

DANIELLE ALVES DA SILVA

**EFEITO DA DENSIDADE DE ADIÇÃO DE *Brachionus plicatilis* (Müller, 1786) NO  
CULTIVO DE PÓS-LARVAS DE *Litopenaeus vannamei* (Boone, 1931) EM SISTEMA  
DE BIOFLOCOS**

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**PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E**  
**AQUICULTURA**

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**DE BIOFLOCOS**

**Danielle Alves da Silva**

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**Danielle Alves da Silva**

Dissertação julgada adequada para obtenção do título de Mestre em Recursos Pesqueiros e Aquicultura. Defendida e aprovada em 14/02/2020 pela seguinte Banca Examinadora.

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## RESUMO

A produção de juvenis de camarões marinhos tem sido utilizada com estratégias de convivência com os patógenos, reduzindo as perdas produtivas na carcinicultura. A aplicação de microrganismos capazes de melhorar o teor nutricional dos flocos microbianos nas fases iniciais de cultivo, contribuindo para o maior crescimento dos camarões e melhora do sistema imune é uma ferramenta bastante promissora nessa fase. Neste sentido, o presente trabalho avaliou o efeito da densidade de adição do rotífero *Brachionus plicatilis* em diferentes densidades no cultivo de pós-larvas de *Litopenaeus vannamei* em sistema de bioflocos incentivado por fermentação de carbono orgânico e à base de mix de bactérias. Para tal, o desenho experimental foi composto por quatro tratamentos e três repetições cada, totalizando 12 unidades experimentais em delineamento inteiramente casualizado durante 42 dias. Os tratamentos foram: BFT (Bioflocos); BFT-10 (Bioflocos com adição de *B. plicatilis* na densidade de 10 organismos mL<sup>-1</sup>), BFT-20 (Bioflocos com adição de *B. plicatilis* na densidade de 20 organismos mL<sup>-1</sup>) e BFT-30 (Bioflocos com adição de *B. plicatilis* na densidade de 30 organismos mL<sup>-1</sup>). As pós-larvas de dez dias (PL<sub>10</sub>) com peso médio de 3,4 ± 0,02 mg foram estocadas na densidade de 3.000 indivíduos m<sup>-3</sup> em unidades experimentais de 40L de volume útil. A adição do rotífero *B. plicatilis* foi realizada no 1º, 10º, 20º e 30º dias. Os camarões foram alimentados com ração comercial com 45% de proteína bruta, quatro vezes ao dia com taxa de alimentação inicial de 35% da biomassa. Durante o experimento foi avaliado o desempenho zootécnico, qualidade da água, caracterização de bactérias do gênero *Vibrio*, comunidade planctônica, composição centesimal e contagem total de hemócitos. Sobre o desempenho zootécnico, podem-se destacar os maiores valores médios de peso final e produtividade nos tratamentos com adição de *B. plicatilis*, nos quais os camarões atingiram de 1,09 a 1,26g e 2,25 a 3,41 kg m<sup>-3</sup>, respectivamente. As variáveis de qualidade de água estiveram dentro do recomendado para camarões marinhos e não foram observadas diferenças significativas entre os tratamentos. O efeito das densidades de adição foi significativo para o consumo de água por quilograma de juvenil produzido, além da redução da porcentagem de colônias de sacarose negativas do gênero *Vibrio* presentes na água e no camarão. Os gêneros mais frequentes observados para fitoplâncton foram: *Oscillatoria* (6,97 a 9,27%), *Aphanocapsa* (6,48 a 7,85%), e para o zooplâncton foram: *Brachionus* sp. (21,49 a 33,73%), *Daphnia* sp. (15,44 a 31,25%) e *Arcella* sp. (12,75 a 20,62%). Os tratamentos com maiores níveis de inclusão do rotífero (BFT-20 e BFT-30) obtiveram os melhores resultados em relação à quantidade de proteína e lipídios. Além disso, a adição de rotífero também proporcionou uma melhor resposta imunológica aos animais cultivados comprovada através da contagem total de hemócitos apresentando altas concentrações de hemócitos antes e após os animais serem submetidos ao teste de estresse salino. Portanto, a adição de rotífero em densidade de 20 a 30 organismos mL<sup>-1</sup> demonstrou incrementar o desempenho zootécnico de juvenis de *Litopenaeus vannamei* na fase berçário em bioflocos.

**Palavras-chave:** *Litopenaeus vannamei*, *Brachionus plicatilis*, Berçário, Bioflocos, *Vibrios*, Crescimento, Composição centesimal

## ABSTRACT

The production of juvenile marine shrimp has used strategies that involve coexistence with pathogens, reducing production losses in shrimp farming. One of these strategies is the application of microorganisms that can improve the nutritional content of microbial biofloc in the early stages of cultivation, contributing to greater growth of shrimp and stronger immune systems. This study evaluated the effect of addition of the rotifer *Brachionus plicatilis* at different densities to the cultivation of post-larvae *Litopenaeus vannamei* in a biofloc system supported by fermented base with a mix of bacteria. The experimental design consisted of four treatments with three replicates of each, generating a total of 12 experimental units in a completely randomized design for 42 days. The treatments included: BFT (Bioflocs); BFT - 10 (Bioflocs with the addition of *B. plicatilis* at a density of 10 organisms mL<sup>-1</sup>), BFT - 20 (Bioflocs with the addition of *B. plicatilis* at a density of 20 organisms mL<sup>-1</sup>) and BFT - 30 (Bioflocs with addition of *B. plicatilis* at a density of 30 mL<sup>-1</sup> organisms). Ten-day-old post-larvae (PL<sub>10</sub>) with an average weight of 3.4 ± 0.02 mg were stocked at a density of 3,000 individuals m<sup>-3</sup> in experimental units of 40L of useful volume. The rotifer *B. plicatilis* were added on the 1<sup>st</sup>, 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> days. The shrimps were fed with a commercial ration with 45% crude protein, four times a day with an initial feed rate of 35% of the biomass. During the experiment, we evaluated the water quality, zootechnical performance, and monitored the zooplankton community, centesimal composition, total hemocyte count, and quantified and characterized the *Vibrio* bacteria. The water quality variables were within the recommendations for marine shrimp and no significant differences were observed between treatments. At the end, regarding zootechnical performance, the highest average values of final weight and productivity were in treatments with the addition of *B. plicatilis*, in which the shrimp reached 1.09 to 1.26 g and 2.25 to 3.41 kg m<sup>-3</sup>. The most frequent phytoplankton genera observed were: *Oscillatoria* (6.97 to 9.27%), *Aphanocapsa* (6.48 to 7.85%), and for and zooplankton were: *Brachionus* sp. (21.49 to 33.73 %), *Daphnia* sp. (15.44 to 31.25%) and *Arcella* sp. (12.75 to 20.62%). The effect of the stocking densities was also significant for water consumption per kilogram of juveniles produced, and on the reduction in the percentage of negative sucrose colonies of the *Vibrio* genus in the water and the shrimp. The treatments with higher levels of rotifer (BFT-20 and BFT-30) had the best results for protein and lipids. The addition of rotifers also strengthened the immune system of the cultivated shrimp, as demonstrated by the total hemocyte count, with high concentrations of hemocytes before and after the animals were subjected to a salt stress test. Therefore, the addition of rotifer at densities of 20 to 30 mL<sup>-1</sup> organisms coupled with an efficient fertilization strategy demonstrated excellent results in the production of *L. vannamei* juveniles.

Keywords: *Litopenaeus vannamei*, *Brachionus plicatilis*, Nursery, Bioflocs, *Vibrios*, Growth, Centesimal composition



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## 1. INTRODUÇÃO

Dentre os principais produtos da aquicultura mundial, está a produção de camarão, ao qual destaca-se em termos financeiros, representando o segundo segmento de maior faturamento com US\$ 69,3 bilhões de dólares em 2018 (27,7%) e, o terceiro segmento em volume de produção, com 9,38 milhões de toneladas produzidas (FAO, 2020). Atualmente, *Litopenaeus vannamei* (Boone, 1931) é a espécie com maior representatividade mundial no setor do cultivo de crustáceos (FAO, 2020) e, no Brasil, sua produção atingiu, em 2018, o valor de 45,8 mil toneladas que corresponde a 20,6% de toda produção aquícola nacional, com R\$ 1,1 bilhão de receita (IBGE, 2019). O principal polo produtor é a Região Nordeste, responsável por quase toda a produção nacional (99,4%), com os estados do Rio Grande do Norte e Ceará se destacando com os melhores índices de produtividade (IBGE, 2019).

O crescimento acelerado do setor nas últimas décadas ocorreu frente a graves problemas ambientais e disseminação de doenças (MOSS et al., 2012; NÓBREGA et al. 2013). Esses surtos de doenças causam a diminuição do consumo alimentar, crescimento reduzido e altas mortalidades, gerando uma acentuada queda na produtividade do setor (SOTO-RODRIGUEZ et al., 2012; TAW, 2012). As perdas econômicas geradas por essas quedas na produtividade foram de aproximadamente US\$ 15,0 bilhões de dólares na produção em 2015, onde pode-se destacar os surtos do Vírus da Síndrome da Mancha Branca como o principal fator (VERBRUGGEN, et al., 2016, NUNES E FEIJÓ, 2016; YU et al., 2017). Além disso, bactérias patogênicas e oportunistas do gênero *Vibrio* encontradas na água também acometem os cultivos de camarão (KUMAR et al., 2014; ZHANG et al., 2014; BOWLER, et al., 2015).

Diante disso, pesquisadores e produtores buscam novas técnicas de manejo, mais biosseguras, que visam minimizar essas perdas produtivas (AVNIMELECH, 2012; PÉREZ-FUENTES et al., 2013). Dentre essas técnicas destaca-se o cultivo de camarões em sistema com mínima troca de água denominado de sistema de bioflocos (BFT), tecnologia alternativa que utiliza altas densidades de estocagem, com fertilizações orgânicas e probióticos para incentivar o crescimento da comunidade microbiana e manutenção da qualidade da água (KRUMMENAUER et al., 2012; EMERENCIANO et al., 2013; ROMANO et al., 2018). A fertilização consiste na adição de uma fonte de carbono orgânica que ajuda a aumentar a relação carbono: nitrogênio, estimulando o crescimento das bactérias heterotróficas no sistema (PANIGRAHI et al., 2018). Essa comunidade assimila os compostos nitrogenados tóxicos presentes na água transformando em proteína microbiana, fornecendo uma fonte de

suplementação alimentar e melhorando a qualidade de água (OTOSHI et al., 2011; FÓES et al., 2012; RAJKUMAR et al., 2016).

Esta adequada relação de C:N pode acelerar a ciclagem dos nutrientes (AHMAD et al., 2017). De acordo com Pérez-Fuentes et al. (2016) a relação C:N deve estar acima de 10:1. Porém relações muito elevadas resultam na predominância de bactérias heterotróficas em detrimento das bactérias nitrificantes que são as mais eficientes na remoção dos compostos nitrogenados como amônia e nitrito (RIOS DA SILVA, 2009; BALLESTER et al., 2010). Além disso, a longo prazo, as relações C:N elevadas propiciam o aumento substancial dos sólidos sedimentáveis que podem causar entupimento das brânquias do camarão e deterioração da qualidade da água (SCHVEITZER et al., 2013). Dessa forma, recomenda-se relações CHO:N (10-12:1) onde a distribuição de bactéria heterotrófica e nitrificantes sejam mais homogênea, também estimulando o crescimento do plâncton e proporcionando, redução na carga orgânica (ROMANO, 2017).

Os bioflocos são compostos por microalgas, bactérias, protozoários, resto de ração, exoesqueletos e fezes, entre outras partículas de matéria orgânica (MANAN et al., 2017). Além de contribuir para a manutenção da qualidade da água, os flocos microbianos (bioflocos) são considerados com uma importante fonte de proteínas, lipídios, minerais e vitaminas (EKASARI et al., 2015). Apesar da presença de proteína e lipídeos na sua composição nutricional, existe uma deficiência de metionina e lisina, ácido linoleico, eicosapentaenóico (EPA) e docasahexanóico (DHA) (CRAB et al., 2010; DANTAS et al., 2016; MAGAÑA-GALLEGOS et al., 2018), que são nutrientes essenciais à sobrevivência e crescimento dos camarões (LIN et al., 2015; NESARA E PATURI, 2018).

Estes valores nutricionais dos flocos microbianos são influenciados pela composição de microorganismos presentes, salinidade, temperatura, oxigênio dissolvido e a fonte de carbono utilizada para incentivar a comunidade bacteriana (JU et al., 2008; MAICA et al., 2012; PHULIA et al., 2012). Então essa deficiência de aminoácidos e ácidos graxos pode ser suplementada de outra forma, com a administração de microorganismos vivos que servirão como fonte de aminoácidos, ácidos graxos e minerais para as fases iniciais do cultivo (SCHAAL et al., 2016; KURMAR et al., 2017).

*Brachionus plicatilis* (MÜLLER, 1786) é uma das espécies de rotíferos mais conhecidas por ser considerada uma fonte rica de aminoácidos, ácidos graxos essenciais (EPA e DHA) e minerais, sendo de notável importância nutricional (JEEJA et al., 2011). A composição deste rotífero pode ser manipulada para se adequar aos requerimentos nutricionais do organismo alvo

do cultivo (LUBZENS et al., 1989). Nesse sentido, a composição bioquímica está relacionada com as dietas fornecidas durante o cultivo, podendo apresentar variações no conteúdo de proteína e ácidos graxos polinsaturados (FERREIRA et al., 2008; 2009).

Além da contribuição nutricional, essa espécie de rotífero apresenta características adequadas para a produção em larga escala como o crescimento rápido, baixa mobilidade na coluna d'água, fácil assimilação de substâncias enriquecedoras e elevada atratibilidade como alimento vivo às pós-larvas, quando comparado ao alimento inerte (CONCEIÇÃO et al., 2010). Porém, os aquicultores ao produzir alimento vivo devem buscar um equilíbrio entre a qualidade do alimento e o custo de produção. Os rotíferos são organismos filtradores não seletivos, dessa forma, o uso de microalgas, como *Nannochloropsis* sp., *Isochrysis galbana* e *Chlorella vulgaris* e soluções comerciais como dietas de enriquecimento são frequentemente utilizadas, desde que seja economicamente viável e proporcione o fornecimento constante de alta qualidade (CARVALHO et al., 2014; FUENTES-GRÜNEWALD et al., 2015; TORZILLO E CHINI ZITELLI, 2015; FERREIRA et al., 2018).

A condição nutricional dos animais cultivados está diretamente relacionada com o seu estado imunológico, podendo determinar a capacidade de resistência a mudanças no ambiente e doenças (CUÉLLAR-ANJEL et al., 2010). Nesse cenário vem sendo proposto além do uso do sistema intensivo com mínima troca de água, a adição de microorganismos capazes de melhorar nutricionalmente a composição dos flocos microbianos, também possibilitando um aumento da resistência imune do animal (MARINHO et al., 2017; JAMALI et al., 2015; BRITO et al., 2016; ABREU et al., 2019).

## 2. OBJETIVO GERAL

Avaliar o efeito da densidade de adição de *Brachionus plicatilis* no cultivo de pós-larvas de *Litopenaeus vanammei* em sistema de bioflocos.

### 2.1 Objetivos Específicos

- Avaliar o efeito da densidade de adição de *B. plicatilis* no desempenho zootécnico de pós-larvas de *L. vanammei* cultivadas em sistema de bioflocos.
- Mensurar e avaliar as variáveis físico-químicas de água ao longo do cultivo de pós-larvas de *L. vanammei* com adição *B. plicatilis* em diferentes densidades;

- Analisar a composição centesimal dos bioflocos e camarões com adição *B. plicatilis* em diferentes densidades;
- Identificar e quantificar a comunidade planctônica ao longo do cultivo de pós-larvas de *L. vanammei* com adição *B. plicatilis* em diferentes densidades;
- Quantificar as Unidades Formadoras de Colônia (UFC) de *Vibrio* spp. ao longo do cultivo na água e no camarão;
- Avaliar a sobrevivência dos camarões a choque osmótico após o cultivo e o status imunológico através da contagem total de hemócitos.

### 3. ARTIGO CIENTÍFICO

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1 **Effects of addition of *Brachionus plicatilis* (rotifer) in different densities to *Litopenaeus***  
2 ***vannamei* reared in a nursery BFT system with anaerobic and aerobic process wheat**  
3 **bran as an organic carbon source: Growth, Water quality, *Vibrio*, plankton and**  
4 **proximal composition**

5  
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26 **ABSTRACT**

27 The objective of this study was to evaluate the effect of the addition of *Brachionus plicatilis*  
28 on the growth, *Vibrio* count, water quality, plankton composition, total hemocyte count and  
29 proximal composition of *Litopenaeus vannamei* raised in a biofloc system. Four treatments  
30 were used in the first phase: BFT (bioflocs); BFT-10 (addition of 10 org mL<sup>-1</sup> of *Brachionus*  
31 *plicatilis*); BFT-20 (addition of 20 org mL<sup>-1</sup> of *Brachionus plicatilis*) and BFT-30 (addition  
32 of 30 org mL<sup>-1</sup> of *Brachionus plicatilis*), all in triplicate. The shrimp (PL<sub>10</sub>, 3.4 ± 0.02 mg)  
33 were reared in a biofloc system (with inorganic fertilization - nitrogen, phosphorus and  
34 silicate and; anaerobic and aerobic process with wheat brain and a microbial mix with 7.7 X  
35 10<sup>8</sup> UFC g<sup>-1</sup> of *Lactobacillus* sp., *Bacillus subtilis* and *B. Licheniformes* strains for 42 days at a  
36 stocking density of 3,000 shrimp m<sup>-3</sup> fed with commercial feed (45% crude protein and 8%  
37 lipids) with an initial feed rate of 35% of shrimp biomass. The rotifers were added every 10  
38 days (from day 1 to 30), and at the end of 42 days, shrimp performance, water quality, *Vibrio*  
39 count, and proximal composition were evaluated and a salinity stress test conducted. The  
40 BFT-20 and BFT-30 treatments had higher performance values, highlighted by final weight  
41 (1.18 and 1.26 g) and yield (3.14 and 3.41 kg m<sup>-3</sup>). There were no significant differences (p>  
42 0.05) in the water quality parameters among the four treatments. The percentage of positive  
43 sucrose colonies increased and of negative sucrose colonies decreased by the end of the  
44 experimental period, with the negative sucrose colonies dropping to 0% in the BFT-30  
45 treatment. The THC was significantly higher (p< 0.05) at the end of culture and after the  
46 salinity stress test for shrimp submitted to treatments with the addition of rotifers than in the  
47 BFT. The proximal composition (g kg<sup>-1</sup> dry weight basis) of crude protein, 203.11 to 298.25,  
48 and crude lipids of 66.19 to 130.01 in the bioflocs were within significant differences (p <  
49 0.05) for BFT-20 and BFT-30 as compared to BFT and the initial value. The best shrimp  
50 performance was observed in treatments with BFT- 30 and 20, indicating the benefits of

51 *Brachionus plicatilis* on growth enhancement and microbial floc content of *L. vannamei*  
52 postlarvae grown in biofloc systems.

53

54 Keywords: juvenile; shrimp; zooplankton; yield; colonies bacterial

55

## 56 1. INTRODUCTION

57 Inadequate management practices in shrimp farming have contributed to the rapid  
58 spread of diseases, causing significant production losses in recent years (New et al., 2010;  
59 Vidal and Ximenes, 2016). In Brazil, shrimp production decreased by more than 50%  
60 between the years 2003 and 2018 (ABCC, 2013; IBGE, 2019). This reduction is linked to  
61 outbreaks of diseases, mainly of viral and bacterial origins, such as White Spot Syndrome  
62 Virus and Infectious Myonecrosis Virus and the presence *Vibrio* bacteria (Seiffert and  
63 Winckler, 2005; Guerrelhas and Teixeira, 2012; Soto-Rodriguez et al., 2014; Rebouças et al.,  
64 2017; Shinn et al., 2018).

65 In this scenario, new technologies are required to make shrimp culture more  
66 sustainable and increase its biosafety, minimizing the amount of exchange water by  
67 maintaining water quality in ponds (Brito et al., 2011). Among these shrimp farming  
68 technologies, biofloc systems with minimal water exchange stand out (Avnimelech, 2015;  
69 Bossier and Ekasari, 2017). The system promotes growth of the microbial community that  
70 assimilates toxic nitrogenous compounds in the water, transforming them into microbial  
71 proteins that serve as food to supplement shrimp nutrition (Crab et al., 2012; Souza et al.,  
72 2014; Avnimelech, 2015). Organic carbon sources such as molasses and sugar are applied to  
73 maintain the C:N ratio above 10:1 (Ray and Lotz, 2014; Pérez-Fuentes et al., 2016). However,  
74 high C:N ratios cause a substantial increase in sedimentable solids, which can deteriorate  
75 water quality and cause gills to clog (Schweitzer et al., 2013). Thus, C:N ratios are

76 recommended in which the distribution of heterotrophic bacteria and nitrifying bacteria are  
77 more homogeneous (C:N 10-12:1) (Xu et al., 2016; 2018; Romano, 2017).

