a inoculação da *Navicula* sp. Resultados semelhantes foram encontrados por Brito et al. (2016), Marinho et al., (2016) e Abreu et al., (2019).

Os bons resultados de desempenho zootécnico observados nos tratamentos com inoculação da alga podem ser relacionados a maior facilidade que os camarões apresentam em digerir as diatomáceas, visto que, elas apresentam baixo teor de fibras (Moss, 2000). Além disso, a adição da microalga ao sistema de cultivo é uma forma de melhorar nutricionalmente o floco, visto que, a *Navicul*a sp. apresenta altas concentrações em peso seco de proteína (494 g Kg^{-1}) e lipídeos (259 g Kg^{-1}), além de PUFA (110 g Kg^{-1}) e DHA (22 g Kg^{-1}) (Khatoon et al., 2009), além de um alevado teor de aminoácidos (JU et al., 2008).

Segundo Abreu et al. (2019) a adição de *Navicula* sp. no sistema de BFT, proporciona maiores concentrações na composição proximal do biofloco de proteína e lípideos, além de também apresentarem valores elevados de ácidos graxos (DHA e EPA). Todos esses fatores, quando combinados, proporcionam um melhor desempenho do camarão.

Os resultados encontrados indicam que quando inoculada a cada 10 dias, totalizando a adição de 4 inóculos (início, 10, 20 e 30 dias de cultivo), o desempenho zootécnico do camarão é mais satisfatório quando comparado com os demais tratamentos. Corrobando com os dados observados por Xu et al. (2016), que relatam que presença de bactérias autotróficas associada a microalgas no sistema melhoram o crescimento dos camarões em sistema de bioflocos.

Conclusão

Conclui-se com o presente estudo que a *Navicula* sp. quando inoculada na frequência de 10 dias culminou com um melhor desempenho zootécnico quando comparado aos demais tratamentos. A adição da microalga não influenciou na qualidade de água do sistema de cultivo.

5. Referências

ADARME-VEGA, T.C.; LIM, D. K. Y.; TIMMINS, M.; VERNEN, F.; LI, Y. & SCHENK, P. M. Microbial Cell Factories volume Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microb. Cell Fact. 11, 96, 2012.

ALEXANDER, H., JENKINS, B.D., RYNEARSON, T.A., DYHRMAN, S.T. 2015. Meta transcripto me analyses indicate resource partitioning between diatoms in thefield. Proc. Natl. Acad.Sci. U. S. A. 112, E2182–E2190, 2015.

APHA, 2012. Standard methods for the examination of water and wastewater, 22nd edition edited by E. W. Rice, R. B. Baird, A. D. Eaton and L. S. Clesceri. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington, D.C., USA.

AVNIMELECH Y. & KOCHBA, M. Evaluation of nitrogen uptake and excretion by tilapia in bio floc tanks, using 15N tracing. Aquaculture 287, 163–168, 2012.

AVNIMELECH, Y. Biofloc Technology －A Practical Guide Book. 3rd ed. The World Aquaculture Society, Baton Rouge, United States. 2015.

AVNIMELECH, Y. Biofloc technology. A practical guide book. The World Aquaculture Society, Baton Rouge. 2009.

AVNIMELECH, Y. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture 176, 227–235. 1999.

BARBIERI, E.; BONDIOLI, A. C. V.; MELO, C. B.; HENRIQUES, M. B. Nitrite toxicity to *Litopenaeus schmitti* (Burkenroad, 1936, Crustacea) at different salinity levels. Aquaculture Research, 47(4): 1260-1268. 2014.

BRITO, L. O., SANTOS, I. G. S., ABREU, J. L., ARAÚJO, M. T., SEVERI, W., & GÁLVEZ, A. O. (2016). Effect of addition of diatoms (*Navicula* spp.) and rotifers (*Brachionus plicatilis*) on growth and water quality of the *Litopenaeus vannamei* postlarvae reared in biofloc system. Aquaculture Research, 47, 3990–3997, 2016.

BRITO, L. O., SANTOS, I. G. S., ABREU, J. L., ARAÚJO, M. T., SEVERI, W., & GÁLVEZ, A. O. Effect of addition of diatoms (*Navicula* spp.) and rotifers (*Brachionus plicatilis*) on growth and water quality of the *Litopenaeus vannamei* postlarvae reared in biofloc system. Aquaculture Research, 47, 3990–3997, 2016.

BROWDY, C.L., BRATVOLD, D., STOKES, A.D., MCINTOSH, R.P. Perspectives on the application of closed shrimp culture systems. In: Browdy, C.L., Jory, D.E. (Eds.). The New Wave, Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture 2001. The World Aquaculture Society, Baton Rouge, USA, pp 20-34, 2001.

CARDONA, E.; LORGOUX, B.; GEFFROY, C.; RICHARD, P.; SAULINIER, D.; GUEGEEN, Y.; GUILLOU, G.; CHIM, L. Relative contribution of natural productivity and compound feed to tissue growth in blue shrimp (*Litopenaeus stylirostris*) reared in biofloc: Assessment by C and N stable isotope ratios and effect on key digestive enzymes. Aquaculture, v. 448. p. 288 – 297. 2015.

CASILLAS-HERNÁNDEZ, R, MAGALLÓN-BARAJAS, F., PORTILLO-CLARCK, G., PÁEZ-OSUNA, F. Nutrient mass balances in semi-intensive shrimp ponds from Sonora, Mexico using two feeding strategies: Trays and mechanical dispersal. Aquaculture 258: 289–

298, 2006.

CHAROONNART, P. et al. Applications of microalgal biotechnology for disease control in aquaculture. Biology. 2018, 7, 24.

CHEN, J. C.; LIN, C. Y. Lethal effects of ammonia on P. chinensis Osbeck juveniles at different salinity levels. J. Exp. Mar. Biol. Ecol., 156:138-148. 1992

CLIFFORD, H.C. Semi-intensive shrimp farming. In: Chamberlain, G.A., Haby,M.H., Miget, R.J. (Eds.), Texas Shrimp Farming Manual. Texas Agricultural.Extension Service Corpus Christi, Texas, USA, pp. IV15–IV42. 1985.

COLT, J.E.; ARMSTRONG, D.A. Nitrogen toxicity to fish, crustaceans and molluscs. In: ALLEN, LJ, EC KINNEY. (eds.) Proceedings of the Bioengeneering Symposium for Fish Culture Section, Americam Fisheries Society, Bethesda, Maryland, p.34-47. 1981.

CORREIA, E. S. et al. Intensive nursery production of the pacific white shrimp *Litopenaeus vannamei* using two commercial feeds with high and low protein content in biofloc-dominated system. Aquacultural Engineering, Oxford, v. 59, n.2, p. 48-54, 2014.

CUELLAR-BERMUDEZ, S.P.; Aguilar-Hernandez, I.; Cardenas-Chavez, D. L.; Ornelas-Soto, N.; Romero-Ogawa, M. A.; Parra-Saldivar, R.. Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins. Microb. Biotechnol. 8, 190–209, 2015.

DE SCHRYVER, P., CRAB, T., DEFROIDT, N., BOON, W.V. The basics of bio-flocs technology: The added value for aquaculture. Aquaculture 277,125-137, 2008.

DRAAISMA, R.B. et al. (2013) Food commodities from microalgae. Curr. Opin. Biotechnol. 24, 169–177

EBELING, J.M., TIMMONS, M.B., BISOGNI, J.J. Engineering analysis of the stoichiometry of hotoautotrophic, autotrophic, and heterotrophic control of ammonia-nitrogen in aquaculture production systems. Aquaculture 257, 346- 358. 2006.

EMERENCIANO, M.G.C.; MARTÍNEZ-CÓRDOVA, L.F.; PORCHAS, M.M.; BAEZA, A.M. Biofloc Technology (BFT): A Tool for Water Quality Management in Aquaculture. 2017.

FAO. 2007. The State of World Fisheries and Aquaculture 2007. World review of Fisheries and Aquaculture. Rome.

FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome.

FRÍAS-ESPERICUETA, M.G.; PAÉZ-OSUNA, F. Toxicidad de los compuestos del nitrógeno en camarones, In: PAÉZ-OSUNA, F. (ed.). Camaronicultura y Medio Ambiente. El Colegio de Sonora, Mazatlán, Sinaloa, México. p.253-276. 2001

FURTADO P. S., GAONA C.A.P., POERSCH L.H. & WASIELESKY W. Application of different doses of calcium hydroxide in the farming shrimp *Litopenaeus vannamei* with the biofloc technology (BFT). Aquaculture International 22, 1009–1023, 2014.

GODOY, L. C. 2008. Desempenho do camarão-branco (*Litopenaeus vannamei*) cultivado em meio de diatomáceas ou flocos microbianos com mínima troca de água. Dissertação

HAKANSON, L. Basic concepts concerning assessments of environmental effects of marine fish farms. Copenhagen: Nordic Council of Ministers, 1988.

HARGREAVES, A. J. Biofloc Production Systems for Aquaculture. Southern Regional Aquaculture Center – SRAC, v. 4503. 2013.

JENSEN, F. Nitrite disrupts multiple physiological functions in aquatic animals. Comparative Biochemistry and Physiology Part A, 135(1): 9–24, 2003.

JI, F.; LIU, Y.; HAO, R.; LI, G.; ZHOU, Y.; DONG, R. Biomass production and nutrients removal by a new microalgae strain *Desmodesmus* sp. in anaerobic digestion wastewater. Bioresource Technology, v. 161, p. 200-207. 2014.

KHATOON, H., BANERJEE, S., YUSOFF, F. M., & SHARIFF, M. Evaluation of indigenous marine periphytic *Amphora*, *Navicula* and *Cymbella* grown on substrate as feed supplement in Penaeus monodon postlarvae hatchery systems. Aquaculture Nutrition, 15, 186–193, 2009.
KROUPOVA, H., MACHOVA, J., SVOBODOVA, Z. Nitrite influence on fish: a review. Veterinarni Medicina –Czech, 50(11):461–471, 2005.

KUHN, D.D., SMITH, S.A., BOARDMAN, G.D., ANGIER, M.W., MARSH, L., FLICK JR., G.J. Chronic toxicity of nitrate to Pacific white shrimp, *Litopenaeus vannamei*: Impacts on survival, growth, antennae length, and pathology. Aquaculture 306,329-333. 2010.

