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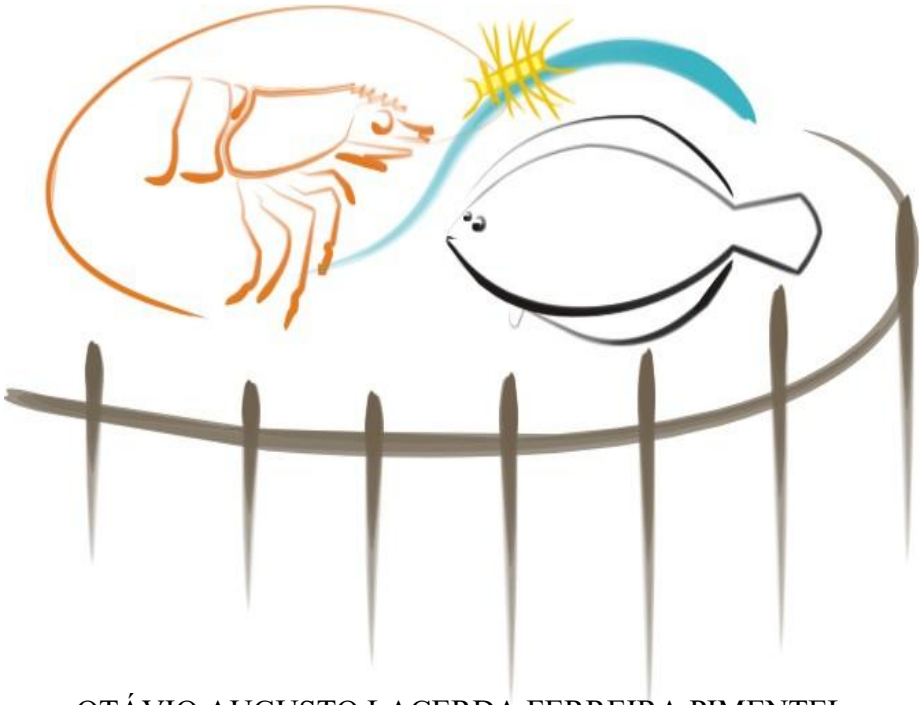
PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA



OTÁVIO AUGUSTO LACERDA FERREIRA PIMENTEL

ESTRATÉGIAS DE FERTILIZAÇÃO PARA O CULTIVO INTENSIVO DE *Penaeus vannamei* COM SISTEMA SIMBIÓTICO

RIO GRANDE, RS

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Discente: Otávio Augusto Lacerda Ferreira Pimentel

Orientador: Prof. Dr. Dariano Krummenauer

Coorientador: Prof. Dr. Wilson Wasielesky Jr.

Tese apresentada como parte dos requisitos para obtenção do grau de Doutor em Aquicultura no Programa de Pós-Graduação em Aquicultura da Universidade Federal do Rio Grande - FURG.

RIO GRANDE, RS

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ATA DE APROVAÇÃO

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ATA DE DEFESA DA 90 ª TESE DE DOUTORADO EM AQUICULTURA
No dia dezessete de fevereiro de dois mil e vinte e cinco, às quatorze horas, reuniu-se a Banca Examinadora de Tese de Doutorado em Aquicultura, de **OTAVIO AUGUSTO LACERDA FERREIRA PIMENTEL**, orientado pelo Prof. Dr. Dariano Krummenauer, composta pelos seguintes membros: Prof. Dr. Dariano Krummenauer (Orientador – IO/FURG), Prof. Dra Wilson Wasiekesky Jr.. (Co-Orientador – IO/FURG), Prof. Dr. Silvio Peixoto (UFRPE), Prof. Dr. Luis Otavio Brito da Silva (UFRPE) e Prof. Dr. Luis Poersch (PPGAq/FURG) Título da Tese: **"ESTRATÉGIAS DE FERTILIZAÇÃO PARA O CULTIVO INTENSIVO DE *Penaeus vannamei* COM SISTEMA SIMBIÓTICO".** Dando início à defesa, o Coordenador do PPGAq Prof. Dr. Ricardo Vieira Rodrigues, passou a presidência da sessão ao Prof. Dr. Dariano Krummenauer, que na qualidade de orientador, passou a palavra para o candidato apresentar a tese. Após ampla discussão entre os membros da Banca e o candidato, a Banca se reuniu sob a presidência do Coordenador. Durante esse encontro ficou estabelecido que as sugestões dos membros da Banca Examinadora devem ser incorporadas na versão final da Dissertação, ficando a cargo do Orientador o cumprimento desta decisão. O candidato **OTÁVIO AUGUSTO FERREIRA PIMENTEL** foi considerado **APROVADO**, devendo a versão definitiva da Tese ser entregue a Secretaria do PPGAq, no prazo estabelecido nas Normas Complementares do Programa. Nada mais havendo a tratar, foi lavrada a presente ata, que após lida e aprovada, será assinada pela Banca Examinadora, pelo candidato e pelo Coordenador do PPGAq.

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ÍNDICE

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RESUMO

Esta tese teve como objetivo analisar o efeito de diferentes estratégias de fertilização do sistema simbiótico sobre a qualidade de água, desenvolvimento da comunidade microbiana e crescimento do camarão *Penaeus vannamei* cultivados em alta densidade de estocagem. O primeiro estudo revisou os diferentes protocolos de fertilização usados no sistema simbiótico e o que vem sendo realizado pela pesquisa para melhorar o manejo desse sistema. O segundo estudo testou o efeito do uso de diferentes farelos vegetais como fonte de carbono orgânico para a fertilização do sistema simbiótico no processo de nitrificação, composição planctônica e crescimento do *P. vannamei* na fase de berçário, bem como compará-lo com o sistema de bioflocos. Este estudo recomendou do uso do farelo de arroz na fertilização do sistema simbiótico, uma vez que ele promoveu um crescimento do camarão *P. vannamei* semelhante aos sistemas de bioflocos e de água clara e superior ao que foi encontrado para o simbiótico fertilizado com farelo de trigo. O farelo de arroz ainda produziu de uma alta carga de microrganismos no sistema, principalmente *Bacillus*. O terceiro estudo avaliou o efeito dos processos de fermentação e respiração, bem como diferentes tempos de processamento do fertilizante do sistema simbiótico, no processo de nitrificação, composição do plâncton e crescimento do *P. vannamei* durante a fase de berçário e comparou esses resultados com o sistema de bioflocos. Neste estudo, o processamento do farelo de arroz por uma fase de fermentação por 12 horas seguido de uma fase de respiração microbiana por 12 horas demonstrou ser a mais eficaz, pois foi capaz de controlar mais rápido a amônia total, acelerou o desenvolvimento da alça microbiana, aumentou a produtividade e reduziu o fator de conversão alimentar do *P. vannamei* quando comparado ao tratamento controle, que usou água clara. O quarto estudo avaliou o efeito do uso de diferentes microrganismos probióticos na fertilização do sistema simbiótico sobre a composição da comunidade microbiana do sistema, processo de nitrificação e crescimento de *P. vannamei* na fase de berçário. Este estudo concluiu que o uso de probióticos compostos de microrganismos do gênero *Bacillus*, *Lactobacillus* e *Pediococcus* na composição do fertilizante proporcionou um controle mais rápido da amônia, maior abundância de bactérias oxidantes de amônia e oxidantes de nitrito, menor abundância de *Vibrio*, maior peso final do *P. vannamei*, maior produtividade e menor fator de conversão alimentar. O quinto estudo avaliou o efeito de diferentes sistemas de cultivo na qualidade da água, composição planctônica e crescimento do *P. vannamei* em água de baixa salinidade e alta densidade de estocagem. Os resultados mostraram que o sistema simbiótico melhorou o controle dos compostos nitrogenados, além de proporcionar o crescimento de mais microrganismos protozoários como ciliados e amebas. Esses resultados foram refletidos em uma maior sobrevivência, produtividade e menor fator de conversão alimentar do camarão.

Palavras-chave: farelo de arroz; fermentação; probióticos; *Bacillus*; RAS; bioflocos.

ABSTRACT

This thesis aimed to analyze the effect of different fertilization strategies of the synbiotic system on water quality, microbial community development, and growth of shrimp *Penaeus vannamei* reared at high stocking density. The first study reviewed different fertilization protocols used in the synbiotic system and what research has been done to improve the management of this system. The second study tested the effect of using different vegetable brans as an organic carbon source for the synbiotic fertilization on the nitrification process, planktonic composition, and growth of *P. vannamei* in the nursery phase, as well as comparing it with the biofloc system. This study recommended the use of rice bran in the synbiotic fertilization, since it promoted growth of shrimp *P. vannamei* similar to the biofloc and clear water systems and superior to what was found for the synbiotic fertilized with wheat bran. The rice bran still produced a high load of microorganisms in the system, mainly *Bacillus*. The third study evaluated the effect of fermentation and respiration processes, as well as different processing times of the synbiotic system fertilizer, on the nitrification process, plankton composition, and growth of *P. vannamei* during the nursery phase and compared these results with the biofloc system. In this study, processing rice bran through a fermentation phase for 12 hours followed by a microbial respiration phase for 12 hours proved to be the most effective, as it was able to control total ammonia faster, accelerating microbial loop development, increased yield, and reduced the feed conversion ratio of *P. vannamei* when compared to the control treatment, which used clear water. The fourth study evaluated the effect of using different probiotic microorganisms in the fertilization of the synbiotic system on the microbial community composition of the system, nitrification process, and growth of *P. vannamei* in the nursery phase. This study concluded that the use of probiotics composed of microorganisms of the genera *Bacillus*, *Lactobacillus*, and *Pediococcus* in the composition of the fertilizer provided faster control of ammonia, higher abundance of ammonia-oxidizing and nitrite-oxidizing bacteria, lower abundance of *Vibrio*, higher final weight of *P. vannamei*, higher yield, and lower feed conversion ratio. The fifth study evaluated the effect of different culture systems on water quality, planktonic composition, and growth of *P. vannamei* in low salinity water and high stocking density. The results showed that the synbiotic system improved the control of nitrogenous compounds, in addition to promoting the growth of more protozoan microorganisms such as ciliates and amoebas. These results were reflected in higher survival, yield, and lower feed conversion ratio of shrimp.

Keywords: rice bran; fermentation; probiotics; *Bacillus*; RAS; biofloc.

ESTRUTURA DA TESE

Esta tese está estruturada em cinco capítulos, os quais estão apresentados no formato de artigos científicos. No capítulo I, que é a introdução geral, foi descrito no formato de revisão os diferentes protocolos de fertilização usados no sistema simbióticos para o cultivo intensivo do *Penaeus vannamei*. Neste estudo foi demonstrada a importância de cada componente do fertilizante, das diferentes estratégias de processamento do farelo vegetal e ainda foi recomendado temas de pesquisas que necessitam ser realizadas para melhorar o manejo desse sistema. Os capítulos seguintes são estudos que buscaram testar diferentes estratégias de fertilização do sistema simbiótico sobre diversos aspectos do cultivo intensivo do *P. vannamei.*

No capítulo II foram testados os efeitos do uso de diferentes farelos vegetais na fertilização do sistema simbiótico sobre o processo de nitrificação, composição da comunidade planctônica e crescimento do camarão *P. vannamei* na fase berçário. Este estudo também se propôs a comparar o sistema simbiótico com os sistemas de bioflocos e de água clara.

No capítulo III foi avaliado o efeito dos processos de fermentação (fase sem aeração) e respiração microbiana (fase com aeração), bem como diferentes tempos de processamento do fertilizante do sistema simbiótico no processo de nitrificação, composição da comunidade planctônica e crescimento do *P. vannamei* na fase de berçário. Além do mais, este estudo comparou os resultados dos tratamentos que usaram sistema simbiótico com o sistema de bioflocos e de água clara.

O capítulo IV foi dedicado a avaliação do efeito da inoculação de diferentes microrganismos probióticos na fertilização do sistema simbiótico sobre a composição microbiana do sistema, processo de nitrificação e crescimento do *P. vannamei* na fase de berçário. Neste estudo, o sistema de água clara foi usado como tratamento controle.

No capítulo V, foi avaliado os efeitos dos sistemas de recirculação (RAS), bioflocos (BFT) e simbiótico sobre a composição da comunidade planctônica e crescimento do *P. vannamei* em cultivos superintensivos com água de baixa salinidade. Neste estudo, foi usada uma estratégia de maturação do sistema simbiótico baseada nos resultados encontrados nos capítulos II, III e IV desta tese e os resultados foram comparados com os dos sistemas RAS e BFT, que também foram previamente maturados.

CAPÍTULO I - INTRODUÇÃO GERAL

The fertilization of synbiotic systems in the intensive culture of *Penaeus vannamei*

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A fertilização de sistemas simbióticos no cultivo intensivo de *Penaeus vannamei*

Resumo expandido

Ao longo dos anos, a intensificação da produção do camarão *Penaeus vannamei* trouxe desafios como surtos de doenças e aumento na carga de nutrientes nos efluentes. Buscando maior sustentabilidade à medida que os sistemas de produção se intensificaram, tornou-se necessário criar sistemas com elevado grau de biossegurança e eficientes no uso da água e do solo. Assim, sistemas microbianos, como o de bioflocos, foram pioneiros para apoiar o crescimento da indústria e a demanda do mercado por camarão. Atualmente, uma derivação do biofloco surgiu e vem ganhando espaço na indústria do camarão, que é o sistema simbiótico. Os sistemas simbióticos são caracterizados pela fertilização da água de cultivo com farelos vegetais (prebióticos) processados por fermentação e/ou respiração microbiana por microrganismos probióticos. O farelo processado funciona como uma fonte de carbono orgânico que vai estimular a assimilação do nitrogênio amoniacal pelas bactérias heterotróficas. Além do mais, esse sistema permite o desenvolvimento da comunidade de bactérias quimioautotróficas, que são responsáveis pela oxidação da amônia e do nitrito para nitrato. O simbiótico cria condições semelhantes às de um ambiente natural, apoiando o desenvolvimento da alça microbiana, controlando a qualidade da água e suprimindo microrganismos nocivos e patogénicos. Por ser um sistema novo, o simbiótico não apresenta uma padronização dos protocolos de fertilização, o que leva a resultados diferentes entre pesquisas laboratoriais e fazendas comerciais. Dessa forma, esta revisão teve como foco descrever os diferentes procedimentos de fertilização utilizados no sistema simbiótico para o cultivo intensivo de *P. vannamei*, a importância de cada componente do fertilizante, bem como as estratégias de processamento. Além disso, neste estudo foram demonstradas perspectivas e recomendações de temas de pesquisas futuras para melhor compreender as vantagens e desvantagens deste sistema de produção. O fertilizante do sistema simbiótico é composto de farelos vegetais (e.g., arroz, trigo ou soja), microrganismos probióticos (e.g., *Bacillus*, *Lactobacillus*, *Pediococcus* e *Saccharomyces*), tampão (i.e., bicarbonato de sódio), uma fonte de carbono orgânico simples (e.g., melaço ou dextrose) e água na razão de 10:1 (volume de água (L):peso de farelo vegetal (Kg)). A concentração de farelo vegetal é calculada de acordo com o volume do tanque de cultivo e ela pode mudar de acordo com as condições de qualidade de água e fase de cultivo. A concentração de

farelo mais usada em cultivos experimentais na fase de berçário é 20 g m^{-3}. Na fase de engorda, a concentração de 10 g m^{-3} geralmente é empregada. Os microrganismos probióticos são usados para quebrar compostos complexos presentes no farelo, como carboidratos fibras e celulose. Essa quebra aumenta a disponibilidade de nutrientes no meio de cultivo. A proporção de probióticos no fertilizante simbiótico pode variar entre 0,2 e 2,5% da quantidade de farelo vegetal. A fonte de carbono simples é usada para estimular a atividade dos microrganismos a iniciar a degradação dos farelos pelos processos de fermentação e respiração microbiana. A proporção da fonte de carbono simples no fertilizante simbiótico é de 10% da quantidade de farelo vegetal. O processamento do farelo é realizado pelos processos de fermentação (fase anaeróbica) e respiração microbiana (fase aeróbica). A fermentação é um processo catabólico anaeróbico onde mudanças químicas são realizadas em um substrato orgânico por meio de enzimas produzidas por microrganismos. Essa fase pode durar entre 12 e 48 horas. A respiração microbiana é um processo aeróbico onde substratos orgânicos reduzidos são oxidados por meio da atividade microbiana, liberando energia, consumindo oxigênio e produzindo dióxido de carbono. Essa fase pode durar entre 12 e 24 horas. O processamento do farelo é realizado em meio aquoso, ocorrendo a produção de dióxido de carbono e secreção de ácidos orgânicos. Dessa forma, ocorre uma redução do pH do meio, o que pode comprometer a atividade das bactérias probióticas. A atividade desses microrganismos ocorre em uma faixa de pH que vai de 4.0 a 9.1. Dessa forma, um agente tampão deve ser adicionado ao meio para estabilizar o pH do meio. A proporção do tampão no fertilizante simbiótico é de 10% da quantidade de farelo vegetal. Em cultivos experimentais, o sistema simbiótico tem demonstrado bons resultados de crescimento, sobrevivência, controle dos compostos nitrogenados, controle de bactérias patogênicas e apresentando alta abundância de zooplâncton. Entretanto, esses estudos apresentam uma alta variabilidade nas estratégias de fertilização, com diferentes tipos de farelos vegetais, diferentes fases e períodos de processamento e uso de diferentes microrganismos probióticos. Essa falta de consenso sobre as estratégias de fertilização é uma lacuna que precisa ser preenchida para melhorar as estratégias de manejo de fertilização e da qualidade de água no sistema simbiótico. Recentemente, esforços têm sido realizados para guiar os regimes de fertilização de cultivos intensivos de *P. vannamei* com sistema simbiótico. O primeiro passo foi determinar o melhor farelo vegetal para a fertilização do simbiótico. Os farelos de arroz, trigo e soja foram testados em berçários intensivos de *P. vannamei* e comparados com o sistema de bioflocos e de água clara. Os resultados demonstraram que o farelo de arroz proporcionou

resultados de crescimento e produtividade semelhantes ao encontrado para os sistemas de bioflocos. Além do mais, o farelo de arroz proporcionou o crescimento de uma elevada carga de microrganismos, principalmente de bactérias *Bacillus* e reduziu a abundância de *Vibrio* quando comparado ao simbiótico usando farelo de trigo. Outro passo importante foi o estabelecimento da melhor estratégia de processamento do farelo de arroz. Os processos de fermentação e respiração microbiana determinam a quantidade de carbono orgânico lábil no sistema, o que tem efeitos significativos sobre o controle dos compostos nitrogenados e desenvolvimento da alça microbiana. Dessa forma, pesquisadores encontraram que o processamento do fertilizante simbiótico por um período de 12 horas fermentação somado a um período de 12h de respiração microbiana foi a melhor estratégia para o controle do nitrogênio amoniacal total. Ainda, essa estratégia proporcionou uma maior produtividade e menor fator de conversão alimentar do que o tratamento controle, que usou um sistema de água clara. Um aspecto importante para a fertilização do sistema simbiótico está na composição dos microrganismos probióticos que são inoculados no fertilizante. O uso de probióticos comerciais composto por cepas com um único gênero de bactéria (*Bacillus*) contra o uso de misturas de vários probióticos (*Bacillus*, *Lactobacillus* e *Pediococcus*) foi testado em berçários intensivos de *P. vannamei*. Além do mais, a inserção da levedura *Saccharomyces cerevisiae* no fertilizante simbiótico foi testada tanto com os *Bacillus* quanto com a mistura de *Bacillus*, *Lactobacillus* e *Pediococcus*. Os resultados demonstraram que o crescimento e a eficiência alimentar do camarão foram melhores no tratamento onde a mistura de bactérias foi usada. Além disso, o uso de *Bacillus*, *Lactobacillus* e *Pediococcus* proporcionou um melhor controle da amônia, maior abundância de bactérias oxidantes de amônia e nitrito e menor abundância de *Vibrio*. Além do mais, os resultados demonstraram que a inoculação da levedura retardou o processo de nitrificação. Finalmente, um aspecto essencial é o processo de maturação da água, que pode ter efeitos significativos sobre o controle dos compostos nitrogenados, principalmente quando se trata de cultivos com água de baixa salinidade. O cultivo superintensivo do *P. vannamei* e foi testado com os sistemas de recirculação, simbiótico, bioflocos em água com salinidade 2 g L^{-1}. No tratamento simbiótico, os autores empregaram uma estratégia de maturação da água com 16 fertilizações diárias de farelo de arroz processado somada a aplicações diárias de 1 mg L^{-1} de cloreto de amônio como uma fonte de nitrogênio inorgânico. Isso ajudou no estabelecimento das bactérias nitrificantes no sistema, o que levou a melhores resultados de controle dos compostos nitrogenados no simbiótico do que no sistema de bioflocos. Os resultados

encontrados neste estudo de revisão demonstraram que o sistema simbiótico refina e diversifica as estratégias de fertilização de cultivos intensivos de *P. vannamei* devido aos seus efeitos positivos sobre a qualidade de água, composição microbiana controle de microrganismos nocivos como Cianobactérias e *Vibrio* e melhoram o crescimento do camarão. Esforços tem sido feito para testar diferentes protocolos de fertilização para esse sistema, entregando resultados práticos para o setor produtivo. Entretanto, isso ainda não é suficiente e estudos ainda necessitam ser realizados para otimizar o processo de produção de camarão com sistema simbiótico. Uso de farelos desengordurados, efeitos das fertilizações iniciais, uso de inoculo, influência de diferentes níveis de sólidos suspensos totais e uso de substratos artificiais são temas que necessitam ser explorados para um melhor manejo do sistema, com maior sustentabilidade.

The fertilization of synbiotic systems in the intensive culture of *Penaeus vannamei*

Abstract

Over the years, the intensification of shrimp *Penaeus vannamei* production has brought challenges such as disease outbreaks and increases in the nutrient loading of effluents. To be more environmentally friendly as production systems intensified, it became necessary to create systems with a high degree of biosecurity and towards efficiency in water and land use. Thus, microbial-based systems, such as the biofloc, were pioneered to support industry growth and market demand for shrimp. Currently, a derivation of biofloc has emerged and is gaining ground in the shrimp industry, which is the synbiotic system. Synbiotic systems are microbially based and are characterized by fertilizing the culture water with vegetable bran (prebiotics) that were processed by fermentation and/or microbial respiration by probiotic microorganisms. The fertilization strategy creates conditions similar to a natural environment by supporting microbial loop development, controlling water quality, and suppressing harmful, and pathogenic microorganisms. The synbiotic system is still new and without standard fertilization protocols, which has leads to dissimilar results between laboratory research and commercial farms. Therefore, this review is focused on describing the different fertilization procedures used in the synbiotic system for *P. vannamei* intensive culture, the importance of each fertilizer component, as well as the processing strategies. Furthermore, in this study we seek to demonstrate perspectives and recommend future research topics to better understand the advantages and disadvantages of this production system.

Keywords: organic carbon; fermentation; microbial respiration; probiotic; nitrogenous compounds; rice bran.

1. Introduction

Aquaculture is the farming of aquatic organisms and has surpassed overall wild fisheries harvests in seafood production [1]. As a result, aquaculture is now playing an ever more important and pivotal role in helping to ensure global food security. Of all groups of organisms produced by aquaculture, crustaceans are the third largest group, representing 13.5 % of total production [1]. Among crustaceans, the shrimp *Penaeus vannamei* is the most produced species, reaching more than 50% of total crustacean aquaculture production in 2022 [1]. The production of *P. vannamei* has spread throughout the world with varied water sources under different water quality conditions, using different culture technologies, stocking densities levels, and associated feed inputs [2].

There has been an increasing trend towards intensification of shrimp production, which has brought about new challenges such as increased transmission of infectious diseases, nutrient loads that can more rapidly deteriorate water quality, and concerns regarding the impact of aquaculture on the environment [3, 4]. This has subsequently brought to light the need to create new production systems that would make it possible for farmers to sustain the growth of the shrimp farming industry to meet increased market demands. Moving forward, culture systems should be more controlled, have excellent biosecurity, and enable higher efficiency in the use of water and land to provide increased productivity with environmental sustainability. These factors were important for the eventual development of a specific type of microbial-based system that is characterized by the manipulation of microorganisms to achieve greater control of water quality for successful culture [4].

In this new microbial-based system, Avnimelech [5] demonstrated that by elevating the carbon:nitrogen (C:N) ratio of water through the addition of a simple organic carbon source effectively controlled the total ammonia nitrogen (TAN) by the rise in the activity and abundance of heterotrophic bacteria. This bacterial group assimilates TAN and uses it to produce bacterial biomass, thus effectively immobilizing this compound that would otherwise be toxic to shrimp [3, 6]. The elevation of the C:N ratio served as the basis for the development of the biofloc system [7], which today is the most widely used microbial-based system for intensive shrimp culture. The recycling of TAN into microbial biomass maintains water quality and provides a consistent supply of natural food for shrimp [8]. Generally, the higher C:N ratios increase the abundance of heterotrophs, but this ratio often varies between 10 and 20:1 in practice [9]. While heterotrophs tend

to dominant at higher C:N ratios, there is generally still nitrifying bacteria that convert TAN to nitrite (NO_2^-), and finally to nitrate (NO_3^-) [6].

In biofloc-based systems, sugar cane molasses, dextrose, and vegetable brans are among the most widely used organic carbon sources to provide the energy to stimulate heterotrophic growth and creation of bioflocs [10, 11]. Carbon is around 40% in most organic carbon sources while N is calculated based on how much protein is added into the system after making several key assumptions. Generally, the choice of carbon is based on availability and cost. For example, molasses is often preferred in sugar cane producing countries such as Brazil, while rice bran (a by-product of rice production) is commonly used in Asia. Simple sugars dissolve more easily in water, and are thus readily available to bacteria, while vegetable brans are often processed via fermentation (phase without oxygen) or microbial respiration (phase with oxygen) by probiotic microorganisms to reduce cellulose content and improve solubility [12]. Based on the use of vegetable bran, recently, a new microbial-based shrimp culture system has been used both in the nursery and grow-out phase of *P. vannamei* production. The microbial processing of vegetable brans gave rise to the "synbiotic system", which can improve water quality (i.e., ammonia and nitrite control) and shrimp growth under culture conditions [13]. Some characteristics of this system differ from biofloc, as described by Khanjani et al. [14], with conservative stocking densities (between 150 and 300 shrimp m^{-2}), no control of the C:N ratio in the water, and the main organic carbon source supplemented is not soluble as in bioflocs, they are grain by-products. While the synbiotic system does involve supplementation of brans to the culture system, it also differs from the "nutritious pond concept", which is a production strategy that involves the use of unprocessed brans as a food source for the animals in combination with commercial feed [15]. In the synbiotic system, bran and microbial aggregates grown in the medium serve as supplementary food sources for shrimp. Despite the success and adoption of the synbiotic system by some commercial producers, recent research has revealed that the fertilization process in this new production system needs to be better understood. This includes optimizing fermentation methods and application of processed vegetable bran.

The culture of *P. vannamei* using the synbiotic system is still new. Since there is no standard fertilization protocol yet for this system, dissimilar results performance may occur among research laboratories and commercial farming operations employing this production technique.

Therefore, this review is focused on describing the different fertilization procedures used in the synbiotic system for *P. vannamei* culture, the importance of each fertilizer component, as well as processing strategies. Elucidation of these aspects may be important for the development of a standard protocol for the culture of shrimp in the synbiotic system. Furthermore, we seek to provide perspectives and recommend future directions for research to better understand how this system can maintain optimal water quality, which is becoming increasingly important as the shrimp industry continues to expand and is heading towards greater intensification.

2. Synbiotic system

A synbiotic system is established when probiotics and prebiotics are added to the system and exert a synergy on water quality and production/health of the host [14]. Unlike biofloc systems, in which various organic carbon sources can be added, synbiotic systems are created when a vegetable bran (prebiotic) is processed with probiotics before being added to the culture system [14]. Thus, there is an addition of both prebiotics and probiotics throughout the production cycle. Another distinction from traditional biofloc systems is that the processed bran does not depend on the proportion of nitrogen input [16]. However, vegetable bran, in addition to contributing to the supplementation of organic carbon, also contains nitrogen in its composition from protein [17] as well as various other compounds that supply microorganisms with essential nutrients for growth. Thus, the contribution of the vegetable bran (prebiotic) and probiotics provides the basis of synbiotics. This allows for stimulation of microbial growth and maintains water quality in suitable ranges of pH, TAN, and NO_2^- [13]. Additionally, the nitrification process was observed in *P. vannamei* intensive nurseries and grow-out with synbiotic systems [18, 19].

This fertilization strategy creates a turbid environment similar to the natural environment of estuaries where shrimp often spend the early part of their lives. This is achieved by creation of a microbial loop and ecosystem that supports the growth and maintenance of high loads of microorganisms including phytoplankton, zooplankton, and bacteria [19]. These various microorganisms in the synbiotic system play a key role not only in controlling water quality, but also in controlling pathogenic microorganisms, such as bacteria from the genus *Vibrio* [20]. This occurs due to the probiotic characteristics of the bacteria used to process the vegetable bran, such as *Bacillus*, *Lactobacillus*, and *Saccharomyces cerevisiae* that produce antimicrobial substances, compete for nutrients and space with other pathogenic microorganisms and, in some cases, quench

quorum sensing among pathogenic bacteria [21]. Moreover, the microbial growth in the synbiotic system creates microbial aggregates which act as a constant supply of dietary supplements for cultivated shrimp. Such availability of supplemental nutrition can improve shrimp growth and health because they not only contain macronutrients, but also can contain bioactive ones [22, 23].

Another factor that can be considered an advantage of the synbiotic system is the contribution of processed vegetable bran as fertilizer, which can also be consumed by shrimp [14]. These processed vegetable bran grains have more available nutrients after the fermentation and microbial respiration processes [24]. Furthermore, microorganisms colonize bran grains and, when ingested, can colonize the shrimp gut, improving feed digestibility, growth, and disease resistance [14]. The greater digestibility of natural foods is called the synergistic digestibility effect and has already been demonstrated in carp by Roy et al. [25].

3. Components of the synbiotic system fertilizer

The main actors in the synbiotic fertilization process are vegetable brans, which are byproducts of the processing of grains such as rice, wheat, and soybeans that are generally low cost and highly available because they do not compete with human nutrition [26]. The amount of vegetable bran to add is often calculated according to the volume of the culture tank. This may change depending on the water quality of the system and phase of culture. For example, a concentration of 20 g m^{-3} is generally used in *P. vannamei* experimental intensive nurseries using a synbiotic system, but it can reach up to 50 g m^{-3} [18, 27-30]. In the grow-out phase, a concentration of 10 g m^{-3} is generally used [31, 32]. The concentration of bran can be adjusted according to the increase and reduction of the stocking density used in the different phases of shrimp culture.

One of the most used vegetable bran sources in synbiotic systems is rice bran, which is a by-product of milling paddy rice and represents approximately 10% of the whole grain [33]. In this process, the outer layer of brown rice is separated and imparts several benefits as a prebiotic and carbon source in synbiotic systems. Rice bran can have a carbohydrate percentage of up to 50%, with a relatively high fiber content (Table 1). For example, in contrast to processed sugar, rice bran has several beneficial vitamins, tocols, and oryzanols, which are compounds that have high antioxidant activity [34]. On the other hand, rice bran has a relatively high fiber content that is mostly insoluble (i.e., lignin and cellulose) [35, 36]. Furthermore, some anti-nutritional factors are present,

such as phytic acid, which can reduce its nutritional value for shrimp. However, fermentation and microbial respiration can substantially reduce these levels [37], and moreover, unlock the encapsulated nutrients for both bacteria and shrimp. It should be noted that the composition of rice bran can vary according to the type of cultivar, climatic conditions, and rice processing method [38]. A disadvantage of this type of bran is the relatively high content of lipid (Table 1) with an elevated activity of lipases and lipoxygenases, which makes it prone to rancidity [39]. Solutions to this issue include the addition of antioxidants and/or defatting to create a more stable product as well as storage in cool dry areas [39].

Wheat bran is obtained from grinding wheat and represents 13 to 19% of the total weight of the grain [40]. It has a high fiber content, which can vary between 33.4% and 63% (Table 1) and more than 90% of the fiber found in wheat bran are insoluble, such as cellulose and lignin [43]. Soluble fibers are also present, such as β-glucan, which can vary between 0.5 and 1.5% [43]. The carbohydrate content can reach 75%, which is higher than what is generally found in rice bran and can be an advantage for this type of bran (Table 1). Furthermore, wheat bran contains compounds such as vitamin B6 and E, and antioxidants such as phenolic compounds [44].

Soybean bran (also known as soybean dregs) is a byproduct obtained from grinding soybeans. This bran has a higher protein content than found in other types of bran, which can exceed 30% (Table 1). The fiber content present in soybean bran is lower than that found in rice and wheat bran (Table 1), but like the other brans, it is mostly composed of lignins and cellulose [45]. A key factor in soybean bran is that they contain many essential and non-essential amino acids, such as glutamic acid, aspartic acid, arginine, alanine, leucine, lysine, and isoleucine [46] that can contribute to shrimp nutrition. Some of the antinutritional factors present in soybean bran include trypsin inhibitors, phytic acid, and tannins that are in high enough levels that they can even be toxic to some livestock [47]. Thus, fermenting soybean products is an old and common process to improve their nutrient availability by both digesting proteins to peptides as well as reducing various antinutritional factors [48]. A common thread among all the types of vegetable brans used as fertilizers in the synbiotic system are insoluble fibers, but these are substantially reduced via microbial processing [49].

Table 1. The nutritional composition of the primary vegetable bran used in the synbiotic system.

Vegetable bran	Protein (%)	Lipids (%)	Carbohydrates (%)	Fibers (%)	Reference
Rice	13	21	50	21	38
Wheat	9.6 – 18.6	3.5	60 – 75	33.4 – 63	40, 41
Soybean	34	22.3	30	6.6	42

The incorporation of probiotic microorganisms is essential in synbiotic fertilization, since they act in the processing of vegetable bran before they are applied to the system. The main probiotics inoculated in synbiotic production systems are *Bacillus*, *Lactobacillus*, *Pseudomonas*, *Pediococcus*, and *Saccharomyces cerevisiae* [14, 20]. These microorganisms act to degrade complex compounds that subsequently increase nutrient availability [50]. Additionally, probiotics perform competitive exclusion, which is a phenomenon that helps control the presence of pathogenic microorganisms in the environment [51]. Furthermore, these microorganisms, such as *Bacillus*, have several mechanisms that can modulate water quality conditions, such as mineralization of organic matter, thus reducing the buildup of sludge that can serve as habitats for pathogens, for example *Vibrio* bacteria [52]. The amount of probiotic inoculated in synbiotic fertilizers can range between 0.2 and 2.5% of the amount of vegetable bran (Table 2). Another component that can be added to improve the processing of synbiotic fertilizers are commercial enzymes. These proteins are characterized by accelerating the speed of reactions, without affecting their balance during fertilizer processing [53]. The amount of enzyme present in the fertilizer is also based on the amount of bran and can vary between 0.1 and 0.2% of the amount of vegetable bran (Table 2).

Table 2. Main inputs and their percentages in relation to vegetable bran used as a fertilization protocol for the synbiotic system.

Input	Percentage quantity (%)
Vegetable bran	100
Molasses, sugar or dextrose	10
Buffer	10
Probiotic	0.2 – 2.5
Enzyme	0.1 – 0.2

Clean water (v:w)	10:1

Source: Adapted from Pereira et al. [54]. v:w: volume of clean water (L): rice bran weight (Kg) ratio.

Vegetable bran processing is conducted by two distinct but complementary processes that include fermentation and microbial respiration. Fermentation is an anaerobic catabolic process in which chemical changes (i.e., degradation of complex compounds) are performed on an organic substrate through enzymes produced by microorganisms [55]. At this stage, the fertilizer is kept for a period without aeration, typically between 12h and 48h, to induce fermentation [14] (Figure 1a). On the other hand, microbial cellular respiration is an aerobic process where reduced organic substrates are oxidized through microbial activity to release energy, consuming oxygen, and producing carbon dioxide [56]. In this phase, the fertilizer is maintained with oxygen, which can be as simple as an air stone, for a period that can vary between 12h and 24h [14] (Figure 1b, and c). Molasses, sugar or dextrose can be used as a simple carbon source to stimulate the activity of microorganisms that kickstart bran degradation by both fermentation and microbial respiration. This entire process takes place in an aqueous environment (Figure 1), which should reflect the salinity of the culture medium to help ensure the survivability of the probiotics. The amount of water can vary between 2 and 10 times the amount of bran [14]. This is a topic which requires further investigation to optimize. Both fermentation and respiration solubilize the complex compounds in vegetable bran, improving their protein, and lipid content [24, 57]. For example, Ribeiro et al. [58] tested the solid fermentation of rice bran with the fungus *Rhizopus oryzae* and found an increase in the content of both proteins and lipids throughout the fermentation period. Similarly, Romano et al. [24] reported a higher increase in protein and lipid content in rice bran subjected to the process of microbial respiration by *Bacillus* spp. in a liquid medium compared to rice bran fermented with the same probiotic.

Since these processes in the vegetable bran involve the production of carbon dioxide, as well as the secretion of organic acids and absorption of basic amino acids, a decrease in pH of the medium is observed. This can potentially compromise the viability and effectiveness of the bacteria because they all have an optimal pH for activity and growth (Table 3). To help counteract this issue, a buffering agent is often added to the fertilizer to stabilize the pH. The most used buffer is sodium bicarbonate, and it can be used at a proportion of 10% of the amount of vegetable bran

to maintain a pH above 4.5, which is adequate for maintaining the activity of most probiotic microorganisms [63] (Table 3).

Table 3. pH range for the growth of the main probiotic microorganisms used in the synbiotic system.

Probiotic microorganism	pH range	Reference
Bacillus subtilis	4.9 – 9.1	59
Lactobacillus sp.	5.5 – 6.2	60
Pediococcus sp.	6.0 – 6.5	61
Saccharomyces cerevisiae	4.0 – 6.0	62

Figure 1. Synbiotic system fertilizer in the fermentation phase located in the Northeast of Brazil (a), in the microbial respiration phase located in Thailand (b), and in the south of Brazil, at the Federal University of Rio Grande – FURG (c). Source: Luis Otavio Brito (a), Nicholas Romano (b), and Otávio Augusto L. F. Pimentel (c).

4. Fertilization strategies in the synbiotic system

Research on synbiotic systems in *P. vannamei* has been carried out mainly in their nursery phase [19, 20, 27-31, 64-66], and a few studies evaluating performance in the grow-out phase [18, 32, 67, 68]. These studies demonstrated suitable results in growth, survival, control of nitrogenous compounds throughout the culture period, reduction in the concentration of sucrose-negative bacteria, and high abundance of zooplankton [13, 27-29, 57, 65, 67-70]. However, these studies also have a high variability in the fertilization strategies, such as different types of vegetable brans, probiotic microorganisms, and bran processing phases (fermentation versus respiration) as well as processing duration (Table 4). Regarding the application of processed vegetable bran, it is generally applied directly to the culture water. In this system, studies generally prepare the water prior to stocking the shrimp with fertilizer applications that can range from 7 to 24 applications [30, 31]. During culture, there is a wide variation in the frequency of fertilizations, ranging from daily applications during the first fifteen days to applications at different weekly frequencies [18, 19, 32]. The lack of consensus on fertilization strategies is a gap that needs to be filled so that better management of fertilization and water quality in the synbiotic system is achievable.

Table 4. Water fertilization protocols for intensive culture of *Penaeus vannamei* with synbiotic system.

Phase	Stocking density	Vegetable bran (concentration used)	Probiotic strains (concentration used)	Enzyme	Simple carbon source (concentration used)	Buffer (concentration used)	Processing	v:w ratio	Reference
Nursery	3000 shrimp m^{-3}	Rice bran (20 to 10 g m^{-3})	*Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus* sp. (0.1 g m^{-3})	-	Molasses (1.5 to 2 g m^{-3})	Sodium bicarbonate (2 to 4 g m^{-3})	24h fermentation + 24h respiration	10:1	27
Nursery	2500 shrimp m^{-3}	Rice bran (20 g m^{-3})	*B. subtilis*, *B. licheniformis*, *Saccharomyces* sp., and *Pseudomonas* sp (0.05 g m^{-3})	-	Molasses (2 g m^{-3})	Sodium bicarbonate (4 g m^{-3})	24h fermentation + 24h respiration	10:1	29
Nursery	3000 shrimp m^{-3}	Rice bran (30 g m^{-3})	*B. subtilis, B. licheniformis,* *Saccharomyces* sp., and *Pseudomonas* (0.5 g m^{-3})	-	Molasses (3 g m^{-3})	Sodium bicarbonate (6 g m^{-3})	24h fermentation + 24h respiration	10:1	71
Nursery	3000 shrimp m^{-3}	Wheat bran (50 g m^{-3})	*Lactobacilus* sp., *B. subtilis,* and *B. licheniformis* (0.5 g m^{-3})	-	Molasses (25 g m^{-3})	Sodium bicarbonate (10 g m^{-3})	48h fermentation + 24h respiration	10:1	28
Nursery	3000 shrimp m^{-3}	Wheat bran (22.5 g m^{-3})	*B. subtilis*, *B. licheniformis*, *Saccharomyces* sp., and *Pseudomonas* sp. (0.5 g m^{-3})	-	Molasses (12 g m^{-3})	Sodium bicarbonate (4.5 g m^{-3})	48h fermentation + 24h respiration	10:1	65
Nursery	2000 shrimp m^{-3}	Rice bran (20 g m^{-3})	*B. subtilis, B. licheniformis,* and *Pseudomonas* sp. (0.5 g m^{-3}), and *Saccharomyces cerevisiae* (0.25 g m^{-3})	-	Sugar (2 g m^{-3})	Sodium bicarbonate (4 g m^{-3})	24h fermentation + 24h respiration	10:1	31
Nursery	2000 shrimp m^{-3}	Rice bran (20 g m^{-3})	*B. subtilis, B. licheniformis,* and *Bacillus* sp. (0.5 g m^{-3})	-	Molasses (2 g m^{-3})	Sodium bicarbonate (4 g m^{-3})	24h fermentation + 24h respiration	10:1	30
Nursery	1000 shrimp m^{-3}	Rice bran	*B. subtilis*	-	-	Sodium bicarbonate	24h fermentation	2:1	57

Nursery	2000 shrimp m^{-3}	Rice bran (20 g m^{-3})	*B. subtilis, B. licheniformis,* and *Bacillus* sp. (0.5 g m^{-3})	-	Sugar (2 g m^{-3})	Sodium bicarbonate (4 g m^{-3})	24h fermentation + 24h respiration	10:1	72
Nursery	2000 shrimp m^{-3}	Rice bran, wheat bran, and soybean bran (10 e 30 g m^{-3})	*B. subtilis* and *B. licheniformis* (0.05 and 0.15 g m^{-3})	-	Molasses (1 and 3 g m^{-3})	Sodium bicarbonate (1 and 3 g m^{-3})	24h fermentation + 24h respiration	10:1	19
Nursery	2000 shrimp m^{-3}	Rice bran (20 g m^{-3})	*B. subtilis, B. licheniformis, Lactobacillus plantarum,* and *Pediococcus acidilactici* (0.4 g m^{-3}), and *S cerevisiae* (0.2 g m^{-3})	-	Molasses (2 g m^{-3})	Sodium bicarbonate (2 g m^{-3})	12h fermentation + 12h respiration	10:1	20
Nursery	2400 shrimp m^{-3}	Rice bran (20 g m^{-3})	*B. subtilis* and *B. licheniformis* (0.2 g m^{-3})	-	Molasses (2 g m^{-3})	Sodium bicarbonate (2 g m^{-3})	12 to 24h fermentation, 12 to 24h respiration, 12 to 24h fermentation plus 12 to 24 h respiration	10:1	64
Nursery	2000 to 6000 shrimp m^{-3}	Rice bran (20 to 10 g m^{-3})	*B. subtilis*, *B. licheniformis*, *B. pumilus*, *L. plantarum*, *L. acidophilus*, and *S. cerevisiae* (0.5 g m^{-3})	-	Brown sugar (1 to 2 g m^{-3})	-	24h fermentation + 24h respiration	10:1	66
Grow-out	125 shrimp m^{-2}	Rice bran	*B. amyloliquefaciens*, *B. licheniformis*, *B. pumilus*, and *B. Subtilis*	β-xylanase	-	-	Overnight fermentation	10:1	23
Grow-out	300 shrimp m^{-3}	Rice bran (10 g m^{-3})	*B. subtilis*, *B. licheniformis*, and *Bacillus* sp. (0.05 g m^{-3})	-	Sugar (1 g m^{-3})	Sodium bicarbonate (2 g m^{-3})	24h fermentation + 24h respiration	10:1	18

Grow-out	416 shrimp m^{-3}	Rice bran (10 a 20 g m^{-3})	*Bacillus subtilis*, *B. licheniformis*, *Bacillus* sp., (0.05 and 0.5 g m^{-3})	-	Sugar (2 g m^{-3})	Sodium bicarbonate (1 to 4 g m^{-3})	24h fermentation + 24h respiration	10:1	32
Grow-out	100 shrimp m^{-2}	Rice bran	*Bacillus* sp., *Pseudomonas putida*, *P. denitrificans*, *Nitrosomonas*, and *Nitrobacter*	-	-	Sodium bicarbonate	24h fermentation + 24h respiration	10:1	73
Grow-out	30 to 120 shrimp m^{-2}	Rice bran	*B. subtilis* and *B. licheniformis*	-	-	-	48h fermentation	10:1	70
Grow-out	500 shrimp m^{-3}	Rice bran (20 g m^{-3})	*Bacillus subtilis*, *B. licheniformis,* and *B. pumilus* (0.4 g m^{-3})	-	Dextrose (2 g m^{-3})	Sodium bicarbonate (2 g m^{-3})	12h fermentation + 12h respiration	10:1	68
Grow-out	125 shrimp m^{-3}	Rice bran (5 g m^{-3})	*Bacillus subtilis*, *B. licheniformis,* and *Bacillus* sp. (0.04 g m^{-3})	-	Raw sugar (0.5 g m^{-3})	-	24h fermentation + 24h respiration	10:1	74

v:w: volume of clean water (L): rice bran weight (Kg) ratio

Recently, efforts have been made to standardize fertilization regimes for the synbiotic system [19, 20, 64]. The first step was to compare rice, wheat, or soybean brans that were processed by probiotic microorganisms on *P. vannamei* nurseries, versus biofloc (using molasses as organic carbon source), and a traditional clear water system. This study revealed that a synbiotic system fertilized with rice bran provided shrimp growth and yield similar to biofloc or clear water systems. Furthermore, the use of rice bran processed by fermentation and microbial respiration produced a higher concentration of microorganisms such as coccoid and *Bacillus* bacteria in the system compared to biofloc and clear water systems while also reducing the abundance of *Vibrio* spp. compared to synbiotic system using wheat bran as organic carbon source [19]. The use of rice bran allowed the growth of the microbial loop, providing an environment containing Chlorophyta, diatoms, protozoa, rotifers, nematodes, and copepods [75, 76]. These microorganisms, such as diatoms and ciliates, are rich in polyunsaturated fatty acids (PUFA), vitamins, and carotenoids. Collectively, these represent an important source of natural food for shrimp and have a positive influence on shrimp growth [77, 78]. Furthermore, the use of the synbiotic system reduced the abundance of Cyanobacteria to levels lower than those found in traditional biofloc systems [76]. This is beneficial because many species of Cyanobacteria produce off-flavors as well as microcystins that are toxic to human consumers. Such reduction in Cyanobacteria was likely caused by being outcompeted with bacteria such as *Bacillus* provided from the periodic synbiotic fertilization. These findings confirmed rice bran as the most effective bran type in the most recent studies with synbiotic systems (Table 4), demonstrating the positive effects of this bran in relation to others in key aspects such as water quality control, microbial composition, pathogen control, and shrimp growth.