78 Applications of organic carbon have been used after anaerobic and aerobic process of  
79 prebiotics (wheat, soybean and rice bran), associated with the use of probiotics, which can  
80 have synergistic effects on the growth environment (Hapsari, 2016; Romano, 2018). This type  
81 of strategy reduces problems related to excess suspended solids, improving the distribution of  
82 heterotrophic and nitrifying bacteria and stimulating plankton growth (Romano, 2017;  
83 Kawahigashi, 2018).

84 In general, microbial aggregates offer a supplemental source of proteins and lipids  
85 (Bakhshi et al., 2018). However, low levels of methionine and lysine, eicosapentaenoic acid  
86 (EPA) and docosahexaenoic acid (DHA) are observed (Zhou et al., 2007; Hu et al., 2011;  
87 Magaña-Gallegos et al., 2018), which are important nutrients for shrimp reared. Thus, some  
88 studies have shown that the addition to microorganisms to shrimp culture can improve  
89 zootechnical performance, probably due to the proximal composition of the microbial flocs  
90 (Marinho et al., 2014; Marinho et al., 2017; Jamali et al., 2015; Brito et al., 2016; Martins et  
91 al., 2016; Abreu et al., 2019).

92 The rotifer *Brachionus plicatilis* (Müller, 1786) has been used in the early stages of  
93 fish and shrimp hatcheries as a supplementary source of protein (amino acids) and lipids (fatty  
94 acids) (Dhert et al., 2001; Jeeja et al., 2011). The morphotypes of the rotifer can be classified  
95 as: “SS” (*Super small*), “S” (*Small*) and “L” (*Large*) based on the length of the lorica, which  
96 may be approximately 90-150, 100-210 or 130-340 (Hagiwara et al., 2001; Mills et al., 2017).  
97 According to Demir and Diken (2011a), *B. plicatilis* has approximately 480 - 590 g of crude  
98 protein and 61 -142 g of lipids per kilogram of dry matter. Jeeja et al. (2011) found amino  
99 acid levels between 241.4 to 411.8 g kg<sup>-1</sup> in *B. plicatilis* fed with different types of  
100 microalgae. Regarding essential fatty acids, Kotani et al. (2017) found mean values between

101 2.52 to 4.83 mg g<sup>-1</sup> dry weight of EPA and DHA in rotifers of the species *B. plicatilis* of the  
102 strain “Large” fed with *Chlorella vulgaris*.

103         These organisms are capable of absorbing the nutritional value of the diet offered,  
104 which must be chosen by considering production cost, nutritional quality and ease of large-  
105 scale production (Torzillo and Chini Zitelli, 2015; Ferreira et al., 2018). To improve the  
106 nutritional content of rotifers, microalgae are used in various forms (frozen, dry, concentrated  
107 or fresh) (Tzovenis et al., 2004; Seychelles et al., 2009; Kotani et al., 2010). One widely used  
108 microalgae is *Nannochloropsis* sp., which contains substantial amounts of EPA (Adissin et  
109 al., 2019). Other forms of enrichment include the use of baker's yeast (*Saccharomyces*  
110 *cerevisiae*) (Penglase et al., 2011), microcapsules (Langdon, 2003) and emulsions (Haché and  
111 Plante, 2011) rich in lipids. The choice of the diet offered is directly related to the  
112 biochemical composition of the rotifers being cultivated (Whyte et al., 1994).

113         In addition to these nutritional aspects, *B. plicatilis* grows quickly, which facilitates  
114 large-scale production, and is combined with its high tolerance to environmental changes that  
115 occur during cultivation, low mobility in the water column and a size suitable for feeding the  
116 postlarvae (Pousao-Ferreira et al., 2003; Seychelles et al., 2009; Jeeja et al., 2011; Dhont et  
117 al., 2013; Yin et al., 2013).

118         The nutritional quality of food supplied to cultivated animals contributes to better  
119 nutritional status and consequently to better immunological strength (Mohapatra et al., 2013;  
120 Jin et al., 2013; Newaj-Fyzul et al., 2014). Healthy animals are more resistant to changes in  
121 the culture system and to possible infections (Cuéllar-Anjel et al., 2010). Greater resistance  
122 may allow them to survive longer when submitted to stress tests, such as osmotic tests  
123 (Álvarez et al., 2004).

124         In this context, it is important to study the addition of microorganisms to improve the  
125 nutritional floc and zootechnical performance (Crab et al., 2012; Shah et al., 2018). The

126 purpose of this study is to evaluate the effects of addition of *Brachionus plicatilis* (rotifer) at  
127 different densities to the production of *L. vannamei* juveniles on zootechnical performance,  
128 water quality; presence of *Vibrio*, plankton composition, proximal composition and total  
129 hemocyte count in a nursery BFT system with the addition of anaerobic and aerobic wheat  
130 bran as an organic carbon source.

131

## 132 2. MATERIALS AND METHODS

### 133 2.1 Experimental conditions

134 An indoor trial was conducted for 42 days and the experimental design was  
135 completely randomized with four treatments: BFT (biofloc); BFT-10 (biofloc with addition of  
136 10 org mL<sup>-1</sup> of *Brachionus plicatilis*); BFT-20 (addition of 20 org mL<sup>-1</sup> of *Brachionus*  
137 *plicatilis*) and BFT-30 (addition of 30 org mL<sup>-1</sup> of *Brachionus plicatilis*), all in triplicate.

138 A matrix tank with water salinity of 35g L<sup>-1</sup> was chlorinated with 13 mg L<sup>-1</sup> of  
139 chlorine and after 72 hours of aeration, it was applied inorganic fertilization (divided in two  
140 applications) composed of urea (4.5 N g m<sup>-3</sup>), triple superphosphate (0.225 g P m<sup>-3</sup>) and  
141 sodium silicate (3.0 g Si m<sup>-3</sup>). After two days, organic fertilization was added through 10  
142 applications of a product for 48h in an anaerobic phase followed by a 24h aerobic phase, with  
143 wheat bran (50 g m<sup>-3</sup>), molasses (25 g m<sup>-3</sup>), sodium bicarbonate (10 g m<sup>-3</sup>) and a bacteria-  
144 based product (0.5 g m<sup>-3</sup>), containing *Lactobacillus* sp., *Bacillus subtilis* e *B. Licheniformes*  
145 com 7.7 x 10<sup>8</sup> UFC g<sup>-1</sup> (Kayros Ambiental e Agrícola, Brazil). Sea water (with 10 ppm of  
146 chlorine) was applied in a 10x proportion of wheat bran. The organic fertilizer was added at  
147 three-day intervals between applications. At the end of this organic fertilization process the  
148 water from a matrix tank (0.45 mg L<sup>-1</sup> Total ammonia nitrogen, 0.3 mg L<sup>-1</sup> N-NO<sub>2</sub>, 1.44 mg L<sup>-1</sup>  
149 N-NO<sub>3</sub>, 140 mg alkalinity CaCO<sub>3</sub> L<sup>-1</sup>, 20.51 mg L<sup>-1</sup> de orthophosphate, pH 8.4 and C:N  
150 11.2) was mixed and distributed equally to fill twelve black-plastic tanks (40 L). The organic

151 fertilizer was added to the experimental treatments every three days during culture until  
152 settleable solids reached 10 mL L<sup>-1</sup>.

153 The experimental units were constantly aerated (> 5.0 mg L<sup>-1</sup>), temperature  
154 maintained at (~31C) with a thermostat (100 W/40L) and light intensity was kept at 27 μmol  
155 m<sup>-2</sup> s<sup>-1</sup> with a natural photoperiod. The water was exchanged during the experimental period  
156 at a mean of 1% day<sup>-1</sup>, with the addition of dechlorinated freshwater to compensate for  
157 evaporation losses. Sodium bicarbonate (relative total neutralization power of 56%) was  
158 added to maintain alkalinity >100 mg L<sup>-1</sup> and pH >7.

159

## 160 **2.2 Production and addition of *Navicula* sp. and *Brachionus plicatilis***

161 Rotifers (*Brachionus plicatilis*) strain “L” (lorica length 198 μm) were cultured in a  
162 transparent glass conical-cylinder with a volume of 2.0 L and 20L and light intensity was  
163 maintained at 37 μmol m<sup>-2</sup> s<sup>-1</sup> using a fluorescent lamp with an integral light photoperiod.  
164 Culture water with 35 g L<sup>-1</sup> of salinity was initially chlorinated with 15 ppm chlorine and  
165 constant water aeration, pH 7.5-8.0, temperature 28 ± 1 ° C. Each week, 0.4 mL L<sup>-1</sup> of B-  
166 complex vitamins (B<sub>1</sub>, B<sub>6</sub> and B<sub>12</sub>) and 0.15 g L<sup>-1</sup> of fish oil (360 mg EPA and 240 mg DHA)  
167 were added, which was suspended 3 days before inoculation with rotifers in the experimental  
168 units. The rotifers were fed with microalgae in exponential growth phase *Nannochloropsis* sp.  
169 (50 x 10<sup>4</sup> cell mL<sup>-1</sup>). The organisms were filtered using a 50 μm mesh (and had approximate  
170 body size between 150 and 250 μm) and their density was estimated with a Sedgewick-Rafter  
171 chamber, before being added to the experimental units. Rotifers were added on the 1<sup>st</sup>, 10<sup>th</sup>,  
172 20<sup>th</sup> and 30<sup>th</sup> days to the BFT-10 (10 org mL<sup>-1</sup>), BFT-20 (20 org mL<sup>-1</sup>) and BFT-30 (30 org  
173 mL<sup>-1</sup>) treatments, with an addition of approximately 0.5 L of rotifers to each experimental  
174 tank, based on Brito et al. (2016).

175

176 **2.3 Shrimp stocking, feeding and monitoring**

177 *L. vannamei* postlarvae ( $3.4 \text{ mg} \pm 0.02 \text{ mg}$ ) were obtained from a commercial shrimp  
178 hatchery (Aquatec LTDA, RN, Brazil) and stocked at a density of  $3,000 \text{ shrimp m}^{-3}$  (120  
179 shrimp by experimental units) for 42 days. The postlarvae were fed four times a day (at 08:00  
180 a.m., 11:00 a.m., 02:00 p.m. and 04:00 p.m.), with a commercial shrimp feed with 45% crude  
181 protein, 8% lipids, 13% moisture, 9.5% crude fiber, 4% mineral matter (In vivo Animal  
182 Nutrition and Health). The daily feeding rate of 35% of body weight used at the start of the  
183 culture was gradually reduced to 10% of body weight after 42 days based on the Van Wyk  
184 (1999) table and adjusted daily according to estimated shrimp consumption and mortality rate.

185 Shrimp weight was monitored weekly after 21 days of culture to determine shrimp  
186 growth and adjust the amount of feed offered. At the end of the experiment, biomass gain,  
187 mean final weight, feed conversion ratio (FCR), survival, and yield and water consumption  
188 (WC) were determined based on the following equations: Biomass gain (g) = *final biomass*  
189 *(g) – initial biomass (g)*; Final weight (g) = *final biomass (g)/number of individuals at the end*  
190 *of evaluation period*; FCR = *feed supplied/biomass gain*; Survival (%) = *(number of*  
191 *individuals at the end of evaluation period/initial number of individuals) × 100*; Yield ( $\text{kg m}^{-3}$ ) = *final biomass (kg)/volume of experimental unit ( $\text{m}^3$ )* and WC = *Total water consumed (L)/*  
192 *Final biomass (kg)*.

194

195 **2.4 Water quality**

196 Dissolved oxygen, temperature, salinity and pH were monitored (YSI model 100,  
197 Yellow Springs, Ohio, USA) twice a day (at 08:00 hours and 16:00 hours). The settleable  
198 solids (Imhoff cone) (Avnimelech, 2015) were monitored three times a week. Total ammonia  
199 nitrogen (APHA, 2012), N-nitrite (APHA, 2012) and alkalinity ( $\text{mg L}^{-1} \text{ CaCO}_3$ ) (APHA,



200 2012) were monitored once a week. N-nitrate (APHA, 2012) and orthophosphate (APHA,  
201 2012) were monitored every fifteen days.

202

### 203 **2.5 C:N ratio**

204 At the end of the experiment the C/N ratio was measured, based on the amount of  
205 organic carbon source (wheat bran, molasses and feed) and nitrogen (feed, urea 45% nitrogen  
206 and wheat bran) used in the matrix tank during the experiment. The total nitrogen feed was  
207 measured considering that 1 kg of the 45% crude protein feed with 6.25%-N has 72 g of  
208 nitrogen. The estimated organic carbon content was determined using the formula presented  
209 by Hart et al., (2007) as follows: Organic carbon (%) =  $(0.53 \times protein) + (0.80 \times lipids) +$   
210  $(0.42 \times nitrogen\ free\ extract)$ .

211

### 212 **2.6 Microbiological samples and Analyses in TCBS medium**

213 Water samples were collected at the beginning and end of the trial from each treatment  
214 with sterile falcon tubes (50 mL) at aerated surface water. For this analysis, each 500  $\mu$ L of  
215 the sample was diluted in 9.0 mL of alkaline peptone water (pH 8.6), thus making six  
216 successive serial dilutions. The shrimp samples were collected at the beginning (50 pL<sub>10</sub>  
217 whole body) and end of the experiment (5 shrimp – hepatopancreas samples (0.14g) from  
218 each culture unit and were immersed in a 70% alcohol solution individually for 15 seconds,  
219 then immersed individually in a 1.5% sodium hypochlorite solution with 0.1% of tween-80  
220 for 15 minutes and washed thoroughly using sterilized water to remove the remaining surface  
221 bacteria and disinfectant. After disinfection, the shrimp or shrimp hepatopancreas were  
222 weighed, macerated and diluted in 9.0 mL of alkaline peptone water ( $10^{-1}$ ). After  
223 homogenization, water and shrimp samples were serially diluted  $10^{-1}$  to  $10^{-6}$  (100  $\mu$ L).  
224 Samples were taken from water (0.1 ml) and shrimp with the help of a sterilized pipette and

225 were applied using the spread-plate method on Thiosulphate Citrate Bile Sucrose (TCBS)  
226 agar and incubated at 30°C for 24h in triplicates. After incubation, the bacterial colonies of  
227 negative sucrose (green *Vibrio* like bacteria) and positive sucrose (yellow *Vibrio* like bacteria)  
228 (CFU mL<sup>-1</sup> and CFU g<sup>-1</sup>) were counted between 30 and 300 colonies using a colony counter.

229

## 230 **2.7 Phytoplankton and Zooplankton Community**

231 Water sampling was performed at the 1<sup>st</sup>, 21<sup>st</sup> and 42<sup>nd</sup> day of culture (500 mL). The  
232 water was filtered through a cylindrical-conical 250, 125 and 70 µm net mesh to reduce the  
233 suspended solids in the sample and then filtered through a 50 µm mesh, to retain zooplankton,  
234 and a 15 µm mesh to retain phytoplankton, which were stored in 25 mL containers. Next, a  
235 2.5 mL aliquot was fixed in 4% formalin and stored for further analysis. A Sedgewick-Rafter  
236 chamber and binocular optical microscope (Olympus CH30) with magnification of 800x  
237 (Pereira-Neto et al., 2008) were used for identification at the genus level, with the aid of  
238 identification keys for phytoplankton (Hoek et al., 1995; Bicudo and Menezes, 2006) and  
239 zooplankton (Bradford-Grieve et al., 1999; Foissner et al., 1999). Phytoplankton was  
240 expressed in cells per milliliter (cell mL<sup>-1</sup>) following the methodology described by Hötzel  
241 and Croome (1999) and zooplankton were expressed in individuals per liter (ind L<sup>-1</sup>)  
242 following the methodology described in APHA (2012).

243

## 244 **2.8 Proximal Composition**

245 Analysis of crude protein, lipids, moisture, ash and fiber contents of whole-body  
246 shrimp, biofloc, wheat bran, molasses and rotifers were performed in triplicate using standard  
247 methods (AOAC, 2012). Biofloc samples were collected to determine their proximal  
248 composition at the beginning from matrix tank and end of the experiment from each tank  
249 using a 50-µm mesh. Shrimp samples were also collected post larvae (pL<sub>10</sub>) at the beginning

250 and from each tank at the end of the experiment, and washed with distilled water to remove  
251 epiphytes and encrusting material. Rotifers were collected to determine their proximal  
252 composition at the end of production and separated using a 50 µm mesh. The biofloc, shrimp  
253 and rotifers were oven-dried at 60°C. For moisture content, the samples were oven-dried at  
254 105°C until constant weight (315 SE model, Fanem). The difference in weight before and  
255 after sample drying was recorded and expressed in percentage. Protein content was  
256 determined by measuring nitrogen (N x 6.25) using the Kjeldahl method (TE 0363 model;  
257 Tecnal, São Paulo, Brazil). Total lipid content was determined by the Soxhlet extraction  
258 method using pure Hexane (98%) solvent (Ma 044/8/50 model, Marconi, São Paulo, Brazil).  
259 The crude fiber content was determined with the Enzymatic-gravimetric method by  
260 measuring the residue after acid and alkaline digestion (Vasconcelos et al., 2010). The ash  
261 was determined by oven incineration at 550°C (Q318 D24 model; Quimis, São Paulo, Brazil).  
262 The carbohydrate was determined by the equation: (% dry weight) = 100 - (%crude protein –  
263 %lipid – %ash- %fiber).

264

## 265 **2.9 Salinity stress test**

266 At the end of the experiment, a salinity stress test was carried out by transferring  
267 juvenile shrimps from each treatment to three replicated units (30 shrimp per unit) containing  
268 freshwater, gently aerated by one air stone per recipient for 30 min. The shrimp were then  
269 transferred to water with salinity of 35 g L<sup>-1</sup>. After an additional exposure of 30 min, all  
270 shrimp not responding to mechanical stimulus were considered dead. Experimental conditions  
271 were 29.0 ± 0.5°C and pH 7.8 ± 0.1, using twelve 2-L plastic bottles (Burbano-Gallardo et al.,  
272 2015).

273

## 274 **2.10 Total haemocyte count**

275           At the end of shrimp culture and after the final salinity stress test a hemolymph sample  
276 was taken according Guertler et al. (2013) with a 1-mL syringe containing 200 µl of  
277 precooled anticoagulant modified Alsever solution (MAS) (336 mmol/L NaCl, 115 mmol/L  
278 glucose, 27 mmol/L sodium citrate, 9 mmol/L EDTA, pH 7.2) at a proportion of 1:2 (v:v). For  
279 total hemocyte counting (THC), triplicates of 100 µl of diluted hemolymph were counted for  
280 the number of hemocytes using a hemocytometer under a light microscope.

281

## 282 **2.11 Statistical analyses**

283           Statistical analyses of the data were performed using Statistica software version 10.0  
284 (StatSoft). Data were checked for homogeneity of variance with the Cochran test (Cochran  $p$   
285  $< 0.05$ ) and normality using the Shapiro-Wilk test ( $p < 0.05$ ). One-way variance analysis  
286 (ANOVA) was conducted to evaluate the zootechnical performance variables, proximal  
287 composition and repeated ANOVAs were used to compare water quality data, followed by the  
288 Tukey test ( $p < 0.05$ ) to compare means. The *Vibrio* and plankton genera were evaluated with  
289 the Kruskal-Wallis non-parametric test followed by Dunn's multiple comparison test ( $p <$   
290  $0.05$ ).

291           The phytoplankton and zooplankton density were previously logarithmized, and  
292 transformation ( $\log(x + 1)$ ), and Cluster analysis (Bray–Curtis similarity) were used to  
293 observe the community similarity on temporal and spatial scales and possible group  
294 formation. The analysis of similarity (ANOSIM) ( $p < 0.05$ ), with 999 permutations, was used  
295 to identify difference within and between groups (Clarke and Warwick, 2001); and the  
296 percentage similarity (SIMPER) to identify the main typifying species of the groups. Data  
297 analyses were performed using PRIMER 6.0 software.

298

299 **3. RESULTS**

300 **3.1 Shrimp zootechnical parameters**

301 The zootechnical parameters of the shrimp during the 42-day experimental period are  
302 summarized in figure 1. The shrimp weight on days 21 and 42 ranged from 0.19 to 0.29 g and  
303 1.09 g to 1.26 g respectively and the BFT-30 had a significant difference ( $p < 0.05$ ) with the  
304 other treatments (Figure 1A). The shrimp survival rates were all above 77% during the 42-day  
305 experimental period and for BFT-30 were significantly higher ( $p < 0.05$ ) than the BFT (Figure  
306 1B). The yield was higher ( $p < 0.05$ ) in BFT-20 ( $3.14 \text{ kg m}^{-3}$ ) and BFT-30 ( $3.42 \text{ kg m}^{-3}$ ) than  
307 in BFT (Figure 1 C). The FCR on days 21 and 42 ranged from 0.67 to 1.05 g and 1.12 g to  
308 1.23 g, respectively with significant difference ( $p < 0.05$ ) in the treatments (Figure 1D).