LIN Y. E CHEN J. Acute toxicity of ammonia on *Litopenaeus vannamei* Boone juveniles at different salinity levels. Journal of Experimental Marine Biology and Ecology. v. 259, p. 109-119, 2001.

LIN YC, CHEN JC. Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone) juveniles at

different salinity levels. Aquaculture. 224:193–201, 2003.

LOURENÇO, S.O. Cultivo de microalgas marinhas: princípios e aplicações. Editora Rima, São Carlos – Brasil. 2006.

LUO, G.; AVNIMELECH, Y.; PAN, Y.F.; TAN, H.X. Inorganic nitrogen dynamics in sequencing Batch reactors using biofloc technology to treat aquaculture sludge. Aquacultural Engineering, 52 (1): 73-79. 2012.

MADRID, R. M. M. A crise econômica da carcinicultura. Revista Panorama da Aquicultura, v. 15, n. 90, 2005.

MARINHO, Y. F., BRITO, L. O., CAMPOS, C. V. F. S., SEVERI, W., ANDRADE, H. A., & GALVEZ, A. O. Effect of the addition of *Chaetoceros calcitrans*, *Navicula* sp. and *Phaeodactylum tricornutum* (diatoms) on phytoplankton composition and growth of *Litopenaeus vannamei* (Boone) postlarvae reared in a biofloc system. Aquaculture Research, 48, 4155–4164. 2016.

MARINHO, Y. F., BRITO, L. O., SILVA, C. V. F., GÀLVEZ, A. O. (2014). Effect of addition of *Navicula* sp. on plankton composition and postlarvae growth of *Litopenaeus vannamei* reared in culture tanks with zero water exchange. Latin American Journal of Aquatic Research, 42, 427– 437, 2014.

MELO, F. P.; FERREIRA, M. G. P.; BRAGA, I. F. M.; CORREIA, E. S. Toxicidade do nitrito para o camarão *Litopenaeus vannamei* cultivado em sistemas de água clara e bioflocos. Boletim do Instituto de Pesca, São Paulo, 42(4): 855-865, 2016.

PHILIPS, S., LAANBROEK, H.J., VERSTRAETE,W. Origin, causes and effects of increased

nitrite concentrations in aquatic environments. Rev. Environ. Sci. Biotechnol. 1,115-141, 2002.

PONCE-PALAFOX, J., MARTINEZ-PALACIOS, C. A., & ROSS, L. G. The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone, 1931. Aquaculture, 157, 107–115, 1997.

PONCE-PALAFOX, J.T., RUÍZ-LUNA, A., GÓMEZ, M.G.U., Esparza-Leal, H.M.,Arredondo-Figueroa, J.L., Martinez-Palacios, C.A., Ross, L.G., Aresponse-surface analysis of the relative importance of the temperature,salinity and body weight on the respiratory metabolism of the white shrimp *Litopenaeus vannamei* (Boone, 1931). Mar. Freshwater Behav. Physiol. 46 (6),399–417, 2013.

RAY, A. J. et al. Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-based feed in minimalexchange, superintensive culture systems. Aquaculture, Amsterdam, v. 299, n.1-4, p. 89-98, 2010.

Reynolds, C.S. (2006) The Ecology of Phytoplankton. Cambridge University Press, Cambridge.

ROCHA, I. P. Carcinicultura Brasileira: Desenvolvimento tecnológico, sustentabilidade ambiental e compromisso social. Revista da Associação Brasileira de Criadores de Camarão (ABCC). Recife, n. 1, p. 16-22, 2007

ROCHA, R. S. Vibrio spp. Na água e sedimento de viveiros de fazenda de carcinicultura no Estado do Ceará-Brasil. 2008. 100f. Dissertação (Mestrado em Ciências Marinhas Tropicais) Universidade Federal do Ceará, Fortaleza, 2008.

SAMOCHA T.M. Use of intensive and super-intensive nursery systems. The shrimp book,

theory and practice of Penaeid shrimp aquaculture. Nottingham, UK, pp 247–280, 2010.

SAMOCHA, T.M.; PRANGNELL, D.I.; HANSON, T.R.; TREECE, G.D.; MORRIS, T.C.; CASTRO, L.F.; STARESINIC, N. 2017. Design and Operation of Super Intensive, Biofloc-Dominated Systems for Indoor Production of the Pacific White Shrimp, Litopenaeus vannamei– The Texas A&M AgriLife Research Experience. Louisiana: The World Aquaculture Society. 368p.

SCHVEITZER, R. et al. Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. Aquacultural Engineering, Oxford, v. 56, n.1, p. 59-70, 2013.

TIMMONS, M.B., EBELING, J.M. Recirculating Aquaculture. 2nd Edition. Cayuga Aqua Ventures, Ithaca, NY, USA. 2010.

TIMMONS, M.B.; EBELING, J.M. Recirculating Aquaculture, 2nd ed. Cayuga Aquaculture Ventures, Ithaca, NY, USA, p.948. 2010.

VAN WYK P. & SCARPA J. Water Quality Requirements and Management. In: Farming marine shrimp in recirculating freshwater systems. (ed. by P. Van Wyk, M. Davis-Hodgkins, R. Laramore, K. L Main, J. Mountain & J. Scarpa), pp. 141-162. Florida Department of Agriculture and Consumer Services - Harbor Branch Oceanic Institute, Florida. 1999.

VAN WYK, P., SCARPA, J. Water Quality and Management. In: VAN WYK, P., ET AL. (Eds.), Farming Marine Shrimp in Recirculating Freshwater Systems. Florida Department of Agriculture and Consumer Services, Tallahassee, pp. 128–138, 1999.

VAN WYK. Nutrition and feeding of *Litopenaeus vannamei* in intensive culture systems. In: P. Van Wyk, M. Davis-Hodgkins, R. Laramore, K. L. Main, J. Mountain, & J. Scarpa (Eds.), Farming marine shrimp in recirculating freshwater systems (pp. 125–140). Fort Pierce, FL: Florida Department of Agriculture and Consumer Services - Harbor Branch Oceanic Institute. 1999.

WALNE, P. R. Experiments in the large scale culture of the larvae of Ostrea edulis. Fish Investigation, 25, 1–53. 1966.

WICKINS, J. F. The tolerance of warm-water prawns to recirculated water. Aquaculture, v. 9, p. 19-37. 1976.

XU, WU-JIE, MORRIS, T. C., SAMOCHA, TZACHI M. Effects of C/N ratio on biofloc development, water quality, and performance of litopenaeus vannamei juveniles in a biofloc-based, high-density, zeroexchange, outdoor tank system. Aquaculture. 453 (169-175), 2016.

Z.Y. JU, I. FORSTER, L. CONQUEST, W. DOMINY, W.C. KUO, F.D. Horgen Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles Aquac. Res., 39 (2008), pp. 118-133

Tabela 1. Parâmetros de qualidade de água durante (35 dias) o cultivo de pós larvas de

Litopenaeus vannamei em sistema de bioflocos com diferentes frequências de inoculação da *Navicula* sp.

Parâmetros	Tratamentos			
	BFT	**BFT 5D**	**BFT 10D**	**BFT 15D**
Salinidade (g L^{-1})	32,66 ± 0,94	33,97 ± 1,07	32,75 ± 0,78	32,74 ± 1,16
Temperatura (ºC)	28,70 ± 0,86	28,79 ± 1,01	28,80 ± 1,14	28,61 ± 1,09
Oxigênio dissolvido (mg L^{-1})	4,36 ± 0,10	4,33 ± 0,11	4,73 ± 1,38	4,42 ± 0,11
pH	7,98 ± 0,11	7,98 ± 0,10	7,98 ± 0,11	7,98 ±0,09
Nitrogênio Total da Amônia NAT (mg L^{-1})	0,47 ± 0,05	0,68 ± 0,79	0,53 ± 0,46	0,50 ± 0,41
N- Nitrito (mg L^{-1})	1,03 ± 0,40	0,87 ± 0,62	1,05 ± 0,07	0,94 ± 0,59
Nitrato (mg L^{-1})	106,08 ± 57,36	95,04 ± 70,75	106,59 ± 95,69	98,31 ± 86,01
Alcalinidade (mg $CaCO_3$ L^{-1})	159,0 ± 17,95	157,33 ± 16,99	160,0 ± 10,52	160,33 ± 8,06
Ortofosfato (mg L^{-1})	2,98 ± 0,37	2,92 ± 0,38	2,74 ± 0,23	2,82 ± 0,16
Sólidos sedimentáveis (mL L^{-1})	$7,12 \pm 6,8^{b}$	$2,64 \pm 2,39^{ab}$	$1,76 \pm 1,46^{a}$	$1,80 \pm 1,5^{a}$

Resultados do teste de Tukey (p) e Kruskal-Wallis (h).Os valores médios na mesma linha com letras diferentes sobrescritas diferem significativamente ($\alpha \leq 0,05$); os dados correspondem à média ± desvio padrão; BFT (sistema de bioflocos sem adição de diatomácea); BFT 5D (sistema de bioflocos com adição da diatomácea a cada 5 dias); BFT 10D (sistema de bioflocos com adição da diatomácea a cada 10 dias) e BFT 15D (sistema de bioflocos com adição da diatomácea a cada 15 dias).

Figura 1. Sobrevivência e produtividade do *Litopenaeus vannamei* cultivado durante 35 dias

em sistema de bioflocos com diferentes frequências de inoculação da microalga *Navicula* sp.

