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PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

EFEITO DA FREQUÊNCIA DO AJUSTE DO EQUILÍBRIO IÔNICO NO DESEMPENHO ZOOTÉCNICO DO *Litopenaeus vannamei* (BOONE, 1931) EM SISTEMA SIMBIÓTICO EM ÁGUAS OLIGOHALINAS

Valdemir Queiroz de Oliveira

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 cultivo do camarão marinho *Litopenaeus vannamei* em água de baixa salinidade é uma prática em crescente desenvolvimento no mundo e Brasil. Essa prática é possível devido as altas concentrações de dureza total e alcalinidade total das fontes de água, além de alguns ajustes realizados nos principais íons necessários para o desenvolvimento do animal. Estudos relatam que esses íons são utilizados pelos camarões para a realização de reações metabólicas, ao processo de ecdise, sendo absorvidos para a mineralização do exoesqueleto do animal, papel importante na ativação da enzima Na^+/K^+-ATPase, responsável pelo transporte ativo de íons entre os meios extra e intracelulares entre outras. Entretanto não se sabe a frequência necessária que estes íons devem ser repostos no ambiente de cultivo, diante disso, o presente estudo visou avaliar o efeito de diferentes frequências de ajuste iônico no cultivo de camarão marinho em baixa salinidade, assim como verificar o efeito desta correção na composição mineral do floco microbiano e da composição corporal do camarão marinho *L. vannamei* cultivado em sistema simbiótico. Foi realizado um cultivo experimental ao longo de 40 dias, onde foram estocadas pós larvas a uma densidade de 2000 cam.m^{-3}, em 5 tratamentos: SW (agua do mar- salinidade 31), SWD (água do mar diluída- salinidade 2.3), 1IA (salinidade 2.3- ajuste de íons para atingir a relação 1:3:1 Ca:Mg:K apenas no 1° dia) – 2IA (salinidade 2.3- ajuste de íons para atingir a relação 1:3:1 Ca:Mg:K no 1° e 20° dia) e 3IA- (salinidade 2.3- ajuste de íons para atingir a relação 1:3:1 Ca:Mg:K no 1°, 10° e 20° dia). Os camarões foram alimentados na frequência de 4x ao dia com ração de 45% de proteína bruta e realizou-se fertilização orgânica com farelo de arroz (20g m^{-3} < 300 micra), açúcar demerara (2 g m^{-3}), bicarbonato de sódio (4 g m^{-3}) e 0.5 g m^{-3} de mix de bactérias contendo: *Bacillus subtilis*, *B. licheniformis*, *Saccharomyces* sp. e *Pseudomonas sp.* in a total of 5.5 to 6.5 × 10^7 CFU g^{-1}, sendo essa mistura foi submetida por 24 horas ao processo anaeróbico e em seguida ao aeróbico (24 horas), realizadas a cada 3 dias. Os ajustes dos íons para manter a relação 1:3:1 Ca:Mg:K foram realizados utilizando cloreto de magnésio (10,5 % Mg), cloreto de potássio (51,4% K) e carbonato de cálcio (30,4%Ca). As variáveis de qualidade de água (NAT, NO_2-N, NO_3-N, PO_4, alcalinidade total, dureza total, cálcio, magnésio, potássio, sódio, sulfato, cloreto) e as biometrias foram mensuradas a cada 10 dias. A composição mineral dos flocos e do camarão foram mensuradas ao final dos 40 dias de cultivo. Os compostos nitrogenados se mantiveram dentro dos níveis recomendados (0,66 mg/L NAT, 0,52 mg/L NO_2-N, 1,91 mg/L NO_3-N) para o cultivo do *L. vannamei* em água

oligohalinas em sistema simbiótico. Os parâmetros de desempenho zootécnico (1,14 a 1,18 g de peso médio; 88,61 a 94,44 % de sobrevivência; 2,10 a 2,15 kg/m^3 de produtividade) entre os tratamentos de baixa salinidade não diferiram significativamente quando comparados entre si, entretanto os resultados foram superiores aos encontrados para o tratamento de alta salinidade (1,02 g de peso médio; 84,72 % de sobrevivência; 1,73 kg/m^3 de produtividade). Ao avaliar a concentração de minerais no floco microbiano e na biomassa do camarão foi observado que a frequência da realização do ajuste iônico também não influenciou significativamente essas concentrações, diferindo apenas quando comparada a composição dos elementos da água marinha. Ao final do cultivo, os camarões foram submetidos a um teste de estresse amoniacal durante 96h para avaliar a resistência dos juvenis, obtendo 100% de sobrevivência após o período de prova em todos os tratamentos. Dessa forma, com base nos resultados obtidos, pode-se produzir juvenis de *L. vannamei* em água do mar diluída (salinidade 2.3; 38,0 mg Ca/L, 96,0 mg Mg/L, 48,3 mg K/L, 92,33 mg Alcalinidade total/L e 490,13 mg dureza total/L, relação Ca:Mg:K 1:2,5:1,3) sem a realização de ajustes iônicos e sem troca de água ao longo de 40 dias na fase de berçário em sistema de simbiótico.

PALAVRAS-CHAVE**: Equilíbrio iônico; água oligohalina; desempenho zootécnico; minerais; farelo de arroz fermentado**.

ABSTRACT

The cultivation of the marine shrimp *Litopenaeus vannamei* in low salinity water is an increasingly developing practice. This practice is possible due to the adjustments made to the main ions necessary for the animal's development. Studies report that these ions are used by shrimp to carry out metabolic reactions, as well as in the biofloc system, they stimulate the stabilization of the system and the formation of microbial flakes. However, the frequency that these ions must be replaced in the system is not known. Therefore, the present study aimed to evaluate the effect of different frequencies of ionic adjustment in the cultivation of marine shrimp in low salinity, as well as to verify the effect of this correction on the composition mineral of the biofloc and body composition of marine shrimp L. vannamei. An experimental cultivation was carried out over 40 days, the larvae were stored after larvae at a density of 2000cam.m-³, in 5 treatments: SW (sea water-salinity 31), SWD (diluted sea water - salinity 2.3), 1IA (salinity 2.3 - adjustment only on the 1st day) - 2IA (salinity 2.3 - adjustment on the 1st and 20th day) and 3IA - (salinity 2.3 - adjustment on the 1st, 10th and 20th day). The water quality variables remained within the recommended levels for the cultivation of *L. vannamei* in a biofloc system. The zootechnical performance parameters between the low salinity treatments did not differ significantly when compared to each other, however the results were superior to those found for the high salinity treatment. When evaluating the concentration of minerals in the biofloc and biomass of shrimp, it was observed that the frequency of the ionic adjustment did not significantly influence these parameters either, differing only when compared to the composition of elements in seawater. At the end of culture, the animals were subjected to an ammonia stress test for 96h, however, all treatments obtained 100% survival after the test period. Thus, based on the results obtained, the use of seawater diluted to salinity 2.3 without performing ionic adjustments throughout the cultivation of *L. vannamei* in the nursery phase does not represent losses to zootechnical indices, and it is possible to obtain the same production rate and cost reduction related to fertilizers used to carry out ionic corrections.

KEYWORDS: Ionic equilibrium; oligohaline water; zootechnical performance; minerals; rice bran fermented.