309

310 **3.2 Water quality**

311 There were no significant differences ( $p > 0.05$ ) in the water quality parameters  
312 between the four treatments (Table 1). The water quality parameters were temperature  $\sim 31^\circ\text{C}$ ;  
313 dissolved oxygen  $\sim 5 \text{ mg L}^{-1}$ ; salinity  $\sim 32 \text{ g L}^{-1}$ ; pH  $\sim 8.06$ ; SS  $7.49 \pm 0.75$  to  $9.13 \pm 0.87 \text{ mL}$   
314  $\text{L}^{-1}$ ; TAN  $0.38 \pm 0.03$  to  $0.47 \pm 0.06 \text{ mg L}^{-1}$ ; N-nitrite  $0.59 \pm 0.07$  to  $0.75 \pm 0.12 \text{ mg L}^{-1}$ ; N-  
315 nitrate  $18.61 \pm 6.71$  to  $23.68 \pm 8.68$ ; orthophosphate  $27.08 \pm 6.97$  to  $33.38 \pm 8.10$ ; and  
316 alkalinity  $98.61 \pm 7.92$  to  $106.67 \pm 5.67 \text{ mg CaCO}_3 \text{ L}^{-1}$ . Water consumption ranged from  
317  $261.34$  to  $372.45 \text{ L kg}^{-1}$  shrimp juvenile and the C:N ratio ranged from 10.57 to 10.73.

318

319 **3.3 Microbiological samples and Analyses in TCBS medium**

320 Microbiological organisms in TCBS (*Vibrio*-like bacteria colonies) in water (CFU  
321  $\text{mL}^{-1}$ ) and shrimp (postlarvae and hepatopancreas) (CFU  $\text{g}^{-1}$ ) were classified as yellow  
322 (positive sucrose) or green (negative sucrose). The *Vibrio*-type bacteria colonies in the water  
323 ranged from  $1.58$  to  $5.66 \times 10^4 \text{ CFU mL}^{-1}$ , without significant differences between treatments,

324 however, the higher the addition of rotifers, the higher the percentage of sucrose positive  
325 colonies. The *Vibrio*-like bacteria colonies in the shrimp ranged from 10.30 to 227.6 x 10<sup>4</sup>  
326 CFU g<sup>-1</sup>, without significant differences between the treatments, however, the percentage of  
327 positive sucrose colonies increased and of negative sucrose colonies decreased by the end of  
328 experimental period, with values ranging from 15 to 100% and 62 to 0%, respectively (Table  
329 2).

330

### 331 **3.4 Phytoplankton and Zooplankton Communities**

332 A total of 26 genera at the beginning and 34 genera at the end were identified and  
333 distributed in the following groups: Cyanophyta (7 and 7), Heterokontophyta (8 and 12),  
334 Chlorophyta (6 and 10), Euglenophyta (2 and 2) and Dinophyta (3 and 3), respectively. The  
335 total cell density of phytoplankton ranged from 3.871 to 13.938 cells mL<sup>-1</sup> (Table 3). The  
336 most frequent genera at the beginning were *Oscillatoria* (Cyanophyta), *Fragilaria*  
337 (Heterokonphyta) and *Ulothrix* (Chlorophyta). At the end, the most frequent were  
338 *Oscillatoria*, *Aphanocapsa* (Cyanophyta) and *Cylindrotheca* (Heterokonphyta) (Table 3). The  
339 analysis of similarity (ANOSIM) showed no significant differences in the phytoplankton  
340 communities among the treatments (R global = 0.019, p=0.29). However, it demonstrated that  
341 there were significant differences in the phytoplankton over the days of culture (R global =  
342 0.767, p<0.001) from the 1<sup>st</sup> to 21<sup>st</sup> and 42<sup>nd</sup> days (Table 3). In a 71,26% cut-off, the cluster  
343 analysis showed the formation of two groups, with group I composed by the beginning (day  
344 0), and group II by days 21 and 42 (Figure 2). The results of the similarity percentage analysis  
345 (SIMPER) reflect the contribution rate of the main species to the similarity between  
346 treatments. The microalgae *Oscillatoria* (6.97 a 9.27%) and *Aphanocapsa* (6.48 a 7.85%)  
347 were the main species responsible for the similarities present in each treatment, without  
348 significant differences (p>0.05) between treatments.

349 A total of 9 genera were identified at the beginning and 12 genera at the end, in the  
350 following groups: Protozoa (2), Cladocera (2), Copepoda (3), Rotifera (4) and Cirripedia (1).  
351 The total density of zooplankton ranged from 2.06 to 4.37 org. mL<sup>-1</sup> (Table 4). The most  
352 frequent genera at the beginning were *Arcella* sp. (Protozoa) and *Daphnia* sp. (Cladocera). At  
353 the end, the most frequent were *Arcella* sp. (Protozoa), *Bosmina* sp. (Cladocera) and  
354 *Brachionus* sp. (Rotifers) (Table 4). The analysis of similarity (ANOSIM) showed no  
355 significant differences in the zooplankton communities among the treatments (R global =  
356 0.069, p=0.11). However, it demonstrated that there were significant dissimilarities in the  
357 phytoplankton during the culture (R global = 0.593, p=0.001) from the 1<sup>st</sup> to 21<sup>st</sup> and 42<sup>nd</sup>  
358 days (Table 4). At a 70% cut-off, the cluster analysis showed the formation of two groups,  
359 with group I composed by the beginning (day 0), group II (by days 21 and 42) (Figure 3). The  
360 results of the similarity percentage analysis (SIMPER) reflect the contribution of the main  
361 species to the similarity between treatments. The zooplankton *Brachionus* sp. (21.49 to  
362 33.73%), *Daphnia* sp. (15.44 to 31.25%) and *Arcella* sp. (12.75 to 20.62%) were the main  
363 species responsible for the similarity in each treatment, with significant differences (p<0.05)  
364 between treatments for Protozoa, Rotifera and Copepoda.

365

### 366 **3.5 Proximal composition**

367 There were significant differences (p < 0.05) in the proximal composition between the  
368 four treatments (Table 5). The shrimp proximal composition (g kg<sup>-1</sup> dry weight basis) ranged  
369 from crude protein 212.10 to 283.07 and crude lipids 41.57 to 111.40, with significant  
370 differences (p< 0.05) between BFT-20 and BFT-30 and BFT and the initial value (postlarvae).  
371 The ash content ranged from 124.15 to 144.29, fiber from 30.04 to 33.27 and carbohydrate  
372 from 90.23 to 117.67 (Table 5). The microbial biofloc proximal composition (g kg<sup>-1</sup> dry  
373 weight basis) of crude protein ranged from 203.11 to 298.25, of crude lipids from 66.19 to

374 130.01, ash from 243.62 to 61.94, fiber from 64.36 to 66.83, and carbohydrates from 31.17 to  
375 48.55, with significant differences ( $p < 0.05$ ) for crude protein and lipids between BFT-20 and  
376 BFT-30 and BFT and the initial amounts (post larvae) (Table 2). The proximal composition of  
377 wheat bran ( $\text{g kg}^{-1}$  dry weight basis) was crude protein  $257.10 \pm 4.84$ , lipids  $14.07 \pm 0.50$ ,  
378 fiber  $172.30 \pm 2.42$ , ash  $59.37 \pm 2.47$  and carbohydrates  $439.45 \pm 13.96$ . The rotifers'  
379 proximal composition ( $\text{g kg}^{-1}$  dry weight basis) were crude protein 483.79 and 194.94 lipids  
380 (Table 5).

381

### 382 **3.6 Salinity stress test and total haemocyte count (THC)**

383 Shrimp survival after the salinity stress test was 100% without a significant difference  
384 ( $p > 0.05$ ) between the treatments with and without the addition of rotifers. The THC was  
385 significantly higher ( $p < 0.05$ ) at the end of culture and after a salinity stress test for shrimp  
386 submitted to treatments with the addition of rotifers ( $32.89$  to  $21.6 \times 10^6$  cells  $\text{mL}^{-1}$ ) than in  
387 the BFT ( $11.15 \times 10^6$  cells  $\text{mL}^{-1}$ ) (Figure 4). Moreover, there was higher reduction in the  
388 percentage of THC (47%) in BFT than BFT-30 (32%) after salinity stress test.

389

## 390 **4. DISCUSSION**

### 391 **4.1 Shrimp zootechnical parameters**

392 The analysis of zootechnical indexes demonstrated a relationship between the stocking  
393 density of the rotifer and the parameters evaluated, confirming the nutritional quality of the  
394 enriched rotifer linked to the biofloc.

395 Studies with BFT (fertilized with fresh molasses at an initial density of  $2,500 \text{ pL m}^{-3}$   
396 and an initial weight of 16.2 mg), and the addition of rotifers ( $30 \text{ org mL}^{-1}$ ) and an average  
397 temperature of  $26.8 \text{ }^\circ\text{C}$  in shrimp nurseries have demonstrated good zootechnical performance  
398 ( $1.76 \pm 0.27 \text{ kg m}^{-3}$ ,  $0.82 \text{ g} \pm 0.09 \text{ g}$  and FCA  $1.32 \pm 0.20$ ) over 35 days (Brito et al., 2016). In



399 addition, the use of BFT with the addition of *Navicula* in tanks with molasses at a temperature  
400 of 28.3°C (initial density of 3,000 pL m<sup>-3</sup> and initial weight of 1.0 mg) also demonstrated  
401 good zootechnical results (2.19 to 2.42 kg m<sup>-3</sup>, 0.80 to 0.86 g and FCA 0.77 to 0.82) over 42  
402 days (Abreu et al., 2019). However, in this study, organic fertilization from anaerobic and  
403 aerobic process of wheat bran and rotifers addition led to higher zootechnical performance  
404 values (2.52 to 3.42 kg m<sup>-3</sup>, final weight of 1.09 to 1.26 g and FCA of 1.23 to 1.12) in the  
405 same laboratory conditions as the studies cited with the addition of molasses as an organic  
406 carbon source. The addition of different densities of the rotifer improved the zootechnical  
407 performance of the treatments. The addition of the microorganisms provides important  
408 nutritional compounds, enriches the biofloc composition and improves digestive enzyme  
409 activity (Anand et al., 2013; Martins et al., 2016, Shah et al., 2018).

410         The control treatment also had good results, probably due to the fertilization protocol  
411 adopted. Studies have found that the anaerobic and aerobic process of bran improves the  
412 availability of nutrients such as proteins and amino acids. Some enzymes produced by  
413 microorganisms during anaerobic and aerobic process improve the digestibility of bran,  
414 indicating transforms complex organic compounds into simpler compounds (Al-Mashhadani,  
415 2011; Kraler et al., 2014; Al-Mashhadani, 2019).

416         Three factors were fundamental to this productive improvement, the maintenance of  
417 temperature close to 31°C, which is one of the environmental factors that affect the growth  
418 and physiological performance of the species (Madeira et al., 2015; Zhang et al., 2019), the  
419 use of a mixture of bacteria, and the fertilization adopted, which provides a beneficial  
420 bacterial community and favors the primary community (Romano, 2018).

421

## 422 **4.2 Water quality**

423           The water quality variables found in this study remained in an ideal range for intensive  
424 farming according to Samocha (2019). The pH dropped from the middle to the end of the  
425 experiment, which was related to an increase in organic matter during the culture and  
426 consumption of inorganic carbon by autotrophic bacteria (Silva et al., 2013; Furtado et al.,  
427 2015; Samocha, 2019). The decrease in pH was not detrimental to shrimp development in any  
428 of the treatments, since this reduction was controlled through weekly additions of sodium  
429 bicarbonate to maintain alkalinity above 100 mg CaCO<sub>3</sub><sup>-1</sup>. Van Wyk and Scarpa (1999) found  
430 that alkalinities  $\geq 100$  mg CaCO<sub>3</sub><sup>-1</sup> favor the stabilization of pH levels and autotrophic  
431 bacteria growth.

432           In intensive systems, high stocking densities are used and the concentrations of  
433 nitrogen compounds tend to increase as a result of the remains of uneaten feed, high biomass  
434 and accumulation of organic matter during culture (Wasiolesky et al., 2006; Furtado et al.,  
435 2011). In all treatments, the TAN and N-nitrite levels were constant during the first five  
436 weeks, however, there was a small increase at the end, although the amounts remained at the  
437 ideal levels (TAN < 3 mg L<sup>-1</sup> and N-nitrite < 10 mg L<sup>-1</sup>) recommended by Samocha (2019).  
438 The increase in N-nitrate concentrations observed at the end of the experiment indicates the  
439 presence of nitrifying bacteria (Zhao et al., 2012; Luo et al., 2013), which was also observed  
440 in other studies with the addition of microalgae and rotifers to shrimp nurseries (Marinho et  
441 al., 2014; Brito et al., 2016; Abreu et al., 2019). Maintaining the levels of nitrogenous  
442 compounds within the ideal ranges for culture of the species is extremely important, since  
443 higher values of nitrogen compounds are a limiting factor for growth and survival (Ebeling et  
444 al., 2006). Thus, the protocol of inorganic and organic fertilization (anaerobic and aerobic  
445 process of wheat bran and a bacterial mix) proved to be an efficient strategy for controlling  
446 nitrogen compounds in a shrimp nursery phase.

447 The minimal exchange of water and high densities practiced in intensive systems  
448 increase the solids in the system (Van Wyk, 2006). Sedimentable solids must be monitored  
449 during culture to avoid problems such as gill clogging and excessive consumption of  
450 dissolved oxygen due to the degradation of this organic matter (Gaona et al., 2011). The mean  
451 values of sedimentable solids in this study remained below the critical limit of 14 mL L<sup>-1</sup> for  
452 the nursery phase (Samocha et al., 2019), ranging from 7.49 to 9.13 mL L<sup>-1</sup>.

453 During culture, freshwater was replaced to compensate for evaporation losses,  
454 reaching a daily replacement rate between 1.05 and 1.17% of the total volume of the  
455 experimental unit at the end of the experiment. In this context, we can measure the  
456 effectiveness of water use by the amount used (replacement + exchange) for the production of  
457 1 kg of shrimp juveniles (Krummenauer et al., 2014). The values obtained in this study were  
458 between 372.45 and 261.34 L kg<sup>-1</sup>, which is close to 352 L kg<sup>-1</sup> reported by Cohen et al.  
459 (2005) and the 200 to 400 L kg<sup>-1</sup> by Hargreaves (2013). This result emphasizes that the  
460 addition of rotifers at the different densities used in this study, does not negatively influence  
461 water quality.

462

#### 463 **4.3 Microbiological samples and Analyses in TCBS medium**

464 Minimal water exchange favors the development of pathogenic and opportunistic  
465 bacteria such as the *Vibrio* genus, due to the large amount of organic matter accumulated  
466 during the culture (Ferreira et al., 2011; Yanong e Erlacher-Reid, 2012). However, it was  
467 observed that at the beginning of culture, 92.77% of these were negative sucrose colonies and  
468 at the end 55.59 to 98.61% were composed of positive sucrose colonies. Concerning the CFU  
469 found in the shrimp, a change in the proportion of negative and positive sucrose from the  
470 beginning to the end of the experiment was also observed, reaching zero negative sucrose  
471 concentration in the BFT-30 treatment.

472           The amount of rotifers added was directly related to increases in the percentage of  
473 positive sucrose. The main species of *Vibrio* reported as pathogenic to shrimp are sucrose  
474 negative, such as *V. parahaemolyticus*, a microorganism that causes mortality in intensive  
475 shrimp systems (Leaño and Mohan, 2012; Gomez-Gil et al., 2014; Hostins et al., 2017). This  
476 inversion of negative and positive sucrose associated with the increased stocking density may  
477 indicate a competition for nutrients in the growing environment or the presence of  
478 antibacterial compounds found in rotifers, which can inhibit the negative sucrose *Vibrio*  
479 (Rimper, 2014; Farisa et al., 2019).

480           The BFT treatment also had a negative to positive inversion of sucrose concentration  
481 in both water and shrimp. This may suggest the influence of the fertilization protocol adopted  
482 (anaerobic and aerobic process of wheat bran with a bacterial mix) used in all treatments,  
483 which reduced the load of this type of bacteria through the addition of beneficial species that  
484 compete for nutrients (Lakshmi et al., 2013). Therefore, the use of a fertilization method that  
485 encourages the increase of beneficial bacterial communities can help minimize impacts  
486 resulting from pathogenic bacteria of the genus *Vibrio* spp. (Costa et al., 2008, Romano et al.,  
487 2018).

488

#### 489 **4.4 Phytoplankton and Zooplankton Community**

490           The planktonic community varies according to the culture system, stocking biomass  
491 and nutrient input (Muangkeow et al., 2007; Casé et al., 2008; Melo et al., 2010). In intensive  
492 systems, nutrients accumulate, mainly inorganic phosphorus and nitrogen (in the form of  
493 nitrate), which are not incorporated into the shrimp biomass and thus do not induce their  
494 growth (Franceschiniet al., 2010; Wang et al., 2015).

495           There was an increase in the density and diversity of the planktonic community along  
496 the culture period, which is correlated to this gradual accumulation of nutrients in the system,

497 as also reported by Marinho et al. (2014) and Campos et al. (2019). These differences were  
498 emphasized in the Cluster and ANOSIN cluster analysis.

499 A wide variety of phytoplankton communities are found in culture water according to  
500 the literature. In this study, there was a predominance of the Cyanophyta group, but according  
501 to the SIMPER analysis, the contribution of phytoplankton to the treatments was not due to  
502 just one genus. The results showed a low contribution of each genus (<10%), which indicates  
503 that there was a wide diversity of genera demonstrating an ecological balance.

504 The relative abundance revealed that about 67% of the phytoplankton was composed  
505 by the Cyanophyta group, as reported by other studies (Green et al., 2014; Marinho et al.,  
506 2014; Campos et al., 2019), however, the densities in this study were lower than those found  
507 in other studies. This predominance of Cyanophyta is related to their capacity to fix nitrogen  
508 present in the environment, favoring their growth in relation to other phytoplankton groups.  
509 They are also well adapted to low light, and can grow at higher temperatures and with stand  
510 the accumulation of phosphorus in the system (Emerenciano et al., 2011; Almanza et al.,  
511 2016). In this study, the genera *Aphanoscapa* and *Oscillatoria* were those most abundant and  
512 commonly found in environments rich in organic matter, such as aquaculture systems (Rosini  
513 et al., 2016).

514 At the end of the experiment there was a significant difference in the average densities  
515 of zooplankton composition, which were lower in the treatments to which *B. plicatilis* was  
516 added than in the control, with an approximately 60% reduction of protozoa and a near 40%  
517 increase in Rotifera, which suggests that the presence of *B. plicatilis* influenced these  
518 communities. Rotifera was observed with greater relative abundance even in the BFT  
519 treatment, which may be related to the adaptation of these organisms to nutrient rich  
520 environments. Similar results were reported by Anand et al. (2013); Marinho et al. (2014);  
521 Gálvez et al. (2015) and Brito et al. (2017). High concentrations of rotifers and protozoa as

522 seen in this study can benefit shrimp performance (Thompson et al., 2002). Loureiro et al.  
523 (2012), analyzed the intestinal content of *L. vannamei* grown in BFT and found protozoa and  
524 rotifers, indicating a preference for these microorganisms that are considered an important  
525 nutritional source.

526

#### 527 **4.5 Proximal composition**

528 In terms of proximal composition, it was observed that the addition of rotifers  
529 contributed to an increase in the levels of protein and lipids in *L. vannamei* juveniles. The  
530 protein and lipid levels increased according to the level of inclusion of rotifers, this increase is  
531 linked to the better nutritional composition of microbial flocs with the addition of rotifers.  
532 Although there is no significant difference between the initial values and the BFT treatment,  
533 we can observe an increase in the lipid and protein concentrations. A study using *L. vannamei*  
534 grown in bioflocs and clear water found better nutritional properties in shrimp grown in  
535 bioflocs (Rajkumar et al., 2016). However, we observed improved proximal composition  
536 (protein and lipids) of whole juvenile shrimp when rotifers were added to the BFT system  
537 than to BFT. This improvement increased with additional rotifer density and is probably  
538 linked to the better nutritional composition of microbial flocs with additional rotifers.

