



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

**EFEITO DA SALINIZAÇÃO ARTIFICIAL NA PRODUÇÃO DE JUVENIS DE
CAMARÕES *Litopenaeus vannamei* (BOONE, 1931) EM SISTEMA DE MÍNIMA
TROCA DE ÁGUA.**

Agatha Catharina Limeira

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 *Litopenaeus vannamei*, popularmente conhecido como camarão branco do pacífico lidera o ranking da espécie de crustáceos mais produzida no mundo, ao qual tem sido cultivado em sua maioria em áreas litorâneas. Entretanto, devido aos altos custos de implantação, problemas de conflitos ambientais, surtos de enfermidades, observa-se uma migração da produção de camarões marinhos para regiões interiores com águas mesohalinas e oligohalinas. As águas destas regiões são provenientes de lagos, poços, rios, açudes e aquíferos subterrâneos, apresentando grande variação em sua composição iônica (cátions e ânions). Entretanto, estes íons precisam que suas proporções sejam ajustadas para valores semelhantes aos da água do mar, para que os camarões tenham um bom desenvolvimento zootécnico durante o cultivo, promovendo um maior conforto osmótico aos animais, equilibrando os gastos energéticos. A compensação iônica realizada por meio de fertilizantes minerais na água pode ser bastante onerosa dependendo do volume aplicado, como uma alternativa para redução desses custos é a utilização de sistemas de mínima troca de água, oferecendo um reaproveitamento dos minerais utilizados, assim como uma redução no descarte de água e um menor reinvestimento na aplicação dos fertilizantes minerais. Portanto, esse estudo teve por objetivo avaliar diferentes formas de salinização artificial da água para a produção de juvenis do camarão marinho *L. vannamei* em sistema simbiótico durante 40 dias, em unidades experimentais de 60 L, com densidade de 2000 PL's m³. Quatro tratamentos em triplicata por meio de um delineamento inteiramente casualizado foram estabelecidos: SD - água do mar diluída; LCSM - Mistura de sais de baixo custo com água doce; CS - Sal marinho comercial e SW - água do mar. Foi realizado um estudo prévio sobre a eficiência minerais quanto os íons disponibilizados na água de cultivo e posteriormente realizada a salinização artificial para obtenção da salinidade de 2,5 g/L e as proporções dos cátions e ânions. Utilizou-se um substrato artificial de conchas de *Anomalocardia brasiliiana* (1% do volume total da unidade experimental) em cada unidade experimental para acelerar os processos de nitrificação. além da adição de mix comercial de bactérias à base de *Bacillus* na água e na ração. Para verificar a resistência dos juvenis de camarões foi realizado um teste de estresse de amônia ao término do cultivo e, também, foram realizadas no início e fim do experimento, análises microbiológicas para contagem total de *Vibrio spp.* e *Fungos* do intestino dos juvenis. A utilização do substrato artificial juntamente com o sistema simbiótico mostrou-se efetivo no controle dos compostos nitrogenados com média de 0,21 mg NAT/L e 0,32 mg N-NO₂/L. Os

valores de cálcio, magnésio e dureza total se mantiveram acima de 30, 75 e 490 mg/L, respectivamente em todos os tratamentos e a alcalinidade total manteve seus valores superiores a 120 mg CaCO₃/L. Com relação aos parâmetros de sobrevivência nos tratamentos com baixa salinidade, observou-se diferenças significativas entre os tratamentos, com uma maior sobrevivência no tratamento LCSM. O peso médio final observados na baixa salinidade foram superiores ao valor encontrado na água do mar, entretanto as demais variáveis de desempenho zootécnico foram similares. O uso de probiótico a base de *Bacillus* na água e ração ofertada aos camarões neste trabalho, mostrou-se eficiente na colonização da microbiota intestinal dos camarões cultivados em todos os tratamentos. Com isso, as diferentes formas de salinização artificial obtiveram semelhantes resultados de desenvolvimento zootécnico e resistência ao estresse de amônia quando comparado à água do mar.

Palavras-chave: baixa salinidade; juvenis; berçários intensivos; simbiótico, proporção iônica.

Abstract

Litopenaeus vannamei, popularly known as Pacific white shrimp, leads the ranking of the most produced crustacean species in the world, which has been cultured mostly in coastal areas. However, due to the high costs of implantation, environmental conflict problems, disease outbreaks there is a migration of production of marine shrimp to inland waters with mesohaline and oligohaline waters. The waters of these regions come from lakes, wells, rivers, dams and underground aquifers, presenting great variation in their ionic composition (cations and anions). However, these ions need their proportions to be adjusted to values similar to those of seawater, so that the shrimp have a good zootechnical development during culture, promoting greater osmotic comfort to the animals, balancing energy expenditure. The ionic compensation performed by means of mineral fertilizers in the water can be quite expensive depending on the volume applied, as an alternative to reduce these costs is the use of systems with minimal water exchange, offering a reuse of the minerals used, as well as a reduction in the disposal of water and less reinvestment in the application of mineral fertilizers. Therefore, this study aimed to evaluate different forms of artificial salinization of water for the production of juveniles of marine shrimp *L. vannamei* in a synbiotic system for 40 days, in experimental units of 60 L, with a density of 2000 PL's m³. Four treatments in triplicate using a completely randomized design were established: SD - diluted seawater; LCSM - Low cost salt mix with freshwater; CS - Commercial sea salt and SW - sea water. A previous study was carried out on the mineral efficiency regarding the ions available in the culture water and later artificially salinized was carried out to obtain a salinity of 2.5 g/L and the proportions of cations and anions. An artificial substrate of *Anomalocardia brasiliensis* shells (1% of the total volume of the experimental unit) was used in each experimental unit to accelerate the nitrification processes. In addition to the addition of a commercial mix of *Bacillus*-based bacteria in water and feed. To verify the resistance of shrimp juveniles, an ammonia stress test was carried out at the end of the culture and, also, microbiological analysis were carried out at the beginning and end of the experiment for the total count of *Vibrio spp.* and *fungi* Juvenile intestinal. The use of the artificial substrate together with the synbiotic system proved to be effective in the control of nitrogen compounds with an average of 0.21 mg NAT/L and 0.32 mg N-NO₂/L. The values of calcium, magnesium and total hardness remained above 30, 75 and 400 mg/L, respectively, in all treatments and the total alkalinity maintained its values above 120 mg CaCO₃/L. Regarding the survival parameters in the treatments with low salinity,

significant differences were observed between the treatments, with a greater survival in the LCSM treatment. The final average weight observed in the low salinity treatments were higher than the value found in the seawater treatment, however the other variables of zootechnical performance were similar. The use of *Bacillus*-based probiotic in the water and feed offered to the shrimp in this work, proved to be efficient in the colonization of the intestinal microbiota of shrimp cultured in all treatments. Thus, the different forms of artificially salinized obtained similar results in terms of zootechnical development and resistance to ammonia stress when compared to seawater.

Key words: low salinity; intensive nursery; synbiotic, ionic ratio.

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1. Introdução

A produção mundial de crustáceos da aquicultura em 2020 foi de aproximadamente 11.237 milhões de toneladas, sendo 4.477 milhões de toneladas provenientes de cultivos em águas interiores (FAO, 2022). No grupo dos crustáceos, o camarão-cinza *Litopenaeus vannamei* (Boone, 1931) lidera o ranking, com 51,7% do total produzido pela aquicultura (5.812 mil de toneladas), sendo cultivada em aproximadamente 80% das fazendas comerciais do mundo (FAO, 2022).

No Brasil, segundo dados da Associação Brasileira de Criadores de Camarão, a produção brasileira de camarões marinhos no ano de 2019 foi de 90.000 toneladas, com projeções para atingir patamares próximos de 120.000 toneladas nos anos seguintes (ABCC, 2020). Essa produção é basicamente realizada na região Nordeste, que é responsável por 99,4% dessa produção (IBGE, 2018), pois possui condições climáticas bastante favoráveis para a prática da carcinicultura.

Toda a produção de camarões marinhos no Brasil é baseada na monocultura do *L. vannamei*, sendo sua grande parcela em áreas litorâneas, porém nos últimos anos observa-se um avanço da produção desta espécie em águas interiores. O camarão *L. vannamei* suporta uma ampla faixa de salinidade (0,5 – 60 ppt), o que contribui para sua adaptação às diversas condições de qualidade de água (Ramiro, 2017).