Sumário

1. INTRODUÇÃO

1.1 – CONTEXTUALIZAÇÃO DA PESQUISA

A produção de camarão marinho afastado da costa utilizando águas oligohalinas (salinidade 0,5 a 3,0 g L^{-1}) e mesohalinas (salinidade 3,0 a 16,5 g L^{-1}) com origem no lençol freático, reservatórios artificiais, lagos e rios ou salinizada artificialmente tem aumentado significativamente no mundo e no Brasil, explorando novas fronteiras do agronegócio (Brito et al, 2019). Diversos autores demonstram viáveis a produção de camarão marinho longe da costa, como: Samocha et al. (1998) (EUA), Van Wyk et al. (1999) (EUA), Nunes e López (2001) (Brasil e Equador), Boyd, 2001 (Tailândia, EUA, Equador), Samocha et al. 2001 (EUA e Israel), Boyd et al. (2002) (Thailandia, Equador, Brasil e EUA), Boyd e Thunjai (2003) (China, Tailândia, Equador e EUA), McNevin et al. (2004) (EUA), entre outros. Em 2018 a produção em áreas interiores já representou aproximadamente 39% (3.653 milhões de toneladas) da produção mundial de crustáceos (FAO, 2020). No Brasil, esta propensão também pode ser observada em estados como Alagoas, Ceará, Paraíba e Sergipe tem aumentado o número de produtores de camarões em empreendimentos distante do mar (IBGE, 2020). Esses resultados em água interiores deve ser a fácil adaptação dos camarões marinhos às condições de diversas salinidades, temperaturas e altas concentrações de compostos nitrogenados (Ponce-Palafox et al., 1997; Laramore et al., 2001; Lin e Chen, 2003).

Apesar de promissores resultados zootécnicos e econômicos, os corpos hídricos naturais interiores podem apresentar grandes variações de íons em sua composição, sendo influenciados por fatores geológicos e climáticos (Boyd e Thunjai, 2003; Boyd, 2015). A água do mar é composta principalmente pelos íons cloreto, sódio, sulfato, magnésio, cálcio, potássio e bicarbonato, o que corresponde à 99,75% da concentração dos minerais presentes, enquanto e outros 46 minerais correspondem à 0,25% (Hem, 1967). Entretanto, em águas interiores ocorrem modificações na concentração desses sais (Boyd, 2007).

A baixa disponibilidade de alguns íons para animais proporcionam uma maior gasto energético para manter as concentrações de sais nas células através da pressão osmótica (Schmidt-Nielsen, 1990; Boyd, 2015), além disso, vários íons são indispensáveis para fisiologia dos camarões *Litopenaeus vannamei*, como cálcio e magnésio para o processo de ecdise (Boyd e Tucker, 1998), sódio e potássio para o crescimento, osmorregulação e imunidade (Liu et al., 2016; Li et al., 2017), podendo em

baixa disponibilidade desses íons proporcionar elevadas taxas de mortalidade e limitações no crescimento dos camarões (Saoud et al., 2003; Roy e Davis, 2010).

Gao et al. (2016) relacionou a diminuição de salinidade com um menor peso médio final, imunodepressão e a redução da quimiotripsina, inibindo a taxa de síntese digestiva em juvenis de camarões. Essa redução de salinidade da água, proporciona um estresse salino que aumenta os níveis transcritos das enzimas osmorregulatórias Na+ K+ ATPase e anidrase carbônica (CA) em brânquias de crustáceos (Gao et al., 2012; 2016).

São necessárias concentração mínima dos íons Na^{+}, Cl^{-}, K^{+}, Ca^{2+}, Mg^{2+}, SO_4^{2-} e HCO_3^{-} e proporção entre eles relativas à água do mar, sendo necessário realizar o ajuste nas proporções entre os íons: Na^{+}: Mg^{+2}: Ca^{+2}: K^{+} de 27:3:1:1, garantindo assim condições para o crescimento e a sobrevivência do *L. vannamei* em sistemas com baixa salinidade (Boyd e Thunjai, 2003; Boyd, 2018). Essas concentrações podem ser ajustadas através da biocompesação mineral, na qual os sais são incorporados a ração do animal ou por meio da compensação na água do sistema, sendo utilizados fertilizantes minerais (Cheng et al., 2006; Liu et al., 2016).

A necessidade de realizar as correções de acordo com o perfil iônico da água podem variar as concentrações de fertilizantes necessárias para realização de tal ajuste. Aumentando assim a importância da determinação da frequência em que esses fertilizantes devem ser reaplicados, pois a frequência de compensação iônica artificialmente irá influenciar os custos de produção e pode tornar inviável a atividade dependendo do preço de comercialização do produto final (Galkanda-Arachchige et al., 2020; Pinto et al., 2020), entretanto esse custo de produção pode ser diluído durante vários ciclos de cultivo, quando utiliza-se os sistemas de mínima troca de água ou reduzidos quando utilizamos berçários, como o objetivo de produzir juvenis mais aclimatadas as baixas concentrações de íons e com melhor status imune.

Porém a utilização de sistema caracterizados pela mínima troca de água podem acarretar o aumento na concentração de compostos nitrogenados (amônia e nitrito) (Burford et al. 2003) provenientes da mineralização do resíduo alimentar e da excreção dos animais (Viadero Jr et al., 2005), principalmente nas primeiras semanas cultivo, quando as bactérias heterotróficas e nitrificantes ainda não estão bem estabelecidas. Elevadas concentrações de compostos nitrogenados são tóxicas para animais aquáticos (Ray et al., 2011), e a utilização de sistema com baixa salinidade pode então aumentar o

efeito da toxicidade da amônia e nitrito influenciando negativamente no crescimento e na sobrevivência do *L. vannamei* (Lin e Chen, 2001; 2003).

Para redução dos compostos nitrogenados, o sistema simbiótico é importante ferramenta para formação dos flocos microbianos, resultantes da fermentação e respiração microbiana de farelos vegetais por bactérias (*Bacillus, Bifidobacterium* e *Lactobacillus*) e/ou leveduras (*Saccharomyces cerevisiae*) (Dos Santos et al., 2022; Pimentel et al., 2022), produzindo ácidos orgânicos, vitaminas do grupo do complexo B ou antibióticos naturais, e melhorando a solubilidade das fontes de carboidratos (monossacarídeos, dissacarídeos e polissacarídeos) (Asgharian et al., 2016).

A utilização dos processos anaeróbicos (fermentação) e/ou aeróbios (respiração) com microorganismos (*Lactobacillus*, *Pediococcus*, *Enterococcus*, *Bacillus*, *Saccharomyces*, entre outros) está entre as formas de aumentar a disponibilidade de nutrientes de uma matéria-prima, especialmente os cereais. Durante a fermentação, há uma redução do teor de fibra e um aumento da biomassa microbiana. Esses microrganismos transformam os constituintes químicos das matérias-primas através da quebra de moléculas orgânicas complexas, como polissacarídeos, em moléculas mais simples, aumentando assim a biodisponibilidade e solubilidade dos nutrientes, tornando-os mais nutritivos (Azevedo et al., 2015) e permitindo a formação de ácidos orgânicos, reduzindo o pH e alterando o equilíbrio da microbiota intestinal reduzindo assim o crescimento de microrganismos patogênicos (Brito et al., 2013; Flesch et al., 2014).

Diante disto, o objetivo deste trabalho foi avaliar o efeito da frequência do ajuste iônico em águas oligohalinas na fase de berçários sobre o desempenho zootécnico, qualidade de água, composição mineral dos flocos microbianos e do camarão *L vannamei* em sistema de simbiótico.

1.2 OBJETIVOS

1.2.1 Objetivo geral:

Determinar a melhor frequência de ajuste do equilíbrio iônico no desempenho zootécnico, do *Litopenaeus vannamei* em águas oligohalinas utilizando sistema simbiótico.