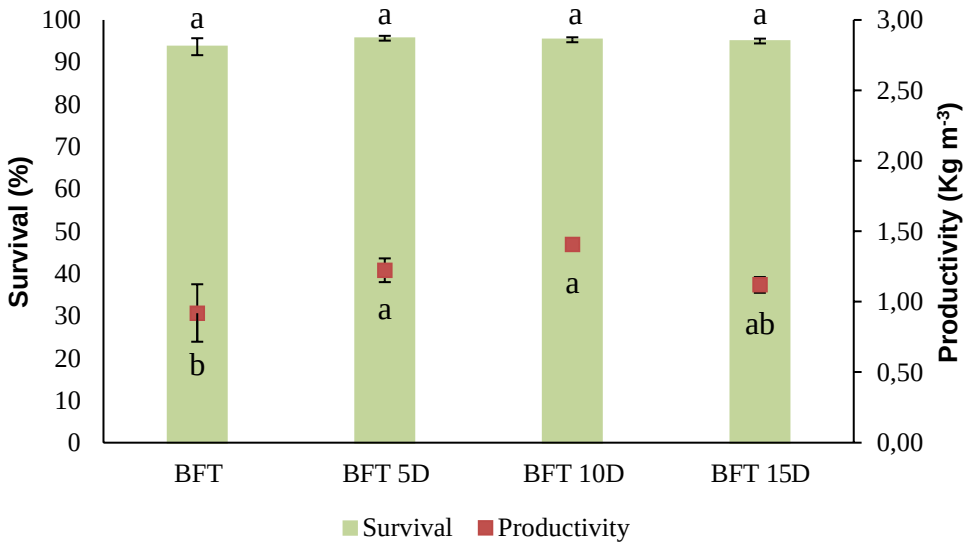


Figura 2. Fator de conversão alimentar e peso médio final do *Litopenaeus vannamei* cultivado durante 35 dias em sistema de bioflocos com diferentes frequências de inoculação da microalga *Navicula* sp.

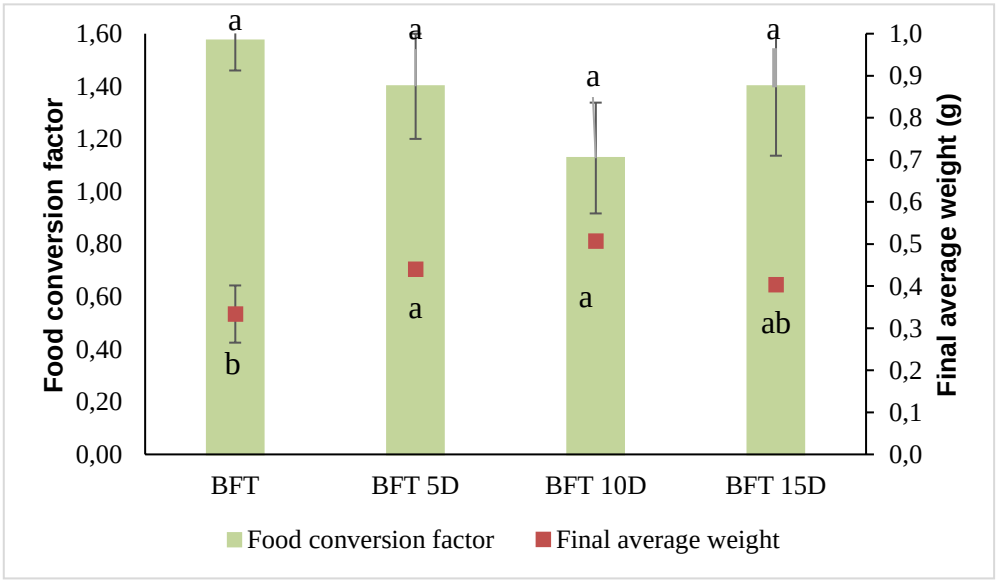
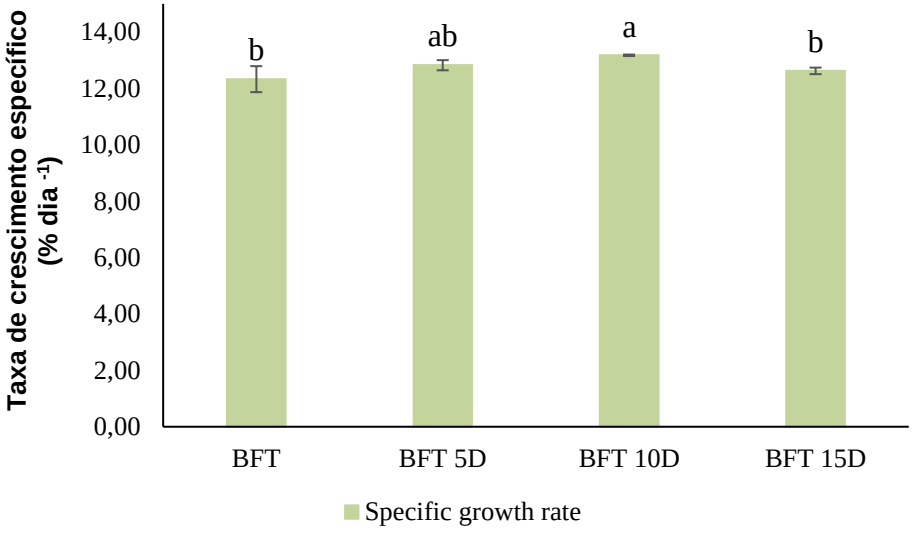


Figura 3. Taxa de crescimento específico do *Litopenaeus vannamei* cultivado durante 35 dias

em sistema de bioflocos com diferentes frequências de inoculação da microalga *Navicula* sp



4. **Considerações finais**

Com os resultados obtidos durante as pesquisas realizadas para o desenvolvimento da tese, é possível concluir que a diatomácea *Navicula* sp. melhora significativamente o desempenho do *L. vannamei* em sistema de bioflocos na concentração 5 $x10^4$ e 10 x 10^4 células.mL e na frequência de inoculação a cada 10 dias, visto que a microalga melhora a qualidade nutricional do flocos aumentando a quantidade de ácidos graxos e, consequetemente, o desempenho zootécnico dos camarões.

Bibliografia

AHMAD, I & R SUBASINGUE. Regional and Global Reviews on Aquaculture. Trends in Global Aquaculture Development. Book of Abstracts, Global Conference on Aquaculture 2010, 22-25 September 2010. FAO/NACA/Thailand Department of Fisheries. Phuket, Thailand. 25-26pp.

AUDELO-NARANJO, J. M.; VOLTOLINA, D.; ROMERO-BELTRÁN, E. Cultural of white shrimp (*Litopenaeus vannamei* Boone, 1931) with zero with exchange and no food addition: an eco-friendly approach. Latin American Journal Aquatic Research, v. 40, n. 2, p. 441-447, 2012.

AVAULT,J. W. JR. Fertilization: Is there a role for it aquaculture. Aquaculture Magazine, Asheville, v. 29, n. 2, p. 47-52, 2003.

AVNIMELECH, Y. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture, v. 176, n. 3-4, p. 227-235, 1999.

BRITO, L. O., SANTOS, I. G. S., ABREU, J. L., ARAÚJO, M. T., SEVERI, W., & GÁLVEZ, A. O. Effect of addition of diatoms (*Navicula* spp.) and rotifers (*Brachionus plicatilis*) on growth and water quality of the *Litopenaeus vannamei* postlarvae reared in biofloc system. Aquaculture Research, 47, 3990–3997, 2016.

COUTTEAU P. Micro-algae. In: Lavens P., Sorgeloos P. (Eds.) Manual on the production and use of live food for aquaculture, Rome, FAO Fisheries Technical Paper, n. 361, 1996, pp. 7-48.

CRAB, R.; DEFOIRDT, T.; BOSSIER, P.; VERSTRAETE, W. Biofloc technology in aquaculture: Beneficial effects and future challenges. Aquaculture, v. 356-357, p. 351- 356,

2012.

DE SCHRYVER ET AL., 2008 P. DE SCHRYVER, R. CRAB, T. DEFOIRDT, N. BOON, W. VERSTRAETE. The basics of bio-flocs technology: the added value for aquaculture Aquaculture, 277 (3), pp. 125-137, 2008.

DECAMP, O.; CONQUEST, L.; CODY, J.; FORSER, I.; TACON, A. G. J. Effect of shrimp stocking density on size-fractionated phytoplankton and ecological groups of ciliated Protozoa within zero-water exchange shrimp culture systems. Journal of the World Aquaculture Society, v. 38, n. 2, p. 395-406, 2007.

FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome.

IBGE – Instituto brasileiro de geografia e estatística. Produção Pecuária Municipal, Rio de Janeiro, v.46, p. 1-8. ISSN 0101-4234

JAIME-CEBALLOS, B.J., HERNANDEZ-LLAMAS, A., GARCIA-GALANO, T., VILLARREAL, H. Substitution of *Chaetoceros muelleri* by *Spirulina platensis* meal in diets for *Litopenaeus schmitii* larvae. Aquaculture 260, 215–220, 2006.

JU, Z. Y., FORSTER, I. P., & DOMINY, W. G. Effects of supplementing two species of marine algae or their fractions to a formulated diet on growth, survival and composition of shrimp (*Litopenaeus vannamei*). Aquaculture, 292, 237–243, 2009.

JU, Z. Y., FORSTER, I., CONQUEST, L., DOMINY, W., KUO, W. C., & HORGEN, F. D. Determination of microbial community structures of shrimp floc culture by biomarkers and analysis of floc amino acid profiles. Aquaculture Research, 39, 118–133, 2008.

KENT, M.; BROWDY, C. L.; LEFLER, J. W. Consumption and digestion of suspended microbes by juvenile pacific white shrimp *Litopenaeus vannamei*. Aquaculture, v. 319, n. 3-4, p. 363 – 368, 2011.

KHATOON, H., BANERJEE, S., YUSOFF, F. M., & SHARIFF, M. Evaluation of indigenous marine periphytic *Amphora*, *Navicula* and *Cymbella* grown on substrate as feed supplement in *Penaeus monodon* postlarvae hatchery systems. Aquaculture Nutrition, 15, 186–193, 2009.

KUHN, D. D.; FLICK, G. J.; BOARDMAN, G. D.; LAWRENCE, A. L. Biofloc: Novel Sustainable ingredient for shrimp feed. Global Aquaculture Advocate, v. 13, n. 2, p. 75-76, 2010.

MARINHO, Y. F., BRITO, L. O., CAMPOS, C. V. F. S., SEVERI, W., ANDRADE, H. A., & GALVEZ, A. O. Effect of the addition of *Chaetoceros calcitrans*, *Navicula* sp. and *Phaeodactylum tricornutum* (diatoms) on phytoplankton composition and growth of *Litopenaeus vannamei* (Boone) postlarvae reared in a biofloc system. Aquaculture Research, 48, 4155–4164, 2016.

MARINHO, Y. F., BRITO, L. O., SILVA, C. V. F., GÀLVEZ, A. O. Effect of addition of *Navicula* sp. on plankton composition and postlarvae growth of *Litopenaeus vannamei* reared in culture tanks with zero water exchange. Latin American Journal of Aquatic Research, 42, 427– 437, 2014.