Entretanto, as variações de salinidade podem afetar o mecanismo de osmorregulação, assim como, alterar a estratégia de absorção de nutrientes e comprometer o crescimento dos camarões (Romano e Zeng, 2012). As consequências do cultivo de crustáceos marinhos em baixas salinidades com perfil iônico inadequado podem incluir baixa sobrevivência, crescimento reduzido e/ou altas taxas de conversão alimentar (Ye et al., 2009), que podem ser minimizadas com ajuste na suplementação mineral (Ca, Mg, Na e K), mesmo com o cultivo dessa espécie em águas com salinidades inferiores a 0,5 ppt (Moraes et al., 2020).

Para a salinização artificial da água é necessário ajustar as proporções iônicas para que fiquem semelhantes à água do mar, proporcionando um maior conforto osmótico aos animais, evitando gastos energéticos (Palheta, 2013). As principais relações de cátions (íons positivos) e ânions (íon negativos) no caso do *L. vannamei* são Na:K, Mg:Ca e Ca:K. A aplicação desses íons na água é com o uso de fertilizantes agrícolas ou por sais marinhos artificiais, podem auxiliar no aumento do crescimento e sobrevivência do camarão em água de baixa salinidade (Boyd, 2002; Oliveira, 2016; Nehru et al., 2018).

Atualmente a produção de camarões marinhos ocorre majoritariamente em regiões litorâneas, entretanto nos últimos anos, incrementa o uso de áreas interiores para a produção de camarões marinhos, com uso de água subterrânea salobra ou que seja feita salinização artificial através de diluição e/ou adição de sal marinho (Tlusty, 2002). Na Tailândia, já se utiliza a mistura de água doce para diluir a salmoura (água hipersalina) para o cultivo dos animais, enquanto nos EUA, utilizam águas salinas subterrâneas e correções iônicas, para que haja suplementação de sais, podendo ocorrer essa adição de sais marinhos na água e/ou na dieta dos animais (Boyd and Thunjai, 2003; Sowers et al., 2006; Roy et al., 2010).Entretanto, dependendo do volume aplicado, as correções iônicas na água são onerosas, podendo inviabilizar a produção, mas podem ser minimizadas em sistemas de mínima troca de água.

Os sistemas de mínima troca de água, através da relação carbono e nitrogênio (C:N) estimula as comunidades de bactérias heterotróficas e nitrificantes que assimilam e oxidam os compostos nitrogenados tóxicos presentes na água (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 a 20:1 (Avnimelech, 1999; Avnimelech, 2007), com a finalidade de estimular o crescimento dessas comunidades microbianas. O perfil microbiano que compõe os agregados é diverso, sendo composto por leveduras, bactérias, protozoários e microalgas (Monroy-Dosta et al., 2013).

Dentro dos sistemas de mínima troca de água, podemos destacar o sistema simbiótico, que consistem na distribuição das bactérias heterotróficas e nitrificantes de forma homogênea, afim de possibilitar a formação de flocos bacterianos e a remoção do nitrogênio residual do sistema, também estimula o crescimento do fito e zooplâncton proporcionando um aumento da disponibilidade de alimento natural, além de reduzir a carga orgânica. Esse sistema também é caracterizado por possuir baixas concentrações de sólidos suspensos e propiciar uma maior estabilidade dos nitrogenados quando comparado a outros sistemas intensivos (Brito, et al., 2019; De Andrade et al., 2021; Pimentel et al., 2022).

A combinação adequada de íons na água e sistemas de mínima troca de água para a produção de juvenis de camarões marinhos ampliar a possibilidade de cultivos de camarões marinhos longe da costa, incrementar a imunidade e resistência dos animais para a segunda etapa da produção de camarões marinhos, aumento na taxa de sobrevivência durante a engorda, aumento do número de ciclos durante o ano, além de proporcionar melhor aclimatação às condições dos viveiros de engorda (Mishra et al., 2008; Nunes, 2020).

Porém, a utilização de berçários de sistema intensivos em águas oligohalinas (salinidades entre 0,5 até 3 g/L) como mínima troca de água, ainda não é bem estabelecido,

devido à alta toxicidade de compostos nitrogenados, que afetam diretamente a produção, uma vez que, em sistemas intensivos a alta densidade de estocagem de animais resulta em uma maior quantidade do alimento por volume de água, que por conseguinte, aumenta a deposição de compostos tóxicos para o camarão (Muhlert et al., 2013) decorrentes da degradação da matéria orgânica proveniente de alimentos não consumidos e da excreção (Alves Neto et al., 2019).

Nesse sentido, o presente estudo tem por finalidade avaliar o efeito de diferentes formas de salinização artificial na produção de juvenis de *L. vannamei* cultivadas em sistema simbiótico.

2. Objetivos do Trabalho

2.1. Geral

Avaliar a influência de diferentes meios de salinização artificial na produção de juvenis de *Litopenaeus vannamei* em sistema simbiótico.

2.1.1 Específicos

- Avaliar a sobrevivência e crescimento de juvenis do *Litopenaeus vannamei* cultivados em água salinizada artificialmente;
- Determinar a contagem presuntiva total de *Vibrio* spp. e fungos no intestino dos juvenis de *Litopenaeus vannamei* em água salinizada artificialmente;
- Avaliar as variáveis físico-químicas de qualidade de água ao longo do cultivo em água salinizada artificialmente;
- Avaliar a resistência aos estresses físico-químicos dos juvenis de *Litopenaeus vannamei* cultivados em água salinizada artificialmente.

1.2. Hipótese

O *L. vannamei* cultivado na fase de berçário em águas oligohalinas salinizadas artificialmente em sistemas simbióticos, apresenta desempenho zootécnico similar aos cultivados em águas marinhas.

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1 **Effects of different forms of artificially salinized in *Penaeus vannamei* low-salinity water**
2 **in the nursery synbiotic system**

3
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13
14 **Abstract**

15 This study aimed to evaluate the effect of different types of artificially salinized on the
16 zootechnical performance, TCBS and Sabouraud Dextrose counts in *Penaeus vannamei*
17 juveniles reared in a synbiotic system, in experimental tanks of 60 L, with a density of 2,000
18 PL m⁻³ for 40 days. Four treatments were established in triplicate using a completely
19 randomized design: SD, diluted seawater; LCSM, low-cost salt mix with freshwater; CS,
20 commercial salt; and SW, seawater. Previously, a study was carried out on mineral efficiency
21 regarding the ions available in the culture water, and later salinization was performed to
22 obtain a salinity close to 3.0 g L⁻¹, and a proportion of cations and anions similar to the values
23 found in seawater. An artificial substrate of *Anomalocardia brasiliiana* shells (1% of the total
24 volume of the experimental unit) was used in each experimental unit. A mix of *Bacillus*-based

25 bacteria was used in the water and feed offered to the animals. An ammonia stress test was
26 performed at the end of the culture, and a microbiological analysis was performed at the
27 beginning and end of the experiment to obtain the total count of *Vibrio* spp. and fungi. The
28 use of the artificial substrate together with the synbiotic system proved to be effective in the
29 control of nitrogen compounds. The values of calcium, magnesium and total hardness
30 remained above 30, 75 and 400 mg L⁻¹, respectively. The main ions remained stable
31 throughout the culture; the total alkalinity values were maintained above 120 mg CaCO₃ L⁻¹,
32 within the recommended range for intensive systems. Better values of final average weight
33 were obtained with the different forms of artificial salinization (LCSM, CS and SW), but
34 survival was lower in low salinity when compared to seawater. There was no influence of
35 different types of salinization on bacterial counts of TCBS and fungi, and on resistance to
36 ammonia stress. Based on the results obtained, it can be concluded that it is possible to rear *P.*
37 *vannamei* juveniles in artificially salinized water of low salinity (3 g L⁻¹) in a synbiotic
38 system.

39

40 **Key words:** Low salinity, Ionic composition, Fungi, *Vibrio*, Ammonium Resistance.

41

42 **Introduction**

43 The world fish market has been fueled by aquaculture since the 1970s, working
44 towards a sustainable economic alternative for human consumption of proteins that aim for
45 quality, high palatability, and affordable value for the final consumer (Valenti, 2021). World
46 aquaculture production in 2020 exceeded 122 million tonnes, of which 54.4 million tonnes
47 came from inland waters and 68.1 million tonnes from coastal regions (FAO, 2022). The third
48 most produced group is crustaceans, with 11.2 million tons in 2020, with 39% of this

49 production being inland (FAO, 2022). Among these, the most reared species is the shrimp
50 *Penaeus vannamei*, representing 51.7% of crustacean production (FAO, 2022).