Choosing the type of processing between fermentation and microbial respiration, is one of the most important factors in the creation of the synbiotic system. Both the fermentation and microbial respiration phases will determine the amount of the most labile organic carbon in the system, which has pronounced effects on the control of nitrogenous compounds and microbial loop development throughout the production cycle. For example, Pimentel et al. [64] evaluated different combinations of fermentation and respiration, as well as different fertilizer processing times (F12: fermentation for 12 hours; F12+R12: fermentation for 12 h + respiration for 12 h; F24: fermentation for 24 h; F24+R24: fermentation for 24 h + respiration for 24 h; R12: respiration for 12 h; R24: respiration for 24) in *P. vannamei* nurseries, which was compared with a biofloc system

and clearwater control. Based on the treatment responses in controlling TAN and creating a microbial loop, the authors understood that fermentation and respiration were equally efficient strategies and that a 12-hour period for each processing phase was sufficient for the system to function properly. Furthermore, this processing approach to fertilization in the synbiotic system provided a higher yield and a reduced feed conversion ratio compared to the clear water system. These findings differed from those found by Santos et al. [63], which based on shrimp growth, recommended a 24-h period rather than 12-h for both processing phases for *Macrobrachium rosenbergii* nurseries. Santos et al. [63] stated that longer duration likely allowed more probiotic growth to enhance prawn performance. Interestingly, it was also found that a combination of respiration with fermentation or only respiration led to better prawn growth compared to rice bran only being fermented [63]. It is worth noting that a simple sugar was not used to process rice bran, which may have led to the longer duration required. This should be further investigated, as reduced processing time by using sugar would be considered more practical and less time consuming for commercial shrimp producers.

Another consideration worth knowing is whether inoculation of commercial probiotics composed of a single genus of bacteria would have a different effect compared to mixes of various probiotics. For example, Pimentel et al. [20] compared the growth performance of *P. vannamei* in a synbiotic system when only using *Bacillus*, a mixture of bacterial probiotics (*Bacillus* + *Lactobacillus* + *Pediococcus*), and a mixture of these bacterial probiotics with yeast (*Saccharomyces cerevisiae*). It was found that *P. vannamei* growth and feeding efficiency was the best when a mixture of bacterial probiotics was used. In addition, the mixture provided rapid control of TAN, provided a higher abundance of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria, and lower abundance of *Vibrio*. This was likely due to complementary actions of the various bacteria on the fertilizer and when being added to the water. Interestingly, inoculation with *Saccharomyces cerevisiae* yeast slowed down nitrification, which was unexpected since these microorganisms are primarily decomposers and could improve environmental conditions [20].

Another aspect to be studied in the synbiotic system is water maturation. In this system, most authors perform initial fertilizations without applying a nitrogen source to stimulate microbial growth [18, 30, 66]. However, this can hamper the establishment of nitrifying bacteria from developing completely, which can lead to problems in controlling nitrogenous compounds later during the

culture cycle when nutrient inputs from food increase. Recently, Pimentel et al. [68] tested the use of the synbiotic system in the super-intensive culture of *P. vannamei* in water with salinity close to 2 g L^{-1}. The authors employed a water maturation strategy, with 16 daily applications of a processed rice bran, plus daily applications of 1 mg L^{-1} of ammonium chloride as a nitrogen source. This helped to establish the community of nitrifying bacteria, causing the system to have fewer events where total ammonia nitrogen exceeded 1 mg L^{-1}, thus water exchanges were not required to control this toxic compound. Furthermore, during the trial, no nitrite spikes were observed, but an accumulation of nitrate occurred, indicating nitrification activity [68]. These findings contributed significantly to improving our understanding of the dynamics of the synbiotic system, paving the way for improvement of fertilization strategies.

5. Final remarks and future directions

The use of the synbiotic system as a microbial-based system can diversify and refine *P. vannamei* intensive culture strategies. This system provides significant positive effects related to water quality, microbial composition, control of harmful microorganisms (e.g., Cyanobacteria and *Vibrio* sp.), and improve shrimp growth performance. Efforts have been made by researchers to test different fertilization protocols for this type of system, delivering practical results to the production sector and significant insights into the effects of different fertilization strategies on different aspects of the system. However, this is not enough to optimize the production of shrimp using synbiotic productions systems.

Studies still need to be developed to investigate the use of different synbiotic fertilization strategies on the grow-out phase of shrimp as well as the use of defatted bran in the fertilization process. The effects of initial fertilization on the nitrification process and microbial composition are another important topic to be explored. This may reveal the impact of carbon addition on the development of chemoautotrophic bacteria in the culture medium and guide the ideal number of fertilizations for better development of microbial processes. The use of inoculum must be studied further as a means to enable the reuse of water from previous culture cycles and thus minimize water use and improve biosecurity. Recent studies show that the use of 15 to 20% of inoculum from previous cycles, combined with fertilization, was sufficient to control nitrogenous compounds in *P. vannamei* cultures with synbiotic system [79]. These results provide guidance on

this issue, but it is necessary to understand the isolated effects of the inoculum, whether it is used based on a percentage of the tank volume or with different concentrations of total suspended solids (TSS).

Another variable that needs to be better understood is the influence TSS has in the synbiotic system. Low concentrations of settleable solids were reported, indicating greater control of organic matter production [80]. However, the TSS reading is more refined and needs to be studied to know what concentration limits can be adopted without affecting system stability and shrimp growth. Finally, the use of artificial substrates should be considered in the synbiotic system because they increase the contact surface for the growth of chemoautotrophic bacteria, reduce the relative stocking density, and provide more natural food for the shrimp that can translate to better survival and growth [81, 82].

Much progress has recently been made in terms of understanding and further optimizing fertilization regimes within the synbiotic system by the research community, but more research is required. Nevertheless, it is important to emphasize that commercial shrimp farmers who plan to use these fertilization approaches in their synbiotic systems must do so in the most efficient way possible to ensure the profitability of their farming operations. Technology transfer and adoption of optimized fertilization regimes by farmers can be further facilitated by validation of these approaches on commercial shrimp farms using proven fertilization regimes. If farmers choose to verify what has been discovered through applied research studies on their own or in collaboration with the research community via on-farm trials, the practical application of these techniques will likely result in further refinement and optimization of existing fertilization regimes under true commercial production conditions.

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Conflict of interest

The authors have no conflict of interest to declare.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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OBJETIVOS

Objetivo geral

Analisar o efeito de diferentes estratégias de fertilização do sistema simbiótico sobre a qualidade de água, desenvolvimento da comunidade microbiana e crescimento do camarão *Penaeus vannamei* em cultivos intensivos.

Objetivos específicos

- Testar o efeito do uso de diferentes farelos vegetais como fonte de carbono orgânico para a fertilização do sistema simbiótico no processo de nitrificação, composição planctônica e crescimento do *Penaeus vannamei* na fase de berçário, bem como compará-lo com o sistema de bioflocos.
- Avaliar o efeito dos processos de fermentação (fase sem aeração) e respiração (fase com aeração), bem como diferentes tempos de processamento do fertilizante do sistema simbiótico, no processo de nitrificação, composição do plâncton e crescimento do *Penaeus vannamei* durante a fase de berçário e comparar esses resultados com o sistema de bioflocos.
- Avaliar o efeito do uso de diferentes microrganismos probióticos na fertilização do sistema simbiótico sobre a composição da comunidade microbiana, processo de nitrificação e crescimento do *Penaeus vannamei* na fase de berçário.
- Analisar o efeito de diferentes sistemas de cultivo na qualidade da água, composição planctônica e crescimento do *Penaeus vannamei* em água de baixa salinidade e alta densidade de estocagem.

CAPÍTULO II

Fertilizing synbiotic system with different vegetable brans: effects on nitrification, plankton composition, and growth of *Penaeus vannamei* in the nursery phase

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Fertilização do sistema simbiótico com diferentes farelos vegetais: efeitos na nitrificação, composição planctônica e crescimento do *Penaeus vannamei* na fase de berçário

Resumo expandido

O sistema simbiótico é caracterizado pela utilização de farelo vegetal (prebiótico) processado por microrganismos probióticos para a fertilização de sistemas intensivos de cultivo de *Penaeus vannamei*. Neste sistema, o farelo vegetal estimula o crescimento de bactérias heterotróficas que utilizam o carbono disponível na água para a imobilização do nitrogênio amoniacal resultante da excreção e alimentação do camarão, aumentando sua biomassa. Atualmente, a utilização do sistema simbiótico vem sendo demonstrada em diversos estudos nas fases de berçário e engorda, apresentando resultados satisfatórios de qualidade de água e crescimento dos camarões. Isso acontece devido composição nutricional de alta qualidade dos farelos, que é melhorada através do processo de fermentação e respiração por microrganismos probióticos. Apesar de demonstrarem efeitos positivos sobre diversos aspectos, esses estudos utilizaram estratégias de fertilização variadas, sendo utilizado diferentes farelos vegetais como fontes de carbono orgânico (e.g., farelo de trigo e arroz) e diferentes concentrações de probiótico. Ainda, há uma escassez de informações sobre o efeito do farelo vegetal sobre o processo de nitrificação do sistema e sua influência sobre a composição da comunidade zooplanctônica e fitoplanctônica. Dessa forma, o objetivo deste estudo foi avaliar o efeito da utilização de diferentes farelos vegetais como fonte de carbono orgânico na fertilização de sistemas simbióticos no processo de nitrificação, composição do plâncton e crescimento do *P. vannamei* na fase de berçário, comparando-o também com o sistema de bioflocos. Foi realizado um berçário estendido durante 53 dias, com uma densidade de 2.000 camarões m^{-3} (peso inicial: 0,03 ± 0,01 g). Foram estabelecidos os seguintes tratamentos, com cinco repetições: CW: água clara (controle); BFT: sistema de bioflocos; RB: sistema simbiótico fertilizado com farelo de arroz; SB: sistema simbiótico fertilizado com farelo de soja; e WB: sistema simbiótico fertilizado com farelo de trigo. O protocolo de fertilização simbiótico utilizou um probiótico comercial compostos de *Bacillus subtilis* e *Bacillus licheniformis*, melaço, bicarbonato de sódio como tampão e água. Os fertilizantes foram processados por uma fase anaeróbica (24 h) e outra aeróbica (24 h). A água dos tratamentos que usaram o sistema simbiótico foi preparada com 8 aplicações do fertilizante por um período de 17 dias. Durante o ensaio foram

realizadas 15 aplicações diárias. O tratamento BFT utilizou melaço como fonte de carbono orgânico, aplicado em uma relação carbono:nitrogênio de 15:1. Neste tratamento foi usado um inoculo que foi diluído para a concentração de 5 mg L^{-1} de sólidos suspensos totais. No tratamento CW, trocas de água diárias foram realizadas em uma taxa de 90% do volume da unidade experimental. Nos tratamentos BFT e CW, probiótico foi aplicado na concentração de 0,35 g m^{-3} nos dias 7 e 14. Isso foi realizado para que todos os tratamentos recebessem a mesma quantidade de probiótico. Ao final do ensaio, o peso final foi maior nos tratamentos CW, BFT e RB do que no WB. Nos tratamentos RB, SB e WB, o nitrogênio amoniacal total (TAN) foi controlado entre os dias 10 e 14 (Figura 1a) e o nitrogênio do nitrito (NO_2^--N) foi controlado a partir do dia 40 do ensaio (Figura 1b), assemelhando-se a um sistema recém-iniciado e demonstrando a atividade das bactérias nitrificantes.

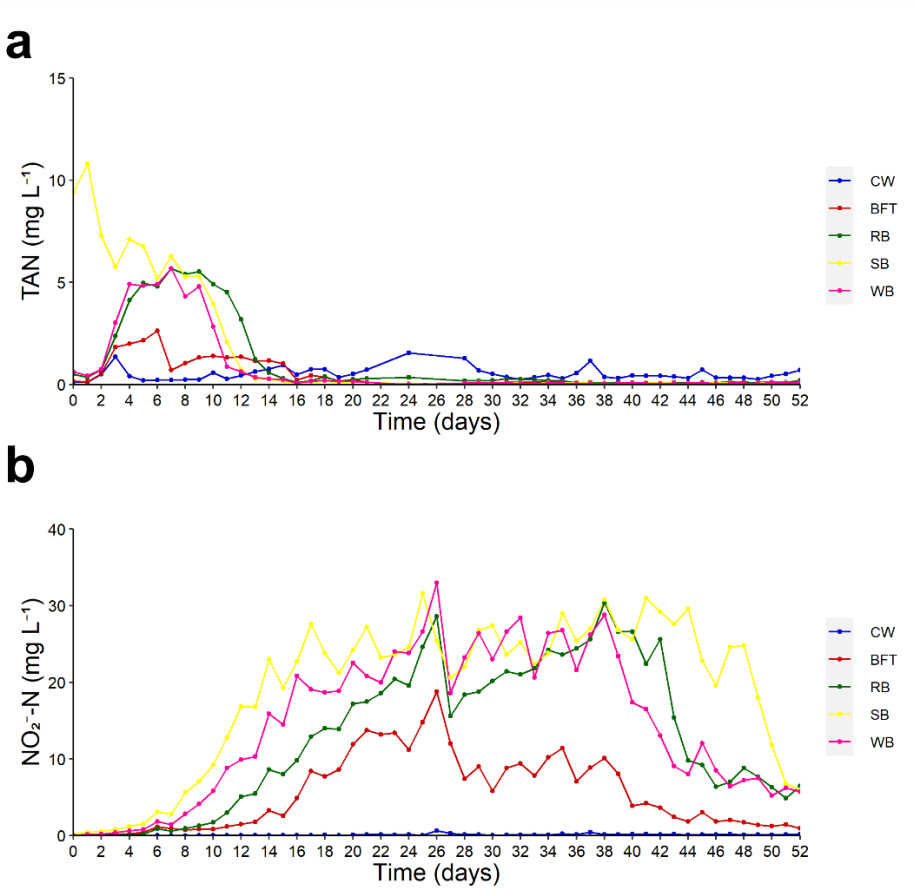

Figura 1. Concentração de TAN (a) e NO_2^--N (b) durante um berçário de *P. vannamei* com simbiótico, fertilizado com diferentes farelos vegetais, sistemas de bioflocos e água clara. CW:

água clara (controle); BFT: sistema de bioflocos; RB: sistema simbiótico fertilizado com farelo de arroz; SB: sistema simbiótico fertilizado com farelo de soja; WB: sistema simbiótico fertilizado com farelo de trigo.

No final do ensaio, uma maior abundância de bactérias cocóides e bacillus foi observada no tratamento RB (Figura 2a e b), enquanto a maior abundância de bactérias vibrio foi observada no WB (Figura 2c).

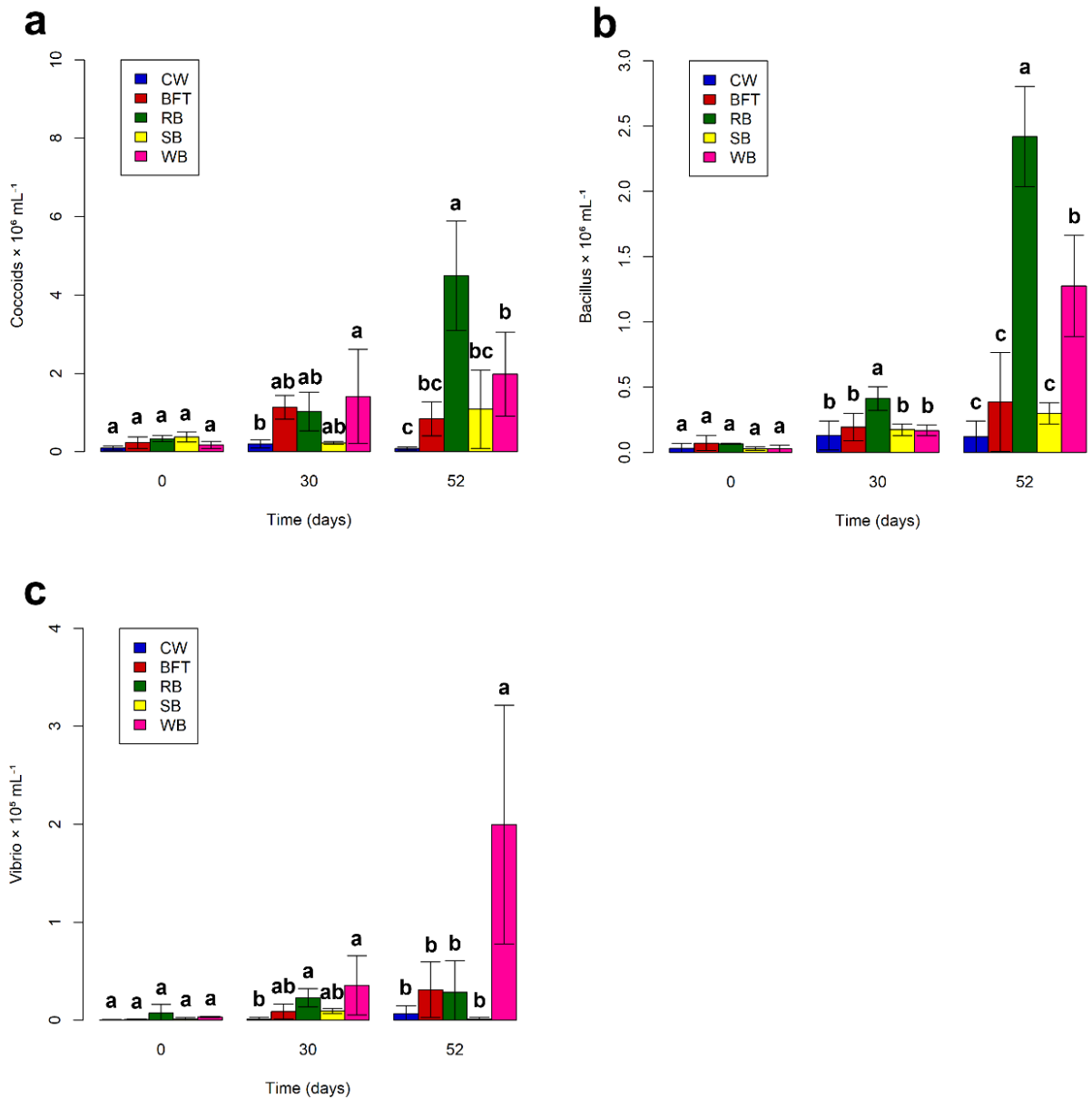

Figura 2. Abundância (organismos mL^{-1}, média ± desvio padrão) de bactérias cocoides (a), bacillus (b) e vibrio (c) durante um berçário de *P. vannamei* com simbiótico, fertilizado com diferentes farelos vegetais, sistemas de bioflocos e água clara. CW: água clara (controle); BFT: sistema de bioflocos; RB: sistema simbiótico fertilizado com farelo de arroz; SB: sistema simbiótico fertilizado com farelo de soja; WB: sistema simbiótico fertilizado com farelo de trigo.

O farelo de arroz demonstrou ser a melhor alternativa para a fertilização simbiótica, pois apresentou peso final (3,27 g) semelhante aos tratamentos BFT e CW, e superior ao WB (2,61 g). Além disso, o uso do farelo de arroz produziu uma elevada carga de microrganismos, principalmente bactérias do morfotipo bacillus, o que pode melhorar o crescimento dos camarões.

Fertilizing synbiotic system with different vegetable brans: effects on nitrification, plankton composition and growth of *Penaeus vannamei* in the nursery phase

Abstract

The aim of this study was to evaluate the effect of using different vegetable brans as organic carbon sources in synbiotic system fertilization on the nitrification process, plankton composition, and growth of *Penaeus vannamei* in the nursery phase, also comparing it with the biofloc system. An extended nursery rearing was carried out for 53 days, at a density of 2000 shrimp m^{-3} (initial weight: 0.03±0.01 g). The following treatments were established, with five repetitions: CW, clear water (control); BFT, biofloc system; RB, synbiotic system fertilized with rice bran; SB, synbiotic system fertilized with soybean bran; and WB, synbiotic system fertilized with wheat bran. The synbiotic fertilization protocol used a commercial blend of *Bacillus subtilis* and *Bacillus licheniformis*, molasses, sodium bicarbonate as buffer, and water. The fertilizers were processed by an anaerobic (24 h) and an aerobic (24 h) phase. BFT treatment used molasses as organic carbon source. At the end of the trial, final weight was higher in CW, BFT, and RB treatments than in WB. In RB, SB, and WB treatments, TAN was controlled between days 10 and 14 and NO_2^--N was controlled from day 40 of the trial, resembling a newly started system. At the end of the trial, a higher abundance of coccoid and bacillus was observed in the RB treatment, while a higher abundance of vibrio bacteria was observed in WB. Rice bran proved to be the best alternative for the synbiotic fertilization, as it presented a final weight (3.27 g) similar to BFT and CW treatments, and higher than WB (2.61 g). Also, the use of rice bran produced a high load of microorganisms, which can improve shrimp growth.

Keywords: rice bran, soybean bran, wheat bran, biofloc system, bacterial community, zooplankton.

1. Introduction

Intensive shrimp production is a good way to increase productivity and economic indices of commercial enterprises (Engle et al. 2017; Samocha 2019). However, it can increase disease outbreaks and have adverse impacts on the environment, such as the release of nutrient-rich effluents with a high biochemical demand for oxygen (Páez-Osuna 2001; Romano and Kumar 2017). Thus, the search for safe and environmentally friendly systems becomes important for turning the development of shrimp farming sustainable.

In this sense, production technologies based on organic carbon supplementation, such as biofloc technology (BFT), were developed with the aim of enabling water reuse, greater stocking density as well as biosecurity, and less area use (Krummenauer et al. 2011, 2014a; Emerenciano et al. 2013; Samocha 2019; Robles-Porchas et al. 2020). In these farming systems, different organic carbon sources are used for fertilization, such as molasses, dextrose, sucrose, cellulose, tapioca four, and vegetable bran (e.g., wheat and rice bran) (Suita et al. 2015; Serra et al. 2015; Wei et al. 2016; Rajkumar et al. 2016; Deng et al. 2018; Romano et al. 2018; Panigrahi et al. 2019; Tinh et al. 2021; Zhu et al. 2021).

Vegetable bran has a high-quality nutritional composition that can be processed and used for the intensive systems fertilization. Rice bran, for instance, is a by-product of rice processing and contains 43.25% carbohydrates, 11.60% lipids, and 13% fibers (Moro et al. 2004). Wheat bran is a product composed by 60 – 75% of carbohydrates and 33 – 63% of fibers (Onipe et al. 2015). Another bran that can be used as an organic fertilizer is soybean bran, which has 29.9% of carbohydrates, 6.6% of fiber, and 22.3% of lipids (Gebrezgi 2019). The use of these brans as an organic fertilizer in intensive marine shrimp farming has already been tested and has shown satisfactory results in controlling water quality conditions (Serra et al. 2015; Tinh et al. 2021). However, Ekasari et al. (2014) suggested that a previous processing of the vegetable bran by an anaerobic process should be carried out so that the more complex compounds, such as fibers and carbohydrates, are made available in the system in a more labile way.

Thus, these brans can be processed by anaerobic (fermentation) and aerobic (microbial respiration) phases by probiotic microorganisms, improving their nutritional content and reflecting in microbial flocs nutritional composition (Romano et al. 2018). Vegetable bran processing by probiotic microorganisms produces, as secondary metabolites after the growth of microorganisms,

bioactive compounds such as antioxidants, organic acids, and enzymes, including protease and amylase, which are responsible for breaking down proteins and starch into simpler molecules (Anson et al. 2012; Rashid et al. 2015; Sadh et al. 2018). This strategy has been widely used in the processing of bran for the water fertilization of shrimp culture with synbiotic system.

The synbiotic system is characterized by using vegetable bran (prebiotic) processed by probiotic microorganisms for the intensive systems fertilization (Romano 2017; Kawahigashi 2018). In this system, vegetable bran stimulates the growth of heterotrophic bacteria that use the carbon available in the water for the immobilization of ammonia nitrogen resulting from shrimp excretion and feed, increasing its microbial biomass. Also, the inoculation of probiotic bacteria through fertilization (e.g., *Bacillus*) can increase the growth and activity of chemoautotrophic bacteria, which are responsible for the nitrification process and act in the oxidation of total ammonia nitrogen (TAN) and nitrite (NO_2^-) to less toxic compounds for animals (i.e., nitrate (NO_3^-)) (Abdel-Tawwab et al. 2020).

This fertilization strategy can be used in a supplementary way to the BFT system, since it shares many of its characteristics, such as the use of an external carbon source, growth of microbial aggregates, reduced water exchange, and high aeration (Khanjani et al. 2023). Some of the main differences between the two systems are the use of the vegetable bran processing prior to the application in the water, no control of the carbon to nitrogen (C:N) ratio, and the growth of a high load of microorganisms (Khanjani et al. 2023). The growth of microorganisms allows the maintenance of water quality, controls pathogenic organisms such as *Vibrio* bacteria, and acts as supplementary source of food for shrimp (Romano 2017; Romano and Kumar 2017; Kawahigashi 2018).

Currently, the use of the synbiotic system has been demonstrated in several studies in the nursery and grow-out phases, showing satisfactory growth, survival, control of nitrogenous compounds throughout the culture time (i.e., TAN and NO_2^-), and reduction in the concentration of sucrose-negative bacteria, in addition to still providing high concentrations of zooplankton (Katalani et al. 2023; Santos 2020; Liñan-Vidriales et al. 2020; Lima et al. 2021; Andrade et al. 2021; Silva et al. 2021; Pimentel et al. 2022). Despite showcasing positive effects on the control of toxic nitrogenous compounds, microbial composition of the system and proximal composition of microbial flocs, these studies had varied fertilization protocols with different vegetable brans

(e.g., wheat and rice bran) and distinct probiotic concentrations (Silva et al. 2021; Andrade et al. 2021). Still, there is a lack of information about the effect of bran on the nitrification process of the system, its influence on the planktonic community composition, and whether the use of the synbiotic produces different results from those found for the biofloc system. Therefore, the aim of this study was to evaluate the effect of using different vegetable brans as organic carbon source for the synbiotic system fertilization on the nitrification process, plankton composition, and growth of *Penaeus vannamei* in the nursery phase, as well as compare it with the biofloc system.

2. Materials and methods

This study was carried out for 53 days at the "Estação Marinha de Aquacultura" of the "Universidade Federal do Rio Grande – FURG," Brazil.

2.1. Design and experimental conditions

P. vannamei (Boone 1931) shrimp post-larvae (0.03 ± 0.01 g) were stocked at a density of 2000 shrimp m^{-3} in experimental units of 50 L (0.05 m^3). The seawater used in the experiment was previously filtered, chlorinated (10 g m^{-3}), and dechlorinated with the ascorbic acid addition (1 g m^{-3}). Thus, five treatments were established, all with five repetitions: CW, clear water (control); BFT, biofloc system; RB, synbiotic system fertilized with rice bran; SB, synbiotic system fertilized with soybean bran; and WB, synbiotic system fertilized with wheat bran. The experimental units were arranged in a completely randomized experimental design. In the CW treatment, daily water changes were performed at a rate of 90% of the experimental unit volume (adapted from Hostins et al. 2017). The water used for the changes was previously chlorinated and dechlorinated with ascorbic acid application.

2.2. Water fertilization

For the treatments RB, SB, and WB, before stocking, a pre-fertilization was performed, with 8 applications over 17 days. Three matrix tanks (one for each treatment using vegetable brans) with 0.35 m^3 of seawater were fertilized, and then the water was distributed among the experimental units. The fertilizer used for the initial fertilization was composed of vegetable bran (< 300 μm; 30 g m^{-3}), powder probiotic (0.15 g m^{-3}; Sanolife PRO W, Inve Aquaculture™. Composition: *Bacillus subtilis* and *Bacillus licheniformis*. Concentration: 5×10^{10} CFU g^{-1}), molasses (3 g m^{-3}), sodium bicarbonate (3 g m^{-3}), and seawater (chlorinated and dechlorinated) in

a ratio of 10× the amount of vegetable bran. After this period, the fertilizer was applied in the experimental units.

During the experimental period, 15 daily applications were performed following methodology adapted from Kawahigashi (2018). After this time, the experimental units received a new fertilizer application only when the total ammonia nitrogen concentration (TAN) exceeded 1 mg L^{-1}. The fertilizer applied during the trial was composed of vegetable bran (< 300 µm; 10 g m^{-3}), powder probiotic (0.05 g m^{-3}), molasses (1 g m^{-3}), sodium bicarbonate (1 g m^{-3}), and seawater (chlorinated and dechlorinated) in a ratio of 10× the amount of vegetable bran. The mixture was processed by an anaerobic (24h) and an aerobic (24h) phase. After this period, the fertilizer was applied in the experimental units.

In the BFT treatment, molasses was used as a carbon source to control TAN, maintaining a carbon:nitrogen (C:N) ratio of 15:1 (Ebeling et al. 2006; Avnimelech 2012). Molasses was only applied when TAN reached 1 mg L^{-1}. In this treatment, an inoculum was diluted in sterilized seawater to reach a final concentration of 5 mg L^{-1} of total suspended solids (Machado 2021). The inoculum used had the following physicochemical characteristics (mean of three replicates ± standard deviation): TAN = 0.06 ± 0.02 mg L^{-1}, NO_2^--N = 0.11 ± 0.01 mg L^{-1}, NO_3^--N = 53.88 ± 5.30 mg L^{-1}, total alkalinity = 163.33 ± 5.77 mg L^{-1}, TSS = 501.66 ± 22.55 mg L^{-1}, and pH = 7.44 ± 0.09.

In the BFT and CW treatments, powder probiotic (Sanolife PRO W, Inve Aquaculture™) was applied in the experimental units at a concentration of 0.35 g m^{-3} on days 7 and 14. This was done so that all treatments received the same amount of probiotic.

2.3. Feed management

During the experimental time, the animals were fed three times a day (8:00 am, 1:00 pm, and 6:00 pm) using commercial feed with 40% crude protein (Guabitech PL and Guabitech J). The amount of feed offered was determined according to Jory et al. (2001).

2.4. Growth performance

Shrimp growth performance was evaluated at the end of the experimental time. Thus, it was possible to determine final weight (g), survival (%) [(number of animals at the end of

experimental time/initial number of animals) × 100], feed conversion ratio (FCR) [(feed supplied/biomass gain)], specific growth rate (SGR, % day^{-1}) 100 × [(ln final weight (g) – ln initial weight (g))/experimental time (days)], and yield (kg m^{-3}) [final biomass (Kg)/ volume of the experimental unit (m^3)].

2.5. Water quality variables

Twice a day, dissolved oxygen (DO, mg L^{-1}) and temperature (°C) (YSI EcoSense DO200A) were measured. Total ammonia nitrogen (TAN, mg L^{-1}) (UNESCO 1983), nitrite nitrogen (NO_2^--N, mg L^{-1}) (Aminot and Chaussepied 1983), and pH (Mettler Toledo seven2Go) were analyzed once a day. In treatments BFT, RB, SB, and WB, when the NO_2^--N concentration exceeded the safe level of 25.7 mg L^{-1} (Lin and Chen 2003), water changes were performed at a rate of 25 – 30% of the experimental unit volume.

Weekly, nitrate nitrogen (NO_3^--N, mg L^{-1}) (García-Robledo et al. 2014), orthophosphate (PO_4^{3-}, mg L^{-1}) (Aminot and Chaussepied 1983), settleable solids (SS, mL L^{-1}) (Eaton et al. 1995), total suspended solids (TSS, mg L^{-1}) (Strickland and Parsons 1972), salinity (g L^{-1}; Hanna multiparameter, model HI98194), total alkalinity (mg L^{-1}) (APHA 2012), and carbon dioxide (CO_2, mg L^{-1}) (Timmons and Ebeling 2013) were analyzed. When necessary, adjustments were made in the alkalinity level to 150 mg L^{-1} with sodium bicarbonate ($NaHCO_3$) application according to Furtado et al. (2011). Also, when TSS exceeded 500 mg L^{-1}, water changes were performed to maintain concentrations of 400 mg L^{-1} (Gaona et al. 2011).

2.6. Plankton community

Water samples were collected from the experimental units on days 0, 30, and 52 of the experimental time, for the quantification and identification of the main groups of microorganisms in the system. Samples were stored in amber flasks and preserved with formalin at a final concentration of 4%.

Identification and quantification of the phytoplankton and zooplankton community were performed in a sedimentation chamber, and 30 fields were randomly counted in an inverted microscope (Nikon, Eclipse TS100) at a final magnification of 200× (Utermöhl 1958). Phytoplankton and zooplankton abundance were expressed in cells mL^{-1} and organisms mL^{-1}, respectively.

Samples for the quantification and identification of the bacterioplankton community were filtered through a polycarbonate membrane filter (nuclepore, 0.2 μm of mean retention and 25 mm diameter) previously darkened with Irgalan black and stained with acridine orange in a final concentration of 0.1% (Hobbie et al. 1977). The bacteria were photographed using a camera coupled to an epifluorescence microscope (Axioplan-Zeiss™), with a final magnification of 1000× for subsequent counting of 20 random fields and determination of the main morphotypes (coccoid, bacillus, free and attached filamentous, vibrio, and prosthecate). Bacterioplankton community abundance was expressed in organisms mL^{-1}.

2.7. Data analysis

Data were tested for normality using the Shapiro-Wilk test and for homoscedasticity using the Levene test. One-way analysis of variance (one-way ANOVA) was applied to shrimp growth performance data to assess significant differences among treatments. Means were considered significantly different when p-value < 0.05. Percentage data (SGR and survival) were arcsine transformed before analysis (Zar 2010). When ANOVA was significant, Tukey's mean comparison test was applied to determine which treatments differed.

For water quality variables, repeated measures ANOVA was applied, followed by the Tukey test to assess differences among the treatments. When necessary, data (temperature afternoon, DO morning and afternoon, pH, TAN, NO_3^--N, PO_4^{3-}, CO_2, alkalinity, TSS, and SS) were transformed to fulfill parametric assumptions. For non-parametric data (temperature morning, NO_2^--N), the Friedman test was performed, followed by the Conover multiple comparison test with Bonferroni correction.

One-way ANOVA was applied to the abundance data of the main groups identified in phytoplankton, zooplankton, and bacterioplankton (separated by time: days 0, 30 and 52). Means were considered significantly different when p-value < 0.05. When ANOVA was significant, Tukey's mean comparison test was applied to determine which treatments differed. When necessary, data (Bacillariophyta, days 0, 30, and 52; Chlorophyta, day 52; flagellates, day 52; ciliates, days 0 and 30; rotifers, days 30 and 52; nematodes, day 52; amoeba, days 0 and 30; free filamentous, day 0; attached filamentous, day 30; vibrio, days 0, 30, and 52; and prosthecate, days 30 and 52) were transformed to fulfill parametric assumptions. For non-parametric data

(Chlorophyta - day 0; nematodes - days 0 and 30; attached filamentous bacteria - day 0, prosthecate bacteria – day 0, and amoeba - day 52), the Kruskal-Wallis test was applied, followed by Dunn's comparison test with Bonferroni correction to assess significant differences among treatments.

Graphs, one-way ANOVA, Kruskal-Wallis, Friedman, and their post hoc tests were performed in R software version 4.2.3 (R Core team 2023) using packages car (Fox and Weisberg 2019), dunn.test (Dinno 2017), PMCMRplus (Pohlert 2022), ggplot2 (Wickham 2016), and Rmisc (Hope 2022). Repeated measures ANOVA and its post hoc were performed using Past 4.03 2020 software (Hammer et al. 2001).

3. Results

3.1. Growth performance

The shrimp final weight was higher in the CW, BFT, and RB treatments than in WB (Table 1). SGR was higher in the CW treatment than in WB. Yield was higher in the CW, BFT, and RB treatments when compared to SB and WB (Table 1).

Table 1. Growth performance of *P. vannamei* after a nursery with synbiotic, fertilized with different vegetable bran, biofloc, and clear water systems.

	Treatments				
	CW	BFT	RB	SB	WB
Initial weight (g)	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Final weight (g)	3.17 ± 0.20^{a}	3.05 ± 0.14^{a}	3.27 ± 0.24^{a}	2.96 ± 0.14^{ab}	2.61 ± 0.22^{b}
Survival (%)	87.80 ± 5.63^{a}	90.60 ± 6.18^{a}	88.40 ± 8.20^{a}	90.40 ± 7.53^{a}	93.60 ± 5.18^{a}
FCR	1.69 ± 0.11^{a}	1.60 ± 0.15^{a}	1.52 ± 0.08^{a}	1.83 ± 0.24^{a}	1.77 ± 0.30^{a}
SGR (% day^{-1})	8.83 ± 0.12^{a}	8.73 ± 0.10^{ab}	8.72 ± 0.17^{ab}	8.71 ± 0.09^{ab}	8.46 ± 0.16^{b}
Yield (Kg m^{-3})	5.36 ± 0.18^{a}	5.49 ± 0.27^{a}	5.67 ± 0.16^{a}	4.69 ± 0.35^{b}	4.45 ± 0.20^{b}

Data corresponds to mean ± standard deviation. Superscript letters correspond to the results of Tukey's test. FCR: feed conversion ratio; SGR: specific growth rate. CW: clear water (control); BFT: biofloc system; RB: synbiotic system fertilized with rice bran; SB: synbiotic system fertilized with soybean bran; WB: synbiotic system fertilized with wheat bran.

3.2. Water quality variables

Water temperature in the morning was maintained at 26 °C in all treatments (Table 2). In the afternoon, mean temperature was between 29 and 30 °C. Mean DO was above 5 mg L^{-1} in all treatments in the morning and afternoon (Table 2).

TAN was higher in treatments RB, SB, and WB than in CW and BFT (Table 2). The SB treatment started the experiment with a TAN concentration of 9.40 mg L^{-1}. Treatments WB and RB reached the highest mean concentration on the 7th (5.68 mg L^{-1}) day of culture. A TAN reduction was observed between days 10 and 14 of the experimental time in treatments RB, SB, and WB (Figure 1a). However, WB and SB showed a faster reduction in the TAN concentration compared to the other treatments.

NO_2^--N was higher in the SB, WB, and RB when compared to BFT and CW treatments (Table 2). The highest concentration of NO_2^- was reached in WB and SB (40.00 mg L^{-1}). A reduction in NO_2^- concentration was observed in BFT, RB, WB, and SB from the 40th day of culture onwards (Figure 1b).

NO_3^--N was higher in the BFT, RB, WB, and SB treatments, when compared to CW (Table 2). From the 40th day of culture (week 6), an increase in the NO_3^--N concentration was observed in all groups, except for CW (Figure 1c).

PO_4^{3-} was higher in RB treatment than in the CW, BFT, and SB (Table 2). SS and TSS were higher in BFT, RB, WB, and SB, when compared to CW (Table 2).

Table 2. Water quality variables during a *P. vannamei* nursery with synbiotic, fertilized with different vegetable brans, biofloc, and clear water systems.

Variables	Treatments				
	CW	BFT	RB	SB	WB
Temperature morning (°C)	26.28 ± 1.32 [a] 26.17 (23.40 – 28.66)	26.06 ± 1.48 [a] 25.89 (23.50 – 28.84)	26.00 ± 1.43 [a] 25.84 (23.50 – 28.77)	26.09 ± 1.42 [a] 25.90 (23.30 – 28.61)	25.93 ± 1.40 [a] 25.80 (23.20 – 28.17)
Temperature afternoon (°C)	29.33 ± 1.60 [a] 29.33 (25.50 – 32.10)	30.00 ± 1.55 [a] 30.29 (26.70 – 32.37)	30.12 ± 1.65 [a] 30.41 (26.30 – 32.30)	29.35 ± 1.56 [a] 29.46 (25.90 – 31.94)	28.85 ± 1.42 [a] 28.73 (26.30 – 32.07)
DO morning (mg L^{-1})	5.99 ± 0.20 [a] 5.98 (5.48 – 6.40)	5.99 ± 0.25 [a] 5.98 (5.44 – 6.70)	5.98 ± 0.23 [a] 5.99 (5.42 – 6.43)	5.94 ± 0.23 [a] 5.96 (5.46 – 6.38)	6.01 ± 0.21 [a] 6.00 (5.57 – 6.44)
DO afternoon (mg L^{-1})	5.66 ± 0.27 [a] 5.66 (4.89 – 6.15)	5.54 ± 0.25 [a] 5.52 (4.98 – 6.06)	5.49 ± 0.23 [a] 5.51 (5.07 – 5.96)	5.55 ± 0.23 [a] 5.53 (5.01 – 6.09)	5.69 ± 0.23 [a] 5.67 (5.10 – 6.21)
pH	8.04 ± 0.08 [a] 8.03 (7.88 – 8.15)	7.91 ± 0.16 [a] 7.87 (7.63 – 8.15)	7.92 ± 0.19 [a] 7.95 (7.41 – 8.18)	7.92 ± 0.18 [a] 7.90 (7.40 – 8.21)	7.89 ± 0.19 [a] 7.88 (7.50 – 8.19)
Salinity (g L^{-1})	33.34 ± 2.15 [a] 33.00	33.53 ± 2.11 [a] 34.00	33.41 ± 1.66 [a] 33.00	33.37 ± 1.81 [a] 33.00	33.58 ± 1.78 [a] 34.00

	(29.00 – 36.37)	(26.72 – 37.43)	(29.00 – 36.87)	(28.00 – 37.40)	(28.00 – 36.36)
TAN (mg L^{-1})	0.51 ± 0.34 [b] 0.46 (0.03 – 1.95)	0.50 ± 0.74 [b] 0.10 (0.00 – 5.80)	1.13 ± 1.88 [a] 0.13 (0.00 – 7.80)	1.65 ± 2.96 [a] 0.09 (0.00 – 11.40)	0.88 ± 1.68 [a] 0.09 (0.00 – 6.60)
NO_2^--N (mg L^{-1})	0.11 ± 0.13 [d] 0.08 (0.00 – 0.99)	5.43 ± 6.56 [c] 2.50 (0.00 – 33.00)	12.86 ± 11.50 [b] 11.00 (0.01 – 38.00)	18.95 ± 10.75 [a] 22.00 (0.17 – 40.00)	14.51 ± 11.47 [b] 15.00 (0.00 – 40.00)
NO_3^--N (mg L^{-1})	0.38 ± 0.31 [b] 0.28 (0.00 – 1.16)	32.60 ± 41.73 [a] 15.00 (0.00 – 147.00)	23.67 ± 40.36 [a] 10.00 (0.00 – 159.00)	17.24 ± 29.36 [a] 7.00 (0.00 – 131.00)	31.14 ± 51.50 [a] 12.00 (0.00 – 217.00)
PO_4^{3-} (mg L^{-1})	0.15 ± 0.12 [c] 0.10 (0.03 – 0.44)	0.55 ± 0.55 [b] 0.27 (0.05 – 2.04)	1.18 ± 0.79 [a] 0.96 (0.25 – 2.90)	0.58 ± 0.47 [b] 0.47 (0.05 – 2.18)	0.77 ± 0.79 [ab] 0.42 (0.15 – 3.16)
Total alkalinity (mg L^{-1})	137.10 ± 16.01 [a] 130.00 (110.00 – 180.00)	117.70 ± 39.37 [a] 120.00 (50.00 – 190.00)	128.60 ± 33.97 [a] 120.00 (60.00 – 190.00)	126.00 ± 41.88 [a] 110.00 (60.00 – 210.00)	124.00 ± 41.31 [a] 110.00 (60.00 – 200.00)
CO_2 (mg L^{-1})	2.91 ± 0.75 [a] 2.67 (1.58 – 4.94)	3.32 ± 0.85 [a] 3.28 (1.92 – 5.56)	3.38 ± 1.07 [a] 3.34 (1.80 – 6.50)	3.48 ± 1.26 [a] 3.23 (1.97 – 7.76)	3.38 ± 1.16 [a] 3.25 (1.64 – 6.97)
SS (mL L^{-1})	0.21 ± 0.17 [b] 0.20 (0.00 – 0.50)	18.17 ± 10.89 [a] 19.00 (0.00 – 40.00)	11.35 ± 11.22 [a] 7.00 (0.50 – 40.00)	7.07 ± 6.54 [a] 6.00 (0.00 – 30.00)	7.53 ± 6.92 [a] 5.50 (0.20 – 23.00)

	104.60 ± 68.58 [b]	493.10 ± 317.78 [a]	430.90 ± 314.28 [a]	313.70 ± 200.97 [a]	385.90 ± 263.84 [a]
TSS (mg L^{-1})	85.00	530.00	430.00	275.00	325.00
	(15.00 – 275.00)	(5.00 – 1,055.00)	(40.00 – 1,505.00)	(5.00 – 825.00)	(120.00 – 1,055.00)

Data corresponds to mean ± standard deviation, median (minimum - maximum). Superscript letters correspond to the results of Tukey's or Conover's multiple comparison tests. DO: dissolved oxygen; TAN: total ammonia nitrogen; NO_2^--N: nitrite nitrogen; NO_3^--N: nitrate nitrogen; PO_4^{3-}: orthophosphate; CO_2: carbon dioxide; SS: settleable solids; TSS: total suspended solids. CW: clear water (control); BFT: biofloc system; RB: synbiotic system fertilized with rice bran; SB: synbiotic system fertilized with soybean bran; WB: synbiotic system fertilized with wheat bran.

