OTÁVIO AUGUSTO LACERDA FERREIRA PIMENTEL

EFEITO DO AJUSTE IÔNICO EM ÁGUA OLIGOHALINA SOBRE O DESEMPENHO ZOOTÉCNICO DO *Litopenaeus vannamei* E RAZÕES ESTEQUIOMÉTRICAS (C:N:P) DO SÉSTON NA FASE DE BERÇÁRIO

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PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

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Otávio Augusto Lacerda Ferreira Pimentel

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

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Resumo

O camarão *Litopenaeus vannamei* é o crustáceo marinho mais cultivado no mundo. Essa espécie se destaca por suas características fisiológicas rústicas, que contribuem para que esta seja a mais cultivada também em água de baixa salinidade. Entretanto, o cultivo de camarão em água oligohalina possui diversos entraves, como a determinação das condições iônicas ideais, que, se não ajustadas, proporcionam um ambiente limitante ao crescimento do animal. Com isso, ajustes nas concentrações e/ou proporções de íons, como o cálcio (Ca^{2+}), magnésio (Mg^{2+}) e potássio (K^{+}), são recomendados para proporcionar maior conforto para os animais. Esses ajustes podem alterar variáveis de qualidade de água, como o pH, alcalinidade total e dureza total. Isso pode criar um ambiente favorável para o melhor desenvolvimento da comunidade microbiana (MC, séston), podendo alterar o seu metabolismo, e consequentemente, a composição química da sua biomassa. Portanto, o objetivo desse trabalho foi testar a influência de diferentes estratégias de ajuste iônico na água oligohalina sobre o desempenho zootécnico do camarão marinho e a composição estequiométrica carbono:nitrogênio:fósforo (C:N:P) do séston na fase de berçário. Foi realizado um cultivo berçário de camarão marinho *Litopenaeus vannamei* durante 35 dias, em unidades experimentais de 60 L, com densidade de 2.000 camarões m^{-3}. A água do mar diluída em água doce até a salinidade 2,5 g L^{-1}, foi utilizada nos seguintes tratamentos: T1 (água na salinidade 2,5 g L^{-1}), T2 (água na salinidade 2,5 g L^{-1} com ajuste de K^{+} para a concentração equivalente à da água do mar) e T3 (água na salinidade 2,5 g L^{-1} com ajuste na relação Ca:Mg:K para 1:3:1), todos em triplicata. O ajuste de K^{+} no tratamento T2 foi realizado no dia 0 do período experimental com a aplicação de cloreto de potássio (KCl) e no T3 os ajustes nas concentrações de Ca^{2+} e de Mg^{2+} foram realizados nos dias 0 e 17, com a aplicação de carbonato de cálcio ($CaCO_3$) e sulfato de magnésio ($MgSO_4$) para manter a relação 1:3:1. Um biofiltro de conchas de *Anomalocardia brasiliana* em um ativador biológico foram adicionados nas unidades experimentais para auxiliar no processo de oxidação dos compostos nitrogenados do sistema. A qualidade de água e as relações C:N:P da MC (nos bioflocos) e fração dissolvida (DF) foram analisadas a partir do fracionamento da amostra bruta em filtros 1,6 µm de retenção média onde MC > 1,6 µm e DF < 1,6 µm. Ao final do período experimental foram realizados testes de estresse osmótico e ao $N\text{-}NH_3$. Os diferentes ajustes iônicos proporcionaram um bom crescimento do camarão, que atingiu peso final de 0,40 a 0,49 g e produtividade média entre 0,7 e 0,81 Kg m^{-3}, sem diferenças significativas entre os tratamentos. Em relação aos testes de estresse osmótico e ao $N\text{-}NH_3$ em todos os tratamentos foi observada uma sobrevivência ≥ 90%. Observou-se uma tendência de estabilidade das relações C:P, C:N e N:P de MC em relação as variações das relações C:N:P da DF, indicando um possível comportamento homeostático da MC, de acordo com o que ocorre em sistemas com alta disponibilidade de nutrientes. As estratégias de ajuste iônico utilizadas não produziram efeito significativo sobre o crescimento dos camarões. Entretanto, os resultados indicam que as concentrações iniciais de Ca^{2+} (25,07 mg L^{-1}), Mg^{2+} (89,75 mg L^{-1}), K^{+} (25,00 mg L^{-1}), alcalinidade total próxima a 100,00 mg L^{-1} e dureza total de 433,30 mg L^{-1} proporcionam condições que não são limitantes para o crescimento de camarões em berçários com água oligohalina, sem a necessidade de seguir a proporção Ca:Mg:K de 1:3:1. A estabilidade das relações C:N:P da MC em relação à DF, pode indicar um possível comportamento homeostático da MC.

Palavras-Chave: Equilíbrio iônico; desempenho zootécnico; minerais; nutrientes; razão estequiométrica.

Abstract

The marine shrimp *Litopenaeus vannamei* is the most widely cultured crustacean in the world. This species stands out for its rustic physiological characteristics, which also contributes to be the most cultivated in low salinity water. Shrimp farming in oligohaline water has several obstacles, such as determining the ideal ionic conditions, which when it is unbalanced, it provides a limiting environment to the animal growth. Therefore, adjustments to the concentrations and/or proportions of ions, such as calcium (Ca^{2+}), magnesium (Mg^{2+}) and potassium (K^{+}), are recommended to provide comfort for the animal's growth. These adjustments can alter water quality variables, such as pH, total alkalinity and total hardness, which can create a favorable environment for the better development of the microbial community (MC, seston). It can also improve microbial metabolism, and consequently, its biomass composition. Therefore, the aim of this work was to test the influence of different ionic adjustment strategies in oligohaline water on marine shrimp zootechnical performance and seston carbon:nitrogen:phosphorus (C:N:P) stoichiometric composition in nursery. A marine shrimp nursery was carried out for 35 days, in 60 L indoor experimental units, with a density of 2,000 shrimp m^{-3}. Seawater was diluted in fresh water until salinity 2.5 g L^{-1} and was used in the following treatments: T1 (water at a salinity of 2.5 g L^{-1}); T2 (water at a salinity of 2.5 g L^{-1} with K^{+} adjustment close to seawater equivalent concentration); T3 (water at a salinity of 2.5 g L^{-1} with Ca:Mg:K ratio adjustment to 1:3:1), in triplicate. The K^{+} adjustment in T2 treatment was made on day 0 of the experimental time with the application of potassium chloride (KCl) and in T3, adjustments in the Ca^{2+} and Mg^{2+} concentration were made on day 0 and 17 with the application of calcium carbonate ($CaCO_3$) and magnesium sulfate ($MgSO_4$) to maintain the ratio at 1:3:1. An *Anomalocardia brasiliana* shells biofilter and a biological activator was added to the experimental units to better support the system's nitrogen compounds oxidation. Water quality and the C:N:P ratios of the MC (in bioflocs) and dissolved fraction (DF) were analyzed from the bulk sample fractionation in 1.6 µm mean retention filters where bioflocs MC > 1.6 µm and DF < 1.6 µm. At the end of the experimental time, osmotic stress and NH_3-N tests were performed. The different ionic adjustments provided a good shrimp growth, which reached a final weight of 0.40 - 0.49 g and average yield of 0.7 - 0.81 kg m^{-3}, without significant differences among treatments. Survival ≥ 90% was observed in osmotic and NH_3-N stress tests in all treatments. A stability trend of the C:P, C:N and N:P bioflocs MC ratios were observed in view of the DF C:N:P ratios variations, indicating a homeostatic behavior of the bioflocs MC, as occurs in systems with high nutrient availability. The ionic adjustment strategies used did not have a significant effect on shrimp growth. However, these results indicate that the initial concentrations of Ca^{2+} (25.07 mg L^{-1}), Mg^{2+} (89.75 mg L^{-1}), K^{+} (25.00 mg L^{-1}), total alkalinity close to 100.00 mg L^{-1} and total hardness of 433.30 mg L^{-1} provide conditions that are not limiting for shrimp growth in nurseries in oligohaline water, but not requiring to follow 1:3:1 Ca:Mg:K ratio. A stability of C:N:P ratios of the bioflocs MC compared to the dissolved fraction, may indicate a possible MC homeostatic behavior under these culture conditions.

Keywords: Ionic equilibrium; zootechnical performance; minerals; nutrients; stoichiometric ratio.

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1. Introdução

O camarão branco do Pacífico *Litopenaeus vannamei* (Boone, 1931) é o crustáceo mais cultivado no mundo, e sua produção correspondeu a mais de 4,9 milhões de toneladas (52,9% de toda produção de crustáceos) no ano de 2018 (FAO, 2020). Essa espécie se destaca por suas características fisiológicas, como: rápido crescimento, tolerância a altas densidades de estocagem, resistência às variações ambientais, como temperatura (24 a 35°C) e alta capacidade de osmorregulação (0,5 a 60 g L^{-1}) (VAN WYK et al., 1999; LIN e CHEN, 2003; SAOUD et al., 2003; BRIGGS et al., 2004; DAVIS, SAMOCHA e BOYD, 2004; XU e PAN, 2012; VALENZUELA-QUIÑÓNEZ et al., 2012; ESPARZA-LEAL et al., 2016). Esta capacidade contribui para que esta espécie de crustáceo seja a mais cultivada em água de baixa salinidade (água oligohalina – 0,5 – 3,0 g L^{-1}, ESTEVES, 2011), apresentando bons índices de desempenho zootécnico (LIMA et al., 2017; VALENCIA-CASTAÑEDA et al., 2017), apesar de resultados de sobrevivência e crescimento variados (ROY et al., 2009; ESPARZA-LEAL et al., 2016).