1.2.2 Objetivos específicos:

- Avaliar o desempenho zootécnico das pós-larvas de *L vannamei* submetidos à diferentes frequências do ajuste no equilíbrio iônico em sistemas simbióticos;
- Avaliar a frequência de ajuste do equilíbrio iônico na qualidade de água durante a fase de berçário de *L vannamei* em sistemas simbióticos;
- Avaliar a resistência das pós-larvas de *L vannamei*, cultivadas com diferentes frequências do ajuste no equilíbrio iônico, ao teste de estresse osmótico e teste de estresse amoniacal ao final da fase de berçário em sistema simbiótico;
- Avaliar o efeito da frequência do ajuste no equilíbrio iônico na composição mineral do floco microbiano em sistemas simbióticos;
- Avaliar o efeito da frequência do ajuste no equilíbrio iônico na composição mineral do *L vannamei* em sistemas simbióticos.

1.2. Hipóteses

- A frequência do ajuste iônico nas relações entre os cátions Ca:Mg:K na água oligohalina proporcionará melhor desempenho zootécnico dos camarões marinhos;
- A frequência do ajuste iônico nas relações entre os cátions Ca:Mg:K na água oligohalina proporcionará modificação nos compostos nitrogenados da água.

2. ARTIGO CIENTÍFICO - Effect of ionic adjustment frequency in low salinity water on zootechnical performance, water quality and mineral composition of *Litopenaeus vannamei* in a synbiotic nursery system

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Abstract

Ions are important for metabolic processes in marine shrimps, but it is not known the need to maintain levels without affecting shrimp growth. The purpose of this work was to evaluate the effect of ionic adjustment frequencies in low salinity water in a synbiotic nursery system as well as verify its effects on the microbial flocs and *Litopenaeus vannamei* mineral composition. The experiment was carried out for 40 days, with a density of 2000 post-larvae m^{-3}. Thus, following treatments were tested, all in triplicate: SW (seawater - salinity 31 g L^{-1}); SWD (seawater diluted to a salinity of 2.3 g L^{-1}); 1IA (salinity 2.3 g L^{-1}- ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 only on the 1st day); 2IA (salinity 2.3 g L^{-1} - ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st and 20th day) and 3IA - (salinity 2.3 g L^{-1} - ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st, 10th and 20th day). Shrimps were fed four times a day with a 45% protein diet. The synbiotic fertilization protocol used a mix of *Bacillus subtilis, B. licheniformis, Saccharomyces* sp. and *Pseudomonas* sp. in a total of 5.5 to 6.5 × 10^7 CFU g^{-1} every 3 days. The nitrogen compounds remained within the recommended levels (NAT = 0.66 mg L^{-1}, NO_2^--N = 0.52 mg L^{-1}, NO_3^--N = 1.91 mg L^{-1}) for *L. vannamei* reared in low salinity water. Mean weight survival and yield (mean weight: 1.14 to 1.18 g; survival: 88.61 to 94.44 % and yield: 2.10 to 2.15 Kg m^{-3}) were not significantly different in low salinity treatments but were higher than those found in SW treatment (mean weight = 1.02 g; survival = 84.72%; and yield = 1.73 Kg m^{-3}). Mineral concentration in microbial floc and shrimp biomass did not differ among the frequencies tested, only differing when compared to SW treatment. To evaluate the juvenile's resistance, an ammoniacal stress test was performed during 96h, obtaining 100% survival for all treatments. According to the results, *L. vannamei* juveniles can be produced in diluted seawater (salinity 2.3; Ca^{2+} = 38.0 mg L^{-1}, Mg^{2+} = 96.0 mg L, K^+ = 48.3 mg L^{-1},

total alkalinity = 92.33 mg L^{-1} and total hardness = 490.13 mg L^{-1}, Ca:Mg:K ratio 1:2.5:1.3) without ionic adjustments and without water exchange for 40 days in the nursery phase using synbiotic system without harming production parameters.

Keywords: growth; stress test; Ca:Mg:K; mineral; nitrogen.

2.1.Introduction

Although the production of *Litopenaeus vannamei* is predominantly carried out in a marine or estuarine environment, there are already several productive results in places far from the sea, such as the Gobi desert in China, considered the furthest place from the sea on earth (FAO, 2020). In 2018, the marine shrimp production in inland areas corresponded to 3.653 million tons (52% of the world production of crustaceans) (FAO, 2020). These farms in areas with such different environmental characteristics are only feasible because *L. vannamei* is an easily adaptable species, supporting an extensive temperature and salinity gradient, as well as high concentrations of nitrogen compounds (Ponce-Palafox et al., 1997; Laramore et al., 2001; Lin and Chen, 2003).

The ease and reduced cost of inland areas are the main motivations for shrimp farming implementation in such lands, when compared to coastal lands, those inland areas present greater flexibility for acquisition of environmental licenses as well as access to reduced energy taxes.

These indoor crops are carried out using oligohaline (0.5 to 3.0 g L^{-1} salinity) and mesohaline (3.0 to 16.5 g L^{-1} salinity) waters from groundwater, artificial reservoirs, lakes and rivers, or artificially salt flats appear as a strategy of using inadequate water resources for agriculture and human consumption, increasing significantly the agribusiness borders.

Despite promising zootechnical and economic results, inland natural water bodies can present great variations in their composition, and this water profile is influenced by geological and climatic factors. (Boyd and Thunjai, 2003; Boyd, 2015).

The low availability of some ions for animals causes a greater energy expenditure to equalize the concentration of salts inside the cell through osmotic pressure (Schmidt-Nielsen, 1990; Boyd, 2015), in addition, several ions are associated with metabolic and chemical reactions. Physiological characteristics of *L. vannamei*, such as: calcium (Ca^{2+})

and magnesium (Mg^{2+}) participate in the ecdysis process (Boyd and Tucker, 1998), sodium (Na^{+}) and potassium (K^{+}) act on growth, osmoregulation and immunity (Liu et al., 2016; Li et al., 2017). When the *L. vannamei* culture is carried out in environments that present a low concentration of these ions, it can cause high mortality rates and limitations in the growth of shrimp. (Saoud et al., 2003; Roy and Davis, 2010).

Minimum concentrations of Na^{+}, chloride (Cl^{-}), K^{+}, Ca^{2+}, Mg^{2+}, sulfate (SO_4^{2-}) and bicarbonate (HCO_3^{-}) ions and a proportion among them relative to seawater are required, and it is necessary to adjust the proportions among the ions: Na^{+}: Mg^{2+}: Ca^{2+}: K^{+} of 27:3:1:1, thus ensuring conditions for the *L. vannamei* growth and survival in systems with low salinity (Boyd and Thunjai, 2003; Boyd, 2020). These concentrations can be adjusted through mineral bio compensation, a process in which the salts are incorporated into the animals feed or into the system's water, using mineral fertilizers (Cheng et al., 2006; Liu et al., 2016).

The concentration of fertilizers required to make the corrections are determined according to the ionic profile of the water. Thus, increasing the importance of determining the frequency at which these fertilizers should be reapplied, as the frequency of ionic compensation will artificially influence production costs and may make the activity unfeasible depending on the marketing price of the final product. (Galkanda-Arachchige et al., 2020; Pinto et al., 2020). However, this production cost can be diluted during several farming cycles, using systems of minimum water exchange, for this, a good control of nitrogenous compounds in low salinity is necessary, due to the toxicity of these compounds to cultivated marine shrimps.

Synbiotic system, on the other hand, results from the decomposition of products of plant origin, which are mostly wheat, soybean or rice bran, by bacteria (*Bacillus*, *Bifidobacterium* and *Lactobacillus*) and/or yeasts (*Saccharomyces cerevisiae*), into a

controlled aerobic and/or anaerobic process, which produces organic acids, B-complex vitamins or natural antibiotics, improving the solubility of carbohydrate sources (monosaccharides, disaccharides and polysaccharides). These microorganisms transform the chemical constituents of raw materials by breaking complex organic molecules, such as polysaccharides, into simpler molecules, thus increasing the bioavailability and solubility of nutrients, making them more nutritious. Consequently, they increase the control of nitrogenous compounds by increasing the microbial biomass in the system (heterotrophic and nitrifying bacteria).