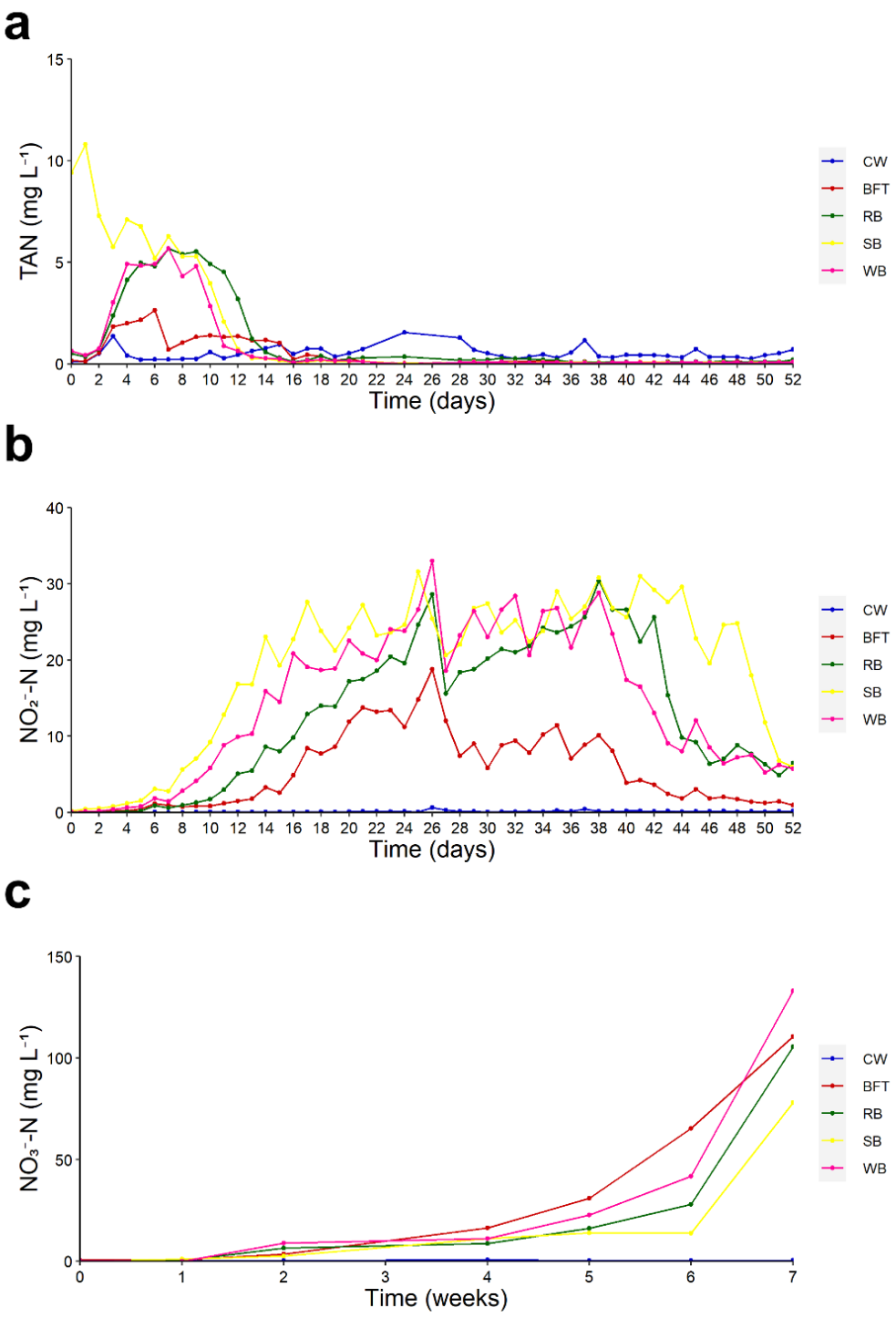

Figure 1. Concentration of TAN (a), NO_2^--N (b), and NO_3^--N (c) during a *P. vannamei* nursery with synbiotic, fertilized with different vegetable bran, biofloc, and clear water systems. CW: clear

water (control); BFT: biofloc system; RB: synbiotic system fertilized with rice bran; SB: synbiotic system fertilized with soybean bran; WB: synbiotic system fertilized with wheat bran.

3.3. Plankton community composition

3.3.1. Phytoplankton community

The abundance of Bacillariophyta was higher in treatments BFT, RB, SB, and WB than in CW on days 30 and 52 of the experimental time (Figure 2a). A reduction in the abundance of Bacillariophyta was also observed between days 30 and 52 (Figure 2a).

On day 30 of the experiment, BFT and RB showed a higher abundance of Chlorophytes than CW, SB, and WB (Figure 2b). On day 52, BFT, RB and SB had a higher abundance of Chlorophytes than CW and WB (Figure 2b).

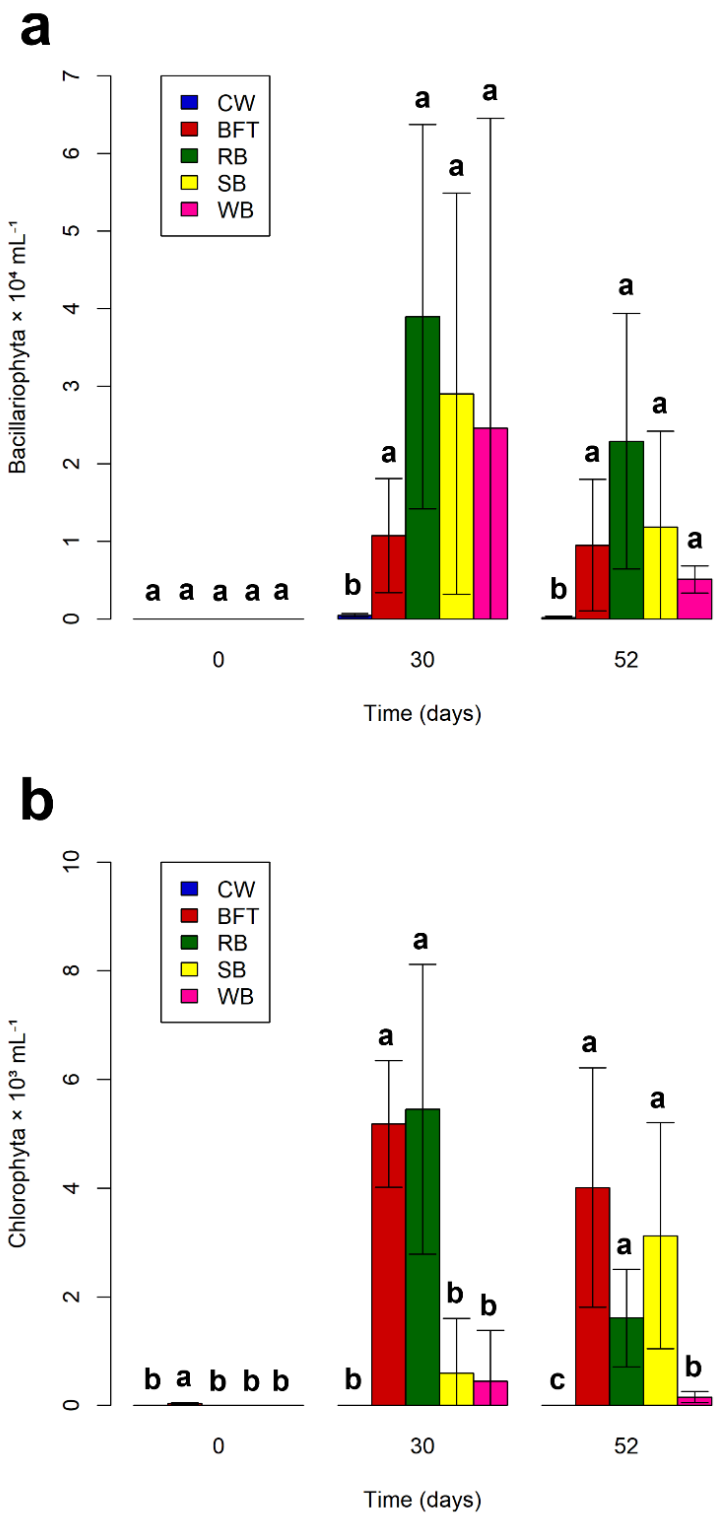

Figure 2. Abundance (cells mL^{-1}, mean ± standard deviation) of Bacillariophyta (a) and Chlorophyta (b) during a *P. vannamei* nursery with synbiotic, fertilized with different vegetable

bran, biofloc, and clear water systems. CW: clear water (control); BFT: biofloc system; RB: synbiotic system fertilized with rice bran; SB: synbiotic system fertilized with soybean bran; WB: synbiotic system fertilized with wheat bran.

3.3.2. Zooplankton community

At the beginning of the experimental time, treatment WB showed a higher abundance of flagellated protozoa than treatment CW and SB (Figure 3a). On days 30 and 52, BFT, RB, SB, and WB showed a higher abundance of flagellates when compared to that found in CW (Figure 3a). On day 30, the abundance of ciliated protozoa was significantly higher in the BFT, RB, SB and WB treatments when compared to CW (Figure 3b). At the end of the experimental time, the abundance of ciliates was higher in the SB treatment than in the CW treatment (Figure 3b). Furthermore, an increase in the abundance of flagellated and ciliated protozoa was observed throughout the experiment (Figure 3a and b).

On day 0, the abundance of rotifers was higher in RB treatment when compared to the other treatments (Figure 3c). On day 30 of the experimental period, the abundance of rotifers was higher in BFT, RB, and WB when compared to CW (Figure 3c). On day 0, the nematodes abundance was higher in WB (Figure 3d). At the end of the experiment, the abundance of nematodes in the BFT treatment was higher than in CW and SB (Figure 3d). At the beginning of the experimental time, the abundance of amoeba was higher in RB than in CW, BFT, and SB (Figures 3e and 5c). At the end of the trial, amoeba abundance was higher in WB than in CW and RB (Figure 3e).

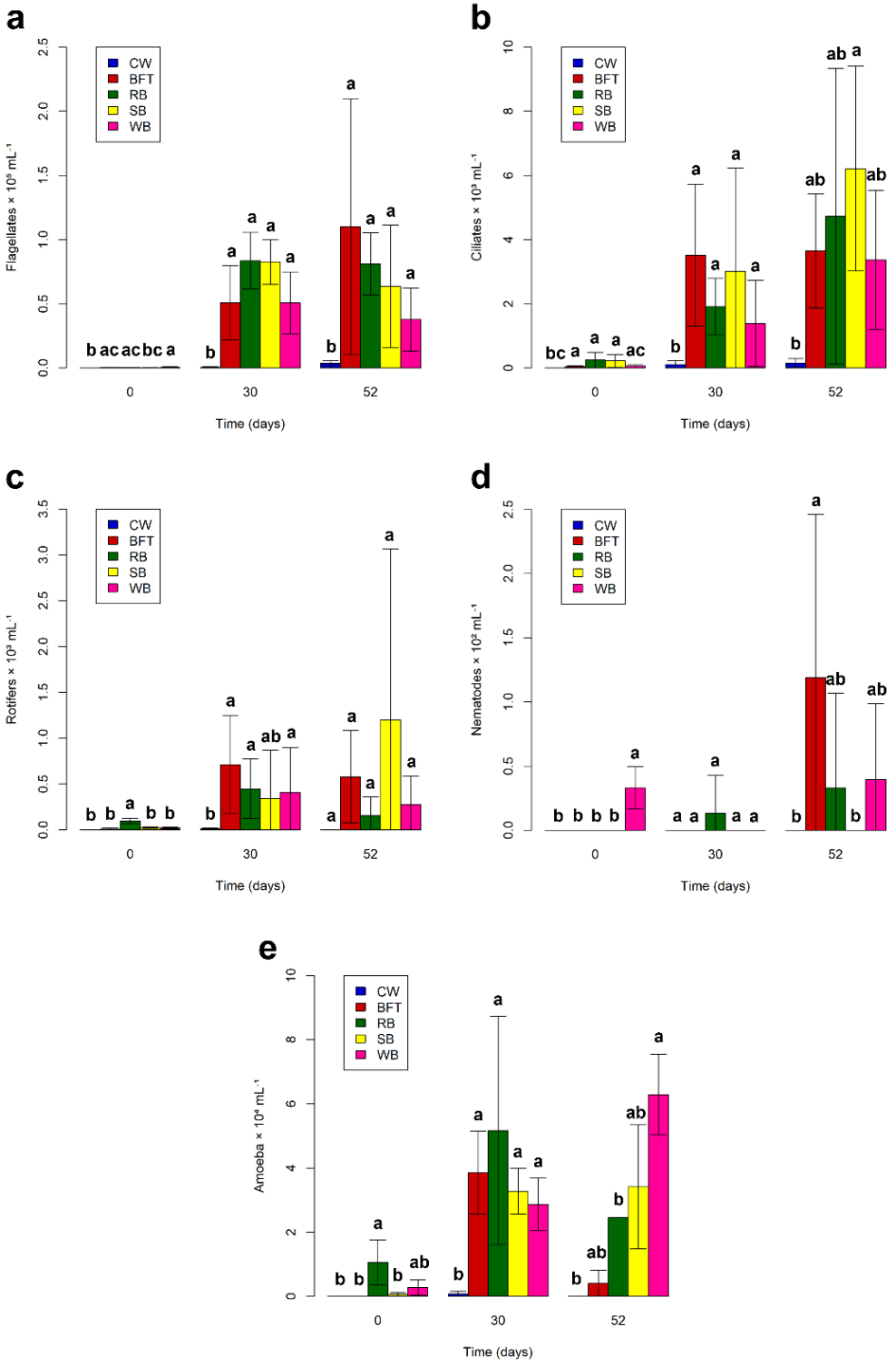

Figure 3. Abundance (organisms mL^{-1}, mean ± standard deviation) of flagellates (a), ciliates (b), rotifers (c), nematodes (d) and amoeba (e) during a *P. vannamei* nursery with synbiotic, fertilized

with different vegetable bran, biofloc, and clear water systems. CW: clear water (control); BFT: biofloc system; RB: synbiotic system fertilized with rice bran; SB: synbiotic system fertilized with soybean bran; WB: synbiotic system fertilized with wheat bran.

3.3.3. Bacterioplankton community

At the end of the experimental time, abundance of coccoid bacteria was higher in RB than in other treatments (Figures 4a and 5b). An increase in the abundance of these bacteria was observed in the RB treatment throughout the experiment (Figure 4a). On days 30 and 52 of the experimental time, there were more bacillus in RB when compared to the other treatments (Figures 4b and 5a). On day 52 of the experiment, the abundance of free filamentous bacteria was higher in the RB than in the other treatments (Figures 4c and 5e). On day 52 of the experimental time, the abundance of attached filamentous bacteria was higher in the WB treatment than in CW and SB (Figure 4d). On day 30 of the experiment, the abundance of prosthecate bacteria was higher in WB than in the other treatments (Figure 4e). On day 52, there were more prosthecate bacteria in RB and SB than in CW, BFT, and WB (Figures 4e and 5d). Regarding vibrio bacteria, on day 52 there was a higher abundance in WB than in the other treatments (Figure 4f).

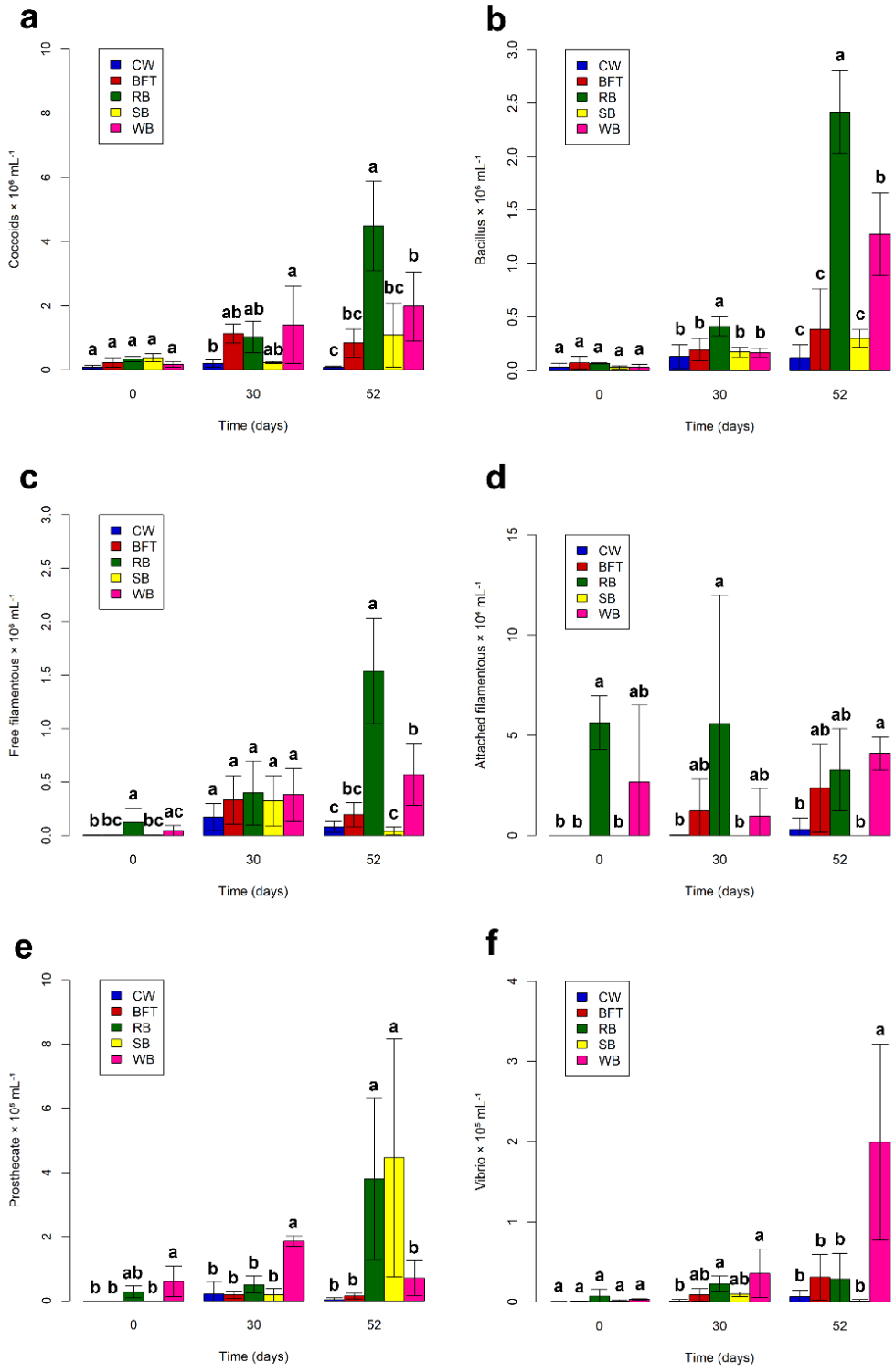

Figure 4. Abundance (organisms mL^{-1}, mean ± standard deviation) of coccoid (a) bacillus (b), free filamentous (c), attached filamentous (d), prosthecate (e) and vibrio (f) bacteria during a *P.*

vannamei nursery with synbiotic, fertilized with different vegetable bran, biofloc, and clear water systems. CW: clear water (control); BFT: biofloc system; RB: synbiotic system fertilized with rice bran; SB: synbiotic system fertilized with soybean bran; WB: synbiotic system fertilized with wheat bran.

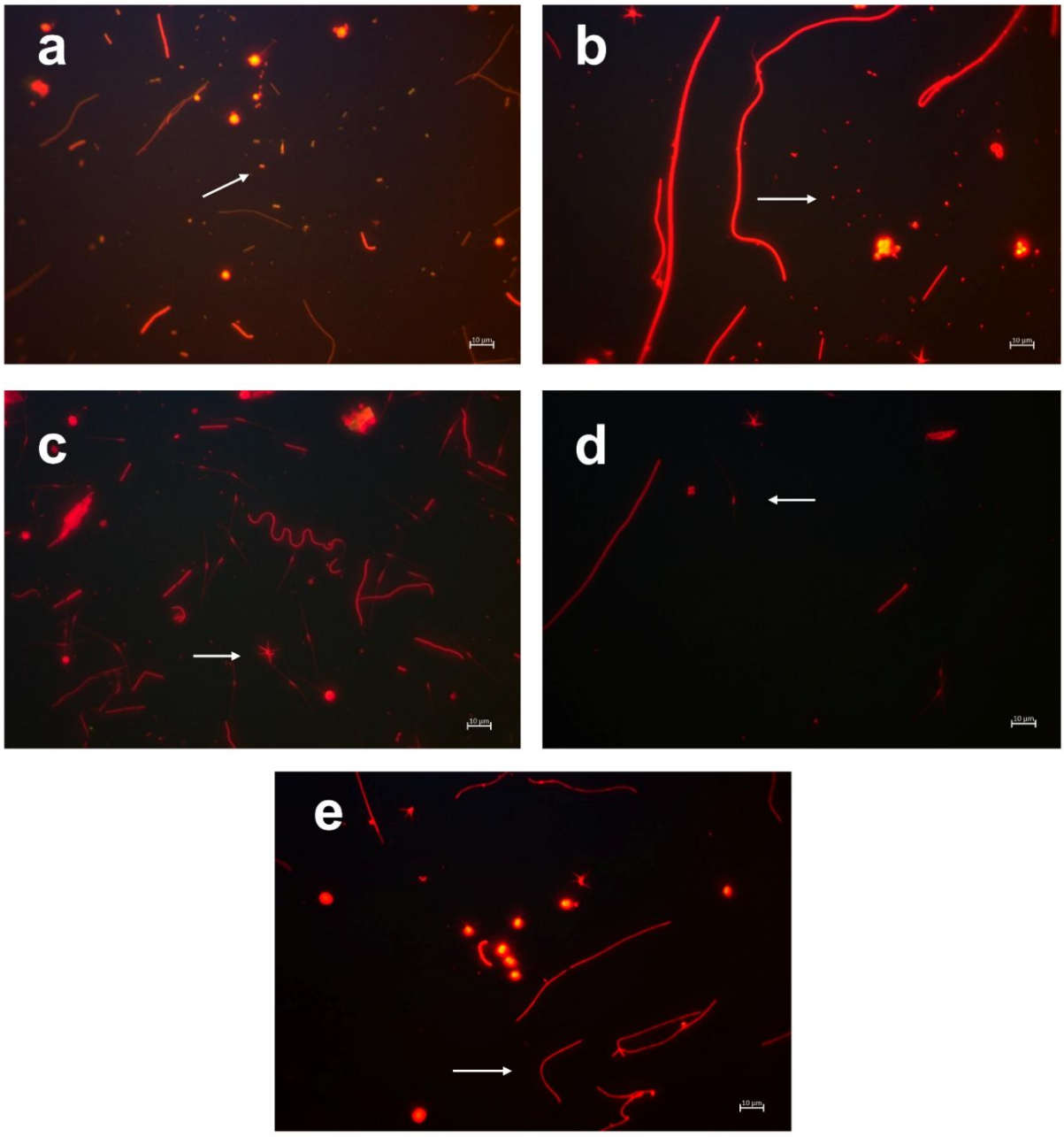

Figure 5. Epifluorescence photomicrographs of microorganisms present in treatments CW (a; arrow - bacillus), BFT (b; arrow - coccoid bacteria), RB (c; arrow - amoeba), SB (d; arrow -

prosthecate bacteria) and WB (e; arrow - filamentous bacteria). CW: clear water (control); BFT: biofloc system; RB: synbiotic system fertilized with rice bran; SB: synbiotic system fertilized with soybean bran; WB: synbiotic system fertilized with wheat bran.

4. Discussion

4.1. Shrimp growth

Nursery is an intermediate phase between hatchery and grow-out where shrimp post-larvae are stocked in a controlled system with high densities to achieve greater survival and growth of juveniles in the grow-out phase (Alday-Sanz 2010). In our study, good survival rates (higher than 85%) and shrimp final weight (close to 3 g) at the end of the experimental time were achieved in all treatments. The final weight achieved complies with what is found for extended nurseries. Samocha (2019) has reported that nurseries lasting six to eight weeks produced juveniles weighing between 1 and 6 g.

In microbial-based systems, the growth of a diverse microbial community mediated by an organic carbon source acts as a supplementary food for the animals (Khanjani et al. 2022), which probably made shrimp growth in the RB and BFT treatments not different. In fact, shrimp growth in synbiotic and BFT systems has been shown to be comparable. Hussain et al. (2021) tested the effect of shrimp culture in a biofloc and synbiotic system, using rice bran, and found a survival rate above 90% in all treatments, with no significant difference between systems.

In the synbiotic system, the use of a prebiotic (vegetable bran) processed by probiotic microorganisms as fertilizer for the water can be an advantage over the BFT system since the fertilizer can be directly ingested by the shrimp (Khanjani et al. 2023). This can greatly influence the culture yield, as the shrimp will be ingesting a more nutritious supplementary food source in addition to the microorganisms that are grown in the system. Our findings suggest that the high yield achieved probably occurred due to the time of the experiment (approximately 7.5 weeks), increasing biomass stocked and, consequently, also increasing yield. Concerning specific growth rate, as a comparative example, Wasielesky et al. (2020), testing the effect of different feeding frequencies in intensive nurseries (2000 shrimp m^{-3}) with BFT system for 40 days, reached a specific growth rate close to that found in our study, in the treatment where the animals were fed three times a day (8.31% day^{-1}).

Based on shrimp growth results, the use of rice bran can be considered the best strategy for the water fertilization in the synbiotic system. This is confirmed by Abdel-Tawwab et al. (2020), who found that the use of rice bran fermented with *Bacillus subtilis* in the *P. vannamei* culture proved to be a good alternative to improve shrimp growth, increase *Bacillus* count, reduce *Vibrio* concentration in the system, and significantly improve water quality.

4.2. Water quality

During the experimental time, variables DO, pH, temperature, CO_2, and TSS were kept within the recommended for *P. vannamei* intensive culture (Van Wyk et al. 1999; Gaona et al. 2011; Samocha 2019). In general, the best water quality conditions were found in the CW treatment (control), which was only possible due to the daily water changes.

In this study, an increase in the concentration of TAN was observed from the second day of the experiment on the BFT, RB, and WB treatments. The accumulation of this compound can be explained by the feed intake with a high crude protein content (40%) and by the animal's excretion, which are the main routes of nitrogen input into aquaculture systems (Boyd and Tucker 1998; Samocha 2019). High concentration of TAN recorded in the SB treatment at the beginning of the experimental time was probably due to the composition of the soybean bran, which has a high percentage of protein that was broken down by fermentation and respiration processes by probiotic microorganisms and made available in the system (Romano et al. 2018). It is estimated that soybean bran contains 34% crude protein, which is higher than what is found in rice bran (12.25%) and wheat bran (9.6 – 18.6%) (Moro et al. 2004; Onipe et al. 2015; Gebrezgi 2019). Furthermore, the use of the synbiotic system fertilized with soybean bran (SB treatment) did not provide a good growth of the nitrifying bacteria community when compared to the other treatments.

Naturally, BFT system had better TAN control due to the exogenous carbon source used (i.e., molasses), as it makes carbon available in a more labile way and, therefore, rapidly stimulates the immobilization of nitrogen by the heterotrophic bacteria community (Ekasari et al. 2014). The TAN reduction between days 10 and 14 of the experimental time in the treatments that used vegetable bran as fertilization strategy was probably due to the establishment of the ammonia-oxidizing bacteria (AOB) community in the system (Abakari et al. 2021). This group of bacteria acts in the presence of oxygen (O_2) and uses reduced nitrogen compounds (i.e., NH_4^+ or NH_3) as

hydrogen donors to carry out the oxidation process to obtain energy for the synthesis of biomolecules, generating NO_2^- (Boyd and Tucker 1998; Esteves 2011). It is estimated that this group of bacteria is established in intensive systems in a period of 4 to 6 weeks (Samocha 2019).

Increase in NO_2^- concentration in the BFT, RB, SB, and WB treatments occurred from day 8 of the experiment, confirming the establishment of the community of AOB bacteria in the system (Abakari et al. 2021). The reduction of NO_2^- from day 40 of the experimental time can be explained by the establishment of a community of nitrite-oxidizing bacteria (NOB) in the system, which oxidize NO_2^-, forming NO_3^- and generating energy (Boyd and Tucker 1998; Esteves 2011). This was confirmed with the increase in NO_3^- concentration from the 6th week of culture. Reis et al. (2019) tested the effect of light restriction on the microbial community in the BFT system and observed the same behavior in NO_2^- and NO_3^- fluctuation in the control treatment, where a natural photoperiod was applied.

This behavior in the nitrogen compounds variation resembles a newly started system, where there is no establishment of a microbial community that can effectively control the nitrogen compounds generated in intensive systems (Samocha 2019). The synbiotic fertilization applied in this study, using different vegetable brans as an organic carbon source, even without controlling the carbon to nitrogen (C:N) ratio at 15:1, as occurs in traditional BFT systems (Ebeling et al. 2006; Avnimelech 2012), proves to be efficient in the control of total ammonia nitrogen by the heterotrophic bacteria community and in the establishment of nitrifying bacteria community in *P. vannamei* intensive nurseries. This efficiency in the nitrification process in synbiotic has already been reported in some studies using seawater, oligohaline water, and freshwater (Hussain et al. 2021; Pimentel et al. 2022; Santos et al. 2022).

Still, in those periods of culture where there was a reduction of TAN and NO_2^- in the system, the activity of the nitrifying bacteria community could be proven with the reduction of alkalinity and pH of the water, requiring the application of mineral fertilizers for correction of these variables to recommended levels for shrimp farming (Furtado et al. 2011). This relationship between chemoautotrophic bacteria activity, alkalinity, and pH variables is described by Ebeling et al. (2006) where they estimate that for the consumption of 1 g of NH_4^+-N by chemoautotrophic bacteria, 7.05 g of inorganic carbon is consumed and 5.85 g of CO_2 is produced, resulting in a decrease in the alkalinity and pH of the system.

4.3. Plankton composition

The differences among treatments BFT, RB, SB, and WB related to the CW treatment observed in the groups of phytoplankton, zooplankton, and bacterioplankton microorganisms can be explained by the absence of fertilizations in treatment CW, causing the growth of the planktonic community to not be stimulated. This was also reinforced by the daily water changes that were performed during the experiment.

In intensive systems, phytoplankton is important in the nitrogen compounds cycling for biomass production, since it has greater affinity for ammonia than bacteria and are better competitors when ammonia concentration is low (Hargreaves 2006; Khanjani et al. 2022). Our results indicated that the higher abundance of microalgae (Chlorophytes and Bacillariophytes) may be linked to the fact that ammonia is the preferred nitrogen substrate to the growth of this group (Hargreaves 2006). Furthermore, it is likely that the increase in nitrate availability over the experimental time stimulated Chlorophyta growth (Hargreaves 1998).

As primary producers, phytoplankton are responsible for transferring energy and matter through the food chain (Khanjani et al. 2022). The high abundance of Bacillariophytes in the BFT, RB, SB, and WB treatments when compared to CW throughout the experimental time shows the efficiency of the fertilization process in the growth of photoautotrophic microorganisms in the system. Studies prove that the presence of diatoms in the culture environment can positively influence growth and yield of *P. vannamei* shrimp in culture systems because they have high nutritional value and help control cyanobacteria (Martins et al.2016; Marinho et al. 2017). Godoy et al. (2012), evaluating the contribution of diatoms on *P. vannamei* growth in an intensive system, found that a medium rich in diatoms increases weight gain and reduces feed conversion rate of the animals. This may be related to the shrimp's preference for this natural food source, as exposed by Abreu et al. (2007) who showed that *Farfantepenaeus paulensis* shrimp have a greater preference for consuming centric diatoms present in biofilms, when cultured in cages and at high density.

The presence of Chlorophyta during the experimental period in the treatments where the synbiotic systems and BFT were tested was less marked than that of heterotrophic microorganisms. In intensive shrimp farming systems, the use of external carbon sources favors the growth of heterotrophic microorganisms (Khanjani and Sharifnia 2020). Thus, throughout the time, the

growth of the phytoplankton community is limited by shading caused by the high production of microbial aggregates that are formed predominantly by those organisms (Hargreaves 2013).

Organic fertilization made large amounts of carbon available in the water, favoring the growth of the bacterial community and consequently the development of protozoa microorganisms (Decamp et al. 1999). Flagellated protozoa are the most abundant microorganisms of protozooplankton (Wetzel 2001) and in fact we observed higher abundances of them compared to the other zooplankton microorganisms. On the other hand, ciliated protozoa have a grazing behavior on bacteria, microalgae (e.g., cyanobacteria and diatoms) and other protozoa (e.g., flagellates), and can exert top-down control over the abundance of these microorganisms, being important in the energy transfer through the microbial loop (Wetzel 2001; Esteves 2011). This same behavior can be observed for rotifers, as they have herbivore, omnivore, and carnivore feeding habits (Esteves 2011).

Top-down control is characterized by the predation of organisms from a higher trophic level (e.g., zooplankton) on a group of low trophic level (e.g., phytoplankton), influencing the structure of this community (Wetzel 2001; Schefer 2004). This effect may have occurred in our study because an increase in the abundance of ciliated protozoa, rotifers (SB treatment), and nematodes (BFT treatment) was observed between days 30 and 52 of the experiment. Xavier et al. (2022) analyzed the effect of a flocculant additive on the microbial community in a *P. vannamei* culture with biofloc systems and found the same temporal behavior in the community of protozoans and rotifers.

The use of the synbiotic system provides conditions similar to those of a natural environment, causing the establishment of a diverse microbial community (Romano2017; Romano and Kumar 2017). The presence of larger microorganisms, such as copepods and cladocerans, was reported by Silva et al. (2021) when they evaluated the zooplankton community using a synbiotic system. However, the absence of these microorganisms in our study may be related to the experimental time, which meant that there were no changes in the planktonic community sufficient to favor the development of larger microorganisms in the system.

In intensive heterotrophic and mixed (i.e., systems based on heterotrophic and chemoautotrophic bacteria) shrimp culture systems, bacteria play a key role in the microbial loop development. These microorganisms are the main components of microbial aggregates, act in the

process of oxidation of nitrogenous compounds and control pathogens in the system (Ferreira 2008; Krummenauer et al. 2014b; Samocha 2019). In this study, treatment RB produced more coccoid bacteria and bacillus at the end of the experimental time, although we added the same amount of probiotic to all treatments. The presence of coccoid bacteria in the culture system can be positive, as they produce mucus that helps in the formation of microbial aggregates (Ferreira 2008). Also, this group of bacteria can act in the system's nutrients cycling, since they have high surface to volume ratio and therefore assimilate nutrients more efficiently (Suita et al. 2015).

Like coccoid bacteria, bacilli are extremely important microorganisms in intensive shrimp culture systems. This is due to its probiotic character, improving animal performance and acting against pathogenic microorganisms such as bacteria of the genus *Vibrio* (Krummenauer et al. 2014b; Ferreira et al. 2015). In fact, vibrio was at a disadvantage in our system, where the concentration was lower than that observed for bacillus even in the WB treatment, where the abundance of this morphotype of bacteria was higher than in other treatments at the end of the experiment. This happens due to the competitive behavior of bacillus for nutrients and space with bacteria of the genus *Vibrio* (Vidal et al. 2018).

Filamentous bacteria are one of the main microorganisms that form microbial aggregates (Liu et al. 2019). The presence of a greater number of filamentous bacteria observed at the end of the experiment in the RB treatment may indicate possible negative impacts on the system management. It is reported that in biofloc systems, the high concentration of these bacteria can make it difficult to control suspended solids and, in extreme cases, cause clogging of the shrimp gills (Hargreaves2013; Khanjani and Sharifnia 2020).

The presence of a high concentration of bacteria in the RB treatment may be related to a greater release of nutrients such as carbon and phosphorus dissolved in the water by fertilization and feed input into the system. This was observed in our results, since RB had a significantly higher concentration of PO_4^{3-} when compared to the CW, BFT, and SB treatments. Regarding carbon availability in water, Pimentel et al. (2022), assessing the effect of different ionic adjustment strategies on the microbial community stoichiometry in a low salinity *P. vannamei* nursery using synbiotic system with rice bran and in a fertilization protocol similar to that used in this study found a trend towards an increase in dissolved carbon concentration over the experimental time in the control treatment, where no ionic adjustment was performed.

Finally, the use of different processed vegetable brans has proven to promote the nitrifying bacteria and the microbial loop development, which is essential for shrimp growth. Therefore, our findings can contribute to the standardization of fertilization strategies in *P. vannamei* intensive nurseries with synbiotic system.

5. Conclusion

The use of rice, wheat, and soybean bran in the synbiotic system fertilization produces a similar effect in the control of nitrogenous compounds throughout the experimental time. However, we recommend the use of rice bran for the fertilization of the synbiotic system, as it provided a shrimp growth and yield similar to the BFT and clear water (CW) systems and higher than synbiotic system using wheat bran as fertilization strategy. Furthermore, the use of rice bran produces a high load of microorganisms, mainly bacillus, into the system.

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Data availability

The data that support the findings of this study are available on request from the corresponding author.

Declarations

Ethics approval and consent to participate

The research undertaken complies with the current animal welfare laws in Brazil. All the authors agree to participate in this experiment.

Consent for publication

All the authors of this article agree to the publication.

Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

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CAPÍTULO III

Effect of different synbiotic fertilizer processing strategies in *Penaeus vannamei* intensive nurseries

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Efeito de diferentes estratégias de processamento de fertilizante simbiótico em berçários intensivos de *Penaeus vannamei*

Resumo expandido

No sistema simbiótico, o farelo vegetal usado como fonte de carbono orgânico pode ser processado por fases de fermentação e respiração microbiana para quebrar os compostos complexos presentes no farelo. A fermentação é uma fase anaeróbica onde mudanças químicas ocorrem em um substrato orgânico por meio da ação de enzimas produzidas por microrganismos. A respiração microbiana é uma fase aeróbica onde um substrato orgânico é oxidado por microrganismos para liberar energia. Estudos demonstram que o uso de ao menos uma dessas fases de processamento é obrigatória no processo de fertilização do sistema simbiótico. Apesar das pesquisas reportarem bons resultados de controle de compostos nitrogenados e crescimento do camarão, diferentes estratégias de processamento do farelo vegetal são empregadas. Os processos de fermentação e respiração são usados separados ou juntos com variados períodos, que podem variar entre 12 e 48 horas. A falta de consenso neste tópico destaca a necessidade de esclarecimentos sobre qual fase e tempo de processamento proporcionam um controle mais eficaz de compostos nitrogenados e uma comunidade microbiana mais diversificada, otimizando o manejo de fertilização do sistema simbiótico. Portanto, o objetivo deste estudo foi avaliar o efeito dos processos de fermentação (F; fase sem aeração) e respiração (R; fase com aeração), bem como dos diferentes tempos de processamento do fertilizante do sistema simbiótico, no processo de nitrificação, composição do plâncton e crescimento de *Penaeus vannamei* durante a fase de berçário comparando-o com um sistema de bioflocos. Um berçário com densidade de estocagem de 2.400 camarões m^{-3} foi conduzido durante 48 dias compreendendo os seguintes tratamentos (com três repetições cada): CW: água clara (controle); BFT: sistema de bioflocos; F12: fermentação por 12 h; F12 + R12: fermentação por 12 h + respiração por 12 h; F24: fermentação por 24 h; F24 + R24: fermentação por 24 h + respiração por 24 h; R12: respiração por 12 h; R24: respiração por 24 h. No tratamento BFT, melaço foi usado como fonte de carbono orgânico, sendo aplicado em uma razão C:N de 15:1. No tratamento CW, 90% do volume da unidade experimental foi trocado diariamente. Nos tratamentos que testaram o sistema simbiótico, o fertilizante foi composto de farelo de arroz, um probiótico comercial em pó composto por *Bacillus subtilis* e *Bacillus licheniformis*, bicarbonato de sódio como tampão, melaço e água. Nos tratamentos CW e BFT, probiótico foi aplicado

semanalmente na concentração de 0,8 g m^{-3}. Os tratamentos F12 + R12 e R24 demonstraram controle mais rápido do nitrogênio amoniacal total (TAN) (Figura 1).

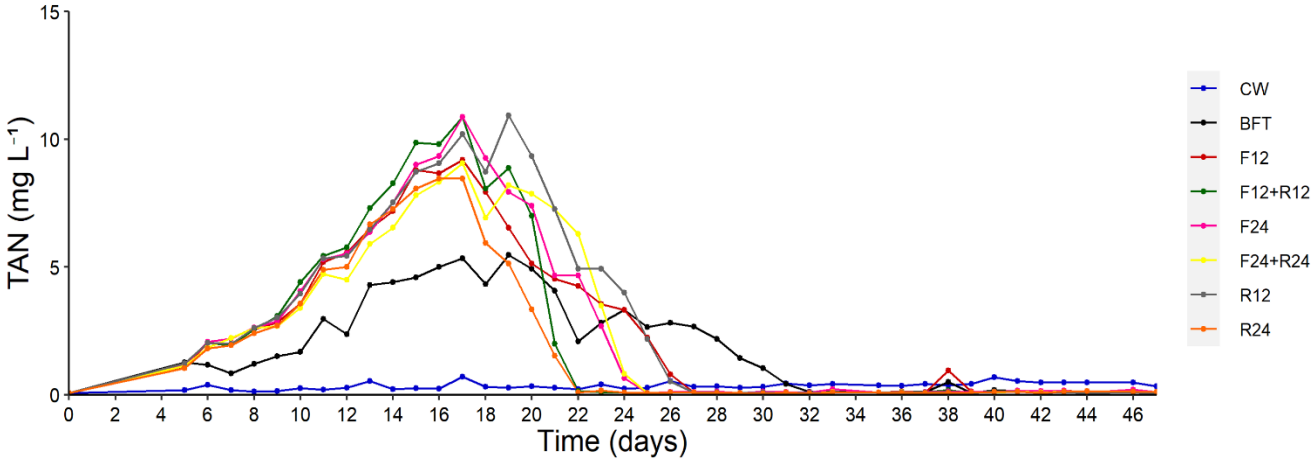

Figura 1. Concentração de TAN durante um berçário de *Penaeus vannamei* com sistema simbiótico utilizando diferentes estratégias de processamento do fertilizante, sistemas de bioflocos e de água clara. CW: água clara (controle); BFT: sistema de bioflocos; F12: fermentação por 12 h; F12 + R12: fermentação por 12 h + respiração por 12 h; F24: fermentação por 24 h; F24 + R24: fermentação por 24 h + respiração por 24 h; R12: respiração por 12 h; R24: respiração por 24 h.

Uma dominância de ameba foi observada nos tratamentos BFT, F12, F12 + R12 e F24. Os tratamentos F12 + R12, F12 e F24 + R24 exibiram maior abundância de ciliados em comparação com CW, BFT, F24 e R12 no final do período experimental (Figura 2a). A abundância de vibrio ao final do ensaio foi maior no tratamento F24 + R24 do que nos demais tratamentos (Figura 2b).

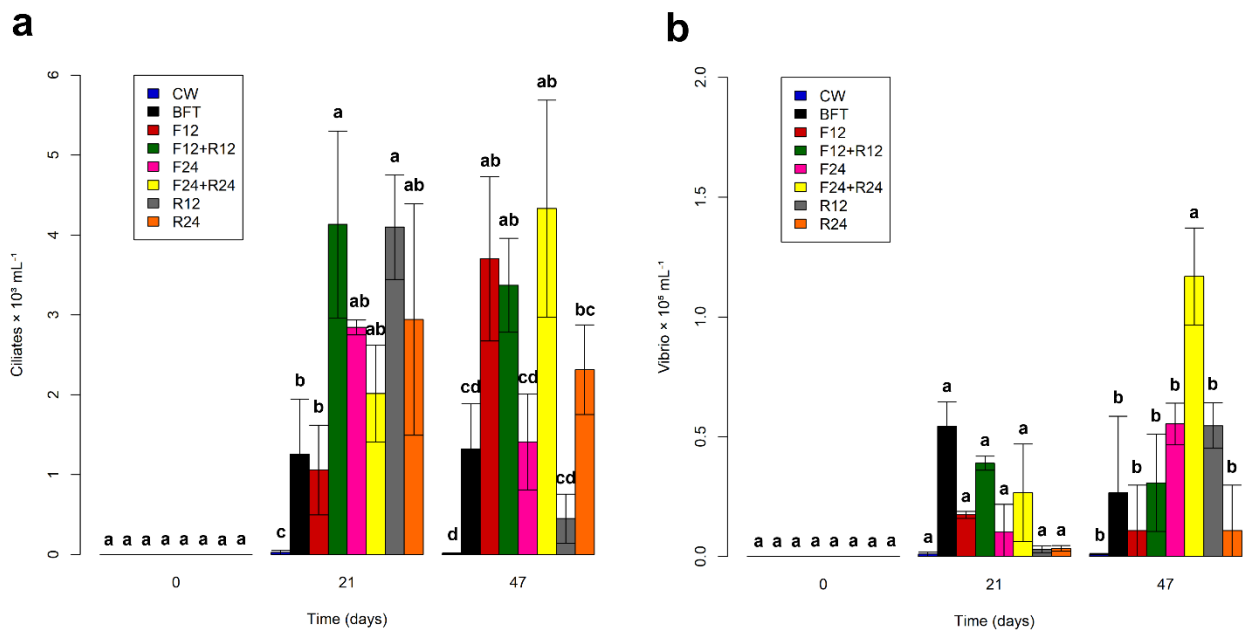

Figura 2. Abundância (organismos mL^{-1}, média ± desvio padrão) de ciliados (a) e vibrio (b) durante um berçário de *Penaeus vannamei* com sistema simbiótico utilizando diferentes estratégias de processamento do fertilizante, sistemas de bioflocos e de água clara. CW: água clara (controle); BFT: sistema de bioflocos; F12: fermentação por 12 h; F12 + R12: fermentação por 12 h + respiração por 12 h; F24: fermentação por 24 h; F24 + R24: fermentação por 24 h + respiração por 24 h; R12: respiração por 12 h; R24: respiração por 24 h.