MOSS, S. M.; LEAMASTER, B. R.; SWEENEY, J.N. Relative abundance and species composition of gram-negative, aerobic bacterial associated with the gut of juvenile white shrimp *Litopenaeus vannmaei* reared in oligotrophic well water and eutrophic ponds water. Journal of the World Aquaculture Society, v. 31, n. 2, p. 255-263, 2000.

NATORI, M.M.; SUSSEL, F.R.; SANTOS, E.C.B. DOS.; PREVIERO, T. DE C.; VIEGAS, E.M.M.; GAMEIRO, A.H. - Desenvolvimento da carcinicultura marinha no Brasil e no mundo: avanços tecnológicos e desafios. Informações Econômicas (ISSN0100- 4409), 41(2):61-73, São Paulo, SP, Brasil. 2011.

OTOSHI, C. A.; MOSS, D.; MOSS, S. M. Growth - enhancing effect of pond water on four size clases of Pacific white shrimp, *Litopenaeus vannamei*. Journal of the World Aquaculture Society, v. 42, n. 3, p. 417-422, 2011.

PÉREZ-FUENTES, J. A., PÉREZ-ROSTRO, C. I., & HERNÁNDEZ-VERGARA, M. P. Pond-reared Malaysian prawn *Macrobrachium rosenbergii* with biofloc system. Aquaculture, 400–401, 105–110, 2013.

PHILLIPS, N.W., Role of different microbes and substrates as potential suppliers of specific essential nutrients to marine detritivores. Bulletin of Marine Science. 35, 283–298, 1984.

SORGELOOS, P. Plenary Lecture I. Resources and technologies for future aquaculture: a needs assessment for sustainable development. Book of Abstracts, Global Conference on Aquaculture 2010, 22-25 September 2010. FAO/NACA/Thailand Department of Fisheries. Phuket, Thailand. 30-31pp.

XU, W. J.; PAN, L. Q. Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. Aquaculture, v. 412-413, p. 117–124, 2013.

XU, W. J.; PAN, L. Q.; SUN, X. H.; HUANG, J. Effects of bioflocs on water quality, and survival, growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zero-water exchange culture tanks. Aquaculture Research, v. 44, n. 7, p. 1093– 1102, 2013.

ZHAO, P., J. HUANG, X.-H. WANG, X.-L. SONG, C.-H. YANG, X.-G. ZHANG, G., WANG, C. The application of bioflocs technology in high-intensive, zero exchange farming systems of *Marsupenaeus japônicas* Aquaculture, 354–355, pp. 97-106, 2012.

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ORIGINAL ARTICLE

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Effects of addition of *Navicula* sp. (diatom) in different densities to postlarvae of shrimp *Litopenaeus vannamei* reared in a BFT system: Growth, survival, productivity and fatty acid profile

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Abstract

The objective of this study was to evaluate the effect of the addition of *Navicula* sp. on the growth and fatty acids profile of *Litopenaeus vannamei* postlarvae in a biofloc system (BFT). Four treatments were used: BFT; BFT 2.5N (addition of 2.5×10^4 cells/ml of *Navicula* sp.); BFT 5N (addition of 5×10^4 cells/ml of *Navicula* sp.) and BFT 10N (addition of 10×10^4 cells/ml of *Navicula* sp.), all in triplicate. The shrimp (1 ± 0.01 mg) were stocked at a density of 3,000 postlarvae/m^3 and fed with commercial feed. The diatom was added every 10 days, and at the end of 42 days, shrimp performance, water quality and proximal composition were evaluated. The BFT 5N and BFT 10N treatments had higher performance values, highlighting the values of productivity (2.30 and 2.42 kg/m^3) and specific growth rate (15.92 and 16.08%/day), which were higher than the other treatments. In addition, the highest levels of fatty acids were observed in treatments with diatom (BFT 5N and BFT 10N), indicating the benefits of *Navicula* sp. on growth enhancement and fatty acid content of *L. vannamei* postlarvae grown in biofloc systems.

KEYWORDS
diatoms, presumptive diagnosis, proximal composition, stress response, water quality

1 | INTRODUCTION

Shrimp farming in Brazil is almost entirely based on the cultivation of *Litopenaeus vannamei* and in 2017 reached production of approximately 41 thousand tonnes (IBGE [Instituto Brasileiro de Geografia e Estatística], 2018). Much of this production is in semi-intensive farming systems, with regular water exchanges and less biosecurity. However, in recent years, problems of outbreaks of viruses such as infectious myonecrosis virus (IMNV) and white spot syndrome virus (WSSV) have resulted in reduced productivity. Therefore, it is necessary to use systems with minimum water changes and emission of effluents (Krummenauer & Seifert, 2012), such as the biofloc system (Crab, Defoirdt, Bossier, & Verstraete, 2012; Pérez-Fuentes, Pérez-Rostro, & Hernández-Vergara, 2013).

The key to biofloc systems is their manipulation of the carbon-nitrogen relationship in the ponds or raceways, stimulating the growth of the microbial community formed by bacteria, phytoplankton, zooplankton, uneaten feed and faeces remains, exoskeletons and other invertebrates (Avnimelech, 2009; De Schryver, Crab, Defoirdt, Boon, & Verstraete, 2008; Samocha et al., 2017), providing an increase in final weight, a higher growth rate and a reduction in feed conversion ratio (FCR) (Avnimelech, 2009; Pérez-Fuentes et al., 2013; Zhao et al., 2012). However, some studies indicate a deficiency in essential amino acids on bioflocs, such as methionine and lysine (Gamboa-Delgado et al., 2017; Valle et al., 2015), and polyunsaturated fatty acids, such as eicosapentae- noic acid (EPA) and docosahexaenoic acid (DHA) (Ekasari, Crab, & Verstraete, 2010).

The presence of microalgae may be beneficial because of its contribution as a food source for the farmed shrimp. Ray, Shuler, Leffler, and Browdy (2009) reported that the biomass in an autotrophic-heterotrophic shrimp system was 17% higher and the FCR was 18% lower than those in a completely heterotrophic system.

The planktonic community could play an important role in shrimp nutrition in biofloc systems because of its good nutritional quality, considering plankton protein, lipid and carbohydrate levels ranging, respectively, from 12% to 35%, 7% to 23% and 4% to 23% of their dry weight, making them a significant food source in the early stages of cultivation (Brito et al., 2016; Hemaiswarya, Raja, Ravikumar, Ganesa, & Anbazhagan, 2011; Marinho, Brito, Silva, Santos, & Gàlvez, 2014; Marinho et al., 2016; Muller-Feuga, Robert, Cahu, Robin, & Divanach, 2003; Richmond, 2004). In terms of microalgal communities, diatoms are distinguished by their high nutritional content and can contribute with essential amino acids and highly unsaturated fatty acids (HUFAs) (Ju et al., 2008; Ju, Forster, & Dominy, 2009).

Navicula sp. is a benthic diatom that presents approximate dry weight levels of 430–490 g/kg of protein, 230–260 g/kg of lipids, which are 30–150 g of EPA g/kg and 20–30 g of DHA g/kg (Khatoon, Banerjee, Yusoff, & Shariff, 2009). Other studies have shown that the lipid content ranges from 4.3% to 31.7% and protein from 12.7% to 13.31% (Courtois, Viera, Huchetteb, & Izquierdo, 2012; Pacheco-Vega, Cadena-Roa, Ascencio, Rangel-Dávalos, & Rojas-contreras, 2015). In this context, some studies using *Navicula* sp. (5×10^4 cells/ml addition every 5 days) in biofloc systems in the nursery phase presented good results regarding the nutritional contribution of this diatom to the growth of *L. vannamei* postlarvae (Brito et al., 2016; Marinho et al., 2014, 2016).

According to Underwood, Boulcott, Raines, and Waldron (2004) bethic diatoms produce fucose, xylose, mannose, galactose, glucose and glucans as intracellular storage compounds. These extracellular polymeric substances are important as adhesive components for the attachment of symbiotic bacteria (Watanabe et al., 2005). Another beneficial effect of microalgal-bacterial interactions is the excretion into the environment of carbon sources or other products by microalgae that have a stimulatory effect on bacteria, including bacterial DNA synthesis (Murray, Cooksey, & Priscu, 1986), and increased bacterial biofilm formation (Espeland, Francoeur, & Wetzel, 2001). This beneficial effect depends on microalgae and bacterial density and species (Natrah, Bossier, Sorgeloos, Yusoff, & Defoirdt, 2014).

Therefore, the objective of this study was to evaluate the effect of the addition of different densities of the diatom *Navicula* sp. on the shrimp performance and fatty acids profile of *L. vannamei* in the nursery phase reared in a biofloc system.

2 | MATERIAL S AND METHODS

2.1 | Experimental conditions

An indoor trial was conducted for 42 days at the Sustainable Mariculture Laboratory (LAMARSU) of the Fisheries and Aquaculture Department (DEPAq) of the Rural Federal University at Pernambuco (UFRPE), Recife, Brazil. The experimental design was completely randomized with four treatments: BFT (biofloc system without addition of *Navicula* sp); BFT with the addition of 2.5×10^4 cells/ml *Navicula* sp. (BFT 2.5N); BFT with the addition of 5×10^4 cells/ml *Navicula* sp. (BFT 5N); and BFT with the addition of 10×10^4 cells/ml *Navicula* sp. (BFT 10N), all in triplicate.

Five days prior to stocking, water from a matrix tank (TAN 0.05 mg/L, $N\text{-}NO_2$ 0.25 mg/L, $N\text{-}NO_3$ 0.5 mg/L, alkalinity 150 mg $CaCO_3$/L, pH 8.8, salinity 30 g/L, P-orthophosphate 0.9 mg/L, TSS 100 mg/L and settleable solids 4.16 ml/L) was mixed and distributed equally to fill 12 black plastic tanks (50 L, 50 × 35 × 23 cm). The experimental units were constantly aerated by three airstones per tank. No water exchange was carried out during the experimental period, except for the addition of dechlo- rinated freshwater to compensate for evaporation losses. Light in- tensity was kept at 27 μmol m^{-2} s^{-1} using a fluorescent lamp with a natural photoperiod.