Therefore, the aim of this study was to evaluate the effect of the ionic adjustment frequency in oligohaline water on the zootechnical performance, water quality, *L. vannamei* mineral and microbial floc composition in a synbiotic nursery system.

2.2.Material and methods

Experimental design

This study was carried out for 40 days at the Shrimp Culture Laboratory (LACAR), of the Fisheries and Aquaculture Department of the Rural Federal University of Pernambuco (UFRPE), Brazil. The experimental design was completely randomized with five treatments: SW (seawater - salinity 31 g L^{-1}); SWD (seawater diluted to a salinity of 2.3 g L^{-1}); 1IA (salinity 2.3 g L^{-1}- ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 only on the 1st day); 2IA (salinity 2.3 g L^{-1} - ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st and 20th day) and 3IA- (salinity 2.3 g L^{-1} - ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st, 10th and 20th day), all in triplicate.

Experimental conditions

The treatments 1IA, 2IA, 3IA e SWD used seawater with 35 g L^{-1} diluted with freshwater 0.16 g L^{-1} to a salinity of 2.3 g L^{-1}. The water inside the experimental units was prepared 15 days prior to animal stocking. This procedure was carried out by filling 15% (9 L) of the microbial floc tanks (TAN = 0.49 mg L^{-1}, N-NO_2^-= 0.70 mg L^{-1}, N-NO_3^-= 0.81 mg L^{-1}, total hardness = 763 mg $CaCO_3$ L^{-1}, total alkalinity = 88 mg $CaCO_3$ L^{-1}), and 85% (51 L) of water treated with sodium hypochlorite in a concentration of 15 g m^{-3} of activated chloride. Then, the synbiotic fertilization was applied. The fertilizer was submitted to an anaerobic process (24 h) and an aerobic phase (24 h) which was composed of 20 g m^{-3} of rice bran (< 200 μm), 2 g m^{-3} of sugar, 4 g m^{-3} of sodium bicarbonate and 0.5 g m^{-3} of commercial bacterial mix [5.5 to 6.5 × 10^7 colony-forming units (CFU) g^{-1}, containing: *Bacillus subtilis*, *Bacillus licheniformis*, *Saccharomyces* sp. e *Pseudomonas* sp. (Kayros Agrícola and Ambiental, Brazil) and chlorinated water [20 mg L^{-1} active chlorine (60%), then unchlorinated through aeration] in a proportion to 10 × the amount of rice bran. Totaling seven applications, with a two-day interval among fertilizations. The organic fertilizer was added to the experimental treatments every three days during culture until settleable solids reached 5 ml L^{-1}.

The experimental units (60 L), were constantly aerated (dissolved oxygen > 5.0 mg L^{-1}), temperature maintained at (~31°C; Hopar Sh-608 heater 100 W), 12:12-h photoperiod and mean luminance of 8.65 μmol photons m^{-2} s (Equitherm Lux-204). No water exchange was performed during the experiment, and dechlorinated freshwater was added to replace evaporation loss four times a week. The artificial substrate 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.

Shrimp stocking, feeding and monitoring

The post larvae (PL10 1.18 ± 0.4 mg) were acquired from a commercial hatchery (Aquatec, Rio Grande do Norte, Brazil), which was produced in water with salinity at 35 g L^{-1}. Prior to experiment, the PL10 were acclimated to salinity condition of 2.5 g L^{-1} for 10 days. At the end of the acclimatization period, the PL 20 (10 ± 3 mg) were randomly selected and stocked at a density of 2000 PL's m^{-3} in experimental units.

The post larvae were fed with commercial feed of 45% crude protein, 7% lipids, (0.3 - 0.6 mm, Inve Aquaculture Inc), during 1 day to 17days, and with 45% crude protein, 9.5% lipids, (0.8 – 1.3 mm, ADM Animal Nutrition Company), between 17 days to 40 days, four times a day (8:00 am, 11:00 am, 2:00 pm and 5:00 pm). Initially, a 27.2% daily feeding rate was adopted, which was gradually reduced until 4.8% of body weight during 40 days. The feeding rate was daily adjusted according to the shrimp feed consumption estimate and mortality rate in each experimental unit (Van Wyk et al., 1999).

Shrimp weight was monitored every 10 days to determine shrimp growth and adjust the amount of feed offered. At the end of the experimental time, biomass gain, mean final weight, specific growth rate (SGR), FCR, survival and yield were determined based on the following equations:

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

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

SGR (% day^{-1}) = 100 × [ln final weight (g) − ln initial weight (g)]/time (days);

FCR = feed supplied/biomass gain;

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

Yield (Kg m^{-3}) = final biomass (Kg)/volume of experimental unit (m^3).

Ionic adjustment

The ionic adjustment was performed in treatments (1IA, 2IA and 3IA), according to the result of water quality variables based on the Ca:Mg:K (1:3:1) ratios proposed by Boyd and Thunjai (2003). For the adjustment of these ions, commercial products were used: potassium chloride (KCl), calcium carbonate ($CaCO_3$) and magnesium chloride hexahydrate ($MgCl_2$. $6H_2O$). The addition of ions were made based on previous tests of the efficiency of the chemical compounds in increasing the concentration of ions in the water (applied in the experimental units in two applications: 50% in the morning and 50% in the afternoon). The test was performed in experimental units with 14 L of water with a salinity of 2.5 g L^{-1}, obtained from seawater diluted in freshwater, under constant aeration. The pH and ion concentration were analyzed before application and 72 h after application of the chemicals at a concentration of 100 g m^{-3} (Table 1).

Location of the table 1

The chemical compounds application on the experimental units to adjust the ionic concentration was performed according to the following equation (Samocha, 2019):

$$Amount\ of\ product = \frac{Fc - Ic}{TI} \times V$$

- Fc is the final concentration;
- Ic is the initial concentration;
- TI is the % increment of the target ion (decimal value);
- *V* is the tank volume (m^3).

After analyzing the water in all treatments, ion concentrations in milliequivalent L^{-1} (mEq L^{-1}) were calculated to check cations and anions equilibrium. This calculation was made by the difference between the sum of the cations mEq L^{-1} (Na^+ = 23 mg mEq^{-1}; K^+ = 39.1 mg mEq^{-1}; Ca^{2+} = 20 mg mEq^{-1} and Mg^{2+} = 12.15 mg mEq^{-1}) and the sum of the anions mEq L^{-1} (HCO_3^- = 61 mg mEq^{-1}; Cl^- = 35.45 mg mEq^{-1} and SO_4^{2-}= 48.03 mg mEq^{-1}) for chemical equilibrium certification (Boyd, 2015). As a standard for certifying the accuracy of the analysis of these major ions, a balance error between cations and anions below 15% 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 (Na^+, K^+, Ca^{2+} e Mg^{2+});
- Σ anions: sum of anions (HCO_3^-, Cl^- e SO_4^{2-}).

Water quality

Dissolved oxygen (DO; mg L^{-1}) and temperature (°C; YSI model 100, Yellow Springs, Ohio, USA), were monitored twice a day (at 08:00 AM and 04:00 PM). Salinity (salinity meter AZ, model 8371), pH (pH meter Akso, model A90), and settleable solids (mL L^{-1}; Imhoff cone) (Avnimelech, 2012) were monitored three times a week. Total ammonia nitrogen (TAN; APHA, 2012), nitrite-N (NO_2^--N; Fries, 1971), nitrate-N (NO_3^--N; APHA, 2012), orthophosphate (PO_4^{3-}; APHA, 2012), total alkalinity (TA; APHA, 2012), total hardness (TH; APHA, 2012), Ca^{2+} (APHA, 2012), Mg^{2+} (APHA, 2012), Na^+ (APHA, 2012), Cl^- (APHA, 2012), SO_4^{2-} (APHA, 2012) and K^+ (Fries and Getrost, 1977) were monitored every ten days.