Ao final do ensaio, os tratamentos F12, F12 + R12, F24 e R12 apresentaram FCA menor que o tratamento CW. A produtividade foi maior no F12, F12 + R12, F24, R12 e R24 quando comparado ao tratamento CW. Os resultados sugerem que a redução do tempo de processamento do fertilizante é viável para a fertilização do sistema simbiótico, otimizando o manejo do sistema. Ambas as fases de fermentação e respiração são igualmente importantes e, portanto, o tratamento F12 + R12 emergiu como a estratégia de fertilização mais eficaz, exibindo efeitos significativos na taxa de conversão alimentar do camarão, no rendimento e na composição do plâncton do sistema.

Effect of different synbiotic fertilizer processing strategies in *Penaeus vannamei* intensive nurseries

Abstract

The aim of this study was to assess the effect of fermentation (F; a phase without aeration) and respiration (R; a phase with aeration) processes, as well as varying processing times of the synbiotic system fertilizer, on the nitrification process, plankton composition, and the growth of *Penaeus vannamei* during the nursery phase comparing it with a biofloc system. A trial with a stocking density of 2400 shrimp m^{-3} was conducted over 48 days comprising the following treatments (with three repetitions each): CW: clear water (control); BFT: biofloc system; F12: fermentation for 12 h; F12 + R12: fermentation for 12 h + respiration for 12 h; F24: fermentation for 24 h; F24 + R24: fermentation for 24 h + respiration for 24 h; R12: respiration for 12 h; R24: respiration for 24 h. Rice bran, a commercial powder probiotic consisting of *Bacillus subtilis* and *Bacillus licheniformis*, sodium bicarbonate as a buffer, molasses, and water were utilized in the fertilizer. Treatments F12 + R12 and R24 demonstrated a more rapid control of TAN. A dominance of amoeba was noted in BFT, F12, F12 + R12, and F24 treatments. Treatments F12 + R12, F12, and F24 + R24 exhibited a higher abundance of ciliates compared to CW, BFT, F24, and R12 at the end of the experimental period. The abundance of vibrio at the end of the experiment was higher in the F24 + R24 treatment than in the other treatments. By the end of the trial, treatments F12, F12 + R12, F24, and R12 showed a lower FCR than the CW treatment. Yield was higher in F12, F12 + R12, F24, R12, and R24 compared to the CW treatment. Our findings suggest that reducing fertilizer processing time is viable for synbiotic system fertilization, optimizing the system management. Both fermentation and respiration phases are equally crucial, and therefore, the F12 + R12 treatment emerged as the most effective fertilization strategy, exhibiting significant effects on shrimp FCR, yield, and plankton composition of the system.

Keywords: aerobic; anaerobic; fermentation; respiration; plankton, nitrification.

1. Introduction

The Synbiotic system is characterized by utilizing vegetable bran (e. g., wheat and rice bran) processed by probiotic microorganisms (e.g., *Bacillus* and yeasts) as a supplementary organic carbon source for system fertilization (Kawahigashi, 2018; Khanjani et al., 2023). The addition of an organic carbon source to the system stimulates the assimilation and oxidation of toxic nitrogenous compounds [un-ionized ammonia (NH_3) and nitrite (NO_2^-)], generated through daily feed intake and animal excretion, by heterotrophic and nitrifying bacteria (Avnimelech, 2012; Samocha, 2019). This process ensures control and stabilization of the concentration of NH_3 and NO_2^-, which are essential water quality variables for successful culture (Robles-Porchas et al., 2020). Therefore, the greater the amount of labile carbon available in the water for microorganisms, the better the control of nitrogenous compounds (Serra et al., 2015). Vegetable bran has more complex components that require processing before being applied to the system (Ekasari et al., 2014). The processing of these brans can be carried out through fermentation and microbial respiration with the aim of improving total ammonia removal and producing nutritious microbial aggregates (Romano et al., 2018).

Fermentation is a process in which chemical changes occur in an organic substrate through the action of enzymes produced by microorganisms (Zhang and Cai, 2014). This process can be employed to enhance the quality of vegetable bran, thereby increasing its nutritional value (Wizna et al., 2012). The inoculation of probiotic microorganisms can contribute to the degradation of complex compounds, such as polysaccharides and crude fiber, into more labile forms. This results in enzyme production, changes in fatty acid composition, an increase in protein content, and an elevation in amino acid content (Silveira and Furlong, 2007; Oliveira et al., 2011; Wizna et al., 2012). Through this process, microorganisms utilize these nutrients and convert them into microbial protein (Khanjani et al., 2022).

Microbial cellular respiration is an aerobic process in which organic substrates are oxidized by microorganisms to release energy (Kirchman, 2008). In this process, microorganisms obtain energy through the breakdown of the glucose molecule ($C_6H_{12}O_6$) to produce Adenosine Triphosphate (ATP) for various cellular functions (Tortora, 2017). During the respiration process, oxygen (O_2) is consumed, and carbon dioxide (CO_2) is produced (Kirchman, 2008; Tortora, 2017). Under aerobic conditions, the energy made available from a glucose molecule is much greater than that released through the fermentation process (Madigan et al., 2019). Therefore, the respiration

process may be significant for the processing of the bran used for the fertilization of the synbiotic system.

As an example of the results of these processes on vegetable brans, Romano et al. (2018) compared the effect of fermentation and respiration processes on rice bran for 24 h. After this period, they observed an 11.12 % increase in protein content in the bran subjected to the respiration process and a 5.07 % increase in the bran subjected to the fermentation process. Similarly, they found a 14.79 % increase in lipid content in bran processed by respiration and a 10.54 % increase in bran processed by fermentation. Additionally, Abdel-Tawwab et al. (2022) utilized rice bran fermented by *Bacillus subtilis* in feeding *P. vannamei* and found, after 24 h of fermentation, a protein percentage of 19.8 % and a lipid percentage of 14.8 %.

The use of a synbiotic system fertilized with vegetable bran subjected to fermentation and respiration processes by probiotic microorganisms has been discussed in several studies focusing on intensive marine shrimp culture during the nursery and grow-out phases. These studies have demonstrated satisfactory results in terms of growth, survival, and control of nitrogen compounds throughout the culture period (Kawahigashi, 2018; Liñan-Vidriales et al., 2020; Lima et al., 2021; Silva et al., 2021a; Andrade et al., 2021; Silva et al., 2021b; Abdel-Tawwab et al., 2022). However, these studies employed different strategies for processing vegetable bran before its application in the system, incorporating varied periods and processing phases (i.e., fermentation and respiration). For instance, in a nursery system lasting 45 days, Silva et al. (2021b) tested the effect of adding the rotifer *Brachionus plicatilis* and *Navicula* sp. on the growth of *P. vannamei*, using wheat bran processed by fermentation and microbial respiration for periods of 48 and 24 h, respectively. The study found survival rates above 90% in all treatments and a yield exceeding 2 Kg m^{-3}. Similarly, Pimentel et al. (2022) explored the effect of different ionic adjustment strategies in oligohaline water on the growth of *P. vannamei*, utilizing rice bran processed by fermentation and microbial respiration for a period of 24 h for each phase. The results indicated good outcomes in terms of survival and control of nitrogenous compounds.

Thus, the lack of consensus regarding the use of different processing phases and periods highlights the need for clarification on this topic. Understanding which phase and processing time yield effective control of nitrogenous compounds and foster a more diverse microbial community is crucial for optimizing the organic fertilization management of the synbiotic system. Consequently, the aim of this study was to assess the effect of fermentation (F; a phase without

aeration) and respiration (R; a phase with aeration) processes, as well as varying processing times of the synbiotic system fertilizer, on the nitrification process, plankton composition, and growth of *Penaeus vannamei* during the nursery phase, and to compare these results with the biofloc system.

2. Materials and methods

A *Penaeus vannamei* nursery was conducted for 48 days at the "Estação Marinha de Aquacultura" of the "Universidade Federal do Rio Grande – FURG", Brazil.

2.1. Design and experimental conditions

Post-larvae of *P. vannamei* (0.01 ± 0.001 g) were stocked at a density of 2400 shrimp m^{-3} in experimental units with a useful volume of 50 L featuring constant aeration and controlled temperature (~29 C; Roxin Q5 heater, 300 W). The seawater used underwent prior filtration, chlorination (10 g m^{-3}), and dechlorination with the addition of ascorbic acid (1 g m^{-3}).

Eight treatments were established, each with three repetitions, in a completely randomized experimental design (Table 1). In the CW treatment, daily water changes were conducted at a rate of 90% of the useful volume of the experimental unit. The water used for these changes was previously chlorinated and dechlorinated.

Table 1. Experimental design of the *Penaeus vannamei* nurseries with synbiotic using different fertilizer processing strategies, biofloc, and clear water systems.

CW	Clear water (control)
BFT	Biofloc system
F12	Fermentation for 12 hours
F12+R12	Fermentation for 12 hours + respiration for 12 hours
F24	Fermentation for 24 hours
F24+R24	Fermentation for 24 hours + respiration for 24 hours
R12	Respiration for 12 hours
R24	Respiration for 24 hours

2.2. Water fertilization

The fertilization process of the experimental units commenced with the stocking of animals and was sustained throughout the experimental period with four applications per week. The fertilizer used for the treatments employing different fermentation and respiration periods had the

following composition, adapted from Kawahigashi (2018) and Pimentel et al. (2022): rice bran (< 300 μm; 20 g m^{-3}), probiotic (0.2 g m^{-3}; sanolife PRO W - Inve Aquaculture. Composition: *Bacillus subtilis* and *Bacillus licheniformis*. Concentration: 5.0×10^{10} colony forming units g^{-1}), molasses (2 g m^{-3}), sodium bicarbonate (2 g m^{-3}), and seawater (chlorinated and dechlorinated) in the proportion of 10 times the amount of rice bran. The fertilizer was processed according to the periods (12 h, 24 h, 12 h + 12 h, and 24 h + 24 h) and processing phases (fermentation, respiration, and fermentation + respiration) determined by each treatment.

In the BFT treatment, molasses served as an organic carbon source with a carbon:nitrogen ratio of 15:1 to regulate total ammonia nitrogen (TAN, Ebeling et al., 2006; Avnimelech, 2012). Molasses was administered to the experimental units when the TAN reached 1 mg L^{-1}.

For both the BFT and CW treatments, probiotic (Sanolife PRO W - Inve Aquaculture) was applied weekly in the experimental units at a concentration of 0.8 g m^{-3}. This standardization ensured that all treatments received the same quantity of probiotic.

2.3. Water quality variables

Twice daily, dissolved oxygen (DO, mg L^{-1}) and temperature (°C; YSI EcoSense DO200A) were measured. Daily assessments included pH (Mettler Toledo seven2Go), total ammonia nitrogen (TAN, mg L^{-1}) (UNESCO, 1983), and nitrite nitrogen (NO_2^--N, mg L^{-1}) (Aminot and Chaussepied, 1983). In the event that the NO_2^--N concentration surpassed the safe level (Lin and Chen, 2003), water changes were performed at a rate of 30% of the useful volume of the experimental unit.

On a weekly basis, nitrate nitrogen (NO_3^--N, mg L^{-1}) (García Robledo et al., 2014), orthophosphate (PO_4^{3-}, mg L^{-1}) (Aminot and Chaussepied, 1983), settleable solids (SS, mL L^{-1}) (Eaton et al., 1995), total suspended solids (TSS, mg L^{-1}) (Strickland and Parsons, 1972), salinity (g L^{-1}), and carbon dioxide (CO_2, mg L^{-1}) (Timmons and Ebeling, 2013) were analyzed. Total alkalinity (mg L^{-1}; APHA, 2012) was measured twice a week. Adjustments to the alkalinity concentration, when necessary, were made to reach 150 mg L^{-1} through the application of sodium bicarbonate ($NaHCO_3$), following Furtado et al. (2011). Additionally, water changes were performed as needed to adjust TSS concentration up to 500 mg L^{-1} (Gaona et al., 2011).

2.4. Plankton composition

To quantify and identify the primary groups of microorganisms in the system, water samples were collected from the experimental units on days 0, 21, and 47 of the experimental period. These samples were preserved with formalin at a final concentration of 4%.

The quantification and identification of the primary phytoplankton and zooplankton groups were conducted by counting 30 random fields in an inverted optical microscope (Nikon, Eclipse TS100) at a final magnification of 200×, using a sedimentation chamber (Utermöhl, 1958). Microorganisms' abundance was expressed in organisms mL^{-1}.

For the quantification and identification of bacterioplankton, samples were filtered through a polycarbonate membrane filter with a mean retention of 0.2 μm, previously darkened with Irgalan black, and stained with acridine orange (Hobbie et al., 1977). Bacteria were photographed using a camera coupled to an epifluorescence microscope (Axioplan Zeiss™) with a final magnification of 1000× for subsequent counting of 20 random fields and determination of the main morphotypes (coccoids, bacillus, free and attached filamentous bacteria, vibrio, and prosthecate). Bacterioplankton community abundance was expressed in organisms mL^{-1}.

2.5. Feed management

Throughout the experimental period, the animals were fed three times a day with a commercial feed containing 40% crude protein (Guabitech PL and Guabitech J). The quantity of feed offered was determined in accordance with Jory et al. (2001).

2.6. Shrimp growth

Shrimp growth performance was assessed at the conclusion of the experiment to determine the following parameters: final weight (g), survival (%) [(number of animals at the end of experimental period/ initial number of animals) × 100], feed conversion ratio (FCR) [(feed supplied/biomass gain)], specific growth rate (SGR, % day^{-1}) calculated as 100 × [(ln final weight (g) – ln initial weight (g))/experimental period (days)] and yield (Kg m^{-3}) calculated as [final biomass (Kg)/ volume of the experimental unit (m^3)].

2.7. Data analysis

Data underwent testing for normality using the Shapiro-Wilk test and homoscedasticity using the Levene test. For water quality variables, a repeated measures analysis of variance test (ANOVA) was applied, followed by the Tukey test to assess differences among treatments. When necessary, data were transformed (temperature morning and afternoon, pH, CO_2, PO_4^{3-}, and SS)

to fulfill parametric assumptions. For variables that did not meet the assumptions of normality and homoscedasticity (DO morning and afternoon, TAN, NO_2^--N, and NO_3^--N), the Friedman test followed by the Conover multiple comparison test with Bonferroni correction was applied to assess significant differences among treatments.

For the abundance of microorganisms (separated by sampling time: days 0, 21, and 47) and shrimp growth data, one-way ANOVA was applied. Prior to ANOVA, data were tested for normality and homoscedasticity. When ANOVA was significant (p-value < 0.05), the Tukey test was applied to identify differing treatments. Data were transformed (coccoids – day 21, vibrio – day 21, flagellates – day 47, free filamentous bacteria – day 47, ciliates – day 21, prosthecate bacteria – day 21, attached filamentous bacteria – day 47, bacillus - day 21, SGR, and survival) as needed to fulfill parametric assumptions. When data did not meet assumptions of normality and homoscedasticity (Bacillariophytes – day 21; flagellates – day 0; amoeba – days 21 and 47; nematodes, coccoids, bacillus, and prosthecates – day 47; free and attached filamentous bacteria – day 21), the Kuskal-Wallis test followed by Dunn's comparison test with Bonferroni correction was applied to assess significant differences among treatments.

Graphs, one-way ANOVA, Kruskal-Wallis, Friedman, and their post hoc analyses were conducted in the R software version 4.2.3 (R core team, 2023) using following packages: car (Fox and Weisberg, 2019), dunn.test (Dinno, 2017), PMCMRplus (Pohlert, 2022), ggplot2 (Wickham, 2016), and Rmisc (Hope, 2022). Repeated measures ANOVA and its post hoc analysis were performed using Past 4.03 2020 software (Hammer et al., 2001).

3. Results

3.1. Water quality variables

Temperature in the morning ranged between 27 and 28 °C across all treatments (Table 2). In the afternoon, the temperature was 28 °C in all treatments (Table 2). Dissolved oxygen levels were maintained above 5 mg L^{-1} in all treatments during both the morning and afternoon periods (Table 2).

Total ammonia nitrogen (TAN) was lower in the R24 treatment compared to the F12, F12+R12, F24, and R12 treatments (Table 2). R24 and BFT showed the lowest TAN variation over the experimental period (excluding CW treatment; Figure 1a). Both treatments reached a maximum concentration of 9.80 mg L^{-1} (Table 2). A reduction in TAN was observed between

days 17 and 27 of the experimental period in treatments F12, F12+R12, F24, F24+R24, R12, and R24 (Figure 1a). However, treatments R24 and F12+R12 showed a faster reduction in TAN concentration than the other treatments (Figure 1a).

Nitrite nitrogen (NO_2^--N) was higher in the R24 treatment compared to the CW and BFT treatments (Table 2). Among the treatments using different synbiotic fertilizer processing strategies, the F12 treatment showed the lowest variation over the experimental period (Table 2). A reduction trend in NO_2^--N concentration was observed in the BFT, F12, F12+R12, F24, F24+R24, R12, and R24 treatments from the 45th day of culture (Figure 1b).

No significant differences among treatments were observed for NO_3^--N. However, from the 40th day of culture (week 6), an increase in NO_3^--N concentration was observed in all treatments, except for the CW treatment (Figure 1c).

Orthophosphate (PO_4^{3-}) was higher in the F12+R12, F24, and F24+R24 treatments than in the CW and BFT treatments (Table 2). Carbon dioxide (CO_2) was higher in the BFT, F12, F12+R12, F24+R24, and R12 treatments than in the CW treatment (Table 2). Settleable solids (SS) and total suspended solids (TSS) were lower in the CW treatment when compared to the other treatments (Table 2).

Table 2. Water quality variables during *Penaeus vannamei* nurseries with synbiotic using different fertilizer processing strategies, biofloc, and clear water systems.

Variables	Treatments							
	CW	BFT	F12	F12+R12	F24	F24+R24	R12	R24
Temperature morning (°C)	28.29 ± 1.59[a]	27.93 ± 1.94[a]	28.37 ± 1.82[a]	28.04 ± 2.13[a]	27.29 ± 2.83[a]	28.07 ± 1.71[a]	27.94 ± 2.42[a]	27.57 ± 1.77[a]
	28.77	28.38	29.11	28.80	28.34	28.48	28.83	28.13
	(23.20 – 29.89)	(21.60 – 30.13)	(21.50 – 30.10)	(22.30 – 30.36)	(21.00 – 30.39)	(22.80 – 29.94)	(20.70 – 30.29)	(22.10 – 29.90)
Temperature afternoon(°C)	28.68 ± 1.14[a]	28.74 ± 1.21[a]	28.76 ± 1.19[a]	28.82 ± 1.23[a]	28.41 ± 1.77[a]	28.84 ± 1.17[a]	28.87 ± 1.41[a]	28.90 ± 1.08[a]
	29.10	29.40	29.26	29.29	29.14	29.25	29.49	29.33
	(26.10 – 30.01)	(25.60 – 30.14)	(24.80 – 30.01)	(26.40 – 30.47)	(24.40 – 30.11)	(26.34 – 30.31)	(25.20 – 30.94)	(26.90 – 30.27)
DO morning (mg L^{-1})	5.62 ± 0.18[a]	5.66 ± 0.23[a]	5.63 ± 0.20[a]	5.64 ± 0.37[a]	5.67 ± 0.31[a]	5.71 ± 0.28[a]	5.65 ± 0.36[a]	5.73 ± 0.34[a]
	5.56	5.60	5.57	5.53	5.63	5.63	5.57	5.63
	(5.32 – 6.05)	(5.33 – 6.37)	(5.38 – 6.40)	(5.30 – 6.87)	(5.40 – 6.48)	(5.40 – 6.80)	(5.32 – 6.74)	(5.41 – 6.90)
DO afternoon (mg L^{-1})	5.62 ± 0.20[a]	5.54 ± 0.19[a]	5.62 ± 0.19[a]	5.54 ± 0.22[a]	5.62 ± 0.27[a]	5.61 ± 0.18[a]	5.52 ± 0.23[a]	5.51 ± 0.17[a]
	5.64	5.51	5.62	5.55	5.52	5.63	5.52	5.51
	(5.27 – 5.97)	(5.22 – 6.02)	(5.36 – 6.14)	(5.17 – 5.88)	(5.23 – 6.24)	(5.35 – 5.92)	(5.17 – 6.06)	(5.23 – 5.80)
pH	8.37 ± 0.06[a]	8.33 ± 0.09[a]	8.33 ± 0.11[a]	8.32 ± 0.10[a]	8.33 ± 0.10[a]	8.32 ± 0.11[a]	8.34 ± 0.11[a]	8.35 ± 0.09[a]
	8.37	8.34	8.34	8.30	8.36	8.32	8.38	8.34
	(8.22 – 8.47)	(8.16 – 8.46)	(8.11 – 8.48)	(8.09 – 8.45)	(8.14 – 8.47)	(7.99 – 8.46)	(8.15 – 8.52)	(8.19 – 8.48)
Salinity (g L^{-1})	34.45 ± 0.95[a]	34.39 ± 1.13[a]	34.25 ± 0.81[a]	34.74 ± 0.83[a]	34.51 ± 0.92[a]	34.01 ± 1.41[a]	34.51 ± 1.43[a]	34.09 ± 1.33[a]
	34.78	34.53	34.31	34.94	34.62	34.44	34.94	34.27
	(32.76 – 35.61)	(32.46 – 37.18)	(32.19 – 35.47)	(33.16 – 36.22)	(32.15 – 36.51)	(30.00 – 35.64)	(29.79 – 35.94)	(31.46 – 36.36)
TAN (mg L^{-1})	0.35 ± 0.15[ab]	1.88 ± 2.39[ab]	2.53 ± 3.56[a]	2.40 ± 3.63[a]	2.59 ± 3.76[a]	2.47 ± 3.53[ab]	2.91 ± 4.16[a]	1.92 ± 2.87[b]
	0.33	0.28	0.14	0.12	0.18	0.13	0.12	0.11
	(0.07 – 0.90)	(0.04 – 9.80)	(0.05 – 12.60)	(0.04 – 13.60)	(0.04 – 14.60)	(0.04 – 14.60)	(0.02 – 14.60)	(0.02 – 9.80)
NO_2^--N (mg L^{-1})	0.11 ± 0.15[c]	10.94 ± 10.58[b]	11.91 ± 10.79[ab]	12.39 ± 10.72[ab]	12.66 ± 11.17[ab]	12.13 ± 11.01[ab]	12.27 ± 11.13[ab]	13.57 ± 11.32[a]
	0.04	9.50	15.00	16.00	17.00	15.00	15.50	18.00
	(0.00 – 1.15)	(0.00 – 28.00)	(0.00 – 30.00)	(0.00 – 33.00)	(0.00 – 40.00)	(0.00 – 38.00)	(0.00 – 33.00)	(0.00 – 38.00)
NO_3^--N (mg L^{-1})	0.29 ± 0.30[a]	2.80 ± 4.33[a]	4.45 ± 10.25[a]	6.31 ± 11.27[a]	3.46 ± 4.76[a]	2.73 ± 4.92[a]	2.44 ± 3.84[a]	2.42 ± 2.98[a]
	0.18	0.12	0.56	2.50	1.50	0.12	0.12	0.56
	(0.06 – 1.57)	(0.00 – 16.00)	(0.00 – 49.00)	(0.00 – 47.00)	(0.00 – 18.00)	(0.00 – 19.00)	(0.00 – 15.00)	(0.00 – 8.00)
PO_4^{3-} (mg L^{-1})	0.23 ± 0.27[c]	1.17 ± 1.36[b]	1.73 ± 1.27[ab]	1.94 ± 1.67[a]	1.89 ± 1.23[a]	2.22 ± 1.79[a]	1.74 ± 1.53[ab]	1.56 ± 1.09[ab]
	0.13	0.39	1.47	1.65	1.75	1.70	1.23	1.35
	(0.01 – 0.90)	(0.05 – 4.70)	(0.08 – 5.00)	(0.08 – 7.00)	(0.08 – 4.35)	(0.08 – 7.80)	(0.08 – 6.60)	(0.08 – 3.90)

Alkalinity (mg L^{-1})	136.90 ± 11.94[a]	140.40 ± 31.47[a]	135.70 ± 27.62[a]	133.90 ± 29.08[a]	136.10 ± 30.83[a]	137.50 ± 24.77[a]	137.40 ± 29.16[a]	134.30 ± 24.53[a]
	140.00	145.00	132.50	135.00	137.50	135.00	137.50	130.00
	(115.00 – 165.00)	(75.00 – 195.00)	(75.00 – 195.00)	(70.00 – 200.00)	(55.00 – 195.00)	(90.00 – 185.00)	(85.00 – 195.00)	(85.00 – 190.00)
CO_2 (mg L^{-1})	1.24 ± 0.28[b]	1.41 ± 0.26[a]	1.39 ± 0.26[a]	1.38 ± 0.20[a]	1.34 ± 0.32[ab]	1.41 ± 0.31[a]	1.35 ± 0.26[a]	1.29 ± 0.26[ab]
	1.12	1.37	1.37	1.37	1.27	1.34	1.29	1.24
	(0.96 – 2.22)	(0.99 – 2.03)	(0.69 – 2.02)	(1.04 – 1.91)	(0.92 – 2.88)	(1.04 – 2.54)	(0.96 – 2.04)	(0.89 – 2.38)
SS (mL L^{-1})	0.16 ± 0.24[b]	4.03 ± 3.08[a]	3.44 ± 4.33[a]	3.89 ± 4.54[a]	2.82 ± 3.39[a]	2.72 ± 3.30[a]	2.48 ± 2.42[a]	2.28 ± 1.99[a]
	0.00	4.25	2.50	2.45	1.25	1.15	2.00	1.75
	(0.00 – 0.90)	(0.00 – 9.00)	(0.00 – 18.00)	(0.00 – 18.00)	(0.00 – 12.00)	(0.00 – 12.00)	(0.00 – 8.00)	(0.00 – 7.00)
TSS (mg L^{-1})	80.48 ± 58.22[b]	207.60 ± 133.76[a]	186.00 ± 109.64[a]	218.80 ± 135.90[a]	176.30 ± 99.37[a]	173.30 ±101.16[a]	170.50 ± 100.60[a]	175.00 ± 93.61[a]
	45.00	225.00	195.00	195.00	190.00	155.00	145.00	180.00
	(20.00 – 230.00)	(35.00 – 510.00)	(35.00 – 425.00)	(35.00 – 530.00)	(35.00 – 340.00)	(35.00 – 350.00)	(35.00 – 340.00)	(35.00 – 345.00)

Data are mean ± standard deviation, median (minimum - maximum). Superscript letters indicate the result of Tukey's test or Conover test. DO: dissolved oxygen; TAN: total ammonia nitrogen; NO_2^--N: nitrite nitrogen; NO_3^--N: nitrate nitrogen; PO_4^{3-}: orthophosphate; CO_2: carbon dioxide; SS: settleable solids; TSS: total suspended solids. CW: clear water (control); BFT: biofloc system; F12: fermentation for 12 hours; F12+R12: fermentation for 12 hours + respiration for 12 hours; F24: fermentation for 24 hours; F24+R24: fermentation for 24 hours + respiration for 24 hours; R12: respiration for 12 hours; R24: respiration for 24 hours.

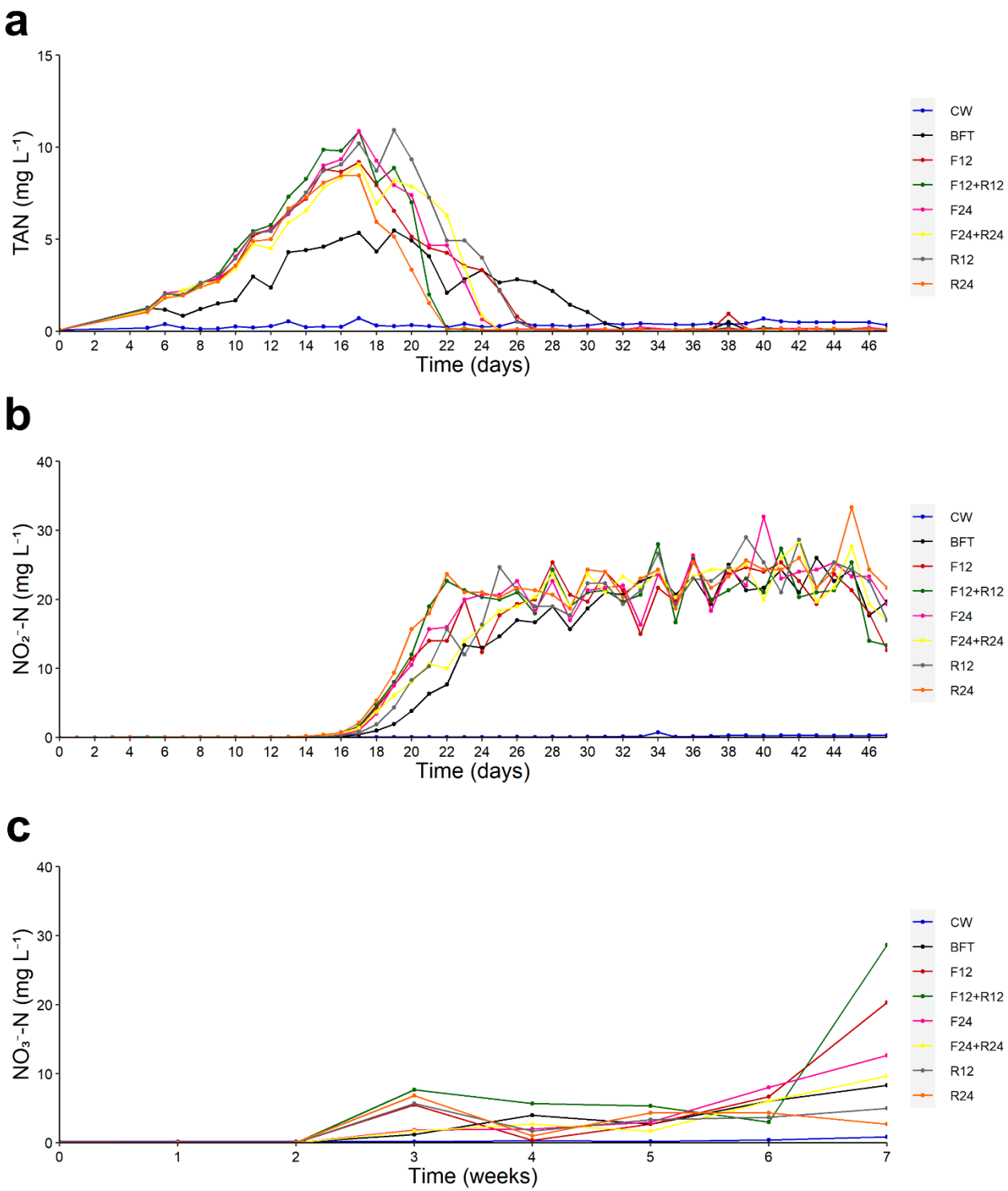

Figure 1. Concentration of TAN (a), NO_2^--N (b) and NO_3^--N (c) during *Penaeus vannamei* nurseries with synbiotic using different fertilizer processing strategies, biofloc, and clear water systems. CW: clear water (control); BFT: biofloc system; F12: fermentation for 12 hours; F12+R12: fermentation for 12 hours + respiration for 12 hours; F24: fermentation for 24 hours;

F24+R24: fermentation for 24 hours + respiration for 24 hours; R12: respiration for 12 hours; R24: respiration for 24 hours.

3.2. Plankton community composition

3.2.1. Phytoplankton

On day 21, the abundance of Bacillariophyta was higher in the R24 treatment than in the CW treatment (Figure 2). On day 47 of the experimental period, the abundance of Bacillariophyta was higher in the F12+R12 treatment compared to the CW, F12, and R12 treatments (Figure 2).

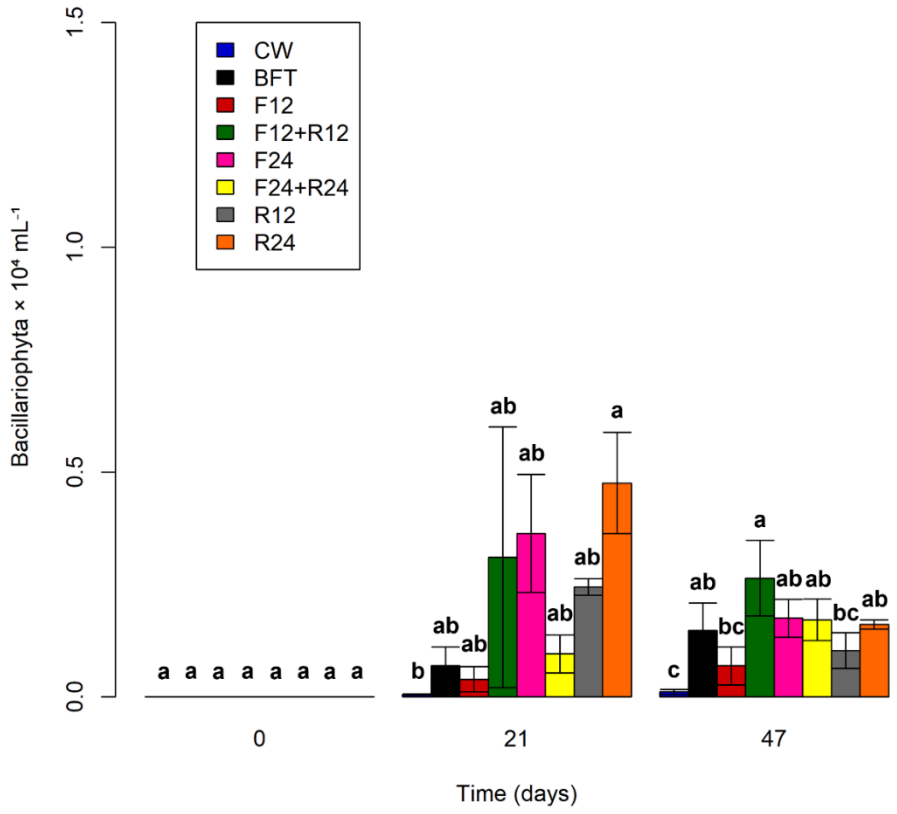

Figure 2. Abundance of Bacillariophyta (organisms mL^{-1}, mean ± standard deviation) during *Penaeus vannamei* nurseries with synbiotic using different fertilizer processing strategies, biofloc, and clear water systems. Superscript letters indicate the result of Tukey's test or Dunn's test. CW: clear water (control); BFT: biofloc system; F12: fermentation for 12 hours; F12+R12: fermentation for 12 hours + respiration for 12 hours; F24: fermentation for 24 hours; F24+R24: fermentation for 24 hours + respiration for 24 hours; R12: respiration for 12 hours; R24: respiration for 24 hours.

3.2.2. Zooplankton

On days 21 and 47 of the experimental period, a higher dominance of amoeba was observed in the BFT, F12, F12+R12, and F24 treatments (Figure 3a). In the R24 treatment, flagellates were the most abundant group on days 21 and 47 of the experiment (Figure 3a). Nematodes were only observed in treatments F12+R12 and F24 at the end of the experimental period (Figure 3a).

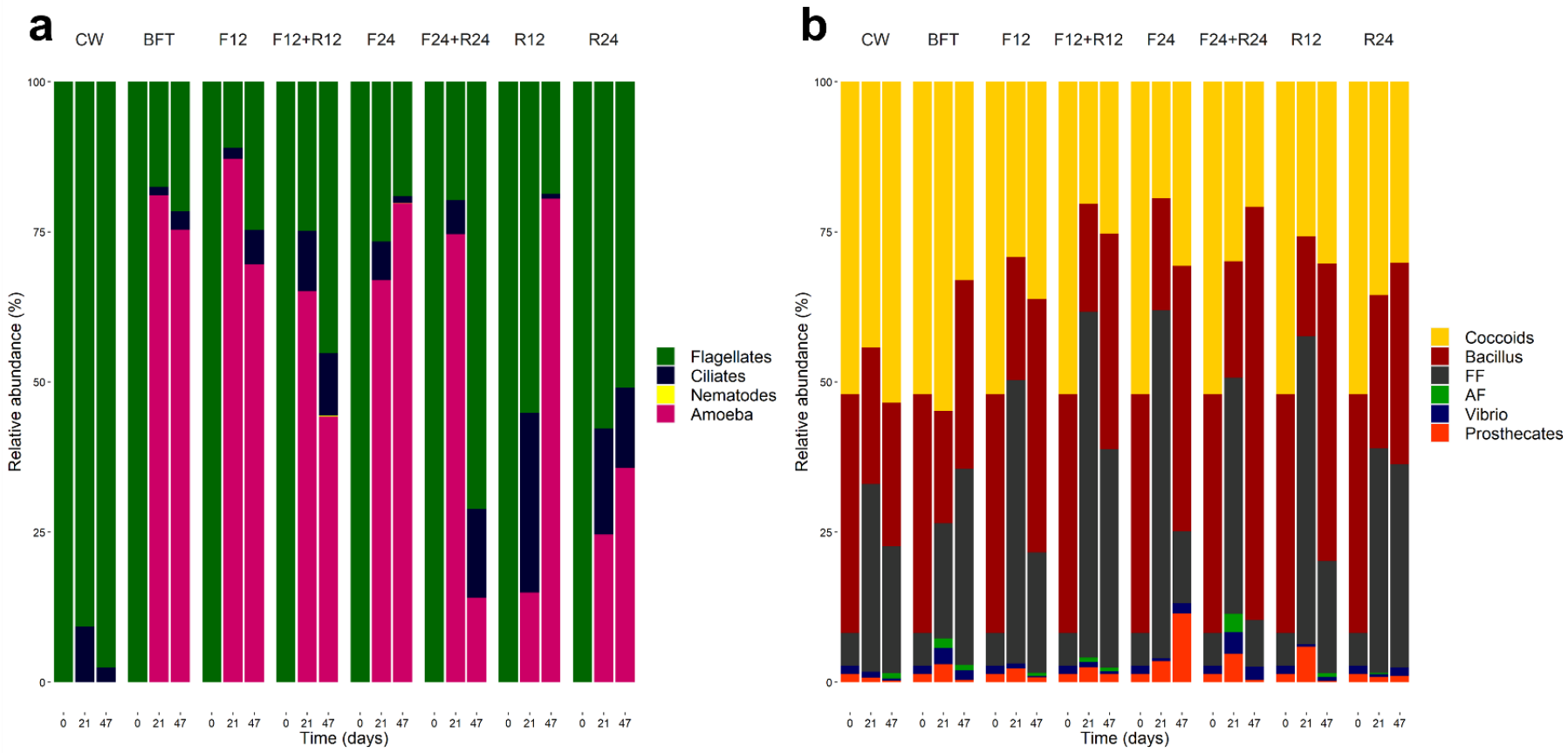

Figure 3. Relative abundance of the main groups of zooplankton (a) and bacterial morphotypes (b) found during *Penaeus vannamei* nurseries with synbiotic using different fertilizer processing strategies, biofloc, and clear water systems. AF: attached filamentous bacteria. FF: free filamentous bacteria. CW: clear water (control); BFT: biofloc system; F12: fermentation for 12 hours; F12+R12: fermentation for 12 hours + respiration for 12 hours; F24: fermentation for 24 hours; F24+R24: fermentation for 24 hours + respiration for 24 hours; R12: respiration for 12 hours; R24: respiration for 24 hours.

On day 21 of the experimental period, the abundance of flagellates in the BFT treatment was higher than in CW, F12, F24+R24, and R12 treatments (Figure 4a). On day 47, the abundance of flagellates was higher in treatments F24 and F24+R24 compared to treatments CW, BFT, R12, and R24 (Figure 4a).

The abundance of ciliates on day 21 of the experiment was higher in treatments F12+R12 and R12 than in treatments CW, BFT, and F12 (Figure 4b). On day 47, treatments F12, F12+R12, and F24+R24 showed a higher abundance of ciliates than treatments CW, BFT, F24, and R12 (Fig. 4b).

On day 47 of the experimental period, the abundance of nematodes was higher in treatments F12+R12 and F24 when compared to treatments CW, BFT, F12, F24+R24, R12, and R24 (Figure 4c).

Amoeba abundance on day 21 of the experimental period was higher in the BFT treatment than in the CW treatments (Figure 4d). On day 47, amoeba abundance was higher in the F24 treatment compared to the CW treatment (Figure 4d).

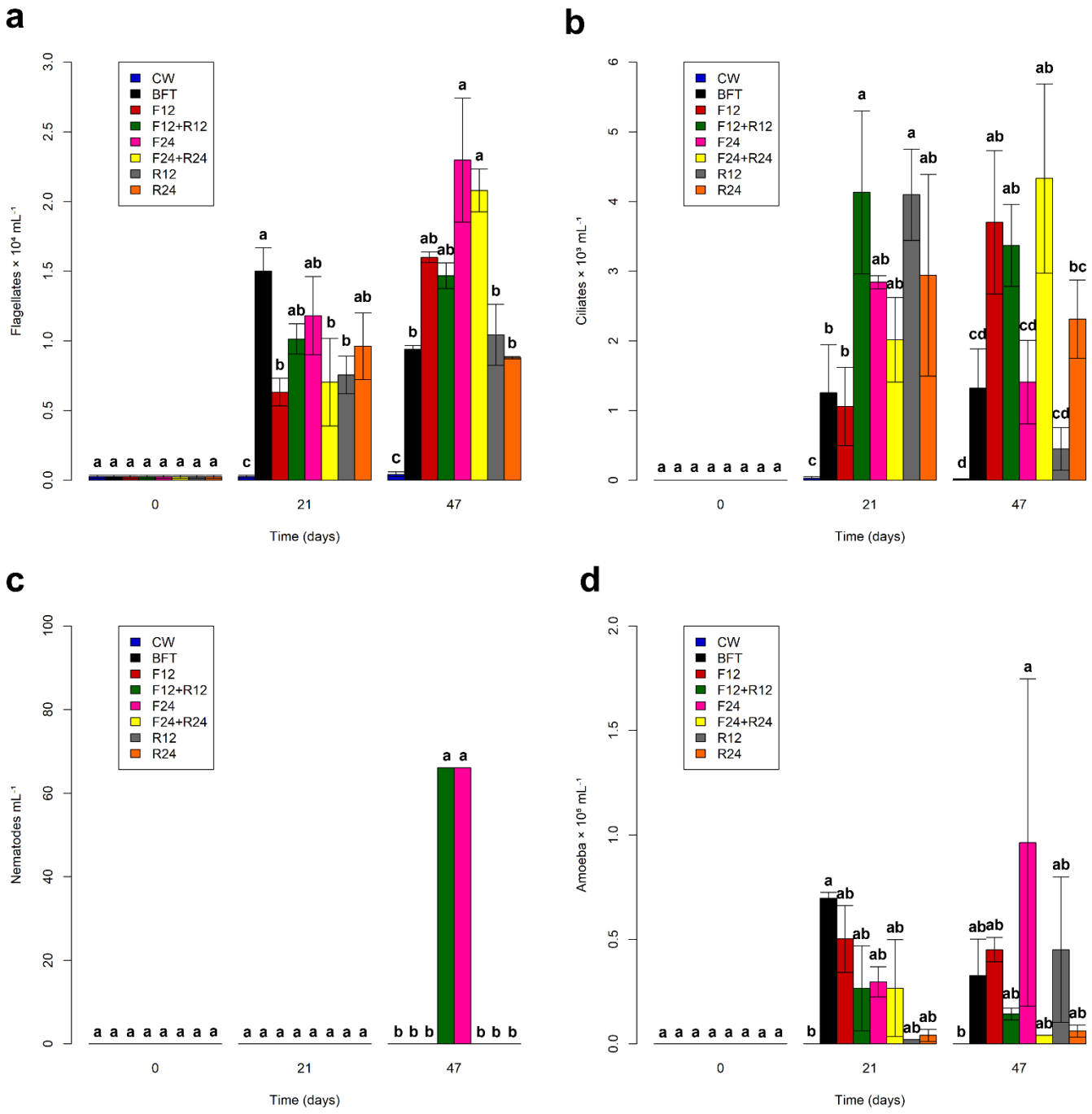

Figure 4. Abundance (organisms mL^{-1}, mean ± standard deviation) of flagellates (a), ciliates (b), nematodes (c) and amoeba (d) during *Penaeus vannamei* nurseries with synbiotic using different fertilizer processing strategies, biofloc, and clear water systems. Superscript letters indicate the result of Tukey's test or Dunn's test. CW: clear water (control); BFT: biofloc system; F12: fermentation for 12 hours; F12+R12: fermentation for 12 hours + respiration for 12 hours; F24: fermentation for 24 hours; F24+R24: fermentation for 24 hours + respiration for 24 hours; R12: respiration for 12 hours; R24: respiration for 24 hours.

