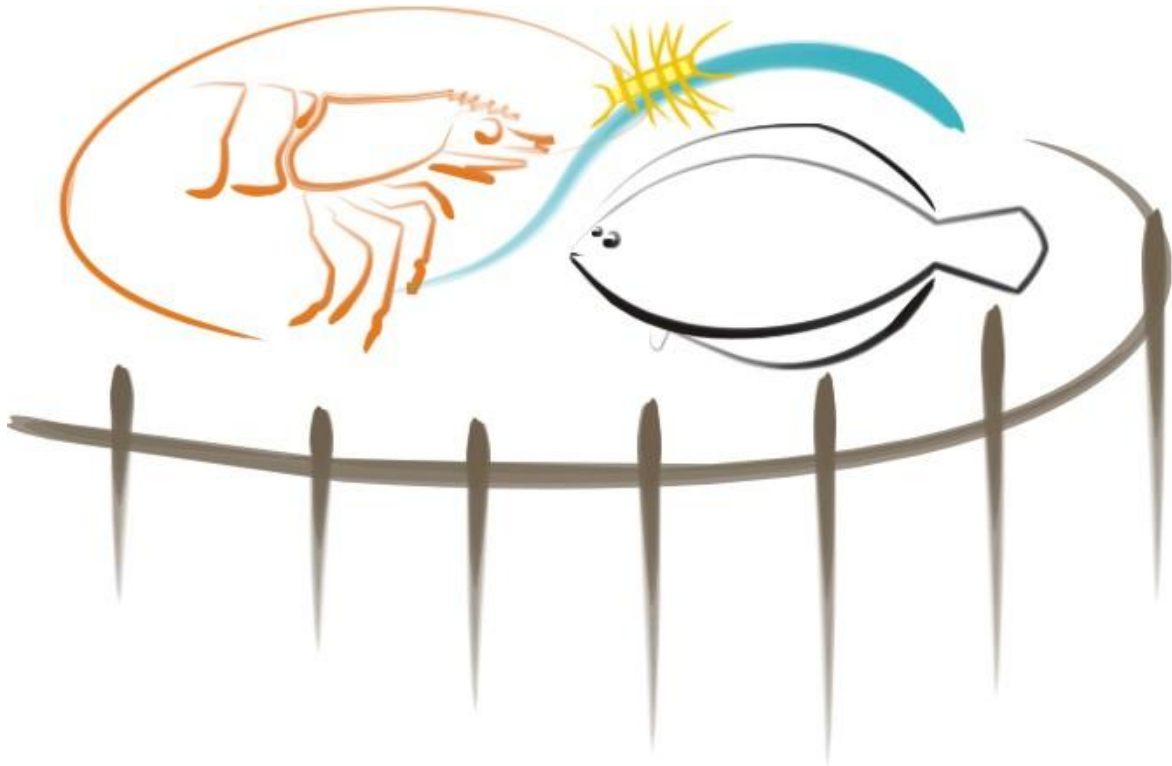


UNIVERSIDADE FEDERAL DO RIO GRANDE
PROGRAMA DE PÓS GRADUAÇÃO EM AQUICULTURA



**RELEVÂNCIA DA ALCALINIDADE, pH E DIÓXIDO DE
CARBONO NA SOBREVIVÊNCIA E CRESCIMENTO DE
Litopenaeus vannamei (Boone 1931) EM SISTEMA DE BIOFLOCOS**

PLÍNIO SCHMIDT FURTADO

FURG

Rio Grande, RS

2014

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Tese apresentada ao Programa de Pós-Graduação em Aquicultura da Universidade Federal do Rio Grande, como parte dos requisitos para obtenção do grau de Doutor em Aquicultura.

Rio Grande - RS - Brasil

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Índice

	Pg.
Dedicatória	VI
Agradecimentos	VII
Resumo Geral	VIII
Abstract	IX
Introdução Geral	01
Objetivos	10
Objetivo geral.....	10
Objetivos específicos.....	10
Referências Bibliográficas	11
Capítulo I: Acute effect of pH and its influences in the survival and the biochemical biomarkers of juvenile white shrimp <i>Litopenaeus vannamei</i>.....	16
Resumo.....	17
Abstract.....	18
Introduction.....	19
Material and Methods.....	20
Results.....	25
Discussion.....	31
Conclusions.....	34
Acknowledgements.....	34
Literature Cited.....	34
Capítulo II: Toxicidade aguda de dióxido de carbono em juvenis de camarão marinho <i>Litopenaeus vannamei</i> (Boone 1931).....	40
Resumo.....	41
Abstract.....	42
Introdução.....	43
Material e Métodos.....	45
Resultados.....	49
Discussão.....	52
Conclusão.....	55
Agradecimentos.....	55

Referências Bibliográficas.....	56
Capítulo III: Avaliação dos efeitos da suspensão da aeração artificial durante o cultivo de <i>Litopenaeus vannamei</i> (Boone 1931) no sistema de bioflocos sobre as concentrações de dióxido de carbono dissolvido	60
Resumo.....	61
Abstract.....	62
Introdução.....	63
Material e Métodos.....	64
Resultados.....	67
Discussão.....	70
Conclusão.....	73
Agradecimentos.....	74
Referências Bibliográficas.....	74
Capítulo IV: Efeito das concentrações de dióxido de carbono dissolvido no consumo de oxigênio do camarão branco do Pacífico <i>Litopenaeus vannamei</i> (Boone 1931).....	78
Resumo.....	79
Abstract.....	80
Introdução.....	81
Material e Métodos.....	82
Resultados.....	87
Discussão.....	92
Conclusão.....	95
Agradecimentos.....	96
Referências Bibliográficas.....	96
Capítulo V: The effect of different alkalinity levels on <i>Litopenaeus vannamei</i> reared with biofloc technology (BFT).....	101
Resumo.....	102
Abstract.....	103
Introduction.....	104
Materials and Methods.....	106
Results.....	110

Discussion.....	118
Conclusion.....	123
Acknowledgements.....	123
References.....	123
Capítulo VI: Application of different doses of calcium hydroxide in the	128
 farming shrimp <i>Litopenaeus vannamei</i> with the biofloc technology (BFT).....	
Resumo.....	129
Abstract.....	130
Introduction.....	131
Materials e Methods.....	133
Results.....	139
Discussion.....	145
Conclusion.....	152
Acknowledgements.....	152
References.....	152
Conclusão Geral.....	157

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RESUMO

Os cultivos de organismos aquáticos em sistemas sem renovação de água que utilizam altas densidades de estocagem, forte aeração e biota predominantemente aeróbia e heterotrófica, formadora de flocos microbianos são chamados de tecnologia de bioflocos (BFT). Estes sistemas são considerados biosseguros e permitem elevar a produtividade do cultivo devido ao suplemento nutricional fornecido pelos bioflocos. Como não há renovação de água ao longo do cultivo e a água pode ser reutilizada por vários ciclos de produção, ocorre a redução dos níveis de alcalinidade e de pH, podendo também ocorrer um acúmulo de dióxido de carbono (CO₂), proveniente dos processos respiratórios dos camarões e dos bioflocos. Alterações na qualidade de água de cultivo geram estresse e reduzem o crescimento e a capacidade de resposta do sistema imunológico, podendo afetar a sobrevivência dos camarões. Este trabalho busca aprimorar as técnicas de cultivo de *Litopenaeus vannamei* em sistemas de bioflocos sem renovação de água, compreendendo e corrigindo as concentrações de dióxido de carbono, alcalinidade e pH. Para tal, foram realizados cinco experimentos na estação marinha de aquicultura da Universidade Federal do Rio Grande (EMA-FURG-Brasil) e um na Universidad Nacional Autónoma do México (UMDI-Sisal-UNAM): 1. Determinação do nível letal do pH ácido e básico (pH_{50-96h}) e avaliação do efeito subletal do pH nos parâmetros de estresse oxidativo em juvenis de *L. vannamei*; 2. Determinação da concentração letal e do nível de segurança de dióxido de carbono para juvenis de *L. vannamei*; 3. Verificação das concentrações de CO₂ em diferentes densidades de cultivo e de sólidos em suspensão quando ocorre falha no sistema de aeração e se aplica peróxido de hidrogênio (H₂O₂) como fonte de oxigênio em sistema BFT; 4. Avaliação do efeito do CO₂ no consumo de oxigênio de juvenis de *L. vannamei* cultivados em sistema BFT; 5. Avaliação do efeito de diferentes concentrações de alcalinidade nos parâmetros de qualidade da água, na formação dos bioflocos e no desempenho zootécnico de *L. vannamei* cultivados em sistema BFT; 6. Determinação da melhor dosagem de hidróxido de cálcio (Ca(OH)₂) nos parâmetros de qualidade da água e desempenho zootécnico de *L. vannamei* cultivados em sistema BFT. Nas condições experimentais descritas no capítulo 1, o pH₅₀ 96 h de pH ácido e básico foram de 4,04 e 9,58 para juvenis de *L. vannamei*. Quanto ao ensaio de estresse oxidativo constatamos que os camarões expostos tanto ao pH 4,5 como ao pH 9,5 apresentaram desequilíbrio na atividade das enzimas antioxidantes. Já no Capítulo 2 encontramos os valores de CL₅₀ e limites de confiança 95% em 24, 48, 72 e 96 h foram de 130,05 (104,2-162,1), 77,2 (73,8-80,02), 69,65 (65,47-74,32), 59,12 (53,08-66,07) mg/L de CO₂, respectivamente. Assim, o nível de segurança calculado para *L. vannamei* foi de 5,9 mg/L de CO₂. No capítulo 3 verificamos que as concentrações de CO₂ se incrementaram com os incrementos de biomassa e de sólidos suspensos totais com o passar do tempo, alcançando valores subletais de CO₂ para os camarões. No capítulo 4 foi verificado um incremento no consumo de oxigênio de juvenis nas concentrações de até 60 mgCO₂/L e uma redução no consumo de oxigênio nas concentrações de 95, 150 e 300 mgCO₂/L seguido de sintomas de anestesia e mortalidade. No Capítulo 5 foi possível formar bioflocos com valores de alcalinidade superiores a 70 mgCaCO₃/L e pH superior a 7,38. Contudo, as melhores taxas de nitrificação e melhor desempenho zootécnico ocorreram nos tratamentos com maior alcalinidade. No capítulo 6 para a correção da alcalinidade, pH e CO₂ pode ser aplicado dosagens de 0,05 g/L de hidróxido de cálcio ou aplicações diárias entre 10 e 20% da quantidade de ração ofertada aos camarões. Os resultados obtidos poderão auxiliar no dia a dia dos produtores de camarões em sistemas BFT.

ABSTRACT

The Biofloc technology (BFT) is the system of production of aquatic organisms in high stocking densities without water renewal, using strong aeration and predominantly aerobic and heterotrophic biota that form microbial flakes. These systems are considered biosafe and raise cultivation productivity due to the nutritional supplement provided by the bioflocs. As there is no renewal of water along the cultivation and the water can be reused for several cycles of production, the levels of alkalinity and pH are reduced and carbon dioxide (CO₂) derived from the respiration of shrimp and bioflocs can be accumulated. Changes in the quality of cultivation water can generate stress and reduce immune system responses and the growth and survival of the shrimp. Thus, this study aimed to improve cultivation techniques of *Litopenaeus vannamei* in biofloc systems without water renewal by means of comprehension and correction of carbon dioxide, alkalinity and pH levels. To this end, five experiments were performed at the Marine Aquaculture Station, Federal University of Rio Grande (EMA - FURG - Brazil) and one experiment was performed at the National Autonomous University of Mexico (UMDI-Sisal-UNAM): 1. Determination of the lethal concentration of acid and basic pH (pH_{50-96h}) and assessment of the sublethal effect of the pH on oxidative stress parameters in juveniles of *L. vannamei*; 2. Determination of the lethal concentration and the safety level of carbon dioxide for juveniles of *L. vannamei*; 3. Verification of the CO₂ concentrations and suspended solids at different stocking densities when there is a failure in the aeration system and hydrogen peroxide (H₂O₂) is applied as a source of oxygen in the BFT system; 4. Evaluation of the effect of CO₂ in the oxygen consumption of *L. vannamei* juveniles reared in the BFT system; 5. Evaluation of the effect of different levels of alkalinity in the parameters of water quality, in the formation of bioflocs and in the zootechnical performance of *L. vannamei* reared in the BFT system; 6. Determination of the optimum dosage of calcium hydroxide (Ca(OH)₂) in the parameters of water quality and growth performance of *L. vannamei* cultivated in the BFT system. Under the experimental conditions described in Chapter 1, the pH_{50 96 h} results of acid and basic pH for *L. vannamei* juveniles were 4.04 and 9.58. As for the oxidative stress test, we found out that the shrimp exposed to both pH 4.5 and pH 9.5 presented an imbalance in the activity of antioxidant enzymes. In Chapter 2, LC₅₀ values with 95% confidence limits in 24, 48, 72 and 96 h were 130.05 (104.2-162.1), 77.2 (73.8-80.02), 69.65 (65.47-74.32), 59.12 (53.08-66.07) mg/L of CO₂, respectively. Thus, the calculated safety level for *L. vannamei* was 5.9 mg/L of CO₂. In Chapter 3 we noted that CO₂ levels increased with the increments of biomass and total suspended solids over time, reaching sublethal levels of CO₂ for the shrimp. In Chapter 4 an increase in oxygen consumption of immature fish was observed at concentrations up to 60 mgCO₂/L and a reduction in the oxygen consumption at the concentrations of 95, 150 and 300 mgCO₂/L, followed by anesthesia symptoms and mortality. In Chapter 5 bioflocs were formed in alkalinity and pH levels greater than 70 mgCaCO₃/L and 7.38, respectively. However, the best rates of nitrification and zootechnical performance occurred in the treatment with the highest levels of alkalinity. In Chapter 6, 0.05 g/L of calcium hydroxide or daily doses of 10 and 20% of the amount of feed offered to the shrimp were applied to correct the alkalinity, pH and CO₂. The results obtained could assist the daily routine of shrimp farmers using BFT systems.

1 INTRODUÇÃO GERAL

2

3 A tecnologia de bioflocos surge para a aquicultura como uma ferramenta
4 sustentável que lida simultaneamente com questões ambientais, sociais e econômicas
5 conjuntamente com seu crescimento (Crab et al. 2012). Os cultivos de organismos
6 aquáticos sem renovação de água utilizando altas densidades de estocagem, com
7 aeração constante e intensa, com grande diversidade de microorganismos aeróbicos
8 (fotoautotróficos, quimioautotróficos e heterotróficos) formadores de flocos
9 microbianos são conhecidos como tecnologia de bioflocos (BFT) (Hargreaves 2006; De
10 Schryver et al. 2008; Avnimelech 2009).