51 The production of *P. vannamei* is carried out mainly in coastal areas, however, in
52 recent years it has expanded to locations far from the coast and in low salinity conditions
53 (Roy et al., 2010a; Fierro et al., 2018). This can be related to the various economic and
54 environmental advantages presented in these regions when compared to coastal zones, such as
55 lower cost of land acquisition, less conflicts arising from the use of common resources, and
56 reduced impact on not very resilient ecosystems such as mangroves (Nunes and Lopez, 2001;
57 Jory, 2017; Lacerda et al., 2021). This is possible because the shrimp *P. vannamei* supports a
58 wide range of salinity (0.5–60 g L⁻¹), although large variations and an inadequate ionic profile
59 can affect the osmoregulation mechanism and change the nutrient absorption strategy of the
60 species.

61 In addition, the culture of marine crustaceans in low salinities with an inappropriate
62 ionic profile can lead to lower animal performance, such as poor survival, reduced growth
63 and/or high feed conversion ratio (Diaz et al., 2001; Li et al., 2007; Ye et al., 2009), which
64 can be minimized by adjusting the mineral supplementation (Ca, Mg, Na and K) so that the
65 water in low salinity becomes similar to the proportions of ions (cations and anions) found in
66 seawater, even with the culture of this species in waters with salinities below 0.5 g L⁻¹
67 (Moraes et al., 2020).

68 When culture in low salinity uses the technique of artificially salinized and/or ionic
69 adjustment of the water, with similarity in the proportions of ions in relation to seawater, it
70 favors greater osmotic comfort for the animals, avoiding energy expenditure and helping to
71 increase shrimp growth and survival (Boyd, 2002; Oliveira, 2016; Nehru et al., 2018). For *P.*
72 *vannamei*, the main ratios of cations are Na:K, Mg:Ca and Ca:K, making it necessary to apply
73 these ions in water, through the use of agricultural fertilizers or artificial sea salts.

74 In order to increase the growth of aquaculture activity in inland regions and minimize
75 the high cost of transporting and/or producing saline water from saline/brackish effluents,
76 techniques such as adding artificial sea salt or diluting the hypersaline water with freshwater,
77 has been widely used, respectively in the USA and Thailand (Boyd and Thunjai, 2003;
78 Sowers et al., 2006, Roy et al., 2010a). However, the use of inappropriate technologies and/or
79 techniques can cause a series of environmental impacts on natural ecosystems (Figueiredo,
80 2005; Araneda et al., 2008). To minimize these impacts and increase the efficiency of water
81 reuse, generating less application of new fertilizers and/or sea salt in the water, systems with
82 minimum water exchange can be used.

83 Among the systems of minimum water exchange, the synbiotic stands out, since it
84 stimulates the growth of phyto and zooplankton and provides an increase in the availability of
85 natural food, reducing the organic load and enhancing the stability of nitrogen when
86 compared to other intensive systems, in addition to having low concentrations of suspended
87 solids (Brito et al., 2019; De Andrade et al., 2021; Pimentel et al., 2022; Oliveira et al., 2022
88 a,b; Santos et al., 2022a,b).

89 The synbiotic used in semi-intensive and intensive shrimp systems with minimal water
90 exchange consists of the use of bran after the fermentation process and/or microbial
91 respiration with probiotic microorganisms (Romano et al., 2018). This process allows a more
92 efficient use of polysaccharides (such as wheat or rice bran), from aerobic and anaerobic
93 processes, providing the diversified growth of microbial aggregates composed of
94 phytoplankton, zooplankton, and autotrophic and heterotrophic bacteria (Romano et al., 2018;
95 De Andrade et al., 2021; Pimentel et al., 2022).

96 The combination of the use of minimal water exchange systems with ion
97 supplementation (artificially salinized) for the production of juvenile shrimp in low salinity
98 increases the possibility of shrimp culture far from the coast, since the population of the grow-

99 out phase with juveniles instead of post-larvae increases the survival rate during grow-out
100 phase and the number of production cycles during the year, in addition to providing better
101 acclimatization and resistance to shrimp to the water quality conditions of the ponds (Mishra
102 et al., 2008; Nunes, 2020).

103 In this sense, the objective of this work is to evaluate the effect of different forms of
104 artificially salinized on zootechnical performance and water quality, and the resistance to
105 ammonia stress in the production of *P. vannamei* juveniles in a synbiotic system.

106

107 **Material and methods**

108 *Experimental design and system*

109 The study was carried out for 40 days at the Laboratório de Carcinicultura (LACAR
110 [Shrimp Culture Lab]), of the Departamento de Pesca e Aquicultura (DEPAq [Fisheries and
111 Aquaculture Department]) of Universidade Federal Rural de Pernambuco (UFRPE [Rural
112 Federal University of Pernambuco]), in Brazil. Four treatments in triplicate using a
113 completely randomized design were established: SD, diluted seawater; LCSM, low-cost salt
114 mixture; CS, commercial salt mixture; and SW, seawater.

115 A matrix tank (0.8 m³ by treatment) with seawater and freshwater was chlorinated with
116 20 mg L⁻¹ of active chlorine and dechlorinated through constant aeration. Twenty days before
117 stocking the shrimp, the synbiotic started being added at two-day intervals, for a total of ten
118 fertilizations. This synbiotic was obtained through an anaerobic (24 h) and aerobic (24 h)
119 process, from a mixture of 20 g m⁻³ of rice bran (< 200 µm), 2 g m⁻³ of sugar, 0.5 g m⁻³ of
120 commercial bacterial mix (*Bacillus subtilis* [2.2 × 10⁹ CFU g⁻¹], *B. licheniformis* [1.8 × 10⁹
121 CFU g⁻¹], *Bacillus* sp. (1.6 × 10⁹ CFU g⁻¹), sodium chloride (NaCl), and magnesium
122 hydroxide [Mg(OH)₂], from Kayros Agrícola and Ambiental, SP, Brazil), 4 g m⁻³ of sodium
123 bicarbonate, and previously chlorinated water (20 mg L⁻¹ of chlorine per 24 hours, followed

124 by dechlorination by aeration) in the proportion of ten times the amount of rice bran. An
125 initial inoculum of 5% of the tank volume with water from a shrimp nursery in a synbiotic
126 system was used.

127 Throughout the culture, the procedure for the preparation of the synbiotic was the
128 same, for four times a week up until reaching 50% of the concentrations of the inputs, apart
129 from the probiotic, which continued at 0.5 g m⁻³. The use of the synbiotic was until settleable
130 solids reached 5 ml L⁻¹, and applications were suspended past this limit.

131 The experimental units (60 L) were constantly aerated (dissolved oxygen > 5.0 mg
132 L⁻¹), with temperature maintained at ~30°C (Hopar Sh-608 heater 100 W), a 12:12 h
133 photoperiod, and mean luminance of 8.65 μmol photons m⁻² (Equitherm Lux-204). No water
134 exchange was performed during the experimental time. Dechlorinated freshwater was added
135 four times a week to compensate for evaporation loss. To assist the growth of the nitrifying
136 bacterial community, artificial substrate (14 × 14 × 3.2 cm) corresponding to 1% of the
137 volume of the experimental units (60 L), made with *Anomalocardia brasiliiana* shells, was
138 added. Alkalinity correction with sodium bicarbonate (NaHCO₃) was also performed every 10
139 days after water analysis to reach values > 150 mg L⁻¹.

140

141 *Salinization adjustment*

142 Prior to the salinization of the treatments, previous studies were carried out on the
143 efficiency of increasing ions in the water by applying chemical products. The tests were
144 performed in experimental units of 14 L, with salinity of ~2.5 g L⁻¹, obtained by diluting
145 seawater in freshwater under constant aeration. The increase in pH and ion concentration was
146 analyzed after 72 hours of application of 100 g m⁻³ of potassium chloride (KCl), calcium
147 carbonate (CaCO₃), magnesium sulfate heptahydrate (MgSO₄7H₂O), magnesium chloride
148 hexahydrate (MgCl₂6H₂O), and *Lithothamnium* (Primasea, Bahia, Brazil) (Table 1).

149 *Table 1.* Increase in percentage of ionic concentration (after 72 hours of
150 application of 100 g m⁻³) of chemical fertilizers used in ionic adjustment.

Fertilizers	KCl	MgSO ₄ 7H ₂ O	MgCl ₂ 6H ₂ O	<i>Lithothamnium</i>	NaCl ⁻	
% target ion increment	pH [#]	0.41 ± 0.04	0.40 ± 0.07	0.17 ± 0.01	0.00 ± 0.00	-
	Ca ²⁺	-	-	-	26.70 ± 1.80	-
	K ⁺	51.10 ± 11.38	-	-	-	-
	Mg ²⁺	-	9.40 ± 0.51	10.52 ± 1.37	5.20 ± 2.00	-
	Na ⁺	-	-	-	-	39.42 ± 0.08
	Cl ⁻	-	-	-	-	60.33 ± 0.02

151 Data correspond to the mean of two replicates ± standard deviation. KCl: potassium chloride;
152 CaCO₃: calcium carbonate; MgSO₄7H₂O: magnesium sulfate heptahydrate; MgCl₂6H₂O:
153 magnesium chloride hexahydrate. [#]pH in absolute value.