Stress tests

At the end of the experimental time, the shrimp juveniles were submitted to resistance tests for osmotic stress and water ammonia nitrogen concentration (NH_3-N). For the osmotic stress test, 10 shrimps were randomly collected in each experimental unit and were stocked in experimental units with 10 L of seawater (35 g L^{-1}), with aeration, for 30 min (SWD, 1IA, 2IA, 3IA) and with 10 L of freshwater (0 g L^{-1}), with aeration, for 30 min (SW). After this time, the animals were exposed to salinity of 2.5 g L^{-1} for 30 min (SWD, 1IA, 2IA, 3IA) and exposed to salinity of 35 g L^{-1} for 30 min (SW). 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, 10 shrimps were randomly collected in each experimental unit and were transferred to experimental units containing 10 L of water salinity 2.5 g L^{-1} (SWD, 1IA, 2IA, 3IA), and 10 L of water salinity 35 g L^{-1} (SW) with NH_3-N concentrations between 0.26 and 0.41 mg L^{-1} (Table 2), water temperature close to 28.2 °C and pH close to 8.0. The NH_3-N concentration was achieved by applying a stock solution of 10g L^{-1} of NH_4Cl. The test was carried out for 96 h, and survival was measured every 24 h (Zhang et al., 2012).

Location of the table 2

Mineral composition (Floc and Shrimp)

At the end of experimental time the floc samples (30 g) were collected with a cylindrical mesh net of 50 μm for solids retention and shrimp whole body, in triplicate for mineral composition analyses. Prior to the processing of the analysis, the microbial flocs samples were dried in an oven until constant weight was obtained and then kept in a desiccator, while the whole shrimp samples were freeze-dried, then both were subjected

to wet digestion through nitro-perchloric mineralization and submitted to the respective readings. Potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn), from atomic absorption spectrophotometry (Agilent Technologies, US - SpectrAA 55B), as for phosphorus (P) and sulfur (S), they were performed with a UV-VIS spectrophotometer (Thermo Scientific, FI – Genesys 10S UV-Vis), according to the methodology described in Nogueira and Souza (1998). The samples were analyzed in the Plant Nutrition Laboratory- Brazilian Agricultural Research Corporation (EMBRAPA).

Statistical analysis

The data were checked for homogeneity of variance with the Cochran test ($p < 0.05$) and normality using the Shapiro–Wilk test ($p < 0.05$). One-way variance analysis (ANOVA) was conducted to evaluate the zootechnical performance variables and proximal composition, and repeated measures ANOVA were used to compare water quality data, followed by the Duncan test ($p < 0.05$). For non-parametric statistical data, Kruskall–Wallis ($p < 0.05$) followed by Dunn's test (for the SGR data) and Friedman's test with Conover multiple comparison test with Holm–Bonferroni correction (for DO, SS, total alkalinity and NO_2^--N data). Data analysis were performed using Statistica 12.5 software.

2.3. Results

Shrimp zootechnical performance

The zootechnical performance data at the end of the 40 days of experimental culture are summarized in Table 3. SW treatment (final weight = 1.02 g; survival = 84.72 %; yield = 1.7 Kg m^{-3} and SGR = 11.49 % day^{-1}) is lower as compared to low salinity treatments (SWD, 1IA, 2IA and 3IA). However, FCR showed no significant differences ($p > 0.05$) among treatments.

Location of the table 3

Water quality

There were significant differences ($p < 0.05$) in the water quality parameters (dissolved oxygen, SS, salinity, pH, total hardness, total alkalinity, TAN and NO_2^--N) among the five treatments (Table 4). The lowest levels of dissolved oxygen (were observed in the SW treatment (4.75 mg L^{-1}) when compared to the low salinity treatments (5.70 mg L^{-1}). The mean SS concentration was higher in the ionic adjustment treatments compared to SW and SDW, and the range was also greater in the low salinity with ionic adjustment. The pH was lower in 1IA, 2IA and 3IA when compared to SW, but they were similar between the low salinity treatments.

Total hardness and total alkalinity were higher in SW treatments compared to low salinity treatments. The total alkalinity in the ionic adjustment treatments did not show significant differences among treatments, but in the total hardness we observed an increase in the concentration with adjustment frequencies, as well as along the culture time. The orthophosphate values were higher than 30 mg L^{-1}, with higher concentrations in the ionic adjustment treatments.

The TAN and NO_2^--N values in the low salinity treatments were lower than SW, but NO_3^--N did not show significant differences among the treatments. The concentrations of TAN and NO_2^--N were higher until the 20th day of culture, followed by decrease.

Location of the table 4

Ions

The ions Ca^{+2} (402.7 mg L^{-1}), K^+ (217.1 mg L^{-1}), SO_4^- (2,817.5 mg L^{-1}), Cl^- (19,970.2 mg L^{-1}) and Na^+ (11,035.5 mg L^{-1}) showed significant differences ($p < 0.05$) between the SW treatment and the other low salinity treatments (Ca^{+2}: 38 - 51,3 mg L^{-1}; Mg^{+2}: 96- 163,5 mg L^{-1}; K^+: 48,3 – 61,5 mg L^{-1}), however, only the Na:K ratio was significantly different among treatments. The Mg:Ca ratio was similar among the SW treatment and the treatments with ions adjustment (1IA; 2IA and 3IA), however they were significantly different to the SWD. Among treatments in oligohaline water, only Mg^{+2} concentration was significantly different between treatments (Table 5).

Location of the table 5

Microbial floc mineral composition

Minerals in microbial floc at the end of the experimental time are summarized in Table 6. The levels of P (1.90 -4.25 g Kg^{-1}), Fe (621.65 - 1167.46 mg. kg^{-1}) and Mn (11.31 - 16.29 mg Kg^{-1}) did not differ significantly among treatments. The concentrations of K, Mg, Na, S, Cu and Zn were higher in the SW treatments as compared to the then low

salinity treatments, however the Ca concentration was similar among the SWand the treatments with ions adjustment of Ca:Mg:K.

Location of the table 6

Mineral in shrimp biomass

The data for the mineral in the shrimp biomass at the end of the experimental time are summarized in Table 7. The levels of P, K, Ca, Mg, Na, S, Cu, Fe, Mn and Zn were not significantly different in the low salinity groups. However, Mg was higher in the SW treatment when compared to SWD and 1IA. Fe was significantly lower in the SW treatment when compared to low salinity treatments.

Location of the table 7

Stress test

At the end (96 hours) osmotic and ammonia nitrogen concentration (NH_3-N). Stress test survival was 100% in all treatments.

2.4. Discussion

Dissolved oxygen, temperature and pH of the water remained, throughout the experimental period, as recommended for *Litopenaeus vannamei* culture in intensive systems with minimal water exchange (Samocha, 2019). The high total hardness concentration found in the SW treatment was expected (due to its high salinity and seawater composition) the difference found among the treatments came from the different ionic adjustment frequencies, since among the products used there was magnesium

chloride and calcium carbonate, thus causing an increase in Ca^{2+} and Mg^{2+} ions, which constitute the total hardness level (Boyd, 2020).