11 A formação dos bioflocos se inicia a partir da manipulação da relação
12 carbono:nitrogênio (C:N), de modo que essa razão em aproximadamente 15-20:1
13 favorece a assimilação do nitrogênio amoniacal pelas bactérias heterotróficas que
14 possuem capacidade de síntese protéica a partir de carbono orgânico e amônia
15 (Avnimelech 1999; Xu et al. 2013). Segundo Samocha et al. (2007), a adição de fontes
16 de carbono orgânico, como o melaço de cana de açúcar, pode ser empregada para
17 ajustar a relação C:N evitando o aumento das concentrações de nitrogênio amoniacal
18 total durante o cultivo de *Litopenaeus vannamei* em sistemas BFT. Por exemplo,
19 Wasielesky et al. (2006a) concluíram que o material particulado suspenso em sistemas
20 de cultivo de *L. vannamei* com tecnologia BFT pode melhorar significativamente a
21 conversão alimentar, reduzindo custos de produção.

22 Os bioflocos melhoraram a sobrevivência e desempenho de crescimento dos
23 camarões cultivados pela melhor qualidade de água, pelo fornecimento de uma fonte de
24 suplemento alimentar e por exercer efeito positivo sobre atividade das enzimas

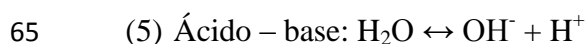
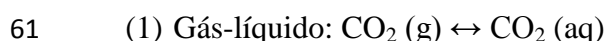
25 digestivas do camarão, melhorando a digestão e utilização desse alimento (Xu & Pan,
26 2012; Xu et al. 2013). Além disso, Silva et al. (2013) ao realizar um balanço de massa
27 do nitrogênio disponível em um sistema de bioflocos observou que os camarões
28 absorvem acima de 40% do nitrogênio proteico disponibilizado, enquanto que no
29 sistema convencional os camarões absorvem de 12 a 15%.

30 Verificando que no sistema BFT ocorre melhor aproveitamento do nitrogênio
31 proteico, torna-se razoável supor que a água de cultivo, rica em bioflocos não deva ser
32 descartada. Pensando nisso, Krummenauer et al. (2012) avaliaram a utilização de
33 diferentes porcentagens de reúso de água de um cultivo e verificaram que um inóculo
34 mínimo (2,5%) acelera a formação dos agregados microbianos em sistemas BFT,
35 evitando concentrações elevadas de amônia e nitrito. Além disso, o tratamento que
36 reutilizou 100% da água apresentou os melhores índices de sobrevivência e
37 crescimento, demonstrando ser uma estratégia adequada a ser adotada no cultivo de
38 camarões marinhos com bioflocos. Durante o cultivo em sistema BFT ocorre
39 incremento dos sólidos em suspensão e matéria orgânica a níveis superiores a 1000
40 mg/L, sendo necessária a remoção do excesso de bioflocos por meio de clarificação,
41 mantendo-os próximos a 500 mg/L, para evitar oclusão das brânquias dos camarões e
42 evitar o excesso de biomassa microbiana consumindo oxigênio (Gaona et al. 2011;
43 Schweitzer et al. 2013).

44 Visto que a melhor estratégia é a reutilização da água por vários ciclos de
45 produção e neste sistema não há renovação de água, a tendência natural é que ocorra o
46 incremento de sólidos em suspensão e a diminuição do material carbonático ao longo do
47 tempo. Conforme Ebeling et al. (2006) para cada grama de nitrogênio amoniacal
48 convertida em biomassa microbiana heterotrófica, são consumido 4,71g de oxigênio
49 dissolvido, 3,57g de alcalinidade e 15,17g de carboidratos, e são produzidos 8,07g de

50 biomassa microbiana e 9,65g de dióxido de carbono. Nos bioflocos e biofilmes estão
51 presentes ainda bactérias nitrificantes que ao oxidar a amônia a nitrato consomem
52 carbono inorgânico na forma de carbonatos e bicarbonatos, reduzindo os valores de
53 alcalinidade (Chen et al. 2006).

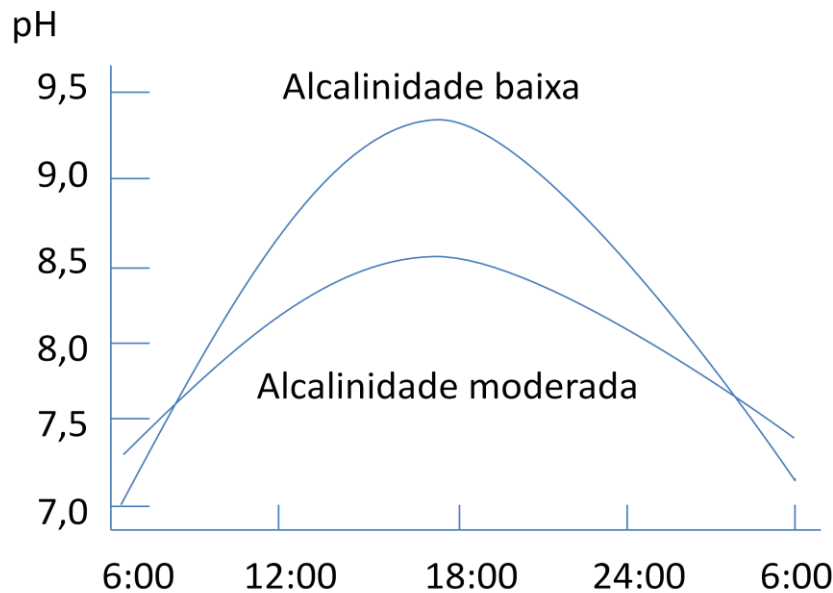
54 A alcalinidade da água se refere à concentração total de bases tituláveis da água
55 capazes de neutralizar cátions de hidrogênio, sendo os íons bicarbonato (HCO_3^-),
56 carbonato (CO_3^{2-}) e hidroxila (OH^-) as principais bases responsáveis pela alcalinidade
57 total da água, e é expressa em equivalentes de carbonato de cálcio ($\text{mg CaCO}_3/\text{L}$). A
58 alcalinidade total também é definida como a capacidade de tamponamento da água, ou
59 seja, a capacidade da água manter o equilíbrio ácido-base, como segue na equação
60 (Summerfelt et al. 2000; Vinatea 2004):



67 Para cada reação de equilíbrio (1, 2, 3, 4, 5) que compõe a equação acima (6)
68 existe uma constante de equilíbrio, por meio das quais é possível calcular as
69 concentrações de CO_2 , uma vez que tenhamos dados adicionais de pH, temperatura,
70 salinidade e alcalinidade (Summerfelt et al. 2000; Timmons & Ebeling 2010)

71 Segundo Boyd (2002) a alcalinidade elevada atenua a oscilação dos valores de
72 pH resultantes da respiração e fotossíntese (figura 1). Para Van Wyk & Scarpa (1999) a

73 alcalinidade da água para o cultivo de *L. vannamei* deve ser superior a 100 mg
74 CaCO_3/L .



75

76 Figura 1 – Oscilações diárias do pH em função da alcalinidade, adaptado de
77 Boyd (2002).

78 Não há um valor ideal de alcalinidade e sim um intervalo de valores adequados
79 para manter o pH com menor oscilação diária. Para Ebeling et al. (2006) em sistemas
80 com limitada troca de água a alcalinidade deve estar entre 100-150 mg CaCO_3/L ,
81 enquanto Boyd & Tucker (1998) sugerem valores superiores a 75 mg CaCO_3/L . Por
82 exemplo, Furtado et al. (2011) verificaram que alcalinidade abaixo de 75 mg CaCO_3/L e
83 de pH abaixo de 7, afetaram negativamente o crescimento dos camarões e a taxa de
84 nitrificação no sistema BFT. Já Xu et al. (2013), avaliando diferentes relações de C/N
85 em cultivos de *L. vannamei* em sistema BFT verificaram a redução da alcalinidade e pH,
86 para elevar esses parâmetros aplicaram bicarbonato de sódio. O bicarbonato de sódio é
87 um produto de pureza elevada e eficaz para elevar a alcalinidade, porém possui um
88 custo mais elevado do que o hidróxido de cálcio (Ca(OH)_2).

89 Para a elevação da alcalinidade e pH Furtado et al. (2011), testaram diferentes
90 compostos alcalinizantes e verificaram que o hidróxido de cálcio (Ca(OH)_2) resultou na
91 melhor relação custo-benefício quando comparado com o carbonato de sódio (Na_2CO_3)
92 e com o bicarbonato de sódio (NaHCO_3), já que proporcionou condições físicas e
93 químicas da qualidade da água favoráveis para o desenvolvimento dos bioflocos e
94 crescimento dos juvenis de *Litopenaeus vannamei*, apresentando menor custo de
95 correção da alcalinidade e do pH.

96 Segundo Villaverde et al. (1997), a faixa ótima de pH para nitrificação pode ser
97 determinada por três diferentes efeitos que o pH pode exercer sobre as bactérias
98 nitrificantes: Ativação-desativação das bactérias nitrificantes; efeitos nutricionais
99 conectados com a alcalinidade (carbono inorgânico); e inibição das bactérias por
100 amônia livre e ácido nitroso livre. No que diz respeito ao processo de nitrificação, o pH
101 ótimo deve estar em uma faixa que varia de acordo com o gênero das bactérias
102 nitrificantes para o crescimento das mesmas. Por exemplo, as *Nitrossomonas*, que
103 oxidam a amônia a nitrito (AOB), a faixa ótima varia entre 7,8 – 8,8 e para as
104 *Nitrobacter*, que oxidam nitrito a nitrato (NOB), variando entre 7,2 – 9,0 (Chen et al.
105 2006).

106 Os camarões marinhos apresentam seu melhor desenvolvimento em águas com
107 pH entre 7,0 e 9,0 (Van Wyk & Scarpa 1999). O pH é definido como o logaritmo
108 negativo da concentração do íon hidrogênio (H^+) a partir do qual se expressa o grau de
109 acidez (<7), neutralidade (7) ou alcalinidade (7-14) de um ambiente (Ville 1967). O pH
110 é um dos principais parâmetros de qualidade da água, pois influencia em quase todas as
111 reações químicas que acontecem na água e inclusive sobre as condições fisiológicas do
112 camarão (Boyd 2001; Vinatea 2004; Lemonnier et al. 2004). O pH também exerce uma
113 forte influência sobre a toxicidade de certos parâmetros químicos, tais como a amônia

114 não ionizada (NH_3) mais tóxica para os camarões, que se torna mais abundante em pH
115 alcalino, e o ácido sulfídrico (H_2S), que aumenta proporcionalmente em pH ácido
116 (Vinatea 2004). Para Boyd (2001) viveiros de cultivo de camarão apresentam melhor
117 produtividade com valores de pH entre 7 e 8,5.

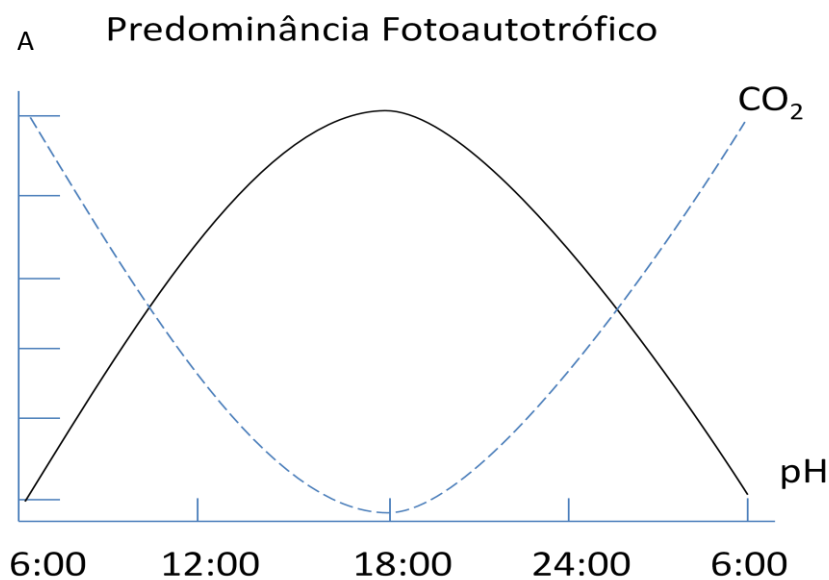
118 Os crustáceos possuem mecanismos de equilíbrio ácido-base da hemolinfa, que
119 está baseado na troca de íons através das brânquias, essas trocas são realizadas pela
120 Na^+/K^+ -ATPase basolateral e possivelmente pela H^+ -ATPase apical (Henry et al. 1981).
121 Juvenis de *L. vannamei* cultivados em sistemas de bioflocos com pH 5.1 apresentaram
122 diminuição no crescimento, entretanto com níveis de sobrevivência de 98% (Wasielesky
123 et al. 2006b).

124 Porém, uma diminuição ou aumento do pH para 6,5 e 10,1 no cultivo de *L.*
125 *vannamei* provoca imunodepressão dos camarões tornando-os mais suscetíveis ao
126 *Vibrio alginolyticus* quando comparados com pH 8,2 (Li & Chen 2008). Variações
127 bruscas de pH além de tornar os camarões mais suscetíveis a patógenos podem ter efeito
128 bactericida, como constatado por Ganguly et al. (1999). Portanto, antes de realizar a
129 correção do pH é necessário conhecer as características químicas (poder de
130 neutralização, taxa de reatividade e poder relativo de neutralização total) do produto a
131 ser aplicado, bem como calcular a dosagem do produto para evitar elevação brusca do
132 pH e seus efeitos bactericidas.

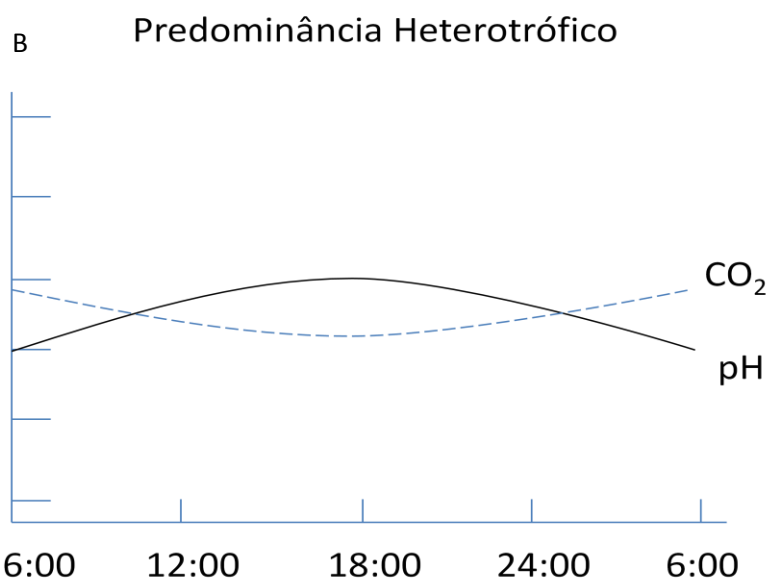
133 O dióxido de carbono dissolvido é tanto um nutriente, como um resíduo em
134 viveiros de cultivo. Uma vez que na fotossíntese serve como fonte de carbono
135 inorgânico que será convertido em carbono orgânico na forma de um açúcar simples. Já
136 na respiração será excretado como produto final, ocasionando uma reação de
137 acidificação da água com liberação de H^+ e HCO_3^- (Boyd 2002, 2008). Inicialmente, as

138 condições de um cultivo são predominantemente fotoautotróficas e os níveis de CO₂
139 podem flutuar entre 5 - 10 mg/L pela manhã, mas podem exceder 20 mg/L em viveiros
140 com biomassa elevada (Boyd 2008).