154

155 In view of the results obtained from the previous study on the increase of ions in the
156 water from the application of mineral fertilizers, the salinization adjustments of the treatments
157 were carried out with commercial products and a commercial salt mixture (Veromix, São
158 Paulo, Brazil) (Table 2), taking into account the salinity of 3.0 g L⁻¹ and the proportions of
159 cations (sodium, potassium, calcium and magnesium) and anions (bicarbonate, chloride,
160 sulfate) (Table 3). The adjustment was based on the proportion factors referring to the salinity
161 of the seawater (Boyd and Thunjai, 2003; Roy et al., 2010a).

162

163 *Table 2.* Fertilizers used in the artificially salinized low-salinity water used in the of *P.*
164 *vannamei* nursery symbiotic system.

Treatment	Inputs	Ions made available
-----------	--------	---------------------

		Sodium, magnesium, potassium, strontium, chlorides, carbonates, bicarbonates, borates, sulfates, bromides, fluorides, and trace elements
Commercial salt mixture (CS)	Commercial salt mixture (830 g m ⁻³ , Veromix) and coarse salt (1,660 g m ⁻³)	
Low-cost salt mixture (LCSM)	Coarse salt (1,951 g m ⁻³), potassium chloride (52.45 g m ⁻³), <i>Lithothamnium</i> (96.67 g m ⁻³), magnesium chloride (466.67 g m ⁻³), magnesium sulfate (515.13 g m ⁻³)	Sodium, chloride, potassium, magnesium, sulfate, and calcium

165

166 *Table 3.* Initial ionic profile in the artificially salinized low-salinity water and seawater used in
 167 the *P. vannamei* nursery in synbiotic system.

Variables	Treatments			
	LCSM	CS	SD	SW
Ca ²⁺ (mg L ⁻¹)	37.87 ± 9.38 ^a	26.67 ± 0.92 ^a	38.40 ± 4.23 ^a	600.00 ± 105.83 ^b
Mg ²⁺ (mg L ⁻¹)	90.07 ± 7.55 ^a	115.67 ± 3.50 ^a	77.76 ± 3.89 ^a	1,514.70 ± 248.21 ^b
K ⁺ (mg L ⁻¹)	34.94 ± 0.98 ^a	22.22 ± 0.90 ^a	26.85 ± 1.06 ^a	322.67 ± 66.89 ^b
Na ⁺ (mg L ⁻¹)	646.46 ± 19.58 ^a	679.11 ± 39.59 ^a	705.23 ± 19.59 ^a	13,323.53 ± 607.77 ^b
SO ₄ ²⁻ (mg L ⁻¹)	230.53 ± 14.37 ^a	257.97 ± 29.90 ^a	212.17 ± 44.86 ^a	3,135.67 ± 212.01 ^b
Cl ⁻ (mg L ⁻¹)	1,169.85 ± 35.45 ^a	1,228.93 ± 71.63 ^a	1,276.20 ± 35.45 ^a	20,561.00 ± 937.92 ^b
TA (mg CaCO ₃ L ⁻¹)	61.67 ± 2.89 ^a	80.00 ± 0.01 ^a	63.33 ± 5.77 ^a	135.00 ± 5.00 ^b

TH (mg CaCO ₃ L ⁻¹)	417.33±20.13 ^a	542.67 ± 16.65 ^a	400.00 ± 16.00 ^a	7,733.33 ± 184.62 ^b
Salinity (g L ⁻¹)	3.01 ± 0.09 ^a	2.99 ± 0.12 ^a	3.12 ± 0.36 ^a	35.07 ± 0.54 ^b
Mg:Ca	2.38	4.34	2.03	2.52
Mg:K	2.58	5.20	2.90	4.69
Ca:K	1.08	1.20	1.43	1.86
Na:K	18.50	30.56	26.26	41.29
TH:TA	6.80	6.78	6.32	57.28
Error (%)	0.88	0.90	2.24	13.88

168 Data correspond to mean (n = 3) ± standard deviation. SD, seawater diluted; LCSM, low-cost
 169 salt mixture; CS, commercial salt mixture; and SW, seawater. TA: total alkalinity; TH: total
 170 hardness.

171

172 After analyzing the water in all treatments, ion concentrations in milliequivalent L⁻¹
 173 (mEq L⁻¹) were calculated to check the cation and anion equilibrium. The calculation was
 174 performed by determining the difference between the sum of the cation mEq L⁻¹ (Na⁺ = 23
 175 mg mEq⁻¹; K⁺ = 39.1 mg mEq⁻¹; Ca²⁺ = 20 mg mEq⁻¹; and Mg²⁺ = 12.15 mg mEq⁻¹) and sum
 176 of the anion mg mEq⁻¹ (HCO₃⁻ = 61 mg mEq⁻¹; Cl⁻ = 35.45 mg mEq⁻¹; and SO₄²⁻ = 48.03 mg
 177 mEq⁻¹) (Boyd, 2020). A balance error lower than 15% between cations and anions was
 178 adopted as a standard for certifying the accuracy of the analysis of these major ions (Boyd,
 179 2002). This error was calculated using the following equation:

180

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

181 In which:

182 ● Σ cations: sum of cations

183 ● Σ anions: sum of anions.

184

185 *Water Quality*

186 Dissolved oxygen and temperature (Asko multiparameter meter, model AZ86031)
187 were monitored twice a day (8 am and 4 pm). Salinity and pH (Asko multiparameter meter,
188 model AZ86031) were monitored twice a week. The settleable solids (Avnimelech, 2009)
189 were monitored three times a week. Total ammonia nitrogen (TAN; APHA, 2012), nitrite-
190 nitrogen (NO_2^- -N; Fries, 1971), nitrate nitrogen (NO_3^- -N; APHA, 2012), orthophosphate
191 (PO_4^{3-} ; APHA, 2012), total alkalinity (TA; APHA, 2012), total hardness (TH; APHA, 2012),
192 Ca^{2+} (APHA, 2012), Mg^{2+} (APHA, 2012), Na^+ (APHA, 2012), Cl^- (APHA, 2012), SO_4^{2-}
193 (APHA, 2012), and K^+ (Fries and Getrost, 1977) were monitored every 10 days. All water
194 samples were previously filtered through a 45 μm paper filter before performing the analyses.

195

196 *Shrimp stocking, feeding, and monitoring*

197 The post-larvae (PL₁₀ ~ 2.0 mg) were acquired in a commercial hatchery (Aquatec,
198 RN, Brazil), produced in water with salinity at 20 g L⁻¹. The batch was divided into two tanks
199 with a useful volume of 800 liters (7,500 PL m⁻³), where a part of the PL was acclimated to a
200 salinity of 3.0 g L⁻¹ and the other to a salinity of 35 g L⁻¹ for 10 days. At the end of the
201 acclimatization period, the PL₂₄ (12 mg \pm 1.0 mg) were randomly selected and stocked at a
202 density of 2,000 PL m⁻³ in experimental units.

203 The post-larvae were fed with a commercial feed of 0.3–0.6 mm granulometry (45%
204 crude protein and 7% lipids, Inve Aquaculture Inc) between day 1 and day 17 of the
205 experimental time. Between day 17 and day 40 of the experimental time, the animals were fed
206 with a commercial feed of 0.8–1.3 mm granulometry (45% crude protein, and 9.5% lipids,

207 ADM Animal Nutrition Company). The feed was offered four times a day (8 am, 11 am, 2 pm
208 and 5 pm). Initially, a 27.2% daily feeding rate was adopted, which was gradually reduced to
209 4.8% of body weight for 40 days. The feeding rate was adjusted daily according to the
210 estimated shrimp feed consumption and mortality rate in each experimental unit (Van Wyk et
211 al., 1999), plus a mix of commercial probiotic (2 g/kg feed) with colony forming units (CFU)
212 g^{-1} containing *Bacillus subtilis* (8.5×10^8 CFU g^{-1}), *B. licheniformis* (8.5×10^8 CFU g^{-1}), *B.*
213 *pumilus* (5.0×10^8 CFU g^{-1}), *B. cereus* var. *toyoi* (8.0×10^8 CFU g^{-1}), *B. amyloliquefaciens*
214 (8.5×10^8 CFU g^{-1}), *Lactobacillus acidophilus* (3.7×10^8 CFU g^{-1}), *L. plantarum* (3.7×10^8
215 CFU g^{-1}), yeast extract, magnesium, and mannan oligosaccharide, in addition to a dispersing
216 agent (Kayros Agrícola and Ambiental, São Paulo, Brazil), manually added to the feed using
217 a commercial binder based on digestive cellulose.

