UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

EFEITO DA ADIÇÃO DE *Navicula* sp. E *Brachionus plicatilis* NA TAXA DE INFECÇÃO DE *Litopenaeus vannamei* DESAFIADOS COM O VÍRUS DA SÍNDROME DE MANCHA BRANCA (WSSV) NA FASE BERÇÁRIO E CULTIVADOS EM SISTEMA INTENSIVO

ALLYNE ELINS MOREIRA DA SILVA

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

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Dedicatória

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família e ao meu noivo. Eles foram
meus incentivadores nesta jornada.

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Resumo

Atualmente na carcinicultura, o sistema intensivo é uma promissora técnica por ter uma maior biosseguridade em relação aos cultivos tradicionais, principalmente quanto aos desafios com o Vírus da Síndrome da Mancha Branca (WSSV). Sendo assim, o objetivo do estudo foi avaliar o efeito da adição conjunta e isolada de *Navicula* sp. e *Brachionus plicatilis* sob o desempenho zootécnico, qualidade da água, concentração de víbrios e resistência ao WSSV de *L. vannamei* cultivados na fase berçário em bioflocos. O trabalho foi dividido em duas fases, a primeira fase consistiu em quatro tratamentos: BFT (sistema biofloco); BFT-N (BFT com a adição de *Navicula* sp); BFT-B (BFT com a adição *Brachionus plicatilis*); BFT-BN (BFT com a adição de *Navicula* sp. e *Brachionus plicatilis*), todos em triplicata e; a segunda fase, constituída pelo desafio viral, também em triplicata. Os camarões (PL_{10}, 2 mg) foram estocados na densidade de 3000 camarões m^{-3} e alimentados quatro vezes ao dia com ração comercial com 45% de proteína bruta. O alimento vivo foi adicionado a cada 10 dias na densidade de 10 x 10^4 cel mL^{-1} para *Navicula* sp. e 30 org mL^{-1} para o *Brachionus plicatilis*. Após 45 dias de cultivo, os animais foram submetidos ao desafio viral, durante 21 dias, na densidade de 300 cam m^{-3}, com todos os tratamentos com e sem a inoculação de WSSV. Os resultados obtidos na primeira fase demonstraram diferença significativa no peso final e produtividade nos animais dos tratamentos que receberam adição isolada e conjunta da diatomácea e rotífero e a predominância de colônias com sacarose positiva na quantificação de *Vibrio* sp. Enquanto na segunda fase, observou-se que o tratamento controle e BFT-B alcançaram mortalidade total primeiramente. Entretanto, em todos os tratamentos houve perda total dos animais e a infecção por WSSV foi confirmada via nested-PCR e histopatologicamente, com a presença de nódulos e deformações teciduais causados pelo vírus. Em suma, embora o acréscimo do plâncton tenha resultado em melhores índices zootécnicos do *L. vannamei* no sistema de berçário intensivo, em termos de resistência à infecção por WSSV, não foram determinadas reduções nas taxas de infecção ou de mortalidade dos animais desafiados e infectados sob as condições experimentais avaliadas.

Palavras-chave: vírus, rotífero, diatomácea, bioflocos, desempenho zootécnico.

Abstract

Currently in shrimp farming, intensive system is a promising technique because it has greater biosecurity in relation to traditional culture, especially regarding the challenges with the White Spot Syndrome Virus (WSSV). The aim of this study was to evaluate the effect of the combined and isolated addition of *Navicula* sp. and *Brachionus plicatilis* on zootechnical performance, water quality, *Vibrio* concentration and WSSV infection of *L. vannamei* reared in nursery with biofloc system. The study was divided into two phases, the first phase consisting of four treatments: BFT (biofloc); BFT-N (BFT with the addition of *Navicula* sp.); BFT-B (BFT with the addition of *Brachionus plicatilis*) and; BFT-BN (BFT with the addition of *Navicula* sp. and *Brachionus plicatilis*), all in triplicate and; the second phase, it was constituted by viral challenge, also in triplicate. Shrimp (PL_{10}, 2 mg) were randomly separated and distributed in the experimental tanks at 3000 shrimp m^{-3} and fed four times a day with a 45% protein commercial shrimp diet. Live food was added every 10 days at a density of 10x 10^4 cel mL^{-1} for *Navicula* sp. and 30 org mL^{-1} for *Brachionus plicatilis*. After 45 days of experiment, the animals were infected with WSSV using minced tissue (21 days) at density of 300 cam m^{-3}. Results indicate that for the first phase, final weight and yield were statistically significant for all treatments with isolated and combined addition of diatoms and rotifers. In this phase, there was a predominance of positive sucrose colonies in *Vibrio* quantification. Massive mortality rate was firstly achieved in control (BFT) and BFT-B treatments in the second phase. However, all treatments achieved 100% of mortality rate and the WSSV infection was confirmed by nested-PCR and histopathologically, with lesions suggestive of WSD (presence of nodules and tissue deformations). Although the addition of live food resulted in best zootechnical indexes of *L. vannamei* in intensive nursery system, in terms of resistance to WSSV infection, there was no reduction in infection and mortality rates in challenged animals under the experimental conditions examined.

Key words: virus, rotifers, diatoms, biofloc, zootechnical performance.

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1. Introdução

Atualmente, a carcinicultura mundial é responsável por produzir 7,8 milhões de toneladas, correspondendo a US$ 57,1 bilhões de dólares, sendo quase sua totalidade concentrada em espécies de crustáceos marinhos, com aproximadamente 62% do total produzido (LUCAS & SOUTHGATE, 2012; FAO, 2018). Das espécies marinhas, o camarão *Litopenaeus vannamei* é a principal espécie de crustáceo cultivada mundialmente, representando em 2016, aproximadamente 4,1 milhões de toneladas (FAO, 2018). No Brasil, o cultivo desta espécie corresponde a 20,6% da produção aquícola nacional, atingindo em 2015, uma produção de 69,86 mil toneladas, com destaque a Região Nordeste, responsável por quase a totalidade da produção nacional (99,3%) (IBGE, 2017).

Entretanto, surtos de doenças de etiologia viral têm ocasionado perdas significativas de aproximadamente US$ 15 bilhões de dólares na produção de *L. vannamei* (COSTA et al., 2011; KARUNASAGAR & ABABOUCH, 2012; MALDONADO et al., 2016; YU et al., 2017). A Síndrome da Mancha Branca (WSD) é a principal responsável por estas perdas produtivas (LIN et al., 2011). No Brasil, os primeiros surtos de WSSV ocorreram em 2005 no estado de Santa Catarina, contudo somente em 2008 o vírus chegou ao Sul da Bahia, se disseminando em 2011 por Sergipe, Pernambuco, Paraíba, Rio Grande do Norte e Ceará, resultando em um decréscimo na produção de 26% entre 2015 e 2016 (SEIFFERT et al., 2005; FEED & FOOD, 2017)

O Vírus da Síndrome da Mancha Branca (WSSV) é um vírus envelopado de DNA de dupla fita (dsDNA) e o agente etiológico da WSD. Caracterizado por alta virulência, este agente patogênico apresenta um ciclo de replicação de aproximadamente 20 horas a 25ºC, sendo capaz de infectar diferentes hospedeiros incluindo crustáceos decápodas, carídeos, moluscos bivalves, poliquetas e outros invertebrados (LO et al., 1996; SÁNCHEZ-PAZ, 2010; VAZQUEZ-BOUCARD et al., 2012). Em *L. vannamei* resulta em taxas de mortalidade de 100% nos camarões infectados no período de 3 a 10 dias, com transmissão horizontal e vertical (SÁNCHEZ-PAZ, 2010; LIGHTNER et al., 2012; CLARK, 2016; OIE, 2018). Os sinais clínicos da doença consistem em manchas brancas múltiplas e descolaração no exoesqueleto entre as tonalidades vermelha a pálida, comportamento letárgico e redução de ingestão de alimentos (TAKAHASHI et al., 1994; SOON et al., 2001; MALDONADO et al., 2016).

Em termos de resposta imunológica a infecções virais, nos crustáceos, as barreiras físicas, como exoesqueleto e trato digestivo quitinosos são os primeiros obstáculos para contenção dos microrganismos. Entretanto, quando há danos a estas estruturas, o patógeno invade o tecido com posterior ativação do sistema imune inato através dos hemócitos

(AGUIRRE-GUZMAN et al., 2009;VAZQUEZ et al., 2009; UNDERWOOD et al., 2013; EKASARI et al., 2014; NIU et al., 2018). No caso de infecção por WSSV, após ingestão de tecido contaminado pelo camarão, o vírus atravessa a membrana basal do trato digestivo e se insere no núcleo dos hemócitos circulantes, com posterior replicação viral nestas células e respectivo declínio significativo em seu número após a infecção (SAHUL-HAMEED et al., 2006; DI LEONARDO et al., 2005), o que torna a análise das variáveis imunológicas e alterações histológicas importantes ferramentas na compreensão da dinâmica viral no hospedeiro.

Dentre as estratégias e estudos para o controle desta virose destacam-se a pré-exposição do camarão a patógenos de etiologia viral de baixa virulência baseando-se na teoria de interferência viral; a manipulação térmica para inibição de replicação viral; a oferta de extratos vegetais para promoção da saúde animal e o uso de dsRNA sintético para indução de resposta antiviral via RNA de interferência (RNAi) (SÁNCHEZ-PAZ, 2010; YAN et al., 2016; NILSEN et al., 2017). Além destas estratégias, a implantação de sistemas produtivos aquícolas com alto grau de biosseguridade, também tem sido avaliada.

Neste sentido, o sistema intensivo é uma tecnologia promissora e consiste em reduzida troca de água do sistema de cultivo diminuindo a probabilidade da entrada de patógenos. Este sistema consegue, por meio do balanço da relação carbono-nitrogênio, promover o desenvolvimento de agregados microbianos (bioflocos), que servirão como fonte de alimento adicional ao animal cultivado (RAJKUMAR et al., 2016). Além disso, o desenvolvimento do biofloco também é responsável pelo controle da qualidade da água, em particular, dos compostos nitrogenados (XU et al., 2016), o que auxilia na manutenção da homeostase do animal.

Para mais, o uso da fertilização orgânica a partir do processo anaeróbio e aeróbio de carboidratos pode otimizar o desenvolvimento de bactérias benéficas, diminuindo as patogênicas no sistema (MARTÍNEZ-CORDOVA et al., 2015). Segundo Romano et al. (2018), o uso desse processo no cultivo de juvenis de *Clarias gariepinus* melhora o desempenho zootécnico do animal e a composição nutricional do floco, além da qualidade da água do sistema como um todo. Esta estratégia seria uma solução viável a problemas como a alta concentração de *Vibrio* sp., que é um microrganismo patogênico oportunista natural, sendo possível, portanto, controlar esse desenvolvimento bacteriano a partir das aplicações do produto gerado a partir do processo anaeróbio e aeróbio da fertilização orgânica de carboidratos (PATEL et al., 2018).

Entretanto, estas características benéficas são fortemente relacionadas à qualidade do agregado bacteriano formado. Rajkumar et al. (2016), ao avaliarem a composição dos

microorganismos que compõem o floco microbiano, observaram presença do plâncton, bactérias e compostos orgânicos podem ser utilizados com fonte complementar de alimento para *L. vannamei*. Contudo, estudos demonstram que os flocos microbianos podem apresentar deficiência na concentração de aminoácidos, principalmente lisina e metionina (DANTAS et al., 2016) e na concentração de ácidos graxos (DANTAS et al., 2014 MAGAÑA-GALLEGOS et al., 2018), sendo estes, fatores potencializadores no desenvolvimento do animal (LIN et al., 2015; NESARA E PATURI, 2018). Neste sentido, estudos voltados à adição de microorganismos com boa qualidade nutricional (aminoácidos e ácidos graxos) no intuito de melhorar a qualidade nutricional do floco microbiano, por conseguinte, o desempenho zootécnico do animal cultivado podem ser estratégias bastante promissoras (CRAB et al., 2012; EKASARI et al., 2014; MALIWAT et al., 2017; SHAH et al., 2018).

Os organismos planctônicos (fitoplâncton e zooplâncton) são fontes ricas de aminoácidos, ácidos graxos essenciais (EPA e DHA) e minerais, sendo importantes nutricionalmente, em particular, para as fases iniciais dos peixes e camarões (JU et al., 2012; MEDINA- FÉLIX et al., 2014; JAMALI et al., 2015; GE et al., 2016; SCHAAL et al., 2016; KURMAR et al., 2017). Esta suplementação é evidenciada no estudo de Abreu et al. (2019) que observaram o aumento de aproximadamente três vezes mais 20:5 n-3 (EPA) e 22:6 n-3 (DHA) nos flocos microbianos com adição da *Navicula* sp. em relação ao controle, sem adição da microalga, além do maior desempenho zootécnico do camarão marinho. Outras pesquisas também demostram que a adição dos microrganismos nos sistemas de cultivo contribuem para maior desempenho zootécnico dos camarões. Brito et al. (2016), demonstraram que o uso de plâncton (*Bacillariophyceae* combinada com *Rotifera*) melhorou o crescimento do camarão e aumentou o seu teor de proteína corporal. Resultados semelhantes inoculando alimento vivo no cultivo de *L. vannamei* podem ser observados nos estudos de Marinho et al. (2014; 2017), Jamali et al. (2015), Martins et al. (2016) e Félix et al. (2017).