During the experimental time, alkalinity was maintained above 75 mg $CaCO_3$ L^{-1}, as recommended by Boyd and Tucker (1998). Alkalinity is an important water quality parameter in marine shrimp culture, being related to the water buffering which causes a lower pH oscillation throughout the day (Boyd et al., 2016), as observed in Table 4. In addition, Alkalinity provides nutrients necessary for the development of chemoautotrophic bacteria which is responsible for cycling nitrogen compounds (Ebeling et al., 2006). In the present study, it is possible that the difference found in this variable among the SWD, 1IA, 2IA and 3IA treatments is related to the nitrifying bacteria communities established in the system, since the differences found in alkalinity reflect the differences observed in TAN concentration. In addition, the presence of nitrifying bacteria can be observed in all treatments due to NO_2^--N and NO_3^--N concentrations in the water as a result of the oxidation process carried out by these communities. The nitrogenous compounds concentrations were maintained as recommended for the marine shrimp culture, indicating a system stabilization (Ramirez-Rochin et al., 2017; Boyd, 2020). The efficient stabilization of the nitrogenous compounds was also reported by Pimentel et al. (2022), Santos et al., (2022) and Romano et al., (2018), who, when performing shrimp culture in low salinity water, attributed the efficiency of nitrogen cycling to the use of rice bran submitted to anaerobic (24h) and aerobic (24h) process.

The use of the synbiotic as an organic carbon source to maintain good water quality conditions by controlling the total ammonia nitrogen and settleable solids concentration contributes to maintaining the C:N ratio close to 6.5:1. This ratio is close to that found by Santos et al. (2022) who used the same fertilization strategy of the present study, where they associated the use of the synbiotic to the reduction of settleable solids

volume. The highest values of settleable solids found in this study were related to the treatments where ionic adjustment was performed. In addition, the shells used as an artificial substrate retained the solids, due to the water circulation between them, until dissociation, after solid saturation on the shell substrate.

Microbial flocs have their composition affected by several factors, such as the system in which they are produced and also by physical and chemical variables, such as salinity (Khanjani et al., 2020), since these particles also have ions in their composition (De Schryver et al., 2008). The use of different frequencies of ionic adjustment in the culture system does not influence the mineral composition of the major ions present in the microbial floc. In the present study, when evaluating the treatments in which the ion adjustment (1IA, 2IA and 3IA) were performed, significant differences were observed only in the levels of Mg^{2+}, with the composition of the other elements evaluated being similar. These minerals can be made available to animals through bioflocs, as these microbial flocs act as a supplemental food source for shrimp (Khanjani et al., 2020). Authors such as Cheng et al., (2005; 2006) observed benefits related to the adoption of mineral supplementation through the feed as a strategy to improve the zootechnical indexes in the culture of *L. vannamei* in low salinity water.

The marine shrimp *L. vannamei* culture in low salinity water has been put to the test, since studies show that the reduction in salinity affects production indices such as survival, average final weight, yield, among others (Esparza-Leal et al., 2016; Laramore et al., 2001). In general, this event is justified by the low salinity and, consequently, the low availability of the major ions for these animals, providing a greater energy expenditure to osmotic and ionic regulation, which can be reflected in the zootechnical performance indices throughout a culture (Roy et al., 2010). However, the different frequencies of ionic adjustment in salinity 2.3 g L^{-1} using a system with minimal water

exchange and synbiotic as organic carbon source do not significantly influence the productive indexes in the *L. vannamei* culture in the nursery phase, since zootechnical results as final mean weight, final biomass, yield and specific growth rate were superior to those found in the treatment using seawater (salinity 31 g L^{-1}).

The shrimp performance results of this study were superior to those found by Esparza-Leal et al., (2016) who evaluated the effect of salinity on the growth of *L. vannamei* and obtained a mortality rate of 100% in salinities 2 and 4 g L^{-1}, while Pimentel et al., (2022), who evaluated the effect of different ionic adjustment strategies on the *L. vannamei* growth performance in salinity 2.5 g L^{-1}, obtained average survival rates lower than those obtained in the present study, reporting an average rate between 82 and 87%. The results of final weight, FCR, SGR and yield reached in the present study exceed those found by the same author. Such differences may be associated with the amount of protein offered to the animals through the feed, since the present study used feed with greater availability of protein. In addition, the mean concentrations of the major ions (Ca^{+2}, Mg^{+2} and K^{+}) that were maintained in the culture reported by the author, are lower than the lowest mean concentrations found in the present study, and although it indicates that the minimum concentrations maintained in that study did not limit the growth of *L. vannamei* in low salinity conditions, it is known that these ions are related to the growth and survival of shrimp when they are in low salinity conditions (Roy et al. 2007; Zacarias, et al., 2018).

Regarding the mineral composition of the *L. vannamei* biomass, the different ionic adjustment frequencies did not influence this parameter. The amount of total minerals can be obtained by determining the amount of ash in a proximal analysis, comparing the composition of *L. vannamei* cultivated in marine water with the biomass of the same species cultivated in low salinity. Liang et al., (2008) did not observe the influence of salinity on the concentration of minerals in the muscle of the animals. It is possible that

the minerals absorbed or consumed by animals are used in metabolic and physiological reactions of the animal, since these ions found in water and feed play a relevant role in metabolic reactions, influencing growth and animal survival.

2.5. Conclusion

The ionic adjustment frequency of the Ca:Mg:K ratio to 1:3:1 did not result in zootechnical differences in the *L. vannamei* nursery using diluted seawater in a synbiotic system. In view of the results of the zootechnical index obtained in the present study, the use of marine water without performing ionic adjustment is equivalent to the results obtained with the performance of different frequencies of this adjustment. In this way, it is possible to carry out *L.vannamei* culture using the synbiotic based on rice bran, in a minimal water exchange system only in salinity 2.3 g L^{-1}, using diluted seawater, saving costs resulting from the ionic adjustments through inorganic fertilization.

Concentrations of Ca^{2+} = 38.0 mg L^{-1}, Mg^{2+} = 96.0 mg L^{-1} and K^{+}= 48.3 mg L^{-1} are sufficient for marine shrimp nurseries in low salinity using synbiotic system.

Although the ionic adjustment is performed in the water, the ratios between the ions found in the microbial flocs has other ratios different from those found in the water and needs to be better studied, as these minerals are ingested by shrimp and may supply some need for osmotic bio compensation.

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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 paper.

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Table 1. Percentage % increment of the target ion (after 72 h of the application of 100 g/m^3) by chemical compounds used for ionic adjustment in intensive nursery synbiotic system.

Commercial product		$MgCl_2.6H_2O$	$CaCO_3$	KCl
% increment of the target ion	pH[a]	0.17 ± 0.01	0.32 ± 0.06	0.41 ± 0.05
	Ca^{2+}	-	30.4 ± 0.73	-
	K^+	-	-	51.1 ± 11.38
	Mg^{2+}	10.52 ± 1.37	-	-

Note: The data correspond to the mean of 2 replicates ± standard deviation. KCl: potassium chloride; $CaCO_3$: calcium carbonate; $MgCl_2.6H_2O$: magnesium chloride hexahydrate. [a]pH in absolute value of the hydrogenic potential; Ca^{2+} : calcium ion; K^+ : potassium ion; Mg^{2+}: magnesium ion.

Table 2. Total ammonia nitrogen (TAN) concentration and non-ionized ammonia (NH3-N) produced in the experimental units from NH_4Cl solution application.

	TAN (mg L^{-1})		$N\text{-}NH_3$ (mg L^{-1})	
	SW	SWD, 1IA, 2IA, 3IA	SW	SWD, 1IA, 2IA, 3IA
Initial	2.40	4.65	0.31	0.31
24hrs	2.20	4.62	0.30	0.26
48hrs	2.50	4.60	0.39	0.37
72hrs	3.23	6.18	0.39	0.41

Note: Data correspond to the mean concentration of TAN and NH_3-N. SW (seawater - salinity 31); SWD (seawater diluted to a salinity of 2.3); 1IA (salinity 2.3- ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 only on the 1st day); 2IA (salinity 2.3 - ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st and 20th day) and 3IA- (salinity 2.3- ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st, 10th and 20th day).

Table 3. Zootechnical performance of *Litopenaeus vannamei* after 40 days in a synbiotic nursery system with different ionic adjustment frequencies in oligohaline water.