218 Shrimp weight was monitored every 10 days to determine growth and adjust the
219 amount of feed offered. At the end of the experimental period, final weight, specific growth
220 rate (SGR), feed conversion ratio (FCR), survival, and yield were determined using the
221 following equations:

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

223 $\text{SGR} (\% \text{ day}^{-1}) = 100 \times [\ln \text{ final weight (g)} - \ln \text{ initial weight (g)}] / \text{time (days)}$

224 $\text{FCR} = \text{feed supplied} / (\text{final biomass} - \text{initial biomass})$

225 $\text{Survival} (\%) = (\text{final number of individuals} / \text{initial number of individuals}) \times 100$

226 $\text{Yield} (\text{Kg m}^{-3}) = \text{final biomass (Kg)} / \text{volume of experimental unit (m}^3\text{)}$.

227

228 *Presumptive total count on Thiosulphate Citrate Bile Sucrose – TCBS (Vibrio spp.) and*
229 *Sabouraud dextrose agar (fungi)*

230 Presumptive total count were performed at the Laboratório de Sanidade de
231 Organismos Aquáticos (LASAq [Aquatic Animal Health Lab]), of the Fisheries and

232 Aquaculture Department] of Rural Federal University of Pernambuco, Brazil for the
233 quantification of the colony-forming units (CFUs g⁻¹) of Thiosulphate Citrate Bile Sucrose –
234 TCBS (*Vibrio* spp.) and Sabouraud dextrose agar (Fungi). Shrimp samples for analysis in
235 TCBS (*Vibrio* spp.) and Sabouraud dextrose agar (Fungi) were collected at the 20th and 40th
236 days (pools of 50 mg gut samples each experimental units) (Vandenberghé et al., 1999).

237 The shrimp were disinfected by immersion in 70% ethanol for 15 seconds, followed
238 by immersion for 15 minutes in sodium hypochlorite solution (1.5%) with 0.1% tween-80 and
239 rinse with sterile distilled water. Subsequently, the biological samples were weighed,
240 macerated and homogenized with a solution of peptone water (2%) in the 1:10 ratio, for the
241 10⁻¹ dilution, serially diluted from 10⁻¹ to 10⁻⁵ and 100 µL were seeded in the plates
242 containing the culture media Thiosulfate Citrate Bile Sucrose and Sabouraud dextrose agar
243 (fungal count made of yeast and filamentous fungi), with the aid of an “L” loop, and then
244 incubated at 30°C for 24 hours (TCBS) and 36°C for 72 hours (Sabouraud dextrose agar), all
245 in triplicate (APHA, 2017). After incubation, total colonies were counted using a colony
246 counter, and macromorphological colonial aspects for the characterization of yeast and
247 filamentous fungi were analyzed based on Trabulsi and Alterthum (2015). The conversion to
248 CFU g⁻¹ for the shrimp samples and gut samples was performed using the following formula:
249
$$\text{CFU g}^{-1} = \text{number of colonies} \times \text{dilution factor} / \text{weight (g) gut of the sample.}$$

250

251 *Stress tests*

252 At the end of the culture time, shrimp were submitted to resistance test for water
253 ammonia nitrogen concentration (NH₃-N). For the NH₃-N resistance test, at the end of the
254 experiment, 10 shrimps were randomly collected in each experimental unit and were stocked
255 to experimental units containing 10 L of water salinity 3.0 g L⁻¹ (LCSM, CS and SD), and 10
256 L of water salinity 35 g L⁻¹ (SW) with NH₃-N concentrations between 0.39 and 0.42 mg L⁻¹

257 (Table 4), water temperature close to 29°C, and pH close to 8.1–8.5. The NH₃-N
 258 concentration was achieved by applying a stock solution of 10 g L⁻¹ of NH₄Cl. The test was
 259 carried out for 96 h, and survival was measured every 24 h (Zhang et al., 2012).

260

261 *Table 4.* Total ammonia nitrogen (TAN) concentration and non-ionized ammonia (NH₃-N)
 262 produced in the experimental units from NH₄Cl solution application.

	TAN (mg L ⁻¹)		NH ₃ -N (mg L ⁻¹)	
	SW	LCSM, CS and SD	SW	LCSM, CS and SD
Initial	2.57	4.91	0.41	0.42
24hrs	2.44	4.79	0.39	0.41
48hrs	2.56	4.88	0.41	0.42
72hrs	2.48	4.77	0.40	0.41
96hrs	2.56	4.80	0.41	0.41

263 Data correspond to the mean concentration of TAN and NH₃-N ± standard deviation.

264

265 *Total hemocyte count (THC)*

266 Hemolymph (10 shrimp per treatment) was collected for the blood analysis before and
 267 after the stress tests of ammonia nitrogen concentration (NH₃-N following the protocol
 268 described by Guertler et al., 2013). For such, the hemolymph (200 µL) was collected from the
 269 hemocoel located in the ventral region of the surviving animals with the aid of a syringe (1 ml)
 270 containing anticoagulant (modified Alsever's solution [MAS: 336 mmol L⁻¹ of NaCl, 115
 271 mmol L⁻¹ of glucose, 27 mmol L⁻¹ of sodium citrate, and 9 mmol L⁻¹ of EDTA, pH 7.2]) at a
 272 proportion of 1:2 (v:v). One aliquot of hemolymph was separated and stored in MAS and 4%
 273 formaldehyde (1:3). The total hemocyte count was performed in a Neubauer chamber in a
 274 similar manner used for white blood cells in triplicate (Hameed et al., 2006).

275

276 *Data analysis*

277 Statistical analyses were performed using the IBM SPSS Statistic 25.0 software. Data
 278 were checked for homogeneity of variances using the Levene test ($p \leq 0,05$) and for normality
 279 using the Shapiro-Wilk test ($p \leq 0,05$). For normal and homogeneous data, parametric one-
 280 way ANOVA (shrimp zootechnical performance and total hemocyte count) and repeated
 281 measures ANOVA (water quality – dissolved oxygen, temperature, pH, salinity, Ca^{2+} , Mg^{2+} ,
 282 Cl^- , K^+ , Na^+ , magnesium hardness, calcium hardness, TH, and TA) were used, followed by
 283 Tukey's mean comparison test ($P \leq 0.05$). Non-parametric data were analyzed using the
 284 Friedman's test with Conover's multiple comparison test with Holm-Bonferroni correction for
 285 TAN, N-NO_2^- , N-NO_3^- , settleable solids, and SO_4^{-2} .

286 TCBS and Sabouraud dextrose agar counts were analyzed using the non-parametric
 287 Kruskal-Wallis test ($P \leq 0.05$) followed by Dunn's post-hoc test with Holm-Bonferroni
 288 correction ($P \leq 0.05$).

289

290 **Results**291 *Ions*

292 Ion concentration had no significant differences ($P > 0.05$) among low salinity
 293 treatments, but it did have ($P < 0.05$) when regarding seawater (SW) (Table 5). The water ions
 294 values were similar between the beginning and the end, however the alkalinity increased
 295 significantly (Table 5).

296

297 *Table 5.* Concentrations (mg L^{-1}) of ions from artificially salinized low-salinity water and
 298 seawater used in the of *P. vannamei* nursery in synbiotic system.

Variables	Treatments			
	LCSM	CS	SD	SW

Ca ²⁺ (mg L ⁻¹)	39.36 ± 5.03 ^b	34.45 ± 6.42 ^b	39.78 ± 5.78 ^b	626.66 ± 46.18 ^a
Mg ²⁺ (mg L ⁻¹)	122.92±20.32 ^b	86.44±19.05 ^b	102.38±19.91 ^b	1,747.80±329.39 ^a
K ⁺ (mg L ⁻¹)	28.33 ± 5.19 ^b	24.00 ± 5.86 ^b	25.37 ± 2.87 ^b	231.69 ± 71.14 ^a
Na ⁺ (mg L ⁻¹)	714.37±347.97 ^b	692.82±294.57 ^b	767.26±359.91 ^b	16,482.12±4,052.30 ^a
SO ₄ ²⁻ (mg L ⁻¹)	333.18 ± 91.95 ^b	317.34 ± 54.52 ^b	316.82±108.13 ^b	3,881.13 ± 691.12 ^a
Cl ⁻ (mg L ⁻¹)	1,127.30±552.83 ^b	1,132.03±455.60 ^b	1,207.66±549.45 ^b	25,169.50±6,371.14 ^a
TA (mg CaCO ₃ L ⁻¹)	165.00 ± 11.92 ^b	151.00 ± 72.35 ^b	157.66 ± 104.80 ^b	200.80 ± 86.65 ^a
TH (mg CaCO ₃ L ⁻¹)	613.33 ± 10.27 ^b	498.67±90.25 ^b	497.33±99.06 ^b	9,500.00±568.37 ^a
Calcium hardness (mg CaCO ₃ L ⁻¹)	96.00 ± 26.54 ^b	81.33 ± 16.13 ^b	97.33 ± 16.46 ^b	1,500.00±110.23 ^a
Magnesium hardness (mg CaCO ₃ L ⁻¹)	512.00 ± 80.30 ^b	390.67±89.86 ^b	400.00±85.92 ^b	8,000.00±464.48 ^a
Mg:Ca	3.12	2.50	2.57	2.79
Mg:K	4.33	3.60	4.03	7.54
Ca:K	1.39	1.43	1.57	2.70
Na:K	25.22	28.87	30.24	71.13
TH:TA	3.72	3.30	3.15	47.31
Error (%)	0.94	0.46	0.79	13.32