Além da contribuição alimentar, o plâncton produz carotenóides, que são pigmentos que contém um efeito positivo na melhora do crescimento e rendimento e redução da taxa de mortalidade dos camarões, por atuarem como imunestimulantes (LÓPEZ-ELÍAS et al., 2016; WADE et al., 2017). Abreu et al. (2019), avaliando a administração dietética de *Navicula* sp. em pós-larvas de camarão marinho, descrevem que a sua presença auxilia no *status* imune dos animais submetidos a estas dietas em comparação com o grupo controle, essa ação é corroborada pelo enriquecimento da composição do floco, como demonstrado no estudo. Sendo assim, novas possibilidades para a realização de investigações sobre o efeito da adição de plâncton em relação à resposta imunológica e infecção viral de *L. vannamei* em sistema de

bioflocos durante a fase de berçário tornam-se cruciais ao entendimento destas suplementações no sistema produtivo.

2. Objetivos

2.1 Objetivo geral

Avaliar o efeito da adição de *Navicula* sp. e *Brachionus plicatilis* na resistência ao Vírus da Síndrome da Mancha Branca em *L. vannamei* cultivados em bioflocos.

2.2 Objetivos específicos

- Avaliar o desempenho zootécnico (sobrevivência; ganho em peso; taxa de crescimento específico e conversão alimentar aparente) dos camarões cultivados em berçários com adição de plâncton pré e pós-desafio com WSSV;
- Determinar as variáveis hematológicas dos camarões desafiados e sobreviventes mantidos em berçário com adição de plâncton;
- Determinar a taxa de infecção dos camarões desafiados por WSSV mantidos em berçário com adição de plâncton.

2.3 Hipótese

A adição de *Navicula* sp. e *Brachionus plicatilis* em conjunto ou isolados no sistema de bioflocos melhora o desempenho zootécnico do *L. vannamei* e aumenta a resistência a infecções virais quando desafiados com WSSV.

3. Artigo científico

Artigo científico a ser encaminhado à Revista Aquaculture (*ISSN: 0044-8486*)

Todas as normas de redação e citação, deste artigo, atendem aquelas estabelecidas pela referida revista.

Effect of *Brachionus plicatilis* and *Navicula* sp on pacific white shrimp growth performance, *Vibrio*, immunological responses and resistance to white spot vírus (WSSV) in nursery biofloc system

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Abstract

The purpose of this study was to evaluate the effect of *Brachionus plicatilis* and *Navicula* sp addition on growth performance, *Vibrio* count, immunological responses and resistance to white spot syndrome virus (WSSV) of *Litopenaeus vannamei* in nursery biofloc system. The study was divided into two phases and four treatments were examined in the first phase: BFT (biofloc); BFT-N (addition of 10×10^4 cells ml^{-1} of *Navicula* sp.); BFT-B (addition of 30 org ml^{-1} of *B. plicatilis*) and; BFT-BN (addition of 10×10^4 cells ml^{-1} of *Navicula* sp. and 30 org ml^{-1}

of *B. plicatilis*), all in triplicate and; the second phase, it was constituted by viral challenge, also in triplicate. Shrimp ($PL_{10,}$ 2 ± 0,01 mg) were reared in biofloc system (fertilized with inorganic and anaerobic and aerobic carbon process with a microbial mix), for 45 days on density at 3,000 shrimp m-³, with controlled temperature, without water exchange and fed with commercial feed. The diatoms and rotifers were added every 10 days, and at the end, shrimp performance, water quality, *Vibrio* count and total hemocyte count were evaluated. After 45 days, 120 shrimp per treatment (300 shrimp m^{-3}) were challenged with WSSV ($2.2x10^7$ copies of WSSV μL^{-1} of DNA) for 21 days. The plankton addition treatments had higher shrimp performance values, highlighting the values of final weight (1.02 to 1.16 g) and yield (2.85 to 3.30 kg m^{-3}) which were higher than the BFT (0.86 g and 2.46 kg m^{-3}). There were no differences in alkalinity, TAN, $N\text{-}NO_2$, $N\text{-}NO_3$ and PO_4 among treatments. *Vibrio* count from water samples was significantly lower at the end of experiment with significantly decrease of sucrose-negative bacteria. However, for hepatopancreas, the *Vibrio* count was significantly higher at the end with significantly decrease of sucrose-negative bacteria (98 to 100%). There is no significant difference between the THC and hyalyne count for all treatments. Massive mortality rate was firstly achieved in BFT and BFT-B treatments in the second phase. However, all treatments achieved 100% of mortality rate and the WSSV infection was confirmed by nested-PCR and histopathologically, with lesions suggestive of WSD (presence of nodules and tissue deformations). The *Navicula* sp. and *B. plicatilis* addition contributed to better zootechnical performance parameters of shrimp in the nursery biofloc system, although there was no significant effect on the WSSV resistance of shrimp. Absence of tissue degeneration in WSSV infected shrimp from BFT-N treatment indicated benefit diatoms uses under standardized conditions.

Key words: rotifers, diatoms, sucrose-positive bacteria, viral resistance, carbon fermentation.

1. Introduction

Shrimp farming is a branch of aquiculture that corresponds to the production of approximately 7.8 million tons, 52.9% of which is related to the production of the whiteleg shrimp, *Litopenaeus vannanmei* (FAO, 2020). However, the emergence of pathogens of a viral and bacterial etiology has played a crucial role in the decline of this productivity (IBGE, 2017; Rebouças et al., 2017). Among such diseases, white spot syndrome virus (WSSV), which is caused by a highly pathogenic virus, has led to economic losses of approximately US$ 15 billion in shrimp farming activities involving *Litopenaeus vannamei* (Costa et al., 2011). WSSV can cause the death of 100% of infected marine shrimp in a period of only three to 10 days. The main clinical signs are multiple white spots, lethargic behavior, discoloration of the exoskeleton, and a reduction in food intake (Takahashi et al., 1994; Soon et al., 2001; Sánchez-Paz, 2010; Lightner et al., 2012; Karunasagar and Ababouch, 2012; Clark, 2016; Maldonado et al., 2016; Yu et al., 2017; OIE, 2018).

A culture system with minimal water exchange and thermal control is promising with regards to minimizing the effects of WSSV (Kakoolaki et al., 2015; Aalimahmoudi et al., 2017). However, certain problems emerge in this type of system due to the excessive increase in the organic load and high temperatures, which can lead to the proliferation of *Vibrio* sp. Despite belonging to the natural flora of the marine environment, *Vibrio* sp. can cause opportunistic infection, resulting in significant economic losses of approximately 10 million dollars (Ching, 2017). This pathogenic agent is harmful when the exposed organism has a suppressed natural defense mechanism and can cause a mortality rate of up to 100% (Biju and Gunalan, 2016; Rebouças et al., 2017; Patel et al., 2018).

Thus, novel concepts have been employed in aquiculture involving a balance between the encouragement of beneficial bacteria through the aerobic and anaerobic process of carbon

and an increase in the plankton community, which results in an improvement in water quality and serves as a complementary food source (Romano et al., 2018). This combination of prebiotics (carbon aerobic and anaerobic process) and probiotic microorganisms releases organic acids that reduce the concentration of sucrose-negative bacteria (*Vibrio* sp.) (Romano et al., 2018).

The adequate selection of microorganisms to introduce into culture systems is essential to the growth performance of the farmed animals, as bacteria and plankton have both antagonistic and symbiotic actions (Erken et al., 2015; Farisa et al., 2019). According to Hoseinifar et al. (2016), the use of *Bacillus* sp. in the water leads to the control of nitrogen compounds and enhances the growth performance of the animals and the use of *Saccharomyces cerevisiae* reduces the development of pathogens responsible for deaths caused by the genus *Vibrio* (Aguilera-Rivera et al., 2014). Likewise, phytoplankton and zooplankton diminish the prevalence of *Vibrio* sp. in the system due to competition for nutrients in the environment (Erken et al., 2015). There is also evidence that plankton inhibits the growth of *Vibrio harveyi* (Farisa et al., 2019).

Diatoms and rotifers can enhance the nutritional quality of microbial flocs (Crab et al., 2012; Ekasari et al., 2014; Maliwat et al., 2017; Shah et al., 2018; Abreu et al., 2019), which are deficient in the amino acids lysine and methionine (Dantas et al., 2016) and fatty acids (EPA and DHA) (Magaña-Gallegos et al., 2018). Examples of this are *Brachionus plicatilis* contains (in dry weight) approximately 40% proteins, with the possibility of the bioencapsulation of polyunsaturated fatty acids and pigments (Jamali et al., 2015; Ferreira et al., 2018) and *Navicula* sp. has (in dry weight) 13 to 50% protein, 10 to 28% carbohydrates, and 4 to 8% lipids (Viçose et al., 2012; Kang et al., 2012), mainly providing essential polyunsaturated fatty acids (Das et al., 2012).

These organisms are important nutritional sources, especially with regards to essential fatty acids (DHA and EPA), which are crucial to the early phases of farmed shrimp (Ju et al., 2012; Medina- félix et al., 2014; Jamali et al., 2015; Ge et al., 2016; Schaal et al., 2016; Kurmar et al., 2017). Abreu et al. (2019) investigated the effects of the addition of *Navicula* sp. on the composition of the biofloc, reporting an approximately threefold increase in 20:5 n-3 (EPA) and 22:6 n-3 (DHA) in comparison to treatments to which this diatom was not added. Brito et al. (2016) evaluated the introduction of *B. plicatilis* and *Navicula* sp. separately and together into shrimp nurseries and found a better growth performance and higher protein content in the meat following the combined addition of these organisms.

Besides being a complementary food source, microalgae have bioactive compounds (carotenoids), such as fucoxanthin, which assist in the development of the animal and contribute to the immune system. Evaluating *L. vannamei* fed commercial diets enriched with *Dunaliella* sp. followed by a WSSV challenge, Médina-Félix et al. (2014) found benefits to the immune response of the challenged animals. Similar results with the use of live feed were reported by Niu et al. (2018), Anaya-Rosas et al. (2019), and Wang et al. (2015). Rotifers are primary consumers and therefore absorb pigments from microalgae, making these substances available to the higher trophic level (Martínez-Cordova et al., 2015; Cezare-Gomes et al., 2019), and therefore can serve as immunostimulants for shrimp (Wang et al., 2015).

The aim of the present study was to evaluate the effect of the addition of *B. plicatilis* and *Navicula* sp. separately and together on growth performance, water quality, the concentration of *Vibrio* sp., and resistance to WSSV in *L. vannamei* in the nursery phase in a biofloc system.

2. Materials and Methods

2.1 Experimental design

The experiment was conducted in two phases: (1) submission of *L. vannamei* post-larvae (PL_{10}) to four treatments [biofloc technology (BFT); BFT-N (BFT with the addition of *Navicula* sp.); BFT-B (BFT with the addition of *Brachionus plicatilis*); and BFT-BN (BFT with the addition of *Navicula* sp. and *B. plicatilis*)] in an entirely randomized design; and (2) submission of animals from these treatments to a viral challenge. Both experiments were conducted in triplicate.

Forty days prior to the stocking of the shrimp for the first phase of the experiment, a matrix tank with 1.2 m³ of working volume was filled with salt water (35 g L^{-1}) that had been previously filtered (30 μm), chlorinated with active chlorine at 15 ppm using sodium hypochlorite, and de-chlorinated through constant aeration. Next, single inorganic fertilization was performed with urea (4.5 g m^{-3} N), triple superphosphate (0.45 g m^{-3} P), and sodium silicate (0.23 g/m³ Si). Fifteen organic fertilizations were then performed with the anaerobic (48 h) and aerobic (24 h) process of wheat bran (22.5 g m^{-3}), sugarcane molasses (12 g m^{-3}), sodium bicarbonate (4.5 g m^{-3}), and 0.5 g m^{-3} of a commercial product composed of *Bacillus subtilis, B. licheniformes*, *Saccharomyces* sp., and *Pseudomonas* sp. in a total of 7.7 x 10^8 colony-forming units (CFUs) g^{-1} (Kayros Ambiental e Agrícola, São Paulo, Brazil), with a two-day interval between fertilizations.

The inputs were homogenized in 500 mL of salt water (30 g L^{-1}) in the anaerobic phase, with the addition of another 500 mL in the aerobic phase. In both phases, the pH was maintained close to 7 with the aid of sodium bicarbonate. Two days prior to the stocking of the shrimp, the water from the matrix tank (total ammoniacal nitrogen 0.07 mg L^{-1}, N-NO_2 0.08 mg L^{-1}, N-NO_3 0.87 mg L^{-1}, alkalinity 175 $CaCO_3$ mg L^{-1}, pH 8.0, salinity 28 g L^{-1}, P-orthophosphate 26.56 mg L^{-1}, and settleable solids 0 mg L^{-1}) was mixed and equally distributed among 12 black polypropylene experimental units (50 L, 50 × 35 × 23 cm).

The experimental units were constantly aerated by three aeration stones per tank, leading to a concentration of approximately 5.20 mg L^{-1} of dissolved oxygen. No exchanges of water were performed during the entire experiment, with the exception of the addition of de-chlorinated freshwater to compensate for loss due to evaporation. The light intensity was maintained at 27 μmol m^{-2} s^{-1}, with a natural photoperiod of approximately 12 h of light/dark, and the temperature was maintained at 30ºC with the use of a thermostat. Sodium bicarbonate ($NaHCO_3$) was used to maintain alkalinity above 150 mg L^{-1} and pH close to 7.8.