A carcinicultura em água oligohalina possui diversos entraves, como a determinação das condições iônicas ideais, dada a ampla variabilidade da composição de íons na água e salinidade entre os locais de cultivo (BOYD e THUNJAI, 2003). Na maior parte dos casos, o perfil iônico da água não atende às exigências dos animais, apresentando desequilibro entre as relações de cátions [potássio (K^+), magnésio (Mg^{2+}), sódio (Na^+) e cálcio (Ca^{2+})] e ânions [sulfato (SO_4^{2-}), cloreto (Cl^-), bicarbonato (HCO_3^-) e carbonato (CO_3^{2-})], favorecendo um ambiente limitante ao crescimento do animal, principalmente quando estão submetidos a altas densidades. Essas condições afetam ainda a composição da microbiota do ambiente de cultivo, pois a carência desses minerais possui influência direta em processos vitais para o fitoplâncton, zooplâncton e o bacterioplâncton, como: manutenção das estruturas da membrana celular, formação da molécula de clorofila, metabolismo intracelular do nitrogênio e troca e transporte de outros íons para os meios intra e extracelular (PIEDAD-PASCUAL, 1989; SAOUD et al., 2003; DAVIS et al.; 2004; REYNOLDS, 2006; ROY e DAVIS, 2010; ESTEVES, 2011; VALENZUELA-MADRIGAL et al., 2017; NEHRU et al., 2018).

Alguns autores sugerem que para a produção de camarão marinho em condições de baixa salinidade, o ajuste das proporções iônicas da água seja necessário, deixando-a com concentrações de Ca^{2+}, Mg^{2+} e K^+ equivalentes à da água do mar, para melhor desenvolvimento das espécies marinhas em condições de conforto osmótico (BOYD e

THUNJAI, 2003; ROY et al., 2010). Dessa forma, pode ser promovido ajuste da deficiência destes íons na água por meio do ajuste das proporções Mg:Ca (3:1), Mg:K (3:1) e Ca:K (1:1) ou ajuste nas concentrações de determinados íons, deixando-os na concentração equivalente à da água do mar (ROY et al., 2010). Em contrapartida, alguns estudos apresentaram resultados satisfatórios de desempenho zootécnico em cultivos intensivos, quando utilizada água do mar diluída, de poços e salinizada artificialmente, porém sem qualquer ajuste nas proporções iônicas (VALENZUELA-MADRIGAL et al., 2017; PINTO et al., 2020; MOURA et al., 2021). Portanto, a ausência de consenso sobre o uso do ajuste iônico na água faz com que ele ainda precise ser elucidado. A compensação da carência de cátions e ânions na água pode ser realizada por meio da aplicação de fertilizantes minerais de origem agrícola na água ou por meio da suplementação destes na ração, podendo auxiliar no aumento do crescimento e sobrevivência do camarão em água de baixa salinidade (ROY e DAVIS, 2010; MAICÁ et al., 2012).

Muitos desses minerais, como Ca^{2+}, Mg^{2+} e K^{+}, são essenciais para os camarões, pois atuam em diversos processos vitais, dos quais podemos citar: a osmorregulação, o processo de ecdise e formação do exoesqueleto, contribuindo, dessa forma, para o crescimento do animal (TACON, 1987; PIEDAD-PASCUAL, 1989). Além de disponibilizar minerais essenciais para o camarão, esse ajuste iônico também se reflete em mudanças nas condições de qualidade de água, como o aumento da alcalinidade total, pH, dureza total, cálcio e magnésio (BOYD e TUCKER, 1998). Essas variáveis de qualidade de água, associada a fatores físicos, como a temperatura, quando estabilizadas em faixas ótimas de crescimento, podem aumentar a produtividade da comunidade microbiana do sistema, como bactérias autotróficas e heterotróficas (LUO et al., 2013; ELDYASTI et al., 2013; AVNIMELECH, 2012; EL-SAYED, 2021). Como consequência, a maior produtividade desses organismos deve acarretar um enriquecimento químico/nutricional [e.g. maiores concentrações relativas de nitrogênio (N) e fósforo (P)] da sua biomassa (hipótese da taxa de crescimento) (STERNER e ELSER 2002; PIMENTEL et al., 2020). Assim, se o ajuste iônico criar condições para aumentar o metabolismo, bem como a taxa de crescimento dos microrganismos, pode-se esperar o enriquecimento de sua composição nutricional (i. e. aumentar as concentrações relativas de N e P em sua biomassa).

O entendimento do efeito do ajuste iônico pode ser considerado uma alternativa para a expansão da atividade da carcinicultura em águas interiores, direcionando-a para áreas mais afastadas do oceano, utilizando água proveniente de rios, poços e salinizada artificialmente. A carcinicultura, associada ao uso de sistemas intensivos com mínima troca de água (por reutilização), aumenta o nível de biosseguridade do sistema, possibilita maior produtividade por hectare, maior densidade de estocagem, aumenta a taxa de crescimento dos animais, aumenta o peso final e promove menor taxa de conversão alimentar, pois os microrganismos presentes no flocos microbianos e no séston contribuem como fonte de proteína para os animais que estão sob cultivo (AVNIMELECH, 2009; AVNIMELECH, 2012; HARGREAVES, 2013; CORREIA et al., 2014; BRITO et al., 2016; MARINHO et al., 2017; JUNG et al., 2017).

Os sistemas intensivos de produção com mínima troca de água baseiam-se na assimilação e oxidação dos compostos nitrogenados tóxicos pela comunidade de bactérias heterotróficas e nitrificantes através da manutenção da adequada proporção carbono e nitrogênio (C:N) na água (AVNIMELECH, 1999; CRAB et al, 2012; XU e PAN, 2012). Inicialmente, é promovido um aumento da razão C:N na água do cultivo, mantendo-a entre 10 e 20:1 (AVNIMELECH, 1999), com a finalidade de estimular a absorção dos compostos nitrogenados disponibilizados no sistema pela ração. Essa manipulação da razão C:N é feita por meio da adição de fontes de carbono orgânico, como melaço de cana, farelo de trigo, farelo de arroz, celulose, ração e farinha de tapioca (BECERRA-DORAME et al., 2014; RAJKUMAR et al., 2016; DENG et al., 2018).

A manipulação da razão C:N também pode ser feita através da fertilização da água com compostos a base de carboidratos complexos, como o farelo de trigo e de arroz. O uso desses carboidratos após os processos anaeróbios (fermentação) e aeróbios (respiração microbiana) proporciona um maior equilíbrio microbiano e um menor aporte de carbono orgânico, quando comparado aos sistemas com uso de melaço in natura (ROMANO et al., 2018). Essa manipulação da razão C:N permite o crescimento de aglomerados microbianos com alta diversidade, compostos por fitoplâncton, zooplâncton, bactérias autotróficas e heterotróficas, fungos, detritos, fezes e exoesqueletos de animais mortos, mantidos constantemente em suspensão na coluna d'água por meio de aeração (HARGREAVES, 2006; DE SCHRYVER et al., 2008; GAO et al., 2012). Durante o processo de maturação do floco no sistema, a oxidação do nitrogênio amoniacal total a nitrato (NO_3^-) se dá por meio da comunidade de bactérias autotróficas (AVNIMELECH,

1999; AVNIMELECH, 2003; EBELING et al., 2006; DA SILVA et al., 2013; BOYD e MCNEVIN, 2015; XU et al., 2016; AHMAD et al., 2017). Este sistema possui grande versatilidade e pode ser aplicado nos modos de cultivo de camarão: monofásico (engorda), bifásico (berçário primário e engorda) e trifásico [berçário primário, berçário secundário (raceway) e engorda].

A fase de berçário é um período entre 15 a 40 dias no qual as pós-larvas de camarão são estocadas a altas densidades em um ambiente biosseguro, com monitoramento de qualidade de água e alimentação, que objetiva alcançar uma maior resistência e melhor desenvolvimento do animal no período inicial da engorda (GARZA DE YTA et al., 2004; MISHRA et al., 2008; CORREIA et al., 2014). Nos cultivos de camarão em água polihalina (16,5 – 30 g L^{-1}) e marinha (30 – 40 g L^{-1}), o sistema de mínima troca de água é muito utilizado na fase de berçário, pois o maior controle das variáveis ambientais dentro dos tanques viabiliza a produção de juvenis durante todas as estações do ano, com elevadas densidades de estocagem, aumentando, dessa forma, o número de ciclos por ano e ainda contribuindo para o rápido crescimento dos animais (MISHRA et al., 2008; EMERENCIANO et al, 2012; WASIELESKY et al., 2013; HARGREAVES, 2013). Contudo, a aplicação desse sistema em água oligohalina ainda não é bem estabelecido devido à maior toxicidade de compostos nitrogenados nessas condições, que afetam o sucesso da produção (LARAMORE et al., 2001; ESPARZA-LEAL et al., 2016).

Dessa forma, entender o efeito de diferentes composições iônicas na água é um expressivo passo para ampliar o uso de berçários intensivos em água oligohalina e consequentemente otimizar a produção dos empreendimentos comerciais. Portanto, o objetivo desse trabalho foi testar a influência de diferentes estratégias de ajuste iônico na água oligohalina sobre o desempenho zootécnico de juvenis de camarão marinho e a composição estequiométrica da comunidade microbiana do séston na fase de berçário.