141 Durante um período de 24 horas ocorrem flutuações dos níveis de oxigênio
142 dissolvido (OD), pH e dióxido de carbono, haja visto que durante a fase clara do dia
143 ocorre a remoção do CO₂ e elevação do pH e OD, decorrentes dos processos de
144 fotossíntese (figura 2A). Já durante a noite cessa a fotossíntese e predomina a respiração
145 com a liberação de CO₂ por parte dos organismos aeróbicos, reduzindo o pH e níveis de
146 OD do meio de cultivo (Vinatea 2004). Com o passar do tempo de cultivo, as
147 concentrações de sólidos suspensos totais aumentam e a comunidade bacteriana
148 heterotrófica passa a ser predominante (figura 2B). Nesta situação ocorre uma atenuação
149 da oscilação diária nos valores de pH, visto que o processo de fotossíntese é reduzido
150 pela turbidez elevada e consequente redução da luminosidade na coluna d'água (Vinatea
151 et al. 2010; Furtado et al. 2011).



152



154 Figura 2 – Oscilações diárias do pH e CO₂, onde A- fase inicial de cultivo com
 155 predominância fotoautotrófico; e B- Incremento de sólidos em suspensão com
 156 predominância heterotrófica.

157 Em cultivos de peixes e camarões as concentrações de CO₂ incrementam-se
 158 principalmente devido a biomassa de organismos cultivados, de matéria orgânica em
 159 decomposição no ambiente de cultivo, pela não renovação da água e ineficiência do
 160 sistema de aeração (Timmons & Ebeling 2010; Furtado et al. 2011). Quando a
 161 concentração de CO₂ é superior a 20 mg/L dificulta a excreção do CO₂ pelo epitélio
 162 branquial, resultando no declínio do pH, afetando negativamente a capacidade do
 163 pigmento respiratório em transportar oxigênio e assim, reduzindo a oxigenação nos
 164 tecidos (hipóxia) e conseqüentemente, aumentando a taxa de ventilação (Taylor &
 165 Whiteley 1989; Van Wyk & Scarpa 1999).

166 Para Van Wyk & Scarpa (1999) níveis de CO₂ na água inferiores a 5 mg/L são
 167 ideais, já 20 mg/L são considerados aceitáveis para peneídeos, enquanto concentrações
 168 entre 20 e 60 mg/L não são letais mas causam interferências na troca de CO₂ nas
 169 brânquias. Em concentrações superiores a 60 mg/L podem ser letais. Para Boyd (2008)

170 organismos aquáticos expostos a concentrações de até 60 mg/L podem sofrer efeitos
171 narcóticos. Por exemplo, Wickins (1984), ao estudar os efeitos da hipercapnia, ou seja,
172 alto nível de CO₂ na hemolinfa, verificou o retardo da muda e a diminuição do
173 crescimento de peneídeos cultivados em água com pH inferior a 7,3.

174 De acordo com Vinatea et al. (2009), em cultivos de *L. vannamei* em sistemas
175 BFT os níveis de oxigênio dissolvido na água, na ausência de aeração, tornam-se
176 críticos em aproximadamente 30 minutos. Nessas condições o peróxido de hidrogênio
177 (H₂O₂) pode ser uma fonte alternativa de oxigênio emergencial para os camarões
178 (Furtado et al. 2014). Porém, como não ocorre a quebra da tensão superficial da água o
179 oxigênio dissolvido consumido é liberado como CO₂ e esse incrementa até o ponto de
180 equilíbrio de troca com a atmosfera, gerando uma condição de
181 hipóxia/normóxia/hipercapnia que favorecem a produção de espécies reativas de
182 oxigênio (EROs), podendo causar estresse oxidativo (Vinatea et al. 2009; Lushchak
183 2011).

184 Em condições de estresse ambiental, um aumento na taxa de respiração pode
185 indicar aumento no consumo energético para a manutenção da homeostase. Conforme
186 Hall & Van Ham (1998), ao incorporar CO₂ na água há uma redução do pH gerando
187 mudanças comportamentais e aumento nos níveis de glicose na hemolinfa de *Penaeus*
188 *monodon*. Os dados de consumo de oxigênio são importantes para o cálculo efetivo da
189 potência de aeração a ser instalada nos tanques de cultivo de uma determinada espécie,
190 além de prover dados sobre o metabolismo dos organismos sob diferentes eventos
191 estressantes que possam surgir durante o cultivo (Bett & Vinatea 2009; Campos et al.
192 2014).

193 No intuito de aprimorar as técnicas de cultivo de camarões em sistemas BFT, é
194 relevante a realização de experimentos que avaliem os efeitos da alcalinidade, pH e
195 dióxido de carbono no cultivo do camarão marinho *Litopenaeus vannamei* com
196 tecnologia de bioflocos. Os resultados desse estudo podem fornecer dados para que
197 produtores de camarão em sistemas BFT melhorem a produtividade dos seus cultivos.

198

199 **OBJETIVOS**

200 **Objetivo geral**

201 Avaliar os efeitos da alcalinidade, pH e dióxido de carbono na qualidade da água
202 de cultivo, no crescimento e na sobrevivência do camarão marinho *Litopenaeus*
203 *vannamei*.

204

205 **Objetivos específicos**

206 - Determinar os níveis letais do pH ácido e básico (pH_{50-96h}) e avaliar o efeito
207 subletal do pH sobre os parâmetros de estresse oxidativo em juvenis de *L.*
208 *vannamei*;

209 - Determinar a concentração letal e o nível de segurança de dióxido de carbono
210 para juvenis de *L. vannamei*;

211 - Verificar as concentrações de CO₂ em diferentes densidades de cultivo e de
212 sólidos em suspensão quando ocorre falha no sistema de aeração e se aplica
213 peróxido de hidrogênio (H₂O₂) como fonte de oxigênio em sistema BFT;

214 - Avaliar o efeito do CO₂ no consumo de oxigênio de juvenis de *L. vannamei*;

- 215 - Avaliar o efeito de diferentes níveis de alcalinidade nos parâmetros de
216 qualidade da água, na formação dos bioflocos e no desempenho zootécnico de *L.*
217 *vannamei* cultivados em sistema BFT;
- 218 - Determinar a melhor dosagem de hidróxido de cálcio (Ca(OH)₂) nos
219 parâmetros de qualidade da água e desempenho zootécnico de *L. vannamei*
220 cultivados em sistema BFT.

221

222 **REFERÊNCIAS BIBLIOGRÁFICAS**

- 223 AVNIMELECH, Y. 1999. Carbon/nitrogen ratio as a control element in aquaculture
224 systems. *Aquaculture*, 176: 227-235.
- 225 AVNIMELECH, Y. 2009. *Biofloc Technology — A Practical Guide Book*. The World
226 Aquaculture Society, Baton Rouge, Louisiana, United States. 182p.
- 227 BETT, C & L VINATEA. 2009. Combined effect of body weight, temperature and
228 salinity on shrimp *Litopenaeus vannamei* oxygen consumption rate. *Braz. J.*
229 *Oceanogr.*, 57(4): 305-314.
- 230 BOYD, C & C TUCKER. 1998. *Pond Aquaculture: Water Quality Management*.
231 Boston: Kluwer Academic Publishers. 700p.
- 232 BOYD, CE. 2001. Water Quality Standards: pH. *Glob. Aquac. Advoc.* February: 42-44.
- 233 BOYD, CE. 2002. Understanding Pond pH. *Glob. Aquac. Advoc.* June: 74-75.
- 234 BOYD, CE. 2008. Carbon Dioxide: Waste, Nutrient. *Glob. Aquac. Advoc.* July/August:
235 62-65.
- 236 CAMPOS, BR, PS FURTADO, F D'INCAO, LH POERSCH & WJr WASIELESKY.
237 2014. The effect of ammonia, nitrite and nitrate on the oxygen consumption of

238 juvenile pinkshrimp *Farfantepenaeus brasiliensis* (LATREILLE 1817)
239 (CRUSTACEA: DECAPODA). J. Appl. Aquacult., 26(1): 94-101.

240 CHEN, S, J LING & JP BLANCHETON. 2006. Nitrification kinetics of biofilm as
241 affected by water quality factors. Aquacult. Eng., 34: 179–197.

242 CRAB, R, T DEFOIRDT, P BOSSIER & W VERSTRAETE. 2012. Biofloc technology
243 in aquaculture: Beneficial effects and future challenges. Aquaculture, 356-357: 351-
244 356.

245 De SCHRYVER, P, R CRAB, T DEFOIRDT, N BOON & W VERSTRAETE. 2008.
246 The basics of bio-flocs technology: the added value for aquaculture. Aquaculture,
247 277: 125–137.

248 EBELING, JM, MB TIMMONS & JJ BISOGNI. 2006. Engineering analysis of the
249 stoichiometry of photoautotrophic, autotrophic, and heterotrophic control of
250 ammonia-nitrogen in aquaculture production systems. Aquaculture, 257: 346-358.

251 FURTADO, PS, LH POERSCH & WJr WASIELESKY. 2011. Effect of Calcium
252 Hydroxide, Carbonate and Sodium Bicarbonate on Water Quality and Zootechnical
253 Performance of Shrimp *Litopenaeus vannamei* Reared in Bio-Flocs Technology
254 (BFT) Systems. Aquaculture, 321: 130-135.

255 FURTADO, PS, FP SERRA, LH POERSCH & WJr WASIELESKY. 2014. Short
256 communication: Acute toxicity of hydrogen peroxide in juvenile white shrimp
257 *Litopenaeus vannamei* reared in biofloc technology systems. Aquacult Int., 22(2):
258 653-659.

259 GANGULY, S, J CHATTERJEE & BB JANA. 1999. Biogeochemical cycling bacterial
260 activity in response to lime and fertilizer applications in pond systems. Aquacult.
261 Int., 7: 413–432.

262 GAONA, CAP, LH POERSCH, D KRUMMENAUER, GK FOES, & WJ
263 WASIELESKY. 2011. The effect of solids removal on water quality, growth and
264 survival of *Litopenaeus vannamei* in a biofloc technology culture system. IJRA, 12:
265 54-73.

266 HALL, MR & EH VAN HAM. 1998. The effects of different types of stress on blood
267 glucose in the giant tiger prawn *Penaeus monodon*. J. World Aquac. Soc., 29(3):
268 290-299.

269 HARGREAVES, JA. 2006. Photosynthetic suspended-growth systems in aquaculture.
270 Aquacul. Eng., 34: 344–363.

271 HENRY, R, G KORMANIK, N SMATRESK & J CAMERON. 1981. The role of
272 CaCO₃ dissolution as a source of HCO₃⁻ for the buffering of hypercapnic acidosis in
273 aquatic and terrestrial decapod crustaceans. J. Exp. Biol., 94: 269-274.

274 KRUMMENAUER, D, CA Jr SEIFERT, LH POERSCH, GK FOES, GR LARA & WJr
275 WASIELESKY. 2012. Cultivo de camarões marinhos em sistema de bioflocos:
276 análise da reutilização da água. Atlantica, 34(2): 103-111.

277 LEMONNIER, H, E BERNARD, E BOGLIO, C GOARANT & J COCHARD. 2004.
278 Influence of sediment characteristics on shrimp physiology: pH as principal effect.
279 Aquaculture, 240: 297-312.

280 LI, C & JC CHEN. 2008. The immune response of white shrimp *Litopenaeus vannamei*
281 and its susceptibility to *Vibrio alginolyticus* under low and high pH stress. Fish &
282 Shellfish Immunol., 25: 701-709.

283 LUSHCHAK, VI. 2011. Environmentally induced oxidative stress in aquatic animals.
284 Aquatic. Toxicol., 101: 13–30.

285 SAMOCHA, TM, S PATNAIK, M SPEED, AM ALI, JM BURGER, RV ALMEIDA, Z
286 AYUB, M HARISANTO, A HOROWITZ & DL BROOK. 2007. Use of molasses as

287 carbon source in limited discharge nursery and grow-out systems for *Litopenaeus*
288 *vanammei*. Aquacult. Eng., 36: 184-191.

289 SCHVEITZER, R, R ARANTES, PFS COSTÓDIO, CME SANTO, LA VINATEA,
290 WQ SEIFFERT & ER ANDREATTA. 2013. Effect of different biofloc levels on
291 microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank
292 system operated with no water exchange. Aquacult. Eng., 56: 59-70.

293 SILVA, KR, WJ WASIELESKY & PC ABREU. 2013. Nitrogen and phosphorus
294 dynamics in the biofloc production of the pacific white shrimp, *Litopenaeus*
295 *vannamei*. J. World Aquacult. Soc., 44: 30-41.

296 SUMMERFELT, ST, BJ VINCI & RH PIEDRAHITA. 2000. Oxygenation and carbon
297 dioxide control in water reuse systems. Aquacult. Eng., 22: 87-108.

298 TAYLOR, EW & NM WHITELEY. 1989. Oxygen transport and acid–base balance in
299 the haemolymph of the lobster, *Homarus gammarus*, during aerial exposure and
300 resubmersion. J. Exp. Biol., 144: 417–436.

301 TIMMONS, MB & JM EBELING. 2010. Recirculating Aquaculture, 2nd edition.
302 Cayuga Aqua Ventures, Ithaca, NY. <http://www.bee.cornell.edu/aquain18/11/2010>.
303 998 p.

304 VAN WYK, P., SCARPA, J., 1999. Water quality and management. In: VAN WYK, P.
305 et al. (Eds.). Farming marine shrimp in recirculating freshwater systems. Florida
306 department of agriculture and consumer services, Tallahassee, Florida, 141-161.

307 VILLE, C. 1967. Biología. Editorial Interamericana S.A. México, 688p.

308 VILLAVERDE, S, PA GARCIA-ENCINA & F FDZ-POLANCO. 1997. Influence of
309 pH over nitrifying biofilm activity in submerged biofilters. Water Res., 31: 1180-
310 1186.

311 VINATEA, LA. 2004. Princípios químicos de qualidade da água em aquicultura: uma
312 revisão para peixes e camarões. 2ª edição revisada e ampliada, Editora UFSC. 231p.

313 VINATEA, L, AO GÁLVEZ, J VENERO, J LEFFLER & C BROWDY. 2009. Oxygen
314 consumption of *Litopenaeus vannamei* juveniles in heterotrophic medium with zero
315 water exchange. *Pesq. Agropec. Bras.*, 44(5): 534-538.

316 VINATEA, L, AO GALVEZ, CL BROWDY, A STOKES, J VENERO, J HAVEMAN,
317 BL LEWIS, A LAWSON, A SHULER & JW LEFFLER. 2010. Photosynthesis,
318 water respiration and growth performance of *Litopenaeus vannamei* in a super-
319 intensive raceway culture with zero water exchange: Interaction of water quality
320 variables. *Aquacult. Eng.*, 42: 17–24.