539 The values of protein (244 to 298 g kg<sup>-1</sup>) and lipids (87 to 130 g kg<sup>-1</sup>) in microbial  
540 flocs were higher than those reported by Abreu et al. (2019) for protein (172 to 206 g kg<sup>-1</sup>)  
541 and lipids (63 to 98 g Kg<sup>-1</sup>) in a nursery to which *Navicula* sp. were added at different  
542 densities and with molasses fertilization. These differences in the bioflocs' nutritional  
543 characteristics may be related to the carbon source, system maturation time, level of total  
544 suspended solids, the planktonic and bacterial communities (Emerenciano et al., 2013) and  
545 the nutritional quality of the rotifer added.

546 The proximate composition of *B. plicatilis* may change according to the type of food  
547 used during its culture, such as microalgae cultures or commercial enrichment products  
548 (Srivastava et al., 2006). Some authors have found values between 482.40 to 592.10 g kg<sup>-1</sup> for  
549 protein and 61.80 to 142.60 g kg<sup>-1</sup> for lipids in rotifers cultivated with different commercial  
550 enrichment products (Demir and Diken, 2011b), while in rotifers fed with microalgae the  
551 reported values were 291.90 to 460.20 g kg<sup>-1</sup> for protein and 159.10 to 361.00 g kg<sup>-1</sup> for lipids  
552 (Jeeja et al., 2011). The results were similar to those observed in this study 483.79 g kg<sup>-1</sup> for  
553 protein and 194.94 g kg<sup>-1</sup> for lipids.

554

#### 555 **4.6 Salinity stress test and total haemocyte count (THC)**

556 Salinity stress tests are used to measure the resistance of the cultured shrimp, and it  
557 consists of an abrupt decrease in salinity over a given time and then a recovery to the original  
558 salinity level (Burbano-Gallardo et al., 2015). The results obtained were 100% for all  
559 treatments, which suggests that the resistance was not only related to the nutritional  
560 supplementation with the rotifer, but also to the BFT system.

561 Although *L. vannamei* tolerates a wide range of salinity, sudden variations require  
562 energy expenditure to maintain the animal's homeostasis and can be considered a stressor (Li  
563 et al. 2007; 2008). Crustaceans, unlike vertebrates, have only innate or non-specific immune  
564 systems, and therefore have no immune memory (Wang and Wang, 2013). The cellular  
565 immune response of crustaceans is mediated by hemocytes, which constitute the first line of  
566 defense and trigger a series of immunological reactions to situations of stress and infections  
567 (Van de Braak et al., 2002; Ekasari et al., 2014; Niu et al. 2018).

568 In this study, we performed a total hemocyte count before the stress test, and the  
569 highest mean values, 36.53 to 48.42 cells mL<sup>-1</sup> x 10<sup>6</sup>, were obtained in treatments with the  
570 addition of rotifers, while the control treatment had 21.3 cells mL<sup>-1</sup> x 10<sup>6</sup>. The results were

571 similar for BFT with the addition of *Navicula*, where values of  $41.25 \text{ cells mL}^{-1} \times 10^6$  were  
572 reported for microalgae addition and  $22.84 \text{ cells mL}^{-1} \times 10^6$  for the BFT treatment (Abreu et  
573 al., 2019).

574 After salinity stress tests, treatments with the addition of rotifer showed concentrations  
575 of  $32.89$  to  $21.60 \text{ cell mL}^{-1} \times 10^6$ , while in the BFT treatment it was  $11.15 \text{ cell mL}^{-1} \times 10^6$ .  
576 Several authors have observed a decrease in the concentration of hemocytes in *L. vannamei*  
577 due to a decrease in salinity, and suggest that it is due to an increase in the volume of  
578 hemolymph in these conditions (Lu-Qing et al., 2005; Wang and Chen et al., 2005).

579 In reaction to an infection or stressful situation, hemocytes migrate to the affected  
580 regions, decreasing the levels of circulating hemocytes (Barracco et al., 2008). This can be  
581 seen in this experiment, where all hemocyte levels decreased after the salt stress test.  
582 However, it was observed that even after the stress test, treatments with the addition of  
583 rotifers had higher hemocyte concentrations under normal conditions than the BFT treatment.  
584 This indicates the importance of the nutritional conditions on shrimp health, through good  
585 immunological indicators such as the hemocyte count (Yeh et al., 2006). According to  
586 Jiravanichpaisal et al. (2006) the increase in THC may indicate better ability to fight  
587 infections.

588

## 589 **5. CONCLUSION**

590 The addition of rotifer (*B. plicatilis*) at a density of  $20\text{-}30 \text{ org mL}^{-1}$  every 10 days  
591 enriched the proximal composition of the bioflocs (protein and lipids), which contributes to  
592 better zootechnical performance. This was accompanied by a better immune response in *L.*  
593 *vannamei* juveniles and a decrease in the bacteria concentration of the negative sucrose *Vibrio*  
594 genus in water and shrimp. The cost of large-scale production must be considered, with  
595 alternatives such as frozen rotifers or the use of commercial emulsions for enrichment.



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604

605 **REFERENCES**

- 606 ABCC, 2013. Levantamento da infraestrutura produtiva e dos aspectos tecnológicos,  
607 econômicos, sociais e ambientais da carcinicultura marinha no Brasil em 2011. (Convênio  
608 ABCC/ MPA – 756578/2011). Natal, RN - Brazil.
- 609 Abreu, J. L., Brito, L. O., Lima, P. C. M., Silva, S. M. B. C., Severi, W., Gálvez, A. O., 2019.  
610 Effects of addition of *Navicula* sp. (diatom) in different densities to postlarvae of shrimp  
611 *Litopenaeus vannamei* reared in a BFT system: Growth, survival, productivity and fatty acid  
612 profile. *Aquaculture Research*, 50, 2231-2239. <https://doi.org/10.1111/are.14104>.
- 613 Adissin, T.O.O., Manabu, I., Shunsuke, K., Saichiro, Y., Moss, A.S., Dossou, S., 2019.  
614 Effects of dietary *Nannochloropsis* sp. powder and lipids on the growth performance and fatty  
615 acid composition of larval and postlarval kuruma shrimp, *Marsupenaeus japonicas*.  
616 *Aquaculture Nutrition*, 26, 186-200. <https://doi.org/10.1111/anu.12980>.
- 617 Almanza, V., Bicudo, C. E. M., Parra, O., Urrutia, R., 2016. Características morfológicas y  
618 limnológicas de las floraciones de *Ceratium furcoides* (Dinophyta) en un lago somero de  
619 Chile. *Limnetica*, 35, 253-268. <https://doi.org/10.23818/limn.35.21>.

- 620 Al-Mashhadani, H.A., 2011. Effect of partial and complete replacement of soyabean meal by  
621 treated vicia faba bean in the diet on production performance of layer hens. The Journal of  
622 Poultry Science, 44, 34-41. <https://doi.org/10.2141/jpsa.44.34>.
- 623 Al-Mashhadani, H.A., Rahem, E.T., Saheab, M.A., 2019. Effect of fermentation of wheat  
624 bran and barley on the improvement of nutritional value. Plant Archives, 19, 4162-4164.
- 625 Álvarez, A.L., Racotta, I.S., Arjona, O., Palacios, E., 2004. Salinity stress test as a predictor  
626 of survival during growout in pacific white shrimp (*Litopenaeus vannamei*). Aquaculture,  
627 237, 237-249. <https://doi.org/10.1016/j.aquaculture.2004.03.029>.
- 628 Anand, P. S. S., Kumar. S., Panigrahi, A., Ghoshal, T. K., SyamaDayal, J., Biswas, G.,  
629 Sundaray, J. K., De, D., Ananda Raja, R., Deo, A. D., Pillai, S. M., Ravichandran, P., 2013.  
630 Effects of C:N ratio and substrate integration on periphyton biomass, microbial dynamics and  
631 growth of *Penaeus monodon* juveniles. Aquaculture International, 21, 511-524.  
632 <https://doi.org/10.1007/s10499-012-9585-6>.
- 633 APHA, 2012. Standard Methods for the Examination of Water and Wastewater, 22th ed.  
634 American Public Health Association, Washington.
- 635 Association of Official Analytical Chemists - AOAC, 2012. Official methods of analysis.  
636 AOAC International, Gaithersburg.
- 637 Avnimelech, Y., 2015. Biofloc technology – A practical guide book. The World Aquaculture  
638 Society, Louisiana.
- 639 Bakhshi, F., Najdegerami, E. H., Manaffar, R., Tukmechi, A., Farah, K. R., 2018. Use of  
640 different carbon sources for the biofloc system during the grow out culture of common carp  
641 (*Cyprinus carpio L.*) fingerlings. Aquaculture, 484, 259-267.  
642 <https://doi.org/10.1016/j.aquaculture.2017.11.036>.
- 643 Barracco, M. A., Perazzolo, L. M., Rosa, R. D., 2008. Inmunologia de crustáceos, con énfasis  
644 en camarones, in: Morales, V., Patología e Inmunología del camaron blanco *Penaeus*

- 645 *vannamei*. Cytel, Panamá.
- 646 Bicudo, C. E., Menezes, M. A., 2006. Gêneros de algas continentais do Brasil: chave para  
647 identificação e descrições, 2<sup>nd</sup> ed., Rima, São Paulo.
- 648 Bossier, P., Ekasari, J., 2017. Biofloc technology application in aquaculture to support  
649 sustainable development goals. *Microbial Biotechnology*, 10, 1012–1016.  
650 doi.org/10.1111/1751-7915.12836.
- 651 Bradford-Grieve, J.M., Markhaseva, E.I., Rocha, C.E.F, Abiahy, B., 1999. Copepoda, in:  
652 Boltoyskoy, D., South Atlantic zooplankton. Backhuys Publishers, Leiden, v.2, pp. 869-1098.
- 653 Brito, L.O., Costa, W.M., Gomes, I.G., Dantas, D.M.M., Pereira Neto, J.B., Soares, R.,  
654 Oliveira, A., 2011. Comparación del efecto de dos regímenes de fertilización sobre la  
655 producción de *Litopenaeus vannamei* em Brasil. *Aqua Cultura*, 84, 31-33.
- 656 Brito, L. O., Santos, I. G. S., Abreu, J. L., Araújo, M. T., Severi, W., Gálvez, A. O., 2016.  
657 Effect of addition of diatoms (*Navicula* spp.) and rotifers (*Brachionus plicatilis*) on growth  
658 and water quality of the *Litopenaeus vannamei* postlarvae reared in biofloc system.  
659 *Aquaculture Research*, 47, 3990–3997. <https://doi.org/10.1111/are.1284>.
- 660 Brito, L. O., Simão, B. R., Pereira Neto, J. B., Cemirames, G., Azevedo, C. M. S, B., 2017.  
661 Densidade planctônica do policultivo de *Litopenaeus vannamei* e *Oreochromis niloticus*.  
662 *Ciência Animal Brasileira*, 18, 1-11, e-16840. <https://doi.org/10.1590/1089-6891v18e-16840>.
- 663 Burbano-Gallardo, E., Imués-Figueroa, M. A., Gonzalez-Legarda, E. A., Brito, L. O., Gálvez,  
664 A. O., Arana, L. A. V., 2015. Supervivencia de poslarvas de *Litopenaeus vannamei* sometidas  
665 a la prueba de estrés osmótico y su relación con el estado de muda. *Revista de Biología*  
666 *Marina y Oceanografía*, 50, 323–329. <https://doi.org/10.4067/S071819572015000300010>.
- 667 Campos, C. V. F. S., Moraes, L. B. S., Farias, R. S., Severi, W., Brito, L. O., Gálvez, A. O.,  
668 2019. Phytoplankton communities in aquaculture system (integration of shrimp and seaweed).  
669 *Chemistry and Ecology*, 35, 903-921. <https://doi.org/10.1080/02757540.2019.1668378>.

- 670 Casé, M., Leça, E. E., Leitão, S. N., Santanna, E. E., Schwamborn, R., Junior, A. T. M., 2008.  
671 Plankton community as an indicator of water quality in tropical shrimp culture ponds. Marine  
672 Pollution Bulletin, 56, 1343-1352. <https://doi.org/10.1016/j.marpolbul.2008.02.008>.
- 673 Clarke, K. R.; Warwick, R. M., 2001. Change in Marine Communities: An Approach to  
674 Statistical Analysis and Interpretation (2nd ed.). Primer-E Limited, United Kingdom.
- 675 Cohen, J. M., Samocha, T. M., Fox, J. M., Gandy, R. L., Lawrence, A. L., 2005.  
676 Characterization of water quality factors during intensive raceway production of juvenile  
677 *Litopenaeus vannamei* using limited discharge and biosecure management tools. Aquacultural  
678 Engineering, 32, 425-442. <https://doi.org/10.1016/j.aquaeng.2004.09.005>.
- 679 Costa, R.A., Vieira, G.H.F., Silva, G.C., Vieira, R.H.S.F., Sampaio, S.S., 2008.  
680 Susceptibilidade “in vitro” a antimicrobianos de estirpes de *Vibrio* spp. isoladas de camarões  
681 (*Litopenaeus vannamei*) e de água de criação destes animais provenientes de uma fazenda de  
682 camarões no Ceará. Brazilian Journal of Veterinary Research Animal Science, 45, 458-462.
- 683 Crab, R., Defoirdt, T., Bossier, P., Verstraete, W., 2012. Biofloc technology in aquaculture:  
684 Beneficial effects and future challenges. Aquaculture, 356-357, 351-356.  
685 <https://doi.org/10.1016/j.aquaculture.2012.04.046>.
- 686 Cuéllar-Anjel, J., Lara, C., Morales, V., Gracia, A., Suárez, O. G., 2010. Manual de buenas  
687 prácticas de manejo para el cultivo del camarón blanco *Penaeus vannamei*. OIRSA-  
688 OSPESCA, Panamá.
- 689 Demir, O., Diken, G. (2011a). Effects of commercial enrichment products on chemical  
690 constitutions of rotifer *Brachionus plicatilis* (O.F. Muller 1786). Journal of Animal and  
691 Veterinary Advances, 10, 3328–3333. <https://doi.org/10.3923/javaa.2011.3328.3333>.
- 692 Demir, O., Diken, G. (2011b). Effects of commercial enrichment products on fatty acid  
693 components of rotifer, *Brachionus plicatilis*. African Journal of Biotechnology, 10, 15065–  
694 15071. <https://doi.org/10.5897/AJB11.3292>.

- 695 Dhert, P., Rombaut, G., Suantika, G., Sorgeloos, P., 2001. Advancement of rotifer culture and  
696 manipulation techniques in Europe. *Aquaculture*, 200, 129-146.  
697 [https://doi.org/10.1016/s0044-8486\(01\)00697-4](https://doi.org/10.1016/s0044-8486(01)00697-4).
- 698 Dhont, J., Dierckens, K., Støttrup, J., Van Stappen, G., Wille, M., Sorgeloos, P., 2013.  
699 Rotifers, Artemia and copepods as live feeds for fish larvae in aquaculture. *Advances in*  
700 *Aquaculture Hatchery Technology*, 242, 157-202.  
701 <https://doi.org/10.1533/9780857097460.1.157>.
- 702 Ebeling, J. M., Timmons, M. B., Bisogni, J. J., 2006. Engineering analysis of the  
703 stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of  
704 ammonia-nitrogen in aquaculture systems. *Aquaculture*, 257, 346–358.  
705 <https://doi.org/10.1016/j.aquaculture.2006.03.019>.
- 706 Ekasari, J., Azhar, M. H., Surawidjaja, E. H., Nuryati, S., de Schryver, P., Bossier, P., 2014.  
707 Immune response and disease resistance of shrimp fed biofloc grown on different carbon  
708 sources. *Fish and Shellfish Immunology*, 41, 332-339.  
709 <https://doi.org/10.1016/j.fsi.2014.09.00>.
- 710 Emerenciano, M., Ballester, E. L. C., Cavalli, R. O., Wasielesky W., 2011. Effect of biofloc  
711 technology (BFT) on the early postlarval stage of pink shrimp *Farfantepenaeus paulensis*:  
712 growth performance, floc composition and salinity stress tolerance. *Aquaculture International*,  
713 19, 891- 901. <https://doi.org/10.1007/s10499-010-9408-6>.
- 714 Emerenciano, M., Gaxiola, G., Cuzon, G., 2013. Biofloc technology (BFT): A review for  
715 aquaculture application and animal food industry, in: Matovic, M. D. (Ed.), *Biomass Now –*  
716 *Cultivation and Utilization*. BoD – Books on Demand, Rijeka, pp. 301–328.
- 717 Farisa, M. Y., Namaskara, K. E., Yusuf, M. B., Desrina, 2019. Antibacterial Potention of  
718 Extract of Rotifers Fed with Different Microalgae to Control *Vibrio harveyi*. *IOP Conference*

- 719 Series: Earth and Environmental Science, 246. <https://doi.org/10.1088/1755->  
720 1315/246/1/012058.
- 721 Ferreira, N.C., Bonetti, C., Seiffert, W.Q., 2011. Hydrological and water quality indices as  
722 management tools in marine shrimp culture. *Aquaculture*, 318, 425-433.  
723 <https://doi.org/10.1016/j.aquaculture.2011.05.045>.
- 724 Ferreira, M., Cortina-Burgueño, A., Freire, I., Otero, A., 2018. Effects of nutritional status  
725 and concentration of *Nannochloropsis gaditana* as enrichment diet for the marine rotifer  
726 *Brachionus* sp. *Aquaculture*, 491, 351-357. <https://doi.org/10.1016/j.aquaculture.2018.03.024>
- 727 Foissner, W., Berger, H., Schaumburg, J., 1999. Identification and ecology of limnetic  
728 plankton ciliates. München: Informationberichte des Bayer Landesamtes für  
729 Wasserwirtschaft.
- 730 Franceschini, I. M., Burliga, A. L., Reviers, B., Prado, J. F., Rézig, S. H., 2010. Algas: uma  
731 abordagem filogenética, taxonômica e ecológica, Artmed, Porto Alegre.
- 732 Furtado, P.S., Poersch, L.H., Wasielesky, W., 2011. Effect of calcium hydroxide, carbonate  
733 and sodium bicarbonate on water quality and zootechnical performance of shrimp  
734 *Litopenaeus vannamei* reared in bioflocs technology (BFT) systems. *Aquaculture*, 321, 130-  
735 135. <https://doi.org/10.1016/j.aquaculture.2011.08.034>.
- 736 Furtado, P. S., Poersch, L. H., Wasielesky, W., 2015. The effect of different alkalinity levels  
737 on *Litopenaeus vannamei* reared with biofloc technology (BFT). *Aquaculture international*,  
738 23, 345-358. <https://doi.org/10.1007/s10499-014-9819-x>.
- 739 Gálvez, A. O., Campos, C. V. F. S., Santos, I. G. S., Marinho, Y. F., Vinatea, L., Brito, L. O.,  
740 2015. Plankton communities in shrimp monoculture, integrated biofloc system. *The Global*  
741 *Aquaculture Advocate*, 1, 36-38.
- 742 Gaona, C. A. P., Poersch, L. H., Krummenauer, D., Foes, G. K., Wasielesky, W., 2011. The  
743 effect of solids removal on water quality, growth and survival of *Litopenaeus vannamei* in a

- 744 biofloc technology culture system. International Journal of Recirculating Aquaculture, 12, 54-  
745 73. <https://doi.org/10.21061/ijra.v12i1.1354>.
- 746 Gomez-Gil, B., Soto-Rodríguez, S., Lozano, R., Betancourt-Lozano, M., 2014. Draft genome  
747 sequence of *Vibrio parahaemolyticus* strain M0605, which causes severe mortalities of  
748 shrimps in Mexico. Genome Announcements, 2, 2. <https://doi.org/10.1128/genomeA.00055->  
749 14.
- 750 Green, B.W., Schrader, K.K., Perschbacher, P.W., 2014. Effect of stocking biomass on solids,  
751 phytoplankton communities, common off-flavors, and production parameters in a channel  
752 catfish biofloc technology production system. Aquaculture Research, 45, 1442-1458.  
753 <https://doi.org/10.1111/are.12096>.
- 754 Guerrelhas, A.C.B.; Teixeira, A.P., 2012. Panorama da situação da mancha branca no  
755 Nordeste. Panorama da Aquicultura, 22, 38 - 41.
- 756 Guertler, C., Rieg, T., Mejía-Ruíz, C. H., Lehmann, M., Barracco, M. A., Perazzolo, L. M.,  
757 2013. Hemograma e sobrevivência de camarões marinhos após silenciamento do WSSV por  
758 RNA de interferência. Pesquisa Agropecuária Brasileira, 48, 983–990.  
759 <https://doi.org/10.1590/S0100-204X2013000800025>.
- 760 Haché, R., Plante, S., 2011. The relationship between enrichment, fatty acid profiles and  
761 bacterial load in cultured rotifers (*Brachionus plicatilis* L-strain) and Artemia (*Artemia salina*  
762 strain *Franciscana*). Aquaculture, 311, 201-208.  
763 <https://doi.org/10.1016/j.aquaculture.2010.11.034>.
- 764 Hagiwara, A., Gallardo, W.G., Assavaaree, M., Kotani, T., De Araujo, A.B., 2001. Live food  
765 production in Japan; Recent progress and future aspects. Aquaculture, 200, 111-127.  
766 [https://doi.org/10.1016/S0044-8486\(01\)00696-2](https://doi.org/10.1016/S0044-8486(01)00696-2).