L. vannamei post-larvae (PL_{10} ± 0.02 mg) negative for WSSV (based on the confirmatory diagnosis) were acquired from a commercial lab (Aquatec LTDA, RN, Brazil) and acclimatized for four hours prior to the onset of the experiment. The animals were stocked at a quantity of 120 per experimental unit, resulting in a density of 300 animals m^{-3}, which is the density found on commercial farms.

The shrimp were fed four times per day (8:00, 11:00, 14:00, and 16:00 horas) using a commercial ration with 45% crude protein and 8% lipids. The feed rate was initiated with 35% of body weight per day and gradually lowered to 10%. Unconsumed ration was not removed. The weight of the animals was determined weekly per tank to monitor growth and adjust the quantity of ration.

Diatoms were added on the 1^{st}, 10^{th}, 20^{th}, and 30^{th} days (10×10^4 cells mL^{-1}) to the BFT-N (BFT with the addition of *Navicula* sp.) and BFT-BN (BFT with the addition of *Navicula* sp. and *B. plicatilis*) treatments, corresponding to the addition of approximately 1.2 L of diatoms in each experimental tank, based on Marinho et al. (2014; 2017), Brito et al. (2016), and Abreu et al. (2019). Rotifers were added on the 1^{st}, 10^{th}, 20^{th}, and 30^{th} days (30 organisms L^{-1}) to the BFT-B (BFT with the addition of *B. plicatilis*) and BFT-BN (BFT with the addition of *Navicula* sp. and *B. plicatilis*) treatments, corresponding to the addition of approximately 1,200organisms L^{-1} in each experimental tank, based on Brito et al. (2016). The following aspects were

evaluated during this first phase: water quality variables, total presumptive count of bacteria of the genus *Vibrio* in the water and in the animals, growth performance of the animals, as well as the total and differential hemocyte counts.

After 45 days of the experiment, the viral challenge was conducted to evaluate the effect on the addition of *Navicula* sp. and *B. plicatilis* on the infection rate and survival of *L. vannamei* cultured in a biofloc system. For this step, the shrimp were maintained at a stocking density of 300 animals m^{-3} (average stocking density used on commercial farms). The same four treatments were used (three replicates each) with and without inoculation with WSSV: (1) BFT-N, (2) BFT-B, (3) BFT-BN, and (4) BFT. The units in this phase had a working volume of 14 L. All units were covered with a screen to contain aerosols and maintained under constant intense aeration, with light intensity of 27 μmol m^{-2} s^{-1}, a 12-h light/dark photoperiod, and a temperature of 28°C, which enables the replication of WSSV (Withyachumnarnkul et al., 2003).

During the challenge, the animals in all treatments were fasted for 24 hours and then fed chopped contaminated tissue [abdominal muscle of *L. vananmei* with 2.2 x 10^7 copies of WSSV/μL of DNA, quantified based on Durand and Lightner (2002)] twice per day at 10% of the biomass per day, with a three-hour interval between inoculations for 24 hours (Pérez et al., 2005). After this period, the animals were fed a commercial feed as described above and were monitored daily for 21 days for the observation of clinical signs and mortality.

All dead and surviving individuals were collected, identified, and stored at -80°C until analysis using nested-PCR for the confirmation of infection by WSSV and the determination of cumulative mortality.

2.2 Production of *Navicula* sp. and *Brachionus plicatilis*

The diatom (*Navicula* sp.) and rotifer (*Brachionus plicatilis*) were acquired from the Live Food Production Lab (LAPAVI, DEPAq, UFRPE). *Navicula* sp. was cultivated in Conway medium (Walne, 1966) with the addition of vitamin C (0.2 mL L^{-1}) C and sodium silicate (2 mL L^{-1}) in water with 30 g L^{-1} salinity (filtered and chlorinated with 15 ppm of active chlorine), pH 8.0, at a temperature of 25 ± 1°C and light intensity maintained at 37 μmol m^{-2} s^{-1} using a fluorescent light with a controlled photoperiod. The diatom had the following composition: 14.32% dry matter, 216.93 g of crude protein/kg of dry matter, and 106.93 g of lipids kg of dry $matter^{-1}$. These values were determined previously.

L-type rotifers (size: 198 μm) were grown in salinity 30 g L^{-1} (with filtered water chlorinated with 15 ppm of active chlorine, as indicated on the product) with the addition of vitamin C (0.4 mL L^{-1}) and fish oil (0.15 g L^{-1}) (360 mg EPA and 240 mg DHA) every two days, which was suspended two days prior to use in the productive units. The culture characteristics of the water were pH 8.0, temperature of 25 ± 1°C, and light intensity maintained at 37 μmol m^{-2} s^{-1} using a fluorescent light with a continuous photoperiod (24 h of light per day). The organisms were filtered through 50-μm mesh (approximate body size: 150 to 250 μm) and density was estimated using a Sedgewick-Rafter chamber to enable the addition of organisms to the experimental units at a density of 30 org mL^{-1}. The rotifer had the following composition: 13.48% dry mater, 483.79 g of crude protein/kg of dry matter, and 194.93 g of lipids/kg of dry matter. These values were determined previously.

2.3 Water quality

Dissolved oxygen, temperature, salinity, and pH were determined (YSI55 model, Yellow Springs, Ohio, USA) twice per day (8:00 and 16:00). Alkalinity ($CaCO_3$ mg L^{-1}) (APHA, 2005), oxygen reduction potential (ORP) (YSI model 55, Yellow Springs, Ohio, USA), settleable solids (SS) (Imhoff cone) (Avnimelech, 2009), and calcium carbonate were

monitored three times per day. Total ammoniacal nitrogen (TAN) and nitrogen-nitrite ($N\text{-}NO_2$) were measured weekly using the methods described by Koroleff (1976) and Golterman, Clymo, & Ohnstad (1978), respectively. Nitrogen-nitrate ($N\text{-}NO_3$) and orthophosphate (PO_4) were monitored twice per week using the methods described by Mackereth, Heron, & Talling (1978) and APHA (2005), respectively. If the SS value surpassed 8 mL L^{-1}, a decanter was used for 15 minutes with a flow of 0.02 L s^{-1} until reaching a minimum of 5 mL L^{-1} and the fertilizations were automatically suspended.

2.4 Counts of bacteria of the genus *Vibrio* in water and shrimp

For the total presumptive determination of *Vibrio* sp., three water samples were withdrawn from each experimental unit of the treatments with the aid of sterile 50-mL Falcon tubes at the onset and end of the first phase of the experiment. The samples were homogenized in a vortex and seeded in peptone water (2%). After homogenization, five successive serial dilutions were performed (1/10). Next, 100 µL of each diluted sample were seeded on dishes containing thiosulfate-citrate-bile salts-sucrose (TCBS) agar. The dishes were incubated at 30ºC for 24 h. The results were expressed in colony-forming units (CFUs ml^{-1}) and differentiated into sucrose-positive and sucrose-negative colonies.

The total *Vibrio* sp. count in the shrimp was determined through an initial collection of 15 shrimp (prior to stocking) (Vandenberghe et al., 1999) and final collection (after 45 days of experiment), during which five animals were removed from each experimental unit using sterilized Falcon tubes. The specimens were disinfected by immersion in 70% ethanol for 15 seconds, followed by immersion in a sodium hypochlorite solution (1.5%) with 0.1% Tween-80 for 15 minutes and washing three times in sterile distilled water. Next, 100 mg of the hepatopancreas of each animal was macerated in 9 mL of peptone water (2%), followed by five successive serial dilutions (1/10) and the seeding of 0.1 ml on TCBS agar. The dishes were

incubated at 30ºC for 24. The results were expressed as CFUsg-1, with the differentiation of sucrose-positive and sucrose-negative colonies.

2.5 Growth performance

The weight of the shrimp was monitored weekly to determine the growth of the animals and adjust the amount of feed offered. At the end of the first phase of the experiment, biomass gain, specific growth rate (SGR), mean final weight (g), weekly growth (g), feed conversion rate (FCR), survival, and productivity (kg m^{-3}) were determined using the following equations: biomass gain (g) = final biomass (g) – initial biomass (g); SGR (% day $^{-1}$) = 100 x [ln peso final (g) – ln peso initial (g)] / time (days); final weight (g) = final biomass (g) / survival; FCR = feed offered (dry weight) / biomass gain; survival (%) = (number of individuals at end of study period / initial number of individuals) * 100; and productivity (kg m^{-3}) = final biomass (kg) / volume of experimental unit (m^3).

2.6 Hematological variables

At the end of the second phase of the experiments, the hemolymph of the animals was collected for the identification and quantification of immunocompetent cells (hemocytes) using the protocol described by Guertler et al. (2013). The hemolymph was obtained through the hemocele of the abdominal region using an anticoagulant solution [modified Alsever's solution (MAS)] (336 mmol L^{-1} of NaCl, 115 mmol L^{-1} of glucose, 27 mmol L^{-1} of sodium citrate, 9 mmol L^{-1} of EDTA, pH 7.2) at a proportion of 1:2 (v:v). The total hemocyte count (THC) was performed with 100 µl of hemolymph collected with the aid of the MAS fixing agent with 4% formaldehyde. The number of cells was estimated in a Neubauer chamber, in triplicate. The differential hemocyte count (DHC) was performed using a protocol adapted from Celi et al. (2013). The classification of hyaline, semi-granular, and granular hemocytes was estimated

from 10 µl, in triplicate, fixed on slides subsequently stained with May–Grünwald–Giemsa stain.

2.7 DNA extraction and nested PCR

At the end of the second phase (viral challenge), all animals were submitted to nested PCR for the confirmation of infection by WSSV. DNA was extracted using the protocol of the Wizard® Genomic DNA Purification kit (Promega) with some modifications, using 100 mg of gill tissue (pool of three animals from each treatment). The extracted DNA was quantified using spectrophotometry and then stored at -20ºC until use in the nested-PCR analysis. The DNA was also qualified in 0.8% agarose gel with ethidium bromide.

The nested-PCR analysis was conducted for the detection of WSSV in the tissue samples used in the preparation of the inoculum and in the challenged animals [pools of three animals from each treatment (living and dead animals throughout the experiment)]. The method recommended by the World Organization of Animal Health (OIE) was used for the confirmatory diagnosis of WSSV in the shrimp, as described by Lo et al. (1996). The reactions from the 1st and 2nd PCRs were conducted with the same concentrations and thermocycling described by Lo et al. (1996). The PCR products were then submitted to electrophoresis in 1% agarose gel stained with ethidium bromide and fragment size was estimated using a 100-bp DNA ladder (Invitrogen). Positive (sample positive for WSSV) and negative (ultrapure water) controls were inserted in all PCRs.

2.8 Histology

Throughout the viral challenge, moribund and healthy (control group) animals in the experimental units were identified (date, collection time, and treatment) and fixed in Davidson AFA solution for 48 hours prior to the histopathological analysis. The analysis of the gills and

stomachs (tissues recommended for the histopathological analysis for WSSV) was conducted based on Bell & Lightner (1948). The tissues were dehydrated in an increasing series of ethanol, followed by clearing in xylol and embedding in paraffin. The tissue blocks were cut in slices measuring 5 µm in thickness, which were stained with hematoxylin-eosin, followed by examination under an optical microscope for the assessment of lesions suggestive of infection by WSSV.

2.9 Statistical analysis

The Cochran and Shapiro-Wilk tests were used for the analysis of equal variances and normality of the data, respectively. Analysis of variance (ANOVA) was used for the comparisons. When significant differences were found between treatments ($P \leq 0.05$), Tukey's test and the Newman-Keuls test were used for the comparison of means ($P \leq 0.05$). The Kruskal-Wallis test ($P < 0.05$) was used for data with non-normal distribution (total *Vibrio* sp. counts), followed by Dunn's post-hoc test ($P \leq 0.05$) when significant differences were detected. All statistical analyses were performed with the aid of ASSISTAT, version 7.6.

3. Results

The data on water quality are displayed in **Table 1**. No significant differences among the treatments were found with regards to nitrogen compounds (TAN, $N\text{-}NO_2$, $N\text{-}NO_3$), alkalinity ($CaCO_3$), orthophosphate (PO_4), or oxygen reduction potential (ORP). In contrast, significant differences were found for dissolved oxygen (DO), salinity, and settleable solids (SS) ($P > 0.05$). The highest DO value was found in the BFT-N treatment (5.29 ± 0.01 mg L^{-1}) and the lowest was found in the BFT-BN treatment (5.21 ± 0.01 mg L^{-1}). The lowest SS value was found in the BFT-N treatment (4.74 ± 0.48 mL L^{-1}).

Regarding the presumptive determination of *Vibrio* sp., all water and animal samples analyzed exhibited bacteria of the genus. In the water, significant differences among treatments were found for the total *Vibrio* sp. count and the characterization of sucrose-positive colonies in the comparison between the initial and final evaluations; the prevalence of sucrose-positive colonies was highest in the BFT-B treatment (96.92%) and the prevalence of sucrose-negative colonies was highest in the BFT-N treatment (36.36%) at the end of the experiment (45 days). In the shrimp, the prevalence of sucrose-positive colonies was highest in the BFT-BN treatment (99.39%) and no sucrose-negative bacteria were recorded in the BFT-B, BFT-N, or BFT treatments at the end of the experiment (45 days) (**Table 2**).