321 WASIELESKY, W, H ATWOOD, A STOKES & CL BROWDY. 2006a. Effect of
322 natural production in a zero exchange suspended microbial floc based super-
323 intensive culture system for white shrimp *Litopenaeus vannamei*. *Aquaculture*, 258:
324 396-408.

325 WASIELESKY, W, H ATWOOD, R KEGL, J BRUCE, A STOKES & CL BROWDY.
326 2006b. Efeito do ph na sobrevivência e crescimento do camarão branco *Litopenaeus*
327 *vannamei* em cultivos superintensivos. *Aquaciência 2006. Anais do congresso*.

328 WICKINS, JF. 1984. The effect of reduced pH on carapace calcium, strontium and
329 magnesium levels in rapidly growing prawns (*Penaeus monodon*). *Aquaculture*, 41:
330 49–60.

331 XU, WJ & LQ PAN. 2012. Effects of bioflocs on growth performance, digestive
332 enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-
333 water exchange tanks manipulating C/N ratio in feed. *Aquaculture*, 356-357: 147–
334 152.

335 XU, WJ, LQ PAN, XH SUN & J HUANG. 2013. Effects of bioflocs on water quality,
336 and survival, growth and digestive enzyme activities of *Litopenaeus vannamei*
337 (Boone) in zero-water exchange culture tanks. Aquacult. Res., 44: 1093–1102.

338

339

CAPÍTULO I

340

341 **Acute Effect of pH and its Influences on the Survival and Biochemical Biomarkers**
342 **of Juvenile White Shrimp Litopenaeus vannamei.**

343

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356

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358 **Resumo**

359 O camarão Branco do Pacífico *Litopenaeus vannamei* é cultivado em diferentes tipos de
360 tanques e densidades de estocagem, com e sem renovação de água, com qualidade de
361 água excelente ou de baixa qualidade em todos os continentes. Mudanças no pH da água
362 podem ocasionar efeitos subletais nos camarões, como alterações fisiológicas teciduais.
363 O objetivo desse estudo foi determinar os níveis letais de pH ácido e básico (pH_{50-96h}) e
364 avaliar os efeitos subletais do pH na atividade enzimática do sistema antioxidante de
365 juvenis de *L. vannamei*. O desenho experimental constituiu de nove tratamentos com
366 três repetições cada (36 camarões por tratamento), sendo os níveis de pH
367 aproximadamente 10,5; 10,0; 9,5; 9,0; 7,0; 5,0; 4,5; 4,0 e 3,5, em que o tratamento 7,0
368 foi considerado como controle. Os valores subletais de pH foram selecionados de
369 acordo com os resultados do ensaio de toxicidade aguda. Três tratamentos com três
370 repetições cada foram utilizados (36 camarões por tratamento): pH ácido- 4,5, pH
371 neutro- 7,0 (controle) e pH básico- 9,5. Para cada pH experimental e ponto de
372 amostragem (tempo inicial ou 0, 12, 24, e 36 h), a hemolinfa de seis camarões foi
373 coletada para ensaio de atividade enzimática de Catalase, Glutathione S- transferase e
374 capacidade antioxidante total. O monitoramento do pH foi conduzido a cada quatro
375 horas, totalizando seis vezes por dia (04:00, 08:00, 12:00, 16:00, 20:00, 24:00 h). Os
376 valores de pH₅₀ 24, 48, 72 e 96 hours, foram de pH 9.82, 9.62, 9.59 e 9.58; e 3.86, 3.92,
377 3.94 e 4.04, respectivamente. Nossos resultados demonstram que os camarões dessa
378 espécie expostos ao estresse de pH ácido e básico apresentaram estresse oxidativo com
379 distúrbio na atividade enzimática do sistema protetor antioxidante para remover
380 peróxido de hidrogênio. Contudo verificamos que os juvenis de *L. vannamei* apresentam
381 maior sensibilidade ao pH básico do que ao pH ácido.

382

383

Abstract

384 The Pacific white shrimp Litopenaeus vannamei is reared in several types of tanks
385 and densities, with and without water exchange and with best or poor water quality. pH
386 changes in water can present sublethal effects in shrimp, causing physiological changes
387 in tissues. The aim of the present study was to determine the lethal level of acidic and
388 basic pH (pH_{50-96h}) and to evaluate the sublethal effects of pH on the oxidative stress
389 parameters in juvenile L. vannamei. The experimental design was composed of nine
390 treatments with three repetitions each (36 shrimps per treatment). The nine treatments
391 consisted of pH levels at approximately 10.5, 10.0, 9.5, 9.0, 7.0, 5.0, 4.5, 4.0 and 3.5,
392 where the treatment at 7.0 was considered the control. The sublethal pH values were
393 selected according to the results of an acute toxicity assay previously performed. Three
394 treatments with three repetitions each were used (36 shrimps per treatment): acidic pH -
395 4.5, neutral pH - 7.0 (control) and basic pH - 9.5. For each experimental pH and
396 sampling point (initial time or 0, 12, 24, and 36 h), the hemolymph of six shrimp were
397 collected to determine catalase and glutathione S-transferase activity and total
398 antioxidant capacity via enzymatic assays. The monitoring pH was performed every
399 four hours, totaling six times per day (0400, 0800, 1200, 1600, 2000 and 2400 h). At
400 pH₅₀ at 24, 48, 72 and 96 h, juvenile L. vannamei had basic pH values equal to 9.82,
401 9.62, 9.59 and 9.58 and acidic pH values of 3.86, 3.92, 3.94 and 4.04, respectively. Our
402 results demonstrated that shrimp exposed to acidic and basic pH stress presented
403 oxidative stress with a disturbance in antioxidant activity to scavenge hydrogen
404 peroxide. L. vannamei juveniles were more sensitive to basic pH.

405

406

407

408 The Pacific white shrimp Litopenaeus vannamei is the most cultivated crustacean
409 worldwide (FAO 2014). This species is reared in traditional rearing systems that occupy
410 large territorial areas in coastal regions and require high rates of water renewal
411 (Sampaio et al. 2010). During the rearing of aquatic organisms, the bottoms of the
412 ponds accumulate nutrients and organic residues, generating anoxic conditions and
413 potentially acidifying the water (Vinatea 2004; Lemonnier et al. 2004). This
414 acidification influences the balance of certain substances in water and soil, such as
415 ammonia, nitrite, hydrogen sulfide, chlorine and some metals (Van Wyk and Scarpa
416 1999; Chen and Cheng 2000). Acidification also influences the physiological conditions
417 of the shrimp (Wang et al. 2002; Li and Chen 2008; Zhou et al. 2009).

418 In rearing nurseries that are predominantly photoautotrophic, pH levels oscillate
419 throughout the day because during the light phase of photosynthesis, dissolved carbon
420 dioxide (CO₂) is removed by phytoplankton, elevating the pH. In the dark phase of
421 photosynthesis, there is no CO₂ removal, but a liberation by the animals and the
422 phytoplankton, reducing the pH (Boyd 2008). In rearing systems with biofloc
423 technology (BFT system), the nurseries are waterproofed with a geomembrane,
424 nullifying the influence of soil, and the organic matter is kept suspended through strong
425 aeration. However, due to high rates of respiration (bioflocs + shrimp) and metabolic
426 processes, pH and alkalinity tend to reduce throughout rearing (Ebeling et al. 2006;
427 Vinatea et al. 2010). Therefore, rearing in a BFT system requires the use of alkalizing
428 compounds (sodium bicarbonate or calcium hydroxide) to correct pH and alkalinity
429 levels throughout rearing (Furtado et al. 2011).

430 According to Van Wyk and Scarpa (1999), marine shrimp development best in
431 waters with a pH ranging from 7 to 9. In addition, decapod crustaceans have the ability
432 to tolerate basic and slightly acidic conditions due to the exchange of Na⁺/H⁺ and Cl⁻

433 /HCO₃⁻ that occurs in the gills of these animals (Henry et al. 1981). Zhou et al. (2009)
434 found a higher mortality in juvenile L. vannamei exposed for 24 h to pH 9.3 than in
435 those exposed to pH 5.6, and no mortality occurred at pH 7.4. According to Allan and
436 Maguire (1992), the pH_{50-96h} for acidic pH in juvenile Penaeus monodon was 3.7
437 (confidence interval of 95%: 3.4 and 4.1) in a salinity of 32 ppt.

438 Juvenile L. vannamei reared in biofloc systems at pH 5.1 decreased in growth
439 but exhibited a survival level of 98% (Wasielesky et al. 2006). However, a decrease or
440 increase of pH to 6.5 or 10.1 during L. vannamei rearing made the animals more
441 susceptible to Vibrio alginolyticus when compared to pH 8.2 (Li and Chen 2008). In
442 addition, a change in pH can create favorable conditions for the production of reactive
443 oxygen species (ROS) in shrimp cells, generating oxidative stress and damaging cellular
444 structures, such as membranes lipid, proteins and DNA (Lushchak 2011; Wang et al.
445 2012). Therefore, the aim of the present study was to determine the lethal level of acidic
446 and basic pH (pH_{50-96h}) and to evaluate the sublethal effects of pH on the oxidative
447 stress parameters in juvenile L. vannamei.

448

449 Materials and Methods

450 Preparation and Stocking

451 The experiment was conducted at the Marine Station of Aquaculture (EMA) of
452 the Institute of Oceanography at the Federal University of Rio Grande (FURG) in
453 Southern Brazil.

454 Shrimp were acquired from the Aquatec Ltda laboratory in Rio Grande do Norte,
455 Brazil. After the arrival of the post-larvae (pl₁₀) L. vannamei at the EMA facilities, the
456 shrimp were kept in the marine and cultivated hatchery shrimp sector. The shrimp were

457 stocked in a nursery (1500pl/m³) in a BFT system and reared until they reached an
458 average weight of 1 g. The shrimp were then harvested and stocked at a density of 300
459 shrimps/m³ until the average weight was 10.37 ± 1.58 g. The shrimp were stocked in a
460 1,000-L tank for a period of seven days to acclimate the animals to laboratory
461 conditions. After the acclimation period, the animals were transferred to the
462 experimental units (polyethylene rectangular tanks with 30 L of useful volume), where
463 12 shrimp were stocked (400 shrimps/m³). Animal feeding was suspended 24 h before
464 the beginning of the experimental period. The shrimp were fasted during exposure to
465 the different pH levels.

466 Acute Toxicity Assay

467 The experimental design was composed of nine treatments with three repetitions
468 each (36 shrimps per treatment), with the pH levels being approximately 10.5, 10.0, 9.5,
469 9.0, 7.0, 5.0, 4.5, 4.0 and 3.5, where treatment 7.0 was considered the control. The assay
470 was performed semi-statically, with partial exchange of the test solution every 24 h.
471 After removing shrimp feces from the tank, 20% of the water volume of the tank was
472 removed and was replaced with water similar to that used in the experimental units. The
473 experimental tanks with 30 L of useful volume had a porous stone to provide aeration
474 from an air blower (4 hp), and the temperature was maintained at 23 ± 1 C in a 27 C air
475 conditioned environment. The salinity was maintained at 25 ± 0.5 ppt, the luminous
476 intensity was maintained at 2,000 lux, and the photoperiod of the experimental room
477 was adjusted to 12 light:12 dark.

478 The water used in the tests was collected from Cassino Beach; the water was
479 filtered through a sand filter and subsequently through a 5-µm Cuno filter. The salinity
480 was adjusted to 25 ppt by diluting the filtered water with freshwater from the municipal

481 supply system, treating the water with a solution of 10 ppm chlorine and dechlorinating
482 the water with intense aeration and the application of one ppm ascorbic acid, two days
483 before using it. Before the shrimp were stocked in the experimental units, the pH for
484 each treatment was stabilized using hydrochloric acid (1 M HCl), with 38% purity, and
485 sodium hydroxide (1 M NaOH).

486 The temperature and oxygen determinations were performed daily, morning and
487 afternoon, using an oximeter model 55 YSI (Yellow Springs Instruments company, OH,
488 USA). Salinity was determined at the beginning and end of the experiment with an
489 optical refractometer (model 103 ATAGO Co., Tokyo, Japan). The concentrations of
490 total ammonia (TAN) ($\text{NH}_3 + \text{NH}_4^+$) and nitrite (NO_2^- -N) and alkalinity were measured
491 at the beginning and at the end of the experiment, according to methodologies
492 recommended by UNESCO (1983), Bendschneider and Robinson (1952) and APHA
493 (1998), respectively.

494 The pH was monitored using a pH meter (model WTW315i, WTW GmbH,
495 Weilheim, Germany) and was adjusted with hydrochloric acid or sodium hydroxide to
496 remain at the desired levels for each treatment. Monitoring was performed every four
497 hours, totaling six times per day (0400, 0800, 1200, 1600, 2000, 2400 h). During pH
498 monitoring, mortality was observed in each experimental unit. Shrimp were considered
499 dead when they did not respond to mechanical stimuli with a glass rod (Lin and Chen
500 2003). Dead shrimp were counted and removed from the tank every 24 h. The
501 cumulative mortality data in the 24, 48, 72 and 96 h periods were used to estimate the
502 average lethal pH (pH_{50}) and its respective confidence interval (95%). The Trimmed
503 Spearman Karber Method was used for this estimation (Hamilton et al. 1977).

504

Enzymatic Assays

505 The juvenile L. vannamei (10.50 ± 0.62 g) were transferred to nine polyethylene,
506 rectangular, 30-L (0.66 x 0.33 x 0.33 m) experimental units (12 shrimp/tank or 400
507 shrimp/m³). Sublethal pH values were selected according to the results of an acute
508 toxicity assay performed previously. Three treatments with three repetitions each were
509 used (36 shrimps per treatment): acidic pH - 4.5, neutral pH - 7.0 (control) and basic pH
510 - 9.5. The water from each treatment was prepared according to the description in Acute
511 Toxicity Assay.

512 For each experimental pH and sampling point (initial time or 0, 12, 24, and 36
513 h), the hemolymph of six shrimp from each treatment was collected directly from the
514 heart of the shrimp using sterile syringes containing an anticoagulant solution (0.1 M
515 glucose, 30 M sodium citrate, 0.026 M citric acid, 0.45 M NaCl, 0.02 M EDTA, pH 7.4)
516 (Sotherhall and Smith 1983) to avoid hemolymph coagulation.

517 For protein quantification and antioxidant enzyme analysis, hemolymph was
518 centrifuged twice at 500 and 900 x g at 4 C for 35 and 15 min, respectively, yielding a
519 cell pellet and cell lysate. After the centrifugation process, the cell lysate was re-
520 suspended in a 4 C buffer solution containing Tris base (20×10^{-3} M), EDTA (1×10^{-3}
521 M), dithiothreitol (1×10^{-3} M), KCl (150×10^{-3} M), and PMSF (0.1×10^{-3} M), with the pH
522 adjusted to 7.6, transferred to a 1.5-mL polyethylene tube and stored at -80 C in an
523 ultra-freezer. All enzymatic measurements were performed at least in triplicate. The
524 total protein content was determined by the Biuret method using a commercial kit
525 (Doles Reagents Ltd., Goiânia, GO, Brazil) and read at 550 nm using a microplate
526 reader (Victor 2, Perkin Elmer).