3.2.3. Bacterioplankton

Throughout the experimental period, the most abundant bacterial morphotypes were coccoid, bacillus, and free filamentous (Figure 3b). On day 47 of the experimental period, the R12 treatment showed a higher abundance of coccoid bacteria and bacillus than the CW treatment (Figure 5a and b). On day 21, the abundance of free filamentous bacteria was higher in the F12+R12 treatment than in the CW treatment (Figure 5c). On day 47, the abundance of free filamentous bacteria was higher in the F12+R12 treatment than in CW, BFT, F24, F24+R24, and R24 treatments (Figure 5c).

Attached filamentous bacteria, on day 47, showed a higher abundance in treatments F12+R12 and R12 when compared to treatments CW, F24, F24+R24, and R24 (Figure 5d). At the end of the experimental period (day 47), the F24+R24 treatment showed a higher abundance of vibrio than the other treatments (Figure 5e). On day 21 of the experimental period, the abundance of prosthecate bacteria in the F12+R12 treatment was higher than in the CW and R24 treatments (Figure 5f).

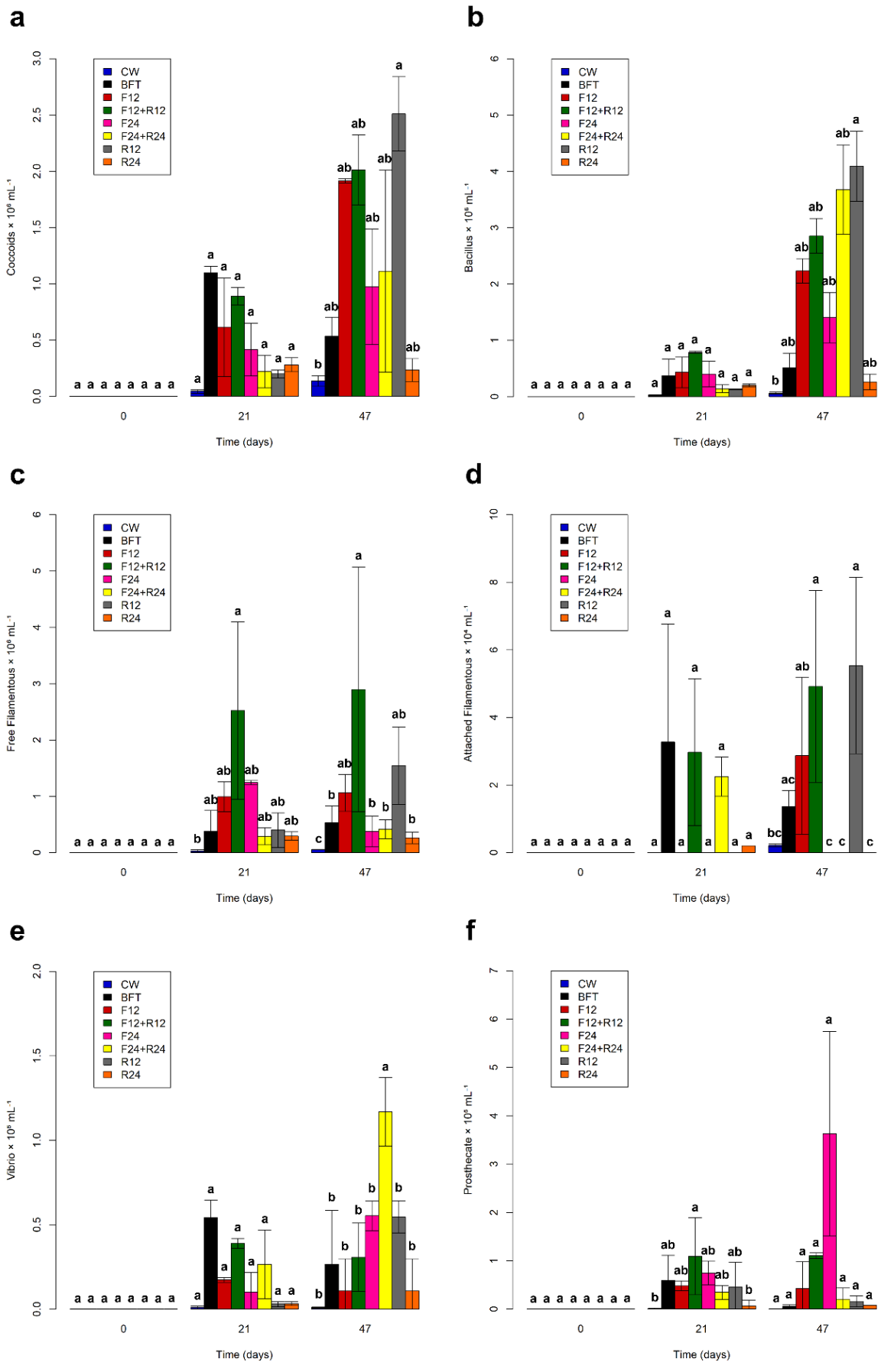
a
b
c
d
e
f
CW
BFT
F12
F12+R12
F24
F24+R24
R12
R24
Coccoids × 10⁶ mL⁻¹
Bacillus × 10⁶ mL⁻¹
Free Filamentous × 10⁶ mL⁻¹
Attached Filamentous × 10⁴ mL⁻¹
Vibrio × 10⁶ mL⁻¹
Prosthecate × 10⁶ mL⁻¹
Time (days)
0
21
47

Figure 5. Abundance (organisms mL^{-1}, mean ± standard deviation) of coccoid (a), bacillus (b), free filamentous (c), attached filamentous (d), vibrio (e) and prosthecate (f) bacteria during *Penaeus vannamei* nurseries with synbiotic using different fertilizer processing strategies, biofloc, and clear water systems. Superscript letters indicate the result of Tukey's test or Dunn's test. CW: clear water (control); BFT: biofloc system; F12: fermentation for 12 hours; F12+R12: fermentation for 12 hours + respiration for 12 hours; F24: fermentation for 24 hours; F24+R24: fermentation for 24 hours + respiration for 24 hours; R12: respiration for 12 hours; R24: respiration for 24 hours.

3.3. Shrimp growth

At the end of the experiment, feed conversion ratio (FCR) was lower in the F12, F12+R12, F24, and R12 treatments compared to the CW treatment (Table 3). Yield was higher in treatments F12, F12+R12, F24, R12, and R24 compared to the CW treatment (Table 3). Survival was higher in F24, R12, and R24 than in the CW treatment (Table 3).

Table 3. Growth of *Penaeus vannamei* at the end of nurseries with synbiotic using different fertilizer processing strategies, biofloc, and clear water systems.

	Treatments							
	CW	BFT	F12	F12+R12	F24	F24+R24	R12	R24
Initial weight (g)	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001
Final weight (g)	1.90 ± 0.46^{a}	1.55 ± 0.21^{a}	1.95 ± 0.08^{a}	1.71 ± 0.11^{a}	1.41 ± 0.05^{a}	1.26 ± 0.19^{a}	1.65 ± 0.20^{a}	1.30 ± 0.04^{a}
Survival (%)	59.58 ± 12.37^{b}	70.83 ± 4.71^{ab}	67.08 ± 0.58^{ab}	79.17 ± 2.35^{ab}	84.44 ± 3.76^{a}	72.78 ± 5.29^{ab}	86.67 ± 10.60^{a}	89.17 ± 12.96^{a}
FCR	1.79 ± 0.21^{a}	1.42 ± 0.10^{ab}	1.25 ± 0.07^{b}	1.25 ± 0.04^{b}	1.34 ± 0.17^{b}	1.56 ± 0.05^{ab}	1.25 ± 0.01^{b}	1.40 ± 0.22^{ab}
SGR (% day^{-1})	11.77 ± 0.47^{a}	11.36 ± 0.30^{a}	11.70 ± 0.27^{a}	11.58 ± 0.13^{a}	11.31 ± 0.23^{a}	11.13 ± 0.40^{a}	11.49 ± 0.26^{a}	11.31 ± 0.52^{a}
Yield (Kg m^{-3})	2.29 ± 0.28^{b}	2.86 ± 0.19^{ab}	3.24 ± 0.19^{a}	3.25 ± 0.11^{a}	3.06 ± 0.38^{ac}	2.41 ± 0.32^{bc}	3.23 ± 0.03^{a}	3.18 ± 0.04^{a}

Data are mean ± standard deviation. Superscript letters indicate the result of Tukey test. FCR: feed conversion ratio; SGR: specific growth rate. CW: clear water (control); BFT: biofloc system; F12: fermentation for 12 hours; F12+R12: fermentation for 12 hours + respiration for 12 hours; F24: fermentation for 24 hours; F24+R24: fermentation for 24 hours + respiration for 24 hours; R12: respiration for 12 hours; R24: respiration for 24 hours.

4. Discussion

Controlling toxic nitrogenous compounds is crucial for the success of intensive shrimp farming systems (Abakari et al., 2021). In this study, the nitrogenous dynamics indicated a quicker establishment of the nitrifying bacteria community in the F12+R12 treatment, as evidenced by a faster reduction of TAN compared to the others and the trend to increase NO_3^--N concentration over the experimental period. In intensive systems, the presence of heterotrophic bacteria is important in the nitrification process, promoting the growth of microbial aggregates that will be colonized by ammonia-oxidizing bacteria (AOB) (Robles-Porchas et al., 2020).

The use of organic carbon at the beginning of the culture was beneficial for controlling total ammonia, both through the action of heterotrophic bacteria, which assimilate ammonia and transform it into microbial biomass, and nitrifying bacteria, which oxidize ammonia to nitrite (Ebeling et al., 2006; Avnimelech, 2012; Robles-Porchas et al., 2020). The fertilizer application throughout the experimental course in this study might not have favored the growth of the nitrite-oxidizing bacteria (NOB) community. NOB has slower growth and reduced activity with increased organic matter in the medium (Abakari et al., 2021). Ebeling et al. (2006) suggest that the percentage of ammoniacal nitrogen removal by autotrophic bacteria decreases with an increase in the carbon:nitrogen (C:N) ratio in the water, indicating that excessive carbon input into the system should be avoided. Therefore, the strategy of fertilizer application until TAN control and stabilization in the system appears to be the most effective and cost-efficient approach for intensive nursery fertilization using a synbiotic system.

Phosphorus accumulation over time is a common occurrence in intensive shrimp production systems as the animals grow and feed increases (Silva et al., 2013). In this study, the concentration of PO_4^{3-} in treatments F12+R12, F24, and F24+R24 was higher than in treatments BFT and CW. This suggests that the rice bran processing strategies used in these treatments provided higher nutrient availability in the system. It also indicates that nutrient cycling is occurring in these treatments, where microorganisms present in the microbial aggregates assimilate and mineralize organic phosphorus, releasing inorganic phosphorus into the system (Luo, 2023). The presence of inorganic phosphorus is crucial for the development of the microbial loop, as it can be easily assimilated by algae and bacteria (Esteves, 2011).

In natural environments, the concentration of nutrients dissolved in water, such as nitrogen and phosphorus, is one of the main factors that regulate phytoplankton productivity (Esteves, 2011). The growth of Bacillariophytes is related to the increase of inorganic phosphorus present in the medium (Finenko and Krupatkina-Akinina, 1974). This relationship

is evident in our study, as Bacillariophyta were more abundant in the F12+R12 treatment compared to the CW (control), F12, and R12 treatment. The presence of these microorganisms in the system may have contributed to the reduction of FCR and increased yield in these treatments, as they have a composition rich in essential nutrients for shrimp growth in the nursery phase (Martins et al., 2016; Abreu et al., 2019).

In intensive marine shrimp production systems using biofloc and synbiotic, the dominance of plankton by protozoan microorganisms such as flagellates, ciliates, and amoeba are frequently reported (Lima et al., 2022; Reis et al., 2023). Pimentel et al. (2023) evaluated the planktonic community of intensive *P. vannamei* nurseries using low salinity water and synbiotic systems and found a dominance of the system by the phyla Ciliophora and Amoebozoa. This dominance is closely related to the input of organic carbon into the system, creating an environment rich in nutrients and with favorable conditions for the rapid development of the microbial loop (Decamp et al., 1999; Lima et al., 2022; Pimentel et al., 2023). In this study, the F12+R12 treatment proved to create the best conditions for zooplankton growth, as it presented a higher abundance of ciliates than the CW, BFT, F24, and R12 treatments and a greater abundance of nematodes than most treatments at the end of the experimental period.

Bacteria play a key role in the cycling and mineralization of nutrients such as carbon, nitrogen, and phosphorus and in the transfer of energy through the food chain (Esteves, 2011). Throughout the entire experimental period and in all treatments, we observed a dominance of bacteria of the coccoid, bacillus and free filamentous morphotypes. This pattern is reported for *P. vannamei* intensive culture systems with biofloc technology (Xavier et al., 2022; Reis et al., 2023). This is due to the efficiency of this type of bacteria, mainly coccoids, to assimilate nutrients, favoring their growth (Suita et al., 2015). The greater abundance of free filamentous bacteria in the F12+R12 treatment than in the CW, BFT, F24, F24+R24, and R24 treatments observed at the end of the experimental period is a disadvantage, as it can trigger operational implications in solids management. High amounts of this bacterial morphotype interfere with floc settling and may hinder the process of clarifying the water (Kotay et al., 2011; Nethaji et al., 2021).

Another morphotype of bacteria that should receive attention in *P. vannamei* intensive culture, mainly in the nursery phase, are the vibrio, due to their pathogenic characteristics. High loads of vibrio in the system can interfere with the growth and survival of the animals (Souza Valente and Wan, 2021). Our findings indicate that the F24+R24 bran processing strategy is the least advantageous in controlling vibrio in the system. However, the abundance of vibrio

was still lower than that found for bacillus bacteria. These bacteria have a probiotic effect, colonize the intestinal tract of animals, improving feed digestibility, immune system, and health status, eliminating pathogenic bacteria, and inducing bioremediation (Ninawe and Selvin, 2009; Olmos et al., 2020).

The findings of this work indicate that the strategy of using fermentation (F; phase without aeration) and respiration (R; phase with aeration) of the fertilizer for a period of 12 h, either separately or in combination, provided a high load of microorganisms and improved the use of feed by the shrimp. This was confirmed by the lower FCR found in the F12+R12, F12, R12, and R24 treatments when compared to the CW treatment (clear water - control), where the microbial community was not stimulated, making the feed the main food source. The treatments employing different synbiotic fertilizer processing strategies created favorable conditions for the growth of microorganisms, as water fertilization with an organic carbon source promotes the growth of heterotrophic and autotrophic microorganisms in microbial aggregates that can act as a supplementary food source for animals. This increases the biomass stocked in the experimental units, reduces FCR, and consequently, lowers feed costs (Wasielesky et al., 2006; Emerenciano et al., 2012). It is estimated that microbial aggregates contribute between 63 and 100% of carbon and 35 to 86% of nitrogen to shrimp biomass in a biofloc system (Krummenauer et al., 2020).

The observed higher yield in treatments F12+R12, F12, R12, F24, and R24 (ranging between 3.06 and 3.25 Kg m^{-3}) compared to the CW treatment (2.29 Kg m^{-3}) suggests that the synbiotic system has demonstrated favorable growth and yield outcomes in intensive marine shrimp nurseries. The study by Lima et al. (2022), which explored the integrated culture of *P. vannamei* with oysters using a synbiotic system fertilized with rice bran, achieved a yield of 2.32 Kg m^{-3} in the control treatment, similar to what was found in this study. Lima et al. also indicates the efficiency of the nitrification process in this system. These results suggest that the synbiotic system supporting a high stocked biomass without compromising animal performance and water quality.

The reduction of processing time in the synbiotic fertilization management, as observed in this study, represents a practical strategy for optimizing the management of this system. The findings contrast with those of Santos et al. (2022), who recommended longer fertilizer processing times for *Macrobrachium rosenbergii* nurseries. It's worth noting that the results reported by Santos et al. were for low salinity water, which could yield different microbial growth outcomes compared to seawater.

The fermentation phase's importance in bran processing is highlighted in this study, emphasizing its role in breaking down complex compounds, increasing protein and vitamin solubility, and releasing beneficial products such as vitamins, amino acids, and enzymes (Dawood and Koshio, 2020). It is important to note that the addition of a respiration phase, following fermentation, can promote the growth of probiotic microorganisms present in the fertilizer. Under aerobic conditions, the balance of ATP produced is greater than in the fermentation process, further contributing to the overall efficiency of the synbiotic system (Madigan et al., 2019). These insights into optimizing the synbiotic system's fertilization process have practical implications for shrimp nursery management in intensive aquaculture settings.

5. Conclusion

The processing of rice bran through a combination of fermentation (anaerobic phase) and respiration (aerobic phase) did not negatively impact shrimp growth and survival. Among the various processing strategies tested, the treatment involving the fermentation of rice bran for 12 h followed by a 12-h respiration phase (F12+R12) emerged as the most effective. This strategy demonstrated faster control of total ammonia nitrogen, accelerated development of the microbial loop, and resulted in higher yield and lower FCR compared to the control treatment and similar to the performance observed in the biofloc system. As a result, the study recommends the adoption of a reduced fertilizer preparation time by combining fermentation and respiration processes, thereby optimizing the fertilization management of the synbiotic system in shrimp nurseries.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Data availability

Data will be made available on request.

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CAPÍTULO IV

Microbial community composition, nitrification process, and growth of *Penaeus vannamei* in a synbiotic nursery system inoculated with different probiotic microorganisms

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Composição da comunidade microbiana, processo de nitrificação e crescimento do *Penaeus vannamei* em berçários com sistema simbiótico inoculado com diferentes microrganismos probióticos

Resumo expandido

No sistema simbiótico, os microrganismos probióticos desempenham um papel chave na quebra dos compostos complexos presentes nos farelos vegetais. Quando induzidos a realizar os processos de fermentação e respiração microbiana, bactérias como as dos gêneros *Bacillus*, *Lactobacillus*, *Pediococcus* e leveduras da espécie *Saccharomyces cerevisiae*, liberam nutrientes de forma mais lábil no sistema e secretam compostos bioativos como ácidos orgânicos e enzimas. Atualmente, as estratégias de fertilização do sistema simbiótico usam uma ampla variedade de probióticos, compostos por apenas uma cepa de bactérias e outros com uma mistura de cepas de probióticos. Alguns estudos inocularam leveduras e reportara bom controle da qualidade de água e crescimento do camarão. Apesar dos resultados promissores, informações sobre a influência desses microrganismos sobre o processo de nitrificação e composição microbiana do sistema ainda são escassos. Portanto, o objetivo deste estudo foi avaliar o efeito da utilização de diferentes microrganismos probióticos na composição do fertilizante do sistema simbiótico sobre a composição microbiana, processo de nitrificação e crescimento do *Penaeus vannamei* na fase de berçário. Foi realizado um cultivo (densidade de estocagem: 2000 camarões m^{-3}) por 40 dias, utilizando protocolos de fertilização simbiótica com diferentes microrganismos probióticos nos seguintes tratamentos: CW – água clara (controle), B – *Bacillus*, BLP – *Bacillus* + *Lactobacillus* + *Pediococcus*, BLPY – *Bacillus* + *Lactobacillus* + *Pediococcus* + levedura (*S. cerevisiae*), BY – *Bacillus* + levedura. Além do probiótico, o fertilizante utilizou farelo de arroz como principal fonte de carbono orgânico, melaço, bicarbonato de sódio (como tampão) e água em sua composição. No tratamento CW, 90% do volume da unidade experimental foi trocado diariamente. A comunidade planctônica foi analisada por contagem direta. A abundância de bactérias oxidantes de amônia (AOB) e oxidantes de nitrito (NOB) foi determinada usando hibridização in situ fluorescente. Durante o período experimental, foi observado um controle mais rápido do nitrogênio amoniacal total (TAN) no tratamento BLP (Figura 1a). Ao longo do ensaio, não foi observado o controle do nitrogênio do nitrito (NO_2^--N) através do processo de nitrificação nos tratamentos onde foram

utilizados sistemas simbióticos (Figura 1b). Nestes tratamentos foram realizadas trocas de água para manter a concentração abaixo do nível seguro para a espécie.

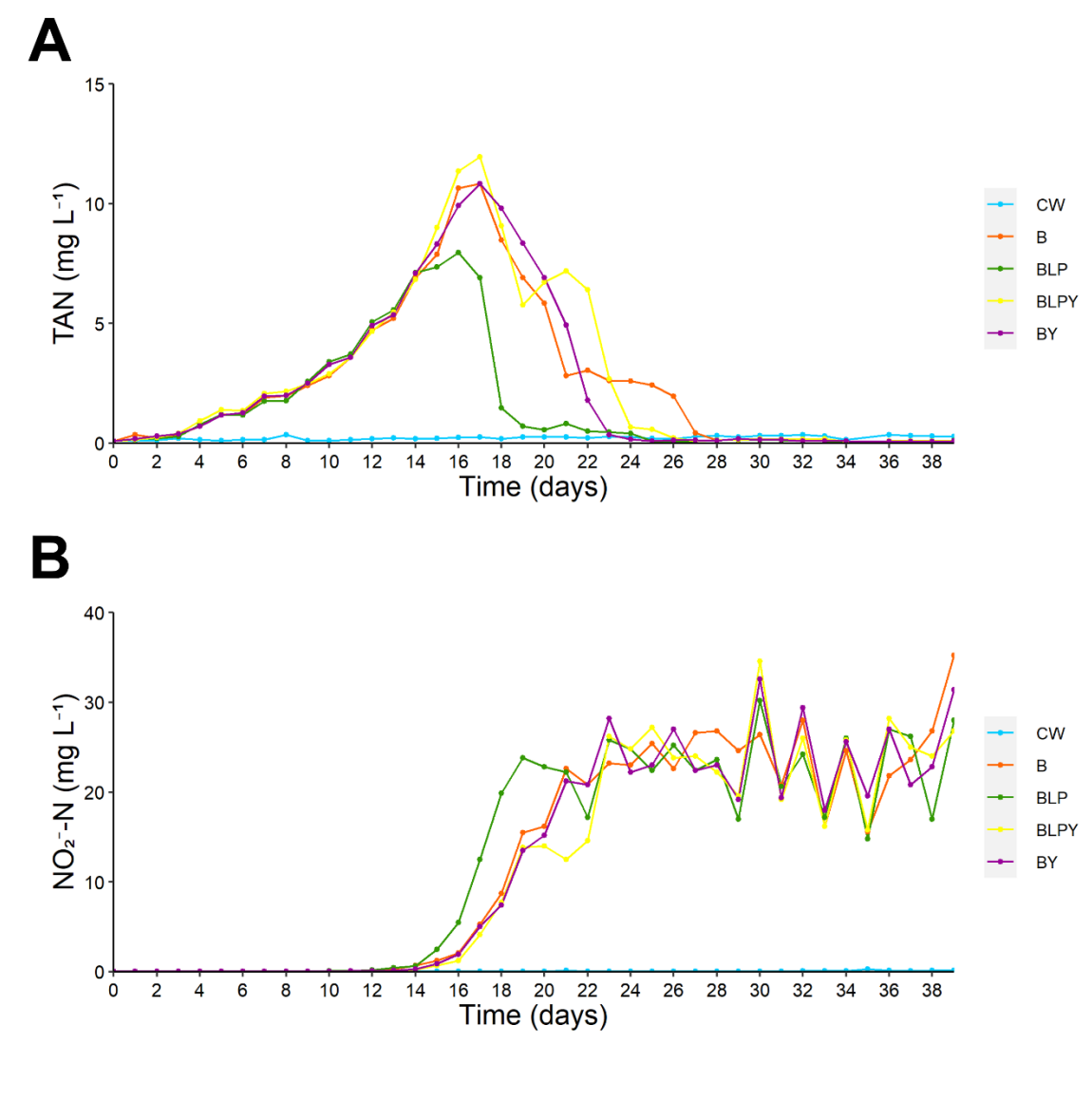

Figura 1. Concentração de TAN (A) e NO_2^--N (B) durante um berçário de *Penaeus vannamei* com sistema simbiótico inoculado com diferentes microrganismos probióticos. CW – água clara (controle), B – *Bacillus*, BLP – *Bacillus* + *Lactobacillus* + *Pediococcus*, BLPY – *Bacillus* + *Lactobacillus* + *Pediococcus* + levedura (*S. cerevisiae*), BY – *Bacillus* + levedura.

As comunidades do fitoplâncton e zooplâncton foram dominadas basicamente por Bacilariofitas e protozoários, respectivamente. No dia 21 do ensaio, o tratamento BLP apresentou maior abundância de bactérias AOB do que nos tratamentos CW, BLPY e BY

(Figura 2a). No dia 39 do experimento, o tratamento BLP apresentou mais NOB do que BLPY e BY (Figura 2b).

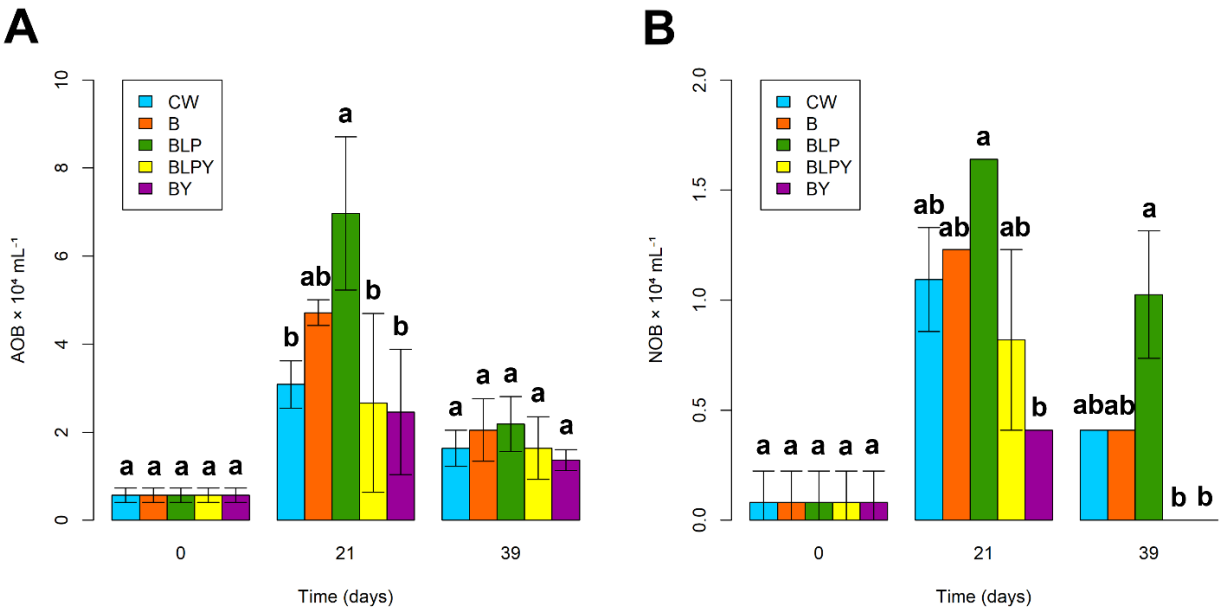

Figura 2. Abundância (organismos mL^{-1}, média ± desvio padrão) de bactérias oxidantes de amônia (AOB) (A) e bactérias oxidantes de nitrito (NOB) (B) durante um berçário de *Penaeus vannamei* com sistema simbiótico inoculado com diferentes microrganismos probióticos. CW – água clara (controle), B – *Bacillus*, BLP – *Bacillus* + *Lactobacillus* + *Pediococcus*, BLPY – *Bacillus* + *Lactobacillus* + *Pediococcus* + levedura (*S. cerevisiae*), BY – *Bacillus* + levedura.

O camarão apresentou maior peso final nos tratamentos CW e BLP do que no BY. A produtividade foi maior nos tratamentos BLP, B e BLPY do que no CW. O uso de *Bacillus*, *Lactobacillus* e *Pediococcus* (tratamento BLP) na fertilização do sistema simbiótico promoveu rápido controle de TAN, maior abundância de AOB e NOB e melhorou o desempenho de crescimento de camarões em berçários intensivos.

Microbial community composition, nitrification process, and growth of *Penaeus vannamei* in a synbiotic nursery system inoculated with different probiotic microorganisms

Abstract

The aim of this study was to evaluate the effect of using different probiotic microorganisms in the composition of the synbiotic system fertilizer on microbial composition, nitrification process, and growth of *Penaeus vannamei* in the nursery phase. An intensive nursery was carried out (stocking density: 2000 shrimp m^{-3}) for 40 days, using synbiotic fertilization protocols with different probiotic microorganisms in the following treatments: CW – clear water (control), B – *Bacillus*, BLP – *Bacillus* + *Lactobacillus* + *Pediococcus*, BLPY – *Bacillus* + *Lactobacillus* + *Pediococcus* + Yeasts (*Saccharomyces cerevisiae*), BY – *Bacillus* + Yeasts. In addition to probiotic, the fertilizer used rice bran as the main organic carbon source, molasses, sodium bicarbonate (as a buffer), and water in its composition. The Plankton community was analyzed by direct counting. The abundance of ammonia-oxidizing (AOB) and nitrite-oxidizing (NOB) bacteria were determined using fluorescent in situ hybridization. During the experimental time, a faster control of total ammonia nitrogen (TAN) was observed in BLP treatment. Throughout the trial, the control of nitrite through the nitrification process was not observed in the treatments where synbiotic systems were used. In these treatments, water changes were carried out to keep the concentration below the safe level for the species. The phytoplankton and zooplankton communities were basically dominated by Bacillariophyta and protozoans, respectively. On day 21 of the trial, BLP treatment had a higher abundance of AOB bacteria than CW, BLPY, and BY treatments. On day 39 of the experiment, BLP treatment had more NOB than BLPY and BY. Shrimp had a higher final weight in CW and BLP treatments than in BY. Yield was higher in BLP, B, and BLPY treatments than in the CW. The use of *Bacillus*, *Lactobacillus*, and *Pediococcus* (BLP treatment) in synbiotic system fertilization promoted rapid control of TAN, a higher abundance of AOB and NOB, and improved shrimp growth performance in intensive nurseries.

Keywords: *Bacillus*, *Lactobacillus*, *Pediococcus*, yeasts, nitrifying bacteria, *Vibrio.*

1. Introduction

The synbiotic system has been widely used among the main shrimp producing countries in the world. This microbial-based system is characterized by the fertilization of water with a vegetable bran (prebiotic) processed with fermentation and/or microbial respiration by probiotic microorganisms (Khanjani et al., 2023). In this system, fertilizers play a key role on water quality and shrimp growth. As an exogenous organic carbon source, it stimulates the assimilation of ammonia by heterotrophic bacteria, and can also act as supplementary source of food, improving the health and growth of animals (Khanjani et al., 2023).

Microorganisms added to the fertilizer, when induced to fermentation process or microbial respiration, use bran as a substrate for their growth, degrading complex compounds (e.g., fibers and carbohydrates) to simpler compounds (Khanjani et al., 2022a). In addition to releasing nutrients in a more labile way into the system, the activity of these microorganisms can result in the production of bioactive compounds such as organic acids and enzymes (James et al., 2021). The main probiotic microorganisms that are used in the synbiotic system fertilization are bacteria of the genus *Bacillus*, *Lactobacillus*, *Pediococcus*, and the yeast *Saccharomyces cerevisiae* (Khanjani et al., 2023).

Bacillus bacteria are widely used in aquaculture due to their probiotic and bioremediation function, being able to control pathogens, act as an immunostimulant, and growth promoter, in addition to degrading organic matter and bioremediating nitrogenous compounds (James et al., 2021). Lactic acid bacteria, such as those of the *Lactobacillus* and *Pediococcus* genus, in addition to having the fermentative ability, can produce antimicrobial substances, organic acids, and improve the immune response of animals against viral pathogens (Naiel et al., 2021; El Saadony et al., 2021). *Saccharomyces cerevisiae* yeasts can improve water quality by eliminating organic substances and inhibiting the growth of pathogenic bacteria by competing for nutrients (Del Valle et al., 2023). Furthermore, these microorganisms colonize the intestinal tract of animals, improving feed digestibility, and contributing to better growth (James et al., 2021; Del Valle et al., 2023).

The application of the synbiotic system using a wide variety of probiotic strains in water fertilization has been reported in several studies with *Penaeus vannamei* culture using seawater and low salinity water with good results for growth and water quality (Hussain et al., 2021; Andrade et al., 2021; Oliveira et al., 2022a). For example, in seawater Silva et al. (2021) tested the effect of adding rotifers on the growth of *P. vannamei* in nurseries with synbiotic system using probiotic strains of *Lactobacillus* and *Bacillus* and found that the use of this fertilization strategy was efficient in controlling nitrogenous compounds, on shrimp growth, and resistance.

Using low salinity water, Oliveira et al. (2022b) testing the effect of ionic adjustment frequency in *P. vannamei* nurseries with synbiotic system using a probiotic composed of *Bacillus* and *Pseudomonas* strains and with addition of *Saccharomyces cerevisiae* proved to control nitrogenous compounds and provide a good growth and survival of the animals. Despite the good results, information on the effects of synbiotic fertilization with the inoculation of different strains of probiotic bacteria and the addition of yeast on the nitrification process, plankton composition, and shrimp growth are still scarce.

It is important to understand the effect of probiotic strains diversity on the synbiotic system to standardize fertilization protocols and improve water quality management in shrimp cultures. Therefore, the aim of this study was to evaluate the effect of using different probiotic microorganisms in the composition of the synbiotic system fertilizer on the microbial community, nitrification process, and growth of *P. vannamei* in the nursery phase.

2. Materials and methods

This study was carried out for 40 days at "Estação Marinha de Aquacultura" of the "Universidade Federal do Rio Grande – FURG", Brazil.

2.1. Design and experimental conditions

A nursery was carried out with the following treatments, all with 5 repetitions and in a completely randomized experimental design: CW – clear water (control); B – *Bacillus*; BLP - *Bacillus* + *Lactobacillus* + *Pediococcus*; BLPY - *Bacillus* + *Lactobacillus* + *Pediococcus* + Yeasts (*Saccharomyces cerevisiae*); BY – *Bacillus* + Yeasts. Shrimp post-larvae 0.01 ± 0.001 g (mean ± standard deviation) were stocked at the density of 2000 shrimp m^{-3} in experimental units of 50 L, with constant aeration. In this study, seawater (salinity 28 g L^{-1}) used was previously filtered (5 μm), chlorinated (10 g m^{-3}), and dechlorinated with ascorbic acid. In the CW treatment, 45 L of water were changed daily in the experimental units. The seawater used for changes was previously filtered, chlorinated, and dechlorinated.

2.2. Water fertilization

The fertilization process of the experimental units started together with the animals stocking. During the trial, the fertilization was performed daily for the first 15 days of culture. The fertilizer used in the treatments had the following composition: rice bran (< 300 μm; 20 g m^{-3}), probiotic (0.4 g m^{-3}), molasses (2 g m^{-3}), sodium bicarbonate (2 g m^{-3}), and seawater (chlorinated and dechlorinated) in a volume of 10 times the amount of rice bran. Yeast (*Saccharomyces cerevisiae*) was used in the BY and BLPY treatments at a concentration of 0.2 g m^{-3}, adapted from Oliveira et al. (2022b). The probiotic used in B and BY treatments was

composed of *Bacillus subtilis* and *Bacillus licheniformis* at a concentration of 5.0×10^{10} colony forming units (CFU) g^{-1}. The probiotic used in the BLP and BLPY treatments was composed of *Bacillus subtilis* (3.4×10^9 CFU g^{-1}), *Lactobacillus plantarum* (1.2×10^9 CFU g^{-1}), and *Pediococcus acidilactici* (1.2×10^9 CFU g^{-1}). The fertilizer was processed by a fermentation phase (12 h) and a microbial respiration phase (12 h), following Pimentel et al. (2024). After this period, it was applied in the experimental units.

2.3. Water quality variables

Dissolved oxygen (DO, mg L^{-1}), temperature (°C; YSI EcoSense DO200A), pH (Mettler Toledo seven2Go), total ammonia nitrogen (TAN, mg L^{-1}) (UNESCO, 1983), and nitrite nitrogen (NO_2^--N, mg L^{-1}) (Aminot and Chaussepied, 1983) were analyzed daily. When the NO_2^--N concentration exceeded the safe level of 25.7 mg L^{-1} (Lin and Chen, 2003), 15 L (30%) of water was changed from the experimental units.

The concentration of nitrate nitrogen (NO_3^--N, mg L^{-1}) (García-Robledo et al., 2014), orthophosphate (PO_4^{3-}, mg L^{-1}) (Aminot and Chaussepied, 1983), settleable solids (SS, mL L^{-1}) (Eaton et al., 1995), total suspended solids (TSS, mg L^{-1}) (Strickland and Parsons, 1972), salinity (g L^{-1}; Hanna multiparameter, model HI98194), and carbon dioxide (CO_2, mg L^{-1}) (Timmons and Ebeling, 2013) were analyzed weekly. Total alkalinity (mg L^{-1}; APHA, 2012) was analyzed twice a week. When necessary, adjustments were made in the alkalinity concentration to 150 mg L^{-1} with the application of sodium bicarbonate ($NaHCO_3$), following Furtado et al. (2011). Also, when TSS exceeded 500 mg L^{-1}, water changes were performed to maintain concentrations below 500 mg L^{-1} (Gaona et al., 2011).

2.4. Feed management

During the trial, shrimp were fed three times a day using commercial feed containing 40% crude protein (Guabitech PL and Guabitech J). The amount of feed offered was determined based on Jory et al. (2001).

2.5. Shrimp growth performance and survival

At the end of the experimental time, shrimp growth was evaluated to determine: final weight (g), survival (%) [(number of animals at the end of experimental time/initial number of animals) × 100], feed conversion ratio (FCR) [(feed supplied/biomass gain)], specific growth rate (SGR, % day^{-1}) 100 × [(ln final weight (g) – ln initial weight (g))/experimental time (days)], and yield (Kg m^{-3}) [final biomass (Kg)/ volume of the experimental unit (m^3)].

2.6. Microbial community assessment

Water samples were collected on days 0 (initial), 21 (middle), and 39 (end of trial) for quantification and identification of the planktonic community of the system. Phytoplankton

and Zooplankton were identified and quantified by direct counting using a sedimentation chamber and inverted microscope (Nikon, Eclipse TS100), with a final magnification of 200 × (Utermöhl,1958). The abundance of microorganisms was expressed in organisms mL^{-1}.

Nitrifying bacteria community and *Vibrio* sp. were identified and quantified using the fluorescent in situ hybridization (FISH), following Cottrell and Kirchman (2003). The samples were previously sonicated (Qsonica sonicators, model Q55) with three pulses of 30 s using a frequency of 10 kHz. An interval of 1 min among pulses was adopted. After, samples were filtered through a polycarbonate membrane with 0.2 μm of mean retention and hybridized with the probes. Oligonucleotide probes labeled with the Cy3 fluorochrome targeting the 16 s rRNA gene were used to identify the genus *Nitrosomonas*, *Nitrosospira*, *Nitrobacter*, *Nitrospira*, and *Vibrio* (Table 1). A negative control (NON) made without specificity for bacteria was used to test the hybridization efficiency (Table 1). The nitrifying bacteria community was divided into ammonia-oxidizing bacteria (AOB = *Nitrosomonas* + *Nitrosospira*) and nitrite-oxidizing bacteria (NOB = *Nitrobacter* + *Nitrospira*). Total abundance of bacteria was determined from the count of bacteria stained with DAPI (4′,6-diamidino-2-phenylindole). Bacteria were photographed using a camera coupled to an epifluorescence microscope (Axioplan-Zeiss™), with a final magnification of 1000 × for subsequent counting. The abundance of microorganisms was expressed in organisms mL^{-1}.

Table 1. Oligonucleotide probes used to identify the groups of bacteria present in a *Penaeus vannamei* synbiotic nursery inoculated with different probiotic microorganisms.

Probe	Taxonomic specificity	Sequence (5'-3')	%F	Reference
NON	Negative control	TAGTGACGCCGTCGA	30	Yokokawa and Nagata, 2005
NEU	*Nitrosomonas*	CCCCTCTGCTGCACTCTA	40	Wagner et al., 1995
Nsv443	*Nitrosospira*	CCGTGACCGTTTCGTTCCG	30	Mobarry et al., 1996
NIT3	*Nitrobacter*	CCTGTGCTCCATGCTCCG	40	Wagner et al., 1996
NSR447	*Nitrospira*	GGTTTCCCGTTCCATCTT	30	Schramm et al., 1998
VIB572a	*Vibrio*	ACCACCTGCATGCGCTTT	40	Huggett et al., 2008

%F: percentage of formamide in the hybridization solution.

2.7. Data analysis

Data were checked for normality and homoscedasticity using the Shapiro-Wilk and the Levene tests, respectively. Repeated measures analysis of variance (ANOVA) was used for water quality data. When repeated measures ANOVA was significant (p-value < 0.05), Tukey's test was used to assess which treatments differed. Temperature, DO, pH, NO_3^--N, alkalinity,

CO_2, and SS data were transformed to fulfill parametric assumptions. For non-parametric data (TAN and NO_2^--N), the Friedman test followed by the Conover multiple comparison test with Bonferroni correction were applied.

One-way ANOVA was applied to the abundance data of the main groups identified in Phytoplankton, Zooplankton, nitrifying bacteria, and *Vibrio* sp. communities (analyzed separately for each day sampled), and shrimp growth data. Data were tested for normality and homoscedasticity using the Shapiro-Wilk and the Levene tests, respectively. When ANOVA was significant (p-value < 0.05), Tukey's test was run to determine which treatments differed. Survival percentage data were arcsine transformed before analysis (Zar, 2010). Chlorophyta (day 39) and SGR data were transformed to fulfill parametric assumptions. For non-parametric data (Flagellates - day 0, rotifers - days 21 and 39, nematodes - day 21, AOB and *Vibrio* - day 0, and NOB – days 0, 21, and 39), the Kruskal-Wallis test followed by the Dunn's comparison test with Bonferroni correction were applied.

Graphs, one-way ANOVA, Kruskal-wallis, Friedman and their post hoc tests were performed in R software version 4.2.3 (R Core team, 2023) using the following packages: car (Fox and Weisberg, 2019), dunn.test (Dinno, 2017), PMCMRplus (Pohlert, 2022), ggplot2 (Wickham, 2016), and Rmisc (Hope, 2022). Repeated measures ANOVA and its post hoc were performed using Past 4.03 2020 software (Hammer et al., 2001).

3. Results

3.1. Water quality variables

During the experimental time, the mean temperature varied between 29.27 and 29.98 °C among the treatments (Table 2). Mean DO was maintained above 5 mg L^{-1} in all treatments. The pH varied between 7.79 and 7.89 during the trial. Salinity was maintained close to 28 g L^{-1} in all treatments throughout the trial.

TAN concentration was higher in BLPY than in BLP (Table 2). CW treatment had a lower concentration than the other treatments (Table 2). BLP treatment showed the lowest TAN variation, being controlled between days 16 and 19 of the experimental time (Fig. 1A). Treatments B, BLPY, and BY showed the highest variation among treatments, with BLPY treatment reaching highest mean concentration (11.96 mg L^{-1}) on day 17 of the trial. TAN control in these treatments occurred from day 17, being completely controlled in BLPY and BY treatments on day 24 of the experimental time (Figure 1A). Treatment B had slower TAN control, being completely controlled on day 27 of the trial (Figure 1A).

NO_2^--N was higher in treatments B and BLP than in BLPY (Table 2). CW treatment showed a lower NO_2^--N concentration than other treatments (Table 2). The increase in NO_2^--N was faster in the BLP treatment than in B, BLPY, and BY (Figure 1B). No reduction in NO_2^--N concentration was observed throughout the experimental time (Figure 1B).

NO_3^--N in treatments B and BLP was higher than in CW (Table 2). An increase pattern in NO_3^--N concentration was observed throughout the experimental time in the treatments B, BLP, BLPY, and BY (Figure 1C).

PO_4^{3-}, SS, and TSS concentration was lower in the CW treatment than in the other treatments (Table 2). Alkalinity varied between 113.5 mg L^{-1} in treatment CW and 128.20 mg L^{-1} in treatment B. CO_2 was maintained at 3 mg L^{-1} in all treatments (Table 2).

Table 2. Water quality variables during a *Penaeus vannamei* synbiotic nursery inoculated with different probiotic microorganisms.