On the temporal scale, sucrose-positive colonies were more prevalent than sucrose-negative colonies in both periods (initial and final evaluation), with significant differences among the treatments in which the inoculation of live feed was performed. The reduction in total counts at the end of the experiment (45 days) was perceptible in all treatments. However, the number of *Vibrio* sp. was the same for all treatments, with a significant difference only regarding the composition of the colonies over time (higher counts of sucrose-positive colonies in all treatments) (**Table 2**).

Regarding growth performance, significant differences were found in final weight, productivity, and specific growth rate (SGR). The best results were achieved with addition of the diatom and rotifer separately and in combination (BFT-N, BFT-B, and BFT-BN). In contrast, no significant difference among treatments was found regarding survival or the FCR (**Table 3**). Moreover, no significant differences were found regarding the total (THC) and differential (DHC) hemocyte counts at the end of the productive cycle of the first phase of the experiment (**Table 4**).

After the viral challenge with WSSV, total mortality was found on the sixth day in the control treatments (without the addition of the rotifer and diatom in phase 1) and the treatments

with the rotifer alone. On the seventh day, the complete loss of the shrimp occurred in the treatments in which only the diatom was administered. Total mortality in the treatments with the combined inoculation of *B. plicatilis* and *Navicula* sp. occurred on the ninth day (**Figure 1**). All infected animals exhibited macroscopic lesions indicative of WSSV, such as white spots on the carapace, opaque musculature, a reddish to brown color, and areas of necrosis.

All challenged animals (second phase of the experiment) were positive for WSSV via nested PCR. Moreover, the histopathological analysis revealed microscopic lesions suggestive of infection by WSSV in treatments, such as basophilic nodules and degeneration of the gill lamellae, in comparison to the non-infected animals (**Figure 2**). In the stomach, the groups without the addition of *Navicula* sp. exhibited basophilic nodules, whereas the infected animals in the BFT-N and BFT-BN treatments did not exhibit these tissue lesions (**Figure 2**).

4. Discussion

The concentrations of the water quality variables (dissolved oxygen, temperature, pH, salinity, and nitrogen compounds) in the treatments were within the ranges recommended for the farming of marine shrimp in an intensive system (Van Wyk & Scarpa, 1999; Samocha et al., 2019). Intensive systems tend to accumulate settleable solids (SS) due to the addition of organic carbon, the minimal water exchange, leftover feed, and fecal matter. It is advisable to maintain the SS value between 10 and 14 mg L^{-1}, as an excess of solids can result in blooms of pathogenic organisms, the clogging of the gills, and a greater biochemical oxygen demand (Samocha et al., 2019). Brito et al. (2016) investigated the influence of the inoculation of *Navicula* sp. and *B. plicatilis* in an intensive system using *L. vannamei* in the nursery phase and found SS values between 5 and 6 mg L^{-1}, reporting no influence of the organisms added. Similar means were found in the present study, but the treatments with the inoculation of *Navicula* sp. had lower values than the other treatments due to the volume of microalgae added to the system,

leading to the dilution of SS and greater water replacement. Moreover, SS values in all treatments were maintained below the critical level reported in the literature (< 10 mL L^{-1}) and, therefore, did not affect the development of the animals.

Regarding the oxygen reduction potential (ORP), which involves chemical and biological phenomena related to water quality and is controlled by the concentration of dissolved oxygen, values between 100 and 300 mV indicate a nitrification process, whereas values between -50 mV and 50 mV suggest a denitrification chain reaction (Vijayan et al., 2017). In the present study, the mean ORP among the treatments ranged from 18.90 to 23.08 mV, which indicates the occurrence of denitrifying bacteria in the culture environment.

Alkalinity consumption occurs through the metabolic activities of the shrimp, phytoplankton, and zooplankton (present in the flocs) as well as the intense metabolic process of bacteria, which need to consume $CaCO_3$ for their development (Furtado et al., 2014; Rajkumar et al., 2016). Therefore, the replacement of inorganic carbon is necessary. In the present study, alkalinity remained near the recommended values for intensive culture systems (140 to 180 mg/L) proposed by Samocha et al. (2019).

The greater production of bacteria of the *Vibrio* sp. with sucrose-positive in the water and shrimp demonstrate the occurrence of bacteria with a lower degree of pathogenicity in the culture environment, as bacteria that cause lethal infections are microbiologically characterized as sucrose-negative species, such as *Vibrio parahaemolyticus* and *V. harveyi* (Ping-Chung et al., 1996; Gomez-Gil et al., 2014; Hostins et al., 2017; Farisa et al., 2019). Regarding the concentration over time, an increase in the total bacterial count and proportions of sucrose-positive species were found in the digestive tract of the animals, likely due to symbiosis between the bacteria and plankton (Erken et al., 2015). This concept is demonstrated in a previous study. Romano et al. (2018) found that the anaerobic and aerobic carbon process encouraged beneficial

bacteria and the plankton community, resulting in a reduction in the load of sucrose-negative bacteria (*Vibrio* sp.).

The beneficial effect of living organisms in the system on growth performance is due to the increase in the digestibility of the ingredients resulting from the addition of plankton, which contributes to an increase in the activity of digestive enzymes (Shah et al., 2018). In the present study, the units to which the diatom and rotifer were added had higher final weight and productivity. This performance is due to the nutritional quality of these organisms, which enrich the microbial flocs, particularly with regards to fatty acids. Studies report EPA and DHA ranging from 5 to 35% and 0.1 to 2.6%, respectively, in *Navicula* sp. as well as from 3 to 10% and 0.5% to 24%, respectively, in *B. plicatilis* (Whitaker and Richardson, 1980; Patil & Anil, 2005; Jiménez-Valera and del Pilar Sánchez-Saavedra, 2016; Hamre et al., 2016; Ferreira et al., 2018). According to Abreu et al. (2019), an important increase occurs in the concentration of n-3 and n-6 chain fatty acid occurs in microbial flocs after the addition of *Navicula* sp., as microbial flocs are reported to be deficient in fatty acids (EPA and DHA) (Liu et al., 2017; Martinez-Porcha et al., 2020)

Abreu et al. (2019) also investigated the effect of the addition of different densities of *Navicula* sp. at a frequency of every 10 days on the growth performance of *L. vannamei* post-larvae and confirmed the benefits of supplementation, such as an improvement in immune status and growth parameters. Brito et al. (2016) and Marinho et al. (2014; 2017) investigated the use of *Bacillariophyceae* combined with *Rotifera* on the growth performance of *L. vannamei* post-larvae and determined that the microorganism assists in maintaining water quality and growth variables, reporting similar productivity and final weight values as those found in the present study. This is likely related to thermal control and use of the culture system.

Besides the advantages with regards to growth, living organisms are reported to have immunostimulant potential, improving immunological variables (Shah et al., 2018). The total

(THC) and differential (DHC) hemocyte counts are indicative of immune status. In a previous study involving the use of a heated extract of *Gracilaria tenuistipitata* for *L. vannamei*, Yeh et al. (2010) found that supplementation assisted in the mitotic index and the proliferation of cells in hematopoietic tissue. However, no significant differences were found among the treatments in the first phase of the experiment in the present study, although the THCs were higher than those reported in studies conducted by Jasmanindar et al. (2018) and Abreu et al. (2019), likely due to the addition of organic carbon after aerobic and anaerobic process, founded on symbiosis (prebiotic plus probiotic), which intensely stimulates the immune system by activating hemocytes more effectively, particularly with regards to respiratory burst and prophenoloxidase (Hamsah et al., 2019). Regarding the differential count, which consists of the quantification of three morphological types of hemocytes (hyaline, semi-granular, and granular), we found values close to those reported by Sudaryono et al. (2018), indicating that the shrimp were in homeostasis, as the cell components are responsible for phagocyting, encapsulating, and forming nodules. If the animals exhibited a condition of infection, lower levels of these cells would be found due to combating the foreign body (Sudaryono et al., 2018).

In the second phase of the experiment (viral challenge), the death of all animals occurred in the first six days after infection in the control group (BFT) and the group with the addition of rotifer alone (BFT-B). Previous studies report that *Brachionus* sp. is a vector of WSSV and is often used as a food source in the nursery phase, which likely favored the rapid propagation of the virus among the animals via ingestion in the present study (Corre et al., 2012)

For the groups with the addition of the diatom either alone or in combination with the rotifer, total mortality was delayed, meaning that it took a longer period of time for all animals to die compared to the treatments that did not receive *Navicula* sp. This may be associated with the capacity of phytoplankton to remove small particles from the water as assimilators of

nutrients for its growth, including viral particles excreted from infected animals, thereby leading to a reduction in the transmission of disease (Zhang et al., 2006).

Infection was confirmed molecularly in 100% of the challenged animals. Moreover, the histopathological analysis revealed that the gills were severely affected, with the degeneration of the lamellae and the occurrence of nodules. The impairment of this tissue leads to the collapse of important functions, such as osmoregulation, gas transport, and the excretion of CO_2 and ammonia (Escobedo-Bonilla et al., 2007). In the stomach, nodule inclusions and deformations were found in the tissues, especially in the treatments in which complete mortality occurred earlier, which were those without the addition of the diatom. The better performance in the treatments with *Navicula* sp. may be linked to the bioactive characteristics of the microalgae, which improved the immune status of the animals, as carotenoids provide better resistance to infection and diminish the occurrence of lipid oxidation, exhibiting protective activity (Abreu et al., 2019; Schleder et al., 2018).

This study shows that use of organic fermentation, supplementation with living organisms, and thermal control ensure water quality and the bloom of beneficial bacteria, providing benefits to productivity and the immune system, which assists in periods of stress.

5. Conclusion

The addition of *B. plicatilis* and *Navicula* sp. in a biofloc culture system contributed to better growth performance of *L. vannamei* in the nursery phase. However, this supplementation did not enhance resistance to WSSV beyond a delay in complete mortality in treatments involving the diatom (BFT-N and BFT-BN), as no reduction in infection or mortality rates occurred in the infected shrimp. However, the use of *Navicula* sp. reduced the damage to the target tissues of WSSV. Thus, further studies are needed involving bioactive ingredients of

adequately processed microalgae in feed for a better understanding of of how it helps shrimp innate immunity to combat WSSV.

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7. References

Aalimahmoudi, M., Azarm, H. M., Mohamadi, Y. Biofloc new technology and shrimp disease in super-intensive aquaculture. International Journal of Fisheries and Aquatic Research. v. 2, n.2, p.17-21.2017.

Abreu, J. L., Brito, L. O., Lima, P. C. M., Silva, S. M. B. C. D., Severi, W., Gálvez, A. O. 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. Aquaculture Research, v. 50, n. 8, p.2231-2239, 2019.

Aguilera-Rivera, D., Prieto-Davó, A., Escalante, K., Chávez, C., Cuzon, G., Gaxiola, G. Probiotic effect of FLOC on Vibrios in the pacific white shrimp *Litopenaeus vannamei*. Aquaculture. v. 424, p.215-219. 2014.

Anaya-Rosas, R. E.; Rivas-Vega, M. E.; Miranda-Baeza, A.; Piña-Valdez, P.; Nieves-Soto, M. Effects of a co-culture of marine algae and shrimp (*Litopenaeus vannamei*) on the growth, survival and immune response of shrimp infected with *Vibrio parahaemolyticus* and white spot virus (WSSV). Fish & shellfish immunology, v.87, p.136-143. 2019.

APHA (2005). Standard methods for the examination of water and wastewater.Washington, DC: American Public Health Association.Avnimelech, Y. Biofloc technology, a practical guidebook. The world. Aquaculture Society, Baton Rouge, Louisiana, EUA, 2009.

Bell, T. A.; Lightner, D.V. A Handbook of Normal Penaeid Shrimp Histology. EUA. World Aquaculture Society. 1948. 114p.

Biju, V. N., Gunalan, B. Prevalence of Vibrio infection in *Penaeus* (*Litopenaeus*) *vannamei* farms. Internacional Journal Science Inventions Today, v.5, n. 6, p.485-493. 2016.

Brito, L.O., Santos, I.G.S., Abreu, J.L., Araujo, M.T., Severi, W., Galvez, A.O. Effect of the addition of diatoms (*Navicula* spp.) and rotifers (*Brachionus plicatilis*) on water quality and growth of the *Litopenaeus vannamei* postlarvae reared in a biofloc system. Aquaculture Research, v. 47, p. 3990-3997, 2016.

Celi, M., Filiciotto, F., Parrinello, D., Buscaino, G., Damiano, M. A., Cuttitta, A., D'Angelo, S., Mazzola, S., Vazzana, M. Physiological and agonistic behavioural response of *Procambarus clarkii* to an acoustic stimulus. Journal of Experimental Biology, v.216, n.4, p.709-718, 2013.

Cezare-Gomes, E. A., Del Carmen Mejia-da-Silva, L., Pérez-Mora, L. S., Matsudo, M. C., Ferreira-Camargo, L. S., Singh, A. K., de Carvalho, J. C. M. Potential of Microalgae Carotenoids for Industrial Application. Applied biochemistry and biotechnology, v.188, n.3, p.602-634. 2019.

Ching, C.A. Estratégias de campo para o controle da síndrome da mancha branca em camarões marinhos. Revista da ABCC. Natal. n.2, p.40-44. nov.2017.

Clark, K.F. Nimaviruses of Crustaceans. In: Kibenge, F. S.B. and Godoy, M.G. Aquaculture Virology, Amsterdam, 2016. cap. 26, p. 397 – 413.

Costa, S. W., Vicente, L. R. M., Souza, T. M., Andreatta, E. R., Marques, M. R. F. Parâmetros de cultivo e a enfermidade da mancha-branca em fazendas de camarões de Santa Catarina. Pesquisa agropecuária brasileira, Brasília, v. 45, n.12, p. 1-10, 2011.