527 Catalase (CAT) catalyzes the dismutation of $2\text{H}_2\text{O}_2$ to O_2 and $2\text{H}_2\text{O}$. The
528 measurement of catalase activity was based on Beutler (1975). Cell lysate (10 μL) was
529 added to 990 μL of a reaction mixture (5 mM EDTA, 1 M Tris-HCl solution, pH 8.0)

530 and read spectrophotometrically at 230 nm. One CAT unit represented the amount of
531 enzyme needed to degrade 1 $\mu\text{mol H}_2\text{O}_2$ per min and per mg of total protein present in
532 the cell lysate at 30 C and pH 8.00.

533 Glutathione-S-transferase (GST) activity was determined by monitoring the
534 formation of a conjugate between 1 mM glutathione and 1 mM 1-chloro-2,4-
535 dinitrobenzene (CDNB) and read at 340 nm using a microplate reader (Victor 2, Perkin
536 Elmer) according to the methods of Habig et al. (1974) and Habig and Jakoby (1981).
537 The results were expressed as GST units mg protein^{-1} , where 1 unit (U) is defined as the
538 amount of enzyme that conjugates to 1 μmol of CDNB per min and per mg protein at 25
539 C and a pH 7.0.

540 The total antioxidant competence against peroxy radicals was analyzed by
541 quantifying ROS in the hemolymph as described by Amado et al. (2009). The total
542 fluorescence production was calculated by integrating the fluorescence units (FU) along
543 the time of the measurement after adjusting the FU data to a second order polynomial
544 function. The results were expressed as the area difference of $\text{FU} \times \text{min}$ in the same
545 sample with and without the addition of 2,2-azobis-2-methylpropionamide
546 dihydrochloride (ABAP) and were standardized to the ROS area without ABAP
547 (background area). The relative difference between the ROS area with and without
548 ABAP was considered a measurement of the antioxidant capacity. A large area
549 difference corresponded to a low antioxidant capacity because high fluorescence levels
550 were obtained after the addition of ABAP, indicating a low competency in neutralizing
551 peroxy radicals.

552

553

Statistical Analysis

554 Data were analyzed using one-way analysis of variance (ANOVA) followed by
 555 Tukey's HSD test and Newman-Keuls post-hoc mean comparisons for the toxicity and
 556 enzymatic assays, respectively. Assumptions of normality (Kolmogorov-Smirnov) and
 557 variance homogeneity (Levene) were previously checked. The significance level was
 558 fixed at $p < 0.05$ for all analyses.

559

560

Results

561 The results of the physical and chemical water quality parameters in this study
 562 are presented in Table 1. The pH differed significantly ($p < 0.05$) among the treatments,
 563 indicating the effectiveness of the control described in the methodology of this study.
 564 The alkalinity was significantly ($p < 0.05$) affected by the manipulation conducted to
 565 control the pH. There were no significant differences among the treatments ($p > 0.05$)
 566 for the other water quality parameters, and they remained in ideal conditions for the
 567 species.

568 Table 1. Water physico-chemical parameters values (means \pm SD) in the experimental
 569 tanks.

Treatments	pH	Temperature (C)	DO (mg/L)	Alkalinity (mgCaCO ₃ /L)	TAN (mg/L)	Nitrite (mg/L)
3.5	3.46 \pm 0.09 ^a	22.92 \pm 1.15	7.46 \pm 0.09	5.00 \pm 0.00 ^a	0.19 \pm 0.15	0.05 \pm 0.03
4.0	4.14 \pm 0.10 ^b	22.85 \pm 1.19	7.39 \pm 0.09	10.00 \pm 4.08 ^b	0.25 \pm 0.11	0.05 \pm 0.01
4.5	4.66 \pm 0.21 ^c	22.88 \pm 1.09	7.38 \pm 0.09	25.00 \pm 4.08 ^c	0.41 \pm 0.35	0.04 \pm 0.02
5.0	5.12 \pm 0.20 ^d	22.96 \pm 1.10	7.36 \pm 0.10	40.33 \pm 2.36 ^d	0.41 \pm 0.41	0.05 \pm 0.03
7.0	7.18 \pm 0.12 ^e	22.86 \pm 1.09	7.36 \pm 0.09	125.83 \pm 5.07 ^e	0.48 \pm 0.40	0.06 \pm 0.02

9.0	8.91±0.09 ^f	22.91±1.19	7.41±0.09	239.17±27.81 ^f	0.49±0.45	0.09±0.04
9.5	9.34±0.19 ^g	22.86±1.08	7.46±0.08	281.67±30.57 ^g	0.51±0.43	0.05±0.02
10.0	9.89±0.16 ^h	22.85±1.04	7.45±0.04	295.0±10.50 ^h	0.21±0.18	0.04±0.01
10.5	10.39±0.08 ⁱ	22.91±1.18	7.41±0.08	338.33±10.35 ⁱ	0.18±0.14	0.02±0.02

570 Different letters in the lines indicate significant differences among treatments by one-
571 way ANOVA ($p < 0.05$). DO: dissolved oxygen, TAN: total ammonia nitrogen

572

573 During the experimental period (Table 2), there was a decrease in survival in the
574 first 24 h of treatment with pH 10.5, 10.0, 4.0 and 3.5. The final survival was above
575 90% at pH levels between 9.5 and 4.5. The mortality and final survival values showed
576 that juvenile L. vannamei presented greater resistance to pH values higher than 4.5 and
577 lower than 9.5 in the 96 h exposure period.

578 Table 2. Cumulative mortality (%) and final survival (%) of juvenile L. vannamei
579 exposed to different pH levels throughout 96 h of exposure.

Treatments (pH)	Cumulative mortality (%)				Survival (%)
	24 h	48 h	72 h	96 h	
3.5	100.0	100.0	100.0	100.0	0.0
4.0	10.0	20.0	26.3	36.6	63.4
4.5	0.0	0.0	0.0	3.00	97.0
5.0	0.0	0.0	0.0	0.0	100.0
7.0	0.0	0.0	0.0	0.0	100.0
9.0	0.0	0.0	0.0	0.0	100.0
9.5	0.0	0.0	0.0	3.00	97.0
10.0	46.6	97.0	100.0	100.0	0.0
10.5	100.0	100.0	100.0	100.0	0.0

580

581

582 At 24, 48, 72 and 96 h in basic and acidic pHs, juvenile L. vannamei had pH₅₀
583 values equal to 9.82, 9.62, 9.59 and 9.58 and 3.86, 3.92, 3.94 and 4.04, respectively.
584 The values of pH₅₀ and the respective confidence limits are shown in Table 3. There
585 were significant differences ($p < 0.05$) between the pH₅₀ values at 24 and 96 hours in
586 acidic pH and basic pH.

587 Table 3. Average lethal value (pH₅₀) of acidic and basic pH and confidence limits of
588 95% calculated for juvenile L. vannamei exposed to different pH values throughout 96
589 hours.

Time (h)	pH ₅₀	Confidence limit (95%)
24	3.86	3.80 - 3.92 ^a
	9.82	9.74 - 9.90 ^A
48	3.92	3.83 - 4.01 ^{ab}
	9.62	9.58 - 9.76 ^{AB}
72	3.94	3.85 - 4.03 ^{ab}
	9.59	9.55 - 9.63 ^{AB}
96	4.04	3.94 - 4.14 ^b
	9.58	9.54 - 9.61 ^B

590 Different superscript letters in lowercase correspond to a significant difference ($p < 0.05$)
591 for the pH₅₀ values for acidic pH; Different superscript letters in uppercase correspond
592 to a significant difference ($p < 0.05$) for the pH₅₀ values for basic pH.

593

594

Enzymatic Assays

595 The results of each treatment were analyzed throughout the experimental period.
596 Shrimp exposed to pH 4.5, 7.0 and 9.5 showed significant differences ($p < 0.05$) in total
597 antioxidant capacity only during 36 hours of exposure. White shrimp subjected to pH

598 4.5 had a reduction in relative area: 158.28%, 205.96% and 211.73% when compared at
599 0 h, 12 h and 24 h, respectively (Fig. 1). L. vannamei exposed to pH 7 (control) at 36 h
600 decreased in relative area: 114.7%, 139.7% and 137.92% when compared at 0 h, 12 h
601 and 24 h, respectively. Shrimp exposed to pH 9.5 showed a lower relative area: 82.98%,
602 124.80 and 48.02% when compared at 0 h, 12 h and 24 h, respectively.

603 Shrimp exposed to pH 4.5 for 12 h exhibited significant differences ($p < 0.05$) in
604 CAT activity, which increased 58.14%, 134.48% and 58.14% when compared at 0 h, 24
605 h and 36 h, respectively (Fig. 2). White shrimp in the control group at 36 h had a
606 decrease in CAT activity: 32.55%, 30.95% and 35.55% when compared at 0 h, 12 h and
607 24 h, respectively. L. vannamei exposed to pH 9.5 showed lower CAT activity at 12 h
608 and 36 h. After 12 h of exposure, the shrimp CAT activity decreased 37.2 and 44.9 %
609 when compared at 0 h and 24 h, respectively. White shrimp exposed to pH 9.5 for 36 h
610 exhibited reduced CAT activity: 32.5% and 40.8% when compared at 0 h and 24 h,
611 respectively.

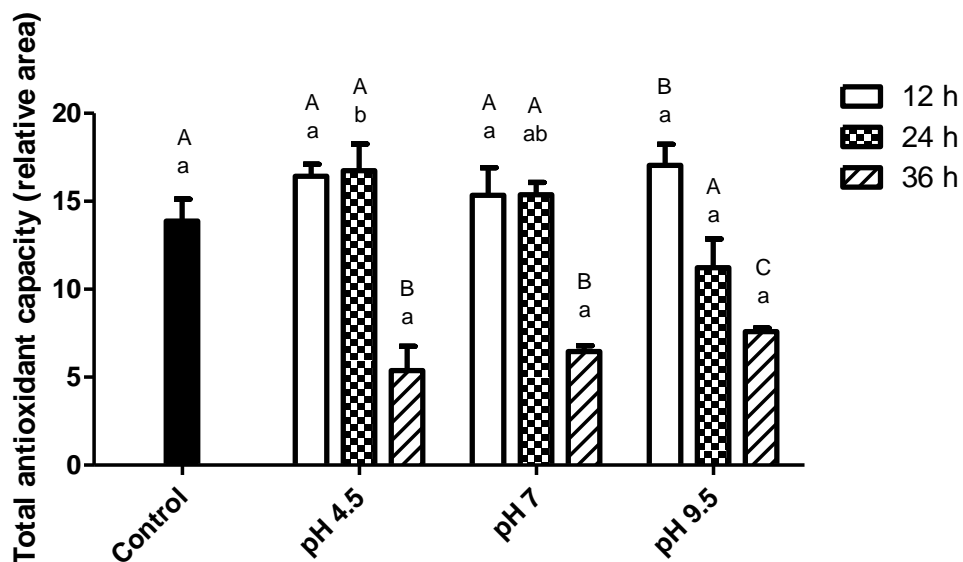
612 The GST activity of shrimp exposed to pH 4.5 for 36 h exhibited a reduction of
613 GST activity of approximately 90%, 95.5% and 92% when compared at 0 h, 12 h and
614 24 h, respectively (Fig. 3). Shrimp in the control group exhibited increased GST activity
615 after 24 h of exposure: 1,387%, 1,090% and 2,875% when compared at 0 h, 12 h and 36
616 h, respectively. L. vannamei exposed to pH 9.5 for 12 h exhibited augmented GST
617 activity of approximately 612% and 280% when compared at 0 h and 24 h, respectively.

618 After analyzing the treatment for each sample point, the lowest total antioxidant
619 capacity and highest relative area were observed in shrimp exposed to pH 4.5 for 24 h,
620 8.9% and 49.2% higher when compared to those exposed to pH 7 and 9.5, respectively.

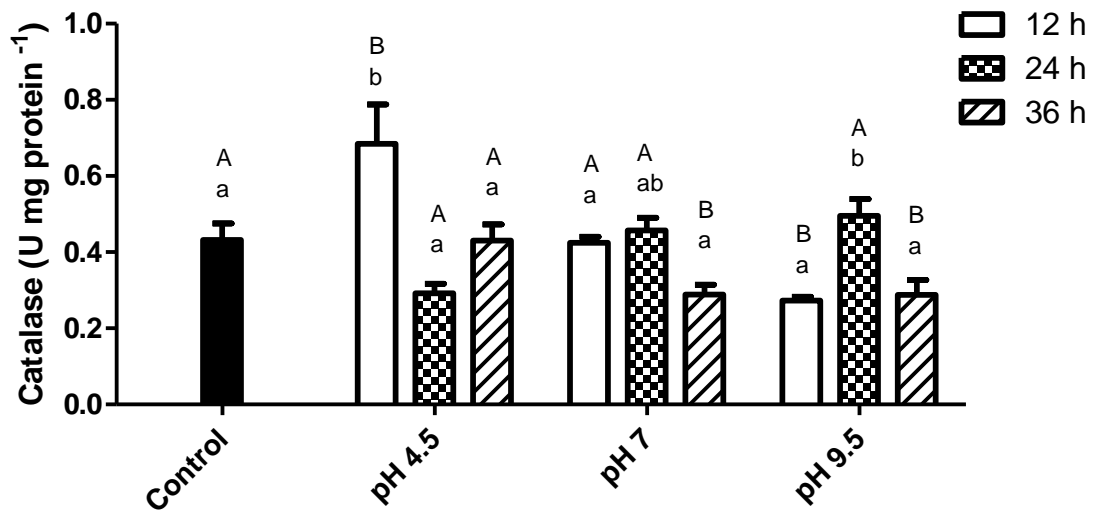
621 The CAT results showed that shrimp exposed to pH 4.5 for 12 h exhibited higher
622 enzyme activities of 61.9% and 151.8% when compared to those of the control group

623 and pH 9.5, respectively. The shrimp exposed to pH 4.5 for 24 h presented a reduction
 624 of CAT activity (55.17% and 68.96%) compared with those of shrimp maintained at pH
 625 7 and 9.5, respectively.

626 The GST activity of shrimp exposed to pH 9.5 for 12 h was approximately
 627 216.6% and 470.0% higher when compared to those of shrimp exposed to pH 4.5 and
 628 7.0, respectively. After 24 h, the highest GST activity was recorded in the control group,
 629 which exhibited an augmented GST activity of approximately 1,090% and 693% when
 630 compared to shrimp kept at pH 4.5 and 9.5, respectively.