- 767 Hapsari, F., 2016. The effect of fermented and non fermented biofloc inoculated with  
768 bacterium *Bacillus cereus* for catfish (*Clarias gariepinus*) juveniles. AACL Bioflux, 9, 334-  
769 339.
- 770 Hargreaves, J.A., 2013. Bioflocs production system for aquaculture, Southern Regional  
771 Aquaculture Center, 4503, 1-12.
- 772 Hart, J. P., Lovis, W. A., Schulenberg, J. K., & Urquhart, G. R. (2007). Paleodietary  
773 implications from stable carbon isotope analysis of experimental cooking residues. Journal of  
774 Archaeological Science, 34, 804–813. <https://doi.org/10.1016/j.jas.2006.08.006>.
- 775 Hoek, C., Mann, D., Jahns, H. M., 1995. Algae: an introduction to Phycology. Cambridge  
776 University Press, Cambridge. <https://doi.org/10.1017/S096702629621100X>.
- 777 Hostins, B., Lara, G., Decamp, O., Cesar, D. E., Wasielesky Jr, W., 2017. Efficacy and  
778 variations in bacterial density in the gut of *Litopenaeus vannamei* reared in a BFT system and  
779 in clear water supplemented with a commercial probiotic mixture. Aquaculture, 480, 58-64.  
780 <https://doi.org/10.1016/j.aquaculture.2017.07.036>.
- 781 Hötzel, G., Croome, R., 1999. A phytoplankton methods manual for Australian Freshwaters.  
782 Land and Water Resources Research and Development Corporation, Canberra.
- 783 Hu, Y., Tan, B., Mai, K., Ai, Q., Zhang, L., Zheng, S., 2011. Effects of dietary menhaden oil,  
784 soybean oil and soybean lecithin oil at different ratios on growth, body composition and blood  
785 chemistry of juvenile *Litopenaeus vannamei*. Aquaculture International, 19, 459-473.  
786 <https://doi.org/10.1007/s10499-010-9361-4>.
- 787 IBGE, 2019. Produção da Pecuária Municipal. Instituto Brasileiro de Geografia e Estatística,  
788 Rio de Janeiro.
- 789 Jamali, H., Ahmadifard, N., Abdollahi, D., 2015. Evaluation of growth, survival and body  
790 composition of larval white shrimp (*Litopenaeus vannamei*) fed the combination of three



- 791 types of algae. International Aquatic Research, 7,115-122. <https://doi.org/10.1007/s40071->  
792 015-0095-9.
- 793 Jeeja, P.K., Imelda, J., Paul, R. R., 2011. Nutritional composition of rotifer (*Brachionus*  
794 *plicatilis* Muller) cultured using selected natural diets. Indian Journal of Fisheries, 58, 59-65.
- 795 Jin, Y., Tian, L., Zeng, S., Xie, S., Yang, H., Liang, G., Liu, Y., 2013. Dietary lipid  
796 requeriment on non-specific immune responses in juvenile grass carp (*Ctenopharyngodon*  
797 *idella*). Fish and Shellfish Immunology, 34, 1202-1208.  
798 <https://doi.org/10.1016/j.fsi.2013.01.008>.
- 799 Jiravanichpaisal, P., Lee, B.L., Soderhall, K., 2006. Cell-mediated in arthropods:  
800 hematopoiesis, coagulation, melanization and opsonization. Immunobiology. 211, 213- 236.  
801 <https://doi.org/10.1016/j.imbio.2005.10.015>.
- 802 Kawahigashi, D., 2018. Synbiotics as a management tool for improving nursery efficiency.  
803 HatcheryFeed, 6, 36-39.
- 804 Kotani, T., Genka, T., Tanabe, M., Miyashima, A., Fushimi, H., 2010. Effect of nutritional  
805 enrichment method on fatty acid contents of rotifer *Brachionus plicatilis*. Journal of the  
806 World Aquaculture Society, 41, 884-892. <https://doi.org/10.1111/j.1749-7345.2010.00431.x>.
- 807 Kotani, T., Haraguchi, T., Yamazaki, Y., Doi, T., Matsui, H., Yokoyama, S., Ishikawa, M.,  
808 Koshio, S., 2017. Effect of the duration of nutritional enrichment on the fatty acid  
809 composition of commonly used rotifers *Brachionus plicatilis* sp. complex and larviculture  
810 performance of red sea bream *Pagrus major*. Aquaculture Science, 65, 133-144.  
811 <https://doi.org/10.11233/aquaculturesci.65.133>.
- 812 Kraler, M., Schedle, K., Domig, K.J., Heine, D., Michlmayr, H., Kneifel, W., 2014. Effects  
813 of fermented and extruded wheat bran on total tract apparent digestibility of nutrients,  
814 minerals and energy in growing pigs. Animal Feed Science and Technology, 197, 121-129.  
815 <https://doi.org/10.1016/j.anifeedsci.2014.07.010>.

- 816 Krummenauer, D., Poersch, L., Romano, L. A., Lara, G. R., Encarnacao, P., Wasielesky Jr,  
817 W., 2014. The effect of probiotics in a *Litopenaeus vannamei* biofloc culture system infected  
818 with *Vibrio parahaemolyticus*. Journal of Applied Aquaculture, 26, 370-379.  
819 <https://doi.org/10.1080/10454438.2014.965575>.
- 820 Lakshmi, B., Viswanath, B., Sai Gopal, D.V.R., 2013. Probiotics as antiviral agents in shrimp  
821 aquaculture. Journal Pathogens, 2013. <https://doi.org/10.1155/2013/424123>.
- 822 Langdon, C., 2003. Microparticle types for delivering nutrients to marine fish larvae.  
823 Aquaculture, 227, 259-275. [https://doi.org/10.1016/S0044-8486\(03\)00508-8](https://doi.org/10.1016/S0044-8486(03)00508-8).
- 824 Leaña, E.M., Mohan, C.V., 2012. Early Mortality Syndrome threatens Asia's shrimp farms.  
825 Global Aquaculture Advocate, 4, 38-39.
- 826 Li, E.C., Chen, L.Q., Zeng, C., Chen, X.M., Yu, N., Lai, Q.M., Qin, J.G., 2007. Growth, body  
827 composition, respiration and ambient ammonia nitrogen tolerance of the juvenile white  
828 shrimp, *Litopenaeus vannamei*, at different salinities. Aquaculture 265, 385–390.  
829 <https://doi.org/10.1016/j.aquaculture.2007.02.018>.
- 830 Li, E.C., Chen, L.Q., Zeng, C., Yu, N., Xiong, Z.Q., Chen, X.F., Qin, J.G., 2008. Comparison  
831 of digestive and antioxidant enzymes activities, haemolymph oxyhemocyanin contents and  
832 hepatopancreas histological of white shrimp, *Litopenaeus vannamei*, at various salinities.  
833 Aquaculture 274, 80–86. <https://doi.org/10.1016/j.aquaculture.2007.11.001>.
- 834 Loureiro, C. K.; Junior, W. W.; Abreu, P. C., 2012. The use of protozoan, rotifers and  
835 nematodes as live food for shrimp raised in bft system. Atlântica, 34, 5-12.  
836 <https://doi.org/10.5088/atl.2012.34.1.5>.
- 837 Luo, G. Z., Avnimelech, Y., Pan, Y.F., Tan, H.X., 2013. Inorganic nitrogen dynamics in  
838 sequencing batch reactors using biofloc technology to treat aquaculture sludge. Aquacultural  
839 Engineering, 52, 73-79. <https://doi.org/10.1016/j.aquaeng.2012.09.003>.

- 840 Lu-Qing, P., Ling-Xu, J., Jing-Jing, M., 2005. Effects of salinity and pH on immune  
841 parameters of the white shrimp *Litopenaeus vannamei*. Journal of Shellfish Research, 24,  
842 1223-1227. [https://doi.org/10.2983/0730-8000\(2005\)24\[1223:EOSAPO\]2.0.CO;2](https://doi.org/10.2983/0730-8000(2005)24[1223:EOSAPO]2.0.CO;2).
- 843 Madeira, D., Mendonça, V., Dias, M., Roma, J., Costa, P.M., Larginho, M., 2015.  
844 Physiological, cellular and biochemical thermal stress response of intertidal shrimps with  
845 different vertical distributions: *Palaemon elegans* and *Palaemon serratus*. Comparative  
846 Biochemistry and Physiology – Part A: Molecular and Integrative Physiology, 183, 107 –  
847 115. <https://doi.org/10.1016/j.cbpa.2014.12.039>.
- 848 Magaña-Gallegos, E., González-Zúñiga, R., Cuzon, G., Arevalo, M., Pacheco, E., Valenzuela,  
849 M. A., Gaxiola, G., Chan-Vivas, E., López-Aguiar, K., Noreña-Barroso, E., 2018. Nutritional  
850 contribution of biofloc within the diet of growout and broodstock of *Litopenaeus vannamei*,  
851 determined by stable isotopes and fatty acids. Journal of the World Aquaculture Society, 49,  
852 1-14. <https://doi.org/10.1111/jwas.12513>.
- 853 Marinho, Y. F., Brito, L. O., Silva, C. V. F., Santos, I. G. S., Gálvez, A. O., 2014. Effect of  
854 addition of *Navicula* sp. on plankton composition and postlarvae growth of *Litopenaeus*  
855 *vannamei* reared in culture tanks with zero water exchange. Latin American Journal of  
856 Aquatic Research, 42, 427-437. <https://doi.org/103856/vol42-issue3-fulltext-4>.
- 857 Marinho, Y. F., Brito, L. O., Silva, C. V. F., Severi, W., Andrade, H. A., Gálvez, A. O., 2017.  
858 Effect of the addition of *Chaetoceros calcitrans*, *Navicula* sp. and *Phaeodactylum*  
859 *tricornutum* (diatoms) on phytoplankton composition and growth of *Litopenaeus vannamei*  
860 (Boone) postlarvae reared in a biofloc system. Aquaculture Research, 48, 4155-4164.  
861 <https://doi.org/10.1111/are.13235>.
- 862 Martins, T. G., Odebrecht, C., Jensen, L. V., D'Oca, M. G. M., Wasielesky, W. Jr., 2016. The  
863 contribution of diatoms to bioflocs lipid content and the performance of juvenile *Litopenaeus*

- 864 *vannamei* (Boone, 1931) in a BFT culture system. *Aquaculture Research*, 47, 1315–1326.  
865 <https://doi.org/10.1111/are.12592>.
- 866 Melo, M. P., Carvalheiro, J. M., Cordeiro, T. A., Queiroz, A. R., Prado, J. P., Borges, I. F.,  
867 2010. Phytoplanktonic composition of three cultivation systems used in *Litopenaeus*  
868 *vannamei* (Boone, 1931) marine shrimp farms. *Acta Scientiarum Biological Sciences*, 32,  
869 223-228. <https://doi:10.4025/actascibiolsci.v32i3.4816>.
- 870 Mills, S.; Alcántara-Rodríguez, A.; Ciroso-Pérez, J.; Gómez, A.; Hagiwara, A.; Hinson, K.;  
871 Galindo, C.; Jersabek, D.; Malekzadeh-Viayeh, R.; Leasi, F.; Lee, J.; Welch, M.; Papakostas,  
872 S.; Riss, S.; Segers, H.; Serra, M.; Shiel, R.; Smolak, R.; Snell, T.; Stelzer, C.-P; Tang, C.;  
873 Wallace, R.; Fontaneto, D., Walsh, E., 2017. Fifteen species in one: deciphering the  
874 *Brachionus plicatilis* species complex (Rotifera, Monogononta) through DNA taxonomy.  
875 *Hydrobiologia*, 796, 39-58. <https://doi.org/10.1007/s10750-016-2725-7>.
- 876 Mohapatra, S., Chakraborty, T., Kumar, V., Deboeck, G., Mohanta, K.N., 2013. Aquaculture  
877 and stress management: a review of probiotic intervention. *Journal of Animal Physiology and*  
878 *Animal Nutrition*, 97, 405-430. <https://doi.org/10.1111/j.1439-0396.2012.01301.x>.
- 879 Muangkeow, B., Ikegima, K., Powtongook, S., Yi, Y., 2007. Effects of white shrimp,  
880 *Litopenaeus vannamei* (Boone) and Nile tilapia, *Oreochromis niloticus* L., stocking density  
881 on growth, nutrient conversion rate and economic return in integrated closed recirculation  
882 system. *Aquaculture*, 269, 363-376. <https://doi.org/10.1016/j.aquaculture.2007.04.002>.
- 883 New, M. B., Valenti, W. C., Tidwell, J. H., D'Abramo, L. R., Kutty, M. N., 2010. Freshwater  
884 prawns: biology and farming, 1st ed. Wiley-Blackwell, England.
- 885 Newaj-Fyzul, A., Al-Harbi, A. H., Austin, B., 2014. Review: Development in the use of  
886 probiotics for disease control in aquaculture. *Aquaculture*, 432, 1-11.  
887 <https://doi.org/10.1016/j.aquaculture.2013.08.026>.

- 888 Niu, J., Xie, S. W., Fang, H. H., Xie, J. J., Guo, T. Y., Zhang, Y. M., Liu, Z. L., Liao, S. Y.,  
889 He, J. Y., Tian, L. X., Liu, Y. J., 2018. Dietary values of macroalgae *Porphyra haitanensis* in  
890 *Litopenaeus vannamei* under normal rearing and WSSV challenge conditions: Effect on  
891 growth, immune response and intestinal microbiota. *Fish & Shellfish immunology*, 81, 135-  
892 149. <https://doi.org/10.1016/j.fsi.2018.06.010>.
- 893 Pereira-Neto, J. B., Dantas, D. M. M., Olivera, A., Brito, L. O., 2008. Avaliação das  
894 comunidades planctônica e bentônica de microalgas em viveiros de camarão (*Litopenaeus*  
895 *vannamei*). *Boletim do Instituto de Pesca*, 34(4), 543-551.
- 896 Pérez-Fuentes, J.A., Hernández-Vergara, M.P., Pérez-Rostro, C.I., Fogel, I., 2016. C:N  
897 rations affect nitrogen removal and production Nile tilapia *Oreochromis niloticus* raised in a  
898 biobloc system under high density cultivation. *Aquaculture*, 452, 247-251.  
899 <https://doi.org/10.1016/j.aquaculture.2015.11.010>.
- 900 Penglase S., Hamre, K., Sweetman, J., Nordgreen, A., 2011. A new method to increase and  
901 maintain the concentration of selenium in rotifers (*Brachionus* spp.). *Aquaculture*, 315, 144-  
902 153. <https://doi.org/10.1016/j.aquaculture.2010.09.007>.
- 903 Pousao-Ferreira, P., Santos, P., Carvalho, A.P., Morais, S., Narciso, L., 2003. Effect of an  
904 experimental microparticulate diet on the growth, survival and fatty acid profile of gilthead  
905 seabream (*Sparus aurata* L.) larvae. *Aquaculture International*, 11, 491-504.  
906 <https://doi.org/10.1023/B:AQUI.00000004190.13871.f3>.
- 907 Rajkumar, M., Pandey, P. K., Aravind R., Vennila, A., Bharti, V., Purushothaman, C. S.,  
908 2016. Effect of different biofloc system on water quality, biofloc composition and growth  
909 performance in *Litopenaeus vannamei* (Boone, 1931). *Aquaculture Research*, 47, 3432-3444.  
910 <https://doi.org/10.1111/are.12792>.

- 911 Ray, A. J., Lotz, J. M., 2014. Comparing a chemoautotrophic-based biofloc system and three  
912 heterotrophic-based systems receiving different carbohydrate sources. *Aquacultural*  
913 *Engineering*, 63, 54-61. <https://doi.org/10.1016/j.aquaeng.2014.10.001>.
- 914 Rebouças, R. H., de Menezes, F. G. R., dos Fernandes Vieira, R. H. S., de Sousa, O. V. *Vibrio*  
915 spp. como patógenos na carcinicultura: alternativas de controle. *Arquivos de Ciências do Mar*.  
916 v.50, n.1, p.163-179. 2017.
- 917 Rimper, J. R. T. S. L., 2014. Deteksi Senyawa Bioaktif Rotifera *Brachionus rotundiformis*  
918 dari Perairan Laut Sulawesi Utara. *Jurnal Ilmu Hewani Tropika*, 3, 17-21.
- 919 Romano. N. 2017. Aquamimicry: um conceito revolucionário para o cultivo de camarão.  
920 *Revista da ABCC*, v.19, n.1, p.26 -28.
- 921 Romano, N., Dauda, A. B., Ikhsan, N., Karim, M., Kamarudin, M. S., 2018. Fermenting rice  
922 bran as a carbon source for biofloc technology improved the water quality, growth, feeding  
923 efficiencies, and biochemical composition of African catfish *Clarias gariepinus* juveniles.  
924 *Aquaculture Research*. 49, 3691-3701. <https://doi.org/10.1111/are.13837>.
- 925 Rosini, E.F., Tucci, A., Carmo, C.F., Rojas, N.E.T., Barros, H.P, Mallasen, M., 2016.  
926 Changes in phytoplankton spatial and temporal dynamics in a Brazilian tropical oligotrophic  
927 reservoir after net cage installation. *Brazilian Journal of Botany.*, 39, 569-581.  
928 <https://doi.org/10.1007/s40415-016-0259-x>.
- 929 Samocha, T.M., 2019. Sustainable Biofloc Systems for Marine Shrimp. Academic Press,  
930 London.
- 931 Schweitzer, R., Arantes, R., Costódio, P.F.S., Espírito Santo, C.M., Arana, L.V., Seiffert,  
932 W.Q., Andreatta, E.R., 2013. Effect of different biofloc levels on microbial activity, water  
933 quality and performance of *Litopenaeus vannamei* in a tank system operated with no water  
934 exchange. *Aquacultural Engineering*, 56, 59-70.  
935 <https://doi.org/10.1016/j.aquaeng.2013.04.006>.

- 936 Seiffert, A.C.B., Winckler, S., 2005. A mancha Branca em Santa Catarina. Panorama da  
937 Aquicultura, 87, 51 - 53.
- 938 Seychelles, L. H., Audet, C., Tremblay, R., Fournier, R., Pernet, F., 2009. Essential fatty acid  
939 enrichment of cultured rotifers (*Brachionus plicatilis*, Müller) using frozen- concentrated  
940 microalgae. Aquaculture Nutrition, 15, 431-439. [https://doi.org/10.1111/j.1365-  
941 2095.2008.00608.x](https://doi.org/10.1111/j.1365-2095.2008.00608.x).
- 942 Shah, M. R., Lutz, G. A., Alam, A., Sarker, P., Chowdhury, M. K., Parsaeimehr, A., Liang,  
943 Y., Daroch, M., 2018. Microalgae in aquafeeds for a sustainable aquaculture industry. Journal  
944 of Applied Phycology, 30, 197-213. <https://doi.org/10.1007/s10811-017-1234-z>.
- 945 Shinn, A.P., Pratoomyot, J., Griffiths, D., Trong, T.Q., Vu, N.T., Jiravanichpaisal, P., Briggs,  
946 M., 2018. Asian shrimp production and the economic costs of disease. Asian Fisheries  
947 Science, 31, 29-58. <https://doi.org/10.33997/j.afs.2018.31.S1.003>.
- 948 Silva, K. R., Wasielesky Jr, W., Abreu, P. C., 2013. Nitrogen and phosphorus dynamics in the  
949 biofloc production of the Pacific white shrimp *Litopenaeus vannamei*. Journal of the World  
950 Aquaculture Society, 44, 30-41. <https://doi.org/10.1111/jwas.12009>.
- 951 Soto-Rodriguez, S.A., Gomez-Gil, B., Lozano-Olvera, R., Betancourt-Lozano, M., Morales-  
952 Covarrubias, M.S., 2014. Field and experimental evidence of *Vibrio parahaemolyticus* as the  
953 causative agent of Acute Hepatopancreatic Necrosis Disease (AHPND) of cultured shrimp  
954 (*Litopenaeus vannamei*) in northwestern Mexico. Applied Environmental Microbiology, 81,  
955 3610-3614. <https://doi.org/10.1128/AEM.03610-14>.
- 956 Souza, D.M., Suita, S.M., Romano, L.A., Wasielesky, W., Ballester, E.L.C., 2014. Use of  
957 molasses as a carbon source during the nursery rearing of *Farfantepenaeus brasiliensis*  
958 (Latreille, 1817) in a Biofloc technology system. Aquaculture Research, 45, 270-277.  
959 <https://doi.org/10.1111/j.1365-2109.2012.03223.x>.