Corre Jr., V., Faisan Jr, J., Carton-Kawagoshi, R. J., Elle, B. J., Traifalgar, R. F., Caipang, C. M. Evidence of WSSV transmission from the rotifer (*Brachionus plicatilis*) to the black tiger shrimp (*Penaeus monodon*) postlarvae and means to control rotifer resting eggs using industrial disinfectants. Aquaculture, Aquarium, Conservation & Legislation, v.5, n.2, p. 64-68, 2012.

Crab, R., Defoirdt, T., Bossier, P., Verstraete, W. Biofloc technology in aquaculture: beneficial effects and future challenges. Aquaculture, v. 356, 351-356,2012.

Dantas jr, E. M., Valle, B. C. S., Brito, C. M. S., Calazans, N. K. F., Peixoto, S. R. M., Soares, R. B. Partial replacement of fishmeal with biofloc meal in the diet of postlarvae of the Pacific white shrimp *Litopenaeus vannamei*. Aquaculture nutrition, v.22, n.2, p.335-342, 2016.

Das, P., Mandal, S. C., Bhagabati, S. K., Akhtar, M. S., Singh, S. K. Important live food organisms and their role in aquaculture. Frontiers in aquaculture. v.5, n.4, p.69-86. 2012.

Durand, S. V., Lightner, D. V. Quantitative real time PCR for the measurement of whitespot syndrome virus in shrimp. Journal of Fish Diseases**.** v.25, p.381–389, 2002.

Escobedo-Bonilla, C. M., Wille, M., Sanz, V. A., Sorgeloos, P., Pensaert, M. B., Nauwynck, H. J. Pathogenesis of a Thai strain of white spot syndrome virus (WSSV) in juvenile, specific pathogen-free *Litopenaeus vannamei*. Diseases of aquatic organisms, v.74, n.2, p.85-94. 2007.

Erken, M., Lutz, C., Mcdougald, D. Interactions of *Vibrio* spp. with zooplankton. Microbiol Spectrum, v.3, n.3, p. 1-15, 2015.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS – FAO. The State of World Fisheries and Aquaculture: Sustainability in action. Fisheries an Aquaculture Technical Paper. Rome, 2020, 206p.

Farisa, M. Y., Namaskara, K. E., Yusuf, M. B. Antibacterial potention of extract of rotifers fed with different microalgae to control *Vibrio harveyi*. IOP Conference Series: Earth and Environmental Science, v. 246, n. 1, p. 12-58, 2019.

Ferreira, M., Cortina-Burgueño, Á., Freire, I., Otero, A. Effect of nutritional status and concentration of *Nannochloropsis gaditana* as enrichment diet for the marine rotifer *Brachionus* sp. Aquaculture, v.491, p.351-357. 2018.

Furtado, P. S., Poersch, L. H., Wasielesky, W. The effect of different alkalinity levels on *Litopenaeus vannamei* reared with biofloc technology (BFT). Aquaculture international, v.23, n. 1, p.345-358, 2015.

Ge, H., Li, J., Chang, Z., Chen, P., Shen, M., Zhao, F. Effect of microalgae with semi continuous harvesting on water quality and zootechnical performance of white shrimp reared in the zero water exchange system. Aquacultural engineering, v. 72, p.70-76, 2016.

Guertler, C., Rieg, T., Mejía-Ruíz, C. H., Lehmann, M., Barracco, M. A., Perazzolo, L. M. Hemograma e sobrevivência de camarões marinhos após silenciamento do WSSV por RNA de interferência. Pesquisa Agropecuária Brasileira, Brasília, v. 48, n.8, p.983-990, 2013.

Golterman, H.J., Clyno, R.S., Ohnstad, M.A. 1978. Methods for physical and chemical analysis of freshwaters. Oxford. Blackwell Scientific Publications, London, England.

Gomez-Gil, B., Soto-Rodríguez, S., Lozano, R., Betancourt-Lozano, M. Draft genome sequence of *Vibrio parahaemolyticus* strain M0605, which causes severe mortalities of shrimps in Mexico. Genome Announc., v.2, n.2, 2014.

Hamre, K. Nutrient profiles of rotifers (*Brachionus* sp.) and rotifer diets from four different marine fish hatcheries. Aquaculture, v. 450, p. 136-142, 2016.

Hamsah, H., Widanarni, W., Alimuddin, A., Yuhana, M., Junior, M. Z., Hidayatullah, D. Immune response and resistance of Pacific white shrimp larvae administered probiotic, prebiotic, and synbiotic through the bio-encapsulation of *Artemia* sp. Aquaculture International, v.27, n.2, p.567-580. 2019.

Hoseinifar, S. H., Ringo, E., Masouleh, A. S., Esteban, M. Á. Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: a review. Reviews in Aquaculture, v.8, n.1, p.89-102. 2016.

Hostins, B., Lara, G., Decamp, O., Cesar, D. E., Wasielesky Jr, W. Efficacy and variations in bacterial density in the gut of *Litopenaeus vannamei* reared in a BFT system and in clear water supplemented with a commercial probiotic mixture. Aquaculture, v.480, p.58-64. 2017.

IBGE (Instituto Brasileiro de Geografia e Estatística). Produção da pecuária municipal, 2017. Disponível em: https://sidra.ibge.gov.br/tabela/3940. Acesso em: 23 de julho de 2017.

Jamali, H., Ahmadifard, N., Abdollahi, D. Evaluation of growth, survival and body composition of larval white shrimp (*Litopenaeus vannamei*) fed the combination of three types of algae. International Aquatic Research, v.7, n.2, p.115-122, 2015.

Jasmanindar, Y., Sukenda, S., Zairin jr, M., Alimuddin, A., Utomo, N. B. Dietary administration of *Gracilaria verrucosa* extract on *Litopenaeus vannamei* immune response, growth, and resistance to *Vibrio harveyi*. Aquaculture, Aquarium, Conservation & Legislation, v.11, n.4, p.1069-1080. 2018.

Jiménez-Valera, S., del Pilar Sánchez-Saavedra, M. Growth and fatty acid profiles of microalgae species isolated from the Baja California Peninsula, México. Latin American Journal of Aquatic Research, v.44, n.4, p.689-702, 2016.

Ju, Z. Y., Deng, D. F., Dominy, W. A defatted microalgae (*Haematococcus pluvialis*) meal as a protein ingredient to partially replace fishmeal in diets of Pacific white shrimp (*Litopenaeus vannamei*, Boone, 1931). Aquaculture, v. 354, p. 50-55, 2012.

Kakoolaki, S., Afsharnasab, M., Sharifpour, I. The relation between temperature and salinity with WSSV occurrence in shrimp farms in Iran: An article review. Survey in Fisheries Sciences, v.2, n.1, p.31-41. 2015.

Kang, K. H., Qian, Z. J., Ryu, B., Kim, D., Kim, S. K. Protective effects of protein hydrolysate from marine microalgae *Navicula incerta* on ethanol-induced toxicity in HepG2/CYP2E1 cells. Food chemistry, v.132, n.2, p.677-685. 2012.

Karunasagar, I., Ababouch, L. Shrimp viral diseases, import risk assessment and international trade. Indian Journal of Virology, v. 23, n.2, p. 141-148, 2012.

Koroleff, F. 1976. Determination of nutrients, in: Grasshoff, K., Ehrhardt, M., Kremling, K. (Eds.), Methods of seawater analysis. Verlag Chemie Weinhein, New York, p. 117-187.

Kumar, S. D., Santhanam, P., Ananth, S., Kaviyarasan, M., Nithya, P., Dhanalakshmi, B., Park, M. S., Kim, M. K. Evaluation of suitability of wastewater-grown microalgae (*Picochlorum maculatum*) and copepod (*Oithona rigida*) as live feed for white leg shrimp *Litopenaeus vannamei* post-larvae. Aquaculture International, v.25, n.1, p.393-411, 2017.

Lightner, D. V., Redman, R. M., Pantoja, C. R., Tang, K. F. J., Noble, B. L., Schofield, P., Mohney, L. L., Nunan, L. M., Navarro, S. A. Historic emergence, impact and current status of shrimp pathogens in the Americas. Journal of invertebrate pathology, v. 110, n.2, p. 174-183, 2012.

Liu, W. C., Luo, G. Z., Li, L., Wang, X. Y., Wang, J., Ma, N. N., Sun, Da-chuan, Tan, H. X. Nitrogen Dynamics and Biofloc Composition using Biofloc Technology to Treat Aquaculture Solid Waste Mixed with Distillery Spent Wash. North American Journal of Aquaculture, v.79, n.1, p.27-35. 2017.

Lo, C.F., Lei, J.H., Ho, C.H., Chen, C.H., Peng, S.E., Chen, Y.T., Chou, C.M., Yeh, P.Y., Huang, C.J., Chou, H.Y., Wang, C.H., Kou, G.H. Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. Diseases of Aquatic Organisms, v.25, p.133-141, 1996.

Magaña-Gallegos, E., González-Zúñiga, R., Cuzon, G., Arevalo, M., Pacheco, E., Valenzuela, M. A, Gaxiola, G, Chan-Vivas, E., López-Aguiar, K., Noreña-Barroso, E. Nutritional

contribution of biofloc within the diet of growout and broodstock of *Litopenaeus vannamei*, determined by stable isotopes and fatty acids. Journal of the World Aquaculture Society, p. 1-14, 2018.

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

Maldonado, M., Rodríguez, J., de Blas, I. EL camarón de cultivo frente al WSSV, su principal patógeno. Revista AquaTIC, n. 21. 2016.

Maliwat, G. C., Velasquez, S., Robil, J. L., Chan, M., Traifalgar, R. F., Tayamen, M., Ragaza, J. A. Growth and immune response of giant freshwater prawn *Macrobrachium rosenbergii* (De Man) postlarvae fed diets containing *Chlorella vulgaris* (Beijerinck). Aquaculture Research, v.48, n.4, p.1666-1676, 2017.

Marinho, Y. F., Brito, L.O., da Silva, C. V. F., dos Santos, I. G. S., 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, v.42, n.3, 2014.

Marinho, Y. F., Brito, L. O., Silva Campos, C. V. F. D., 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, v. 48, n.8, p. 4155-4164, 2016.

Martínez-Córdova, L. R., Emerenciano, M., Miranda-Baeza, A., Martínez-Porchas, M. Microbial-based systems for aquaculture of fish and shrimp: an updated review. Reviews in Aquaculture, v. 7, n.2, p.131-148. 2015.

Martinez-Porchas, M., Ezquerra-Brauer, M., Mendoza-Cano, F., Chan-Higuera, J. E., Vargas-Albores, F., Martinez-Cordova, L. R. Effect of supplementing heterotrophic and

photoautotrophic biofloc, on the production response, physiological condition and post-harvest quality of the whiteleg shrimp, *Litopenaeus vannamei*. Aquaculture Reports, v.16, p. 1-4. 2020.

Medina-Félix, D., López-Elías, J. A., Martínez-Córdova, L. R., López-Torres, M. A., Hernández-López, J., Rivas-Vega, M. E., Mendoza-Cano, F. Evaluation of the productive and physiological responses of *Litopenaeus vannamei* infected with WSSV and fed diets enriched with *Dunaliella* sp. Journal of Invertebrate Pathology, v.117, p.9-12, 2014.

Niu, J., Xie, Shi-Wei, Fang, Hao-Hang, Xie, Jia-Jun, Guo, Tian-Yiu, Zhang, Yan-Mei, Liu, Zhen-Lu, Liao, Shi-Yu, He, Ju-Yun, Tian, Li-Xia, Liu, Y. J. Dietary values of macroalgae *Porphyra haitanensis* in *Litopenaeus vannamei* under normal rearing and WSSV challenge conditions: Effect on growth, immune response and intestinal microbiota. Fish & Shellfish immunology, v. 81, p. 135-149, 2018.

Patel, M. P., Vadher, K. H., Tank, P. R. Virulence of *Vibrio* spp. to pacific white shrimp *Litopenaeus vannamei* juveniles. Journal of Entomology and Zoology Studies, v.6, n.6, p.1258-1262. 2018.

Patil, J. S.; Anil, A. C. Quantification of diatoms in biofilms: standardisation of methods. Biofouling, v.21, *n.*3-4, p.181-188. 2005.

Pérez, F., Volckaert, F. A., Calderón, J. Pathogenicity of white spot syndrome virus on postlarvae and juveniles of *Penaeus* (*Litopenaeus*) *vannamei*. Aquaculture, v. 250, n.3, p. 586-591, 2005.

Ping-Chung, L., Kuo-Kau, L., Kah-Ching, Y., Guang-Hsiung, K., Shiu-Nan, C. (1996). Isolation of *Vibrio harveyi* from diseased kuruma prawns *Penaeus japonicas*. Current Microbiology, v.33, n.2, p.129-132, 1996.

Rajkumar, M.; Pandey, P. K.; Aravind, R.; Vennila, A.; Bharti, V.; Purushothaman, C. S. Effect of different biofloc system on water quality, biofloc composition and growth performance in *Litopenaeus vannamei* (Boone, 1931). Aquaculture Research, v.47, n.11, p.3432-3444, 2016.

Rebouças, R. H., de Menezes, F. G. R., dos Fernandes Vieira, R. H. S., de Sousa, O. V. *Vibrio* spp. como patógenos na carcinicultura: alternativas de controle. Arquivos de Ciências do Mar. v.50, n.1, p.163-179. 2017.