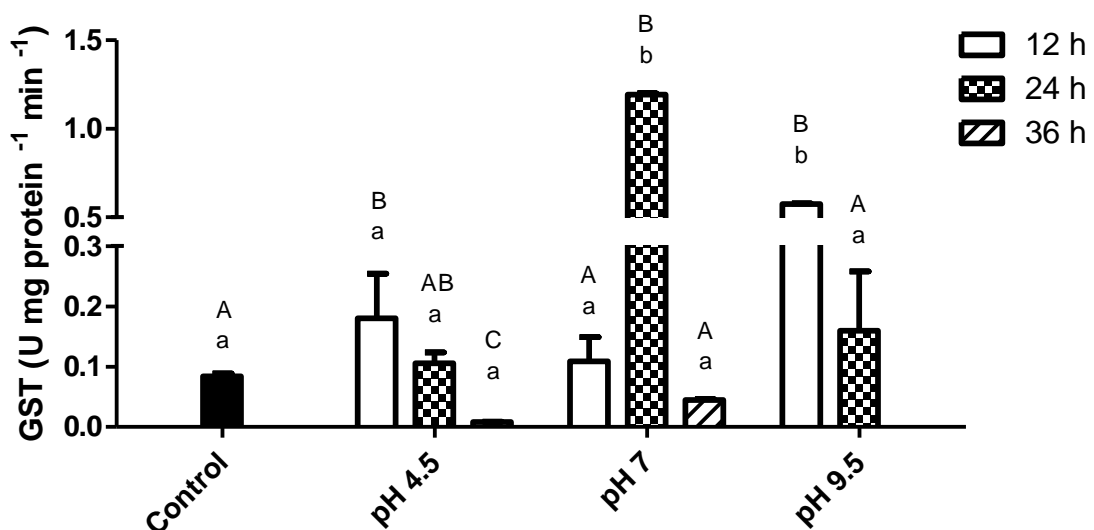


631
 632 Figure 1. Total antioxidant capacity against peroxy radicals in the hemolymph of *L.*
 633 *vannamei* juveniles ($N=6$) exposed to pH 4.5, 7.0 and 9.5 in 0, 12, 24 and 36 h. The data
 634 are expressed as the means \pm SEM. Different lower case letters indicate significant
 635 differences ($p < 0.05$) among treatments over the same time period. Different capital
 636 letters indicate significant differences ($p < 0.05$) in the same treatment at different
 637 sample times throughout the experimental period.



638
639

640 Figure 2. Catalase activity in the hemolymph of *L. vannamei* juveniles ($N=6$) exposed
641 to pH 4.5, 7.0 and 9.5 in 0, 12, 24 and 36 h. The data are expressed as the means \pm
642 SEM. Different lower case letters indicate significant differences ($p < 0.05$) among
643 treatments over the same time period. Different capital letters indicate significant
644 differences ($p < 0.05$) in the same treatment at different sample times throughout the
645 experimental period.



646
647

648 Figure 3. GST activity in the hemolymph of *L. vannamei* juveniles ($N=6$) exposed to
649 pH 4.5, 7.0 e 9.5 in 0, 12, 24 e 36 h. The data are expressed as the means \pm SEM.

650 Different lower case letters indicate significant differences ($p < 0.05$) among treatments
651 over the same time period. Different capital letters indicate significant differences ($p <$
652 0.05) in the same treatment at different sample times throughout the experimental
653 period.

654 Discussion

655 The physical and chemical water quality parameters that were measured
656 throughout the experimental period remained at the appropriate levels for the survival of
657 the shrimp (Ponce-Palafox et al. 1997; Castille and Lawrence 1981; Van Wyk and
658 Scarpa 1999; Lin and Chen 2001, 2003), except for the experimental pH values.

659 According to Buikema et al. (1982), an acute toxicity test provides information
660 about the relative lethality of a toxin, but it cannot predict the concentration that has
661 sublethal and chronic effects on the organisms. Wang et al. (2002) verified the effects of
662 five levels of pH (6.0, 7.0, 7.6, 8.0 and 8.5) on Fenneropenaeus chinensis,
663 demonstrating that this shrimp was more sensitive to acidic pH than was P. monodon,
664 which presented mortality in pH 5.1 (Allan and Maguire 1992). In this study, juvenile
665 L. vannamei presented mortality in pH 4.5, as they were more resistant to acidic pH
666 than other studied species of penaeid. We verified that the juvenile L. vannamei
667 presented lower tolerance to basic pH (10.0) than to acidic pH (4.5) This statement is
668 supported by the results found by Zhou et al. (2009). They also observed that 12 h after
669 the shrimp were exposed to acidic pH and basic pH, the hemolymph pH decreased and
670 increased significantly from 7.72 ± 0.02 to 7.58 ± 0.03 and 7.82 ± 0.04 , respectively.
671 Therefore, this species was sensitive to basic pH conditions and, to a lesser degree,
672 acidic conditions.

673 According to Heinzz (1967), in a variety of aquatic organisms, the active
674 transportation of Na⁺ and K⁺ is inhibited at pH levels below 4.6-5.0, while the passive
675 permeability to Na⁺ of the mucosal barrier increases. In a study with F. chinensis, it was
676 evident that slightly acidic (6.0) or basic (8.5) water pH resulted in metabolic
677 depression, inhibiting the activity of Na⁺-K⁺ ATPase (Wang et al. 2002). This
678 deficiency in the active transport mechanism of Na⁺ through the gill epithelium can be
679 the main cause of shrimp deaths in acidic and basic waters. These changes in the pH of
680 the rearing water of the animals can also present sublethal effects, which cause
681 physiological changes in the tissue.

682 In the present study, the results showed an increase of antioxidant enzymes
683 activities due to the potential oxidative damage caused by the increasing ROS.
684 Moreover, the antioxidant capacity was affected and restored after 36 h of exposure.
685 Wang et al. (2009) observed that an increase of antioxidant enzymes in L. vannamei
686 after pH stress showed that enzymatic defense mechanisms were used for ROS
687 scavenging. ROS, especially H₂O₂, is considered not only toxic to cellular metabolites
688 but also a signaling molecule that mediates responses to various stimuli (Ji 1995). In
689 particular, signal transduction pathways are known to be activated by ROS, and they
690 lead to the transcription of genes involved in cell growth regulatory pathways that
691 include antioxidant enzyme genes, such as CAT, GPx and SOD (Meyer et al. 1993; Sen
692 and Packer 1996; Ji and Leichtweis 1997). Our results demonstrated that shrimp
693 exposed to acidic pH and basic pH stress presented a disturbance to the antioxidant
694 activity to scavenge hydrogen peroxide. CAT activity was affected at pH 4.5 and pH
695 9.5. In addition, this activity was not restored in basic pH stress, confirming the
696 sensitivity of L. vannamei to basic pH.

697 Due to the open circulating system of shrimp, significant changes in hemolymph
698 pH may also affect the function of other shrimp tissues. Gas exchanges at low pH may
699 cause a decrease of hemolymph pH and may promote ROS accumulation. In the present
700 study, shrimp exposed to pH 4.5 and pH 9.5 for 36 h presented minimal or not recorded
701 GST activity, and at pH 9.5, catalase activity was unstable and was not restored to the
702 control level. The responses to acidic and basic exposures induced oxidative stress in
703 the Pacific white shrimp *L. vannamei*, confirming that DNA damage may be a suitable
704 biomarker for assessing the potential ecological risk caused by pH stress (Wang et al.
705 2009).

706 It is likely that the increase in CAT activity (pH 4.5 after 12 h) in the
707 hemolymph observed in this study indicated an accumulation of H₂O₂ in the
708 hemolymph. This result was also observed by Garcia et al. (2011), who exposed silver
709 catfish juveniles infected with *Ichthyophthirius multifiliis* to pH 5.0 and observed a rise
710 in CAT activity in the gills of these animals. Some researchers reported that elevated
711 GST activity serves to re-establish the balance between pro-oxidants and antioxidants to
712 alleviate ROS-induced oxidative damage (Garcia et al. 2011). In the present study, we
713 observed a rise in GST activity at pH 9.5 after 12 h, likely due to basic pH stress to re-
714 establish basal ROS levels.

715 Our results suggest that shrimp exposed to pH stress present an imbalance of
716 antioxidant enzyme activities. Due to this imbalance, the animals most likely spend
717 more energy to attenuate ROS production, and consequently, low growth is expected in
718 a culture system. The imbalance also could be affecting the process of lipoperoxidation,
719 which could affect the quality of the product in terms of taste.

720

721

722

Conclusions

723 The results obtained in the acute toxicity assay showed greater resistance of
724 shrimps to acidic pH than to basic pH. In the experimental conditions described in this
725 study, the pH_{50} after 96 h of acidic and basic pH exposure were 4.04 and 9.58,
726 respectively, for juvenile L. vannamei. As for the oxidative stress assay, we found that
727 the shrimp exposed to both pH 4.5 and pH 9.5 presented an imbalance in antioxidant
728 enzyme activity. The Pacific white shrimp Litopenaeus vannamei should be reared in
729 systems with better pH control (lower daily amplitude and without abrupt changes),
730 facilitating optimal physiological performance and ensuring better growth, survival and
731 productivity throughout rearing.

732

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737

Literature Cited

738 **Amado, L. L., M. L. Garcia, P. B. Ramos, R. F. Freitas, B. F. Zafalon, J. L. R.**
739 **Ferreira, J. S. Yunes and J. M. Monserrat.** 2009. A method to measure total
740 antioxidant capacity against peroxy radicals in aquatic organisms: application to
741 evaluate microcystins toxicity. *Science of the Total Environment* 407:2115–2123.

742 **APHA (American Public Health Association).** 1998. Standard methods for the
743 examination of water and wastewater, 20 ed. Washington, D.C. USA.

- 744 **Allan, G. L. and G. B. Maguire.** 1992. Effects of pH and salinity on survival, growth
745 and osmoregulation in Penaeus monodon Fabricius. *Aquaculture* 107:33–47.
- 746 **Bendschneider, K. and R. J. Robinson.** 1952. A new spectrophotometric method for
747 the determination of nitrite in sea water. *Journal of Marine Research* 11:87-96.
- 748 **Beutler, E.** 1975. The preparation of red cells for assay. In: Beutler E, ed. *Red cell*
749 *metabolism: a manual of biochemical methods.* New York, NY: Grune & Straton;
750 p. 8–18.
- 751 **Boyd, C. E.** 2008. Carbon Dioxide: Waste, Nutrient. *Global Aquaculture Advocate*
752 July/August: 62-65.
- 753 **Buikema, A. L., R. R. Niederlehner and J. J. Cairns.** 1982. Biological monitoring
754 Part IV. Toxicity testing. *Water Research* 16:239-262.
- 755 **Castille, F. L. and A. L. Lawrence.** 1981. The effect of salinity on the osmotic, sodium
756 and chloride concentrations in the hemolymph of euryhaline shrimps of the genus
757 Penaeus. *Comparative Biochemistry and Physiology A* 68:75–80.
- 758 **Chen, J. C. and S. Y. Cheng.** 2000. Recovery of Penaeus monodon from functional
759 anaemia after exposure to sublethal concentration of nitrite at different pH levels.
760 *Aquatic Toxicology* 50:73-83.
- 761 **Ebeling, J. M., M. B. Timmons and J. J. Bisogni.** 2006. Engineering analysis of the
762 stoichiometry of photoautotrophic, autotrophic, and heterotrophic control of
763 ammonia-nitrogen in aquaculture in aquaculture production systems. *Aquaculture*
764 257:346-358.

- 765 **FAO.** 2014. The state of world fisheries and aquaculture. In: www.fao.org. Accessed in
766 09/08/14.
- 767 **Furtado, P. S., L. H. Poersch and W. J. Wasielesky.** 2011 Effect of Calcium
768 Hydroxide, Carbonate and Sodium Bicarbonate on Water Quality and Zootechnical
769 Performance of Shrimp Litopenaeus vannamei Reared in Bio-Flocs Technology
770 (BFT) Systems. Aquaculture 321:130-135.
- 771 **Garcia, L. O., A. G. Becker, M. A. Cunha and B. Baldisserotto.** 2011. Effects of
772 Water pH and Hardness on Infection of Silver Catfish, Rhamdia quelen,
773 Fingerlings by Ichthyophthirius multifiliis. Journal of the World Aquaculture
774 Society 42(3):399-405.
- 775 **Habig, W. H., M. J. Pabst and W. B. Jakoby.** 1974. Glutathione S-transferases, the
776 first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry
777 249:7130–7139.
- 778 **Habig, W. H. and W. B. Jakoby.** 1981 Glutathione S-Transferase (rat and human).
779 Methods in Enzymology 77(27): 218-239.
- 780 **Hamilton, M. A., R. C. Russo and R. V. Thurston.** 1977. Trimmed Spearman-Kaber
781 Method for estimating Median Lethal Concentrations on Toxicity Bioassays.
782 Environmental Science Technology 11(7):714-719.
- 783 **Heinz, E.** 1967. Transport through biological membranes. Annual Review Physiology
784 29:21–58.
- 785 **Henry, R., G. Kormanik, N. Smatresk and J. Cameron.** 1981. The role of CaCO₃
786 dissolution as a source of HCO₃⁻ for the buffering of hypercapnic acidosis in

787 aquatic and terrestrial decapod crustaceans. Journal of Experimental Biology
788 94:269-274.

789 **Ji, L. L.** 1995. Exercise and free radical generation. Role of cellular antioxidant
790 systems. Pages 135-166 in Holoszy J. ed., Exercise and Sports Science Review.
791 InWilliams and zWilkins Co, Baltimore, Maryland, USA.

792 **Ji, L. L. and S. L. Leichtweis.** 1997. Exercise and oxidant stress: source of free
793 radicals and their impact on antioxidant systems. AGE 20:91–106.

794 **Lemonnier, H., E. Bernard, E. Boglio, C. Goarant and J. Cochard.** 2004. Influence
795 of sediment characteristics on shrimp physiology: pH as principal effect.
796 Aquaculture 240:297-312.

797 **Li, C. C. and J. C. Chen.** 2008. The immune response of white shrimp Litopenaeus
798 vannamei and its susceptibility to Vibrio alginolyticus under low and high pH
799 stress. Fish & Shellfish Immunology 25:701-709.

800 **Lin, Y. C. and J. C. Chen.** 2001. Acute toxicity of ammonia on Litopenaeus vannamei
801 (Boone) juveniles at different salinity levels. Journal of Experimental Marine
802 Biology and Ecology 259:109-119.

803 **Lin, Y. C. and J. C. Chen.** 2003. Acute toxicity of nitrite on Litopenaeus vannamei
804 (Boone) juveniles at different salinity levels. Aquaculture 224:193-201.

805 **Lushchak, V. I.** 2011. Environmentally induced oxidative stress in aquatic animals.
806 Aquatic Toxicology 101:13–30.