- 960 Srivastava, A., Hamre, K., Stoss, J., Chakrabarti, R., Tonheim, S. K., 2006. Protein content  
961 and amino acid composition of the live feed rotifer (*Brachionus plicatilis*): With emphasis on  
962 the water soluble fraction. *Aquaculture*, 254, 534-543.  
963 <https://doi.org/10.1016/j.aquaculture.2005.11.014>.
- 964 Thompson, F. L., Abreu, P. C., Wasielesky, W., 2002. Importance of biofilm for water quality  
965 and nourishment in intensive shrimp culture. *Aquaculture*, 203, 263-278.  
966 [https://doi.org/10.1016/S0044-8486\(01\)00642-1](https://doi.org/10.1016/S0044-8486(01)00642-1).
- 967 Torzillo, G., Chini Zittelli, G., 2015. Tubular Photobioreactors. In: Prokop A., Bajpai, R.,  
968 Zappi, M. (Eds.), *Algal Biorefineries vol. 2*. Springer International Publishing, Cham, pp.187-  
969 212. <https://doi.org/10.1007/978-3-319-20200-6>.
- 970 Tzovenis, I., Triantaphyllidis, G., Naibong, X., Chatzinikolaou, E., Papadopoulou, K., Xouri,  
971 G., Tafas, T., 2004. Cryopreservation of marine microalgae and potential toxicity of  
972 cryoprotectants to the primary steps of the aquaculture food chain. *Aquaculture*, 230, 457-  
973 473. [https://doi.org/10.1016/S0044-8486\(03\)00444-7](https://doi.org/10.1016/S0044-8486(03)00444-7).
- 974 Van de Braak, C.B., Botterblom, M.H.A., Taverne, N., Van Muiswinkel, W.B., Rombout,  
975 J.H.W.M., Van der Knaap, W.P.W., 2002. The roles of haemocytes and the lymphoid organ  
976 in the clearance of injected *Vibrio* bacteria in *Penaeus monodon* shrimp. *Fish and Shellfish*  
977 *Immunology*, 13, 293-309. <https://doi.org/10.1006/fsim.2002.0409>.
- 978 Van Wyk, P., 2006. Production of *Litopenaeus vannamei* in recirculating aquaculture  
979 systems: Management and design considerations, in: *Proceedings of the 6th International*  
980 *Conference Recirculating Aquaculture*, 38-47 Virginia Tech University, Blacksburg.
- 981 Van Wyk, P., 1999. Nutrition and feeding of *Litopenaeus vannamei* in intensive culture  
982 systems, in: Van Wyk, P., Davis-Hodgkins, M., Laramore, R., Main, K. L., Mountain, J.,  
983 Scarpa, J., (Eds.), *Farming marine shrimp in recirculating freshwater systems*. Florida

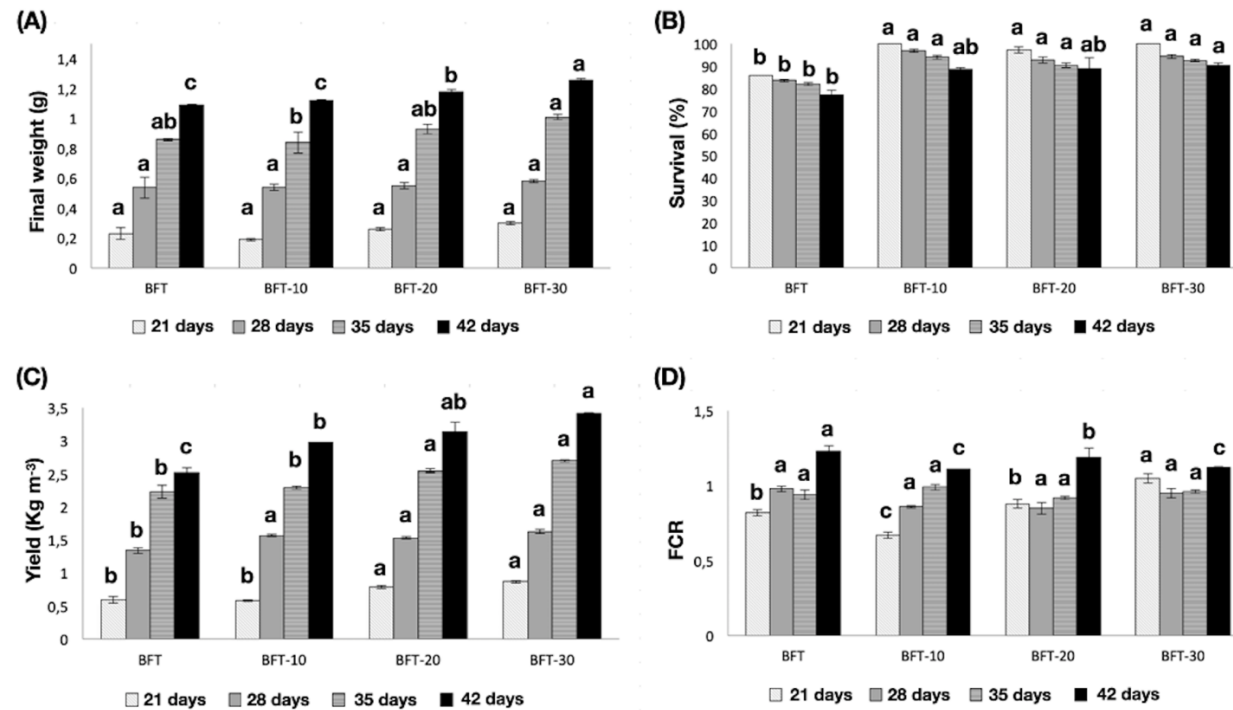


- 984 Department of Agriculture and Consumer Services - Harbor Branch Oceanic Institute, Fort  
985 Pierce, FL, pp. 125–140.
- 986 Van Wyk, P., Scarpa, J., 1999. Water quality requirements and management, in: Van Wyk,  
987 P., Davis-Hodgkins, M., Laramore, R., Main, K. L., Mountain, J., Scarpa, J., (Eds.), Farming  
988 marine shrimp in recirculating freshwater systems. Florida Department of Agriculture and  
989 Consumer Services - Harbor Branch Oceanic Institute, Fort Pierce, FL, pp. 141–620.
- 990 Vasconcelos, C. M., Silva, C. O., Teixeira, L. J. Q., Chaves, J. B. P., 2010. Determinação da  
991 fração da fibra alimentar solúvel em raiz e farinha de yacon (*Smallanthus sonchifolius*) pelo  
992 método enzimático-gravimétrico e cromatografia líquida de alta eficiência. Revista do  
993 Instituto Adolfo Lutz, 69, 188-93.
- 994 Vidal, M. F., Ximenes, L. J. F., 2016. Carcinicultura no Nordeste: velhos desafios para  
995 geração de emprego e de renda sustentáveis, até quando? Caderno Setorial do Escritório  
996 Técnico de Estudos Econômicos do Nordeste - ETENE, 1, 41-45.
- 997 Wang, C., Pan, L., Zhang, K., Xu, W., Zhao, D., Mei, L., 2015. Effects of different carbon  
998 sources addition on nutrition composition and extracellular enzymes activity of bioflocs, and  
999 digestive enzymes activity and growth performance of *Litopenaeus vannamei* in zero  
1000 exchange culture tanks. Aquaculture Research, 47, 3307-3318.  
1001 <https://doi.org/10.1111/are.12784>.
- 1002 Wang, L.U., Chen, J.C., 2005. The immune response of white shrimp *Litopenaeus vannamei*  
1003 and its susceptibility to *Vibrio alginolyticus* at different salinity levels. Fish and Shellfish  
1004 Immunology, 18, 269-278. <https://doi.org/10.1016/j.fsi.2004.07.008>.
- 1005 Wang, X. W., Wang, J. X., 2013. Diversity and multiple functions of lectins in shrimp  
1006 immunity. Developmental and Comparative Immunology, 39, 27-38.  
1007 <https://doi.org/10.1016/j.dci.2012.04.009>.

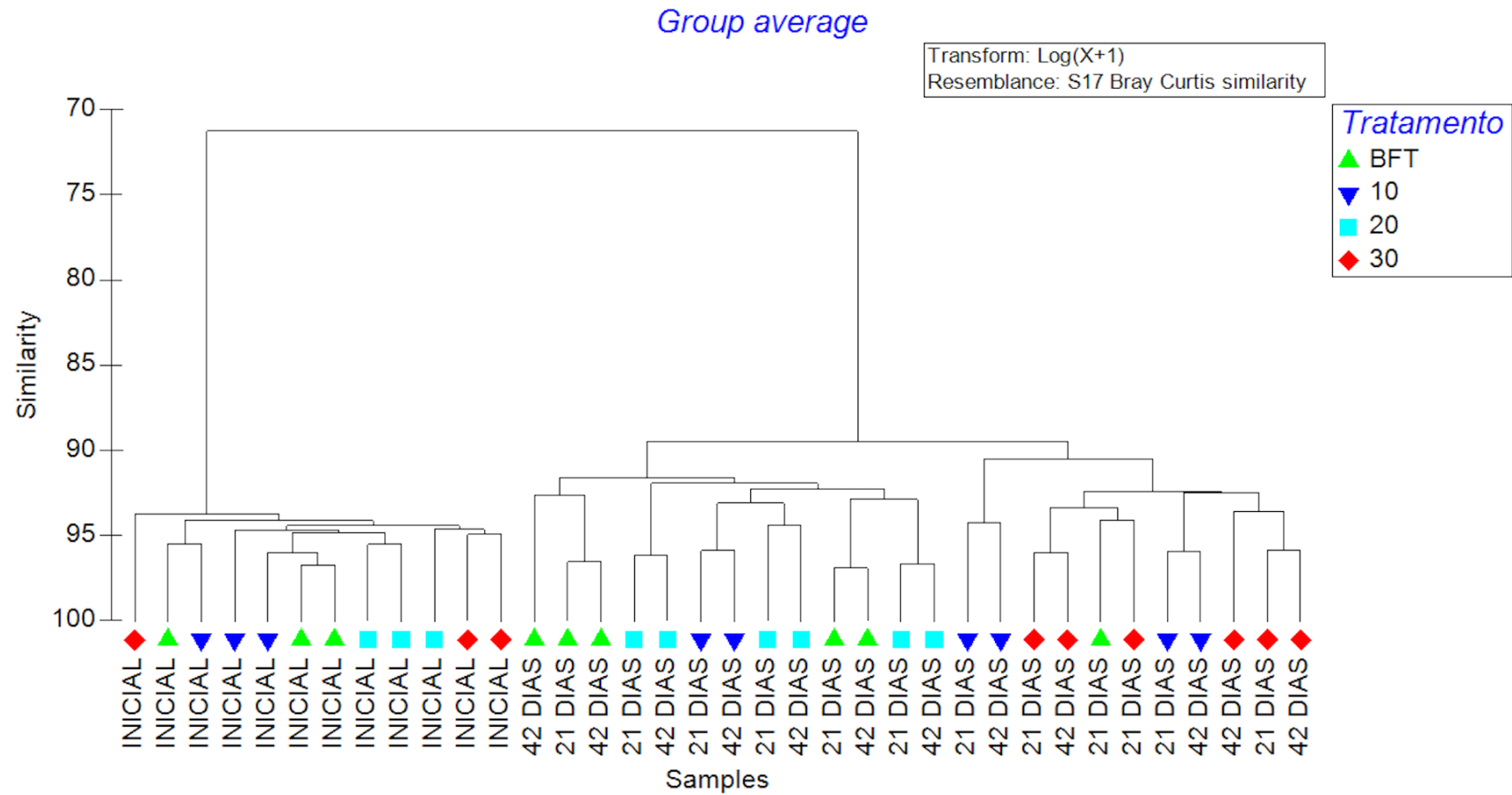
- 1008 Wasielesky, W.J., Atwood, H.I., Stokes, A., Browdy, C.L., 2006. Effect of natural production  
1009 in brown water super-intensive culture system for white shrimp *Litopenaeus vannamei*.  
1010 *Aquaculture*, 258, 396-403. <https://doi.org/10.1016/j.aquaculture.2006.04.030>.
- 1011 Whyte, N.J.C., Clarke, W.C., Ginther, N.G., Jensen, J.O.T., Townsend, L.D., 1994. Influence  
1012 of composition of *Brachionus plicatilis* and *Artemia* on growth of larval sablefish  
1013 (*Anoplopoma fimbria Pallas*). *Aquaculture*, 119, 47-61. <https://doi.org/10.1016/0044->  
1014 8486(94)90443-X.
- 1015 Xu, W.J., Morris, T.C., Samocha, T.M., 2016. Effects of C/N ration on biofloc development,  
1016 water quality, and performance of *Litopenaeus vannamei* juveniles in a biobloc-based, high-  
1017 density, zero-exchange, outdoor tank system. *Aquaculture*, 453, 169-175.  
1018 <https://doi.org/10.1016/j.aquaculture.2015.11.021>.
- 1019 Xu, W.J., Morris, T.C., Samocha, T.M., 2018. Effects of two commercial feeds for semi-  
1020 intensive and hyper-intensive culture and four C/N rations on water quality and performance  
1021 of *Litopenaeus vannamei* juveniles at high density in biobloc-based, zero-exchange outdoor  
1022 tanks. *Aquaculture*, 490, 194-202. <https://doi.org/10.1016/j.aquaculture.2018.02.028>.
- 1023 Yanong, R. P., Erlacher-Reid, C., 2012. Biosecurity in aquaculture, part 1: An overview.  
1024 *South. Reg. Aquac. Cent.* 4707, 1-16.
- 1025 Yeh, S., Liu, C., Sung, T., Lee, P., Cheng, W., 2006. Effect of Saponin on Hematological and  
1026 Immunological Parameters of the Giant Freshwater Prawn, *Macrobrachium rosenbergii*.  
1027 *Aquaculture*, 261, 1432-1439. [doi.org/10.1016/j.aquaculture.2006.08.051](https://doi.org/10.1016/j.aquaculture.2006.08.051).
- 1028 Yin, X. W., Min, W. W., Lin, H. J., Chen, W., 2013. Population dynamics, protein content,  
1029 and lipid composition of *Brachionus plicatilis* fed artificial macroalgal detritus and  
1030 *Nannochloropsis* sp. diets. *Aquaculture*, 380, 62-69.  
1031 <https://doi.org/10.1016/j.aquaculture.2012.11.018>.

- 1032 Zhang, W., Chen, B., Niu, C., Yuan, L., Jia, H., Storey, K. B., 2019. Response of the Chinese  
1033 soft-shelled turtle to acute heat stress: insights from the systematic antioxidant defense.  
1034 *Frontiers in Physiology*, 10, 710. <https://doi.org/10.3389/fphys.2019.00710>.
- 1035 Zhao, P., Huang, J., Wang, X. H., Song, X. L., Yang, C., H., Zhang, X. G., Wang, G. C.,  
1036 2012. The application of bioflocs technology in high-intensive, zero exchange farming  
1037 systems of *Marsupenaeus japonicus*. *Aquaculture*, 354-355, 97-106.  
1038 <https://doi.org/10.1016/j.aquaculture.2012.03.034>.
- 1039 Zhou, Q.C., Li, C.C., Liu, C.W., Chi, S.Y., Yang, Q.H., 2007. Effects of dietary lipid sources  
1040 on growth and fatty acid composition of juvenile shrimp, *Litopenaeus vannamei*. *Aquaculture*  
1041 *Nutrition*, 13, 222-229. <https://doi.org/10.1111/j.1365-2095.2007.00470.x>.

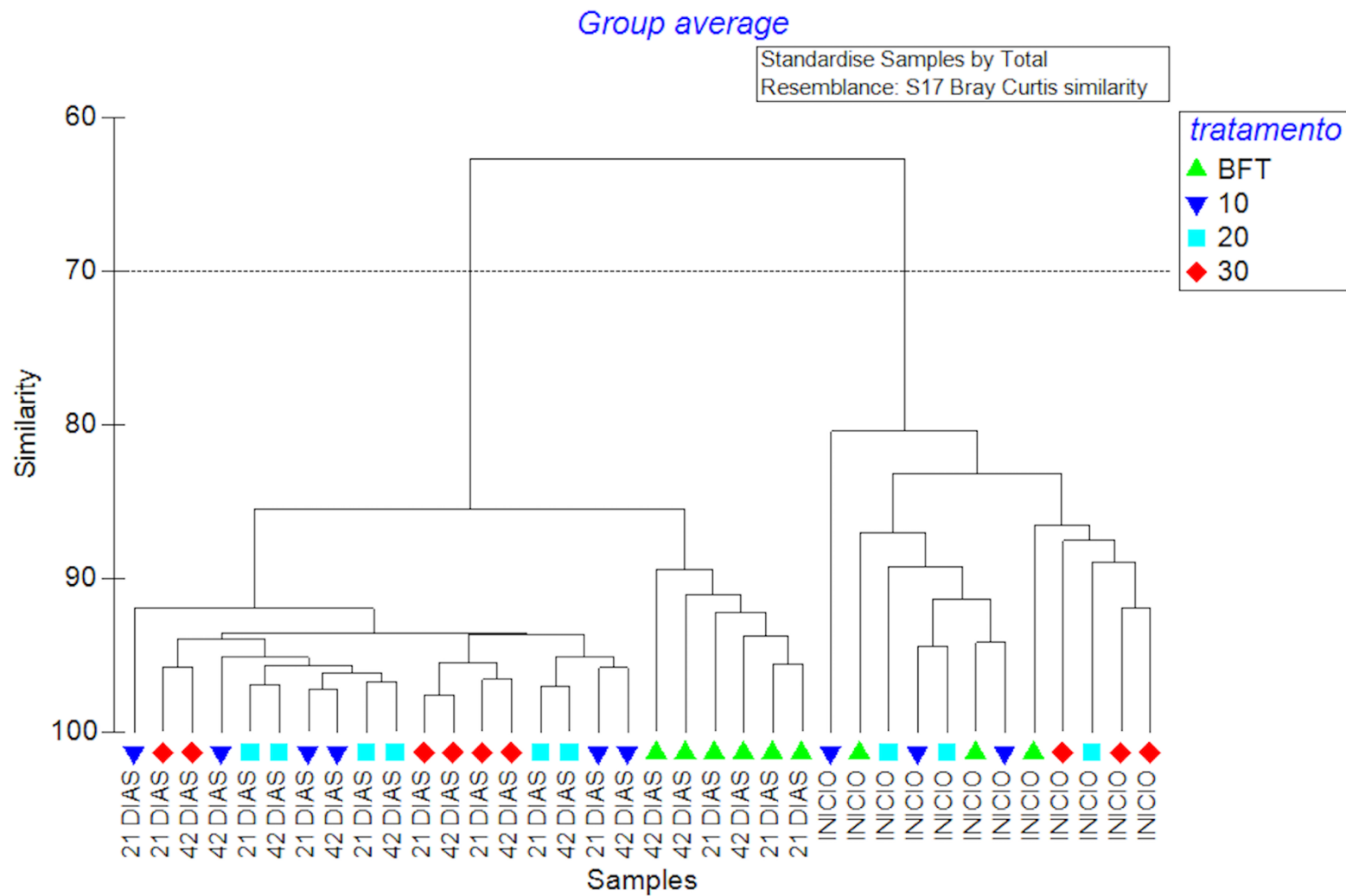
## 8. APPENDICES



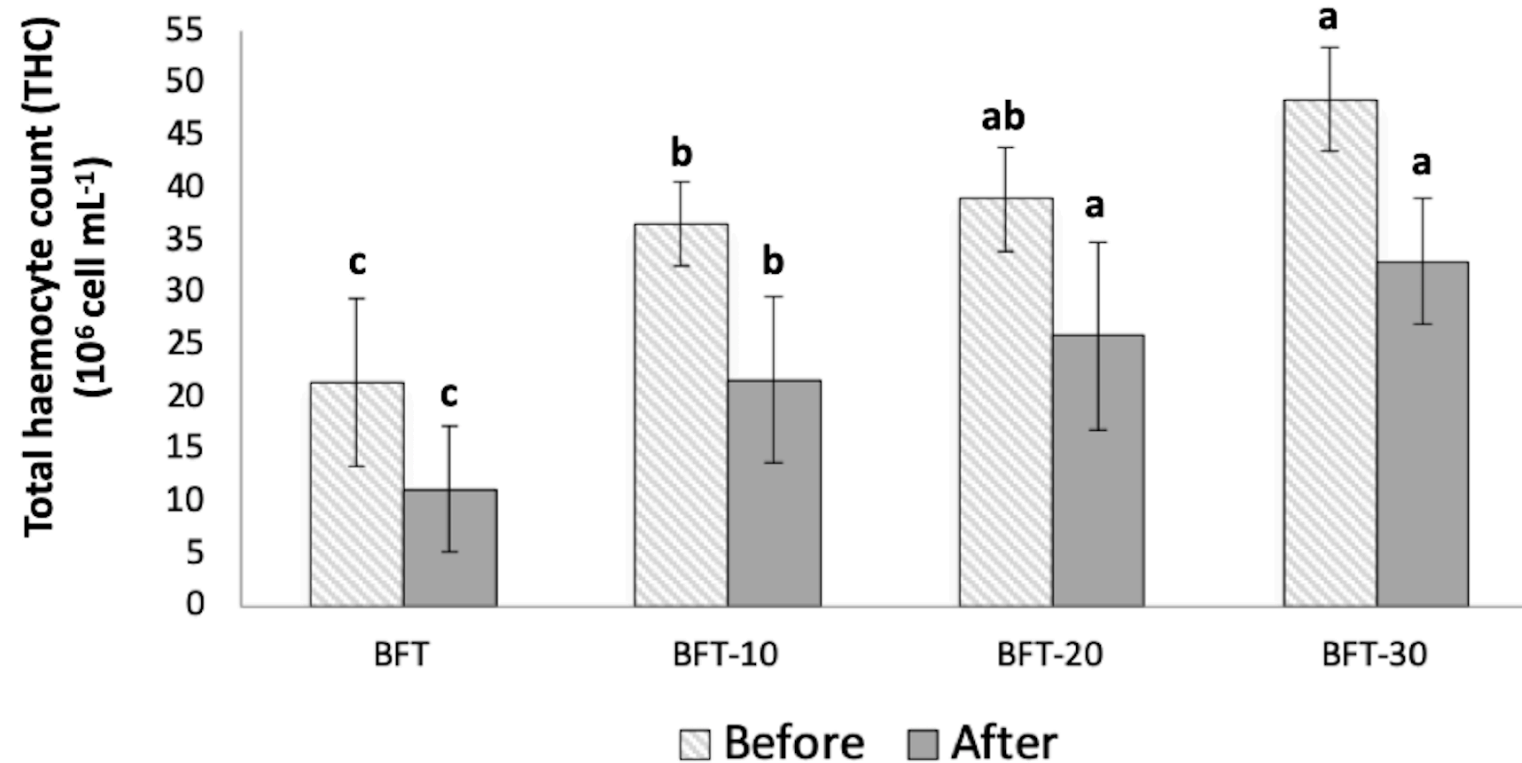
**Figure 1.** Nursery shrimp zootechnical parameters (final weight -A, survival -B, yield- C and FCR -D) with rotifers added at different densities during a 42-day experimental period. The data correspond to the mean  $\pm$  SD. Results were analyzed by performing ANOVA one way and the Tukey's test. Mean values (column and time) with different superscripts differ significantly ( $P < 0.05$ ). BFT (biofloc); BFT-10 (addition of 10 org mL<sup>-1</sup> of *B. plicatilis*); BFT-20 (addition of 20 org mL<sup>-1</sup> of *B. plicatilis*) and BFT-30 (addition of 30 org mL<sup>-1</sup> of *B. plicatilis*).