299 Data correspond to mean ± SD. The results were analyzed by means of a repeated measures
300 ANOVA ($P \leq 0.05$) followed by the Tukey test, Friedman's test and Conover's multiple
301 comparison test ($P \leq 0.05$) for non-parametric data. SD, seawater diluted; LCSM, low-cost
302 salt mixture; CS, commercial salt mixture; and SW, seawater. TH, total hardness; TA, total
303 alkalinity.

304

305 *Water quality*

306 Data on water quality variables are summarized in Table 6. No significant differences
307 among the treatments were found regarding nitrogen compounds (TAN, N-NO₂, N-NO₃).

308 However, significant differences were found for salinity, pH, and SS. The higher values for
 309 SS were found in CS treatment ($1.64 \pm 0.80 \text{ ml L}^{-1}$) and the lowest in SW treatment ($0.20 \pm$
 310 0.08 ml L^{-1}).

311

312 *Table 6.* Water quality variables in the artificially salinized low-salinity water and seawater
 313 used in the of *P. vannamei* nursery in synbiotic system.

Variables	Treatments			
	LCSM	CS	SD	SW
Temperature (°C)	30.50 ± 0.46^a	30.15 ± 0.33^a	30.30 ± 0.44^a	30.01 ± 0.15^a
Oxygen (mg L^{-1})	5.28 ± 0.30^a	5.35 ± 0.22^a	5.21 ± 0.29^a	4.67 ± 0.15^a
Salinity (g L^{-1})	3.00 ± 0.07^b	2.96 ± 0.15^b	2.99 ± 0.34^b	36.50 ± 0.03^a
pH	8.89 ± 0.01^a	8.86 ± 0.08^a	8.90 ± 0.08^a	8.11 ± 0.22^b
TAN (mg L^{-1})	0.18 ± 0.12^a	0.20 ± 0.31^a	0.24 ± 0.12^a	0.23 ± 0.27^a
N-Nitrite (mg L^{-1})	0.42 ± 0.90^a	0.24 ± 0.50^a	0.23 ± 0.10^a	0.42 ± 0.90^a
N-Nitrate (mg L^{-1})	2.84 ± 1.44^a	2.48 ± 1.60^a	3.00 ± 1.66^a	0.82 ± 1.24^a
Settleable solids (ml L^{-1})	1.43 ± 0.56^a	1.64 ± 0.80^a	0.49 ± 0.62^{ab}	0.20 ± 0.08^b

314 Data correspond to mean \pm standard deviation. The results were analyzed by means of a
 315 repeated measures ANOVA ($P \leq 0.05$) followed by the Tukey test for the parametric data, and
 316 Friedman's test and Conover's multiple comparison test ($P \leq 0.05$) for non-parametric data. SD,
 317 seawater diluted; LCSM, low-cost salt mixture; CS, commercial salt mixture; and SW,
 318 seawater.

319

320 *Shrimp zootechnical performance*

321 The shrimp zootechnical performance data at the end of the 40 days of experimental
 322 culture are summarized in Table 7. Significant differences among the treatments were found
 323 in final weight, with highest value in LCSM ($0.87 \pm 0.24 \text{ g}$) and lowest in SW ($0.65 \pm 0.10 \text{ g}$),

324 however, regarding survival, LCSM ($70.00 \pm 5.00\%$) showed lower values when compared to
 325 SW ($90.55 \pm 10.58\%$). No significant differences among the treatments were found regarding
 326 yield, FCR and SGR (Table 7).

327

328 *Table 7.* Zootechnical performance of *P. vannamei* juveniles from artificially salinized low-
 329 salinity water and seawater in synbiotic system.

Variables	Treatments			
	LCSM	CS	SD	SW
Final weight (g)	0.84 ± 0.18^a	0.84 ± 0.04^a	0.72 ± 0.03^a	0.65 ± 0.10^b
Survival (%)	70.00 ± 5.00^b	78.61 ± 3.15^{ab}	82.77 ± 3.93^{ab}	90.55 ± 10.58^a
FCR	1.04 ± 0.31^a	1.18 ± 0.01^a	1.25 ± 0.01^a	1.16 ± 0.05^a
Yield (Kg m ⁻³)	1.01 ± 0.42^a	1.14 ± 0.02^a	1.05 ± 0.06^a	1.09 ± 0.05^a
SGR (% dia ⁻¹)	4.25 ± 0.36^a	4.34 ± 0.81^a	4.56 ± 0.56^a	4.52 ± 0.27^a

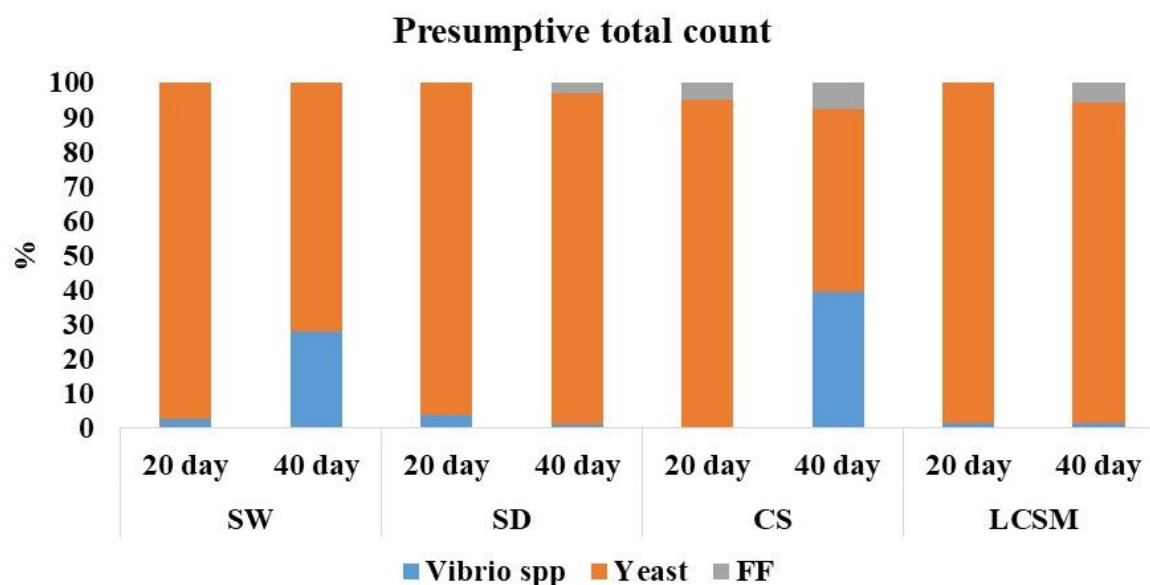
330 Data correspond to mean \pm standard deviation. The results were analyzed by means of
 331 ANOVA ($P \leq 0.05$) followed by the Tukey test. Mean values on the same line with different
 332 superscripts differ significantly. SD, seawater diluted; LCSM, low-cost salt mixture; CS,
 333 commercial salt mixture; and SW, seawater. FCR, feed conversion ratio; and SGR, specific
 334 growth rate.

335

336 *Thiosulphate Citrate Bile Sucrose (TCBS, Vibrio spp.) and Sabouraud dextrose agar (fungi)*

337 The *Vibrio* spp. samples from the shrimp gut at 20 days of culture, were highest in the
 338 SW ($7.44 \pm 9.73 \times 10^4$ CFU g⁻¹), and the lowest in CS ($0.91 \pm 1.89 \times 10^4$ CFU g⁻¹). The
 339 counts at the end of the culture cycle ranged from $15.6 \pm 15.5 \times 10^4$ CFU g⁻¹ (LCSM) to 0.72
 340 $\pm 1.27 \times 10^4$ CFU g⁻¹ (SD), however there were no significant differences between treatments
 341 (Figure 1).