Molasses (30% organic carbon) was added once a day as a carbohydrates source to maintain the CHO:N ratio at 12:1, and was calculated based on Ebeling, Timmons, and Bisogni (2006). Calcium hydroxide ($Ca(OH)_2$) with high neutralizing power (132%), reactivity of 62% and relative total neutralization power of 81% was added at 10% (by weight) of the daily feed lot throughout the study.

2.2 | Shrimp stocking, feeding and monitoring

The postlarvae (1 ± 0.01 mg) of *L. vannamei* were obtained from a commercial laboratory (Aquasul, RN, Brazil) and stocked at a density of 3,000 postlarvae/m^3 (150 shrimp by experimental units) until 42 days. The postlarvae were fed four times a day (at 08:00 a.m., 11:00 a.m., 02:00 p.m. and 04:00 p.m.), with a commercial shrimp feed (0.4–1 mm in diameter) with 40% crude protein and 8% lipids (In vivo Animal Nutrition and Health). The daily feeding rate of 35% of body weight used at the start of the culture was gradually reduced to 10% of body weight after 42 days based on the Van Wyk (1999) table and adjusted daily according to estimated shrimp consumption and mortality rate.

Shrimp weight was monitored weekly after 15 days of culture to determine shrimp growth and adjust the amount of feed offered. At the end of the experiment, biomass gain, mean final weight, specific growth rate (SGR), FCR, survival and productivity were determined based on the following equations:

Biomass gain (g) = final biomass (g) − initial biomass (g);

Final weight (g) = final biomass (g)/number of individuals at the end of evaluation period;

SGR (%/day) = 100 × [ln final weight (g) − ln initial weight (g)]/time (days);

FCR = feed supplied/biomass gain;

Survival (%) = (number of individuals at the end of evaluation period/initial number of individuals) × 100;

Productivity (kg/m^3) = final biomass (kg)/volume of experimental unit (m^3).

2.3 | Navicula addition

The benthic diatom *Navicula* sp. was obtained from the Live Food Production Laboratory (LAPAVI) – DEPAq – UFRPE, and cultured in a Conway medium (Walne, 1966) with $FeCl_3 \cdot 6H_2O$ 1.30; $MnCl_2 \cdot 4H_2O$ 0.36; H_3BO_3 33.6; EDTA 45.0; $NaH_2PO_4 \cdot 2H_2O$ 20.0; $NaNO_3$ 100.0; $ZnCl_2$ 1.1; $CoCl_2 \cdot 6H_2O$ 1.0; $(NH_4)6M_{O7}O_{24} \cdot 4H_2O$ 0.45; $CuSO_4 \cdot 5H_2O$ 1.0; $Na_2SiO_3 \cdot 5H_2O$ 2.0; B_{12} 0.1; B_1 1.0 which was used in a 1.0 ml/L solution, maintained in water with 30 g/L salinity, pH 8.0, temperature 25 ± 1°C, and light intensity kept at 37 μmol m^{-2} s^{-1} using a fluorescent lamp with a 12-hr light/dark photoperiod. Diatoms were added on the 1st, 10th, 20th and 30th days of experimental time in each experimental tank, regardless of the wastes of unconsumed diatoms, based on Marinho et al. (2014), Marinho et al. (2016) and Brito et al. (2016).

2.4 | Water quality

Dissolved oxygen, temperature, salinity and pH (YSI model 100, Yellow Springs, Ohio, USA) were monitored twice a day (at 08:00 a.m. and 04:00 p.m.). Settleable solids (SS) (Imhoff cone) (Avnimelech, 2009) were monitored three times a week. Total ammonia nitro- gen (TAN), nitrogen-nitrite ($N\text{-}NO_2$), nitrogen-nitrate ($N\text{-}NO_3$), total suspended solids (TSS), phosphorus - orthophosphate ($P\text{-}PO_4^3$) and alkalinity (mg/L $CaCO_3$) were monitored once a week, following the methods described by Koroleff (1976), Golterman, Clymo, and Ohnstad (1978), Mackereth, Heron, and Talling (1978), APHA (2005) and Felföldy, Szabo, and Tothl (1987) respectively. Alkalinity and dissolved nutrients were analysed from effluent samples filtered with 0.45 μm pore size HAWP Millipore membranes, while samples for analysing suspended solids were filtered through prehydrated 0.7 μm pore size AP40 Millipore glass fibre filters. For TSS determination, membranes were weighed after being dried at 105°C.

2.5 | Proximate composition and fatty acids profile

Biofloc samples were collected for proximal composition at the end of the experiment from each tank using a 100-μm mesh, and oven-dried at 60°C. Shrimp samples were also collected from each tank at the end of the experiment, washed with distilled water to remove epiphytes and encrusting material, and oven-dried at 60°C. The dried samples, in triplicate, were ground and processed for biochemical analysis following AOAC (2000) at the Physical Analysis and Food Chemistry Laboratory, UFRPE, Recife, Brazil. For moisture content, the quantities of samples were oven-dried at 105°C until constant weight (315 SE model, Fanem). The dif- ference in weight before and after sample drying was taken and expressed in percentage. Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method (TE 0363 model, Tecnal), and ash by oven incineration at 550°C (Q318 D24 model, Quimis).

The crude lipids were extracted for analyses of their fatty acid profile by a transesterification procedure of extracted fatty acids or quantification of the lipid content as methyl ester derivatives (FAME) (Comeau, Hall, & Oldham, 1988; Wychen & Laurens, 2013).

2.6 | Salinity stress test

At the end of the experiment, a salinity stress test was carried out. This test consisted of transferring the juvenile shrimps from each treatment to three replicated units (10 shrimp per unit) containing freshwater, gently aerated by one air stone per recipient for 30 min. The shrimp were then transferred to a salinity of 35 g/L. After an additional exposure of 30 min, all shrimp not responding to mechanical stimulus were considered dead. Experimental conditions were 29.0 ± 0.5°C and pH 7.8 ± 0.1, using twelve 2-L plastic bottles as experimental units (Burbano-Gallardo et al., 2015).

2.7 | Presumptive diagnosis and total haemocyte count

The measurement of presumptive analysis (Morales-Covarrubias, 2010) and total haemocyte count (THC) (Guertler et al., 2013) were performed for 40 shrimp at the end of the rearing period (day 42). To measure presumptive analysis, the following scores were considered: degree of intestinal repletion—(0) empty; (1) slightly full; (2) moderately full; and (3) full; hepatopancreas–Tubules: (0) shows no signs of infection or deformation; (1) low presence of tubular deformation (1–5/field/organism); (2) moderate presence of tubular deformation (6–10/field/organism), atrophy, melanization and tubular necrosis; and (3) high presence of tubular deformation (11–16/field/organism), with moderate to severe lesions, with melanization, necrosis and tubular atrophy; epicomensals in the gills lamellae—(0) does not present lesions caused by epicomensals; (1) low presence of protozoa (1–5/lamella/organism) and lesions caused by epicomensals; (2) moderate presence of protozoa (6–10/lamella/organism) and increased epicomensal lesions (melanization and formation of haemocytic nodules); and (3) high presence of protozoa (10–15/lamella/organism), moderate to severe lesions caused by epicomensals (multifocal melanized areas and formation of haemocytic nodules).

The haemolymph sample was taken with a 1-ml syringe containing 200 μl of precooled anticoagulant solution (modified Alsever solution (MAS) (336 mmol/L NaCl, 115 mmol/L glucose, 27 mmol/L sodium citrate, 9 mmol/L EDTA, pH 7.2) in the proportion of 1:2 (v:v). For total haemocyte counting, duplicates of 0.8 ml of diluted haemolymph were counted for the number of haemocytes using a haemocytometer under a light microscope.

2.8 | Statistical analysis

A parametric one-way ANOVA was used to analyse production parameters and stress salinity analysis, after confirming homocedasticity (Cochran $p < 0.05$) and normality (Shapiro–Wilk $p < 0.05$). Tukey's test ($p < 0.05$) was performed to compare and rank means from the three treatments and the control. Water quality parameters were analysed by performing repeated ANOVA measures. For

nonparametric statistics data (temperature, pH, TAN, N-nitrite, N-nitrate and P-orthophosphate), the Kruskall-Wallis ($\alpha < 0.05$) and Dunn tests ($\alpha < 0.05$) were used to compare and rank medians from the three treatments and the control. For the THC (date log transformation), the *t* test was used. Data analyses were performed using ASSISTAT Version 7.7 (Assistat Analytical Software).

3 | RESULTS

The water quality parameters of the culture treatments are presented in Table 1. Water temperature was maintained at 28°C, dissolved oxygen above 5 mg/L, pH at 8.3, alkalinity between 140.5 and 158.9 mg $CaCO_3$/L, P-orthophosphate around 1 mg/L, and TSS between 147 and 182 mg/L, with no significant difference ($\alpha \geq 0.05$) between treatments. The results for dissolved inorganic nitrogen compounds were TAN < 0.2 mg/L, N-NO_2 < 0.4 mg/L and N-NO_3 < 1 mg/L with no significant difference ($\alpha \geq 0.05$) between treatments. There was no difference in salinity among the treatments with *Navicula* (31.7–32.0 g/L), but all were slightly higher than the control (31.1 g/L).

The proximal compositions of the bioflocs are presented in Table 2. Moisture results varied from 87.6 to 88.9 g/kg, crude pro- tein from 172.0 to 225.5 g/kg, crude lipids from 63 to 150 g/kg and ash from 553.9 to 587.2 g/kg, with a significant difference ($\alpha \geq 0.05$) between treatments for crude protein, lipids and ash.

The fatty acids profile in the biofloc is presented in Table 2. In general, an increase in microalgae led to an increase in linoleic acid, EPA and DHA. The BFT 10N treatment presented high amounts of EPA and DHA of 3.140 and 5.163 g/kg respectively. It is also noteworthy that the BFT 5N and BFT 2.5N treatments also presented high values of EPA (1.281 and 2.529 g/kg) and DHA (1.088 and 3.690 g/kg). The highest values for linolenic acid and arachidonic acid were found in treatment BFT 2.5N at 1.156 and 1.035 g/kg respectively. In addition, the ratio (n-3)/(n-6) was higher in BFT 5N and BFT 10N. (Table 2).