1.1. Objetivos

Objetivo geral

Testar a influência de diferentes estratégias de ajuste iônico na água oligohalina sobre o desempenho zootécnico do camarão marinho *Litopenaeus vannamei* e a composição estequiométrica (C:N:P) do séston na fase de berçário.

Objetivos específicos

- Avaliar a influência do ajuste iônico sobre a sobrevivência; peso final; taxa de crescimento específico, produtividade e conversão alimentar aparente dos camarões cultivados;
- Avaliar a influência do ajuste iônico sobre as razões estequiométricas C:N:P do séston;
- Avaliar a influência do ajuste iônico sobre o controle dos compostos nitrogenados tóxicos durante o período experimental.

1.2. Hipóteses

- O ajuste nas relações entre os cátions Ca:Mg:K na água oligohalina proporcionará melhor desempenho zootécnico dos camarões marinhos;
- O ajuste nas relações entre os cátions Ca:Mg:K aplicado na água oligohalina contribui para a redução das razões C:P, C:N e N:P do séston.

2. Artigo científico

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The effect of different ionic adjustment strategies in low salinity water on *Litopenaeus vannamei* growth and on the C:N:P stoichiometry of the biofloc microbial community in nursery system

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Abstract

The aim of this study was to test the effect of different ionic adjustment strategies in oligohaline water on the growth of *Litopenaeus vannamei* and on the stoichiometric carbon:nitrogen:phosphorus (C:N:P) ratios of the biofloc microbial community (MC) in nursery system. A 35-day culture (2,000 PL's m^{-3}) was carried out in 60 L indoor experimental units, with three treatments: T1 - seawater diluted to a salinity of 2.5 g L^{-1} (control), T2 – water with a salinity of 2.5 g L^{-1} with potassium (K^+) adjusted to be equivalent to the concentration in seawater, and T3 –water at a salinity of 2.5 g L^{-1} with

its calcium:magnesium:potassium (Ca:Mg:K) ratio adjusted to 1:3:1, each in triplicate. The K^+ adjustment in the T2 treatment was made on day 0 of the experiment with the application of potassium chloride (KCl). In T3, the adjustments of Ca^{2+} and Mg^{2+} concentrations were made on days 0 and 17 by adding calcium carbonate ($CaCO_3$) and magnesium sulfate ($MgSO_4$) to maintain the ratio at 1:3:1. A biofilter composed of *Anomalocardia brasiliana* shells and a biological activator were added to the experimental units to better support the system's oxidation of nitrogen compounds. Water quality and the C:N:P ratios of the bioflocs MC and dissolved fraction (DF) were analyzed from the bulk sample fractionation in 1.6 µm mean retention filters, where biofloc MC > 1.6 µm and DF < 1.6 µm. At the end of the experimental time, osmotic and ammonia concentration (NH_3-N) stress tests were performed. The juveniles reached a final weight of 0.40 - 0.49 g and an average yield of 0.7 - 0.81 Kg m^{-3}, without significant differences among the treatments. Survival ≥ 90% was observed in osmotic and NH_3-N stress tests in all of the treatments. A stabilizing trend in biofloc MC ratios of C:P, C:N and N:P was observed considering the variations of C:N:P in the DF, indicating a homeostatic behavior of the biofloc MC, as occurs in systems with high nutrient availability. The ionic adjustment strategies used did not have a significant effect on shrimp growth. However, these results indicate that the initial concentrations of Ca^{2+} (25.07 mg L^{-1}), Mg^{2+} (89.75 mg L^{-1}), and K^+ (25.00 mg L^{-1}), total alkalinity close to 100.00 mg L^{-1} and total hardness of 433.30 mg L^{-1} provide conditions that do not limit shrimp growth in nurseries with oligohaline water, without needing to use a 1:3:1 Ca:Mg:K ratio. The greater stability observed in the C:N:P ratios of the MC than the DF may indicate a possible homeostatic behavior of the bioflocs MC under these culture conditions.

Keywords: Ionic equilibrium; oligohaline water; shrimp performance; minerals; nutrients.

1. Introduction

Litopenaeus vannamei farming is carried out in several countries worldwide, such as Brazil, Ecuador, Mexico, China, Thailand and Vietnam, under different salinity conditions (Samocha et al., 2001; McNevin et al., 2004). However, despite the high capacity of the shrimp to adapt to different environmental conditions, several obstacles exist to shrimp farming in oligohaline water (0.5 - 3.0 g L^{-1}, Esteves, 2011) such as the need to determine ideal ionic conditions of water, given the wide variability of ion composition and salinity among farming sites (Boyd and Thunjai, 2003). In most cases, the ionic profile of the water has low levels of cations [potassium (K^+), magnesium (Mg^{2+}), sodium (Na^+) and calcium (Ca^{2+})] and/or anions [sulfate (SO_4^{2-}), chloride (Cl^-) and bicarbonate (HCO_3^-)]. The low levels of these ions create a limiting environment for shrimp growth, due to the vital importance of these minerals to processes such as: osmoregulation, ecdysis, and exoskeleton composition, contributing to animal growth (Naik, 2012; Nehru et al., 2018a).

The use of ionic supplementation with agricultural mineral fertilizers is a technique used to compensate for ion deficiency in water, by adjusting Ca^{2+}, Mg^{2+} and K^+ concentrations or cation ratios Na:K (28:1), Mg:Ca (3:1), Mg:K (3:1) and Ca:K (1:1) to be equivalent to the ratio found in seawater. It can provide an osmotically more comfortable environment for animals, which influences their zootechnical performance (Roy et al., 2010; Roy and Davis, 2010). This supplementation, associated with the use of intensive systems with minimal water exchange, allows: higher yield per m^{-3}, higher

stocking density, higher animal growth rate, and a lower feed conversion ratio (Avnimelech, 2012; Correia et al., 2014; Brito et al., 2016; Marinho et al., 2016; Jung et al., 2017).

Adjusting the concentration of ions in water, such as K^+, can benefit shrimp growth and survival, since it plays a role in Na^+, K^+ - ATPase enzyme activation. This enzyme promotes the active transport of ions through cell membranes, allowing the maintenance of cell metabolism homeostasis, regulating cell volume and pH, and improving the uptake of extracellular nutrients such as glucose, amino acids, phosphorus and vitamins (Davis and Lawrence 1997; Cheng et al., 2005; Roy et al. 2007; Naik, 2012). This is reflected in better growth results for shrimps in nurseries and grow-out farming that used low salinity water with higher K^+ supplementation, but with a high variability in survival rates (Roy et al., 2007; Antony et al., 2015). Despite the fact that maintaining Ca:Mg:K:Na ratios in low salinity water may be necessary in shrimp farming, the growth and survival results for nurseries using biofloc technology in low salinity water, with different Ca:Mg:K ratios, presented quite different results (Roy et al., 2009; Zacarias et al., 2018). Thus, it is important to determine appropriate minimum concentrations for marine shrimp growth and survival in shrimp farming using low salinity water.

Ionic adjustment through the addition of ions such as K^+, Ca^{2+} and Mg^{2+}, in addition to providing essential minerals for shrimp, causes changes in water quality conditions, such as increased total alkalinity, pH, total hardness, calcium and magnesium (Boyd and Tucker, 1998).These water quality variables, associated with physical factors, such as temperature, when stabilized in optimal growth ranges, can increase productivity of the system's microbial community, such as autotrophic and heterotrophic bacteria (Avnimelech, 2012; Luo et al., 2013; Eldyasti et al., 2013; El-Sayed, 2021). Therefore,

the higher productivity of these organisms must lead to chemical/nutritional enrichment [e.g., higher absolute concentrations of carbon (C) and phosphorus (P)] in their biomass (Pimentel et al., 2020). Thus, if the ionic adjustment creates conditions to increase the metabolism and growth rate of the microorganisms, one can expect the enrichment of their nutritional composition [i.e., an increase in the relative concentrations of nitrogen (N) and P in their biomass]. However, the influence of different ionic adjustment strategies on the stoichiometric ratios of the microbial community in a shrimp nursery with minimal water exchange have not yet been evaluated.

Thus, understanding the effect of different ionic compositions of water is an important step to allow expanding the use of intensive nurseries with oligohaline water and consequently optimize commercial production. Therefore, this study tested the influence of different ionic adjustment strategies in oligohaline water on the zootechnical performance of marine shrimp *L. vannamei* postlarvae and stoichiometric C:N:P ratios of the biofloc microbial community in nursery system.

2. **Material and methods**

2.1. Experimental design

This study was carried out during 35 days at the Shrimp Culture Laboratory (LACAR), of the Fisheries and Aquaculture Department of the Rural Federal University of Pernambuco (UFRPE), Brazil.

The experiment was designed to test the effect of two different ionic adjustment strategies on the zootechnical performance of shrimp *L. vannamei* postlarvae. Three treatments, each in triplicate, were established with different ionic water compositions obtained by diluting seawater with freshwater (to salinity of 2.5 g L^{-1}) and applying

mineral fertilizers: T1 - seawater diluted to a salinity of 2.5 g L^{-1} (control), T2 - seawater diluted to a salinity of 2.5 g L^{-1} with K^+ adjustment in the water to approximate the equivalent concentration in seawater (Roy et al., 2010) and T3 - seawater diluted to a salinity of 2.5 g L^{-1} with Ca:Mg:K ratio adjusted to 1:3:1 (Boyd, 2020).