Variables	Treatments				
	SW	**SWD**	**1IA**	**2IA**	**3IA**
Final weight (g)	1.02^{B} ± 0.06	1.16^{A} ± 0.04	1.18^{A} ± 0.02	1.15^{A} ± 0.05	1.14^{A} ± 0.10
Yield (Kg m^{-3})	1.73^{B} ± 0.08	2.13^{A} ± 0.05	2.10^{A} ± 0.20	2.13^{A} ± 0.15	2.15^{A} ± 0.15
Survival (%)	84.72^{B} ± 0.03	91.67^{AB} ± 1.67	88.61^{AB} ± 7.19	92.22^{AB} ± 2.92	94.44^{A} ± 2.68
FCR	1.02 ± 0.05	0.97 ± 0.02	0.99 ± 0.10	0.97 ± 0.07	0.96 ± 0.06
SGR (% day $^{-1}$)	11.49^{B} ± 0.15	11.81^{A} ± 0.09	11.86^{A} ± 0.05	11.80^{A} ± 0.10	11.76^{A} ± 0.22

Data correspond to the mean (n=3) ± standard deviation. Different letters correspond to One-Way ANOVA, followed by Duncan's test ($p < 0.05$) among treatments. SW (seawater - salinity 31); SWD (seawater diluted to a salinity of 2.3); 1IA (salinity 2.3- ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 only on the 1st day); 2IA (salinity 2.3 - ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st and 20th day) and 3IA- (salinity 2.3- ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st, 10th and 20th day). FCR: feed conversion ratio; SGR: specific growth rate.

Table 4. Water quality variables measured during *Litopenaeus vannamei* synbiotic nursery system with different ionic adjustment frequencies in oligohaline water.

Variables		Treatments											
		SW		SWD		1IA		2IA		3IA			
DO** (mg L^{-1})	Mean ± SD	4.75^{B}	± 0.50	5.72^{A}	± 0.67	5.72^{A}	± 0.64	5.71^{A}	± 0.64	5.71^{A}	± 0.70		
	Range	4.26	- 5.24	5.07	- 6.47	5.10	- 6.74	5.07	- 6.73	5.07	- 6.44		
Temperature* (°C)	Mean ± SD	30.85	± 0.57	30.75	± 0.68	30.69	± 0.53	30.90	± 0.73	30.98	± 0.84		
	Range	30.32	- 31.65	30.36	- 31.07	30.30	- 31.46	30.38	- 31.40	30.24	- 31.49		
SS** (mL L^{-1})	Mean ± SD	0.08^{B}	± 0.15	0.63^{Bb}	± 1.14	3.95^{Aa}	± 4.42	3.96^{Aa}	± 3.97	2.23^{ABab}	± 2.90		
	Range	0.00	- 0.28	0.00	- 2.00	0.70	- 13.67	0.10	- 12.67	0.30	- 11.00		
Salinity* (g L^{-1})	Mean ± SD	31.17^{A}	± 1.05	2.26^{Bb}	± 0.06	2.39^{Bb}	± 0.06	2.59^{Ba}	± 0.17	2.74^{Ba}	± 0.19		
	Range	29.98	- 32.82	2.15	- 2.33	2.32	- 2.48	2.37	- 2.93	2.47	- 2.93		
pH*	Mean ± SD	8.13^{A}	± 0.20	8.04^{ABab}	± 0.22	7.96^{Bab}	± 0.29	7.90^{Bb}	± 0.33	7.98^{Bab}	± 0.28		
	Range	7.98	- 8.35	7.82	- 8.36	7.59	- 8.31	7.37	- 8.38	7.48	- 8.28		
TA** (mg $CaCO_3$ L^{-1})	Mean ± SD	171.33^{A}	± 30.96	92.33^{Ba}	± 16.35	87.33^{BCab}	± 19.35	83.33^{Cab}	± 17.49	87.33^{BCb}	± 15.91		

TH* (mg L^{-1})	Range	125.00	225.00	65.00	- 120.00	55.00	- 115.00	55.00	- 110.00	55.00	- 115.00		
	Mean ± SD	6,160.00^{A}	± 104.31	490.13^{Ed}	± 33.19	586.67^{Dc}	± 61.59	710.13^{Cb}	± 216.32	801.07^{Ba}	± 238.88		
	Range	5,960.00	- 6,240.00	452.00	- 588.00	452.00	- 648.00	452.00	- 988.00	452.00	- 1,088.00		
PO_4^{3-}* (mg L^{-1})	Mean ± SD	32.65^{C}	± 8.21	33.81^{BCb}	± 7.71	38.00^{Aa}	± 10.82	36.05^{ABab}	± 9.47	37.69^{Aa}	± 10.25		
	Range	23.83	- 45.36	23.00	- 47.08	23.00	- 52.05	23.00	- 49.40	23.00	- 50.82		
TAN* (mg L^{-1})	Mean ± SD	0.44^{B}	± 0.51	0.61^{A}	± 0.56	0.55^{AB}	± 0.50	0.55^{AB}	± 0.46	0.66^{A}	± 0.53		
	Range	0.00	- 1.45	0.04	- 1.81	0.00	- 1.54	0.07	- 1.61	0.24	- 1.66		
NO_2^--N (mg L^{-1})**	Mean ± SD	11.60^{A}	± 12.26	0.52^{B}	± 0.45	0.46^{B}	± 0.15	0.49^{B}	± 0.28	0.50^{B}	± 0.30		
	Range	1.20	- 33.00	0.22	- 1.60	0.32	- 0.76	0.20	- 1.30	0.20	- 1.40		
NO_3^--N* (mg L^{-1})	Mean ± SD	1.37	± 1.04	1.73	± 1.26	1.58	± 0.84	1.91	± 1.36	1.61	± 0.90		
	Range	0.20	- 3.32	0.13	- 3.80	0.13	- 3.36	0.13	- 4.70	0.13	- 2.98		

Data correspond to the mean (n=3) ± standard deviation. Different capital letters represent significant differences ($p < 0.05$) among the treatments (considering all treatments) and lowercase letters represent significant differences ($p < 0.05$) among low salinity treatments (excluding SW treatment). * Repeated measures ANOVA results followed by Duncan's test. ** Friedman test results followed by Dunn's test. SW (seawater - salinity 31); SWD (seawater diluted to a salinity of 2.3); 1IA (salinity 2.3- ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 only on the 1st day);

2IA (salinity 2.3 - ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st and 20th day) and 3IA- (salinity 2.3- ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st, 10th and 20th day). SD: standard deviation; DO: dissolved oxygen; SS: settleable solids; pH: hydrogen potential; TA: total alkalinity; TH: total hardness; PO_4^{3-}: orthophosphate; TAN: total ammonia nitrogen; NO_2^--N: nitrite nitrogen; NO_3^--N: nitrate nitrogen.

Table 5. Ion concentration (mg L^{-1}) in *Litopenaeus vannamei* culture water using different ionic adjustment frequencies in a synbiotic nursery system.