Table 3 summarizes the shrimp performance during the 42-day experimental period. Shrimp survival rates were all above 93% and FCR above 0.8 with no significant difference ($\alpha \geq 0.05$) between treatments. In terms of final weight, the different treatments with *Navicula* sp. had similar results, but the BFT 2.5N and BFT 5N treatments were also similar to the control BFT and only the BFT 10N treatment (0.86 ± 0.03 g) was higher to the control BFT (0.69 ± 0.03 g). However, for biomass gain, productivity and SGR, the treatments with *Navicula* sp. had similar results, and while the BFT 2.5N did not differ from the BFT control, the results of BFT 5N and BFT 10N treatments were higher than the control BFT ($\alpha \leq 0.05$). Shrimp survival after the salinity stress test was above 90% without a significant difference ($\alpha \geq 0.05$) between the treatments.

The results of the presumptive diagnosis determined that 80% of all the animals in the treatments with the addition of *Navicula* sp. (BFT 2.5N, BFT 5N and BFT 10N) had a high degree of gut tissue repletion (grade 3), as opposed to 57% of the animals in the control group. In relation to the hepatopancreas, 95% of the animals showed no or very-low tubular deformity (degrees of severity between 0 and 1) in all of the treatments, and 71–91% of the animals submit- ted to the treatments with an addition of diatoms had high rates of lipid droplets (grade 3). In contrast, 28% of the control group (BFT) had a low to moderate presence of protozoa and melanization in the gill lamellae, with a maximum of 15% in the tested groups (BFT 2.5N, BFT 5N and BFT 10N) (Table 4). The THC was significantly higher ($\alpha < 0.05$) in the animals submitted to treatments with the addition of diatoms (41.25×10^6 cells/ml) than in the control group (22.84×10^6 cells/ml) (Figure 1).

TABLE 1 Water quality parameters in the culture of *Litopenaeus vannamei* under nursery biofloc system with and without the addition of diatoms

	Treatments				
Parameters	**BFT**	**BFT 2.5N**	**BFT 5N**	**BFT 10N**	
Salinity (g/L)	31.07 ± 0.57^{b}	31.72 ± 0.39^{ab}	31.94 ± 0.39^{ab}	32.05 ± 0.32^{a}	$p = 0.016$
Temperature (°C)	28.23 ± 0.12	28.42 ± 0.10	28.39 ± 0.37	28.38 ± 0.03	$h = 1.3204$
Dissolved oxygen (mg/L)	5.12 ± 0.02	5.09 ± 0.04	5.08 ± 0,03	5.01 ± 0.03	$p = 0.196$
pH	8.30 ± 0.03	8.31 ± 0.05	8.31 ± 0.02	8.30 ± 0.02	$h = 0.2156$
TAN (mg/L)	0.19 ± 0.03	0.20 ± 0.06	0.08 ± 0.06	0.08 ± 0.02	$h = 6.3385$
N-Nitrite (mg/L)	0.40 ± 0.01	0.40 ± 0.01	0.38 ± 0.01	0.38 ± 0,01	$h = 1.9183$
N-Nitrate (mg/L)	0.97 ± 0.02	0.95 ± 0.01	0.97 ± 0.01	0.98 ± 0.01	$h = 0.5906$
Alkalinity (mg $CaCO_3$/L)	158.93 ± 11.48	140.53 ± 11.60	156.53 ± 19.61	157.60 ± 9.47	$p = 0.406$
Orthophosphate (mg/L)	1.18 ± 0.14	1.11 ± 0.12	1.19 ± 0.06	1.17 ± 0.09	$h = 0.7978$
TSS (mg/L)	170 ± 44	147 ± 65	159 ± 65	182 ± 76	$p = 0.449$

Note: Results from Tukey's test (*p*) and Dunn (*h*). Values in the same row with different superscripts differ significantly ($\alpha < 0.05$); BFT (biofloc system without addition of *Navicula* sp.); BFT 2.5N (addition of 2.5×10^4 cells/ml of *Navicula* sp.); BFT 5N (addition of 5×10^4 cells/ml of *Navicula* sp.) and BFT 10N (addition of 10×10^4 cells/ml of *Navicula* sp.).
Abbreviations: TAN, total ammonia nitrogen; TSS, total suspended solids.

TABLE 2 Proximate composition of microbial floc samples and fatty acids profiles at the end of the experimental period of *Litopenaeus vannamei* postlarvae reared in biofloc system without addition of diatoms and with different densities of diatom addition

	Treatments				
	BFT	**BFT 2.5N**	**BFT 5N**	**BFT 10N**	**Recommended levels**
Proximal composition[1]					
Moisture	89.57 ± 0.66	88.87 ± 0.35	88.68 ± 2.83	87.98 ± 0.84	
Crude protein	225.5 ± 1.66^{a}	206.7 ± 0.81^{a}	220.7 ± 0.09^{a}	172.0 ± 1.28^{b}	45%–50%[2]
Crude lipids	150 ± 0.03^{a}	98 ± 0.03^{b}	97 ± 0.01^{b}	63 ± 0.00^{c}	9%–15%[2]
Ash	553.9 ± 4.2^{c}	567.0 ± 3.4^{bc}	579.9 ± 8.9^{ab}	587.2 ± 1.8^{a}	
Fatty acids					
C18:2n-6	4.301	6.390	8.484	12.466	0.4%[2]
C18:3n-3	0.767	1.156	0.613	0.699	0.3%[2]
C20:4n-6	0.775	1.035	0.538	0.884	
C20:5n-3	0.835	1.281	2.529	3.140	0.4%[2]
C22:6n-3	0.709	1.088	3.690	5.163	0.4%[2]
Σ Saturated	15.268	20.911	23.717	30.034	
Σ Monounsaturated	7.328	9.346	6.066	7.177	
Σ n-3	2.312	3.526	6.833	9.003	
Σ n-6	5.076	7.425	9.023	13.350	

[1]Except for moisture (%), the other values are in terms of g/kg on a dry matter basis. The data correspond to the mean of three replicates ± standard deviation. Results from one-way ANOVA and Tukey test. Mean values in the same row with different superscripts differ significantly ($\alpha \leq 0.05$). BFT (biofloc system without addition of *Navicula* sp.); BFT 2.5N (addition of 2.5 × 10^4 cells/ml of *Navicula* sp.); BFT 5N (addition of 5 × 10^4 cells/ml of *Navicula* sp.) and BFT 10N (addition of 10 × 10^4 cells/ml of *Navicula* sp.).
[2]Van Wyk (1999).

TABLE 3 Shrimp performance in the culture (42 days) of *Litopenaeus vannamei* under nursery biofloc system with and without the addition of diatoms

	Treatment					
Parameters	**BFT**	**BFT 2.5N**	**BFT 5N**	**BFT 10N**	**Probability (*p*)**	***F* ratio**
Final weight (g)	0.69 ± 0.03^{b}	0.79 ± 0.03^{ab}	0.80 ± 0.08^{ab}	0.86 ± 0.03^{a}	$p = 0.0149$	$F = 6.5730^{*}$
Biomass gain (g)	97.25 ± 5.94^{b}	109.51 ± 5.69^{ab}	114.59 ± 8.37^{a}	120.97 ± 4.90^{a}	$p = 0.0103$	$F = 7.4926^{*}$
Productivity (kg/m^3)	1.95 ± 0.11^{b}	2.19 ± 0.12^{ab}	2.30 ± 0.17^{a}	2.42 ± 0.10^{a}	$p = 0.0096$	$F = 7.6790^{**}$
Survival (%)	93.6 ± 2.52^{a}	95.6 ± 2.52^{a}	95.3 ± 3.21^{a}	93.6 ± 5.77^{a}	$p = 0.8646$	$F = 0.2426^{ns}$
SGR (%/day)	15.56 ± 0.10^{b}	15.88 ± 0.08^{ab}	15.92 ± 0.23^{a}	16.08 ± 0.08^{a}	$p = 0.0103$	$F = 7.5064^{*}$
FCR	0.84 ± 0.66^{a}	0.77 ± 0.03^{a}	0.82 ± 0.01^{a}	0.80 ± 0.04^{a}	$p = 0.7847$	$F = 0.3585^{ns}$
Salinity stress test	100 ± 0^{a}	90 ± 10^{a}	100 ± 0^{a}	95.5 ± 5.77^{a}	$p = 0.3289$	$F = 1.0526^{ns}$

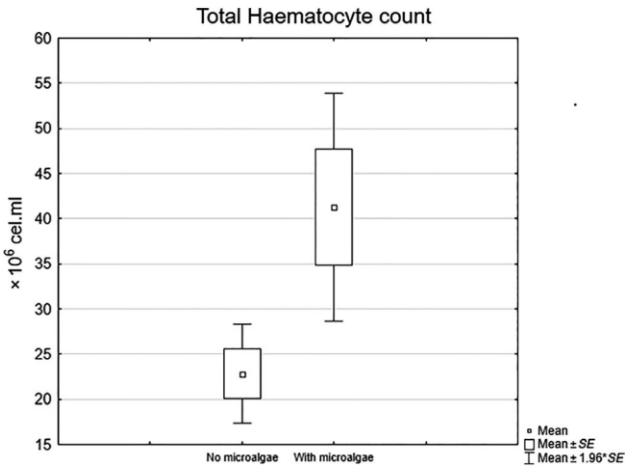
Note: Results from Tukey's test. Values in the same row with different superscripts differ significantly. BFT (biofloc system without addition of *Navicula* sp.); BFT 2.5N (addition of 2.5 × 10^4 cells/ml of *Navicula* sp.); BFT 5N (addition of 5 × 10^4 cells/ml of *Navicula* sp.) and BFT 10N (addition of 10 × 10^4 cells/ml of *Navicula* sp.).
Abbreviations: SGR, specific growth rate; FCR, feed conversion ratio; ns, not significant.
*$\alpha \leq 0.05$.
**$\alpha \leq 0.01$.