2.2.Experimental conditions

The post-larvae (PL_{10} 3.00 mg ± 0.12 mg) were acquired from a commercial hatchery (Aquasul camarão marinho, Rio Grande do Norte, Brazil) with a salinity of 35 g L^{-1}, and were acclimated to a salinity of 2.5 g L^{-1} for 14 days prior to experiment. The shrimps were stocked at PL_{24} (9.24 mg ± 1.38 mg), at a density of 2,000 PL's m^{-3} in experimental units with a useful volume of 60 L (0.06 m^3), under constant aeration (dissolved oxygen > 5.0 mg L^{-1}), constant temperature (29 °C; Hopar Sh-608 heater 100 w), 12:12hrs photoperiod, and mean luminance of 8.65 µmol photons m^{-2} s^{-1} (Equitherm Lux-204).

The post-larvae were fed with commercial feed of 40% crude protein, 11% lipids, 4% crude fiber, and 14% mineral matter (Guabitech Inicial, Guabi, Brazil), 4 times a day (8:00 am, 11:00 am, 2:00 pm and 5:00 pm). Initially, a 33.75% daily feeding rate was adopted, which was gradually reduced to 10.5% of body weight after 35 days, according to Van Wyk (1999), and adjusted daily according to the shrimp feed consumption estimate and mortality rate.

2.3.Water fertilization

Twenty-four days before stocking, the nine experimental units were fertilized daily using a methodology adapted from Romano et al. (2018). The fertilizer was obtained from an anaerobic process (24 hrs) and another aerobic phase (24 hrs) from the mixture of 20 g m^{-3} of rice bran (< 200 μm), 2 g m^{-3} of molasses, 4 g m^{-3} of sodium bicarbonate and 0.5 g m^{-3} of commercial bacterial mix ($6.5x10^7$ Colony Forming Units (CFU) g^{-1}, containing: *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus* sp., sodium chloride (NaCl) and magnesium hydroxide ($Mg(OH)_2$)) (Kayros Agrícola and Ambiental, Brazil) and chlorinated water (20 mg L^{-1} active chlorine (60%), then unchlorinated through aeration) in proportion to 10 × the amount of rice bran. During the experimental time, the fertilizer described above was applied four times a week, and was suspended when the settleable solids exceeded 5 ml L^{-1}.

2.4. Ionic adjustment

The ionic adjustment was performed based on previous tests of the efficiency of the chemical compounds used, in experimental units with 14 L of water volume with salinity of 2.5 g L^{-1}, obtained from seawater diluted in fresh water, under constant aeration. The pH and ion concentration were analyzed before application and 72 hrs after application of the chemicals at a concentration of 100 g m^{-3} (Table 1).

Insert table 1

The application of chemical components to the experimental units to adjust the ion concentration was performed according to the following equation:

$$Amount\ of\ product = \frac{Fc - Ic}{Increment\ of\ the\ desired\ ion} \times V$$

Where:

- Fc: Final concentration;
- Ic: Concentration in the experimental unit;
- V: tank volume (m^3).

In the T1 treatment, the seawater was diluted with freshwater to reach a salinity of 2.5 g L^{-1}. The seawater used had the initial following ionic profile (mean of three replicates ± standard deviation): Ca^{2+} = 437.33 ± 36.95 mg L^{-1}, Mg^{2+} = 1,380.24 ± 25.72 mg L^{-1}, K^{+} = 364.80 ± 4.33 mg L^{-1}, Na^{+} = 13,843.37 ± 299.24 mg L^{-1}, Cl^{-} = 25,051.33 ± 250.67 mg L^{-1}, SO_4^{2-} = 977.87 ± 10.25 mg L^{-1}, total alkalinity = 173.33 ± 2.89 mg L^{-1}, total hardness = 6,773.33 ± 180.37 mg L^{-1}.

In the T2 treatment, at the beginning of the experimental time, K^{+} supplementation was carried out according to the estimated seawater concentration. These ion optimal concentrations in farming ponds or tanks, called equivalent concentration to seawater, are determined by multiplying water salinity in the tank by the ratio of ion concentration in seawater and the mean seawater salinity (Boyd and Thunjai, 2003; Roy et al., 2010). The K^{+} concentration was adjusted from 17.7 mg L^{-1} to 25 mg L^{-1} by adding potassium chloride (KCl).

In the T3 treatment, adjustments in the Ca:Mg:K ratio were performed, keeping it at 1:3:1, then, the seawater ratio was simulated (Boyd, 2020). At the start of the experiment (day 0), calcium carbonate ($CaCO_3$) and magnesium sulfate ($MgSO_4$) were used, increasing the concentration of Ca^{2+} from 26.1 mg L^{-1} to 35 mg L^{-1} and Mg^{2+} from 95.9 mg L^{-1} to 105 mg L^{-1}. At day 17, Ca^{2+} was increased from 36.27 mg L^{-1} to 47 mg

L^{-1}, by adding $CaCO_3$, and the Mg^{2+} concentration from 121.18 mg L^{-1} to 140 mg L^{-1}, by adding $MgSO_4$, to achieve the desired Ca:Mg:K ratio of 1:3:1.

After analyzing the water in all of the treatments, ion concentrations in milliequivalent L^{-1} (mEq L^{-1}) were calculated to check cation and anion equilibrium. This calculation was made by the difference between the sum of the cations mEq L^{-1} (Na^+ = 23 mEq; K^+ = 39 mEq; Ca^{2+} = 20 mEq and Mg^{2+} = 12,15 mEq) and the sum of the anions mEq L^{-1} (HCO_3^- = 61 mEq; Cl^- = 35,45 mEq and SO_4^{2-} = 48 mEq) for chemical equilibrium certification (Boyd, 2020). As a standard for certifying the accuracy of the analysis of these major ions, a balance error between cations and anions below 10% was adopted (Custodio and Llamas, 1983). This error was calculated using the following equation:

$$Error\ (\%) = \frac{|\Sigma\ cations - \Sigma\ anions|}{\Sigma\ cations + \Sigma\ anions} \times 200$$

Where:

- Σ cations: sum of cations;
- Σ anions: sum of anions.

To aid development of the microbial community and the nitrification process, an artificial substrate (biofilter) was added to the experimental units when water fertilization began. The biofilter was composed of mollusk shells (*Anomalocardia brasiliana*), which covered ≈ 28,12% of the bottom area (25 × 24 × 5 cm, width × height × depth) and corresponded to ≈ 3,36% of the experimental unit's useful volume. A biological activator was also applied to the system (Seachem, EUA) over 7 days, at

the beginning of the water fertilization time, with an initial application of 7.5 mL m^{-3} and six consecutive daily applications of 3.75 mL m^{-3}.

2.5.Zootechnical performance

From the 15th day of the experiment weekly calculations were made of specific growth rate (SGR, % day^{-1}) [100×(ln final weight (g) - ln initial weight (g)) ÷ experimental time (days)]; final weight (FW, g) [(final biomass (g) ÷ number of shrimps at the end of experimental time)]; feed conversion ratio (FCR) [(feed supplied ÷ biomass gain)]; survival (%) [(number of shrimps at the end of experimental time ÷ initial number of shrimps)×100] and Yield (Kg m^{-3}) [final biomass (Kg) ÷ experimental units volume (m^3)].

2.6.Stress Tests

At the end of the experiment, the shrimp juveniles were submitted to tests of resistance to osmotic stress and of water ammonia concentration (NH_3-N). For the osmotic stress test, 30 shrimps were collected per treatment (10 shrimps per repetition) and stocked in experimental units with 10 L of seawater (35 g L^{-1}), with aeration, for 30 minutes. After this time, the animals were exposed to salinity of 2.5 g L^{-1} for 30 minutes. Survival was then evaluated for each treatment (Burbano-Gallardo et al., 2015).

For the NH_3-N resistance test, at the end of the experiment, the animals were transferred to experimental units containing 10 L of water (salinity 2.5 g L^{-1}), with NH_3-N concentrations between 0.30 and 0.60 mg L^{-1} (Table 2), room temperature 29.1 °C and pH close to 8.0. The test was carried out for 96 hrs and survival was measured

every 24 hrs (Zhang et al., 2012). The NH_3-N concentration was achieved by applying a stock solution of 10,000.00 mg L^{-1} of NH_4Cl.

Insert table 2

2.7.Water quality, elemental composition (C, N and P) and stoichiometric ratios

During the experimental period, the following environmental variables were analyzed daily, twice a day (morning and afternoon): dissolved oxygen (DO, mg L^{-1}) and temperature (°C) with Yellow Springs multiparameter, model 55; pH (pH-689) and salinity (g L^{-1}) (salinity meter AZ, model 8371). The settleable solids (SS, mL L^{-1}) were estimated three times a week with an Imhoff cone (Avnimelech, 2012).

The water from experimental units was sampled weekly (1.5 L) and filtered in 1.6 µm glass fiber filters (Whatman). Material > 1,6 µm concentrated in the filters was considered as the bioiflocs' microbial community (MC), and the material < 1,6 µm as the dissolved fraction (DF). From the MC fraction were determined total particulate carbon (TPC), total particulate nitrogen (TPN) and total particulate phosphorus (TPP). From the DF were determined total dissolved carbon (TDC), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), total alkalinity, total ammonia nitrogen (TAN), nitrite-N (NO_2^--N), nitrate-N (NO_3^--N), orthophosphate (PO_4^{3-}), total hardness, Ca^{2+}, Mg^{2+}, Na^+, Cl^-, SO_4^{-2} and K^+. Total phosphorus (TP) was determined from the bulk sample.