**Figure 2.** Cluster analysis of the phytoplankton community found of shrimp *L. vannamei* reared with and without different densities rotifers addition.



**Figure 3.** Cluster analysis of the zooplankton community found of shrimp *L. vannamei* reared with and without different densities rotifers addition.



**Figure 4.** Total haemocyte count (THC) before and after salinity stress test in *Litopenaeus vannamei* under nursery biofloc system with *Brachionus plicatilis* added at different densities. The data correspond to the mean  $\pm$  SD. Results were analyzed by performing ANOVA one way and the Tukey's test. Mean values in the same color column with different superscripts differ significantly ( $P < 0.05$ ). BFT (biofloc); BFT-10 (addition of 10 org mL<sup>-1</sup> of *B. plicatilis*); BFT-20 (addition of 20 org mL<sup>-1</sup> of *B. plicatilis*) and BFT-30 (addition of 30 org mL<sup>-1</sup> of *B. plicatilis*).

**Table 1.** Water quality parameters in the culture of *Litopenaeus vannamei* under nursery biofloc system with and *Brachionus plicatilis* added at different densities during a 42-day experimental period.

Parameters	Treatments			
	Controle	BFT-10	BFT-20	BFT-30
Temperature (M) (C°)	31.44±0.14 <sup>a</sup>	30.97±0.13 <sup>a</sup>	31.22±0.14 <sup>a</sup>	31.05±0.13 <sup>a</sup>
Temperature (A) (C°)	31.90±0.09 <sup>a</sup>	31.59±0.08 <sup>a</sup>	31.71±0.12 <sup>a</sup>	31.57±0.11 <sup>a</sup>
Dissolved oxygen (M) (mg L <sup>-1</sup> )	5.25±0.04 <sup>a</sup>	5.34±0.04 <sup>a</sup>	5.29±0.04 <sup>a</sup>	5.32±0.05 <sup>a</sup>
Dissolved oxygen (A) (mg L <sup>-1</sup> )	5.16±0.05 <sup>a</sup>	5.23±0.05 <sup>a</sup>	5.18±0.07 <sup>a</sup>	5.21±0.07 <sup>a</sup>
Salinity (g L <sup>-1</sup> )	32.90±0.25 <sup>a</sup>	32.74±0.30 <sup>a</sup>	32.65±0.31 <sup>a</sup>	32.47±0.32 <sup>a</sup>
pH (M)	8.31±0.06 <sup>a</sup>	8.29±0.05 <sup>a</sup>	8.26±0.06 <sup>a</sup>	8.28±0.06 <sup>a</sup>
pH (T)	8.27±0.06 <sup>a</sup>	8.24 ± 0.06 <sup>a</sup>	8.21±0.07 <sup>a</sup>	8.21±0.07 <sup>a</sup>
TAN (mg L <sup>-1</sup> )	0.38±0.03 <sup>a</sup>	0.38±0.04 <sup>a</sup>	0.47±0.06 <sup>a</sup>	0.43±0.04 <sup>a</sup>
N-Nitrite (mg L <sup>-1</sup> )	0.75±0.12 <sup>a</sup>	0.66±0.09 <sup>a</sup>	0.59±0.07 <sup>a</sup>	0.65±0.14 <sup>a</sup>
N-Nitrate (mg L <sup>-1</sup> )	23.68±8.68 <sup>a</sup>	20.31±8.29 <sup>a</sup>	18.61±6.71 <sup>a</sup>	22.61±7.78 <sup>a</sup>
Ortofosfato (mg L <sup>-1</sup> )	27.75±6.98 <sup>a</sup>	30.04±7.68 <sup>a</sup>	33.38±8.10 <sup>a</sup>	27.08±6.97 <sup>a</sup>
Alkalinity (mg CaCO <sub>3</sub> L <sup>-1</sup> )	106.67±5.67 <sup>a</sup>	103.89±6.41 <sup>a</sup>	98.61±7.92 <sup>a</sup>	100.56±6.38 <sup>a</sup>
SS (mL L <sup>-1</sup> )	9.13±0.87 <sup>a</sup>	7.49±0.75 <sup>a</sup>	8.26±0.77 <sup>a</sup>	8.08±1.07 <sup>a</sup>
Water consumption (L Kg <sup>-1</sup> )	372.45±11.17 <sup>a</sup>	309.21±12.27 <sup>a</sup>	297.58±16.82 <sup>a</sup>	261.34±13.27 <sup>a</sup>
C:N ratio	10.68±0.05 <sup>a</sup>	10.73±0.13 <sup>a</sup>	10.59±0.05 <sup>a</sup>	10.57±0.05 <sup>a</sup>

The data correspond to the mean ± SD. Results were analyzed by performing repeated ANOVA measures and the Tukey's test. The C:N ratio were analyzed by performing ANOVA one way and the Tukey's test. Mean values in the same row with different superscripts differ significantly ( $P < 0.05$ ). BFT (biofloc); BFT-10 (addition of 10 org mL<sup>-1</sup> of *Brachionus plicatilis*); BFT-20 (addition of 20 org mL<sup>-1</sup> of *Brachionus plicatilis*) and BFT-30 (addition of 30 org mL<sup>-1</sup> of *Brachionus plicatilis*). M -Morning; A -afternoon; DO - dissolved oxygen; TAN - total ammonia nitrogen; SS - Settleable solids.



**Table 2.** *Vibrio* density in the culture of *Litopenaeus vannamei* under nursery biofloc system with *Brachionus plicatilis* added at different densities initial and at the end (42-day experimental period).

	<b>Initial</b>	<b>BFT</b>	<b>BFT-10</b>	<b>BFT-20</b>	<b>BFT-30</b>
<b>Water (10<sup>4</sup> UFC mL<sup>-1</sup>)</b>					
Positive Sucrose	0.04 <sup>b</sup>	2.50 <sup>a</sup>	1.09 <sup>a</sup>	3.94 <sup>a</sup>	5.58 <sup>a</sup>
(%)	(7.23)	(55.59)	(68.84)	(82.49)	(98.61)
Negative Sucrose	0.534 <sup>a</sup>	1.99 <sup>a</sup>	0.49 <sup>a</sup>	0.84 <sup>a</sup>	0.08 <sup>a</sup>
(%)	(92.77)	(44.41)	(31.16)	(17.51)	(1.39)
<b>Total</b>	0.58 <sup>a</sup>	4.49 <sup>a</sup>	1.58 <sup>a</sup>	4.77 <sup>a</sup>	5.66 <sup>a</sup>
<b>Shrimp (10<sup>4</sup> UFC g<sup>-1</sup>)</b>					
Positive Sucrose	0.01 <sup>b</sup>	12.60 <sup>a</sup>	34.70 <sup>a</sup>	81.30 <sup>a</sup>	10.30 <sup>a</sup>
(%)	(1.23)	(37.05)	(15.25)	(54.64)	(100)
Negative Sucrose	1.00 <sup>a</sup>	21.50 <sup>a</sup>	192.90 <sup>a</sup>	67.50 <sup>a</sup>	0 <sup>b</sup>
(%)	(98.77)	(62.95)	(84.75)	(45.36)	0
<b>Total</b>	1.01 <sup>a</sup>	34.10 <sup>a</sup>	227.60 <sup>a</sup>	148.80 <sup>a</sup>	10.30 <sup>a</sup>

The data correspond to the mean. Results were analyzed by performing Kruskal-Wallis and the Dunn test. Means in the same row with different superscripts differ significantly ( $p < 0.05$ ). BFT (biofloc); BFT-10 (addition of 10 org mL<sup>-1</sup> of *Brachionus plicatilis*); BFT-20 (addition of 20 org mL<sup>-1</sup> of *Brachionus plicatilis*) and BFT-30 (addition of 30 org mL<sup>-1</sup> of *Brachionus plicatilis*).

**Table 3.** Phytoplankton composition (initial and final) of *Litopenaeus vannamei* under nursery biofloc system with *Brachionus plicatilis* added at different densities initial and at the end (42-day experimental period period).

Division/Genera	Initial	Final			
		BFT	BFT-10	BFT-20	BFT-30
<b>Chlorophyta (cells mL<sup>-1</sup>)</b>	<b>1,316.79</b>	<b>1,603.12<sup>a</sup></b>	<b>1,654.22<sup>a</sup></b>	<b>1,367.94<sup>a</sup></b>	<b>1,459.90<sup>a</sup></b>
(%)	<b>34.01</b>	<b>11.50</b>	<b>12.57</b>	<b>10.18</b>	<b>11.67</b>
<i>Botryococcus</i>	0.00	159.92 <sup>a</sup>	177.31 <sup>a</sup>	239.36 <sup>a</sup>	200.15 <sup>a</sup>
<i>Chlorella</i>	0.00	1.49 <sup>a</sup>	2.82 <sup>a</sup>	1.37 <sup>a</sup>	1.70 <sup>a</sup>
<i>Dunaliella</i>	0.15	0.00 <sup>a</sup>	1.33 <sup>a</sup>	0.95 <sup>a</sup>	0.86 <sup>a</sup>
<i>Haematococcus</i>	0.70	0.41 <sup>a</sup>	1.04 <sup>a</sup>	2.26 <sup>a</sup>	1.33 <sup>a</sup>
<i>Mychonastes</i>	286.00	755.23 <sup>a</sup>	486.78 <sup>a</sup>	451.59 <sup>a</sup>	659.76 <sup>a</sup>
<i>Planctonema</i>	342.40	327.54 <sup>a</sup>	455.62 <sup>a</sup>	259.46 <sup>a</sup>	248.71 <sup>a</sup>
<i>Pyramimonas</i>	0.00	2.09 <sup>a</sup>	1.94 <sup>a</sup>	0.90 <sup>a</sup>	1.96 <sup>a</sup>
<i>Spirogyra</i>	223.20	3.53 <sup>b</sup>	11.38 <sup>a</sup>	4.28 <sup>b</sup>	1.91 <sup>b</sup>
<i>Tretadesmus</i>	0.00	0.00 <sup>a</sup>	0.47 <sup>a</sup>	0.48 <sup>a</sup>	0.82 <sup>a</sup>
<i>Ulothrix</i>	464.34	352.90 <sup>a</sup>	525.01 <sup>a</sup>	400.20 <sup>a</sup>	340.33 <sup>a</sup>
<b>Dinophyta (cells mL<sup>-1</sup>)</b>	<b>0.71</b>	<b>7.25<sup>a</sup></b>	<b>6.30<sup>a</sup></b>	<b>8.06<sup>a</sup></b>	<b>7.04<sup>a</sup></b>
(%)	<b>0.02</b>	<b>0.05</b>	<b>0.05</b>	<b>0.06</b>	<b>0.06</b>
<i>Gymnodinium</i>	0.18	2.85 <sup>a</sup>	2.34 <sup>a</sup>	3.49 <sup>a</sup>	2.74 <sup>a</sup>
<i>Peridinium</i>	0.20	2.40 <sup>a</sup>	1.63 <sup>a</sup>	1.82 <sup>a</sup>	1.91 <sup>a</sup>
<i>Pyrophacus</i>	0.33	2.01 <sup>a</sup>	2.34 <sup>a</sup>	2.75 <sup>a</sup>	2.40 <sup>a</sup>
<b>Euglenophyta (cells mL<sup>-1</sup>)</b>	<b>5.35</b>	<b>6.56<sup>a</sup></b>	<b>4.07<sup>a</sup></b>	<b>4.52<sup>a</sup></b>	<b>4.08<sup>a</sup></b>
(%)	<b>0.14</b>	<b>0.05</b>	<b>0.03</b>	<b>0.03</b>	<b>0.03</b>
<i>Euglena</i>	0.04	1.42 <sup>a</sup>	0.79 <sup>a</sup>	0.77 <sup>a</sup>	0.67 <sup>a</sup>
<i>Trachelomonas</i>	0.14	5.14 <sup>a</sup>	3.28 <sup>a</sup>	3.76 <sup>a</sup>	3.41 <sup>a</sup>
<b>Heterokonphyta (cells mL<sup>-1</sup>)</b>	<b>1,282.85</b>	<b>3,481.44<sup>a</sup></b>	<b>2,754.39<sup>a</sup></b>	<b>2,721.13<sup>a</sup></b>	<b>2,328.33<sup>a</sup></b>
(%)	<b>33.13</b>	<b>24.98</b>	<b>20.93</b>	<b>20.26</b>	<b>18.63</b>
<i>Chaetoceros</i>	0.00	4.13 <sup>a</sup>	3.46 <sup>a</sup>	3.32 <sup>a</sup>	2.53 <sup>a</sup>
<i>Chloridella</i>	21.41	1.72 <sup>b</sup>	2.07 <sup>a</sup>	2.93 <sup>a</sup>	2.33 <sup>a</sup>
<i>Cylindrotheca</i>	21.70	1,478.70 <sup>a</sup>	1,343.98 <sup>a</sup>	1,357.02 <sup>a</sup>	1,349.65 <sup>a</sup>
<i>Diatoma</i>	0.00	15.99 <sup>a</sup>	15.39 <sup>a</sup>	29.17 <sup>a</sup>	23.56 <sup>a</sup>
<i>Fragilaria</i>	489.18	20.42 <sup>a</sup>	12.28 <sup>a</sup>	11.01 <sup>a</sup>	22.20 <sup>a</sup>
<i>Hemiaulus</i>	33.11	598.82 <sup>a</sup>	699.86 <sup>a</sup>	661.44 <sup>a</sup>	467.16 <sup>a</sup>

<i>Navicula</i>	28.04	17.58 <sup>a</sup>	27.09 <sup>a</sup>	30.28 <sup>a</sup>	24.54 <sup>a</sup>
<i>Orthoseira</i>	188.37	9.35 <sup>a</sup>	8.12 <sup>a</sup>	16.22 <sup>a</sup>	8.93 <sup>a</sup>
<i>Phaeodactylum</i>	0.00	1.08 <sup>a</sup>	1.63 <sup>a</sup>	1.53 <sup>a</sup>	2.07 <sup>a</sup>
<i>Rhabdonema</i>	188.41	0.00	0.00	0.00	0.00
<i>Skeletonema</i>	312.65	1,333.63 <sup>a</sup>	640.46 <sup>a</sup>	608.18 <sup>a</sup>	425.32 <sup>a</sup>
<i>Thalassiosira</i>	0.00	0.00	0.06 <sup>a</sup>	0.05 <sup>a</sup>	0.03 <sup>a</sup>
<b>Cyanophyta (cells mL<sup>-1</sup>)</b>	<b>1,266.18</b>	<b>8,840.49<sup>a</sup></b>	<b>8,741.12<sup>a</sup></b>	<b>9,332.47<sup>a</sup></b>	<b>8,700.76<sup>a</sup></b>
<b>(%)</b>	<b>32.70</b>	<b>63.42</b>	<b>66.45</b>	<b>69.47</b>	<b>69.61</b>
<i>Anabaena</i>	24.79	206.40 <sup>a</sup>	212.03 <sup>a</sup>	247.99 <sup>a</sup>	252.41 <sup>a</sup>
<i>Aphanocapsa</i>	433.17	2,153.81 <sup>a</sup>	1,665.09 <sup>a</sup>	2,077.72 <sup>a</sup>	2,399.53 <sup>a</sup>
<i>Merismopedia</i>	35.42	0.00 <sup>b</sup>	168.45 <sup>ab</sup>	0.00 <sup>b</sup>	348.79 <sup>a</sup>
<i>Oscillatoria</i>	614.93	6,169.55 <sup>a</sup>	6,388.07 <sup>a</sup>	6,789.62 <sup>a</sup>	5,399.92 <sup>a</sup>
<i>Plectonema</i>	64.57	26.64 <sup>a</sup>	86.59 <sup>a</sup>	61.62 <sup>a</sup>	32.97 <sup>a</sup>
<i>Pseudanabaena</i>	58.36	257.33 <sup>a</sup>	183.63 <sup>a</sup>	126.74 <sup>a</sup>	231.93 <sup>a</sup>
<i>Spirulina</i>	34.94	26.75 <sup>a</sup>	37.27 <sup>a</sup>	28.77 <sup>a</sup>	35.14 <sup>a</sup>
<b>Total Phytoplankton (cells mL<sup>-1</sup>)</b>	<b>3,871.89</b>	<b>13,938.85<sup>a</sup></b>	<b>13,160.13<sup>a</sup></b>	<b>13,434.12<sup>a</sup></b>	<b>12,500.11<sup>a</sup></b>

The data correspond to the mean  $\pm$  SD. Results were analyzed by performing Kruskal-Wallis test. Mean values in the same color column with different superscripts differ significantly ( $P < 0.05$ ). BFT (biofloc); BFT-10 (addition of 10 org mL<sup>-1</sup> of *Brachionus plicatilis*); BFT-20 (addition of 20 org mL<sup>-1</sup> of *Brachionus plicatilis*) and BFT-30 (addition of 30 org mL<sup>-1</sup> of *Brachionus plicatilis*).

**Table 4.** Zooplankton composition (initial and final) of *Litopenaeus vannamei* under nursery biofloc system with *Brachionus plicatilis* added at different densities initial and at the end (42-day experimental period period).