4 | DISCUSSION

The temperature, dissolved oxygen concentration and pH of the water remained within the ideal range for the cultivation of the species during the whole experimental period (Ponce-Palafox, Martinez-Palacios, & Ross, 1997; Van Wyk & Scarpa, 1999). In biofloc systems, the increase in the microbial biomass and respective consumption of alkalinity causes a drop in pH levels (Silva, Wasielesky, & Abreu, 2013). To avoid pH fluctuations, alkalinity should be kept above 100 mg $CaCO_3$/L, since it acts on the system as a buffer

TA B LE 4 Presumptive diagnosis of nursery culture of juvenile *Litopenaeus vannamei* in a biofloc system with and without the addition of diatoms

		Gut repletion degree				Hepatopancreas Tubules				Epicomensals on gill lamellae			
Treatments	***N***	**0**	**I**	**2**	**3**	**0**	**I**	**2**	**3**	**0**	**I**	**2**	**3**
BFT	14	–	1	5	8	10	4	–	–	10	4	–	–
BFT 2.5N	11	–	–	1	10	9	1	1	–	9	1	1	–
BFT 5N	7	–	1	1	5	4	2	–	–	6	–	1	–
BFT 10N	8	–	–	2	6	5	3	–	–	7	1	–	–



and 2.42 kg/m^3) than the other treatments. Brito et al. (2016) reported productivity of 1.76 ± 0.51 kg/m^3, using a *Navicula* sp. concentration of 5 × 10^4 cells/ml in an *L. vannamei* culture in a biofloc system for 35 days. Such result is lower than that observed in this study, indicating that higher density inoculation of diatoms increases productivity. Regarding the SGR, the BFT 5N and BFT 10N treatments differed significantly from the other treatments. The results were similar to those observed with the addition of microalage in bioflocs by Marinho et al. (2014), who found a rate of 14.87 ± 0.61%/day, and higher than that attained by Brito et al. (2016), who found a rate of 9.41 ± 0.41%/day. For FCR and survival, no significant differences were observed between treatments, indicating that the density of *Navicula* sp. did not influence these parameters. The better shrimp performance in the BFT 10N treatment may be associated with the fatty acids in the bioflocs. Courtois et al. (2012) evaluated the nutritional value of four diatom species for shrimp, one of them being *Navicula incerta*, and observed 4.35 ± 0.17% of lipids in dry weight, of which 21.8% was EPA and 0.07% DHA, indicating that diatoms can contribute nutritionally to the performance of *L. vannamei*.

The hepatopancreas is an important indicator of shrimp health because it is extremely sensitive to different diets and water pollutants, and can be observed directly under a microscope (Manan, Zhong, Othman, & Ikhwanuddin, 2015). In this study, by means of fresh examination, it was possible to determine that the animals in the treatments with the addition of microalgae (BFT 2.5N, BFT 5N and BFT 10N) had hepatopancreas with tubules full of lipid droplets and without significant lesions, as well as a filled intestine, revealing the positive effect of the inclusion of *Navicula* sp. on the health of the shrimp.

As for the presence of epicomensals on the gill lamellae, 28% of the animals in the control group had 1–10 protozoa per lamella, while the animals in the treatments BFT 2.5N, BFT 5N and BFT 10N an occurrence of at most 14%–18% was observed at the same counting interval. In general, high concentrations of organic material (low water quality) increases the epicomensal counts in the gill lamellae (Cuéllar-Anjel, 2008), and the number of unhealthy shrimp (Guzmán & Valle, 2000). In this study, no significant differences were found in the water quality parameters evaluated, suggesting that the differences in the epicomensal counts could be linked to the better health conditions recorded in the treatments inoculated with diatoms.

Regarding the THC, higher mean values were found in the animals of the treatments with the addition of *Navicula* sp. in relation to BFT. According to Krupesha, Seema, Philipose, and Radhkrishnan (2009), higher THC provides higher immunological status, since its increase may provide the crustaceans greater protection against infections since haemocytes are the main immunocompetent cell in crustaceans (Jiravanichpaisal, Lee, & Soderhall, 2006).

5 | CONCLUSION

It is concluded that the diatom *Navicula* sp. inoculated at a den- sity of 5 × 10^4 cells/ml (BFT 5N) and 10 × 10^4 cells/ml (BFT 10N) provides benefits for the development of *L. vannamei* postlarvae, since it presented higher values in the performance variables for final mean weight, productivity, biomass gain, SGR and shrimp health status. In addition, the inoculation with diatoms improved the nutritional quality of bioflocs by increasing the amount of fatty acids.

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R EFER EN CE S

AOAC (2000). *Official methods of analysis of AOAC. International* (17th ed.). Gaithersburg, MD: USA Association of Analytical Communities.

APHA (2005). *Standard methods for the examination of water and wastewater*. Washington, DC: American Public Health Association.

Avnimelech, Y. (2009). *Biofloc technology – A practical guide book*. Baton Rouge, LA: The World Aquaculture Society.

Brito, L. O., Santos, I. G. S., Abreu, J. L., Araújo, M. T., Severi, W., & Gálvez, A. O. (2016). Effect of addition of diatoms (*Navicula* spp.) and rotifers (*Brachionus plicatilis*) on growth and water quality of the *Litopenaeus vannamei* postlarvae reared in biofloc system. *Aquaculture Research*, *47*, 3990–3997. https://doi.org/10.1111/ are.12849

Burbano-Gallardo, E., Imués-Figueroa, M. A., Gonzalez-Legarda, E. A., Brito, L. O., Galvez, A. O., & Arana, L. A. V. (2015). Supervivencia de poslarvas de *Litopenaeus vannamei* sometidas a la prueba de estrés osmótico y su relación con el estado de muda. *Revista de Biología Marina y Oceanografía*, *50*, 323–329. https://doi.org/10.4067/S0718-19572015000300010

Comeau, Y., Hall, K. J., & Oldham, W. K. (1988). Determination of poly-β-hydroxybutyrate and poly-β-hydroxyvalerate in activated sludge by gas liquid chromatography. *Applied and Environmental Microbiology*, *54*, 2325–2327. 0099-2240/88/092325-03$02.00/0

Courtois, V. G., Viera, M. P., Huchetteb, S., & Izquierdo, M. S. (2012). Improving nursery performances of *Haliotis tuberculata coccinea*: Nutritional value of four species of benthic diatoms and green macroalgae germlings. *Aquaculture*, *334–337*, 124–131. https://doi.org/10.1016/j.aquaculture.2011.12.040

Crab, R., Defoirdt, T., Bossier, P., & Verstraete, W. (2012). Biofloc technology in aquaculture: Beneficial effects and future challenges.

Aquaculture, *356–357*, 351–356. https://doi.org/10.1016/j.aquaculture.2012.04.046

Cuéllar-Anjel, J. (2008). Enfermedade por parasitos. In: V. Morales, & Y. J. Cuéllar (Eds.), Guía Técnica – Patología e Inmunología de Camarones Penaeidos. Programa CYTED-Red II-D: Vannamei (pp. 137–157). Panamá, Rep Panamá.

De Schryver, P., Crab, R., Defoirdt, T., Boon, N., & Verstraete, W. (2008). The basics of bioflocs technology: The added value for aquacul- ture. *Aquaculture*, *277*, 125–137. https://doi.org/10.1016/j.aquaculture.2008.02.019

Ebeling, J. M., Timmons, M. B., & Bisogni, J. J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture*, *257*, 346–358. https://doi.org/10.1016/j.aquaculture.2006.03.019

Ekasari, J., Angela, D., Waluyo, S. H., Bachtiar, T., Surawidjaja, E. H., Bossier, P., & De Schryver, P. (2014). The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. *Aquaculture*, *426–427*, 105–111. https://doi.org/10.1016/j.aquaculture.2014.01.023

Ekasari, J., Crab, R., & Verstraete, W. (2010). Primary nutritional content of bio-flocs cultured with different organic carbon sources and salinity. *HAYATI Journal of Biosciences*, *17*, 125–130. https://doi.org/10.4308/hjb.17.3.125

Espeland, E. M., Francoeur, S. N., & Wetzel, R. G. (2001). Influence of algal photosynthesis on biofilm bacterial production and associated glucosidase and xylosidase activities. *Microbial Ecology*, *42*, 524–530. https://doi.org/10.1007/s00248-001-1022-8

Felföldy, L., Szabo, E., & Tothl, L. (1987). *A biológiai vizminösités*. Budapest, Hungary: Vizügyi Hodrobiológia Vizdok.

Gamboa-Delgado, J., Oca, G. A. R. M., Reyes, J. C. R., Villarreal-Cavazos, D. A., Nieto-Lopez, M., & Cruz-Suarez, L. E. (2017). Assessment of the relative contribution of dietary nitrogen from fish meal and bio- floc meal to the growth of Pacific white shrimp (*Litopenaeus vanna- mei*). *Aquaculture Research*, *48*, 2963–2972. https://doi.org/10.1111/are.13129

Golterman, H. J., Clymo, R. S., & Ohnstad, M. A. (1978). *Methods for physical and chemical analysis of freshwaters*. Oxford/London, UK: Blackwell Scientific Publications.

Guertler, C., Rieg, T., Mejía-Ruíz, C. H., Lehmann, M., Barracco, M. A., & Perazzolo, L. M. (2013). Hemograma e sobrevivência de camarões marinhos após silenciamento do WSSV por RNA de interferência. *Pesquisa Agropecuária Brasileira*, *48*, 983-990. https://doi.org/10.1590/S0100-204X2013000800025

Guzmán, G. A., & Valle, F. A. (2000). Infectious disease in shrimp species with aquaculture potential. *Recent Research Developments in Microbiology*, *4*, 333–348.

Hemaiswarya, S., Raja, R., Ravikumar, R., Ganesa, N. V., & Anbazhagan, C. (2011). Microalgae: A sustainable feed source for aquaculture. *World Journal Microbiology and Biotechnology*, *27*, 1737–1746. https://doi.org/10.1007/s11274-010-0632-z

IBGE (Instituto Brasileiro de Geografia e Estatística). (2018). Produção da Pecuária Municipal. 45, 1–8, Rio de Janeiro, Brazil.