Weekly analyses were conducted of TPC, TPN, TDC, TDN (carbon and nitrogen analyzer TOC-V; Shimadzu), TPP, TDP (Mackereth et al., 1978; Carmouze, 1994), total suspended solids (TSS, Wetzel and Likens, 2000), TAN (APHA, 2012), NO_2^--N (Fries, 1971), NO_3^--N (APHA, 2012), PO_4^{3-} (APHA, 2012) and total alkalinity (APHA, 2012).

Total hardness, Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-}, SO_4^{-2} (APHA, 2012), K^{+} (Fries and Getrost, 1977) and TP (Mackereth et al., 1978) were determined during the experiment on days 0, 17 and 35.

Mg:Ca, Mg:K, Ca:K, Na:K and total hardness:alkalinity were calculated in mg L^{-1}:mg L^{-1}. The C:P, C:N and N:P ratios in the MC and DF factions were calculated in mmol g^{-1}:mmol g^{-1} and µM:µM, respectively.

2.8. Data analysis

The data were tested for normality, using the Shapiro-Wilk test, and homoscedasticity using the Levene test. Subsequently, a one-way ANOVA was applied to zootechnical performance, Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-}, SO_4^{-2}, K^{+} and HCO_3^{-} concentrations, Ca:K, Mg:Ca, Mg:K and Na:K ratios, total hardness and total hardness:alkalinity ratio, to evaluate the existence of significant differences among the treatments. The means were considered significantly different when $p < 0.05$. When ANOVA was significant, Tukey's means comparison test ($p < 0.05$) was performed to determine which treatments differed.

For water quality variables (DO, temperature, pH, salinity, TAN, NO_2^{-}-N, NO_3^{-}-N, PO_4^{3-}, TP, TSS, SS and alkalinity), C, N and P absolute composition and C:N:P stoichiometric ratios, a Friedman's nonparametric test was performed to evaluate the existence of significant differences among treatments over experimental time. When medians were considered significantly different ($p < 0.05$), a Conover multiple comparison test with Holm-Bonferroni correction ($p < 0.05$) was applied. Linear regressions were constructed between the absolute composition of nutrients and C:N:P ratios × time. The test assumptions of normality and homoscedasticity have been tested. When necessary, data were cosine transformed (N – MC – T1, P – MC – T2, C:P – MC

– T2, C:N – MC – T2 and C:N – DF - T1), sine and sine + 1 transformed (N – MC – T2, C:N – MC – T1, N:P MC – T1, N:P – MC – T2, C – DF – T2 and N:P – MC – T3) and log transformed (P – DF – T2) to fulfill parametric statistics assumptions.

A Spearman correlation was calculated to verify the existence of correlations between the zootechnical performance data and ions Ca^{2+}, Mg^{2+}, Na^{+}, Cl^{-}, SO_4^{-2}, K^{+}, total alkalinity and total hardness in treatments.

All statistical and graphical tests were performed using the statistical software R, version 4.0.2 (2020), with the packages: car (Fox and Weisberg, 2019), PMCMR (Pohlert, 2014), gvlma (Pena and Slate, 2019), corrplot (Wei and Simko, 2017) and Hmisc (Harrell Jr et al., 2020).

3. Results

3.1.Zootechnical performance

At the end of the experimental period, none of the zootechnical variables showed significant differences among the treatments with different ionic compostions ($p > 0.05$). The final weight of the animals varied between 0.40 and 0.49 g at the end of the experiment (Table 3), SGR remained above 10.0% day^{-1}, the survival rate above 80%, FCR above 1.5 and average yield remained between 0.69 and 0.81 kg m^{-3} (Table 3).

Insert table 3

3.2.Stress Test

In the osmotic stress test, survival was 100% in all treatments. Regarding the NH_3-N resistance test, after 96 hrs, 100% survival was observed for T3 and T1 and 90% survival in the T2 treatment.

3.3.Major ions and cation ratios

The concentrations of calcium, potassium and magnesium ions varied during the trial, with significant differences among treatments ($p < 0.05$; Table 4). Higher concentrations ($p < 0.05$) of Ca^{2+} (initial), Mg^{2+} (initial and final), SO_4^{2-} (final) and higher Mg:Ca and Mg:K (final) ratios in T3 were observed when compared to T1 and T2 (Table 4). The total hardness was maintained above 400 mg $CaCO_3$ L^{-1}, with a total hardness:alkalinity ratio ($CaCO_3$ L^{-1}) close to 4, with a significant difference among treatments at the end of the experimental period (Table 4).

Insert table 4

3.4. *Correlation between zootechnical performance, total alkalinity and calcium*

Total alkalinity was positively correlated with yield, FW and SGR and negatively with survival. Calcium was positively correlated with yield, FW, SGR and FCR and negatively correlated with survival (Figure 1).

Insert figure 1

3.5. Environmental and water quality variables

Mean temperature in all treatments was 29 °C during the experiment. The average dissolved oxygen remained between 6.0 and 6.1 mg L^{-1} during the morning and afternoon (Table 5) ($p > 0.05$). Mean TAN varied between 0.15 and 0.2 mg L^{-1} ($p > 0.05$). NO_2^--N was lower in T3 than in T1 and T2 ($p = 0.042$), NO_3^--N was higher in the T2 and T3 treatments than in T1 ($p = 0.049$). PO_4^{3-} was higher in T2 than in treatments T1 and T3 ($p = 0.030$) and total alkalinity was higher in T1 and T2 than in T3 ($p = 0.042$, Table 5). Mean SS varied between 7.1 and 8.2 mL L^{-1} among the treatments ($p > 0.05$).

Insert table 5

3.5. *Absolute elemental composition (C, N and P) and stoichiometric ratios (C:N:P) of the microbial community and dissolved fraction*

Significant differences ($p = 0.006$) were observed among the treatments for P in the dissolved fraction, with T2 > T3 > T1 (Table 6). Significant differences were observed ($p = 0.030$) for the C:P ratio of the dissolved fraction, with T1 and T3 higher than T2 and for the N:P ratio of the dissolved fraction ($p = 0.005$), for which T1 > T3 > T2 (Table 6).

A stability trend of the C:P, C:N and N:P ratios of the microbial community and an increasing trend in the C:P ratios of the dissolved fraction was observed in all treatments. The dissolved fractions revealed an increase in C:N ratios in treatments T2 and T3 and a reduction in the T1 treatment and a stability of the N:P ratios in the MC and DF of all of the treatments (Table 6).

Insert table 6

4. Discussion

During the experimental period, adjustments to K^+ and Mg:Ca, Mg:K and Ca:K ratios did not influence *L. vannamei* zootechnical performance in nursery with zero water exchange, use of a biofilter, and with seawater diluted with freshwater with major ion concentrations of Ca^{2+} (25.07 mg L^{-1}), Mg^{2+} (89.75 mg L^{-1}), K^+ (25.00 mg L^{-1}), total alkalinity close to 100 mg L^{-1} $CaCO_3$ and total hardness close to 433.30 mg L^{-1}. In addition, an increasing concentration of these ions was observed in the water over 35 days, even in the control treatment (T1), which may have been caused by the addition of feed and presence of *A. brasiliana* shells in the system. The absence of significant differences between the ionic adjustment strategies used in this study shows the possibility for intensive marine shrimp farming in low salinity water without the need for supplementation to maintain the recommended ionic concentrations and ratios throughout the farming time. However, without dispensing the use of a biofilter, biological activator, and fertilization with fermented rice bran. This suggests that providing minimum acceptable concentrations of cations and anions at the beginning of the experiment created conditions that do not limit the growth of *L. vannamei* in nursery system in salinity close to 2.5 g L^{-1}. This understanding should consider the functions of ions such as Ca^{2+} and Mg^{2+} in shrimp growth and physiology and the system's microbiota, since they use minerals to perform vital functions such as maintaining metabolism, osmoregulation, ecdysis, mineralization of exoskeleton, chlorophyll molecule and cell membrane formation (Tacon, 1987; Boyd and Tucker,1998; Roy and Davis, 2010; Valenzuela-Madrigal et al., 2017; Nehru et al., 2018b).

The final weight achieved in this study (0.40 - 0.49 g) is similar to that found in Brito et al. (2016) in a BFT system with 2,500 shrimp m^{-3} and salinity of 35 g L^{-1} (35 days), which reached a mean of 0.68 g in the control treatment. Another issue that should be highlighted is the survival rate (> 80%), which is high when compared to other studies; i.e., Laramore et al. (2001) and Esparza-Leal et al. (2016) in a *L. vannamei* nursery in low salinity obtained 0% of survival for intensive systems with and without water exchange.

The FCR found in this study is similar to that observed by Galkanda-Arachchige et al. (2021) who tested the effect of different Mg^{2+} levels on the growth of *L vannamei* juveniles in water with 3 g L^{-1} salinity for 42 days and found an FCR of 1.7 in treatments with concentrations of 12 and 30 mg Mg^{2+} L^{-1}. Higher FCR values may be linked to the higher energy expenditure of shrimp to perform osmoregulation in low salinity water (Sá, 2012).