- 807 **McCord, J. M. and I. Fridovich.** 1969. Superoxide dismutase: an enzymatic function
808 for erythrocyte hemoglobin (hemocyanin). *Journal of Biological Chemistry* 244:6049–
809 6055.
- 810 **Meyer, M. R., R. Schreck and P. A. Rauerle.** 1993. H₂O₂ and antioxidants have
811 opposite effects on activation of NF- κ B and AP-1 in intact cells: AP-1 as
812 secondary antioxidant responsive factor. *The EMBO Journal* 2:2005–2015.
- 813 **Ponce-Palafox J., C. A. Martínez-Palacios and L. G. Ross.** 1997. The effect of
814 salinity and temperature on the growth and survival rates of white shrimp, *Penaeus*
815 *vannamei*, Boone, 1931. *Aquaculture* 157:107–115.
- 816 **Sampaio, L. A., M. B. Tesser and W. J. Wasielesky.** 2010. Avanços da maricultura na
817 primeira década do século XXI: piscicultura e carcinocultura marinha. *Revista*
818 *Brasileira de Zootecnia* 39:102-111.
- 819 **Sen, C. K. and L. Packer.** 1996. Antioxidant and redox regulation of gene
820 transcription. *The FASEB Journal* 10:709–720.
- 821 **Söderhäll, K. and V. J. Smith.** 1983. Separation of the haemocyte populations of
822 *Carcinus maenas* and other marine decapods, and prophenoloxidase distribution.
823 *Developmental and Comparative Immunology* 7: 229–239.
- 824 **UNESCO (United Nations Educational, Scientific and Cultural Organization).**
825 1983. Chemical methods for use in marine environmental monitoring. Manual and
826 Guides 12, Intergovernmental Oceanographic Commission. Paris, France.
- 827 **Van Wyk, P. and J. Scarpa.** 1999. Water quality and management. Pages 141-161 In
828 **Van Wyk, P. et al.** (Eds.). *Farming marine shrimp in recirculating freshwater*

829 systems. Florida department of agriculture and consumer services, Tallahassee,
830 Flórida, USA.

831 **Vinatea, L. A.** 2004. Princípios químicos de qualidade da água em aquicultura: uma
832 revisão para peixes e camarões. 2ª edição revisada e ampliada, Editora UFSC.
833 Florianópolis, Santa Catarina, Brasil.

834 **Vinatea, L., A. O. Galvez, C. L. Browdy, A. Stokes, J. Venero, J. Haveman, B. L.**
835 **Lewis, A. Lawson, A. Shuler and J. W. Leffler.** 2010. Photosynthesis, water
836 respiration and growth performance of Litopenaeus vannamei in a super-intensive
837 raceway culture with zero water exchange: interaction of water quality variables.
838 *Aquacultural Engineering* 42:17–24.

839 **Wasielesky, W., H. Atwood, R. Kegl, J. Bruce, A. Stokes and C. L. Browdy.** 2006.
840 Efeito do pH na sobrevivência e crescimento do camarão branco Litopenaeus
841 vannamei em cultivos superintensivos. *Aquaciência* 2006. Anais do congresso.

842 **Wang, W. N., A. L. Wang, L. Chen, Y. Liu and R. Y. Sun.** 2002. Effects of pH on
843 survival, phosphorus concentration, adenylate energy charge and $\text{Na}^+\text{-K}^+$ ATPase
844 activities of Penaeus chinensis Osbeck juveniles. *Aquatic Toxicology* 60:75-83.

845 **Wang, W. N., J. Zhou, P. Wang, T. T. Tian, Y. Zheng, Y. Liu, W. J. Mai and A. L.**
846 **Wang.** 2009. Oxidative stress, DNA damage and antioxidant enzyme gene
847 expression in the Pacific white shrimp, Litopenaeus vannamei when exposed to
848 acute pH stress. *Comparative Biochemistry and Physiology C* 150:428–435.

849 **Wang, W. N., B. S. Li, J. J. Liu, L. Shi, M. J. Alam, S. J. Su, J. Wu, L. Wang and**
850 **A. L. Wang.** 2012. The respiratory burst activity and expression of catalase in
851 white shrimp, Litopenaeus vannamei, during long-term exposure to pH stress.
852 *Ecotoxicology* 21:1609–1616.

853 **Zhou, J., W. N. Wang, A. L. Wang, W. Y. He, Q. T. Zhou, Y. Liu and J. Xu.** 2009.
854 Glutathione S-transferase in the white shrimp *Litopenaeus vannamei*:
855 Characterization and regulation under pH stress. Comparative Biochemistry and
856 Physiology C 150:224-230.

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CAPÍTULO II

859

860

861 **TOXICIDADE AGUDA DE DIÓXIDO DE CARBONO EM JUVENIS DE**
862 **CAMARÃO MARINHO *Litopenaeus vannamei* (Boone 1931).**

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876 **Resumo**

877 Durante o cultivo e o transporte de organismos aquáticos são geralmente registrados
878 valores elevados de dióxido de carbono dissolvido (CO₂) e reduzidos de pH na água.
879 Essa condição é conhecida como hipercapnia e pode gerar um desajuste no equilíbrio
880 ácido-base intra ou extra celular no camarão marinho *Litopenaeus vannamei*, afetando
881 negativamente a capacidade da hemocianina em transportar oxigênio, prejudicando o
882 crescimento e sobrevivência dos camarões. O objetivo do presente estudo foi determinar
883 as concentrações letais e o nível de segurança do CO₂ para juvenis de camarão branco
884 *L. vannamei*. Camarões juvenis (1,76±0,36 g) foram expostos por 96 horas a seis
885 concentrações de dióxido de carbono dissolvido (14,5, 23,8, 59,0, 88,0, 115,0, 175,0
886 mg/L) mais o controle (sem adição de CO₂) e a sobrevivência foi monitorada por 96h.
887 Os resultados encontrados para valores de CL₅₀ e limites de confiança 95% em 24, 48,
888 72 e 96 h foram de 130,05 (104,2-162,1), 77,2 (73,8-80,02), 69,65 (65,47-74,32), 59,12
889 (53,08-66,07) mg/L de CO₂, respectivamente. O nível de segurança calculado foi de 5,9
890 mg/L de CO₂ e NOAEC foi de 23,8 mg/L de CO₂, recomendamos que os níveis de CO₂
891 sejam mantidos dentro do nível de segurança calculado.

892 **Palavras-Chave:** toxicidade, dióxido de carbono, nível de segurança, pH.

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899 **Abstract**

900 Elevated dissolved carbon dioxide (CO₂) and reduced pH levels are observed during
901 cultivation and transportation of aquatic organisms. This condition is known as
902 hypercapnia and can generate an acid-base intra or extracellular imbalance in the marine
903 shrimp *Litopenaeus vannamei*, affecting negatively the ability of the respiratory
904 pigment to transport oxygen and the growth and survival of the shrimp. The objective of
905 the present study was to determine lethal concentration and safety levels of CO₂ for
906 juvenile white shrimp *L. vannamei*. To this end, *L. vannamei* juveniles (1.76 ± 0.36 g)
907 were exposed for 96 hours to six concentrations of dissolved carbon dioxide (14.5, 23.8,
908 59.0, 88.0, 115.0, 175.0 mg/L) plus one control group (without addition of CO₂) and
909 their survival was monitored for 96 h. The values of LC₅₀ values and 95% confidence
910 limits in 24, 48, 72 and 96 h were 130.05 (104.2 -162.1), 77.2 (73.8 -80.02), 69.65
911 (65.47 -74.32) and 59.12 (53.08 -66.07) mg/L of CO₂, respectively. The calculated
912 safety level was 5.9 mg/L of CO₂ and NOAEC was 23.8 mg/L of CO₂. We recommend
913 that CO₂ levels should be kept within the safety level obtained in this study.

914 **Keywords:** toxicity, carbon dioxide, safety level, pH.

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923 **Introdução**

924 Recentemente pesquisadores avaliaram o aumento das concentrações de dióxido
925 de carbono dissolvido (CO₂) nos oceanos e verificaram uma redução de 0.1 unidade de
926 pH nas águas superficiais desde a era pré-industrial (Caldeira & Wickett 2003). A
927 previsão para o ano de 2100 é que o pH dos oceanos reduza 0,2 a 0,3 unidades (Feely et
928 al. 2009).

929 Devido a acidificação dos oceanos, diversos estudos foram realizados para
930 analisar seus efeitos em formas larvais, jovens e adultas de invertebrados (Kurihara et
931 al. 2008; Bierbower & Cooper 2010; Carter et al. 2013). Segundo resultados obtidos por
932 Kurihara et al. (2008) a exposição crônica do camarão *Palaemon pacificus* às
933 concentrações de CO₂ previstas para ocorrerem durante os próximos séculos pode
934 reduzir a sobrevivência, crescimento e possivelmente a reprodução desses organismos.

935 Durante o transporte de organismos aquáticos em ambientes fechados como
936 bolsas plásticas, ocorre o consumo de oxigênio e liberação de CO₂, gerando uma
937 condição estressante de hipóxia e hipercapnia (Jensen et al. 2014). Em cultivos com
938 peixes e camarões as concentrações de CO₂ se incrementam principalmente devido a
939 biomassa de organismos cultivados e de matéria orgânica em decomposição no
940 ambiente de cultivo (Timmons & Ebeling 2010). O camarão branco do Pacífico
941 *Litopenaeus vannamei* tem sido a espécie de camarão mais utilizada em sistemas de
942 cultivo superintensivos com tecnologia de bioflocos (BFT), nos quais se trabalham com
943 densidades de estocagem entre 150 e 600 camarões/m² e biomassas de 2 a 8 kg/m²
944 (Silva et al. 2013).

945 No sistema BFT, quando o número de microrganismos heterotróficos excede o
946 de fotoautotróficos, a produção de dióxido de carbono excede a produção de oxigênio

947 dissolvido, podendo gerar acúmulo de CO₂ e reduzir os níveis de pH da água de cultivo
948 (Vinatea et al. 2010; Furtado et al. 2011). Em condições de falha no sistema de aeração
949 ou falta de energia elétrica, as concentrações de oxigênio dissolvido diminuem
950 rapidamente nesses sistemas, o CO₂ se incrementa e a água se acidifica (Furtado et al.
951 2014). Para Van Wyk & Scarpa (1999), concentrações de CO₂ na água inferiores a 5
952 mg/L são ideais; já 20 mg/L são considerados aceitáveis para peneídeos, enquanto
953 concentrações entre 20 e 60 mg/L não são letais mas causam interferências na troca de
954 CO₂ nas brânquias. Em concentrações superiores a 60 mg/L podem ser letais.
955 Dissanayake & Ishimatsu (2011) verificaram um efeito sinérgico de concentrações
956 elevadas de CO₂ e de temperatura, que pode comprometer a capacidade de respiração e
957 habilidade de natação dos peneídeos.

958 O dióxido de carbono é fonte de carbono essencial para a fotossíntese, mas
959 também o produto final da respiração dos organismos aquáticos (Boyd 2008).
960 Inicialmente, as condições de um cultivo em sistema BFT são predominantemente
961 fotoautotróficas e as concentrações de CO₂ podem flutuar entre 5 - 10 mg/L pela manhã,
962 mas podem exceder 20 mg/L em viveiros com biomassa elevada (Boyd 2008). Durante
963 um período de 24 horas ocorrem flutuações de oxigênio dissolvido (OD), pH e dióxido
964 de carbono, haja visto que durante a fase clara do dia ocorre a remoção do CO₂ e
965 elevação do pH e OD, decorrentes dos processos de fotossíntese. Já durante a noite
966 cessa a fotossíntese e predomina a respiração com a liberação de CO₂ por parte dos
967 organismos aeróbicos, reduzindo o pH e OD do meio de cultivo (Vinatea 2004). Ao
968 longo do tempo, com domínio das comunidades heterotróficas no sistema BFT, ocorre a
969 produção permanente de CO₂ e mínima produção de OD por parte do fitoplâncton.

970 Concentrações de CO₂ superiores a 20 mg/L dificultam a excreção do CO₂ no
971 epitélio branquial resultando no declínio do pH da hemolinfa, afetando negativamente a

972 capacidade da hemolinfa em transportar oxigênio e assim, reduzindo a oxigenação nos
973 tecidos e aumentando a taxa de ventilação (Taylor & whiteley 1989; Van Wyk &
974 Scarpa 1999). Um desajuste no equilíbrio ácido-base intra ou extracelular pode
975 prejudicar o metabolismo dos camarões (Wang et al. 2012), mesmo que os crustáceos
976 decápodes possuam habilidades para tolerar condições básicas e ligeiramente ácidas,
977 devido ao intercâmbio de Na^+/H^+ e $\text{Cl}^-/\text{HCO}_3^-$ que ocorre nas brânquias destes animais
978 (Henry et al. 1981).

979 Os estudos sobre os efeitos do CO_2 em camarões peneídeos são escassos quando
980 comparados ao número de pesquisas desenvolvidas com peixes (Grottum & Sigholt
981 1996; Santos et al. 2013). O objetivo do presente estudo foi determinar as concentrações
982 letais e o nível de segurança de dióxido de carbono para juvenis de camarão branco *L.*
983 *vannamei*.

984

985 **Material e Métodos**

986 *Local e instalações*

987

988 O estudo foi conduzido na Estação Marinha de Aquacultura Prof. Marcos
989 Alberto Marchiori (EMA) do Instituto Oceanográfico, da Universidade Federal do Rio
990 Grande (FURG), localizada na praia do Cassino, Rio Grande (32°11'S; 52°10'W), Rio
991 Grande do Sul, Brasil.

992 *Água, camarões e delineamento experimental*

993 A água utilizada para o experimento foi bombeada da praia, filtrada através de
994 filtro de areia e posteriormente de filtro de cartucho com 5 μm de poro. No decorrer do

995 estudo não houve renovação de água, apenas reposição do que foi perdido por
996 evaporação, através da adição de água doce sem cloro.

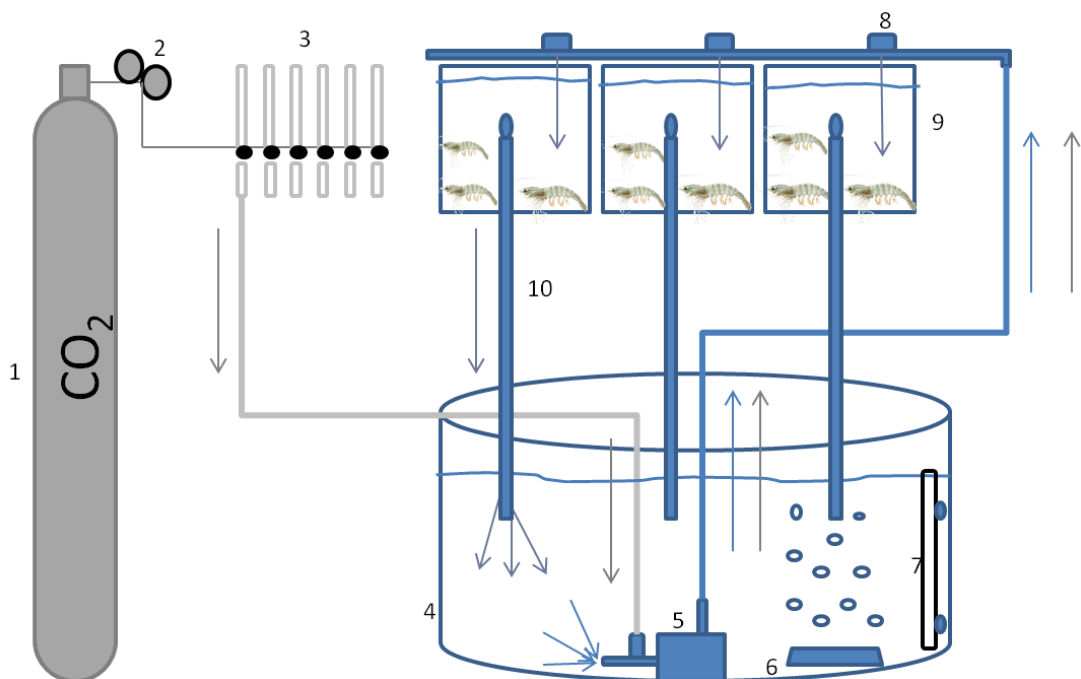
997 Os camarões utilizados no presente estudo foram adquiridos no laboratório
998 Aquatec Ltda (Canguaretama, Rio Grande do Norte, Brasil). Após a chegada dos
999 náuplios de *L. vannamei* nas instalações da EMA, estes foram mantidos no setor de
1000 larvicultura de camarões marinhos até pós-larva 15 (pL15). Posteriormente, os mesmos
1001 foram cultivados em um berçário (1500 pL/m²) em sistema BFT até o peso médio de
1002 1,76±0,36 g. Os 210 juvenis de *L. vannamei* no estágio intermuda (2 dias pós muda)
1003 foram aclimatados por 48 horas as condições experimentais para reduzir o estresse da
1004 captura e manuseio. Nesse período foram alimentados duas vezes por dia com dieta
1005 comercial (Guabi® 38% PB e 8% extrato etéreo) na proporção de 8% da biomassa/dia.
1006 O fotoperíodo experimental adotado foi de 12h luz:12h escuro e temperatura de 27°C.