Variables	Treatments				
	SW	**SWD**	**1IA**	**2IA**	**3IA**
Ca^{2+}	402.7^{A} ± 39.7	38.0^{B} ± 6.7	37.5^{B} ± 3.9	46.9^{B} ± 12.2	51.3^{B} ± 11.2
Mg^{2+}	$1,252.3^{A}$ ± 21.7	96.0^{E} ± 6.2	119.8^{D} ± 15.7	144.1^{C} ± 46.4	163.5^{B} ± 52.0
K^{+}	217.1^{A} ± 61.3	48.3^{B} ± 10.5	52.8^{B} ± 11.5	57.7^{B} ± 14.6	61.5^{B} ± 17.0
SO_4^{2-}	$2,817.5^{A}$ ± 339.0	231.9^{B} ± 23.6	242.9^{B} ± 30.9	258.9^{B} ± 52.7	294.4^{B} ± 65.2
Cl^{-}	$19,970.2^{A}$ ± 2,137.8	$1,201.8^{B}$ ± 66.0	$1,260.8^{B}$ ± 64.6	$1,323.5^{B}$ ± 111.2	$1,425.1^{B}$ ± 139.5
Na^{+}	$11,035.5^{A}$ ± 1,181.4	664.1^{B} ± 36.5	696.7^{B} ± 35.7	731.3^{B} ± 61.5	787.5^{B} ± 77.1
Mg:Ca	$3.1:1^{A}$	$2.5:1^{B}$	$3.2:1^{A}$	$3.1:1^{A}$	$3.2:1^{A}$
Na:K	$51.7:1^{A}$	$13.7:1^{B}$	$13.2:1^{B}$	$12.7:1^{B}$	$12.8:1^{B}$
Ca:Mg:K	1:3.1:0.5	1:2.5:1.3	1:3.2:1.4	1:3.1:1.2	1:3.2:1.2

Data correspond to the mean (n=15) ± standard deviation. Different letters represent repeated measures ANOVA, followed by Duncan's test ($p < 0.05$). SW (seawater - salinity 31); SWD (seawater diluted to a salinity of 2.3); 1IA (salinity 2.3- ionic adjustment to achieve Ca:Mg:K ratio of

1:3:1 only on the 1st day); 2IA (salinity 2.3 - ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st and 20th day) and 3IA- (salinity 2.3- ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st, 10th and 20th day). Ca^{2+} : calcium ion; Mg^{2+}: magnesium ion; K^{+} : potassium ion; SO_4^{2-}: sulfate ion; Cl^{-}: chloride ion; Na^{+}: sodium ion; Mg:Ca: ratio of magnesium and calcium ions; Na:K: ratio of sodium and potassium ions; Ca:Mg:K: ratio of calcium, magnesium and potassium ions.

Table 6. Microbial floc mineral concentration (g Kg^{-1}; dry weight) in the *Litopenaeus vannamei* synbiotic nursery using different ionic adjustment frequencies in oligohaline water.

Minerals	**Treatments**				
(microbial floc)	**SW**	**SWD**	**1IA**	**2IA**	**3IA**
P (g Kg^{-1})	4.25 ± 1.07	2.34 ± 0.47	1.90 ± 0.10	2.05 ± 0.20	1.87 ± 0.29
K (g Kg^{-1})	0.63^{A} ± 0.10	0.15^{B} ± 0.01	0.19^{B} ± 0.02	0.22^{B} ± 0.01	0.24^{B} ± 0.03
Ca (g Kg^{-1})	20.15^{A} ± 3.84	8.88^{B} ± 1.79	10.02^{AB} ± 1.21	11.78^{AB} ± 0.59	9.40^{AB} ± 1.32
Mg (g Kg^{-1})	1.52^{A} ± 0.10	0.38^{D} ± 0.06	0.44^{CD} ± 0.03	0.52^{BC} ± 0.03	0.58^{B} ± 0.03
Na (g Kg^{-1})	5.73^{A} ± 1.13	0.63^{B} ± 0.06	0.61^{B} ± 0.08	0.69^{B} ± 0.04	0.68^{B} ± 0.01
S (g Kg^{-1})	0.94^{A} ± 0.08	0.32^{B} ± 0.07	0.31^{B} ± 0.03	0.36^{B} ± 0.07	0.40^{B} ± 0.02
Cu (mg Kg^{-1})	7.05^{A} ± 0.35	3.87^{B} ± 1.08	3.63^{B} ± 0.71	3.99^{B} ± 0.35	4.27^{B} ± 0.18
Fe (mg Kg^{-1})	1,167.46 ± 50.66	827.19 ± 253.28	621.65 ± 54.86	703.45 ± 79.40	672.55 ± 103.28
Mn (mg Kg^{-1})	11.31 ± 1.52	11.85 ± 2.42	13.94 ± 4.77	16.29 ± 4.68	15.02 ± 2.30
Zn (mg Kg^{-1})	73.70^{A} ± 10.66	41.02^{B} ± 7.70	38.50^{B} ± 3.34	37.06^{B} ± 12.38	39.67^{B} ± 5.67

Data correspond to the mean (n = 3) ± standard deviation. Different letters represent One-Way ANOVA, followed by Duncan's test ($p < 0.05$). SW (seawater - salinity 31); SWD (seawater diluted to a salinity of 2.3); 1IA (salinity 2.3- ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 only on the 1st day); 2IA (salinity 2.3 - ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st and 20th day) and 3IA- (salinity 2.3- ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st, 10th and 20th day). P: phosphor; K: potassium; Ca: calcium; Mg: magnesium; Na: sodium; S: sulfur; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc.

Table 7. Marine shrimp *Litopenaeus vannamei* biomass mineral concentration (g Kg^{-1}; dry weight) using different ionic adjustment frequencies in a synbiotic nursery system.

Minerals (Shrimp)	Treatments				
	SW	**SWD**	**1IA**	**2IA**	**3IA**
P (g Kg^{-1})	12.54 ± 0.06	13.31 ± 0.58	12.31 ± 0.56	12.67 ± 0.32	12.60 ± 1.27
K (g Kg^{-1})	12.03 ± 0.11	13.07 ± 0.73	13.10 ± 1.00	12.35 ± 0.76	11.44 ± 1.21
Ca (g Kg^{-1})	45.88 ± 4.19	42.92 ± 4.12	55.61 ± 6.03	48.83 ± 0.85	51.80 ± 12.31
Mg (g Kg^{-1})	3.90^{A} ± 0.04	2.87^{B} ± 0.17	2.87^{B} ± 0.03	3.13^{AB} ± 0.02	3.26^{AB} ± 0.12
Na (g Kg^{-1})	11.91^{A} ± 0.47	10.40^{AB} ± 0.70	9.79^{AB} ± 0.49	9.38^{B} ± 0.34	9.67^{AB} ± 0.01
S (g Kg^{-1})	6.27^{AB} ± 0.84	6.31^{AB} ± 0.72	5.11^{B} ± 0.54	6.95^{AB} ± 0.03	7.69^{A} ± 0.76
Cu (mg Kg^{-1})	9.83 ± 1.39	11.98 ± 0.60	15.10 ± 0.83	15.75 ± 2.81	15.28 ± 1.14
Fe (mg Kg^{-1})	102.52^{B} ± 3.33	226.85^{A} ± 14.68	226.50^{A} ± 37.44	235.38^{A} ± 40.20	182.80^{A} ± 53.03
Mn (mg Kg^{-1})	3.95^{B} ± 0.33	7.20^{A} ± 0.44	7.05^{A} ± 1.52	6.88^{A} ± 1.52	7.85^{A} ± 0.23
Zn (mg Kg^{-1})	61.22^{B} ± 1.38	64.50^{AB} ± 1.18	64.30^{AB} ± 0.85	66.77^{A} ± 3.03	62.90^{AB} ± 3.40

Data correspond to the mean (n=3) ± standard deviation. Different letters represent One-Way ANOVA, followed by Duncan's test ($p < 0.05$). SW (seawater - salinity 31); SWD (seawater diluted to a salinity of 2.3); 1IA (salinity 2.3- ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 only on the 1st day); 2IA (salinity 2.3 - ionic adjustment to achieve Ca:Mg:K ratio of 1:3:1 on the 1st and 20th day) and 3IA- (salinity 2.3- ionic adjustment

to achieve Ca:Mg:K ratio of 1:3:1 on the 1st, 10th and 20th day). P: phosphor; K: potassium; Ca: calcium; Mg: magnesium; Na: sodium; S: sulfur; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc.

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