The availability of ions such as Mg^{2+} and K^{+} in marine shrimp farming with low salinity water is important because these ions act as cofactors and activators of the enzyme Na^{+}, K^{+}-ATPase, respectively (Naik, 2012; Galkanda-Arachchige et al., 2021). The deficiency of these ions in water can impair the shrimps' osmoregulatory process, affecting their metabolism and survival (Roy et al., 2007). However, the initial concentrations maintained in T1 and T2 did not prove to be limiting for the growth and survival of shrimps.

The yield results (0.7 and 0.8 Kg m^{-3}) indicate that the different ionic compositions, together with the management strategy used were efficient, considering that studies in salinity of 8 g L^{-1} indicated a mean yield of 0.11 Kg m^{-3} (Esparza-Leal et al., 2016). In addition, the osmotic stress test and resistance to NH_3-N results, with survival ≥ 90%

showed the shrimps' resistance to stressful factors, such as transfers at the end of the nursery period to grow-out ponds.

The significant positive correlations between total alkalinity and calcium with final weight, specific growth rate and yield, can be explained by their role in shrimp growth, since they are linked to the process of ecdysis, which absorbs large quantities of calcium for the animal's exoskeleton mineralization (Tacon, 1987; Boyd and Tucker, 1998). This indicates that the adjustment of total alkalinity and calcium to minimum levels in water with low salinity is important for obtaining good development of animals during the farming cycle (Samocha, 2019). Total alkalinity adjustment is also reflected in water quality, providing a greater buffer effect, reducing the daily pH fluctuation (Furtado et al., 2015; Boyd, 2020). Although total alkalinity differed among treatments, this variable was kept close to 100 mg L^{-1} $CaCO_3$ in all of the units throughout the experiment, which provided daily pH variations of around 0.1 between morning and afternoon. This buffer effect can be explained by the addition of the artificial substrate of *A. brasiliana* shells, which are formed by layers of calcite ($CaCO_3$), aragonite ($CaCO_3$) and crystallized calcium carbonate ($CaCO_3$), which were hydrolyzed and released into the water over time (Gosling, 2003; Boyd et al., 2016).

We observed that the nitrification process was efficient during the experimental period, with higher concentrations of NO_3^--N compared to TAN and NO_2^--N. This efficiency is related to the combination of fertilizations using aerobic and anaerobic carbon decomposition and bacteria (Romano et al., 2018). In addition to the use of an artificial substrate and biological activator that probably have accelerated the system's nitrification processes (Silva Neto et al., 2012; Santos et al., 2019). The NO_3^--N reduction in weeks 3 and 5, in all treatments, may be linked to the consumption of this

compound by algae or by anaerobic bacteria activity responsible for the denitrification process present in the water column of experimental units and anaerobic extracts of the biofilter (Egna and Boyd, 1997; Samocha, 2019; Boyd, 2020).

During the experimental time, the mean concentrations of TAN and NO_2^--N were maintained within the recommended concentration range for systems that use low salinity water (< 0.81 mg TAN L^{-1} and < 0.45 mg NO_2^--N L^{-1}) (Lin and Chen, 2001; Gross et al., 2004; Valencia-Castañeda et al., 2018). The control of nitrogen compounds in farming using low salinity water is an important factor for a higher survival rate of farmed animals, because under these conditions, the toxicity of nitrogen compounds, especially nitrite, becomes greater for marine shrimp (Ramírez-Rochín et al., 2016). The high toxicity of these compounds is confirmed by Esparza-Leal et al. (2016) who found 100% mortality on the 14th day of culture, when the water presented 0.5 mg TAN L^{-1} and 5.0 mg NO_2^--N L^{-1} in an *L. vannamei* nursery with a biofloc system at low salinity (2 and 4 g L^{-1}).

In natural aquatic and aquaculture ecosystems, changes in water quality variables, such as total alkalinity, pH and total hardness can alter productivity and, consequently, the elemental and relative chemical composition of a system's microbial community (Sterner and Elser 2002; Esteves, 2011; Luo et al., 2013; Eldyasti et al., 2013). The different ionic adjustment strategies used in this study had no effect on the stoichiometric ratios of the system's microbial community, because they most likely did not alter microbial growth or because their culture environment already had fast growing conditions in all of the treatments. In fact, the mean C:N ratio for this community in all treatments was close to 10:1, indicating that the microbial community reflects the recommended C:N ratio for fertilizing intensive systems with minimal water exchange (Avnimelech, 2012; Samocha, 2019). The low N:P ratios of the microbial

communities may be caused by the increased availability of P in the system in relation to N (which, when available in water as ammonia, is oxidized and immobilized by autotrophic and heterotrophic bacteria, respectively), resulting from the provision of feed and fertilizers, and absence of P immobilization in the sediment and water exchange. In semi-intensive shrimp farming systems, the sediment is responsible for accumulating between 42 - 45% of added P (Casillas-Hernández et al., 2006). This is reflected in growth of the microbial community, which allows a better cycling and immobilization of this nutrient in microbial biomass, and it is also retained in the shrimp internal biomass (Da Silva et al., 2013; Jung et al., 2017).

In this study, there was a lower variability of the C:N:P ratios in the microbial community during the experiment than in the C:N:P ratios of the dissolved fraction. This may indicate the presence of a fast-growing microbial community with a homeostatic behavior despite variations in the absolute and relative C, N and P composition of dissolved fraction (Godwin and Cotner, 2014). This chemical composition in the microbial community is consistent with systems where there is a high availability of nutrients, which reflects high metabolic rates of microorganisms. Under these conditions, they absorb these elements and direct them to cellular functions, such as ATP production and RNA synthesis to produce proteins demanded by the increased growth rate. This mechanism is known as the growth rate hypothesis (Elser et al., 1996; Sterner and Elser, 2002; Godwin et al., 2017) and has been demonstrated in shrimp pond systems (Pimentel et al., 2020). Over time, there was a gradual increase in concentrations of suspended solids, even though the C:N:P ratios did not change in these particles.

The constant organic carbon supply in the system (conducted to maintain the high C:N ratio recommended for intensive systems with minimal water exchange) and feed

cause high concentrations of dissolved C, N and P during the experiment. However, the homeostatic stoichiometric signature of the biofloc microbial community indicates a possible independence from the frequent nutrients supply, mainly of C. Therefore, maintaining high concentrations of these nutrients in the dissolved fraction during culture may not be needed to maintain the microbial community and the system water quality.

5. Conclusions

The ionic adjustment strategies used in this study did not have a significant effect on marine shrimp growth. However, it was observed that maintenance of initial concentrations of major ions [Ca^{2+} (25.07 mg L^{-1}), Mg^{2+} (89.75 mg L^{-1}), K^{+} (25.00 mg L^{-1}), total alkalinity close to 100 mg L^{-1} $CaCO_3$ and total hardness close to 433.30 mg L^{-1}], along with use of an *Anomalocardia brasiliana* biofilter, biological activator, and fertilization with fermented rice bran provide conditions that do not limit the growth of marine shrimp in nurseries using low salinity water (2.5 g L^{-1}), from diluted seawater, with high stocking density, with no need for ionic adjustment over the experimental period.

The K^{+} adjustment and Ca:Mg:K ratio in the water had no direct influence on the C:N:P ratio of the biofloc microbial community. The stability of the C:N:P ratios in the microbial biomass over the experimental time, given the variation in the C:N:P ratios of the dissolved fraction, suggests a homeostatic behavior of the microbial community.

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Table 1. Efficiency of chemical components (increase after 72 hours of the application of 100 g m^{-3}) used to ionic adjustment in the *Litopenaeus vannamei* nursery system.

Chemical component		KCl	$CaCO_3$	$MgSO_4$
Concentration (g m^{-3})		100	100	100
Increment	pH	0.41 ± 0.04	0.32 ± 0.05	0.40 ± 0.07
	Ca^{2+} (g m^{-3})	-	30.4 ± 0.73	-
	K^{+} (g m^{-3})	51.1 ± 11.38	-	-
	Mg^{2+} (g m^{-3})	-	-	9.4 ± 0.51

The data correspond to the mean of 2 replicates ± standard deviation (mg L^{-1}). KCl: potassium chloride; $CaCO_3$: calcium carbonate; $MgSO_4$: magnesium sulfate.

Table 2. Total ammonia nitrogen (TAN) concentration and non-ionized ammonia (NH_3-N) produced in the experimental units from NH_4Cl solution application.

	TAN (mg L^{-1})	NH_3-N (mg L^{-1})
Initial	3.75	0.446 ± 0.038
24hrs	4.00	0.300 ± 0.004
48hrs	7.10	0.576 ± 0.068
72hrs	7.00	0.601 ± 0.091

Data correspond to the mean concentration of TAN and NH_3-N ± standard deviation.

Table 3. Zootechnical performance of *Litopenaeus vannamei* intensive nursery system in oligohaline water with different ionic adjustment strategies after 35 days.

	Treatments			
	T1	T2	T3	p-value
FW (g)	0.41 ± 0.12	0.49 ± 0.04	0.40 ± 0.09	0.437
SGR (% day^{-1})	10.82 ± 0.83	11.38 ± 0.21	10.74 ± 0.58	0.415
Survival (%)	87.22 ± 6.25	82.50 ± 6.29	86.39 ± 3.94	0.575
FCR	1.89 ± 0.60	1.69 ± 0.03	1.93 ± 0.41	0.761
Yield (Kg m^{-3})	0.73 ± 0.25	0.81 ± 0.02	0.69 ± 0.18	0.734

Data correspond to the mean ± standard deviation. p-value correspond to the one-way ANOVA result among treatments. T1 (seawater diluted to a salinity of 2.5 g L^{-1}); T2 (water at a salinity of 2.5 g L^{-1} with K^+ adjustment); T3 (water at a salinity of 2.5 g L^{-1} with Ca:Mg:K ratio adjustment). FW: final weight; SGR: specific growth rate; FCR: feed conversion ratio.