	Treatments					*p*-value
	CW	B	BLP	BLPY	BY	
Temperature (°C)	29.30 ± 1.35	29.98 ± 1.10	29.76 ± 0.83	29.27 ± 0.88	29.46 ± 1.21	0.058
	29.53	30.10	29.60	29.27	29.64	
	(26.44 – 31.31)	(27.80 – 32.35)	(28.64 – 31.79)	(27.05 – 30.77)	(25.35 – 31.39)	
DO (mg L^{-1})	5.74 ± 0.28	5.61 ± 0.24	5.61 ± 0.21	5.69 ± 0.22	5.66 ± 0.22	0.056
	5.68	5.56	5.59	5.68	5.66	
	(5.25 – 6.55)	(5.24 – 6.28)	(5.30 – 6.13)	(5.34 – 6.41)	(5.37 – 6.23)	
pH	7.79 ± 0.07	7.89 ± 0.08	7.88 ± 0.07	7.88 ± 0.12	7.89 ± 0.11	0.667
	7.78	7.89	7.87	7.85	7.86	
	(7.68 – 7.93)	(7.74 – 8.05)	(7.78 – 8.01)	(7.64 – 8.10)	(7.62 – 8.10)	
Salinity (g L^{-1})	28.50 ± 0.80	28.66 ± 1.31	28.83 ± 1.16	28.59 ± 0.77	28.57 ± 1.00	0.531
	28.54	29.01	29.01	28.55	28.62	
	(27.03 – 29.84)	(23.34 – 30.66)	(26.75 – 31.20)	(26.82 – 30.02)	(25.74 – 30.92)	
TAN (mg L^{-1})	0.22 ± 0.12^{c}	2.60 ± 4.19^{ab}	1.63 ± 2.58^{b}	2.77 ± 4.13^{a}	2.50 ± 3.82^{ab}	0.006
	0.21	0.22	0.16	0.43	0.34	
	(0.06 – 1.36)	(0.04 – 18.40)	(0.03 – 14.80)	(0.03 – 16.40)	(0.02 – 14.80)	
NO_2^--N (mg L^{-1})	0.06 ± 0.05^{c}	12.63 ± 12.41^{a}	13.02 ± 12.01^{a}	11.98 ± 12.68^{b}	12.47 ± 12.30^{ab}	< 0.001
	0.04	12.25	14.00	7.50	13.00	
	(0.02 – 0.35)	(0.02 – 37.00)	(0.02 – 44.00)	(0.02 – 52.00)	(0.02 – 44.00)	

NO_3^--N (mg L^{-1})	0.23 ± 0.04[b]	2.03 ± 3.71[a]	3.05 ± 5.28[a]	1.07 ± 2.18[ab]	1.01 ± 1.88[ab]	0.044
	0.23	0.07	0.17	0.02	0.03	
	(0.17 – 0.30)	(0.00 – 13.00)	(0.00 – 21.00)	(0.00 – 9.00)	(0.00 – 6.00)	
PO_4^{3-} (mg L^{-1})	0.25 ± 0.41[b]	1.70 ± 1.17[a]	1.67 ± 1.32[a]	1.81 ± 1.45[a]	1.71± 1.28[a]	< 0.001
	0.15	1.40	1.37	1.57	1.48	
	(0.09 – 2.42)	(0.13 – 4.20)	(0.13 – 6.60)	(0.13 – 6.60)	(0.13 – 4.40)	
Alkalinity (mg L^{-1})	113.50 ± 15.49	128.20 ± 22.58	127.00 ± 21.65	127.80 ± 22.18	128.00 ± 24.82	0.315
	110.00	130.00	130.00	130.00	130.00	
	(90.00 – 150.00)	(80.00 – 180.00)	(80.00 – 170.00)	(60.00 – 160.00)	(80.00 – 190.00)	
CO_2 (mg L^{-1})	3.69 ± 0.73	3.51 ± 0.76	3.42 ± 0.62	3.52 ± 1.14	3.30 ± 1.00	0.128
	3.75	3.44	3.28	3.47	3.47	
	(1.66 – 5.17)	(1.96 – 5.95)	(2.40 – 4.78)	(1.30 – 6.51)	(1.44 – 6.17)	
SS (mL L^{-1})	0.23 ± 0.28[b]	2.35 ± 2.17[a]	2.64 ± 3.02[a]	3.88 ± 7.74[a]	2.45 ± 2.73[a]	< 0.001
	0.20	2.00	1.50	1.50	1.40	
	(0.00 – 1.50)	(0.00 – 10.00)	(0.00 – 12.00)	(0.00 – 30.00)	(0.00 – 10.00)	
TSS (mg L^{-1})	102.50 ± 72.06[b]	181.10 ± 78.48[a]	193.00 ± 96.01[a]	180.20 ± 89.13[a]	204.30 ± 137.98[a]	0.003
	90.00	200.00	205.00	180.00	180.00	
	(30.00 – 405.00)	(30.00 – 320.00)	(30.00 – 535.00)	(30.00 – 415.00)	(10.00 – 545.00)	

Data are mean ± standard deviation, median (minimum - maximum). DO: dissolved oxygen; TAN: total ammonia nitrogen; NO_2^--N: nitrite nitrogen; NO_3^--N: nitrate nitrogen; PO_4^{3-}: orthophosphate; CO_2: carbon dioxide; SS: settleable solids; TSS: total suspended solids. CW – clear water (control); B – *Bacillus*; BLP - *Bacillus* + *Lactobacillus* + *Pediococcus*; BLPY - *Bacillus* + *Lactobacillus* + *Pediococcus* + Yeasts (*Saccharomyces cerevisiae*); BY – *Bacillus* + Yeasts.

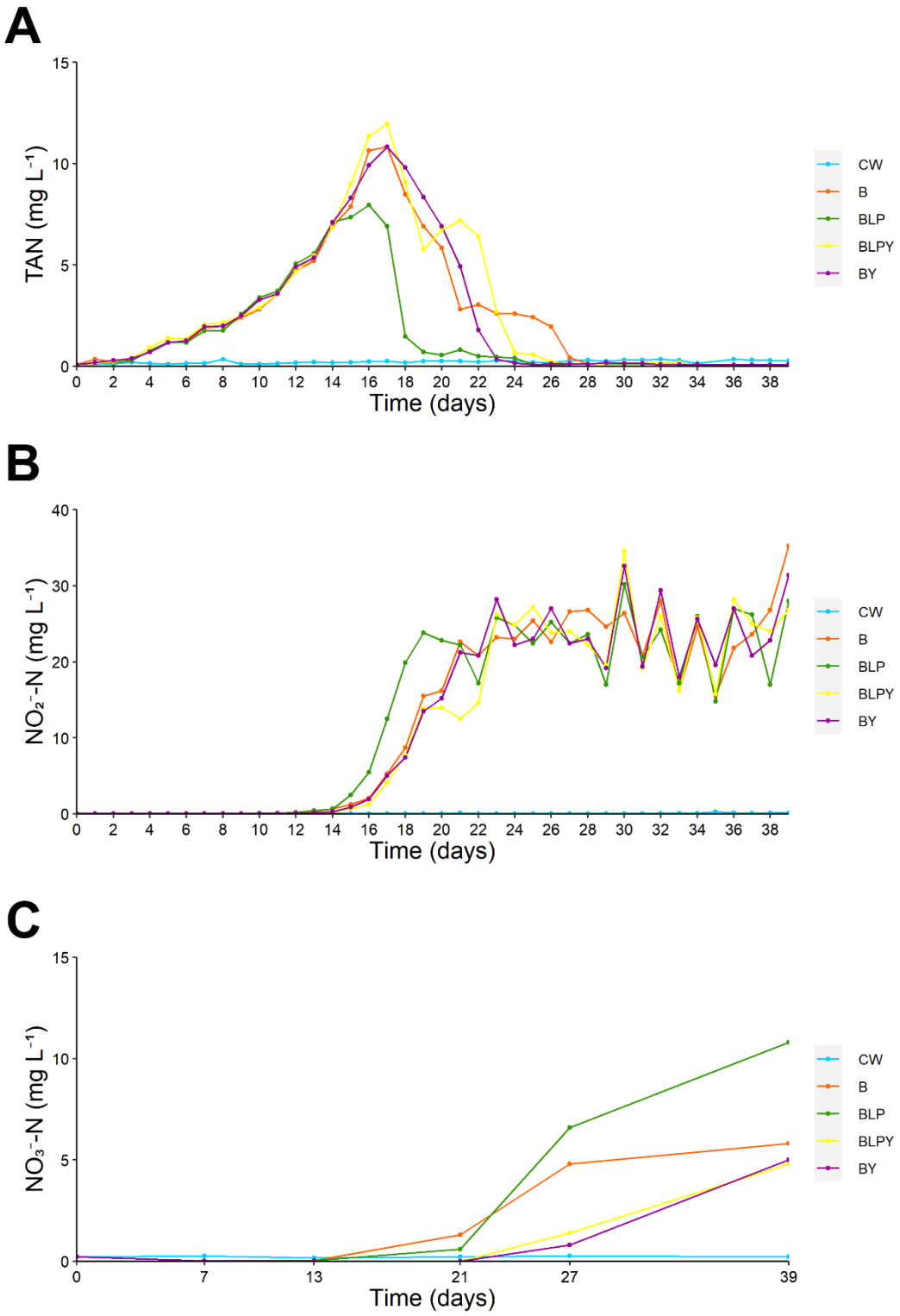

Figure 1. Concentration of TAN (A), NO_2^--N (B), and NO_3^--N (C) during a *Penaeus vannamei* synbiotic nursery inoculated with different probiotic microorganisms. CW – clear water

(control); B – *Bacillus*; BLP - *Bacillus* + *Lactobacillus* + *Pediococcus*; BLPY - *Bacillus* + *Lactobacillus* + *Pediococcus* + Yeasts (*Saccharomyces cerevisiae*); BY – *Bacillus* + Yeasts.

3.2. Composition of Phytoplankton and Zooplankton community

In the treatments CW, B, BLP, and BY, the phytoplankton community was dominated by Bacillariophyta, on days 21 and 39 of the experimental time (Figure 2A). On day 39, Chlorophyta were dominant in BLPY treatment (Figure 2A). On day 21, the abundance of Chlorophyta was higher in BY treatment than in CW (p-value = 0.025; Figure 3A). At the end of the trial (day 39), higher abundance of Chlorophyta was observed in BLPY treatment when compared to the others (p-value < 0.001; Figure 3A). On day 21, the abundance of Bacillariophyta was higher in BY treatment than in the other treatments (p-value < 0.001; Figure 3B). On day 39, all treatments showed a higher abundance of Bacillariophyta than CW (p-value = 0.002; Figure 3B).

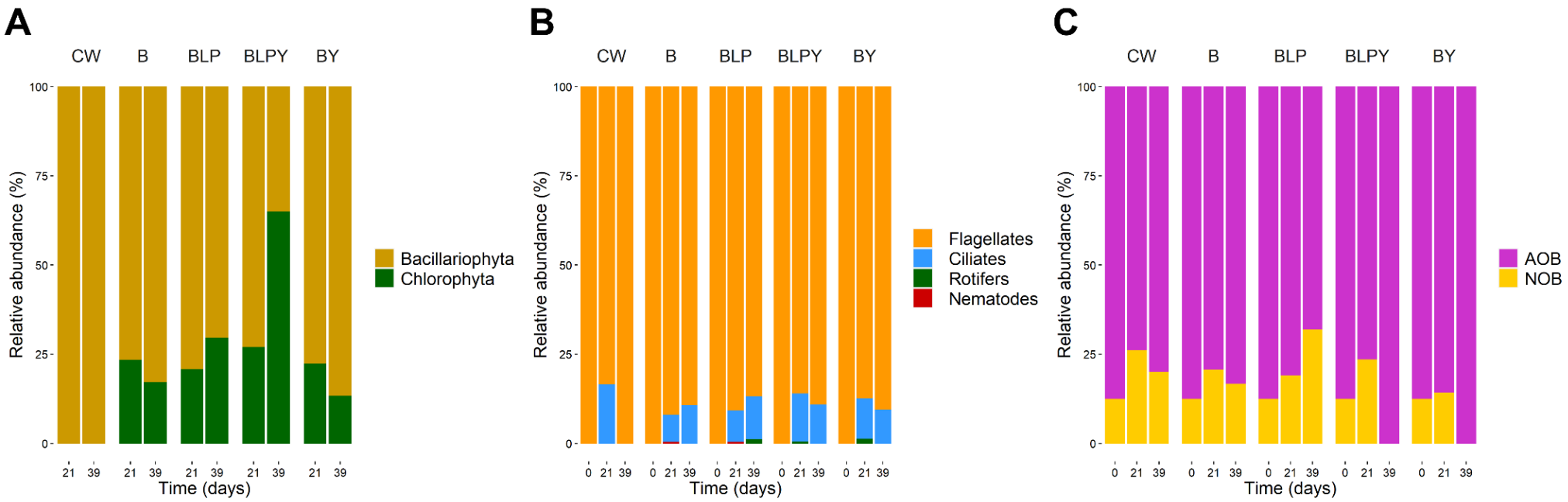

Figure 2. Relative abundance of the main groups of phytoplankton (A), zooplankton (B), and nitrifying bacteria (C) found during a *Penaeus vannamei* synbiotic nursery inoculated with different probiotic microorganisms. CW – clear water (control); B – *Bacillus*; BLP - *Bacillus* + *Lactobacillus* + *Pediococcus*; BLPY - *Bacillus* + *Lactobacillus* + *Pediococcus* + Yeasts (*Saccharomyces cerevisiae*); BY – *Bacillus* + Yeasts.

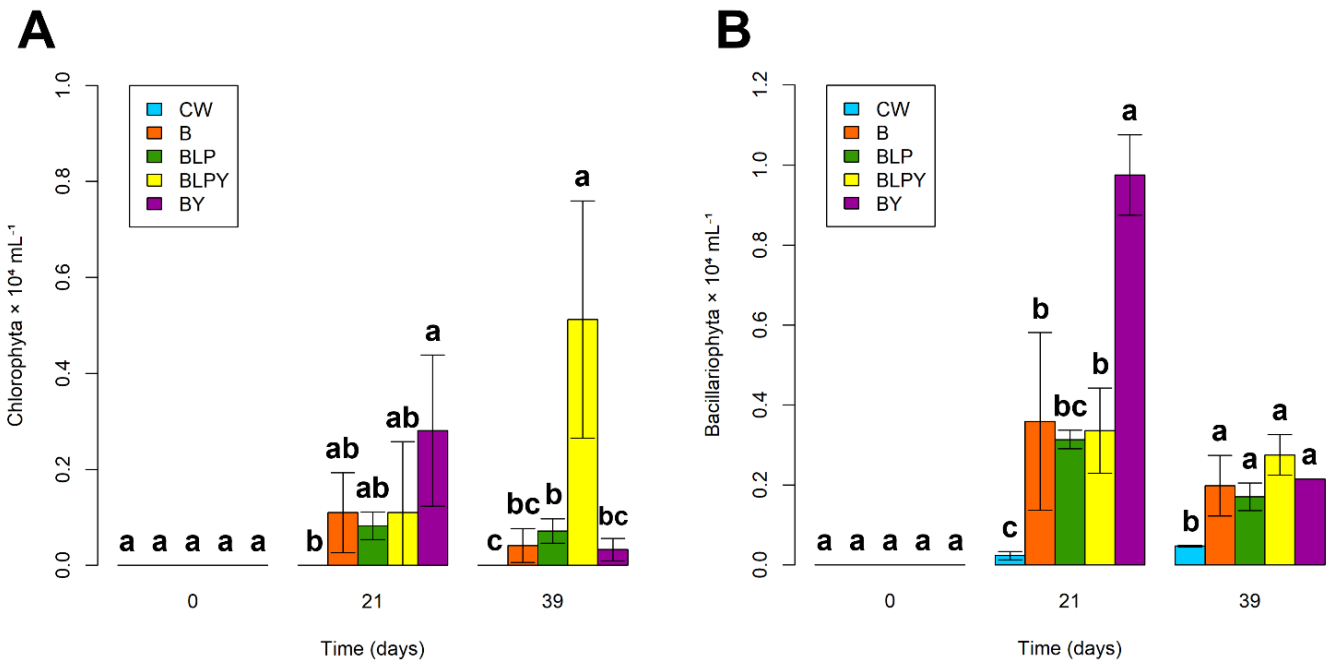

Figure 3. Abundance (organisms mL^{-1}, mean ± standard deviation) of Chlorophyta (A) and Bacillariophyta (B) during a *Penaeus vannamei* synbiotic nursery inoculated with different probiotic microorganisms. CW – clear water (control); B – *Bacillus*; BLP - *Bacillus* + *Lactobacillus* + *Pediococcus*; BLPY - *Bacillus* + *Lactobacillus* + *Pediococcus* + Yeasts (*Saccharomyces cerevisiae*); BY – *Bacillus* + Yeasts.

Flagellates were dominant in all treatments, in the three times sampled throughout the trial (Figure 2B). Rotifers were observed on day 21 of the experimental time in the BLPY and BY treatments, and on day 39 in the BLP treatment (Figure 2B). Nematodes were observed on day 21, in treatments B and BLP (Figure 2B). Flagellates, on day 39, were more abundant in B, BLP, BLPY, and BY treatments than in CW (p-value < 0.001; Figure 4A). Ciliates, on day 21 of the experiment, were more abundant in BLPY and BY treatments than in CW (p-value = 0.009; Figure 4B). On day 39 of the trial, treatments B and BLPY had more ciliates than CW (p-value = 0.040; Figure 4B). On day 21 of the experimental time, rotifers were more abundant in BLPY and BY treatments than in CW and BLP (p-value = 0.002; Figure 4C). On day 39, more rotifers were found in BLP treatment than in the others (p-value < 0.001; Figure 4C). Nematodes were only found on day 21, where they were more abundant in treatments B and BLP than in CW, BLPY, and BY (p-value < 0.001; Figure 4D).

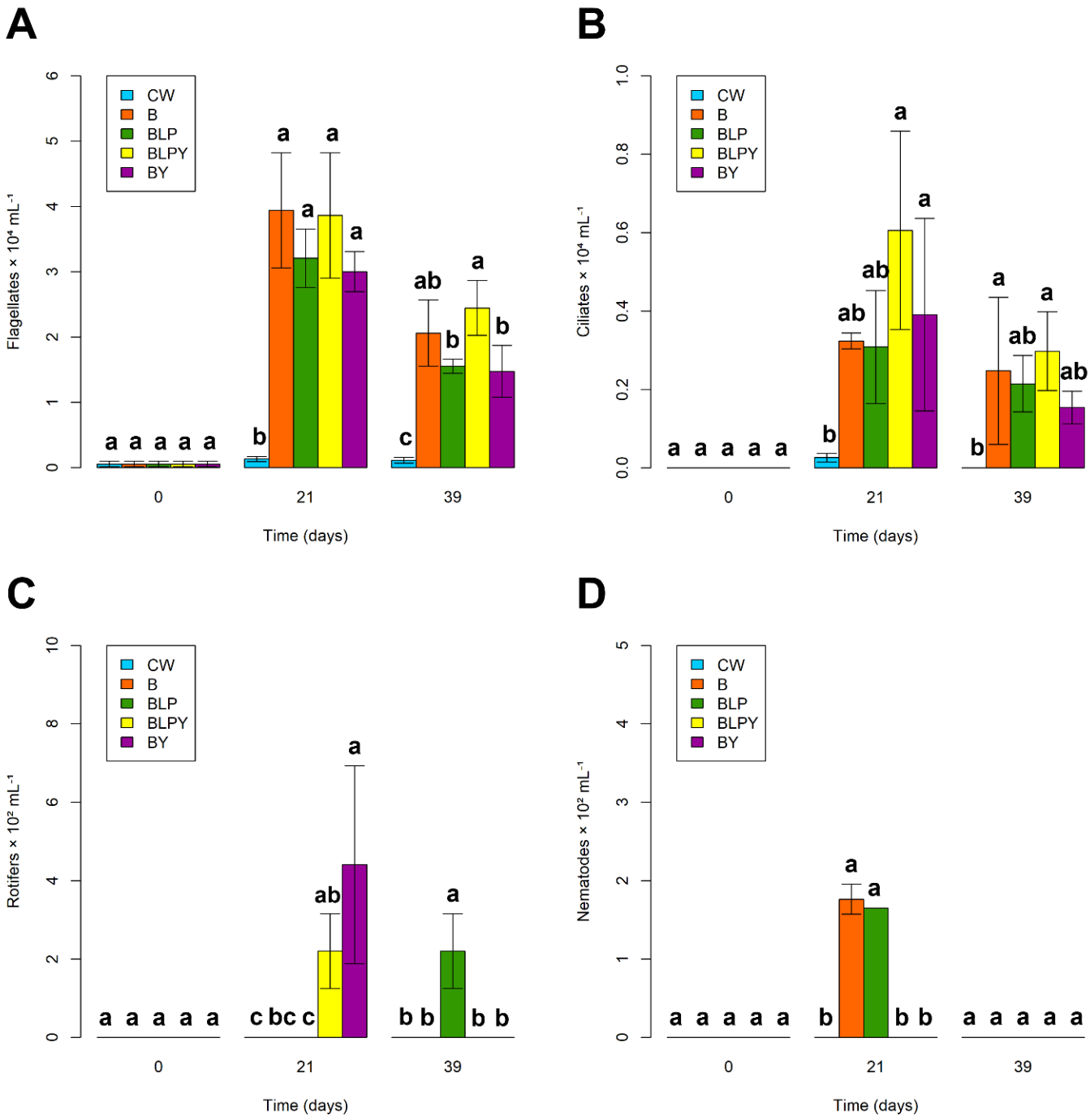

Figure 4. Abundance (organisms mL^{-1}, mean ± standard deviation) of flagellates (A), ciliates (B), rotifers (C), and nematodes (D) during a *Penaeus vannamei* synbiotic nursery inoculated with different probiotic microorganisms. CW – clear water (control); B – *Bacillus*; BLP - *Bacillus* + *Lactobacillus* + *Pediococcus*; BLPY - *Bacillus* + *Lactobacillus* + *Pediococcus* + Yeasts (*Saccharomyces cerevisiae*); BY – *Bacillus* + Yeasts.

3.3. Composition of nitrifying bacteria and Vibrio sp.

AOB were the dominant group of nitrifying bacteria throughout the experimental time, in all treatments (Figure 2C). On day 39, NOB was not observed in the BLPY and BY treatments (Figure 2C). The relative abundance of AOB and NOB in relation to the total bacterial abundance in the CW treatment reached 2.19% and 1.31% on days 21 and 39 of the trial, respectively. In treatments B, BLP, BLPY, and BY, the relative abundance of AOB and NOB in relation to the total abundance of bacteria was less than 1%, on days 21 and 39 of the trial.

On day 21, BLP treatment showed a higher abundance of AOB than CW, BLPY, and BY (p-value = 0.023; Figure 5A). On day 21 of the experiment, BLP treatment had higher abundance of NOB than BY (p-value = 0.024; Figure 5B). On day 39, BLP treatment had more NOB when compared to BLPY and BY (p-value = 0.007; Figure 5B).

On day 21 of the trial, the BY Treatment had less *Vibrio* than the other treatments (p-value = 0.005; Figure 5C). On day 39, CW, BLPY, and BY treatments had higher abundance of *Vibrio* bacteria than BLP treatment (p-value = 0.004; Figure 5C).

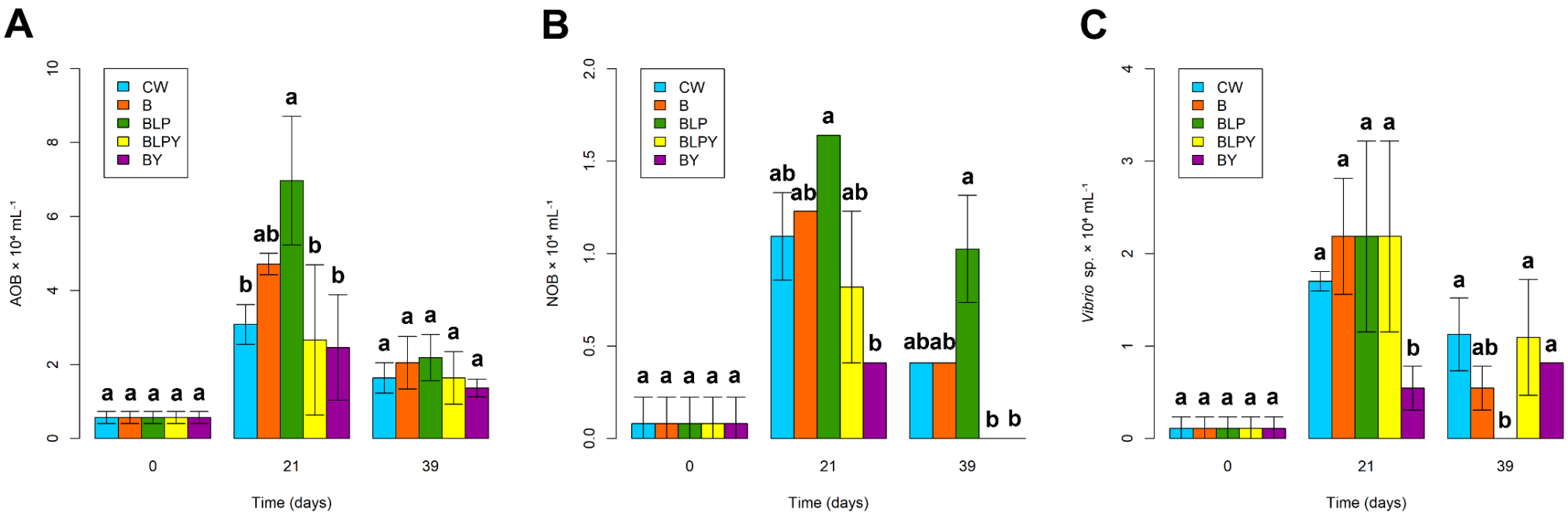

Figure 5. Abundance (organisms mL^{-1}, mean ± standard deviation) of ammonia-oxidizing bacteria (AOB) (A), nitrite-oxidizing bacteria (NOB) (B) and *Vibrio* sp. bacteria (C) during a *Penaeus vannamei* synbiotic nursery inoculated with different probiotic microorganisms. CW – clear water (control); B – *Bacillus*; BLP - *Bacillus* + *Lactobacillus* + *Pediococcus*; BLPY - *Bacillus* + *Lactobacillus* + *Pediococcus* + Yeasts (*Saccharomyces cerevisiae*); BY – *Bacillus* + Yeasts.

3.4. Shrimp growth and survival

Shrimp reached a higher final weight in CW and BLP treatments than BY (Table 3). Survival was higher in BLPY and BY treatments than CW and BLP (Table 3). FCR was lower in treatments B and BLP than CW and BY (Table 3). SGR was higher in CW, B, and BLP treatments when compared to BY (Table 3). Yield was higher in treatments B, BLP, and BLPY than CW (Table 3).

Table 3. *Penaeus vannamei* growth and survival at the end of a synbiotic nursery inoculated with different probiotic microorganisms.

	Treatments					*p*-value
	CW	B	BLP	BLPY	BY	
Initial weight (g)	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	-
Final weight (g)	1.69 ± 0.08^{a}	1.53 ± 0.18^{ab}	1.76 ± 0.11^{a}	1.55 ± 0.08^{ab}	1.39 ± 0.03^{b}	0.006
Survival (%)	76.00 ± 2.00^{b}	81.67 ± 3.05^{ab}	77.67 ± 5.51^{b}	87.67 ± 3.21^{a}	87.33 ± 1.52^{a}	0.003
FCR	1.46 ± 0.08^{a}	1.18 ± 0.06^{b}	1.22 ± 0.04^{b}	1.23 ± 0.06^{bc}	1.38 ± 0.07^{ac}	< 0.001
SGR (% day^{-1})	12.93 ± 0.12^{a}	12.93 ± 0.22^{a}	13.09 ± 0.05^{a}	12.70 ± 0.13^{ab}	12.68 ± 0.47^{b}	0.018
Yield (Kg m^{-3})	2.23 ± 0.12^{b}	2.77 ± 0.15^{a}	2.68 ± 0.09^{a}	2.64 ± 0.13^{ac}	2.37 ± 0.12^{bc}	< 0.001

Data are mean ± standard deviation. FCR: feed conversion ratio; SGR: specific growth rate. CW – clear water (control); B – *Bacillus*; BLP - *Bacillus* + *Lactobacillus* + *Pediococcus*; BLPY - *Bacillus* + *Lactobacillus* + *Pediococcus* + Yeasts (*Saccharomyces cerevisiae*); BY – *Bacillus* + Yeasts.

4. Discussion

Probiotic microorganisms have played a key role in the aquaculture development for their beneficial effect on shrimp, balancing gut microbiota, improving feed use, and increasing the animal's resistance against pathogens (Kumar et al., 2016). Thus, the use of this tool helps to overcome and prevent bacterial diseases outbreaks in shrimp farming worldwide (Hai and Fotedar, 2010). In addition to their unprecedented effect on animal health and growth, the bioremediation effect of probiotic strains on water and soil of aquaculture systems is also demonstrated (Ringø et al., 2020). Bacteria, such as those of the genus *Bacillus* and *Pediococcus*, can improve water quality by removing toxic nitrogenous compounds such as ammonia, nitrite, and nitrate, and reducing biochemical demand for oxygen (Khademzade et al., 2020; Hlordzi et al., 2020).

Throughout the experimental course, the variables of temperature, DO, pH, CO_2, and TSS were within acceptable levels for the *P. vannamei* culture (Van Wyk et al., 1999; Furtado et al., 2017; Gaona et al., 2017). Our findings indicated that the treatment in which *Bacillus*, *Lactobacillus*, and *Pediococcus* strains were used (BLP treatment) without *Saccharomyces cerevisiae* yeasts in the fertilizer preparation proved to be the best strategy for the TAN control in intensive nurseries with synbiotic system. The BLP treatment provided less variation and faster TAN control than the other treatments. This can be confirmed with the highest abundance of AOB in this treatment on day 21 of the trial. AOB are a group of chemoautotrophic bacteria that act in the biological oxidation of ammonia to nitrite, contributing to nitrogen cycling in intensive shrimp farming systems (Robles-Porchas et al., 2020). Higher abundance of AOB bacteria in the BLP treatment was reflected in a faster accumulation of nitrite than in the other treatments.

Although no trend of nitrite control was observed in the final stages of the trial, the treatment in which the oxidation of nitrite to nitrate would probably occur more quickly would be BLP. In this treatment, a higher abundance of NOB was observed at the end of the experiment than in BLPY and BY. In addition to environmental variables, the activity of many groups of bacteria is mediated through quorum sensing, which allows bacteria to communicate through the release of cell-cell chemical signaling molecules called autoinducers (Reading and Sperandio, 2006). This signaling stimulates population density and when the quorum is reached, the expression of certain genes is induced, generating a response behavior (Waters and Bassler, 2005), such as the nitrification process. The lack of quorum sensing may have been one of the factors that contributed to the fact that the nitrite to nitrate oxidation process by NOB was not observed.

In general, the treatments where the yeast *S. cerevisiae* was used had a slower nitrification process. These results are unexpected, since yeasts are primarily decomposers, colonize substrates rich in nutrients and can act in the degradation of organic carbon (e.g., sugars. Starmer and Lachance, 2011; Bai et al., 2022), which can improve water quality conditions. Studies with synbiotic system using *S. cerevisiae* as one of the fertilizer components reported good nitrogenous compounds control in *P. vannamei* cultures using low salinity water (Oliveira et al., 2022b) and in *Macrobrachium rosenbergii* cultures (Santos et al., 2022).

The inoculation of probiotic microorganisms in shrimp cultures can change the composition of the phytoplankton community (Kawman et al., 2022). In this study, the system was generally dominated by Bacillariophyta algae. The dominance of the phytoplankton community by Bacillariophytes has already been reported for *P. vannamei* production systems treated with probiotic microorganisms (e.g., *Bacillus*, *yeasts*, and *Pediococcus*) (Lukwambe et al., 2015). These microorganisms are beneficial to the system, as they can be a supplementary natural food source for animals, make microbial aggregates more nutritious, and control the growth of harmful Cyanobacteria (Martins et al., 2016; Marinho et al., 2017; Abreu et al., 2019). The higher dominance of Chlorophyta in BLPY treatment at the end of the trial may be a disadvantage for this treatment. The nutritional content of these microorganisms may be lower than that of Bacillariophyta and their assimilation by zooplankton and shrimp may be ineffective (Khanjani et al., 2022b).

In all treatments, the observed pattern of dominance by protozoan microorganisms (e.g., flagellates, ciliates, and amoebae) has already been reported for intensive shrimp culture systems with biofloc technology and synbiotic (Reis et al., 2023; Pimentel et al., 2023). This is due to the external organic carbon source that is used to stimulate the growth of heterotrophic bacteria community for the nitrogenous compounds control (Khanjani and Sharifinia, 2020). This ends up boosting the microbial loop development, which was also observed in treatments B, BLP, BLPY, and BY with the presence of microorganisms such as rotifers and nematodes during the trial.

In microbial-based systems, the growth of bacteria of the genus *Vibrio* can be increased with the organic matter accumulation in the system (Khanjani and Sharifinia, 2020). In this sense, the use of probiotic microorganisms in the system can contribute to reducing *Vibrio* abundance in the system (Krummenauer et al., 2014). This happens not only because of the bioremediation characteristic of probiotic microorganisms, as is the case with bacteria of the genus *Bacillus*, but also because these bacteria produce antimicrobial compounds or compete for nutrients with opportunistic pathogens, such as *Vibrio* bacteria (Madhana et al., 2021; Knipe

et al., 2021). In this study, BLP treatment proved to be the best at controlling these bacteria in the system. This can be reflected in good shrimp growth and survival (Souza et al., 2012; Krummenauer et al., 2014; Panigrahi et al., 2022).

In fact, all these factors influenced shrimp growth. The blend of probiotic microorganisms inoculated in the BLP treatment improved the final weight of the animals, the yield, and reduced FCR. The positive effect of microorganisms on shrimp can be proven by the higher FCR in the CW, since in this treatment the feed was the main source of food. This was consequently reflected in the yield of the system, which was reduced in this treatment compared to the others. The use of probiotics can promote shrimp growth by colonizing the digestive tract and improving the digestibility of the feed that is consumed (Ninawe and Selvin, 2009; Madhana et al., 2021). This can be enhanced in shrimp cultures with a synbiotic system, since the shrimp can ingest the bran grains (Khanjani et al., 2023) that may have been colonized by the probiotic bacteria at the moment of fertilizer preparation (fermentation and respiration processes).

Finally, our findings indicate that the use of a probiotic with a more diverse composition of microorganisms in the synbiotic system fertilizer can be effective in maintaining water quality conditions, controlling pathogenic organisms, and even influencing the growth of shrimp reared in intensive systems.

5. Conclusion

The treatment that used microorganisms of the genus *Bacillus*, *Lactobacillus*, and *Pediococcus* in the fertilizer composition (BLP treatment) proved to be the best strategy for the fertilization of *P. vannamei* intensive nurseries with synbiotic system. BLP treatment promoted rapid control of TAN, higher abundance of AOB and NOB, lower abundance of *Vibrio* bacteria, and higher shrimp final weight, higher yield, and lower FCR.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Data availability

Data will be made available on request.

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CAPÍTULO V

The super-intensive culture of *Penaeus vannamei* in low salinity water: A comparative study among recirculating aquaculture system, biofloc, and synbiotic systems

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O cultivo superintensivo de *Penaeus vannamei* em água de baixa salinidade: um estudo comparativo entre sistema de recirculação, bioflocos e sistema simbiótico

Resumo expandido

O cultivo de *Penaeus vannamei* em regiões interiores utilizando água de baixa salinidade é uma realidade em diversas regiões do mundo. Isso acontece devido a rusticidade do camarão, que suporta grandes variações de salinidade e densidades de estocagem elevadas. A disseminação de técnicas de cultivo que permitam alta densidade de estocagem e redução do uso de água podem ser utilizadas para otimizar o processo produtivo, mas requerem estudos comparativos para melhor compreender o funcionamento desses sistemas nessas condições de salinidade e os impactos deles sobre o controle dos compostos nitrogenados e composição microbiana do meio de cultivo. Portanto, este estudo teve como objetivo analisar os efeitos de diferentes sistemas de cultivo, que foram sistema de recirculação (RAS), tecnologia de bioflocos (BFT) e sistema simbiótico (Synbiotic) na qualidade da água, composição planctônica e crescimento de *P. vannamei* em água de baixa salinidade (2 g L^{-1}) e alta densidade de estocagem (500 camarões m^{-3}) por 30 dias. Os camarões foram estocados com peso médio de 1,27 ± 0,06 g. No RAS a água foi preparada com duas aplicações de 5 mg L^{-1} de cloreto de amônio por 10 dias para auxiliar no processo de maturação do sistema. No BFT, a dextrose foi utilizada como fonte de carbono orgânico e administrada na razão carbono:nitrogênio de 15:1. Nesse tratamento um inoculo de um cultivo anterior de *P. vannamei* com salinidade 28 g L^{-1} foi usado. O inoculo foi aclimatado para salinidade 2 g L^{-1} por 13 dias com a redução 2 partes de salinidade por dia. No Simbiótico, o farelo de arroz processado por microrganismos probióticos foi utilizado como estratégia de fertilização orgânica. A preparação da água desse tratamento foi realizada com 16 fertilização diárias somada a aplicações diárias de 1 mg L^{-1} de cloreto de amônio como uma fonte de nitrogênio inorgânico para auxiliar no desenvolvimento das bactérias nitrificantes. No tratamento RAS, todas as espécies de nitrogênio permaneceram estáveis durante todo o ensaio, sem picos e com concentrações de amônia e nitrato inferiores às do BFT e do Simbiótico (Figura 1). O tratamento BFT teve mais eventos quando o nitrogênio amoniacal total (TAN) ultrapassou 1 mg L^{-1} (Figura 1a), necessitando de trocas de água. O tratamento Simbiótico apresentou melhor controle de compostos nitrogenados e acúmulo do nitrogênio do nitrato (NO_3^--N) mais acentuado em relação ao BFT (Figura 2b), sugerindo nitrificação mais eficaz.

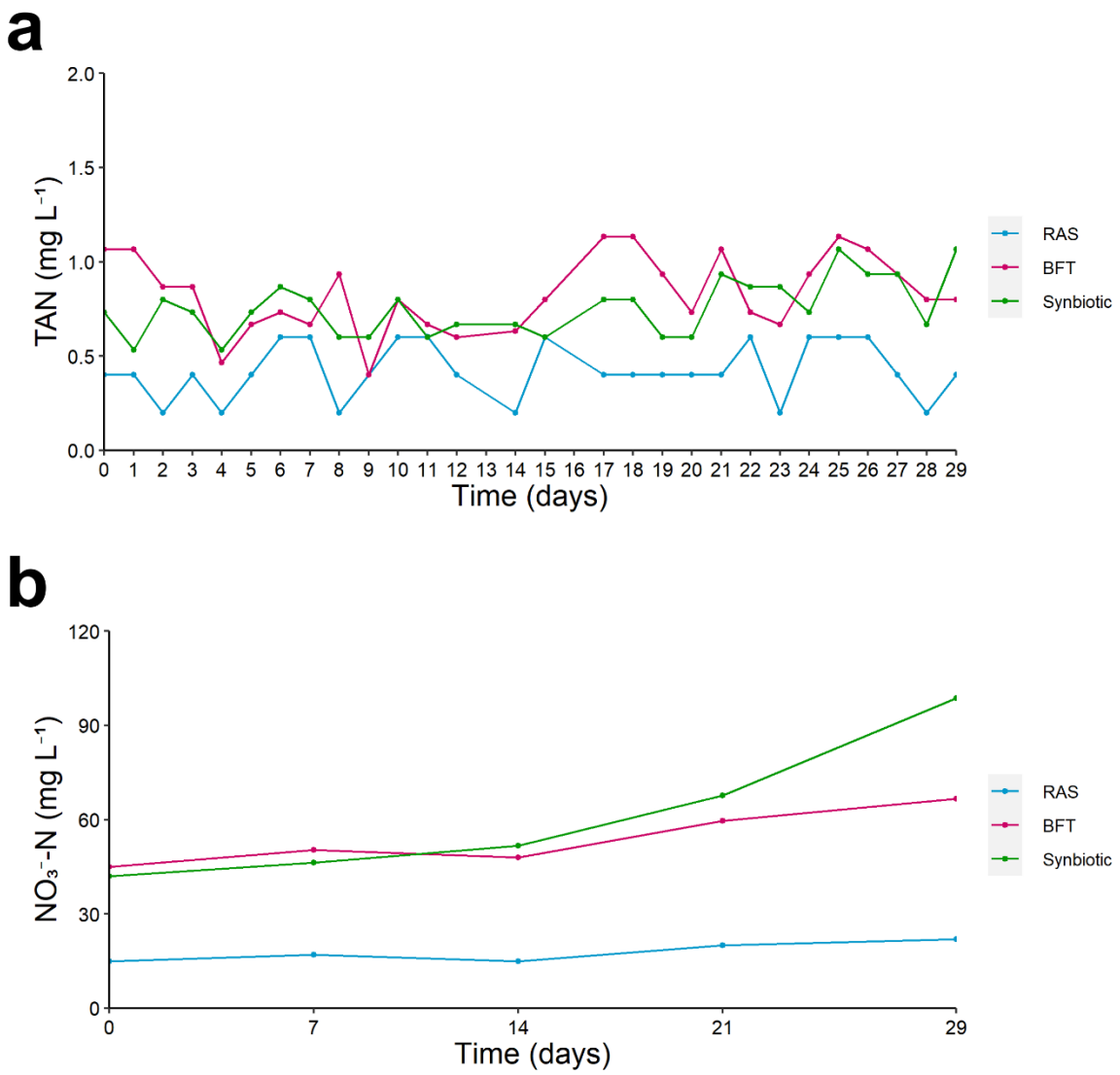

Figura 1. Concentração do TAN (a) e NO_3^--N (b) durante um cultivo superintensivo de *Penaeus vannamei* com sistema de recirculação (RAS), tecnologia de bioflocos (BFT) e sistema simbiótico (Synbiotic) com água de baixa salinidade por 30 dias.

O tratamento BFT teve mais microalgas que Simbiótico e RAS. No entanto, a abundância de zooplâncton foi maior que a do fitoplâncton nos tratamentos BFT e Simbiótico, indicando uma dominância de organismos heterotróficos. O Simbiótico apresentou maior abundância de ciliados e amebas do que os demais tratamentos (Figura 2a e b).

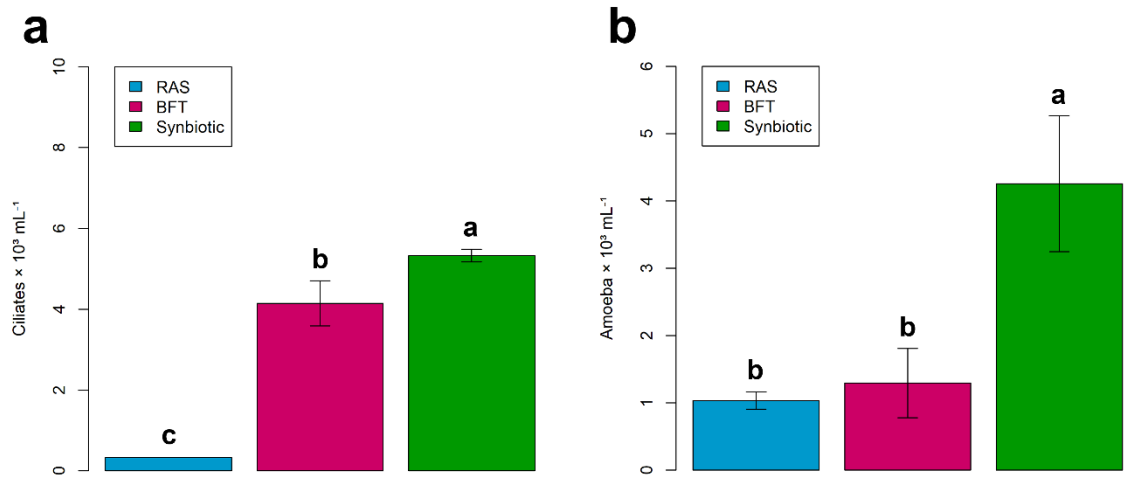

Figura 2. Abundância (organismos mL^{-1}, média ± desvio padrão) de ciliados (a) e ameba (b) durante um cultivo superintensivo de *Penaeus vannamei* com sistema de recirculação (RAS), tecnologia de bioflocos (BFT) e sistema simbiótico (Synbiotic) com água de baixa salinidade por 30 dias.

Além disso, o tratamento simbiótico proporcionou maior sobrevivência, produtividade e menor fator de conversão alimentar do que o BFT e o RAS. Os resultados indicaram que o sistema Simbiótico pode ser considerado como uma alternativa para o cultivo superintensivo de *P. vannamei* em águas de baixa salinidade, pois apresentou melhor controle de compostos nitrogenados comparado ao BFT, maior abundância de ciliados e amebas e proporcionou melhores taxas de produção ao cultivo.

The super-intensive culture of *Penaeus vannamei* in low salinity water: A comparative study among recirculating aquaculture system, biofloc, and synbiotic systems

Abstract

The culture of *Penaeus vannamei* in inland regions using low salinity water is a reality in several regions of the world. The dissemination of culture techniques that allow high stocking density and reduced water use can be used to optimize the production process, but they require comparative studies to better understand the functioning of these systems in those salinity conditions. Therefore, this study aimed to analyze the effects of different culture systems, which were recirculating aquaculture system (RAS), biofloc technology (BFT), and synbiotic system (Synbiotic) on the water quality, plankton composition, and growth of *P. vannamei* in low salinity (2 g L^{-1}) and high stocking density (500 shrimp m^{-3}) for 30 days. The shrimp were stocked at a mean weight of 1.27 ± 0.06 g. In the BFT, dextrose was used as the organic carbon source and administered at a carbon: nitrogen ratio of 15:1. In the Synbiotic, rice bran processed by probiotic microorganisms was used as an organic fertilization strategy. In the RAS treatment, all nitrogen species remained stable throughout the trial, without spikes, and with ammonia and nitrate concentrations lower than in BFT and Synbiotic. The BFT treatment had more events when total ammonia nitrogen exceeded 1 mg L^{-1}, requiring water changes. The Synbiotic treatment had better control of nitrogenous compounds, and a more accentuated accumulation of nitrate compared to BFT, suggesting more effective nitrification. The BFT treatment had more microalgae than Synbiotic and RAS. However, the abundance of zooplankton was higher than that of phytoplankton in BFT and Synbiotic, indicating a dominance by heterotrophic organisms. The Synbiotic treatment had a higher abundance of ciliates and amoeba than the other treatments. Furthermore, the Synbiotic treatment provided higher survival, yield, and lower FCR than BFT and RAS. Our findings indicate that the Synbiotic system can be considered as an alternative for the *P. vannamei* super-intensive culture in low salinity water, as it presented better control of nitrogenous compounds compared to BFT, higher abundance of ciliates and amoeba, and provided better production rates to the culture.

Keywords: RAS; BFT; Water quality; Shrimp growth; Phytoplankton; Zooplankton.