342 Regarding fungi, the SW treatment presented the highest concentration levels ($260.0 \pm$
 343 244.0×10^4 CFU g^{-1}) at 20 days of culture. At the end, an increase in filamentous fungi was
 344 observed in all low salinity treatments, but they were still less representative when compared
 345 to yeasts. The final counts varied between $323.0 \pm 287.0 \times 10^4$ CFU g^{-1} (LCSM) and $40.2 \pm$
 346 41.1×10^4 CFU g^{-1} (SW), however, there were no significant differences between treatments
 347 (Figure 1).

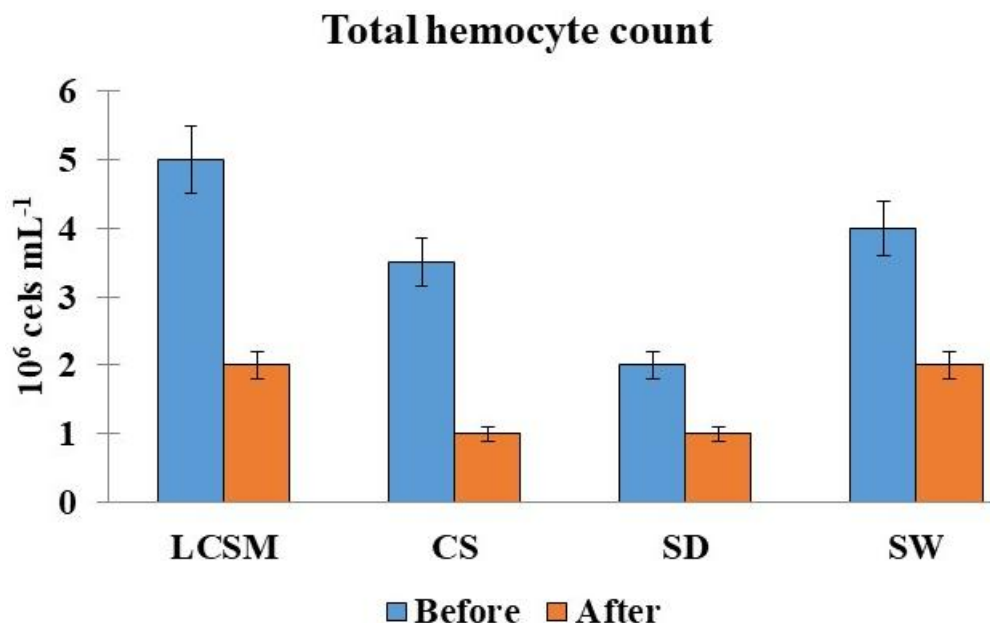


348
 349 *Figure 1.* Presumptive total count for Thiosulphate Citrate Bile Sucrose (TCBS, *Vibrio* spp.)
 350 and Sabouraud dextrose agar (fungi) in *P. vannamei* gut. SD, seawater diluted; LCSM, low-
 351 cost salt mixture; CS, commercial salt mixture; and SW, seawater. FF = filamentous fungi.

352

353 *Stress tests*

354 Regarding the N-NH₃ resistance test, no significant differences were observed
 355 between treatments. After being submitted to the stress test, 100% survival was observed in
 356 all treatments, and total hemocyte counts did not differ significantly between treatments.
 357 Juveniles had hemocyte counts ranging between $5.00 \pm 1.77 \times 10^6$ cells mL^{-1} (LCSM) and
 358 $2.00 \pm 1.17 \times 10^6$ cells mL^{-1} in the SD treatment (Figure 2).



359

360 *Figure 2.* Total hematocyte count (THC) of *Penaeus vannamei* juveniles under stress for
361 water ammonia nitrogen concentration (NH₃-N) after nursery in the artificially salinized low-
362 salinity water and seawater used synbiotic system. SD, seawater diluted; LCSM, low-cost salt
363 mixture; CS, commercial salt mixture; and SW, seawater.

364

365 **Discussion**

366 Regarding ions, the different forms of artificially salinized used in this experiment
367 managed to maintain appropriate levels during the nursery phase, even with the observed
368 ecdysis processes, unlike Huong et al. (2010), who described a reduction of ions (calcium and
369 magnesium) in the culture water during the growth of shrimp by the ecdysis process, as they
370 have a high demand for shell formation. This observed maintenance of ions is probably
371 related to the use of the natural substrate of *A. brasiliiana* and rice bran as a source of organic
372 carbon. This maintenance of calcium and magnesium ions provides greater total water
373 hardness and, in addition to higher concentrations of total alkalinity, favor the growth of
374 shrimp. According to Pimentel et al. (2022), there are positive correlations for the

375 concentrations of total alkalinity and calcium regarding final average weight and specific
376 growth rate of *P. vannamei* juveniles in oligohaline water.

377 The ratio of potassium to sodium in seawater is approximately 28:1, although the
378 shrimp *P. vannamei* support variations of up to 10 points without interfering with its
379 osmoregulatory capacity (Sowers et al., 2006), especially when alkalinity is greater than or
380 equal to 100 mg CaCO₃ L⁻¹ (Pimentel et al., 2022). Potassium is one of the main ions related
381 to the growth and survival of shrimp in low salinity, in addition to influencing osmoregulation
382 when associated with sodium, contributing to the activation of the Na⁺, K⁺-ATPase enzyme,
383 responsible for the active transport of ions through the animal's cell membrane (Roy et al.,
384 2007; Galkanda-Arachchige et al., 2021). The Na:K ratio in the low salinity and total
385 alkalinity treatments during the shrimp nursery remained close to the recommended and may
386 have contributed to the activation of the Na⁺, K⁺-ATPase enzyme, allowing the maintenance
387 of cellular metabolism homeostasis and improving the uptake of glucose and amino acids by
388 shrimp (Galkanda-Arachchige et al., 2021).

389 Dissolved oxygen, temperature and pH remained within the ideal values for the
390 production of *P. vannamei* (Van Wyk and Scarpa, 1999; Samocha, 2019). The levels of
391 dissolved oxygen in the SW treatment were lower than 5 mg L⁻¹, due to the higher
392 concentrations of salts, generating lower solubility of dissolved oxygen (Fiorucci and Filho,
393 2005). However, these concentrations were not limiting for a good development of the shrimp
394 and the transformation of nitrogen compounds by the bacteria in the culture system.

395 The pH values showed a significant difference between the low salinity treatments
396 (LCSM, CS and SD) in relation to that of seawater (SW). The application of mineral
397 fertilizers for artificially salinized, in addition to the use of sodium bicarbonate in low salinity,
398 contributed to increase the pH of the water. According to Brito et al. (2021), low salinity
399 sodium bicarbonate behaves differently when compared to its use in a marine water system,

400 as it has greater power to increase the pH. Alkalinity adjustments with sodium bicarbonate
401 (NaHCO₃) every ten days in the nursery phase maintained mean concentrations greater than
402 150 mg CaCO₃ L⁻¹, within the recommended range for intensive systems (Van Wyk and
403 Scarpa, 1999; Avnimelech et al., 2015), helping to keep the oxidation of nitrogen compounds
404 via nitrifying bacteria and/or the transformation of ammonia into microbial biomass (Ebeling
405 et al., 2006; Ferreira et al., 2011), however, it is necessary to pay attention to the pH values.

406 In addition to contributing to the control of nitrogen compounds, total alkalinity and
407 total hardness (calcium and magnesium) are of paramount importance for a good development
408 of shrimp, as they directly influence the animal's ecdysis process, since the amount of calcium
409 consumed by shrimp will supply the exoskeleton mineralization process (Boyd and Tucker,
410 1998; Samocha, 2019).

411 At low salinity, nitrogen compounds are more toxic, causing large mortalities of
412 shrimp. This problem with nitrogen concentrations can be more accentuated in intensive
413 systems with minimal water exchange in the first days of culture, due to the high
414 concentrations of ammonia and nitrite. According to Furtado et al. (2011), high densities
415 contribute to the accumulation of organic matter from feed waste (leached or not consumed)
416 and high biomass, among others, in addition to the reduced concentrations of nitrifying and
417 heterotrophic bacteria at the beginning of the production cycles. In all treatments in this study,
418 TAN and N-NO₂ levels were maintained within the ideal levels for low salinity treatments (<
419 0.81 mg TAN L⁻¹ and < 0.45 mg N-NO₂ L⁻¹) and for the treatment of seawater (< 3 mg TAN
420 L⁻¹ and < 10 mg N-NO₂ L⁻¹) (Valencia-Castañeda et al., 2018; Samocha, 2019).