Table 4. Major ions concentration (mg L^{-1}), total hardness, cation ratios, total hardness:alkalinity and analysis error during a *Litopenaeus vannamei* intensive nursery system in oligohaline water with different ionic adjustment strategies for 35 days.

Ion/Ratio	Treatments								
	Day 0			Day 17			Day 35		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Ca^{2+}	25.6 ± 1.60^{b}	25.07 ± 1.85^{b}	35.00 ± 0.00^{a}	31.47 ± 5.14^{b}	34.67 ± 5.14^{ab}	47.00 ± 0.00^{a}	45.33 ± 7.56	40.53 ± 5.61	57.07 ± 7.56
Mg^{2+}	89.75 ± 1.48^{b}	97.85 ± 5.70^{ab}	105.00 ± 0.00^{a}	122.10 ± 18.37	109.50 ± 1.48	140.00 ± 0.00	94.28 ± 7.59^{b}	108.22 ± 17.12^{b}	190.80 ± 15.25^{a}
K^{+}	30.60 ± 0.00	25.00 ± 0.00	35.60 ± 9.20	38.73 ± 2.96^{b}	46.52 ± 1.09^{a}	47.10 ± 2.74^{a}	40.80 ± 2.09	45.49 ± 2.42	43.32 ± 1.74
Na^{+}	744.41 ± 19.59	656.25 ± 33.93	682.37 ± 81.56	750.90 ± 49.29	764.00 ± 51.82	734.60 ± 44.88	760.7 ± 62.97	754.2 ± 115.48	764.00 ± 76.50
SO_4^{2-}	240.40 ± 0.00	203.27 ± 30.81	268.33 ± 10.69	291.00 ± 16.73	260.10 ± 56.36	265.10 ± 12.36	286.50 ± 30.92^{b}	284.40 ± 41.51^{b}	573.90 ± 34.85^{a}
Cl^{-}									

	1,347.10 ± 35.45	1,187.58 ± 61.40	1,234.84 ± 147.60	1,359.00± 89.21	1,383.00 ± 93.79	1,329.00 ± 81.23	1,377.00 ± 113.95	1,365.00 ± 208.97	1,383.00 ± 138.44
HCO_3^-	124.03 ± 12.70	126.10 ± 3.52	115.90 ± 6.10	158.60 ± 6.10	146.40 ± 27.95	136.20 ± 7.05	162.70 ± 12.69	181.00 ± 15.35	170.8 ± 16.14
TH	433.30 ± 4.62	465.30 ± 25.40	460.00 ± 56.00	581.30 ± 62.78	537.30 ± 8.33	589.30 ± 40.06	501.30 ± 46.70^{b}	546.70 ± 80.43^{b}	928.00 ± 48.49 ^{a}
TH:Alk	4.29 ± 0.41	4.50 ± 0.12	4.87 ± 0.83	4.47 ± 0.47	4.59 ± 0.90	5.29 ± 0.52	3.78 ± 0.55^{b}	3.69 ± 0.49^{b}	6.65 ± 0.29^{a}
Mg:Ca	3.52 ± 0.26^{ab}	3.91 ± 0.30^{a}	3.00 ± 0.00^{b}	4.03 ± 1.36	3.21 ± 0.55	2.98 ± 0.00	2.11 ± 0.28^{b}	2.68 ± 0.35^{ab}	3.40 ± 0.66^{a}
Ca:K	0.78 ± 0.00	1.00 ± 0.07	1.03 ± 0.25	0.81 ± 0.15	0.75 ± 0.12	1.00 ± 0.06	1.12 ± 0.23	0.88 ± 0.07	1.32 ± 0.22
Mg:K	2.98 ± 0.00	3.91 ± 0.22	3.08 ± 0.75	3.16 ± 0.53^{a}	2.35 ± 0.07^{b}	2.98 ± 0.17^{ab}	2.32 ± 0.27^{b}	2.37 ± 0.29^{b}	4.41 ± 0.33^{a}

Na:K	24.33 ± 0.00^{ab}	26.25 ± 1.36^{a}	19.63 ± 2.74^{b}	19.40 ± 0.24^{a}	16.44 ± 1.39^{ab}	15.66 ± 1.79^{b}	18.70 ± 2.12	16.54 ± 1.89	17.69 ± 2.36
Error (%)	7.65 ± 0.00	3.32 ± 2.05	3.21 ± 0.69	3.78 ± 2.52	3.60 ± 2.49	3.83 ± 0.65	7.30 ± 1.04^{a}	5.46 ± 1.45^{ab}	1.89 ± 2.12^{b}

Superscript letters correspond to the result of the Tukey test among treatments, per week. Concentration represented after ionic adjustment at day 0 and 17 (mean ± standard deviation). TH: total hardness; TH:Alk: total hardness:alkalinity. T1 (seawater diluted to a salinity of 2.5 g L^{-1}); T2 (water at a salinity of 2.5 g L^{-1} with K^{+} adjustment); T3 (water at a salinity of 2.5 g L^{-1} with Ca:Mg:K ratio adjustment).

Table 5. Water quality variables measured during *Litopenaeus vannamei* intensive nursery system in oligohaline water with different ionic adjustment strategies for 35 days.

Variables	Treatments			p-value
	T1	T2	T3	
DO morning (mg L^{-1})	6.14 (6.60 – 5.80)	6.12 (6.53 – 5.83)	6.15 (6.50 – 5.91)	0.513
DO afternoon (mg L^{-1})	6.07 (6.74 – 5.79)	6.05 (6.72 – 5.72)	6.08 (6.74 – 5.82)	0.311
Temperature (ºC)	29.45 (29.87 – 28.80)	29.39 (29.89 – 29.04)	29.36 (29.70 – 29.00)	0.513
Salinity (g L^{-1})	2.62 (2.84 – 2.40)	2.55 (2.88 – 2.36)	2.64 (3.05 – 2.40)	0.135
pH morning	7.90 (8.20 – 7.79)	7.92 (8.20 – 7.78)	7.89 (8.30 – 7.81)	0.119
pH afternoon	7.86 (8.10 – 7.63)	7.86 (8.00 – 7.68)	7.81 (8.20 – 7.67)	0.069
TAN (mg L^{-1})	0.00 (1.88 – 0.00)	0.00 (1.10 – 0.00)	0.00 (2.47 – 0.00)	0.678
NO_2^-- N (mg L^{-1})	0.20 (0.80 – 0.00) [a]	0.22 (0.70 – 0.04) [a]	0.14 (0.50 – 0.06) [b]	0.042
NO_3^-- N (mg L^{-1})	10.5 (18.70 – 0.15) [b]	23.40 (62.50 – 9.00) [a]	16.80 (54.00 – 3.30) [a]	0.049
PO_4^{3-} (mg L^{-1})	17.35 (21.50 – 9.00) [b]	18.35 (32.00 – 15.10) [a]	15.50 (43.50 – 11.20) [b]	0.030
TP (mg L^{-1})	0.84 (1.16 – 0.75)	0.98 (1.31 – 0.71)	0.88 (0.99 – 0.72)	0.716
TSS (mg L^{-1})	168.29 (311.00 – 74.03)	172.70 (388.00 – 80.00)	180.30 (384.00 – 76.80)	0.513
SS (ml L^{-1})	7.56 (13.50 – 1.00)	7.94 (14.50 – 1.30)	7.50 (16.00 – 1.20)	0.846
Alkalinity (mg L^{-1})	117.50 (145.00 – 75.00) [a]	115.00 (160.00 – 90.00) [a]	107.50 (155.00 – 80.00) [b]	0.042

Data correspond to the median of repetitions over the experimental time (maximum - minimum). p-value correspond to the Friedman test result and superscript letters correspond to the Conover multiple comparison test results ($p < 0.05$). T1 (seawater diluted to a salinity of 2.5 g L^{-1}); T2 (water at a salinity of 2.5 g L^{-1} with K^+ adjustment); T3 (water at a salinity of 2.5 g L^{-1} with Ca:Mg:K ratio adjustment). DO: dissolved oxygen;

TAN: total ammonia nitrogen; NO_2^--N: nitrite nitrogen; NO_3^--N: nitrate nitrogen; PO_4^{3-}: phosphate; TP: total phosphorus; TSS: total suspended solids; SS: settleable solids.

Table 6. Absolute elemental composition (C, N and P) and stoichiometric ratios C:N:P of the microbial community (MC) and dissolved fraction (DF) in the intensive *Litopenaeus vannamei* nursery system in oligohaline water with different ionic adjustment strategies for 35 days.