Romano, N., Dauda, A. B., Ikhsan, N., Karim, M., Kamarudin, M. S. Fermenting rice bran as a carbon source for biofloc technology improved the water quality, growth, feeding efficiencies, and biochemical composition of African catfish *Clarias gariepinus* juveniles. Aquaculture research, v.49, n.12, p.3691-3701.2018.

Samocha, T. M. Sustainable Biofloc Systems for Marine Shrimp. Academic Press: EUA. 2019. 463 p.

Sánchez-Paz, A. White spot syndrome virus: an overview on an emergent concern. Veterinary research, v. 41, n. 6, p. 1-34, 2010.

Schaal, G., Leclerc, J. C., Droual, G., Leroux, C., Riera, P. Biodiversity and trophic structure of invertebrate assemblages associated with understorey red algae in a *Laminaria digitata* bed. Marine Biology Research, v.12, n.5, p.513-523, 2016.

Schleder, D. D., Peruch, L. G. B., Poli, M. A., Ferreira, T. H., Silva, C. P., Andreatta, E. R., Leila Hayashi, L, Vieira, F. N. Effect of brown seaweeds on Pacific white shrimp growth performance, gut morphology, digestive enzymes activity and resistance to white spot virus. Aquaculture, v.495, p.359-365. 2018.

Shah, M. R., Lutzu, G. A., Alam, A., Sarker, P., Chowdhury, M. K., Parsaeimehr, A., Liang, Y., Daroch, M. Microalgae in aquafeeds for a sustainable aquaculture industry. Journal of Applied Phycology, v.30, n.1, p.197-213, 2018.

Soon, L.T., Lee, K.L., Shariff, M., Hassan, M.D., Omar, A.R. Quantitative analysis of an experimental white spot syndrome virus (WSSV) infection in *Penaeus monodon Fabricius* using competitive polymerase chain reaction. Journal of Fish Diseases, v. 24, n. 6, p. 315-323, 2001.

Sudaryono, A., Chilmawati, D., & Susilowati, T. Oral Administration of Hot-water Extract of Tropical Brown Seaweed, *Sargassum cristaefolium*, to Enhance Immune Response, Stress Tolerance, and Resistance of White Shrimp, *Litopenaeus vannamei*, to *Vibrio parahaemolyticus*. Journal of the World Aquaculture Society, v.49, n.5, p.877-888. 2018.

Takahashi, Y., Itami, T., Kondo, M., Maeda; M., Fujii, R., Tomonaga, S., Supamattaya, K., Boonyaratpalin, S. Electron microscopic evidence of bacilliform virus infection in kuruma shrimp (*Penaeus japonicus*). Fish Pathology, v.29, n. 2, p. 121-125, 1994.

Van Wyk, P. Nutrition and feeding of *Litopenaeus vannamei* in intensive culture systems. In: Van Wyk, P., Davis-Hodgkins, M., Laramore, R, Main, K.L., Mounta, J., Scarpa,,J. Farming Marine Shrimp in Recirculating Freshwater Systems. Florida Department of Agriculture and Consumer Services – Harbor Branch Oceanic Institute, Tallahassee, Florida. 1999, pp. 125–140.

Vandenberghe, J., Verdonck. L., Robles-Arozarena, R., Rivera G; Bolland, A., Balladares, M., Gomez-Gil, B., Calderon, J., Sorgeloos, P., Swings, J. Vibrios associated with *Litopenaeus vannamei* larvae, postlarvae, broodstock, and hatchery probionts. Applied and Environmental Microbiology. v.65, n. 06, p.2592–2597, 1999.

Viçose, G. C., Viera, M. P., Huchette, S., Izquierdo, M. S. Improving nursery performances of *Haliotis tuberculata coccinea*: nutritional value of four species of benthic diatoms and green macroalgae germlings. Aquaculture, v.334-337, p.124-131. 2012.

Vijayan, G., Saravanane, R., & Sundararajan, T. Studies on Impact of Variants Influencing the Performance of SBR. Open Science Publishers, v.4, n.2, p.9-20. 2017.

Wang, L., Chen, Y., Huang, H., huang, Z., Chen, H., Shao, Z. Isolation and identification of *Vibrio campbellii* as a bacterial pathogen for luminous vibriosis of *Litopenaeus vannamei*.

Whitaker, T. M., Richardson, M. G. Morphology and chemical composition of a natural population of an ice-associated antarctic diatom *Navicula glaciei.* Journal of Phycology, v.16, n.2, p.250-257, 1980

Withyachumnarnkul, B., Boonsaeng, V., Chomsoong, R., Flegel, T.W., Muangsin, S., Nash, G.L. Seasonal variation in white spot syndrome virus-positive samples in broodstock and post-larvae of *Penaeus monodon* in Thailand. Diseases of Aquatic Organisms. v.53, p. 167–171. 2003.

World Organisation for Animal Health – OIE. Manual of Diagnostic Tests for Aquatic Animals. Office international des epizooties, França. 2018, cap. 2.2.8, p. 1-16.

Yeh, S. T., Lin, Y. C., Huang, C. L., Chen, J. C. White shrimp *Litopenaeus vannamei* that received the hot-water extract of *Gracilaria tenuistipitata* showed protective innate immunity and up-regulation of gene expressions after low-salinity stress. Fish & Shellfish Immunology, v.28, n.5-6, p.887-894. 2010.

Yu, Y., Liu, J., Li, F., Zhang, X., Zhang, C., Xiang, J. Gene set based association analyses for the WSSV resistance of Pacific white shrimp *Litopenaeus vannamei*. Scientific Reports, v. 7, p.10, 2017.

Zhang, J. S., Dong, S. L., Tian, X. L., Dong, Y. W., Liu, X. Y., Yan, D. C.Studies on the rotifer (*Brachionus urceus* Linnaeus, 1758) as a vector in white spot syndrome virus (WSSV) transmission. Aquaculture, v.261, n.4, p.1181-1185. 2006.

8. Appendices

Table 1. Water quality parameters in the culture of *Litopenaeus vannamei* under nursery biofloc system with and without the addition of *Navicula* sp. and *B. plicatilis* during a 45-day experimental period.

VARIABLE	TREATMENT			
	BFT	**BFT -B**	**BFT-N**	**BFT-BN**
Dissolved oxygen (mg L^{-1})	5.24 ± 0.01^{b}	5.23 ± 0.01^{b}	5.29 ± 0.01^{a}	5.21 ± 0.01^{b}
Temperature (Cº)	31.74 ± 0.16^{a}	31.50 ± 0.06^{a}	31.65 ± 0.04^{a}	31.61 ± 0.05^{a}
pH	7.97 ± 0.01^{a}	7.94 ± 0.01^{a}	7.99 ± 0.01^{a}	7.94 ± 0.01^{a}
Salinity (g L^{-1})	30.52 ± 0.10^{c}	30.88 ± 0.11^{bc}	31.11 ± 0.10^{ab}	31.30 ± 0.12^{a}
SS (mL L^{-1})	7.14 ± 0.51^{a}	7.01 ± 0.47^{a}	4.74 ± 0.48^{b}	6.03 ± 0.46^{ab}
Alkalinity (mg $CaCO_3$ L^{-1})	130 ± 5.92^{a}	126 ± 6.20^{a}	130 ± 6.16^{a}	133 ± 6.75^{a}
TAN (mg L^{-1})	0.22 ± 0.04^{a}	0.21 ± 0.02^{a}	0.18 ± 0.01^{a}	0.18 ± 0.02^{a}
N-NO_2 (mg L^{-1})	1.44 ± 0.49^{a}	2.41 ± 0.66^{a}	2.37 ± 0.58^{a}	2.18 ± 0.61^{a}
N-NO^3 (mg L^{-1})	1.21 ± 0.09^{a}	1.24 ± 0.09^{a}	1.25 ± 0.12^{a}	1.12 ± 0.1^{a}
PO^4 (mg L^{-1})	33.94 ± 3.15^{a}	39.70 ± 1.20^{a}	40.20 ± 6.47^{a}	37.28 ± 4.58^{a}
ORP (mv)	23.08^{a}	20.49^{a}	22.62^{a}	18.90^{a}

The data correspond to the mean ± standard deviation. The results were analyzed by performing repeated measures of ANOVA and the Tukey's test. Values in the same row with different superscripts differ significantly ($\alpha < 0.05$). BFT (biofloc); BFT-N (addition of 10×10^4 cells ml^{-1} of *Navicula* sp.); BFT-B (addition of 30 org ml^{-1} of *Brachionus plicatilis*); BFT-BN (addition of 10×10^4 cells ml^{-1} of *Navicula* sp. and 30 org ml^{-1} of *Brachionus plicatilis*). Abbreviations: SS settleable solids. TAN- total ammonia nitrogen; ORP - oxidation reduction potencial

Table 2. *Vibrio* density in the culture of *Litopenaeus vannamei* under nursery biofloc system with and without the addition of *Navicula* sp. and *B. plicatilis* during a 45-day experimental period.

WATER	**INITIAL**					**FINAL**				
(UFC mL^{-1})	**GVLB**	**%**	**YVLB**	**%**	**T**	**GVLB**	**%**	**YVLB**	**%**	**T**
BFT	$5.5x10^{3Aa}$	58.2	$4.5x10^{3Aa}$	41.8	$9.23x10^{3Aa}$	$0.4x10^{3Ba}$	36.36	$0.8x10^{3Bb}$	63.64	$1.1x10^{3Bb}$
BFT-B	$5.5x10^{3Aa}$	58.2	$4.5x10^{3Aa}$	41.8	$9.23x10^{3Aa}$	$0.1x10^{3Ba}$	3.08	$3.4x10^{3Aa}$	96.92	$4.8x10^{3Aa}$
BFT-N	$5.5x10^{3Aa}$	58.2	$4.5x10^{3Aa}$	41.8	$9.23x10^{3Aa}$	$1.0x10^{3Ba}$	36.36	$1.7x10^{3Bab}$	63.64	$2.7x10^{3Bab}$
BFT-BN	$5.5x10^{3Aa}$	58.2	$4.5x10^{3Aa}$	41.8	$9.23x10^{3Aa}$	$0.2x10^{3Ba}$	21.74	$0.9x10^{3Bb}$	78.26	$1.1x10^{3Bb}$
SHRIMP	**INITIAL**					**FINAL**				
(UFC g^{-1})	**SNC**	**%**	**SPC**	**%**	**T**	**SNC**	**%**	**SPC**	**%**	**T**
BFT	$2.2x10^{3Aa}$	50.9	$1.7x10^{3Ba}$	49.1	$3.78x10^{3Ba}$	$0.0x10^{3Bb}$	0.0	$120x10^{3Aa}$	100	$120x10^{3Aa}$
BFT-B	$2.2x10^{3Aa}$	50.9	$1.7x10^{3Ba}$	49.1	$3.78x10^{3Ba}$	$0.0x10^{3Bb}$	0.0	$78.8x10^{3Ab}$	100	$78.8\ x10^{3Ab}$
BFT-N	$2.2x10^{3Aa}$	50.9	$1.7x10^{3Ba}$	49.1	$3.78x10^{3Ba}$	$0.0x10^{3Bb}$	0.0	$47.7x10^{3Ac}$	100	$47.7x10^{3Ac}$
BFT-BN	$2.2x10^{3Aa}$	50.9	$1.7x10^{3Ba}$	49.1	$3.78x10^{3Ba}$	$3.25x10^{3Aa}$	1.52	$163x10^{3Aa}$	98.48	$165x10^{3Aa}$

The data correspond to the median. The results were analyzed by performing repeated measures of ANOVA and the Student-Newman-Keuls test. Values in the same row with different superscripts differ significantly ($\alpha < 0.05$) a – treatment and A -over time. BFT (biofloc); BFT-N (addition of 10×10^4 cells ml^{-1} of *Navicula* sp.); BFT-B (addition of 30 org ml^{-1} of *Brachionus plicatilis*); BFT-BN (addition of 10×10^4 cells ml^{-1} of *Navicula* sp. and 30 org ml^{-1} of *Brachionus plicatilis*). Abbreviations: Sucrose-negative colonies (SNC) (YVLB). sucrose-positive colonies (SPC) and total (T).

Table 3. Shrimp performance in the culture of *Litopenaeus vannamei* under nursery biofloc system with and without the addition of *Navicula* sp. and *B. plicatilis* during a 45-day experimental period.

VARIÁVEIS	TRATAMENTO			
	BFT	**BFT-N**	**BFT-BN**	**BFT -B**
Final weight (g)	0.86 ± 0.05^{b}	1.06 ± 0.04^{a}	1.02 ± 0.03^{a}	1.16 ± 0.02^{a}
Survival (%)	91.9 ± 2.64^{a}	95 ± 2.2^{a}	93.3 ± 0.96^{a}	94.72 ± 1.11^{a}
FCR	1.33 ± 0.14^{a}	1.26 ± 0.07^{a}	1.23 ± 0.02^{a}	1.22 ± 0.10^{a}
Yield (Kg m^{-3})	2.46 ± 0.14^{c}	3.02 ± 0.17^{ab}	2.85 ± 0.06^{b}	3.30 ± 0.02^{ab}

[1]The data correspond to the mean ± standard deviation. The results were analyzed by performing one-way ANOVA and Student-Newman-Keuls test. Values in the same row with different superscripts differ significantly ($\alpha < 0.05$). BFT (biofloc); BFT-N (addition of 10×10^4 cells ml^{-1} of *Navicula* sp.); BFT-B (addition of 30 org ml^{-1} of *Brachionus plicatilis*); BFT-BN (addition of 10×10^4 cells ml^{-1} of *Navicula* sp. and 30 org ml^{-1} of *Brachionus plicatilis*).