421 This control of nitrogen in different forms of artificial salinization of water is related
422 to the combination of the use of a 5% water reuse inoculum, a synbiotic system and an
423 artificial substrate, as also observed by Pimentel et al. (2022) and Oliveira et al. (2022b), with
424 production of *P. vannamei* juveniles in oligohaline water. These results contribute to the

425 installation of intensive nurseries with minimal water exchange in oligohaline condition, with
426 salinity close to 3 g L⁻¹, reducing production costs with artificial salinization, since many
427 researches and productions in artificial waters use higher or close salinity to 10 g L⁻¹ (Pinto et
428 al., 2020; Fleckenstein et al., 2022), which makes the cost per m³ of water economically
429 onerous for the production of shrimp in artificially salinized water in some countries.

430 The synbiotic system, when compared to other systems with application of molasses
431 in natura in the nursery phase, produces less settleable solids, due to the application of the
432 source of organic carbon after the processes of fermentation and/or microbial respiration,
433 which make it more soluble in water (Santos et al., 2022a). Higher concentrations of solids in
434 a culture system can cause accumulation of organic matter in the gills of cultivated shrimp,
435 which can affect the diffusion of oxygen and suppress the growth of organisms (Gaona et al.,
436 2011; Schweitzer et al., 2013), requiring the control for different concentrations according to
437 the shrimp growth phase.

438 The concentrations of settleable solids were not different between the forms of
439 artificially salinized, however they were higher than the concentrations with seawater, mainly
440 in the LCMS and CS treatments, due to the application of minerals, similar to the data
441 observed by Oliveira et al. (2022b), in synbiotic with oligohaline water. These results are
442 probably related to the lower solubility of these ions in water, as well as the increase in pH,
443 which hinders the solubility of some ions, especially calcium carbonate (Boyd, 2020).

444 Regarding the zootechnical performance of *P. vannamei* juveniles, we observed
445 significant differences in survival between treatments, with the lowest survival in LCSM
446 (70%). Expressive result in relation to those observed by Pinto et al. (2020), who compared
447 only mineral fertilizers and obtained a low survival (15%) compared to artificial sea salt
448 (81%). The application of *Lithothamnium* (marine algae fossils), which has some important
449 macro and micro minerals for shrimp, probably contributed to the survival of close to 70% in

450 the LCSM treatment, and its application may be increased in future evaluations, since
451 artificial sea salts have a series of micro-constituents and trace elements (Atkinson and
452 Bingman, 1997), which contribute to better survival (Pinto et al., 2020).

453 The final weight was significantly higher in the low salinity treatments (LSCM, CD to
454 SD) when compared to seawater, which may be related to the higher survival rates found in
455 the same treatment (SW). However, the other variables of zootechnical performance were
456 similar without the effect of the form of **artificially salinized**, demonstrating the possibility of
457 producing juveniles in low salinity synbiotic system. The final weight and productivity results
458 were similar to those observed by Pimentel et al. (2022), with marine water diluted to
459 oligohaline water (3 g L⁻¹), with and without the correction of ionic ratios (Ca:Mg:K), by
460 Oliveira et al. (2022b) with diluted marine water (3 g L⁻¹), with different frequencies of
461 correction of the ionic ratios (Ca:Mg:K) and by Brito et al. (2016) with marine water with
462 plankton application as a food supplement.

463 The FCR is an important variable from an economic point of view in the production of
464 juveniles, and the values observed were satisfactory for intensive nurseries, in the different
465 forms of artificially salinized, even in the LCSM treatment with lower survival, since it was
466 compensated by the final weight. FCR values at low salinity can usually be higher, due to the
467 energy expenditure that shrimp use for osmoregulation, needing to use diets with higher
468 carbohydrate contents or application of mineral supplements (Roy and Davis, 2010b).
469 However, in the system of minimum water exchange as the synbiotic in conditions of low
470 salinity, the complementation with natural food has contributed to maintain the values in
471 adequate ranges (Pimentel et al., 2022; Oliveira et al., 2022b).

472 The genus *Vibrio* is present in the shrimp gut in different farming systems,
473 contributing negatively to food digestion and mortality (Fan et al., 2019a; Fan et al., 2019b;
474 Fan and Li, 2019c; Gao et al., 2019). These bacteria are pathogenic and opportunistic and can

475 cause disease and mortality in shrimp when they are physiologically debilitated (Valente and
476 Wan, 2021).

477 In a system with minimal water exchange, *Vibrio* spp. have their development favored
478 due to the large amount of organic matter present in the crop cycle (Ferreira et al., 2011;
479 Yanong and Erlaher-Reid, 2012). This increase in organic matter is very common due to the
480 high C:N ratios used for the development of heterotrophic bacteria (Avnimelech, 2007), in
481 addition to the carbon sources such as the hexose, glucose and fructose sugars used
482 (Emerenciano et al., 2017).

483 Thus, in a synbiotic system, we can reduce the proportion of *Vibrio* in shrimp, as rice
484 bran (prebiotic) used as a source of organic carbon contributes to the supply of beneficial
485 microorganisms (probiotics), and can therefore alter the host's microbiota, increasing
486 competition with pathogens and production of enzymes and organic acids, in addition to the
487 reduction of intestinal pH (Huynh et al., 2017; Dawood and Koshio, 2020), which can be used
488 as a preventive tool to control *Vibrio* (Valente and Wan, 2021). However, artificially salinized
489 of water does not seem to have a direct influence on *Vibrio* spp. concentration in the shrimp
490 gut when compared to seawater, probably due to the use of the feed with the application of
491 commercial probiotic and the synbiotic system.

492 As well as bacteria, the presence of fungi (filamentous and yeasts) is observed in
493 synbiotic systems (water and hepatopancreas) of shrimp, however with yeast dominance (de
494 Andrade et al., 2021). These data are similar to the observed in this study, with a synbiotic
495 system and application of probiotics via nutrition, with no significant effect of salinity and the
496 type of artificially salinized. This dominance of yeasts in different forms of artificially
497 salinized and seawater is important, because yeasts have a high protein value and are rich in B
498 vitamins, with an important effect on the digestive system, helping the functioning of the gut,

499 and playing a role in the defense of the organism against pathogens (Agboola et al., 2021), as
500 the health of shrimp is closely linked to the gut microbiota (Holt et al., 2021).

501 Shrimp can be in constant challenge in intensive systems in relation to nitrogen
502 compounds, until the bacterial community is stabilized, or even when the amounts of feed
503 used are rapidly increased, generating toxic compounds more quickly. According to Perazollo
504 et al. (2002), we can use total hemocyte count (THC) as a health indicator for shrimp, as its
505 concentration responds to factors such as ecdysis, environmental stress or infections.
506 Furthermore, higher concentrations of circulating hemocytes in the hemolymph indicate lower
507 effects of environmental stress or infection (Le Moullac et al., 1998).

508 After 96 hours of the ammonia stress test, the juveniles showed 100% survival, with
509 reduced circulating THC in the hemolymph, indicating an effect of N-NH₃ levels on the
510 immune response. However, with the survivals obtained, we can infer that the shrimp
511 tolerated the evaluated concentrations and the exposure time well, even though the N-NH₃
512 values were approximately 3 to 4 times higher than the safety limit (concentrations equivalent
513 to 10% of the PL₅₀) for *P. vannamei* (post-larvae and juveniles) in low salinity (0.12 mg N-
514 NH₃ L⁻¹; Valencia-Castaneda et al., 2018) and higher salinity (0.16 mg N-NH₃ L⁻¹; Lin and
515 Chen, 2001).

516

517 **Conclusion**

518 The different forms of artificially salinized used in the present study were efficient for
519 the maintenance of the main water ions. Regarding the production of *P. vannamei* juveniles in
520 nurseries with a synbiotic system in artificially salinized water, they obtained similar
521 zootechnical results and resistance to ammonia stress when compared to seawater. Regarding
522 the presumptive count of bacteria in TCBS (*Vibrio* spp.) the use of the synbiotic and the

523 commercial probiotic in the feed were efficient to keep the concentrations low, however the
524 artificially salinized increased the participation of *Vibrio* spp.

525

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539 **Credit author statement**

540 **Agatha Catharina Limeira:** investigation, conceptualization, methodology, formal analysis,
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542 methodology, formal analysis. **Gisely Karla de Almeida Costa:** data curation, methodology,
543 formal analysis. **Suzianny Maria Bezerra Cabral da Silva:** methodology, writing (review
544 and editing). **Alfredo Oliveira Galvez:** methodology, writing (review and editing). **Luis**
545 **Otavio Brito:** supervision, methodology, resources, writing (review and editing).

546

547 **Declaration of competing interest**

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

550

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