1. Introduction

The production of crustaceans by aquaculture around the world is dominated by marine shrimp of the species *Penaeus vannamei* (FAO, 2024). This production in inland regions represented a total of 8.7 % of world production in 2022 (FAO, 2024). The interiorization of *P. vannamei* culture is possible due to the hardiness characteristics of the animal, which supports a wide variation in salinity and high stocking densities (Van Wyk et al., 1999; Prangnell et al., 2019a). This makes it possible to disseminate activity with the use of intensive systems, such as Recirculating Aquaculture System (RAS), Biofloc Technology System (BFT), and the Synbiotic System.

RAS is a system with controlled conditions, where water is treated before being recirculated among the culture tanks, allowing the establishment of different setups and with high design flexibility (Ahmed and Turchini, 2021). The water treatment takes place mainly through mechanical and biological filtration, and oxygenation (Nugraha et al., 2023). In RAS, the constant water filtration process for solids retention makes bacteria the main microorganisms that grow in this system (Rurangwa and Verdegem, 2015). One of the advantages of using RAS in shrimp production is the maintenance of clear water, good control of nitrogenous compounds, through the nitrification process, and reduction of water use (Nugraha et al., 2023). However, the long-term accumulation of nitrate and the use of electrical energy to keep the system running increase the production cost and can be considered a disadvantage for the RAS (Badiola et al., 2018).

The BFT is a microbial system based on the use of a simple organic carbon source (e.g., molasses) to control ammonia through the growth of heterotrophic bacteria (Avnimelech, 2012). Adjusting the carbon:nitrogen (C:N) ratio of water largely mediates the growth and activity of heterotrophic bacterial communities in the biofloc system (Ebeling et al., 2006). In addition to heterotrophic bacteria, chemoautotrophic bacteria also grow in the BFT system and play a key role in controlling nitrogenous compounds through the oxidation of ammonia to nitrite and nitrate (Abakari et al., 2021). The growth of microorganisms over time creates microbial aggregates that act as water quality controllers and as a supplementary source of food (Martínez-Córdova et al., 2015; Krummenauer et al., 2020). The comparative culture of *P. vannamei* between RAS and BFT was carried out by Tierney and Ray (2018) using seawater, where the authors found better water quality conditions in the RAS treatment than in the BFT. Furthermore, *P. vannamei* growth in BFT systems has been shown to be superior to RAS in

seawater (Ramiro et al., 2024a). In the case of shrimp, BFT may be superior to RAS, however this does not work for all species, as reported by Romano et al. (2020) in the culture of largemouth bass (*Micropterus salmoides*). The authors reported in the BFT a stressful condition for the fish that caused a reduction in feed intake, reducing the final weight and leading to the understanding that this system was unsuitable for the culture of that species (Romano et al., 2020).

Recently, the Synbiotic system has been growing with the potential to expand the management tools for intensive shrimp production systems. The Synbiotic is characterized by the fertilization of water with vegetable bran processed by probiotic microorganisms (Kawahigashi, 2018). In this system, the processed bran has a dual function, acting to control water quality and as a nutritious supplementary food source for shrimp, since probiotic microorganisms colonize the bran grains during the processing phase (Khanjani et al., 2023). Furthermore, this culture strategy promotes the growth of microorganisms. Synbiotic generally employs stocking densities that can vary between 150 and 300 shrimp m^{-2} (Khanjani et al., 2023). The stocking densities used in the Synbiotic are lower than those used in the BFT, which have already been tested with up to 2250 shrimp m^{-3} in the grow-out phase (Silveira et al., 2022). Therefore, it is important to assess its performance using higher densities so that it is possible to understand the behavior of this system and design more efficient management strategies for it. The Synbiotic system, together with the BFT, can be advantageous compared to RAS mainly due to the strong development of phytoplankton, zooplankton, and bacterial communities. This is one of the factors that make a difference in these systems, as it can improve shrimp growth, reduce feed use, and improve the animals' immune status (Khanjani et al., 2022).

It is important to highlight the lack of comparative studies among these culture systems using water with salinity 2 g L^{-1} (i.e., oligohaline water) and the impacts of their use on the control of nitrogenous compounds and the microbial composition of the system. Therefore, the aim of this study was to analyze the effect of different culture systems, which were recirculating aquaculture system (RAS), biofloc technology (BFT), and synbiotic system (Synbiotic), on the water quality, plankton composition, and growth of *P. vannamei* in low salinity water and high stocking density.

2. Materials and Methods

2.1. Experimental design and conditions

This study was carried out at the Virginia Seafood Agricultural Research and Extension Center at Virginia Polytechnic Institute and State University, USA. A *P. vannamei* culture with low salinity water was conducted for 30 days in experimental units of 0.1 m^3 testing the following treatments, all with three repetitions: Recirculating Aquaculture System (RAS), Biofloc Technology System (BFT), and Synbiotic System (Synbiotic).

The water used in the experiment was prepared by mixing dechlorinated tap freshwater with artificial salt (Instant Ocean Sea Salt) to reach a salinity 2 g L^{-1}. In the RAS treatment, the water was constantly recirculated using a centrifugal pump with a flow rate of approximately 180 L h^{-1} (Doheny's, model 2601, 0.75 HP), filtered through a mechanical filter (Bubble bead filter), and a biological filter (with K1 Kaldnes Biological Media constantly aerated), before returning the experimental units. The RAS system had a total volume of approximately 0.6 m^3. Backwashes were carried out once a week to clean the mechanical filter. Each experimental unit of the RAS treatment received one air stone for oxygen supplementation. The BFT and Synbiotic treatments were static and received pillows, measuring 15 ×15 cm, containing artificial substrates that were inserted into the experimental units to assist in the nitrification process. The pillows were composed of K1 Kaldnes Biological Media. The RAS did not receive pillows with biological media because it contains a biofilter as a water treatment step. All experimental units were illuminated with LED light with a spectrum of 5000 K + 660 nm of red light. The lights were placed 33 cm above the tanks and followed a photoperiod of 12 h light and 12 h dark. In all treatments, the water temperature was maintained close to 30 °C using heaters. Each experimental unit of the BFT and Synbiotic treatments received four air stones for oxygen supplementation.

2.2. Source of shrimp, acclimation, and stocking

The shrimp post-larvae were acquired in a commercial hatchery and initially maintained in a nursery, with salinity 28 g L^{-1}, for 35 days, at a density of 1500 shrimp m^{-3} until they reached a mean weight of 1.27 ± 0.06 g. During the nursery, the post-larvae were fed with commercial feed containing 50 % crude protein and 15 % lipids (Zeigler Bros). Then, the juveniles were gradually acclimatized over seven days until they reached a salinity 2 g L^{-1}. After this period, the animals were stocked in the experimental units at a density of 500 shrimp m^{-3}.

2.3. Fertilization

Prior to the animals' stocking, the RAS treatment received two applications of 5 mg L^{-1} of ammonium chloride (NH_4Cl) over 10 days to assist in the system's maturation process. The BFT treatment reused water from a previous culture at 28 g L^{-1} salinity. The biofloc water had the following physicochemical characteristics (mean of three repetitions ±standard deviation): total ammonia nitrogen (TAN): 1.68 ±0.33 mg L^{-1}, nitrite nitrogen (NO_2^--N): 37.63 ±0.68 mg L^{-1}, nitrate nitrogen (NO_3^--N): 170.00 ±26.45 mg L^{-1}, alkalinity: 160.00 ±0.00 mg L^{-1}, total suspended solids (TSS): 252.66 ±17.92 mg L^{-1}, and pH: 7.70 ± 0.00. The salinity of the biofloc water was reduced to 2 g L^{-1} over 13 days, with a reduction of 2 parts of salinity per day. Dextrose was used as an organic carbon source for the BFT treatment and was applied at a carbon:nitrogen (C:N) ratio of 15:1 (Ebeling et al., 2006; Avnimelech, 2012) when TAN reached 1 mg L^{-1}. In the RAS and BFT treatments, weekly applications of probiotic (INVE aquaculture, Sanolife Mic) were carried out at a concentration of 0.4 g m^{-3}.

The Synbiotic treatment received 16 daily organic fertilizations prior to shrimp stocking. The fertilizer used in the treatments had the following composition: rice bran (20 g m^{-3}), powder probiotic (0.4 g m^{-3}. Sanolife Mic, INVE aquaculture. Composition: *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus pumilus*. Concentration: 5 $\times 10^{10}$ colony forming units (CFU) g^{-1}), dextrose (2 g m^{-3}), sodium bicarbonate (2 g m^{-3}), and water at salinity 2 g L^{-1} (chlorinated and dechlorinated) in a proportion of ten times the amount of rice bran. The fertilizer was processed through a fermentation phase (12h) and a microbial respiration phase (12 h), following Pimentel et al. (2024a). After this period, it was applied in the system. Along with organic fertilization, the experimental units received daily applications of 1 mg L^{-1} of NH_4Cl as a nitrogen source to assist in the nitrifying bacteria development in the system. During the experimental time, daily applications of organic fertilizer were made during the first fifteen days. After this period, organic fertilizations were carried out three times a week. The initial water quality conditions of the RAS, BFT, and Synbiotic treatments are described in detail in Table 1.

Table 1. Water quality variables at the beginning of a *Penaeus vannamei* super-intensive culture in a recirculating aquaculture system (RAS), biofloc technology (BFT), and synbiotic system (Synbiotic) with low salinity water for 30 days.

Variables	Treatments		
	RAS	BFT	Synbiotic

Temperature (°C)	30.60 ± 0.00	29.53 ± 0.38	29.23 ± 0.15
DO (mg L^{-1})	6.10 ± 0.00	6.30 ± 0.00	6.36 ± 0.23
pH	7.80 ± 0.00	7.83 ± 0.06	8.00 ± 0.10
Salinity (g L^{-1})	2.40 ± 0.00	2.16 ± 0.06	2.13 ± 0.06
TAN (mg L^{-1})	0.40 ± 0.00	1.06 ± 0.11	0.73 ± 0.11
NO_2^--N (mg L^{-1})	0.12 ± 0.00	0.26 ± 0.02	0.19 ± 0.03
NO_3^--N (mg L^{-1})	15.00 ± 0.00	45.00 ± 1.00	42.00 ± 3.46
Alkalinity (mg L^{-1})	140.00 ± 0.00	113.33 ± 11.55	140.00 ± 0.00
CO_2 (mg L^{-1})	4.43 ± 0.00	3.32 ± 0.41	2.84 ± 0.65
TSS (mg L^{-1})	0.00 ± 0.00	161.00 ± 45.97	99.33 ± 13.05
SS (mL L^{-1})	0.00 ± 0.00	9.16 ± 1.04	3.66 ± 1.75

Data are mean ± standard deviation. DO: dissolved oxygen; TAN: total ammonia nitrogen; NO_2^--N: nitrite nitrogen; NO_3^--N: nitrate nitrogen; CO_2: carbon dioxide; TSS: total suspended solids; SS: settleable solids.

2.4. Water quality

Temperature (°C), dissolved oxygen (DO, mg L^{-1}; Multiparameter YSI, model Pro2030), pH (portable pH meter Hanna, model HI98107), and TAN (mg L^{-1}; Hach method 8038) were analyzed daily. Twice a week, NO_2^--N (mg L^{-1}; Hach method 8507), alkalinity (mg L^{-1}; APHA, 2012), and carbon dioxide (CO_2, mg L^{-1}; Timmons and Ebeling, 2013) were measured. Salinity (g L^{-1}; Multiparameter YSI, model Pro2030), NO_3^--N (mg L^{-1}; Hach method 8039), TSS (mg L^{-1}; Hach portable multiparameter colorimeter, model DR900), and settleable solids (SS, mL L^{-1}; Eaton et al., 1995) were analyzed weekly. Calcium (Ca^{2+}, mg L^{-1}. Salifert Ca Profi Test), Magnesium (Mg^{2+}, mg L^{-1}. Salifert Mg Profi Test), Mg:Ca ratio, and total hardness (mg $CaCO_3$ L^{-1}; Boyd, 2020) were analyzed at the beginning and end of the trial. If TAN exceeded 1 mg L^{-1}, water changes were carried out at a rate of 10 % of the experimental unit volume. When necessary, applications of sodium bicarbonate were made to maintain alkalinity at 150 mg L^{-1}, following Furtado et al. (2011).

2.5. Plankton community composition

At the end of the experimental time, water samples were collected and preserved with ethanol at a final concentration of 70 % for subsequent identification and quantification of the main groups of Phytoplankton and Zooplankton. The analysis was carried out through direct counting under an optical microscope with a Sedgewick rafter chamber (APHA, 2017).

Phytoplankton and Zooplankton abundance were expressed in cells mL^{-1} and organisms mL^{-1}, respectively.

2.6. Feed management

During the trial, the animals were fed twice a day by hand with a commercial diet containing 35 % crude protein and 7 % lipids (Zeigler Bros). The daily amount of feed was calculated according to Jory et al. (2001).

2.7. Shrimp growth

At the end of the trial, shrimp were sampled to determine the following parameters: final weight (g), survival (%) [(number of animals at the end of experimental period/initial number of animals) × 100], feed conversion ratio (FCR) [(feed supplied/biomass gain)], weekly growth rate (WGR, g $week^{-1}$) [(final weight – initial weight)/ time (weeks)], and yield (Kg m^{-3}) calculated as [final biomass (Kg)/volume of the experimental unit (m^3)].

2.8. Data analysis

Water quality data were tested for normality and homoscedasticity with the Shapiro-Wilk and the Levene tests, respectively. Differences among groups were assessed with repeated measures analysis of variance (ANOVA), followed by the Tukey's test. When necessary, data were transformed to fulfill parametric assumptions.

Data on ionic profile of the water (analyzed separately for each sampled time), total abundance and the main groups of phytoplankton and zooplankton, and shrimp growth were tested for normality, with the Shapiro-Wilk test, and homoscedasticity with the Levene test. One-way ANOVA followed by Tukey's test were used to analyze the differences among treatments. When necessary, the data were transformed to fulfill parametric assumptions. Survival percentage data were transformed into arcsine before analysis (Zar, 2010). Ca^{2+} (final sample) non-parametric data was tested with Kruskal-Wallis followed by Dunn's test with Bonferroni correction.

A significance level of $\alpha = 0.05$ was adopted in all tests. The graphs, the one-way ANOVA, Kruskal-Wallis, and their post hoc were performed in the software R 4.3.1 (R Core Team, 2023), using the following packages: ggplot2 (Wickham, 2016), Rmisc (Hope, 2022), car (Fox and Weisberg, 2019), and Dunn.test (Dinno, 2017). The repeated measures ANOVA and its post hoc were performed using PAST 4.03 2020 software (Hammer et al., 2001).

3. Results

3.1. Water quality

The temperature was kept at approximately 30 °C in all treatments (Table 2). The DO was above 5 mg L^{-1}. TAN, NO_3^--N, and TSS were higher in the BFT and Synbiotic treatments than in the RAS (p-value < 0.01) (Table 2). The SS was higher in the BFT treatment than in the RAS and Synbiotic (p-value < 0.05) (Table 2).

During the trial, the BFT treatment had more events where the mean TAN exceeded 1 mg L^{-1} (Figure 1a). The mean concentration of NO_2^--N was less than 0.5 mg L^{-1} in all treatments, with no spikes throughout the experimental time (Table 2, Figure 1b). A pattern of increasing NO_3^--N concentration was observed throughout the trial in the BFT and Synbiotic treatments (Figure 1c).

Table 2. Water quality variables during a *Penaeus vannamei* super-intensive culture in a recirculating aquaculture system (RAS), biofloc technology (BFT), and synbiotic system (Synbiotic) with low salinity water for 30 days.

Variables	Treatments		
	RAS	BFT	Synbiotic
Temperature (°C)	30.29 ± 0.19	29.49 ± 0.25	29.76 ± 0.49
DO (mg L^{-1})	6.00 ± 0.15	6.02 ± 0.25	5.81 ± 0.41
pH	8.01 ± 0.16	8.06 ± 0.16	8.06 ± 0.14
Salinity (g L^{-1})	2.36 ± 0.05	2.24 ± 0.18	2.19 ± 0.11
TAN (mg L^{-1})	0.42 ± 0.14^b	0.83 ± 0.25^a	0.75 ± 0.18^a
NO_2^--N (mg L^{-1})	0.14 ± 0.02	0.19 ± 0.06	0.17 ± 0.03
NO_3^--N (mg L^{-1})	17.80 ± 2.88^b	53.93 ± 9.07^a	61.27 ± 21.68^a
Alkalinity (mg L^{-1})	135.60 ± 8.47	125.90 ± 23.41	134.10 ± 14.48
CO_2 (mg L^{-1})	2.91 ± 1.56	2.37 ± 1.26	2.49 ± 1.00
TSS (mg L^{-1})	0.00 ± 0.00^b	147.50 ± 30.57^a	136.60 ± 30.97^a
SS (mL L^{-1})	0.00 ± 0.00^c	16.99 ± 8.26^a	9.20 ± 5.74^b

Data are mean ± standard deviation. DO: dissolved oxygen; TAN: total ammonia nitrogen; NO_2^--N: nitrite nitrogen; NO_3^--N: nitrate nitrogen; CO_2: carbon dioxide; TSS: total suspended solids; SS: settleable solids.

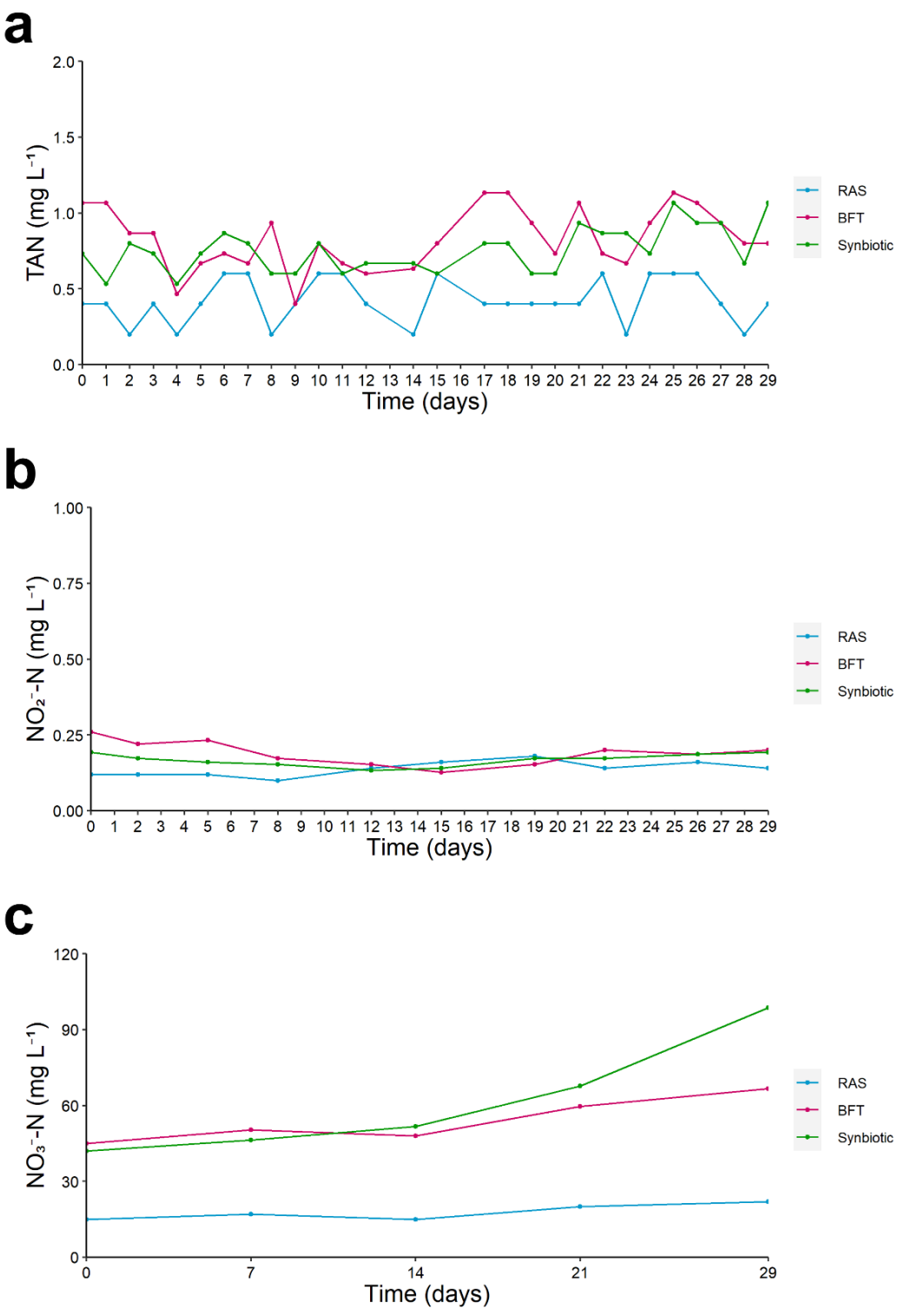

Figure 1. Concentration of total ammonia nitrogen (TAN, a), nitrite nitrogen (NO_2^--N, b), and nitrate nitrogen (NO_3^--N, c) during a *Penaeus vannamei* super- intensive culture in a recirculating aquaculture system (RAS), biofloc technology (BFT), and synbiotic system (Synbiotic) with low salinity water for 30 days.

At the beginning of the trial, Ca^{2+} was higher in RAS than in BFT (Table 3). Mg^{2+} and the Mg:Ca ratio were higher in BFT and Synbiotic than in RAS. Total hardness was higher in BFT than in RAS (Table 3). At the end of the experiment, Ca^{2+} was higher in RAS than in BFT and total hardness was higher in RAS and Synbiotic than in BFT (Table 3).

Table 3. Water ionic profile during a *Penaeus vannamei* super-intensive culture in a recirculating aquaculture system (RAS), biofloc technology (BFT), and synbiotic system (Synbiotic) with low salinity water for 30 days.

	Initial			Final		
	Treatments			Treatments		
	RAS	BFT	Synbiotic	RAS	BFT	Synbiotic
Ca^{2+} (mg L^{-1})	100.00 ± 0.00^{a}	83.33 ± 5.77^{b}	90.00 ± 10.00^{ab}	110.00 ± 0.00^{a}	80.00 ± 10.00^{b}	90.00 ± 0.00^{ab}
Mg^{2+} (mg L^{-1})	90.00 ± 0.00^{b}	140.00 ± 17.32^{a}	110.00 ± 17.32^{a}	120.00 ± 0.00	100.00 ± 17.32	130.00 ± 17.32
Mg:Ca	0.90 ± 0.00^{b}	1.68 ± 0.19^{a}	1.23 ± 0.25^{a}	1.09 ± 0.00	1.26 ± 0.25	1.44 ± 0.19
TH (mg L^{-1})	620.80 ± 0.00^{b}	785.10 ± 79.56^{a}	678.20 ± 75.61^{ab}	769.40 ± 0.00^{a}	612.00 ± 75.61^{b}	760.60 ± 71.36^{a}

Data are mean ± standard deviation. Ca^{2+}: calcium; Mg^{2+}: magnesium; Mg:Ca: magnesium:calcium ratio; TH: total hardness.

3.2. Plankton composition

3.2.1. Phytoplankton

The BFT treatments had a higher total phytoplankton abundance than the Synbiotic and RAS treatments (p-value < 0.01) (Figure 2a). The RAS treatment was dominated by Bacillariophyta, while the BFT and Synbiotic treatments had a dominance of Chlorophyta (Figure 3a). The BFT treatment had a higher abundance of Chlorophyta and Bacillariophyta than the Synbiotic and RAS (p-value < 0.01) (Figure 4).

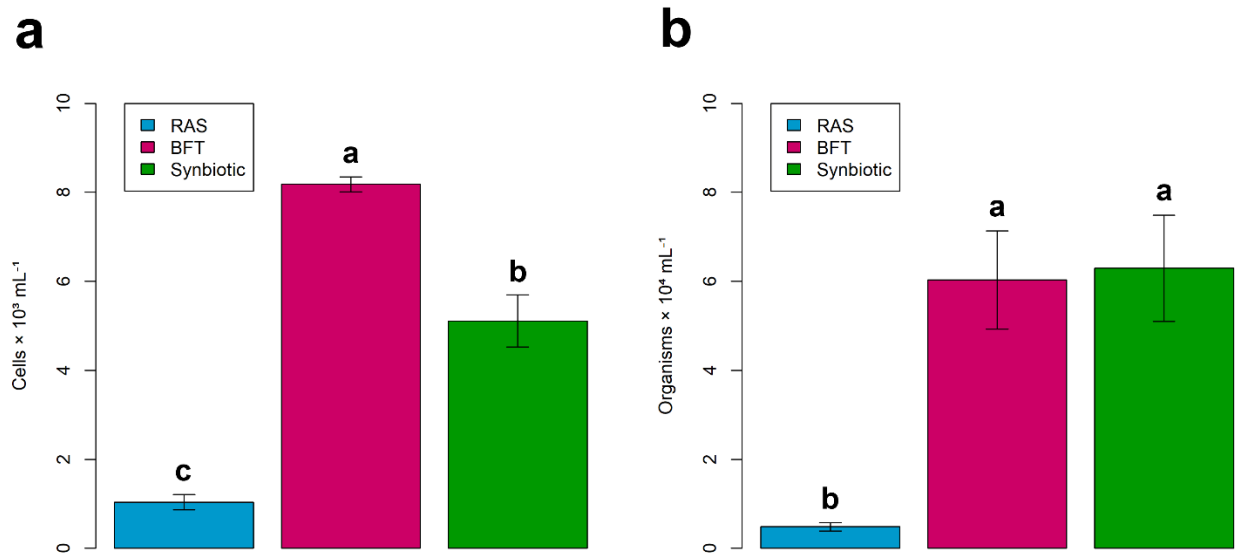

Figure 2. Total abundance (mean ± standard deviation) of phytoplankton (a) and zooplankton (b) at the end of a *Penaeus vannamei* super-intensive culture in a recirculating aquaculture system (RAS), biofloc technology (BFT), and synbiotic system (Synbiotic) with low salinity water for 30 days.

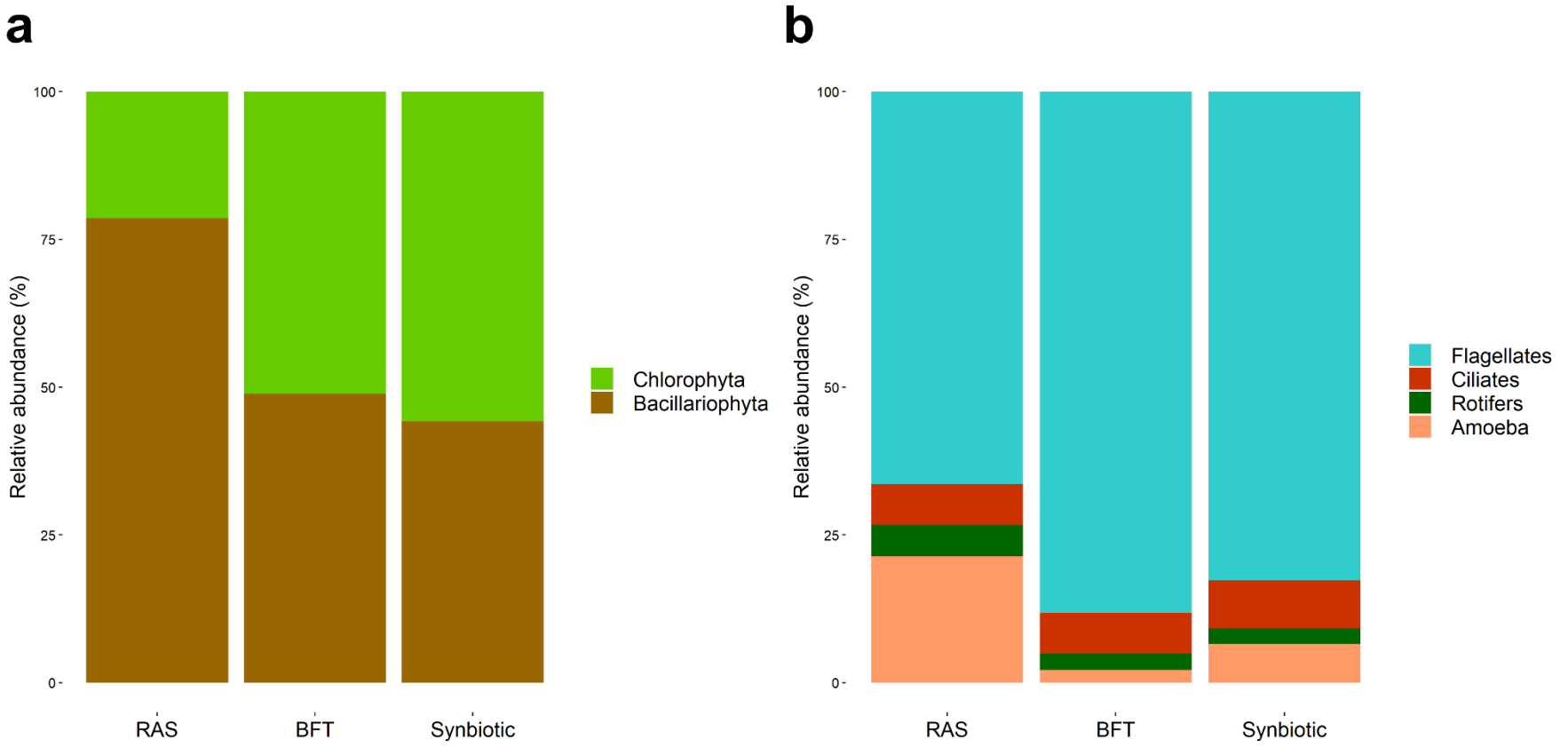

Figure 3. Relative abundance of phytoplankton (a) and zooplankton (b) at the end of a *Penaeus vannamei* super-intensive culture in a recirculating aquaculture system (RAS), biofloc technology (BFT), and synbiotic system (Synbiotic) with low salinity water for 30 days.

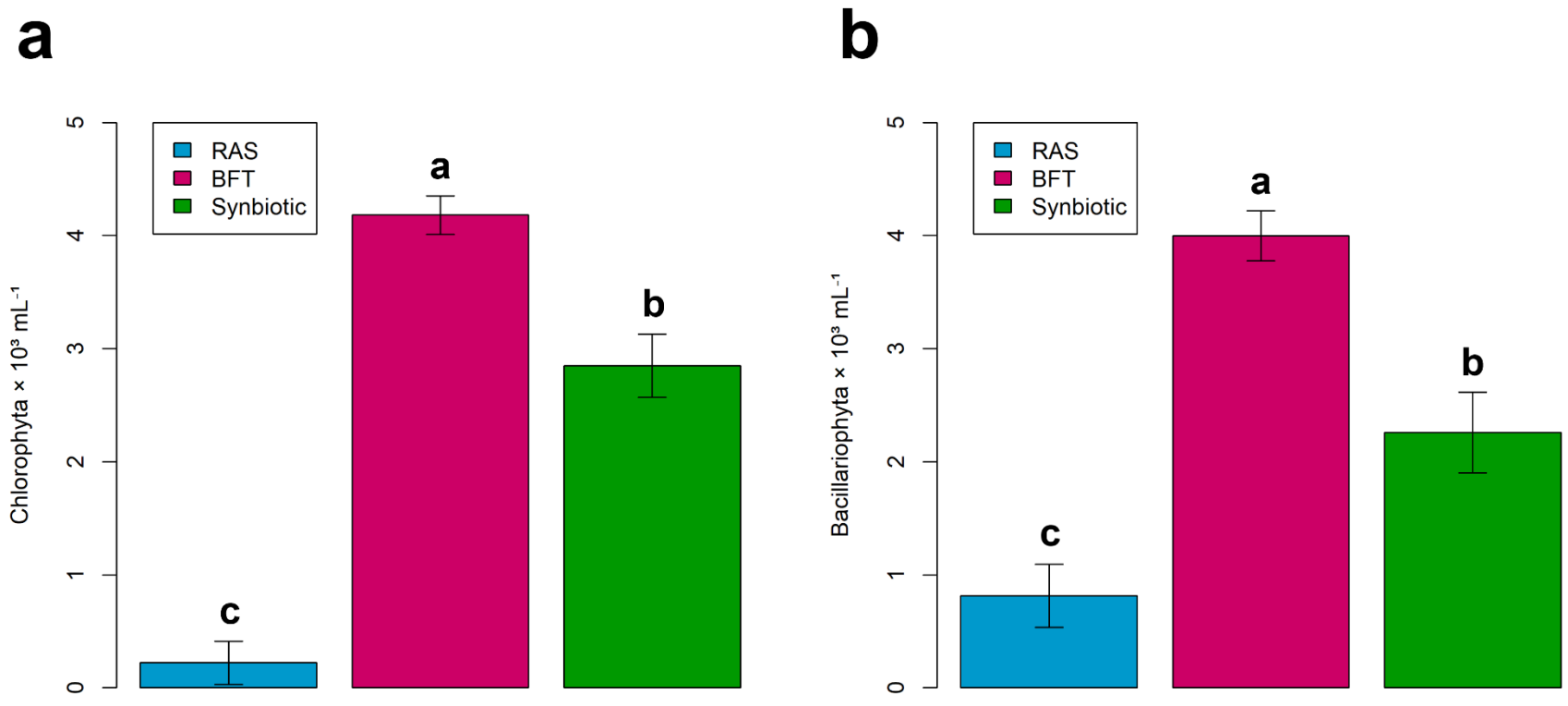

Figure 4. Abundance (mean ± standard deviation) of Chlorophyta (a) and Bacillariophyta (b) at the end of a *Penaeus vannamei* super-intensive culture in a recirculating aquaculture system (RAS), biofloc technology (BFT), and synbiotic system (Synbiotic) with low salinity water for 30 days.

3.2.2. *Zooplankton*

The BFT and Synbiotic treatments had a higher total abundance of zooplankton than the RAS treatment (p-value < 0.01) (Figure 2b). All treatments had a dominance of flagellated protozoa (Figure 3b). The BFT and Synbiotic treatments showed a similar pattern in the zooplankton relative abundance (Figure 3b). The BFT and Synbiotic treatments had a higher abundance of flagellates and rotifers than the RAS treatment (p-value < 0.01) (Figure 5a and c). The Synbiotic treatment had a higher abundance of ciliates and amoeba than the RAS and BFT (p-value < 0.01) (Figure 5b and d).

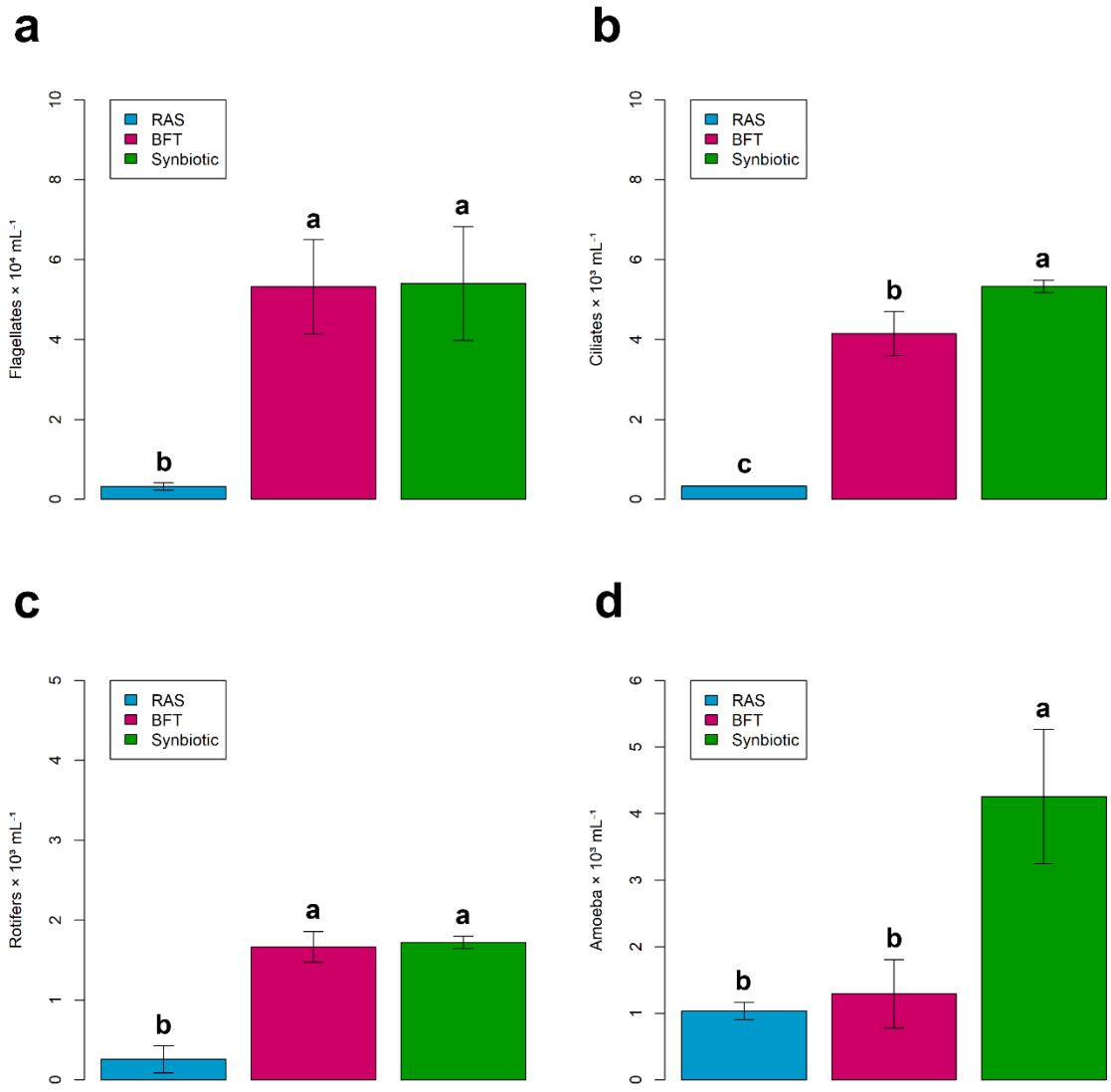

Figure 5. Abundance (mean ±standard deviation) of flagellates (a), ciliates (b), rotifers (c) and amoeba (d) at the end of a *Penaeus vannamei* super-intensive culture in a recirculating

aquaculture system (RAS), biofloc technology (BFT), and synbiotic system (Synbiotic) with low salinity water for 30 days.

3.3. Shrimp growth

The final weight and weekly growth rate were higher in the BFT and Synbiotic treatments (p-value < 0.05) (Table 4). Survival and yield were higher in the Synbiotic treatment (p-value < 0.01 and < 0.05) (Table 4). The FCR was lower in the synbiotic treatment (p-value < 0.05) (Table 4).

Table 4. Growth of *Penaeus vannamei* at the end of a super-intensive culture in a recirculating aquaculture system (RAS), biofloc technology (BFT), and synbiotic system (Synbiotic) with low salinity water for 30 days.

	Treatments		
	RAS	BFT	Synbiotic
Initial weight (g)	1.27 ± 0.06	1.27 ± 0.06	1.27 ± 0.06
Final weight (g)	4.45 ± 0.14^{b}	5.14 ± 0.22^{a}	4.99 ± 0.14^{a}
Survival (%)	80.67 ± 7.02^{b}	85.33 ± 5.77^{b}	98.00 ± 2.83^{a}
WGR (g week^{-1})	0.75 ± 0.03^{b}	0.91 ± 0.05^{a}	0.87 ± 0.03^{a}
FCR	2.01 ± 0.17^{a}	1.80 ± 0.23^{a}	1.38 ± 0.09^{b}
Yield (Kg m^{-3})	1.79 ± 0.10^{b}	1.93 ± 0.16^{b}	2.31 ± 0.11^{a}

Data are mean ± standard deviation. FCR: feed conversion ratio; WGR: weekly growth rate.

4. Discussion

During the experimental time, the variables temperature, dissolved oxygen, pH, CO_2, TSS, SS, and total hardness were within the recommended limits for the *Penaeus vannamei* culture in intensive systems (Van Wyk et al., 1999; Samocha and Prangnell, 2019). The concentration of Ca^{2+} and Mg^{2+} observed during the trial were above the minimum concentration recommended for intensive shrimp culture in low-salinity water (Pimentel et al., 2022). Although the Mg:Ca ratio did not follow the recommended 3:1 (Samocha and Prangnell, 2019), this was not a limiting factor for the shrimp growth and has already been reported in other studies. These results are reinforced by those found by Moura et al. (2021) who tested the partial inclusion of seawater in the *P. vannamei* intensive cultures using low salinity water and found no differences in shrimp growth at Mg:Ca ratios between 1.34:1 (salinity 1.72 g L^{-1}) and 2:1 (salinity 5.03 g L^{-1}). RAS was the treatment that provided the best conditions for controlling nitrogenous compounds. In the RAS, periodic cleaning of the mechanical filter is

essential to maintain the proper functioning of the system (Xiao et al., 2019). In this way, much of the nitrate produced by the nitrification process is discarded. This probably happened in this study, where backwashing was carried out weekly, causing the nitrate concentration to be lower than in other treatments. On the other hand, the high microbial activity in the BFT and Synbiotic treatments may have contributed to a higher nitrate accumulation.

The patterns found in the BFT suggest that the community of ammonia-oxidizing bacteria was not completely established, as it had more events where the mean TAN exceeded 1 mg L^{-1}, causing water changes to be carried out to maintain the concentration of this compound within acceptable limits for the species. For salinity 3 g L^{-1}, the safe level of total ammonia nitrogen for *P. vannamei* juveniles is estimated to be 0.93 mg L^{-1} (Li et al., 2007). It is important to highlight that in low salinity water the toxicity of ammonia and nitrite to shrimp is higher (Prangnell et al., 2019b) and that spikes in the concentration of these compounds must be avoided so that they do not cause damage to production. For example, high concentrations of ammonia can reduce shrimp growth, while high concentrations of nitrite also reduce growth, and can reduce shrimp respiratory functions (Romano and Zeng, 2013).

The Synbiotic treatment had better control of nitrogenous compounds throughout the experimental time compared to BFT, as there were fewer events where the mean TAN exceeded 1 mg L^{-1}. Another factor that contributed to this conclusion was the more pronounced increase in nitrate in the Synbiotic than in the BFT treatment, starting on day 14, and reaching a concentration of 98.67 mg L^{-1} at the end of the trial. These findings showed the efficiency of the protocol adopted for the maturation of the Synbiotic system, which included daily organic fertilization of the water with rice bran processed by probiotic microorganisms associated with inorganic fertilization with ammonium chloride until the nitrogen compounds were stabilized. This process took 16 days and proved to be effective for the development of ammonia-oxidizing and nitrite-oxidizing bacteria in the system, which can be confirmed with low nitrite concentrations and no spikes throughout the experimental course. Our results differ from those reported by Pimentel et al. (2024b), where the authors required 24 daily fertilizations to mature *P. vannamei* nurseries using low salinity water. The maturation process may have been faster in this study due to inorganic fertilization carried out prior to shrimp stocking, providing a source of nitrogen to stimulate the growth of nitrifying bacteria in addition to the carbon source contributed by the synbiotic fertilization.

Regarding plankton composition, the BFT treatment had a higher abundance of Chlorophyta and Bacillariophyta than that found in the synbiotic treatment. This may be

another factor that helps explain the ammonia spikes in BFT, since they release organic nitrogen into the environment when they die, and this is recycled in the form of ammonia by heterotrophic bacteria (Hargreaves, 2006). In systems dominated by photoautotrophic organisms, variations in the concentration of ammonia, dissolved oxygen, and pH are frequent and make system management difficult (Ebeling et al., 2006). Despite the higher abundance of microalgae in the BFT, Zooplankton were more abundant than Phytoplankton in both BFT and Synbiotic. This may indicate heterotrophic dominance in both treatments. Stimulating dominance by heterotrophic microorganisms in intensive shrimp farming systems is important to achieve better control of Phytoplankton, water quality, and nutrient cycling (Khanjani et al., 2022). Systems dominated by heterotrophic organisms are a striking feature of BFT and Synbiotic systems (Pimentel et al., 2024c; Ramiro et al., 2024b).

The BFT and Synbiotic treatments demonstrated a dominance by flagellated protozoa. This reinforces the idea of heterotrophic dominance in these treatments, since the growth of flagellates is mediated by the availability of bacteria in the system, which are the main source of food for these protozoa (Wetzel, 2001). Zooplankton diversity patterns showed the development of the microbial loop in both treatments. The microbial loop is characterized by the incorporation of dissolved organic matter into bacterial biomass that is consumed by heterotrophic protozoa (Sanders, 2022). This was further reinforced in the Synbiotic treatment, where the abundance of ciliates and amoeba was higher than in the other treatments. Like flagellates, amoebas feed primarily on bacteria (Sanders, 2022) and the presence of these microorganisms has already been reported in Synbiotic systems (Andrade et al., 2021). Ciliates have an important ecological function in aquatic ecosystems, consuming flagellated protozoa, microalgae, and bacteria (Sanders, 2009) and can be a nutritious food source for shrimp cultivated in intensive and super-intensive systems.

Overall, the BFT and Synbiotic treatments had a higher abundance of microorganisms than the RAS. This was expected, due to the operational characteristics of the systems. The presence of microorganisms can be considered an advantage, as it clearly influenced shrimp growth. Of the three treatments, the Synbiotic presented the best survival and yield indicators and the lowest FCR. This demonstrates the positive effect of microorganisms as a supplementary natural food source for shrimp, which can improve the animals' immune status, increase system yield, and reduce feed costs. These factors are important when we are dealing with the culture of marine shrimp in low salinity water since this is an adverse condition for the animal. Therefore, creating a comfortable environment in terms of water quality and rich

in microorganisms can be considered an alternative to the *P. vannamei* super-intensive culture in inland regions with low-salinity water. It is important to highlight that these results demonstrate a short period of a grow-out and that a complete cycle must be tested to confirm the positive effect of the Synbiotic system in *P. vannamei* super-intensive culture systems. Furthermore, as a comparative example, considering the stocking density and experimental time, our findings are superior to those reported by Oliveira et al. (2022) who tested the *P. vannamei* grow out in water with salinity 2.7 g L^{-1} for 56 days at a density of 300 shrimp m^{-3} reached a mean weight varying between 3.8 and 4.7 g.