Table 4. Haematocyte count of juvenile *Litopenaeus vannamei* under after nursery culture in a biofloc system with and without the addition of *Navicula* sp. and *B. plicatilis*.

TREATMENT	BFT	BFT-B	BFT-N	BFT-BN	REFERENCE INTERVAL	REFERENCE
THC (x 10^7 mL^{-1})	1.64[a]	1.58[a]	1.47[a]	1.12[a]	4.0 x 10^6 mL^{-1} a 2.1 x $10^7 mL^{-1}$	Liu et al.. 2018 Hamsah et al.. 2019
HC (%)	32.6[a]	32.4[a]	32.9[a]	32.3[a]	0 – 20%	Sudaryono et al.. 2018
SGC (%)	0.62[a]	0.69[a]	0.31[a]	0.92[a]	0 - 20%	Zubaidah et al.. 2015
LGC (%)	0.11[a]	0.15[a]	0.06[a]	0.36[a]	0 – 20%	Zubaidah et al.. 2015

The data correspond to the mean. The results were analyzed by performing one-way ANOVA and Student-Newman-Keuls test. Values in the same row with different superscripts differ significantly ($\alpha < 0.05$). BFT (biofloc); BFT-N (addition of 10×10^4 cells ml^{-1} of *Navicula* sp.); BFT-B (addition of 30 org ml^{-1} of *Brachionus plicatilis*); BFT-BN (addition of 10×10^4 cells ml^{-1} of *Navicula* sp. and 30 org ml^{-1} of *Brachionus plicatilis*). Abbreviations: total hemocyte count (THC). hyaline count (HC). semi-granular count (SGC) and granular count (LGC).

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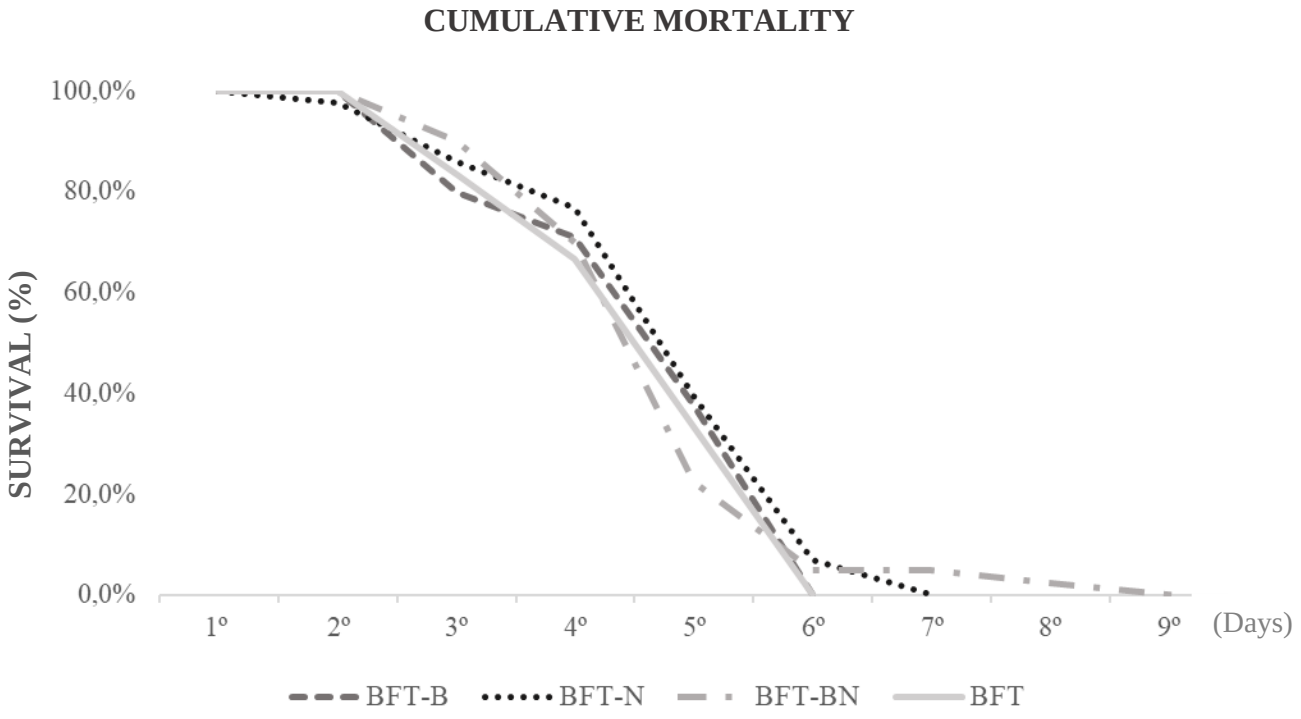

Figure 1. Cumulative mortality of *Litopenaeus vannamei* challenged with WSSV with and without the addition of *Navicula* sp. and *Brachionus plicatilis*

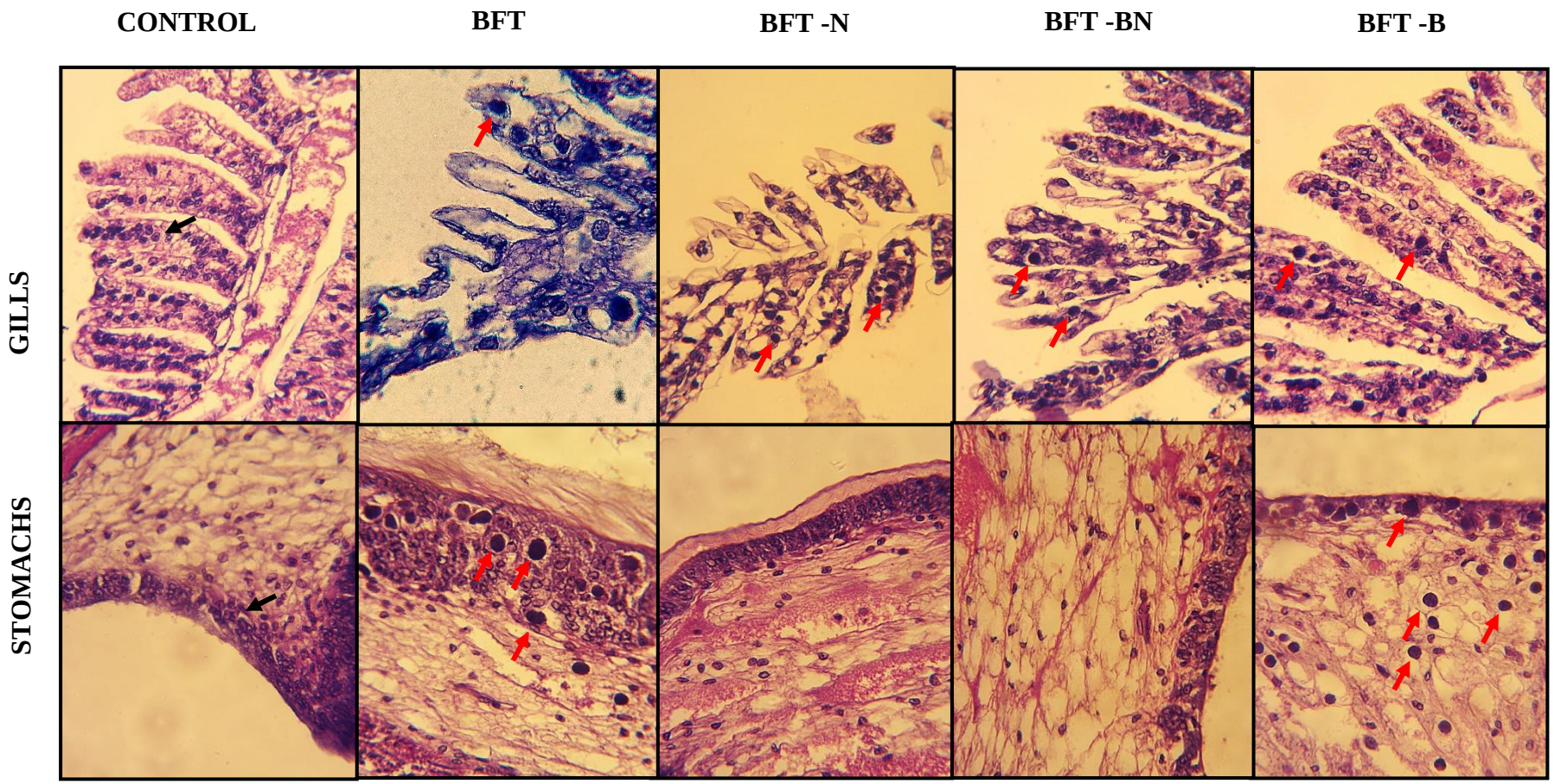

Figure 2. Histopathology of *L. vannamei* gills and stomachs challenged with WSSV with and without the addition of *Navicula* sp. and *B. plicatilis*, showing tissue degeneration and nodules of inclusion (red arrows), while normal cells are indicated with black arrows (H&E, 400 X).

4. CONSIDERAÇÕES FINAIS

A suplementação da *Navicula* sp. e *Brachionus plicatilis* na fase berçário de *Litopenaeus vannamei* em sistema de bioflocos utilizando o processo anaeróbio e aeróbio do farelo de trigo como fonte de carbono demonstra resultados promissores na qualidade da água e no desempenho zootécnico do animal. além da influência da caracterização do *Vibrio* sp. no ambiente de cultivo para bactérias sacarose positiva. Contudo. a contribuição dessa adição sobre a resistência ao WSSV não pode ser evidenciada. apenas o retardo da mortalidade total em tratamentos que houve a adição da diatomácea (BFT-N e BFT-BN). indicando que a microalga pode conter compostos que ajudem a melhorar o sistema imune dos camarões. Com isso. outros estudos que possibilitem maior compreensão sobre a resistência ao WSSV e os benefícios da microalga na resposta imunológica no camarão são necessários.

5. REFERÊNCIAS

ABREU. J. L.; BRITO. L. O.; LIMA. P. C. M.; SILVA. S. M. B. C. D.; SEVERI. W.; GÁLVEZ. A. O. 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. Aquaculture Research. v. 50. n. 8. p.2231-2239. 2019.

AGUIRRE-GUZMAN. G.; SANCHEZ-MARTINEZ. J. G.; CAMPA-CORDOVA. A. I.; LUNA-GONZALEZ. A.; ASCENCIO. F. Penaeid shrimp immune system. Thai Journal of Veterinary Medicine. v.39. n.3. p.205-215. 2009.

BRITO. L.O.; SANTOS. I.G.S.; ABREU. J.L.; ARAUJO. M.T.; SEVERI. W.; GALVEZ. A.O. Effect of the addition of diatoms (*Navicula* spp.) and rotifers (*Brachionus plicatilis*) on water quality and growth of the *Litopenaeus vannamei* postlarvae reared in a biofloc system. Aquaculture Research. v. 47. p. 3990-3997. 2016.

COSTA. S. W.; VICENTE. L. R. M.; SOUZA. T. M.; ANDREATTA. E. R.; MARQUES. M. R. F. Parâmetros de cultivo e a enfermidade da mancha-branca em fazendas de camarões de Santa Catarina. Pesquisa Agropecuária Brasileira. v. 45. n.12. p. 1-10. 2011.

CLARK. K.F. Nimaviruses of Crustaceans. In: KIBENGE. F. S.B. & GODOY. M.G. Aquaculture Virology. 2016. cap. 26. p. 397 – 413.

CRAB. R.; DEFOIRDT. T.; BOSSIER. P.; VERSTRAETE. W. Biofloc technology in aquaculture: beneficial effects and future challenges. Aquaculture. v. 356. 351-356. 2012.

DANTAS JR. E. M.. VALLE. B. C. S.. BRITO. C. M. S.. CALAZANS. N. K. F.. PEIXOTO. S. R. M.. SOARES. R. B. Partial replacement of fishmeal with biofloc meal in the diet of postlarvae of the Pacific white shrimp *Litopenaeus vannamei*. Aquaculture nutrition. v.22. n.2. p.335-342. 2016.

DI LEONARDO. V. A.; BONNICHON. V.; ROCH. P.; PARRINELLO. N.; BONAMI. J. R. Comparative WSSV infection routes in the shrimp genera *Marsupenaeus* and *Palaemon*. Journal of Fish Diseases. v.28. n.9. p.565-569. 2005.

EKASARI. J.; AZHAR. M. H.; SURAWIDJAJA. E. H.; NURYATI. S.; DE SCHRYVER. P.; BOSSIER. P. Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. Fish & Shellfish Immunology. v.41. n. 2. p. 332-339. 2014.

FÉLIX. D. M.; ELÍAS. J. A. L.; CÓRDOVA. Á. I. C.; CÓRDOVA. L. R. M.; GONZÁLEZ. A. L.; JACINTO. E. C.; ALDAZ. N. H.; MENDOZA. F. C.; ZAZUETA. M. G. B. Survival of *Litopenaeus vannamei* shrimp fed on diets supplemented with *Dunaliella* sp. is improved after challenges by *Vibrio parahaemolyticus*. Journal of Invertebrate Pathology. v. 148. p.118-123. 2017.