Jiravanichpaisal, P., Lee, L. B., & Soderhall, K. (2006). Cell- mediated immunity in arthropods: Hematopoiesis, coagulation, melaniza- tion and opsonization. *Immunobiolog*, *211*, 213–236. https://doi.org/10.1016/j.imbio.2005.10.015

Ju, Z. Y., Forster, I., Conquest, L., Dominy, W., Kuo, W. C., & Horgen, F. D. (2008). Determination of microbial community structures of shrimp floc culture by biomarkers and analysis of floc amino acid profiles. *Aquaculture Research*, *39*, 118–133. https://doi.org/10.1111/j.1365-2109.2007.01856.x

Ju, Z. Y., Forster, I. P., & Dominy, W. G. (2009). Effects of supplement- ing two species of marine algae or their fractions to a formulated diet on growth, survival and composition of shrimp (*Litopenaeus vannamei*). *Aquaculture*, *292*, 237–243. https://doi.org/10.1016/j.aquaculture.2009.04.040

Khatoon, H., Banerjee, S., Yusoff, F. M., & Shariff, M. (2009). Evaluation of indigenous marine periphytic *Amphora*, *Navicula* and *Cymbella* grown on substrate as feed supplement in *Penaeus monodon* post- larvae hatchery systems. *Aquaculture Nutrition*, *15*, 186–193. https://doi.org/10.1111/j.1365-2095.2008.00582.x

Koroleff. (1976). Determination of nutrients. In K. Grasshoff (Ed.), *Methods of seawater analysis* (pp. 97–115). New York, NY: Verlag Chemie Weinhein.

Krummenauer, D., Seifert, C. A. Jr, Poersch, L. H., Foes, G. K., Lara, G. R., & Wasielesky, W. Jr (2012). Cultivo de camarões marinhos em sistema de bioflocos: análise de reutilização da água. *Atlântica, Rio Grande do Norte*, *34*, 103-111. https://doi.org/10.5088/atlântica.v34i2.3118

Krupesha, S. R., Seema, J., Philipose, K. K., & Radhkrishnan, E. V. (2009). Effect of salinity an on pH selected immune functions of the Indian white shrimp, *Fenneropenaeus indicus* (H. Milne Edwards, 1937). *Indian Journal of Fisheries*, *56*, 183–187.

Lim, C., Ako, H., Brown, C. L., & Hahn, K. (1997). Growth response and fatty acid composition of juvenile *Penaeus vannamei* fed different sources of dietary lipid. *Aquaculture*, *151*, 143–153. https://doi.org/10.1016/S0044-8486(96)01500-1

Mackereth, F. J. H., Heron, J., & Talling, J. F. (1978). *Water analysis: Some revised methods for limnologists*. Oxford/London, UK: Blackwell Scientific Publications.

Manan, H., Zhong, J. M. H., Othman, F., & Ikhwanuddin, M. H. D. (2015). Histopathology of the hepatopancreas of pacific white shrimp, *Penaeus vannamei* from none early mortality syndrome (EMS) Shrimp Ponds. *Journal of Fisheries and Aquatic Science*, *10*, 562–568. https://doi.org/10.3923/jfas.2015.562.568

Marinho, Y. F., Brito, L. O., Campos, C. V. F. S., Severi, W., Andrade, H. A., & Galvez, A. O. (2016). Effect of the addition of *Chaetoceros calcitrans*, *Navicula* sp. and *Phaeodactylum tricornutum* (diatoms) on phytoplankton composition and growth of *Litopenaeus vannamei* (Boone) postlarvae reared in a biofloc system. *Aquaculture Research*, *48*, 4155–4164. https://doi.org/10.1111/are.13235

Marinho, Y. F., Brito, L. O., Silva, C. V. F., ... Gàlvez, A. O. (2014). Effect of addition of Navicula sp. on plankton composition and postlarvae growth of Litopenaeus vannamei reared in culture tanks with zero water exchange. *Latin American Journal of Aquatic Research*, *42*, 427-437. 103856/vol42-issue3-fulltext-4

Martins, T. G., Odebrecht, C., Jensen, L. V., D'Oca, M. G. M., & Wasielesky, W. Jr (2016). The contribution of diatoms to bioflocs lipid content and the performance of juvenile *Litopenaeus vannamei* (Boone, 1931) in a BFT culture system. *Aquaculture Research*, *47*, 1315–1326. https://doi.org/10.1111/are.12592

Morales-Covarrubias, M. S. (2010). *Enfermidades del camarón: detectión mediante análisis en fresco e histopatología (Diseases shrimp detection by means of gross analysis and histopathology)* (2nd^a ed.). Mexico City: Trillas.

Muller-Feuga, A., Robert, R., Cahu, C., Robin, J., & Divanach, P. (2003). Uses of microalgae in aquaculture In J. G. Støttrup, & L. A. McEvoy (Eds.), *Live feeds in marine aquaculture* (pp. 253–299). Berlin, Germany: Blackwell Publishing Company.

Murray, R. E., Cooksey, K. E., & Priscu, J. C. (1986). Simulation of bacterial DNA synthesis by algal exudates in attached algal-bacterial consortia. *Applied and Environmental Microbiology*, *52*, 1177–1182. 0099-2240/86/111177-06$02.00/0

Natrah, F. M. I., Bossier, P., Sorgeloos, P., Yusoff, F. M., & Defoirdt, T. (2014). Significance of microalgal-bacterial interactions for Aquaculture. *Reviews in Aquaculture*, *6*, 48–61. https://doi.org/10.1111/raq.12024

Pacheco-Vega, J. M., Cadena-Roa, M. A., Ascencio, F., Rangel-Dávalos, C., & Rojas-contreras, M. (2015). Assessment of endemic microalgae as potential food for *Artemia franciscana* culture. *Latin American*

Journal of Aquatic Research., *43*, 23–32. https://doi.org/10.3856/vol43-issue1-fulltext-3

Patil, V., Kallqvist, T., Olsen, E., Vogt, G., & Gislerod, H. R. (2007). Fatty acid composition of 12 microalgae for possible use in aquaculture feed. *Aquaculture International*, *15*, 1–9. https://doi.org/10.1007/s10499-006-9060-3

Pérez-Fuentes, J. A., Pérez-Rostro, C. I., & Hernández-Vergara, M. P. (2013). Pond-reared Malaysian prawn *Macrobrachium rosenbergii* with biofloc system. *Aquaculture*, *400–401*, 105–110. https://doi.org/10.1016/j.aquaculture.2013.02.028

Ponce-Palafox, J., Martinez-Palacios, C. A., & Ross, L. G. (1997). The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Boone, 1931. *Aquaculture*, *157*, 107–115. https://doi.org/10.1016/S0044-8486(97)00148-8

Ray, A. J., Shuler, A. J., Leffler, J. W., & Browdy, C. L. (2009). Microbial ecology and management of biofloc systems. In B. L. Craig, & D. Jory (Eds.), *The rising tide, proceedings of the special session on sustainable shrimp farming, world aquaculture 2009* (pp. 255–266). Baton Rouge, LA: The World Aquaculture Society.

Richmond, A. (2004). *Handbook of microalgal culture: Biotechnology and applied phycology*. Ames, IA: Blackwell Science Ltd a Blackwell Publishing Company.

Samocha, T. M., Prangnell, D. I., Hanson, T. R., Treece, G. D., Morris, T. C., Castro, L. F., & Staresinic, N. (2017). *Design and operation of super intensive, biofloc-dominated systems for indoor production of the Pacific White Shrimp,* Litopenaeus vannamei *– The Texas A&M AgriLife Research Experience*. Baton Rouge, LA: The World Aquaculture Society.

Shiau, S. Y. (1998). Nutrient requirements of penaeid shrimps. *Aquaculture*, *164*, 77–93. https://doi.org/10.1016/S0044-8486(98)00178-1.

Silva, K. R., Wasielesky, J. R., & Abreu, P. C. (2013). Nitrogen and phosphorus dynamics in the biofloc production of the Pacific White Shrimp, *Litopenaeus vannamei*. *Journal of the World Aquaculture Society*, *44*, 30–41. https://doi.org/10.1111/jwas.12009

Underwood, G. J. C., Boulcott, M., Raines, C. A., & Waldron, K. (2004). Environmental effects on exopolymer production by marine benthic diatoms: Dynamics, changes in composition and pathways of production. *Journal of Phycology*, *40*, 293–304.

Valle, B. C. S., Dantas, E. M. Jr, Silva, J. F. X., Bezerra, R. S., Correia, E. S., Peixoto, S. R. M., & Soares, R. B. (2015). Replacement of fishmeal by fish protein hydrolysate and biofloc in the diets of *Litopenaeus vannamei* postlarvae. *Aquaculture Nutrition*, *21*, 105–112. https://doi.org/10.1111/anu.12149

Van Wyk, P. (2006). Production of *L. vannamei* in recirculating aquaculture systems: Management and design considerations. In: T. T. Rakestraw, L. S. Douglas, L. Marsh, L. Granata, A. Correa, & G. J. Flick (Eds.), Proceedings of the 6th international conference on recirculation aquaculture (pp. 38–47). Roanoke, VA.

Van Wyk, P., Scarpa, J., et al. (1999). Water quality and management. In P. Van Wyk (Ed.), *Farming marine shrimp in recirculating freshwater systems* (pp. 128–138). Tallahassee, FL: Department of Agriculture and Consumer Services.

Walne, P. R. (1966). Experiments in the large scale culture of the larvae of Ostrea edulis. *Fish Investigation*, *25*, 1–53.

Watanabe, K., Takihana, N., Aoyagi, H., Hanada, S., Watanabe, Y., Ohmura, N., et al. (2005). Symbiotic association in *Chlorella* culture. *FEMS Microbiology Ecology*, *51*, 187–196.

Wickins, J. F., & Lee, D. O'C. (2002). *Crustacean farming – Ranching and culture* (2nd ed.) Oxford, UK: Blackwell Science.

Wychen, V. S., & Laurens, L. M. L. (2013). Determination of total lipids as fatty acid methyl esters (FAME) by in situ transesterification. NREL.

Van Wyk. (1999). Nutrition and feeding of Litopenaeus vannamei in intensive culture systems. In: P. Van Wyk, M. Davis-Hodgkins, R. Laramore, K. L. Main, J. Mountain, & J. Scarpa (Eds.), *Farming marine shrimp in recirculating freshwater systems* (pp. 125–140). Fort Pierce, FL: Florida Department of Agriculture and Consumer Services - Harbor Branch Oceanic Institute.

Zhao, P., Huang, J., Wang, X. H., Song, X. Z., Yang, C. H., Zhang, X. G., & Wang, G. C. (2012). The application of bioflocs technology in high-intensive, zero exchange farming systems of *Marsupenaeus japoni- cus*. *Aquaculture*, *354–355*, 97–106. https://doi.org/10.1016/j.aquaculture.2012.03.034

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