Division/Genera	Initial	Final			
		BFT	BFT-10	BFT-20	BFT-30
<b>Protozoa (org mL<sup>-1</sup>)</b>	<b>0.58</b>	<b>1.05<sup>a</sup></b>	<b>0.67<sup>b</sup></b>	<b>0.59<sup>b</sup></b>	<b>0.66<sup>b</sup></b>
(%)	<b>28.15</b>	<b>29.33</b>	<b>16.63</b>	<b>15.53</b>	<b>15.10</b>
<i>Arcella</i> sp.	0.46	0.83 <sup>a</sup>	0.45 <sup>b</sup>	0.42 <sup>b</sup>	0.44 <sup>b</sup>
<i>Leprotintinnus</i> sp.	0.12	0.23 <sup>a</sup>	0.22 <sup>a</sup>	0.16 <sup>a</sup>	0.22 <sup>a</sup>
<b>Cladocera (org mL<sup>-1</sup>)</b>	<b>0.62</b>	<b>0.77<sup>a</sup></b>	<b>1.11<sup>a</sup></b>	<b>0.97<sup>a</sup></b>	<b>0.86<sup>a</sup></b>
(%)	<b>30.10</b>	<b>21.51</b>	<b>27.54</b>	<b>25.53</b>	<b>19.68</b>
<i>Bosmina</i> sp.	0.08	0.38 <sup>a</sup>	0.51 <sup>a</sup>	0.41 <sup>a</sup>	0.47 <sup>a</sup>
<i>Daphnia</i> sp.	0.54	0.39 <sup>a</sup>	0.60 <sup>a</sup>	0.55 <sup>a</sup>	0.72 <sup>a</sup>
<b>Copepoda (org mL<sup>-1</sup>)</b>	<b>0.19</b>	<b>0.40<sup>b</sup></b>	<b>0.43<sup>ab</sup></b>	<b>0.39<sup>b</sup></b>	<b>0.56<sup>a</sup></b>
(%)	<b>9.22</b>	<b>11.17</b>	<b>10.67</b>	<b>10.26</b>	<b>12.81</b>
<i>Clausocalanus</i> sp.	0.04	0.16 <sup>ab</sup>	0.24 <sup>a</sup>	0.13 <sup>b</sup>	0.19 <sup>ab</sup>
<i>Euterpina</i> sp.	0.14	0.15 <sup>a</sup>	0.08 <sup>a</sup>	0.17 <sup>a</sup>	0.21 <sup>a</sup>
<i>Harpacticus</i> sp.	0	0.07 <sup>b</sup>	0.11 <sup>ab</sup>	0.09 <sup>ab</sup>	0.15 <sup>a</sup>
<b>Rotifera (org mL<sup>-1</sup>)</b>	<b>0.67</b>	<b>1.22<sup>b</sup></b>	<b>1.64<sup>a</sup></b>	<b>1.71<sup>a</sup></b>	<b>1.77<sup>a</sup></b>
(%)	<b>32.52</b>	<b>34.08</b>	<b>40.69</b>	<b>45.00</b>	<b>40.50</b>
<i>Asplanchna</i> sp.	0.04	0.25 <sup>a</sup>	0.31 <sup>a</sup>	0.34 <sup>a</sup>	0.28 <sup>a</sup>
<i>Brachionus</i> sp.	0.61	0.60 <sup>b</sup>	0.89 <sup>a</sup>	0.91 <sup>a</sup>	1.04 <sup>a</sup>
<i>Filinia</i> sp.	0	0.19 <sup>a</sup>	0.10 <sup>b</sup>	0.17 <sup>ab</sup>	0.08 <sup>ab</sup>
<i>Keratella</i> sp.	0.02	0.15 <sup>b</sup>	0.34 <sup>a</sup>	0.28 <sup>a</sup>	0.35 <sup>a</sup>
<b>Cirripedia (ind. mL<sup>-1</sup>)</b>	<b>0</b>	<b>0.14<sup>a</sup></b>	<b>0.19<sup>a</sup></b>	<b>0.15<sup>a</sup></b>	<b>0.20<sup>a</sup></b>
(%)	<b>0</b>	<b>3.91</b>	<b>4.71</b>	<b>3.95</b>	<b>4.58</b>
Nauplios	0	0.14 <sup>a</sup>	0.19 <sup>a</sup>	0.15 <sup>a</sup>	0.20 <sup>a</sup>
<b>Total Zooplankton (org mL<sup>-1</sup>)</b>	<b>2.06</b>	<b>3.58<sup>b</sup></b>	<b>4.03<sup>ab</sup></b>	<b>3.80<sup>b</sup></b>	<b>4.37<sup>a</sup></b>

The data correspond to the mean  $\pm$  SD. Results were analyzed by performing Kruskal-Wallis test. Mean values in the same column with different superscripts differ significantly ( $P < 0.05$ ). BFT (biofloc); BFT-10 (addition of 10 org mL<sup>-1</sup> of *Brachionus plicatilis*); BFT-20 (addition of 20 org mL<sup>-1</sup> of *Brachionus plicatilis*) and BFT-30 (addition of 30 org mL<sup>-1</sup> of *Brachionus plicatilis*).

**Table 5.** Proximal composition of shrimp and biofloc in the culture of *Litopenaeus vannamei* under nursery biofloc system with and *Brachionus plicatilis* added at different densities during a 42-day experimental period.

Proximal composition <sup>1</sup>	Treatments				
	Initial	BFT	BFT-10	BFT-20	BFT-30
<b>Shrimp</b>					
Moisture (%)	89.43±0.55 <sup>a</sup>	79.56±0.34 <sup>b</sup>	78.05±0.20 <sup>b</sup>	76.58±0.77 <sup>b</sup>	79.10±0.67 <sup>b</sup>
Crude Protein	172.21±5.33 <sup>b</sup>	212.10±6.11 <sup>b</sup>	252.52±1.27 <sup>ab</sup>	274.45±1.60 <sup>a</sup>	283.07±2.82 <sup>a</sup>
Lipids	30.99±1.97 <sup>b</sup>	41.57±0.50 <sup>b</sup>	63.44±3.09 <sup>ab</sup>	89.04±2.10 <sup>a</sup>	111.40±1.77 <sup>a</sup>
Ash	-	144.29±1.35 <sup>a</sup>	124.15±1.46 <sup>b</sup>	138.26±0.42 <sup>a</sup>	139.09±1.71 <sup>a</sup>
Fiber	-	30.04±1.74 <sup>a</sup>	31.59±0.78 <sup>a</sup>	33.27±0.35 <sup>a</sup>	32.14±2.56 <sup>a</sup>
Carbohydrate	-	117.67±0.48 <sup>a</sup>	110.92±1.24 <sup>a</sup>	108.77±3.73 <sup>a</sup>	90.23±4.90 <sup>b</sup>
<b>Biofloc</b>					
Moisture (%)	93.84±0.10 <sup>a</sup>	90.22±1.80 <sup>ab</sup>	89.78±0.15 <sup>ab</sup>	88.87±0.17 <sup>b</sup>	88.20±1.31 <sup>b</sup>
Crude Protein	179.23±3.19 <sup>c</sup>	203.11±9.28 <sup>b</sup>	244.32±9.25 <sup>b</sup>	279.65±8.98 <sup>ab</sup>	298.25±5.38 <sup>a</sup>
Lipids	43.60±1.81 <sup>c</sup>	66.19±1.08 <sup>b</sup>	87.64±2.25 <sup>ab</sup>	117.39±12.52 <sup>a</sup>	130.01±4.62 <sup>a</sup>
Ash	191.51±21.9 <sup>a</sup>	243.62±18.50 <sup>a</sup>	261.94±14.90 <sup>a</sup>	258.48±9.05 <sup>a</sup>	253.03±7.94 <sup>a</sup>
Fiber	55.49±1.22 <sup>a</sup>	66.83±1.44 <sup>a</sup>	66.55±3.07 <sup>a</sup>	65.71±0.96 <sup>a</sup>	64.36±0.36 <sup>a</sup>
Carbohydrate	32.84±1.26	48.55±6.72 <sup>a</sup>	34.67±1.25 <sup>b</sup>	31.30±6.81 <sup>b</sup>	31.17±8.37 <sup>b</sup>

<sup>1</sup> Except for moisture (%), the other values are in terms of dry weight (g 100 g<sup>-1</sup> dry weight). The data correspond to the mean ± SD. Results were analyzed by performing ANOVA one way and a Tukey's test. Mean values in the same row with different superscripts differ significantly (P < 0.05). BFT (biofloc); BFT-10 (addition of 10 org mL<sup>-1</sup> of *Brachionus plicatilis*); BFT-20 (addition of 20 org mL<sup>-1</sup> of *Brachionus plicatilis*) and BFT-30 (addition of 30 org mL<sup>-1</sup> of *Brachionus plicatilis*).

#### 4. CONSIDERAÇÕES FINAIS

No presente estudo foi constatado que a adição do rotífero *Brachionus plicatilis* contribui positivamente no cultivo de *Litopenaeus vannamei* na fase berçário em sistema de bioflocos. As densidades de adição de 20-30 org mL<sup>-1</sup> proporcionaram os maiores valores desempenho zootécnico, melhor composição centesimal e melhores concentrações de hemócitos após um teste de estresse salino. Atrelado a adição do rotífero, podemos atrelar o sucesso do cultivo a eficiente estratégia de fertilização adotada, o que possibilitou a manutenção da qualidade de água durante todo o cultivo. Contudo, outros estudos devem ser conduzidos para testar novas formas de ofertar os rotíferos.

#### 5. REFERÊNCIAS

- ABREU, J. L.; BRITO, L. O.; LIMA, P. C. M.; SILVA, S. M. B. C.; 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, p. 2231-2239, 2019.
- AHMAD, I.; RANI, A. B.; VERMA, A. K.; MAQSOOD, M. Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition. **Aquaculture International**, v. 25, n. 3, p. 1215- 1226, 2017.
- AVNIMELECH, Y. **Biofloc Technology**: a practical guidebook. 2nd ed. Baton Rouge: LA. World Aquaculture Society, 2012. 272p.
- BALLESTER, E.; ABREU, P.; CAVALLI, R. O.; EMERENCIANO, M.; ABREU, L. WASIELESKY, W. Effect of practical diets with different protein levels on the performance of *Farfantepenaeus paulensis* juveniles nursed in a zero exchange suspended microbial flocs intensive system. **Aquaculture Nutrition**, v. 16, n. 2, p. 163-172, 2010.
- BOWLER, B.; LIMSUWAN, C.; CHUCHIRD, N.; ESPINOZA, C.; ROJAS, P. Use of functional diets improves survival of *Vibrio*-infected shrimp. **The Global Aquaculture Advocate**, p. 26-27, 2015.
- BRITO, L. O.; SANTOS, I. G. S.; ABREU, J. L.; ARAÚJO, M. T.; SEVERI, W.; GÁLVEZ, A. O. Effect of addition of diatoms (*Navicula* spp.) and rotifers (*Brachionus plicatilis*) on growth and water quality of the *Litopenaeus vannamei* postlarvae reared in biofloc system. **Aquaculture Research**, v. 47, p. 3990-3997, 2016.
- CARVALHO, J.; MATSUDO, M.; BEZERRA, R.; FERREIRA-CAMARGO, L.; SATO, S.; 2014. Microalgae Bioreactors, in: Bajpai, R., Prokop, A., Zappi, M. (Eds.), *Algal Biorefineries*, vol 1. Springer Netherlands, Dordrecht, pp. 83-126. <https://doi.org/10.1007/978-94-007-7494-0>

CONCEIÇÃO, L. E.; YÚFERA, M.; MAKRIDIS, P.; MORAIS, S.; DINIS, M. T. Live feeds for early stages of fish rearing. **Aquaculture Research**, vol. 41, n.5, p. 613-640, 2010.

CRAB, R.; CHIELENS, B.; WILLE, M.; BOSSIER, P.; VERSTRAETE, W. The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. **Aquaculture Research**, v. 41, n. 4, p. 559-567, 2010.

CUÉLLAR-ANJEL, J. LARA, C., MORALES, V., GRACIA, A., SUÁREZ, O. G., 2010. Manual de buenas prácticas de manejo para el cultivo del camarón blanco *Penaeus vannamei*. OIRSA-OSPESCA, Panamá.

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.

EKASARI, J.; RIVANDI, D. R.; FIRDAUSI, A. P.; SURAWIDJAJA, E. H.; ZAIRIN JR., M.; BOSSIER, P.; DE SCHRYVER, P. Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance. **Aquaculture**, v. 441, p. 72-77, 2015.

EMERENCIANO, M.; GAXIOLA, G; CUZON, G. Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry. In: MATOVIC, M.D. Biomass Now - Cultivation and Utilization. Croatia: InTech, 2013. p. 301-328.

FAO. **The State of World Fisheries and Aquaculture**: sustainability in action. Rome. Fisheries and Aquaculture Technical Paper, 2020. 244p.

FÓES, G.K.; GAONA, C.A.P.; POERSCH, L.H. Cultivo em bioblocos (BFT) é eficaz na produção intensiva de camarões. **Visão Agrícola**, n. 11, p. 28-32, 2012.

FERREIRA, M.; FÁBREGAS, J.; OTERO, A.; 2008. Enriching rotifers with "premium" microalgae. *Isochrysis* aff. *galbana* clone T-ISO. *Aquaculture* 279, 126-130. <https://doi.org/10.1016/j.aquaculture.2008.03.044>.

FERREIRA, M.; COUTINHO, P.; SEIXAS, P.; FÁBREGAS, J.; OTERO, A. 2009. Enriching rotifers with "Premium" microalgae. *Nannochloropsis gaditana*. *Mar. Biotechnol.* 11, 585-595.

FERREIRA, M.; CORTINA-BURGUENO, A.; 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, abril 2018

FUENTES-GRÜNEWALD, C.; BAYLISSA, C.; ZANAIN, M.; POOLEY, C.; SCOLAMACCHIA, M.; SILKINA, A.; 2015. Evaluation of batch and semi-continuous culture of *Porphyridium purpureum* in a photobioreactor in high latitudes using Fourier Transform Infrared spectroscopy for monitoring biomass composition and metabolites production. *Bioresource Technol.* 189, 357-363. <https://doi.org/10.1016/j.biortech.2015.04.042>.

IBGE (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA). Produção da Pecuária Municipal. **Infográfico**, v.44, p.51, 2019.

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.

JEEJA, P.K.; JOSEPH I.; RAJ R.P. Nutritional composition of rotifer (*Brachionus plicatilis* Muller) cultured using selected natural diets. **Indian Journal Fish**, v. 58, p. 59-65, 2011.

JU, Z. Y.; FORSTER, I.; CONQUEST, L.; DOMINY, W.; KUO, W. C.; HORGAN, F. D. Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profile. **Aquaculture Research**, v. 39, p. 118-133, 2008.

KRUMMENAUER, D.; SEIFERT JR, C.A.; POERSCH, L.H.; FOES, G.K.; LARA, G.R.; WASIELESKY JR, W. Cultivo de camarões marinhos em sistema de bioflocos: análise de reutilização da água. **Atlântica, Rio Grande**, v. 34, n. 2, p. 103-111, 2012.

KUMAR, B. K.; DEEKSHIT, V. K.; RAJ, J. R. M.; RAI, P.; SHIVANAGOWDA, B. M.; KARUNASAGAR, I.; KARUNASAGAR, I. Diversity of *Vibrio parahaemolyticus* associated with disease outbreak among cultured *Litopenaeus vannamei* (Pacific white shrimp) in India. **Aquaculture**, v. 433, p. 247-251, 2014.

KURMAR, 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.

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.

LUBZENS, E.; TANDLER, A.; MINKOFF, G.; 1989. Rotifers as food in aquaculture. **Hydrobiologia**, 186/187: 387- 400.

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, EUA**, p. 1-14, 2018.

MAICA, P. F.; BORBA, M. R.; WASIELESKY JR, W. Effect of low salinity on microbial floc composition and performance of *Litopenaeus vannamei* (Boone) juveniles reared in a zero-water-exchange super-intensive system. **Aquaculture Research**, v. 43, n. 2, p. 361-370, 2012.

MARINHO, Y. F.; BRITO, L. O.; SILVA, C. V. F.; SEVERI, W.; ANDRADE, H. A.; GALVEZ, A. O. Effect of the addition of *Chaetoceros calcitrans*, *Navicula* sp. and *Phaeodactylum tricorutum* (diatoms) on phytoplankton composition and growth of *Litopenaeus vannamei* (Boone) postlarvae reared in a biofloc system. **Aquaculture Research**, v. 48, p. 4155-4164, 2017.

MANAN, H.; MOH, J. H. Z.; KASAN, N. A.; SURATMAN, S.; IKHWANUDDIN, M.



Identification of biofloc microscopic composition as the natural bioremediation in zero water exchange of Pacific white shrimp, *Penaeus vannamei*, culture in closed hatchery system. *Applied Water Science*, v. 7, n. 5, p. 2437-2446, 2017.

MOSS, S.M.; MOSS, D.R.; ARCE, S.M.; LIGHTNER, D.V.; LOTZ, J.M. The role of selective breeding and biosecurity in the prevention of disease in penaeid shrimp aquaculture. **Journal of Invertebrate Pathology**, v. 110, n. 1, p. 247-250, 2012.

NESARA KM; 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.

NÓBREGA, G.N.; FERREIRA, T.O.; ROMERO, R.E.; MARQUES, A.G.B.; OTERO, X.L. (2013) - Iron and sulfur geochemistry in semi-arid mangrove soils (Ceará, Brazil) in relation to seasonal changes and shrimp farming effluents. *Environmental Monitoring and Assessment*, 185(9):7393–7407.

NUNES, A. J. P.; FEIJÓ, R. G. Convivência com o vírus da mancha branca no cultivo de camarão marinho no Brasil. **Associação Brasileira de Criadores de Camarão**, n. 2, p. 30-36, 2016.

OTOSHI, C.A.; RODRIGUEZ, N.; MOSS, S.M. Establishing nitrifying bacteria in super-intensive biofloc shrimp production. **Global Aquaculture Advocate**, v. 14, n. 4, p. 24-26, 2011.

PANIGRAHI, A.; SARANYA, C.; SUNDARAM, M.; KANNAN, S. V.; DAS, R. R.; KUMAR, R. S.; RAJESH.; OTTA, S. K. Carbon: Nitrogen (C: N) ratio level variation influences microbial community of the system and growth as well as immunity of shrimp (*Litopenaeus vannamei*) in biofloc based culture system. **Fish & Shellfish immunology**, v. 81, p. 329-337, 2018.

PÉREZ-FUENTES, J. A.; HERNÁNDEZ-VERGARA, M. P.; PÉREZ-ROSTRO, C. I.; FOGEL, I. C:N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. **Aquaculture**, v. 452, n. 1, p. 247-251, 2016.

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

PHULIA, V.; MANDAL, B.; BERA, A.; SINGH, S.; DAS, R.; JAMWAL, A. Factors controlling biofloc characteristics. **World Aquaculture**, v. 43, p. 57-59, 2012.

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*, EUA, v.47, n.11, p.3432-3444, 2016.

RIOS DA SILVA, K. Dinâmica do nitrogênio e do fósforo no cultivo superintensivo de *Litopenaeus vannamei* e *Farfantepenaeus paulensis* sem renovação de água. 2009. 68p. **Dissertação (Mestrado)** - Universidade Federal do Rio Grande (PPGAq-FURG).

ROMANO, N. Aquamimicry: a revolutionary concept for shrimp farming. **Global Aquaculture Advocate**, 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.

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*, Dinamarca, v.12, n.5, p.513-523. 2016.

SCHVEITZER, R.; ARANTES, R.; COSTÓDIO, P.F.S.; ESPÍRITO SANTO, C.M.; ARANA, L.V.; SEIFFERT, W.Q.; ANDREATTA, E.R. Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. **Aquacultural Engineering**, v. 56, n. 1, p. 59-70, 2013.

SOTO-RODRIGUEZ, S.A.; GOMEZ-GIL, B.; LOZANO, R.; RIO-RODRÍGUEZ, R.; DIEGUEZ, A.L.; ROMALDE, J.L. Virulence of *Vibrio harveyi* responsible for the “Bright-red” Syndrome in the Pacific white shrimp *Litopenaeus vannamei*. **Journal of Invertebrate Pathology**, v. 109, n. 3, p. 307-317, 2012.

TAW, N. Recent developments in biofloc technology - Biosecure systems improve economics, sustainability. **Global Aquaculture Advocate**, v. 15, n. 5, p. 28-29, 2012.

TORZILLO, G.; CHINI ZITTELLI, G.; 2015 Tubular Photobioreactors. In: Prokop A., Bajpai, R., Zappi, M. (Eds.), *Algal Biorefineries vol. 2*. Springer International Publishing, Cham, pp.187-212. <https://doi.org/10.1007/978-3-319-20200-6>.

VERBRUGGEN, B.; BICKLEY, L.K.;VAN, A.R.;BATEMAN, K.S.;STENTIFORD, G.D.;SANTOS, E.M.;TYLER, C.R. Molecular mechanisms of white spot syndrome virus infection and perspectives on treatments. **Viruses**, v.8, n.1, p.1–29, 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.

ZHANG, X.; YAN, B.; BAI, X.; BI, K.; GAO, H.; QIN, G. Isolation and characterization of *Vibrio parahaemolyticus* and *Vibrio rotiferianus* associated with mass mortality of chinese shrimp (*Fenneropenaeus chinensis*). **Journal Shellfish Research**, v. 33, n. 1, p. 61-68, 2014.