	Treatments			Friedman	Treatments	Linear regression (Nutrients/Ratios × Time)		
	T1	T2	T3	p-value		r^2	p-value	Equation
C - MC (mg g^{-1})	278.90 (338.9 – 238.2)	267.9 (335.7 – 235.5)	268.9 (332.2 – 236.6)	0.311	T1	0.085	0.292	y = 5.222 x + 258.247
					T2	0.053	0.409	y = 2.728 x + 252.304
					T3	0.0087	0.739	y = 265.905 – 0.825x
N - MC (mg g^{-1})	30.81 (70.66 – 25.61)	29.90 (56.46 – 24.34)	31.54 (45.27 – 26.72)	0.846	T1	0.058	0.385	y = 0.269 – 0.112 x
					T2	0.138	0.172	y = - 0.3303 – 0.136 x
					T3	0.00011	0.970	y = 30.756 + 0.018 x
P - MC (mg g^{-1})	22.99 (30.06 – 5.93)	22.85 (25.74 – 5.64)	22.72 (29.67 – 6.20)	0.311	T1	0.161	0.138	y = 0.935 x + 21.200
					T2	0.0031	0.844	y = 0.026 x + 0.027
					T3	0.254	0.055	y = 1.192 x + 19.658
C - DF (mg L^{-1})	25.24 (37.30 – 16.85)	26.08 (38.29 – 18.15)	22.67 (39.80 – 13.93)	0.311	T1	0.864	< 0.001	y = 13.716 + 3.899 x
					T2	0.027	0.557	y = 0.078 x – 0.465
					T3	0.889	< 0.001	y = 9.809 + 5.539 x
N - DF (mg L^{-1})	13.63 (19.87 – 6.72)	14.90 (19.96 – 7.42)	14.11 (20.87 – 7.54)	0.223	T1	0.519	< 0.01	y = 18.584 – 1.189 x
					T2	0.118	0.209	y = 16.826 – 0.480 x
					T3	0.125	0.195	y = 16.905 – 0.708 x

P - DF (mg L^{-1})	2.69 (4.04 – 1.14) [c]	3.16 (4.73 – 1.24) [a]	2.99 (3.51 – 1.20) [b]	0.006	T1	0.218	0.079	y = 3.398 – 0.171 x
					T2	0.106	0.236	y = 1.392 – 0.042 x
					T3	0.021	0.603	y = 3.083 – 0.032 x
C:P (mmol g^{-1}:mmol g^{-1}) - MC	30.88 (138.56 – 20.47)	29.86 (149.50 – 24.77)	30.10 (136.38 – 23.50)	0.606	T1	0.052	0.412	y = 32.367 – 0.779 x
					T2	0.0079	0.751	y = 0.045 x – 0.155
					T3	0.238	0.065	y = 34.979 – 1.679 x
C:N (mmol g^{-1}:mmol g^{-1}) - MC	10.29 (12.34 – 5.60)	10.08 (12.75 – 5.22)	9.89 (12.08 – 7.88)	0.606	T1	0.042	0.463	y = 0.067 x – 0.706
					T2	0.222	0.076	y = 0.212 x – 0.998
					T3	0.008	0.745	y = 10.213 – 0.059 x
N:P (mmol g^{-1}:mmol g^{-1}) – MC	2.99 (17.30 – 1.89)	2.95 (15.77 – 2.27)	2.97 (15.10 – 2.34)	0.311	T1	0.085	0.289	y = 0.076 x + 0.0021
					T2	0.041	0.471	y = 0.064 x – 0.046
					T3	0.165	0.132	y = 0.060 x – 0.804
C:P (µM:µM) - DF	28.94 (58.53 – 13.21) [a]	21.75 (41.85 – 10.30) [b]	26.37 (46.30 – 11.26) [a]	0.030	T1	0.871	< 0.001	y = 10.087 + 4.496 x
					T2	0.781	< 0.001	y = 8.572 + 4.179 x
					T3	0.879	< 0.001	y = 8.681 + 4.804 x
C:N (µM:µM) - DF	2.51 (3.89 – 1.11)	2.60 (3.69 – 1.29)	2.41 (3.85 – 1.08)	0.606	T1	0.831	< 0.001	y = 0.681 – 0.344 x
					T2	0.739	< 0.001	y = 0.972 + 0.412 x
					T3	0.753	< 0.001	y = 0.502 + 0.568 x
N:P (µM:µM) - DF				0.005	T1	0.058	0.387	y = 12.624 – 0.307 x

	11.74 (16.45 – 8.73) [a]	10.45 (14.32 – 6.46) [c]	11.42 (14.94 – 8.43) [b]	T2	0.0063	0.777	y = 9.424 + 0.125 x
				T3	0.098	0.253	y = 12.337 – 0.449 x

Data correspond to the median of repetitions (maximum - minimum) over the weeks of experimental time. p-value correspond to the Friedman test result and the superscript letters correspond to the results of the Conover multiple comparison test with Holm-Bonferroni correction ($p < 0.05$). T1 (seawater diluted to a salinity of 2.5 g L^{-1}); T2 (water at a salinity of 2.5 g L^{-1} with K^+ adjustment); T3 (water at a salinity of 2.5 g L^{-1} with Ca:Mg:K ratio adjustment). C: carbon; N: nitrogen; P: phosphorus.

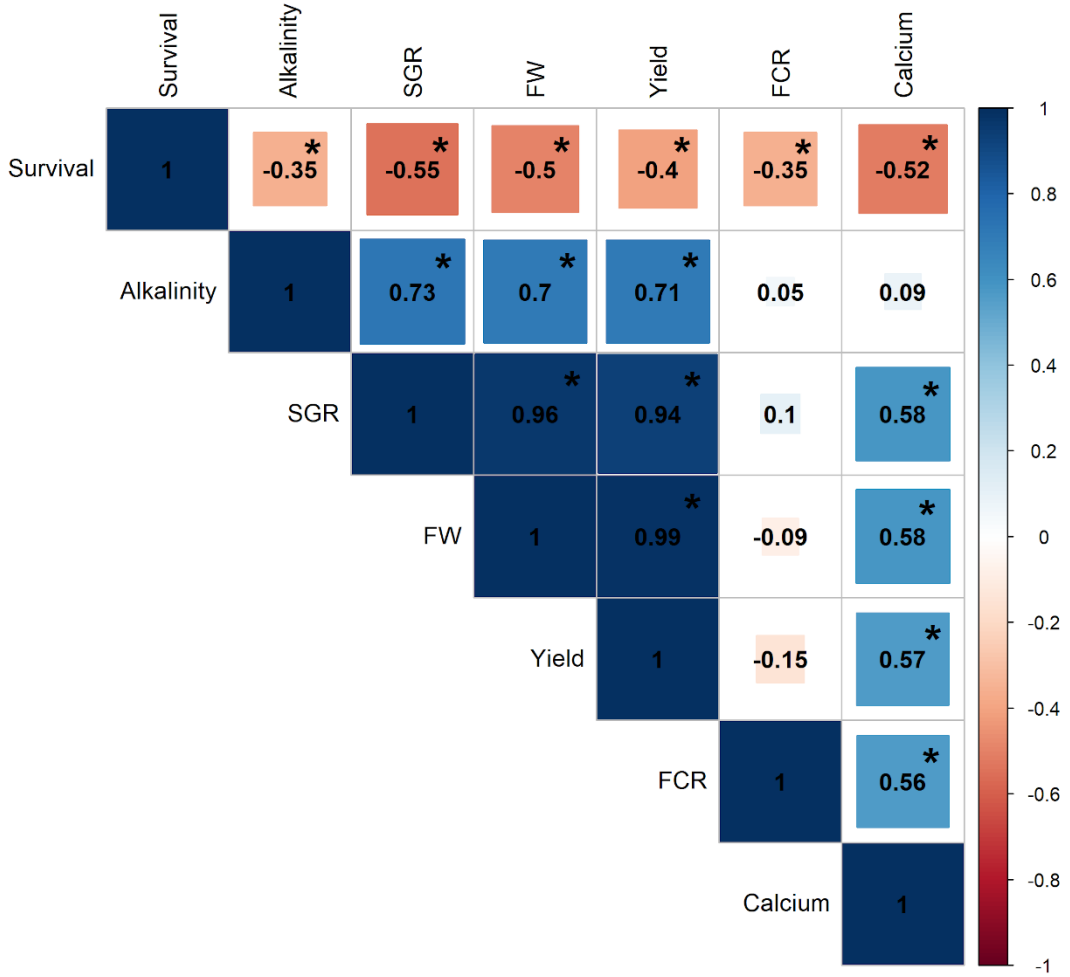

Figure 1. Spearman correlation matrix between calcium, alkalinity, and zootechnical performance of marine shrimp *Litopenaeus vannamei*. Numbers inside the boxes correspond to the coefficient of correlation. * Significant correlations ($p < 0.05$). FCR: feed conversion ratio; FW: final weight; SGR: specific growth rate.

3. Considerações finais

As estratégias de ajuste iônico utilizadas neste estudo não afetaram significativamente o crescimento do camarão marinho *Litopenaeus vannamei*. No entanto, foi observado que a manutenção das concentrações iniciais dos principais íons, o uso do biofiltro de *Anomalocardia brasiliana,* do ativador biológico e da fertilização com fermentado de farelo de arroz proporcionaram condições que não limitaram o crescimento do camarão marinho em berçários utilizando água de baixa salinidade (2,5 g L^{-1}), com água do mar diluída, alta densidade de estocagem, sem a necessidade de ajuste iônico ao longo do período experimental.

O ajuste de K^+ e da razão Ca:Mg:K na água não possui influência direta na relação C:N:P da comunidade microbiana. A estabilidade das relações C:N:P ao longo do período experimental, frente a variação nas relações C:N:P da fração dissolvida, sugere um comportamento homeostático da comunidade microbiana.

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