FEED AND FOOD: Porta-voz da agroindústria da cadeia de proteína animal. Sorocaba: Ciasulli. n. 123. 2017.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS – FAO. The State of World Fisheries and Aquaculture: Meeting the sustainable development goals. Fisheries an Aquaculture Technical Paper. Rome. 2018. 210p.

GE. H.; LI. J.; CHANG. Z.; CHEN. P.; SHEN. M.; ZHAO. F. Effect of microalgae with semi continuous harvesting on water quality and zootechnical performance of white shrimp reared in the zero water exchange system. Aquacultural Engineering. v. 72. p.70-76. 2016.

IBGE (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA). Produção da pecuária municipal. 2017. Disponível em: https://sidra.ibge.gov.br/tabela/3940. Acesso em: 23 de julho de 2017.

JAMALI. H.; AHMADIFARD. N.; ABDOLLAHI. D. Evaluation of growth. survival and body composition of larval white shrimp (*Litopenaeus vannamei*) fed the combination of three types of algae. International Aquatic Research. v.7. n.2. p.115-122. 2015.

JU. Z. Y.; DENG. D. F.; DOMINY. W. Adefatted microalgae (*Haematococcus pluvialis*) meal as a protein ingredient to partially replace fishmeal in diets of Pacific white shrimp (*Litopenaeus vannamei*. Boone. 1931). Aquaculture. v. 354. p. 50-55. 2012.

KARUNASAGAR. I.; ABABOUCH. L. Shrimp viral diseases. import risk assessment and international trade. Indian Journal of Virology. v. 23. n.2. p. 141-148. 2012.

KUMAR. S. D.; SANTHANAM. P.; ANANTH. S.; KAVIYARASAN. M.; NITHYA. P.; DHANALAKSHMI. B.; PARK. M. S.; KIM. M. K. Evaluation of suitability of wastewater-grown microalgae (*Picochlorum maculatum*) and copepod (*Oithona rigida*) as live feed for white leg shrimp *Litopenaeus vannamei* post-larvae. Aquaculture International. v.25. n.1. p.393-411. 2017.

LIGHTNER. D. V.; REDMAN. R. M.; PANTOJA. C. R.; TANG. K. F. J.; NOBLE. B. L.; SCHOFIELD. P.; MOHNEY. L. L.; NUNAN. L. M.; NAVARRO. S. A. Historic emergence. impact and current status of shrimp pathogens in the Americas. Journal of Invertebrate Pathology. v. 110. n.2. p. 174-183. 2012.

LIN. Y. R.; HUNG. H. C.; LEU. J. H.; WANG. H. C.; KOU. G. H.; LO. C. F. The role of aldehyde dehydrogenase and HSP70 in suppression of White Spot Syndrome Virus replication at high temperature. Journal of Virology. v. 85. n.7. p 3517-3525. 2011.

LIN. H.; CHEN. Y.; NIU. J.; ZHOU. C.; HUANG. Z.; DU. Q.; ZHANG. J. Dietary methionine requirements of pacific white shrimp *Litopenaeus vannamei*. of three different sizes. The Israeli Journal of Aquaculture-Bamidgeh. v. 67. p. 10. 2015.

LO. C.F.; LEI. J.H.; HO. C.H.; CHEN. C.H.; PENG. S.E.; CHEN. Y.T.; CHOU. C.M.; YEH. P.Y.; HUANG. C.J.; CHOU. H.Y.; WANG. C.H.; KOU. G.H. Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. Diseases of Aquatic Organisms. v.25. p.133-141. 1996.

LÓPEZ-ELÍAS. J. A.; MEDINA-FÉLIX. D.; CAMPA-CÓRDOVA. Á. I.; MARTÍNEZ-CÓRDOVA. L. R.; HERNÁNDEZ-LÓPEZ. J.; MENDOZA-CANO. J. F.; RIVAS-VEGA. M. E. Optimización de la supervivencia y respuesta inmune de *Litopenaeus vannamei* alimentado con dietas ricas en carotenos e infectado con el Síndrome de Mancha Blanca. Latin American Journal of Aquatic Research. v. 44. n.2. p. 305-312. 2016.

LUCAS. J.S.; SOUTHGATE. P.C. Aquaculture Farming Aquatic Animals and Plants. Second edition. Nova Jersey: Wiley-Blackwell. 2012.

MAGAÑA-GALLEGOS. E.; GONZÁLEZ-ZÚÑIGA. R.; CUZON. G.; AREVALO. M.; PACHECO. E.; VALENZUELA. M. A; GAXIOLA. G; CHAN-VIVAS. E.; LÓPEZ-AGUIAR. K.; NOREÑA-BARROSO. E. Nutritional contribution of biofloc within the diet of growout and broodstock of *Litopenaeus vannamei*. determined by stable isotopes and fatty acids. Journal of the World Aquaculture Society. p. 1-14. 2018.

MALDONADO. M.; RODRÍGUEZ. J.; DE BLAS. I. EL camarón de cultivo frente al WSSV. su principal patógeno. Revista AquaTIC. n. 21. 2016.

MALIWAT. G. C.; VELASQUEZ. S.; ROBIL. J. L.; CHAN. M.; TRAIFALGAR. R. F.; TAYAMEN. M.; RAGAZA. J. A. Growth and immune response of giant freshwater prawn *Macrobrachium rosenbergii* (De Man) postlarvae fed diets containing *Chlorella vulgaris* (Beijerinck). Aquaculture Research. v.48. n.4. p.1666-1676. 2017.

MARINHO. Y. F.; BRITO. L.O.; DA SILVA. C. V. F.; DOS SANTOS. I. G. S.;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. v.42. n.3. 2014.

MARINHO. Y. F.; BRITO. L. O.; SILVA CAMPOS; C. V. F. D.; 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. v. 48. n.8. p. 4155-4164. 2017.

MARTÍNEZ-CÓRDOVA. L. R.; EMERENCIANO. M.; MIRANDA-BAEZA. A.; MARTÍNEZ-PORCHAS. M. Microbial-based systems for aquaculture of fish and shrimp: an updated review. Reviews in Aquaculture. v. 7. n.2. p.131-148. 2015.

MARTINS. T. G.; ODEBRECHT. C.; JENSEN. L. V.; D'OCA. M. G.; WASIELESKY. W. The contribution of diatoms to bioflocs lipid content and the performance of juvenile *Litopenaeus vannamei* (Boone. 1931) in a BFT culture system. Aquaculture Research. v.47. n.4. p. 1315-1326. 2016.

MEDINA-FÉLIX. D.; LÓPEZ-ELÍAS. J. A.; MARTÍNEZ-CÓRDOVA. L. R.; LÓPEZ-TORRES. M. A.; HERNÁNDEZ-LÓPEZ. J.; RIVAS-VEGA. M. E.; MENDOZA-CANO. F. Evaluation of the productive and physiological responses of *Litopenaeus vannamei* infected with WSSV and fed diets enriched with *Dunaliella* sp. Journal of Invertebrate Pathology. v.117. p.9-12. 2014.

NESARA. K.M.; PATURI. A.P. Nutritional requirement of fresh water prawn and shrimps: A review. Journal of Entomology and Zoology Studies. v.6. n.4. p.1526-1532. 2018.

NILSEN. P.; KARLSEN. M.; SRITUNYALUCKSANA. K.; THITAMADEE. S. White spot syndrome virus VP28 specific double-stranded RNA provides protection through a highly focused siRNA population. Scientific Reports. v. 7. n.1028. p. 1-14. 2017.

NIU. J.; XIE. Shi-Wei; FANG. Hao-Hang; XIE. Jia-Jun; GUO. Tian-Yiu.; ZHANG. Yan-Mei. LIU. Zhen-Lu; LIAO. Shi-Yu; HE. Ju-Yun; TIAN. Li-Xia; LIU. Y. J. Dietary values of macroalgae *Porphyra haitanensis* in *Litopenaeus vannamei* under normal rearing and WSSV challenge conditions: Effect on growth. immune response and intestinal microbiota. Fish & Shellfish Immunology. v. 81. p. 135-149. 2018.

PATEL. M. P.; VADHER. K. H.; TANK. P. R. Virulence of *Vibrio* spp. to pacific white shrimp *Litopenaeus vannamei* juveniles. Journal of Entomology and Zoology Studies 2018; v.6. n.6. p.1258-1262. 2018.

RAJKUMAR. M.; PANDEY. P. K.; ARAVIND. R.; VENNILA. A.; BHARTI. V.; PURUSHOTHAMAN. C. S. Effect of different biofloc system on water quality. biofloc composition and growth performance in *Litopenaeus vannamei* (Boone. 1931). Aquaculture Research. v.47. n.11. p.3432-3444. 2016.

ROMANO. N.; DAUDA. A. B.; IKHSAN. N.; KARIM. M.; KAMARUDIN. M. S. Fermenting rice bran as a carbon source for biofloc technology improved the water quality. growth. feeding efficiencies. and biochemical composition of African catfish *Clarias gariepinus* juveniles. Aquaculture research. v.49. n.12. p.3691-3701.2018.

SÁNCHEZ-PAZ. A. White spot syndrome virus: an overview on an emergent concern. Veterinary Research. v. 41. n. 6. p. 1-34. 2010.

SAHUL HAMEED. A.S.; SAR ATHI. M.; SUDHAKARAN. R.; BALASUBRAMANIAN. G.; SYED MUSTHAQ. S. Quantitative assessment of apoptotic hemocytes in white spot syndrome virus (WSSV)-infected penaeid shrimp. *Penaeus monodon* and *Penaeus indicus*. by flow cytometric analysis. Aquaculture. v. 256. n. 1-4. p. 111-120. 2006.

SCHAAL. G.; LECLERC. J. C.; DROUAL. G.; LEROUX. C.; RIERA. P. Biodiversity and trophic structure of invertebrate assemblages associated with understorey red algae in a *Laminaria digitata* bed. Marine Biology Research. v.12. n.5. p.513-523. 2016.

SEIFFERT. W.; COSTA. S.W.; MAGGIONI. D.A mancha branca em Santa Catarina. Revista Panorama da Aquicultura. Rio de Janeiro. v.15. n.. 87. p.51-53. 2005.

SHAH. M. R.; LUTZU. G. A.; ALAM. A.; SARKER. P.; CHOWDHURY. M. K.; PARSAEIMEHR. A.; LIANG. Y.; DAROCH. M. Microalgae in aquafeeds for a sustainable aquaculture industry. Journal of Applied Phycology. v.30. n.1. p.197-213. 2018.

SOON. L.T.; LEE. K.L.; SHARIFF; M.; HASSAN; M.D.; OMAR. A.R. Quantitative analysis of an experimental white spot syndrome virus (WSSV) infection in *Penaeus*

monodon Fabricius using competitive polymerase chain reaction. Journal of Fish Diseases. v. 24. n. 6. p. 315-323. 2001.

TAKAHASHI. Y.; ITAMI. T.; KONDO. M.; MAEDA; M.; FUJII. R.; TOMONAGA. S.; SUPAMATTAYA. K.; BOONYARATPALIN. S. Electron microscopic evidence of bacilliform virus infection in kuruma shrimp (*Penaeus japonicus*). Fish Pathology. v.29. n. 2. p. 121-125. 1994.

UNDERWOOD. D. J.; COWLEY. J. A.; JOHNSON. K. N. Antiviral immunity and protection in penaeid shrimp. Invertebrate Immunity. v.1. p. 2-14. 2013.

VAZQUEZ. L.; ALPUCHE. J.; MALDONADO. G.; AGUNDIS. C.; PEREYRA-MORALES. A.; ZENTENO. E. Immunity mechanisms in crustaceans. Innate immunity. v.15. n.3. p.179-188. 2009.

VAZQUEZ-BOUCARD. C.; ESCOBEDO-FREGOSO. C.; DURAN-AVELAR. M. D. J.; MERCIER. L.; LLERA-HERRERA. R.; ESCOBEDO-BONILLA. C.; VIBANCO-PEREZ. N. *Crassostrea gigas* oysters as a shrimp farm bioindicator of white spot syndrome virus. Diseases of aquatic organisms. v.98. n.3. p.201-207. 2012.

WADE. N. M.; GABAUDAN. J.; GLENCROSS. B. D. A review of carotenoid utilisation and function in crustacean aquaculture. Reviews in Aquaculture. v. 9. n.2. p.141-156. 2017.

WORLD ORGANISATION FOR ANIMAL HEALTH – OIE. Manual of Diagnostic Tests for Aquatic Animals. Office international des epizooties. França. 2018. cap. 2.2.8. p. 1-16.

XU. WU-JIE; MORRIS. T.C.; SAMOCHA. T.M. Effects of C/N ratio on biofloc development. water quality. and performance of *Litopenaeus vannamei* juveniles in a biofloc-based. high-density. zero-exchange. outdoor tank system. Aquaculture. v.453. p. 169-175. 2016.

YAN. D.C.; HUANG. J.; YANG. B.; SUN. H.S.; WANG. Y.Y.; LIU. X. Competition of infectious hypodermal and haematopoietic necrosis virus (IHHNV) with white spot syndrome virus (WSSV) for binding to shrimp cellular membrane. Journal of Fish Diseases. v.39. n.10. p.1225-1229. 2016.

YU. Y.; LIU. J.; LI. F.; ZHANG. X.; ZHANG. C.; XIANG. J. Gene set based association analyses for the WSSV resistance of Pacific white shrimp *Litopenaeus vannamei*. Scientific reports. v. 7. p.10. 2017.