5. Conclusion

The Synbiotic system can be considered for the *Penaeus vannamei* super-intensive culture with low salinity water. The Synbiotic provided better control of nitrogenous compounds throughout the experimental time than the BFT and a higher abundance of ciliates and amoeba than the RAS and BFT. This was reflected in the growth rates of the shrimp, which in this treatment achieved higher survival, yield, and lower FCR than the RAS and BFT.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Data availability

Data will be made available on request.

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DISCUSSÃO GERAL

O uso do sistema simbiótico atualmente é marcado por uma grande disparidade nas estratégias de fertilização, indicando que estudos precisam ser realizados para melhorar o entendimento do funcionamento do sistema. As diversas estratégias de fertilização usadas na presente tese demonstraram que o uso do simbiótico em cultivos intensivos de *P. vannamei* proporciona uma melhoria nas condições de qualidade de água, composição microbiana e crescimento do camarão. Ainda, os achados da presente tese contribuem para uma melhor padronização do uso desse sistema, uma vez que foram analisados diversos passos do processo de fertilização passando pelo uso de diferentes farelos, fases de processamento e inoculação de microrganismos probióticos, chegando ao uso do protocolo em cultivos superintensivos usando água com baixa salinidade.

No capítulo II foi testado o efeito de diferentes farelos vegetais na fertilização de berçários intensivos de *P. vannamei* com sistema simbiótico. Os padrões temporais dos compostos nitrogenados produzidos pelo uso dos farelos de arroz, trigo e soja, demonstraram o estabelecimento de um sistema recém iniciado, apesar de ter recebido fertilizações previas ao início do experimento. O sistema simbiótico, mesmo não controlando a relação C:N para 15:1 como acontece no sistema de bioflocos (Ebeling et al., 2006; Avnimelech, 2012), provou controlar os compostos nitrogenados. Isso provavelmente aconteceu devido a atividade das bactérias heterotróficas e nitrificantes estimulada pelas fertilizações orgânicas através do farelo vegetal processado pelos microrganismos probióticos.

No que diz respeito a composição planctônica, o sistema simbiótico criou um meio com condições para o desenvolvimento de microalgas Clorofitas e Bacillariofitas, que tem seu crescimento mediado por compostos como a amônia e nitrato (Hargreaves 1998; Hargreaves, 2006). Além do mais, esses microrganismos servem de base para o crescimento da alça microbiana, transferindo energia e matéria para os níveis tróficos mais altos (Khanjani et al., 2022). Isso pode ser comprovado pelo aumento na abundância de microrganismos protozoários observado em todos os tratamentos, com exceção do controle. Nesse estudo, o tratamento que usou farelo de arroz foi capaz de produzir mais bactéria cocoides e bacillus, que são importantes na formação dos agregados microbianos, na ciclagem de nutrientes e no controle de microrganismos patogênicos (Ferreira, 2008; Krummenauer et al., 2014; Ferreira et al., 2015; Suita et al., 2015). Ao final do ensaio, o tratamento que usou farelo de trigo foi o menos eficiente no controle do *Vibrio*, apresentando uma abundância superior aos demais. As

bactérias do gênero *Vibrio* são patogênicas e quando infectam um cultivo, reduzem o crescimento do camarão, causam opacidade do musculo, letargia e mortalidade (Dash et al., 2017). Esse grupo de bactérias é predominantemente responsável por cerca 20% das perdas no cultivo de camarão (Abdel-Latif et al., 2022). Dessa forma, o controle desses microrganismos é essencial para alcançar o sucesso da carcinicultura.

Em relação ao crescimento do camarão, o uso de farelo de arroz provou ser a melhor estratégia, apresentando resultados de crescimento comparáveis ao sistema de bioflocos e superior ao encontrado para o tratamento que usou farelo de trigo. De fato, o sistema simbiótico já tinha sido comparado ao sistema de bioflocos e apresentado resultados semelhantes de sobrevivência (Hussain et al., 2021). Os bons resultados de crescimento do camarão em sistema simbiótico também foram confirmados por Abdel-Tawwab et al. (2022) que encontrou um melhor desempenho dos camarões em um sistema suplementado com farelo de arroz fermentado por *Bacillus subtilis*. O uso de farelos vegetais no cultivo de camarão pode ser considerada uma alternativa sustentável para a atividade, uma vez que eles são subprodutos do processamento dos grãos, possuem baixo custo, alta disponibilidade e não competem com a alimentação humana.

No capítulo III, foi avaliado o efeito da fermentação e da respiração microbiana e diferentes tempos de processamento em berçários intensivos de *P. vannamei*. O tratamento que usou a fermentação e a respiração associadas por um período de 12h cada (F12+R12) demonstrou um estabelecimento mais rápido das bactérias nitrificantes, o que foi evidenciado pela redução mais rápida da amônia em relação aos demais tratamentos. Além do mais, uma tendencia de aumento mais acentuada do nitrato foi observada nesse tratamento, o que reforça as evidências de atividade das bactérias nitrificantes. O comportamento do nitrito ao longo do ensaio, sem um padrão marcado de controle, demonstrou que a estratégia de fertilizar as unidades experimentais na frequência de quatro vezes na semana não favoreceu o desenvolvimento da comunidade de bactérias nitrito-oxidantes. O percentual de remoção do nitrogênio pelas bactérias quimioautotróficas reduz com o aumento da relação carbono:nitrogênio do sistema (i.e., aumento da concentração de carbono do meio) (Ebeling et al., 2006). Além do mais, quantidade de matéria orgânica no sistema também reduz a atividade das bactérias nitrificantes (Abakari et al., 2021). Isso indica que a adição continua de fertilizante deve ser evitada.

Em relação a comunidade microbiana, o tratamento F12+R12 apresentou uma abundância maior de Bacilariofitas, ciliados e nematoides, demonstrando ter as melhores condições para o desenvolvimento da alça microbiana. Além do mais, ao longo do ensaio, todos os tratamentos foram dominados por bactérias cocoides, bacillus e filamentosas livres. Esses padrões de dominância também foram reportados para sistemas intensivos de cultivo de *P. vannamei* com sistema de bioflocos (Xavier et al., 2022; Reis et al., 2023). O crescimento de uma maior carga de microrganismos no tratamento F12+R12 foi capaz de reduzir em 30,17% o fator de conversão alimentar quando comparado ao controle, onde um sistema de água clara foi usado. No controle, o crescimento microbiano não foi estimulado, o que fez com que a ração fosse a principal fonte de alimento para os camarões. Isso comprovou o efeito positivo dos microrganismos na redução do uso de ração e que pode reduzir o custo de produção do camarão. A fase de fermentação juntamente com a fase de respiração é igualmente importante no processamento do farelo vegetal, uma vez que elas atuam na quebra de compostos complexos em compostos mais simples e promovem o crescimento dos microrganismos probióticos no fertilizante, respectivamente (Madigan et al., 2019; Dawood e Koshio, 2020). Os achados deste capítulo demonstraram que a fermentação por 12h somada ao processo de respiração por 12h foi a melhor estratégia de fertilização, controlando a amônia mais rapidamente, produzindo mais microrganismos e reduzindo o fator de conversão alimentar do camarão. Esses resultados representam uma estratégia prática para a otimização do manejo do sistema simbiótico

No capítulo IV onde foi avaliado o uso de diferentes microrganismos probióticos na composição do fertilizante simbiótico, o tratamento que usou as cepas de *Bacillus*, *Lactobacillus* e *Pediococcus* (BLP) foi a melhor estratégia para o controlar a amônia. O BLP proporcionou menor variação da amônia, o que foi confirmado com a maior abundância de bactérias oxidantes de amônia no meio do experimento e acúmulo mais rápido de nitrito nesse tratamento. Apesar são ter sido observada nenhuma tendencia de controle do nitrito nos estágios finais do ensaio, o tratamento BLP provavelmente controlaria esse composto mais rapidamente. Nesse tratamento, uma maior abundância de bactérias oxidantes de nitrito foi observada ao final do período experimental quando comparado aos tratamentos que usaram *Bacillus*, *Lactobacillus*, *Pediococcus* e leveduras (*Saccharomyces cerevisiae*) (BLPY) e o que usou somente *Bacillus* e leveduras (BY) na composição do fertilizante simbiótico. No geral, os tratamentos que usaram a levedura tiveram um processo de nitrificação mais lento, o que foi

inesperado pelo fato das leveduras atuarem na decomposição de carbono orgânico (Starmer e Lachance, 2011; Bai et al., 2022).

Neste estudo, o sistema foi dominado por algas Bacilariofitas. Esses microrganismos são benéficos para o sistema, uma vez que podem servir como fonte suplementar de alimento natural para os camarões e controlar microrganismos nocivos como as cianobactérias (Martins et al., 2016; Marinho et al., 2017; Abreu et al., 2019). A dominância do zooplâncton por microrganismos protozoários pode ser explicada pela estratégia de fertilização, que disponibiliza altas quantidade de carbono orgânico no sistema, favorecendo o desenvolvimento de bactérias, que são a principal fonte de alimento para o protozooplâncton (Khanjani e Sharifinia, 2020). Em relação a presença de *Vibrio*, o tratamento BLP provou ser o melhor no controle desses microrganismos patogênicos, o que pode se refletir em um bom crescimento e sobrevivência do camarão (Krummenauer et al., 2014; Panigrahi et al., 2022).

De fato, o tratamento BLP melhorou o peso final dos camarões, produtividade e reduziu o fator de conversão alimentar. O uso de probióticos promove a colonização o trato digestivo do camarão, melhorando a digestibilidade da ração (Ninawe e Selvin, 2009; Madhana et al., 2021). Isso pode ser melhorado com o uso do sistema simbiótico, uma vez que os probióticos colonizam os grãos de farelo durante os processos de fermentação e respiração, que podem ser consumidos pelos camarões quando aplicados no sistema de cultivo (Khanjani et al., 2023). Esses achados mostraram que o uso de um probiótico com uma composição mais variada de cepas de bactérias no fertilizante simbiótico é efetiva no controle da qualidade de água, controle de organismos patogênicos e crescimento do camarão.

No capítulo V, foram avaliados os efeitos de diferentes sistemas de cultivos na qualidade de água, composição planctônica e crescimento de *P. vannamei* em água com baixa salinidade (2 g L^{-1}) e alta densidade de estocagem (500 camarões m^{-3}). Nesse estudo foram testados um sistema de recirculação (RAS), sistema de bioflocos (BFT) e o sistema simbiótico. No tratamento simbiótico, o protocolo de fertilização usado foi estabelecido com base nos achados dos capítulos anteriores desta tese. Além do mais, uma fertilização previa a estocagem dos animais juntamente com a adição de uma fonte de nitrogênio inorgânica foi adotada para estimular o crescimento da comunidade de bactérias nitrificantes, tendo em vista as condições de salinidade. Os resultados demonstraram que o RAS proporcionou as melhores condições de controle dos compostos nitrogenados. No tratamento BFT, a comunidade de bactérias oxidantes de amônia não estava completamente estabelecida, uma vez que teve mais eventos

onde a amônia excedeu 1 mg L^{-1} e trocas de água foram necessárias para manter a concentração daquele composto dentro dos limites recomendados para o *P. vannamei*. O tratamento simbiótico teve as melhores condições de controle dos nitrogenados comparado ao BFT, com poucos eventos onde trocas de água foram necessárias. Isso mostrou que o protocolo de fertilização adotado foi eficiente no estabelecimento microbiano do sistema.

A composição do fitoplâncton demonstrou uma elevada abundância de Clorofitas e Bacilariofitas no BFT comparado com o tratamento simbiótico. Isso pode ter sido mais um fator que levou a picos de amônia nesse tratamento, já que sistemas dominados por microrganismos fotoautotróficos são mais propensos a ter maiores variações na concentração de amônia, oxigênio e pH (Ebeling et al., 2006). O zooplâncton foi mais abundante no BFT e simbiótico do que o fitoplâncton, indicando uma dominância desses sistemas por microrganismos heterotróficos. Isso se reforçou com a maior presença de protozoários flagelados nesses tratamentos. Os padrões do zooplâncton demonstraram o desenvolvimento da alça microbiana (Khanjani et al., 2022), principalmente no tratamento simbiótico, onde a abundância de ciliados e amebas foi maior do que os demais tratamentos.

Dos três tratamentos, o simbiótico demonstrou a maior sobrevivência e produtividade e menor fator de conversão alimentar. Esses achados mais uma vez demonstraram o efeito dos microrganismos como fonte de alimento suplementar, o que pode melhorar a saúde dos animais, melhorar a produtividade e reduzir os custos com ração. Esses fatores são importantes quando se trata do cultivo de camarões marinhos em águas de baixa salinidade, pois esta é uma condição adversa para o animal.

Os resultados e conclusões desta tese, como o uso de farelo de arroz como fonte de carbono orgânico, o processamento do fertilizante por 12h de fermentação somada a 12h de respiração, a inoculação de um probiótico com uma composição mais diversa de microrganismos no fertilizante e a maturação do sistema com uma fonte de nitrogênio inorgânico são resultados práticos que contribuem para otimização do manejo de fertilização do sistema simbiótico. As estratégias de fertilização usadas na presente tese provaram produzir uma alta carga de microrganismos heterotróficos e proporcionaram o crescimento de bactérias oxidantes de amônia e nitrito, contribuindo para o controle da qualidade de água e de *Vibrio* no sistema. Além do mais, esses microrganismos podem servir como fonte de alimento natural para o *P. vannamei* cultivados com sistema simbiótico em alta densidade de estocagem.

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CONSIDERAÇÕES FINAIS

O uso de farelo de arroz é recomendo para a fertilização do sistema simbiótico, pois promove o crescimento do camarão *P. vannamei* semelhante aos sistemas de bioflocos e de água clara e superior ao que encontrado para o simbiótico fertilizado com farelo de trigo. Além do mais, o farelo de arroz produziu uma alta carga de microrganismos no sistema, principalmente bactérias *Bacillus*.

O processamento do farelo de arroz por uma fase de fermentação por 12 horas seguido de uma fase de respiração microbiana por 12 horas demonstrou ser a mais eficaz na fertilização de berçários intensivos de *P. vannamei* com sistema simbiótico. Esta estratégia foi capaz de controlar a amônia total mais rapidamente, acelerou o desenvolvimento da alça microbiana e resultou em uma maior produtividade e menor fator de conversão alimentar do camarão quando comparado ao tratamento controle, que usou água clara.

O uso de probióticos compostos de microrganismos do gênero *Bacillus*, *Lactobacillus* e *Pediococcus* na composição do fertilizante foi a melhor estratégia para a fertilização de berçários intensivos de *P. vannamei* com sistema simbiótico. O uso desses microrganismos proporcionou um controle mais rápido da amônia, maior abundância de bactérias oxidantes de amônia e oxidantes de nitrito, menor abundância de *Vibrio*, maior peso final do *P. vannamei*, maior produtividade e menor fator de conversão alimentar.

O sistema simbiótico pode ser considerado para o cultivo superintensivo de *P. vannamei* em água de baixa salinidade. O simbiótico provou melhorar o controle dos compostos nitrogenados, além de proporcionar o crescimento de mais microrganismos protozoários como ciliados e amebas. Esses resultados foram refletidos em uma maior sobrevivência, produtividade e menor fator de conversão alimentar do camarão.

Esses resultados têm implicações práticas nas técnicas de manejo de fertilização do sistema simbiótico, melhorando a qualidade de água, produzindo uma alta carga de microrganismos que podem servir de alimento natural para o camarão e controlam a presença de *Vibrio*, otimizando o cultivo intensivo do *P. vannamei* principalmente na fase de berçário. Entretanto, estudos ainda precisam ser realizados para aprimorar ainda mais o processo de fertilização desse sistema. O uso de farelos desengordurados na fertilização pode reduzir a carga de nutrientes nesse sistema, o que pode influenciar na qualidade de água e composição microbiana. Os efeitos da fertilização inicial no processo de nitrificação e na composição microbiana do sistema é outro tópico importante a ser explorado. Isto pode revelar o impacto da adição de carbono no desenvolvimento de bactérias quimioautotróficas no meio de cultura

e orientar o número ideal de fertilizações para melhor desenvolvimento dos processos microbianos. O uso de inóculo deve ser melhor estudado, possibilitando o reaproveitamento de água de ciclos de cultivo anteriores, reduzindo o uso de água e melhorando a biosseguridade. Estudos recentes mostram que a utilização de 15 a 20% de inóculo de ciclos anteriores, aliado à fertilização, foi suficiente para controlar compostos nitrogenados em cultivos de *P. vannamei* com sistema simbiótico. Esses resultados orientam essa questão, mas é necessário compreender os efeitos isolados do inóculo, seja ele utilizado com base em um percentual do volume do tanque ou com diferentes concentrações de sólidos suspensos totais (SST).

Outra variável que precisa ser melhor compreendida são os SST no sistema simbiótico. No simbiótico são relatadas baixas concentrações de sólidos sedimentáveis, indicando maior controle da produção de matéria orgânica. Porém, a leitura do SST é mais refinada e precisa ser estudada para saber quais limites de concentração podem ser adotados no sistema simbiótico sem afetar a estabilidade do sistema e o crescimento do camarão. Finalmente, o uso de substratos artificiais deve ser considerado no sistema simbiótico. Essa estratégia aumenta a superfície de contato para o crescimento de bactérias quimioautotróficas, reduz a densidade relativa de estocagem e fornece alimento natural para o camarão, melhorando o crescimento.

Muito progresso foi feito recentemente em termos de compreensão e otimização adicional dos regimes de fertilização dentro do sistema simbiótico pela comunidade de pesquisa. Da mesma forma, ainda há mais pesquisas que precisam ser feitas nesta área de investigação. Apesar disso, é importante enfatizar que os produtores de camarão que planejam utilizar essas abordagens de fertilização em seus sistemas devem fazê-lo da maneira mais eficiente possível para garantir a lucratividade e sustentabilidade da sua operação. A transferência de tecnologia e a adoção de estratégias de fertilização pelos agricultores podem ser facilitadas pela validação dessas abordagens em fazendas comerciais de camarão usando regimes de fertilização comprovados pelas pesquisas.

ANEXO

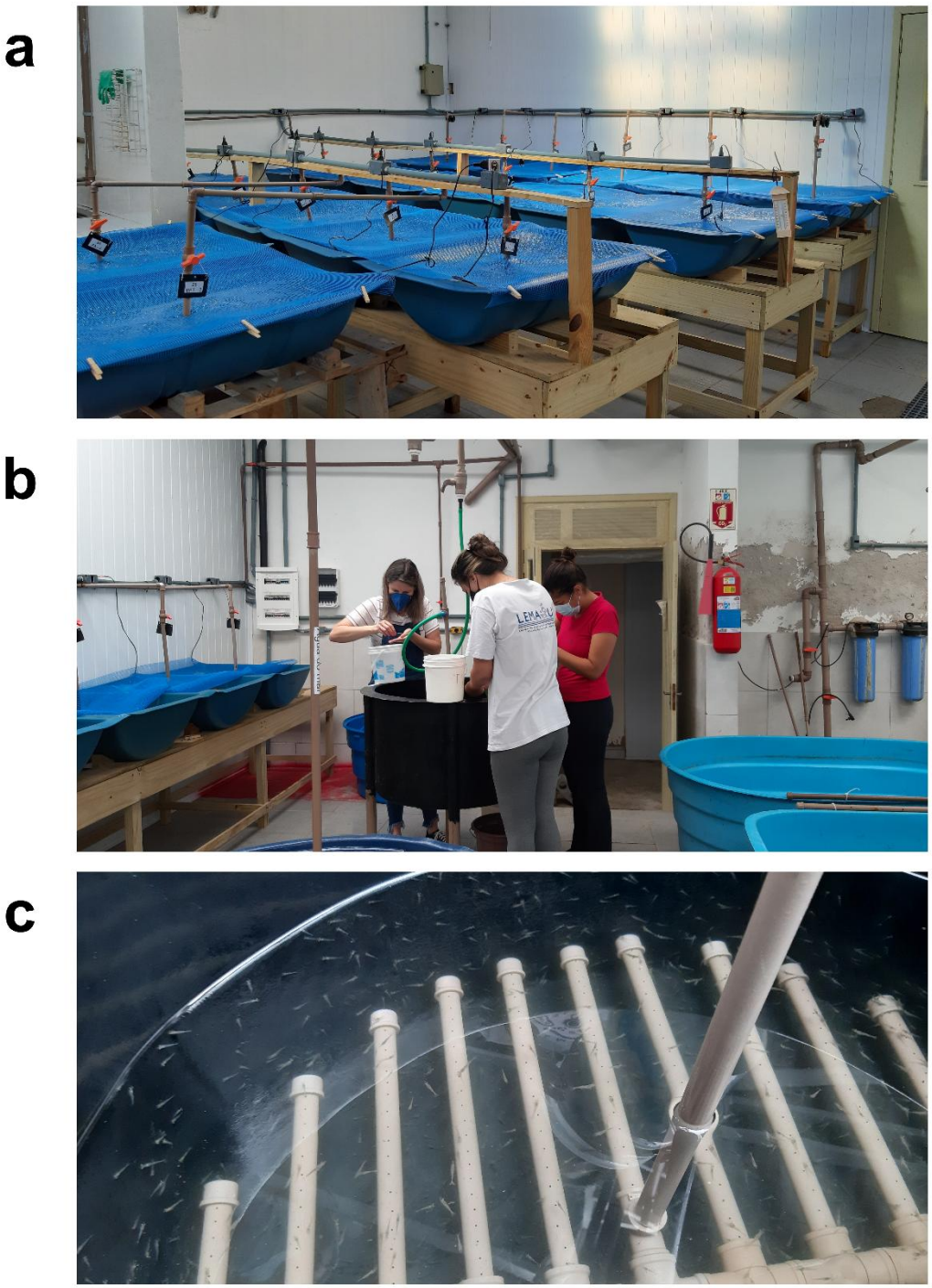

Figura 1. Estrutura usada para a realização dos experimentos referente aos capítulos II, III e IV (a), contagem dos camarões para estocagem do experimento referente ao capítulo II (b) e pós-larvas de *Penaeus vannamei* usadas para o experimento do capítulo II (c) onde foi testado o efeito de diferentes farelos vegetais como fonte de carbono orgânico para a fertilização do sistema simbiótico. Fotos: Otávio Augusto L. F. Pimentel.

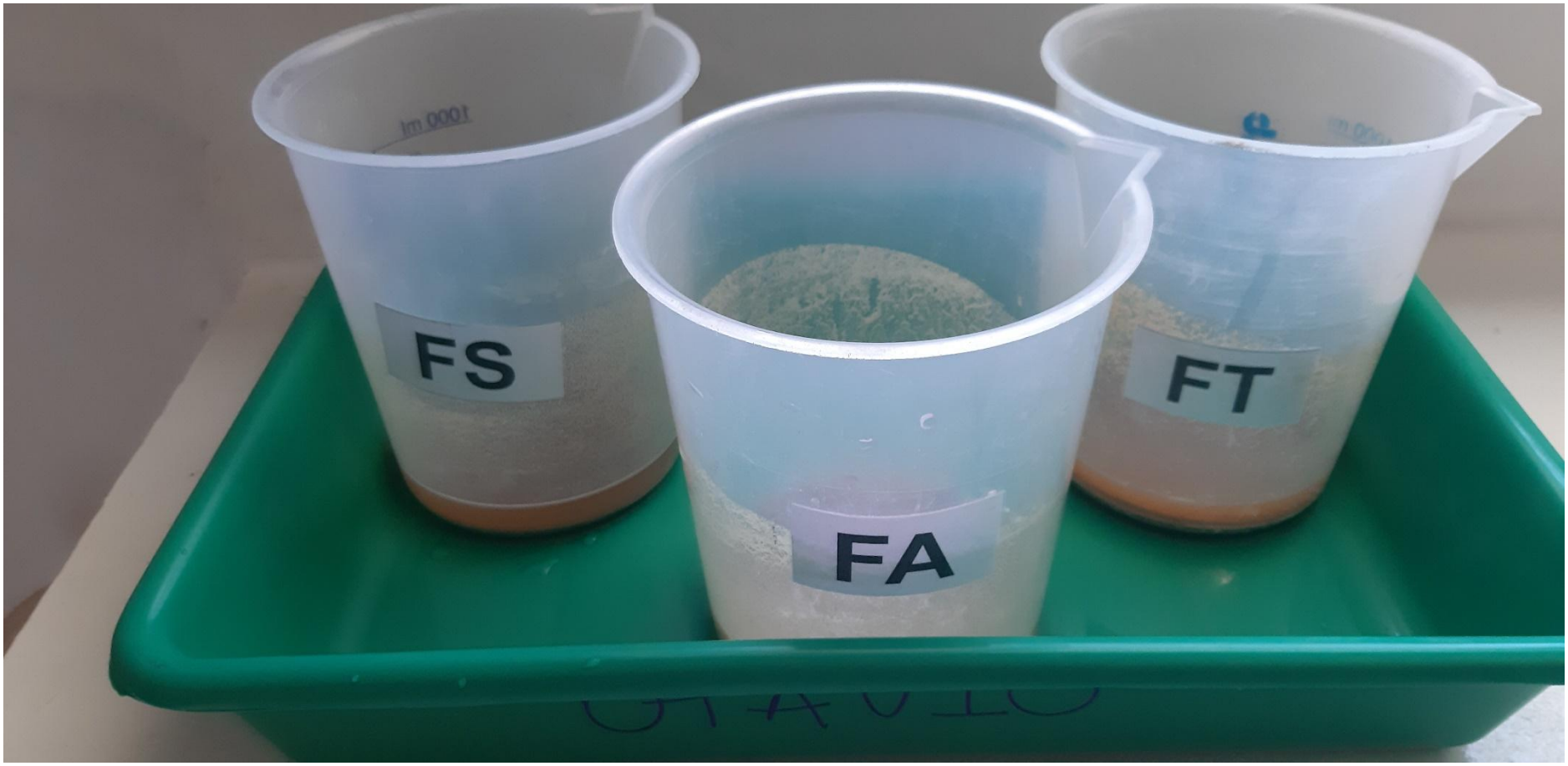

Figura 2. Fase de fermentação dos farelos vegetais usados como fonte de carbono orgânico para a fertilização de berçários intensivos de *P. vannamei* com sistema simbiótico (capítulo II). Foto: Otávio Augusto L. F. Pimentel

Figura 3. Juvenis de *P. vannamei* produzidos ao final do ensaio onde foi testado o efeito de diferentes farelos vegetais como fonte de carbono orgânico para a fertilização do sistema simbiótico (capítulo II). Fotos: Otávio Augusto L. F. Pimentel.

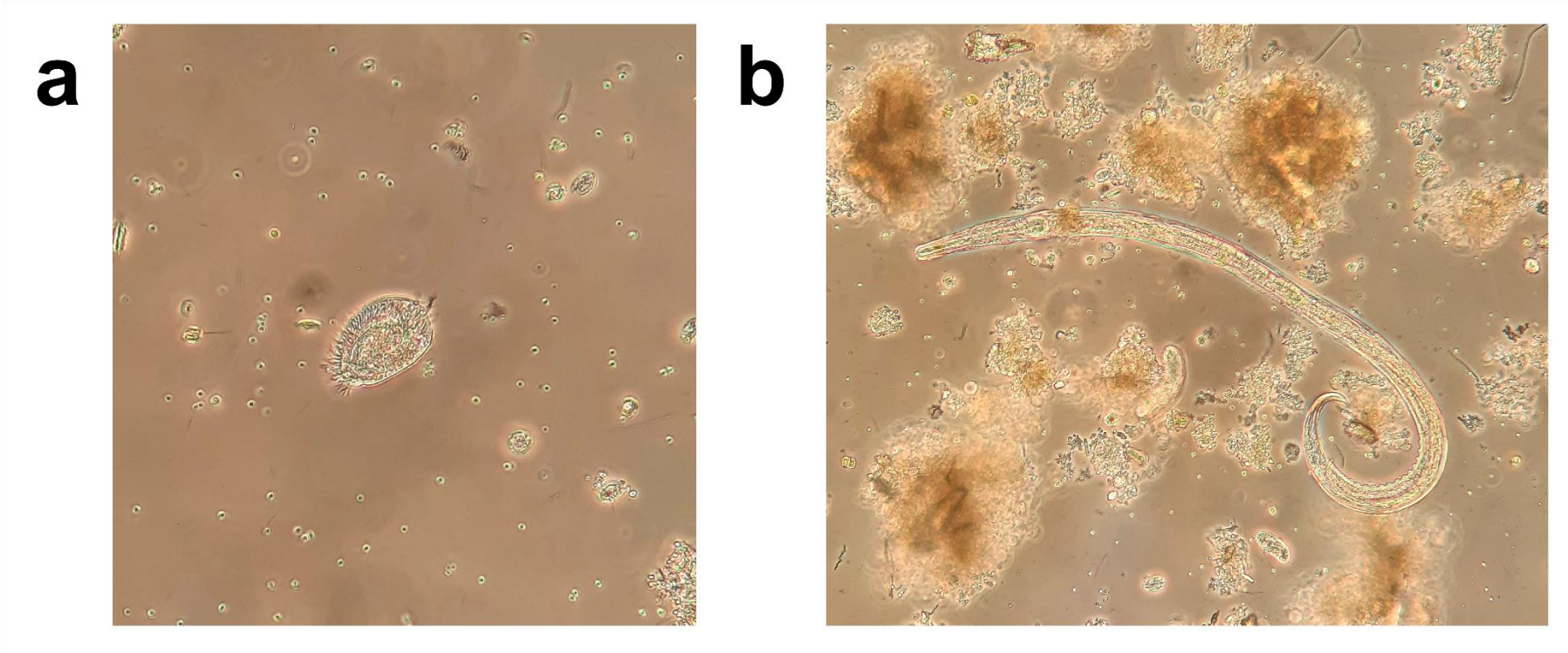

Figura 4. Protozoário ciliado (a) e nematoide (b) encontrado na água de cultivos intensivos de *P. vannamei* com sistema simbiótico fertilizado com diferentes farelos vegetais, sistemas de bioflocos e de água clara (capítulo II). Fotos: Otávio Augusto L. F. Pimentel.

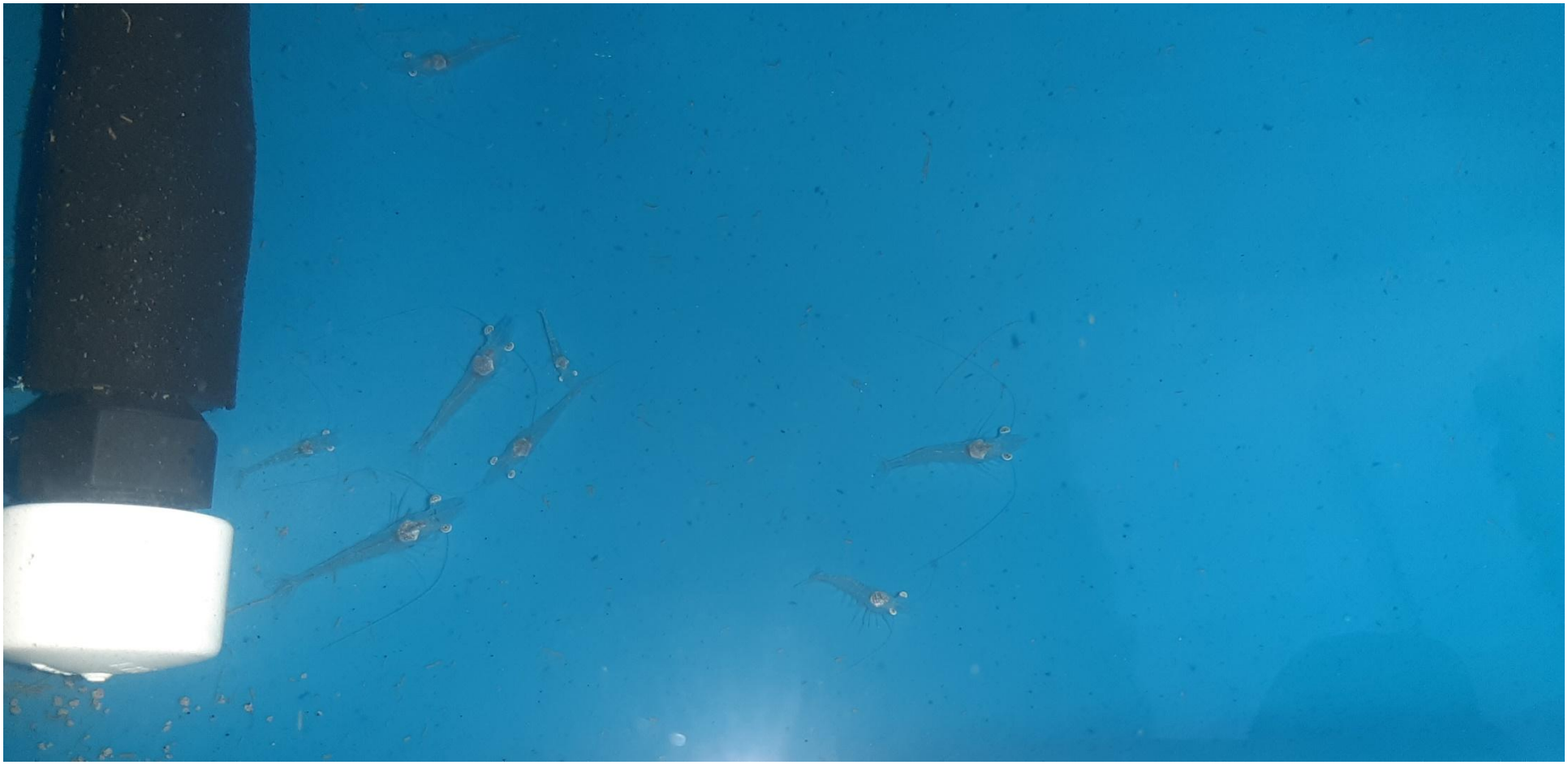

Figura 5. Pós-larvas de *P. vannamei* no tratamento de água clara do ensaio que testou o efeito de diferentes estratégias de processamento do fertilizante do sistema simbiótico e comparou com os sistemas de bioflocos e de água clara (capítulo III). Foto: Otávio Augusto L. F. Pimentel.

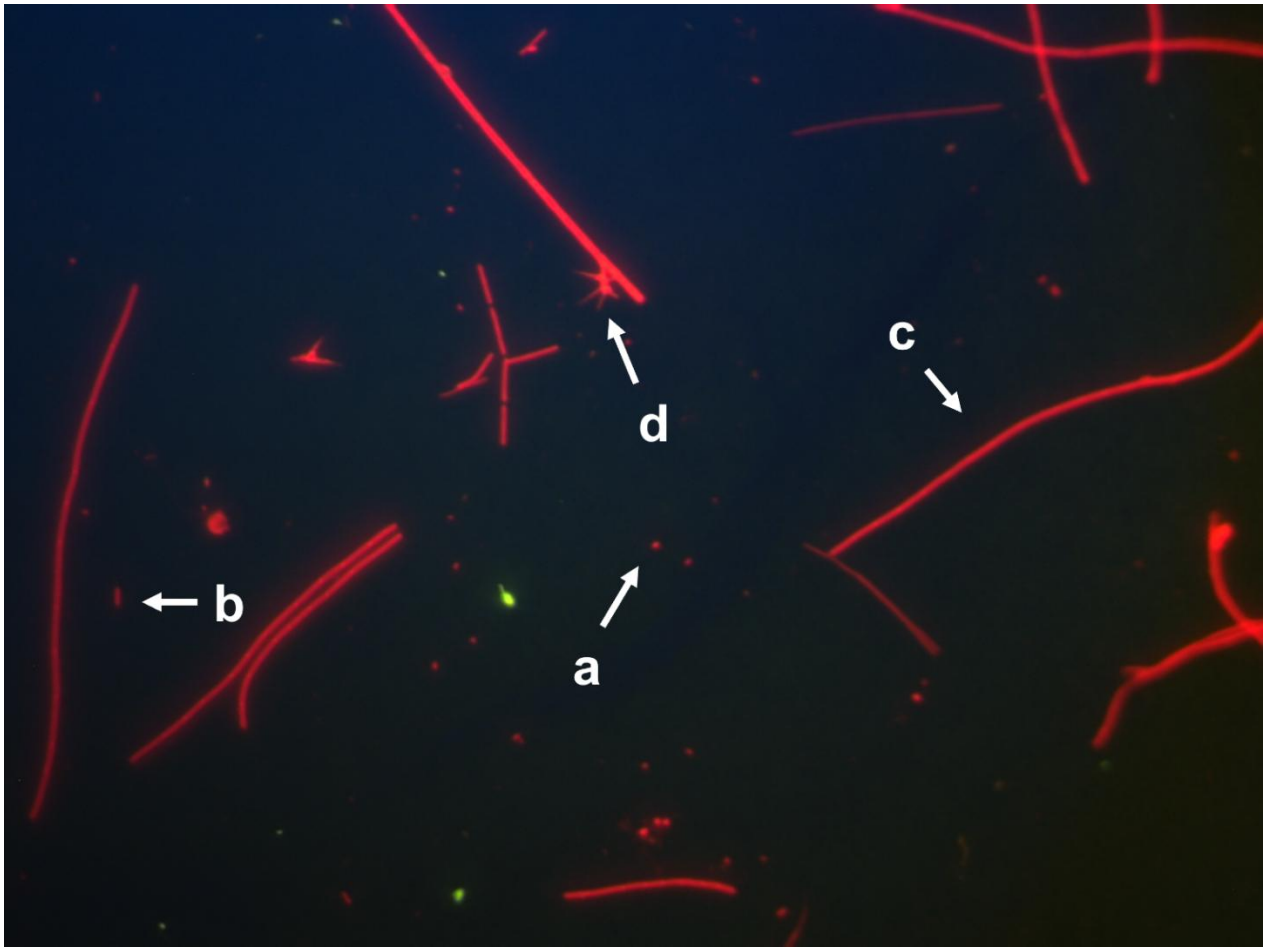

Figura 6. Bactérias cocoides (a), bacillus (b), filamentosas livres (c) e ameba (d) durante um berçário de *P. vannamei* que testou o efeito de diferentes estratégias de processamento do fertilizante do sistema simbiótico e comparou com os sistemas de bioflocos e de água clara (capítulo III). Foto: Otávio Augusto L. F. Pimentel.

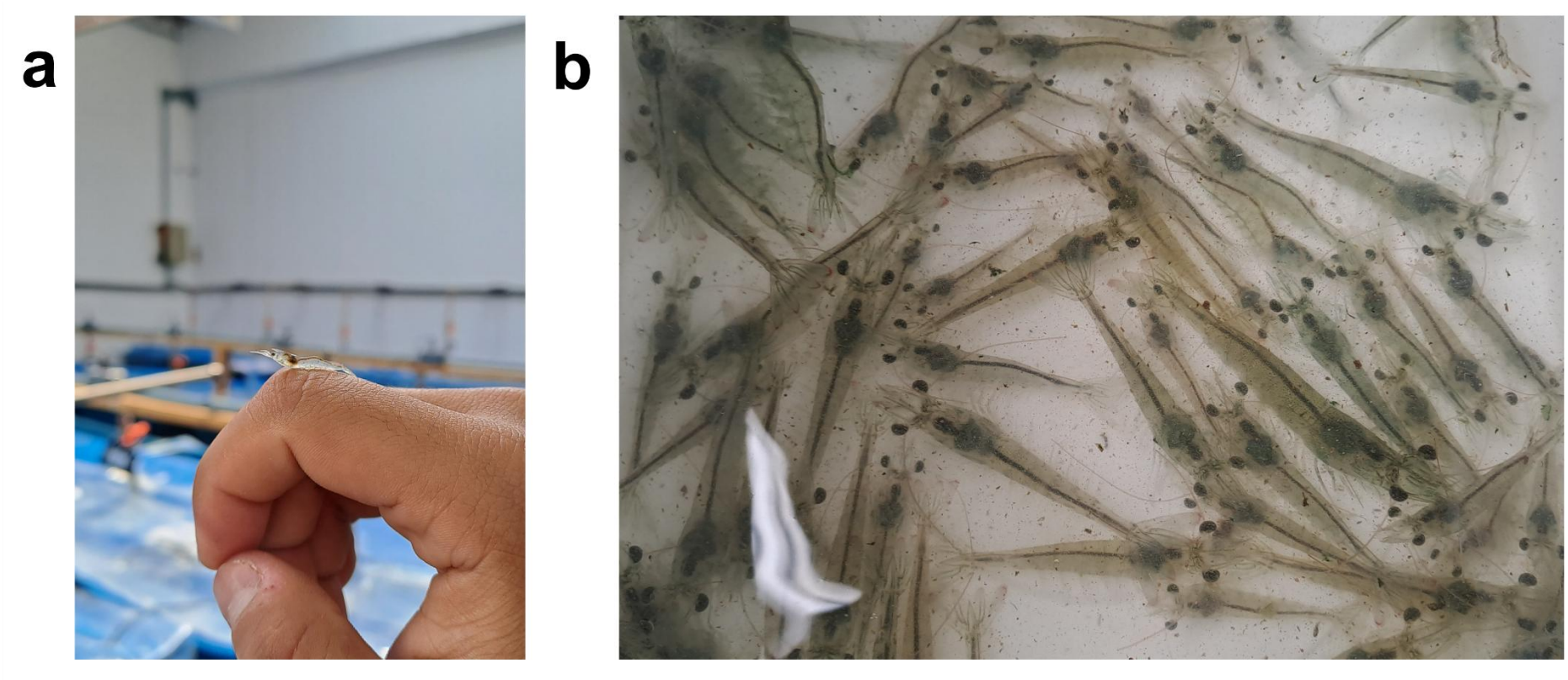

Figura 7. Pós-larvas (a) e juvenis (b) de *P. vannamei* usados no ensaio que testou a inoculação de diferentes microrganismos probióticos no fertilizante do sistema simbiótico (capitulo IV). Fotos: Otávio Augusto L. F. Pimentel.

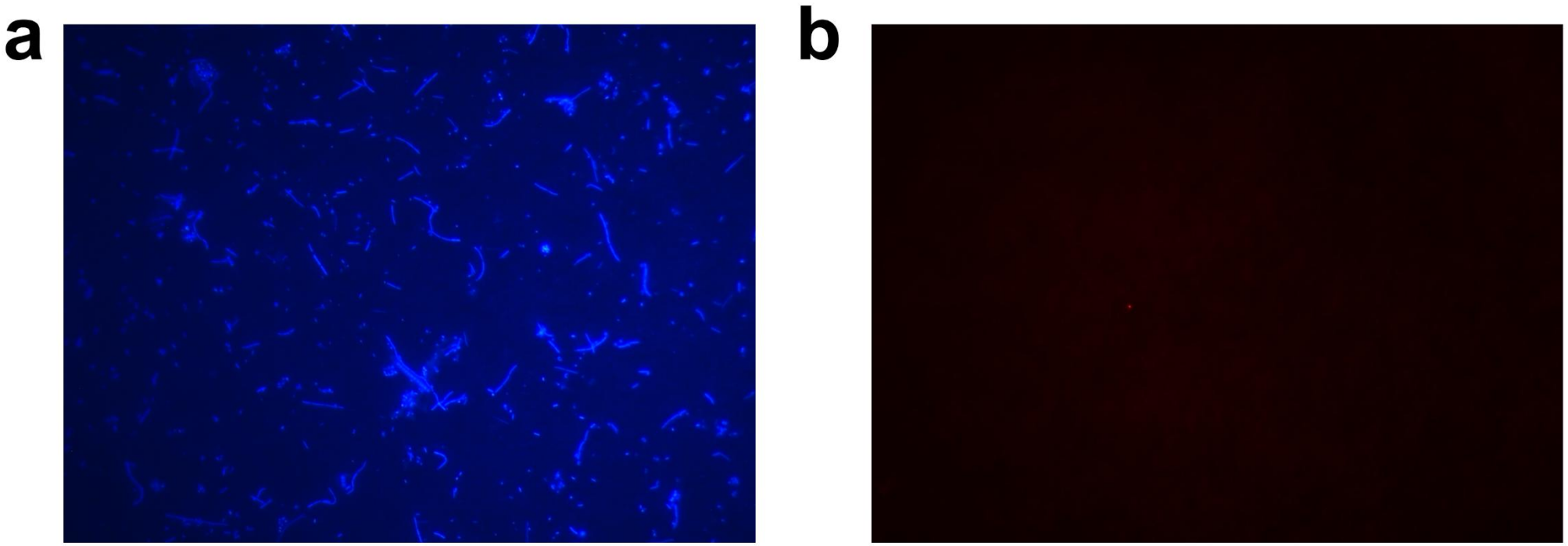

Figura 8. Microbioma (a) e bactéria hibridizada com a sonda NEU (*Nitrosomonas*) (b) presente na água de um berçário de *P. vannamei* que que testou a inoculação de diferentes microrganismos probióticos no fertilizante do sistema simbiótico (capítulo IV). Fotos: Otávio Augusto L. F. Pimentel.

Figura 9. Estrutura usada para a realização do cultivo superintensivo de *P. vannamei* com sistema de recirculação (RAS), tecnologia de bioflocos (BFT) e sistema simbiótico (Synbiotic) com água de baixa salinidade (2 g L^{-1}) por 30 dias. Foto: Otávio Augusto L. F. Pimentel.

Figura 10. *Penaeus vannamei* produzido ao final do ensaio que testou o cultivo superintensivo do camarão em sistema de recirculação (RAS), tecnologia de bioflocos (BFT) e sistema simbiótico (Synbiotic) com água de baixa salinidade (2 g L^{-1}) por 30 dias. Fotos: Otávio Augusto L. F. Pimentel.