1007 O teste agudo para determinação da concentração letal mediana (CL₅₀ 96h) foi
1008 realizado em sistema semiestático e durante o teste os animais não foram alimentados.
1009 Em cada tanque retangular, com volume útil de 30 L, foram colocados 10 camarões.
1010 Cada tratamento contou com 3 repetições (n=30 camarões). Foram testadas seis
1011 concentrações de dióxido de carbono (14,5, 23,8, 59,0, 88,0, 115,0, 175,0 mg/L) mais
1012 tratamento controle (sem adição de CO₂).

1013 Para injetar o CO₂ nas unidades experimentais foi necessário um cilindro de gás
1014 pressurizado de 30 kg com manômetro, válvula reguladora e conjunto de fluxômetros
1015 especiais para dióxido de carbono (escala de 0 a 15 L/min). Para cada tratamento foi
1016 montado um sistema de recirculação composto por três tanques retangulares de 30L e
1017 um reservatório circular com 200L, no qual havia uma bomba submersa (capacidade
1018 para 1000L/h) com Venturi para injeção de CO₂ proveniente do cilindro (Figura 1). A

1019 bomba transferia água do reservatório para os tanques retangulares, solubilizando o
1020 dióxido na água dos tanques onde estavam os camarões, a taxa de renovação de água
1021 em cada unidade experimental era de 150L/h, retornando por gravidade dos tanques
1022 retangulares para o reservatório. Em cada reservatório de 200L havia seis difusores de
1023 ar para prover aeração e também foi instalado um aquecedor com termostato a fim de
1024 manter a temperatura em 27°C.

1025



1026 Figura 1 - Esquema ilustrativo de um tratamento experimental, onde 1: cilindro de
1027 dióxido de carbono (CO₂); 2: manômetro e válvula reguladora; 3: fluxômetros; 4:
1028 tanque circular com volume útil de 200L; 5: bomba submersa com tubo de Venturi para
1029 entrada de CO₂; 6: difusor de ar para oxigenação da água; 7: aquecedor com termostato;
1030 8: registro para ajuste de fluxo de água; 9: tanques experimentais com 30L de volume
1031 útil onde os camarões eram expostos ao CO₂; 10: tubulação de retorno de água das
1032 unidades experimentais ao tanque circular central.

1033 As determinações de pH, temperatura e oxigênio foram realizados a cada 2h,
1034 com auxílio de pH-metro modelo 100 YSI[®] (Yellow Springs Instruments, USA) e
1035 oxímetro modelo 55 YSI[®] (Yellow Springs Instruments, USA), respectivamente. A
1036 salinidade foi verificada no início e no fim do experimento, com refratômetro óptico
1037 (Atago[®], Japan). A cada 24h foram mensurados as concentrações de amônia total (N-
1038 AT) (NH₃ + NH₄⁺) e de alcalinidade, conforme metodologias preconizadas pela
1039 UNESCO (1983) e APHA (1998), respectivamente. As concentrações de dióxido de
1040 carbono dissolvido foram mensurados conforme APHA (1998) e calculados com auxílio
1041 do software CO₂ Analysis Salt[®] (Timmons & Ebeling 2010). O CO₂ foi monitorado
1042 12x/dia de 2 em 2 horas durante as 96h, assim como a sobrevivência dos camarões. Os
1043 camarões eram considerados mortos quando estavam imóveis e não respondiam a
1044 estímulos mecânicos feitos com bastão de vidro (Lin & Chen 2003). Os camarões
1045 mortos eram removidos do tanque assim que detectados.

1046 Os dados cumulativos de mortalidade nos períodos de 24, 48, 72 e 96 horas
1047 foram utilizados para estimar a concentração letal mediana (CL₅₀) e seus respectivos
1048 limites de confiança (95%), para tal foi utilizado “software” Trimmed Spearman Karber
1049 Method (Hamilton et al. 1977). O nível de segurança foi calculado usando fator de 0.1,
1050 conforme Sprague (1971). A NOAEC (= maior concentração que não apresenta
1051 sobrevivência significativamente diferente do controle) (EPA 2002) foi calculada por
1052 comparação entre o número de sobreviventes em cada tratamento no término do teste.

1053 Para a análise estatística dos dados foi utilizado o software STATISTICA 7.0[®]
1054 (StatSoft Inc. 2004, Tulsa, Oklahoma, USA). Depois de verificada a homocedasticidade
1055 das variâncias (Levene) e a normalidade de distribuição dos dados (Kolmogorov-
1056 smirnov), foi realizada uma análise de variância de uma via (ANOVA, one-way), para
1057 verificação de diferença significativa entre os dados obtidos. Quando detectada

1058 diferença significativa entre os tratamentos ($p < 0,05$), o teste de Tukey de comparação
1059 de médias foi utilizado.

1060

1061 **Resultados**

1062 Os valores médios \pm desvio padrão dos parâmetros de qualidade da água
1063 monitorados ao longo do teste são apresentados na Tabela 1. Quanto aos resultados de
1064 salinidade, temperatura e alcalinidade não houve diferenças significativas ($p > 0,05$) entre
1065 os tratamentos. Enquanto foram verificadas diferenças significativas ($p < 0,05$) para
1066 oxigênio dissolvido, pH e nitrogênio amoniacal total (N-AT). Os valores médios de
1067 oxigênio dissolvido foram superiores a 6 mg/L nas concentrações inferiores a 59,0 mg/L
1068 de CO_2 , diferindo significativamente dos demais tratamentos com maiores
1069 concentrações de CO_2 , de modo que o tratamento 175,0 mg/L apresentou o menor valor
1070 de oxigênio dissolvido (5,6 mg/L). Quanto ao pH houve diferença significativa entre
1071 todos os tratamentos, marcando uma relação inversa entre os valores de CO_2 e os de pH.
1072 Assim quanto maior a concentração de CO_2 menor o valor de pH. Já as concentrações
1073 mensuradas de N-AT são maiores desde o tratamento 0,6 até 88,0 mg/L, diferindo dos
1074 tratamentos 115 e 175 mg/L.

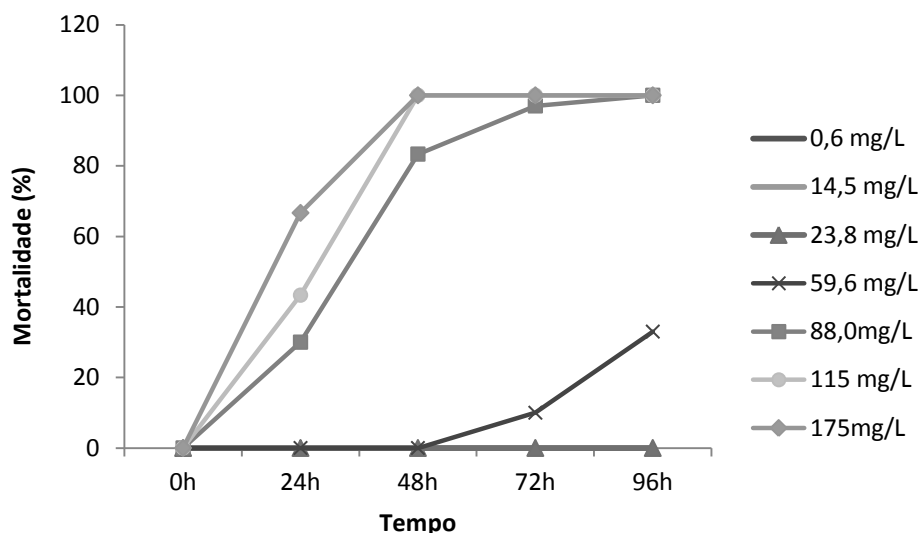
1075 Tabela 1- Parâmetros de qualidade de água (Média \pm DP) mensurados durante as 96 h de
1076 teste com diferentes concentrações de dióxido de carbono (CO_2) nos tanques com
1077 juvenis *L. vannamei*.

Tratamento	Salinidade	Temperatur	OD	pH	N-AT	Alcalinidade
CO_2 (mg/L)	(‰)	a (°C)	(mg/L)		(mg/L)	(mgCaCO ₃ /L)

0,6±0,3 ^a	30,2±0,3	27,2±0,6	6,24±0,18 ^a	8,06±0,05 ^a	0,20±0,13 ^a	105,2±1,4
14,5±0,9 ^b	30,3±0,3	27,0±0,54	6,21±0,15 ^a	6,79±0,08 ^b	0,14±0,09 ^a	105,1±1,7
23,8±1,5 ^c	30,5±0,2	26,9±0,87	6,22±0,14 ^a	6,57±0,13 ^c	0,18±0,11 ^a	104,7±2,6
59,0±3,6 ^d	30,1±0,6	26,96±0,7	6,0±0,12 ^{ab}	6,17±0,1 ^d	0,12±0,06 ^a	104,7±2,5
88,0±6,5 ^e	30,4±0,3	27,1±0,70	5,75±0,25 ^b	6,03±0,04 ^e	0,08±0,04 ^{ab}	104,6±2,5
115,0±10,5 ^f	30,6±0,3	26,95±0,9	5,65±0,27 ^b	5,88±0,1 ^f	0,06±0,03 ^b	104,2±2,0
175,0±15,6 ^g	30,8±0,5	26,81±0,7	5,61±0,3 ^b	5,7±0,05 ^g	0,05±0,01 ^b	103,9±1,5

1078 Letras diferentes sobrescritas em cada coluna indicam diferença significativa ($p < 0,05$)
1079 entre os tratamentos. Oxigênio dissolvido (OD), nitrogênio amoniacal total (N-AT).

1080 Na Figura 2 são apresentados os dados acumulados de mortalidade ao longo das
1081 96h de teste. Todos os juvenis de *L. vannamei* expostos a concentração de 175 mg/L de
1082 CO₂ morreram nas primeiras 36h de exposição, a 115 e 88 mg/L morreram em até 48h e
1083 84h de exposição, respectivamente. Os camarões expostos a 59,0 mg/L de CO₂
1084 apresentaram mortalidade após 64h de exposição e ao final das 96h apresentaram 33%
1085 de mortalidade, enquanto que nos tratamentos 0,6, 14,5 e 23,8 mg/L não foram
1086 verificadas mortalidades ao longo do teste.



1087

1088 Figura 2 - Mortalidade acumulada durante 96 h de exposição as diferentes
 1089 concentrações de CO₂.

1090 A CL₅₀ 96 h foi calculada em 59,12 mg/L de CO₂ com intervalo de confiança
 1091 95% de 53,08 a 66,07 mg/L de CO₂, sendo que os valores de CL₅₀ 24, 48, 72 e 96 h
 1092 estão apresentados na Tabela 2. O nível de segurança calculado foi de 5,9 mg/L de CO₂,
 1093 aproximadamente 25% da maior concentração testada que não causou mortalidade
 1094 (NOAEC) que foi 23,8 mg/L de CO₂.

1095 Tabela 2 - Dados de concentração letal mediana (CL₅₀) de CO₂ para juvenis de camarão
 1096 marinho *L. vannamei*. Limites de confiança são de 95%.

Tempo (h)	CL ₅₀ CO ₂ (mg/L)	Limites de Confiança (mg/L)
24	130,05 ^a	(104,2-162,1)
48	77,2 ^b	(73,8-80,02)
72	69,65 ^{bc}	(65,47-74,32)
96	59,12 ^c	(53,08-66,07)

1097 Letras diferentes sobrescritas na mesma coluna indicam diferença significativa
1098 ($p < 0,05$).

1099

1100 **Discussão**

1101 Os parâmetros químicos e físicos de qualidade da água mensurados neste estudo,
1102 com exceção dos tratamentos com concentrações superiores a 23,8 mg/L de CO₂,
1103 permaneceram dentro da faixa considerada adequada para a sobrevivência de juvenis de
1104 *Litopenaeus vannamei* (Ponce-Palafox et al. 1997; Van Wyk & Scarpa 1999; Lin &
1105 Chen 2001).

1106 A concentração de N-AT aumentou com o passar do tempo, no entanto não
1107 atingiu níveis elevados para afetar a sobrevivência dos camarões (Li & Chen 2001).
1108 Houve diferença quanto aos níveis de N-AT devido a mortalidade precoce dos camarões
1109 nos tratamentos com concentrações superiores a 88,0 mg/L de CO₂, de modo que os
1110 tratamentos com menores concentrações de CO₂ apresentaram concentrações maiores
1111 de N-AT, pois os camarões sobreviveram e excretaram por um tempo maior.

1112 Conforme Van Wyk & Scarpa (1999) concentrações superiores a 5,0 mg/L de
1113 oxigênio dissolvido são considerados ideais para o cultivo de camarões. No presente
1114 estudo as concentrações de OD permaneceram acima de 5,0 mg/L em todos os
1115 tratamentos, mesmo naqueles com maiores concentrações de CO₂. Foi verificada uma
1116 redução de OD com o incremento do CO₂, o mesmo foi verificado por Hall & Van Ham
1117 (1998). Em condições de hipoxia a toxicidade do CO₂ é potencializada, pois interfere na
1118 capacidade de absorção do oxigênio (Boyd & Tucker 1998). Para evitar concentrações
1119 letais de CO₂ durante o transporte de camarões, que em geral apresenta condições de

1120 hipóxia, a biomassa deve ser reduzida e o pH pode ser ajustado com compostos
1121 alcalinizantes (Jensen et al. 2014)

1122 Os valores de pH considerados adequados para o cultivo de camarões peneídeos
1123 são na faixa de 7 a 9 (Van Wyk & Scarpa 1999), porém os peneídeos possuem
1124 resistência a condições ácidas, geradas pela adição de ácido clorídrico, apresentando
1125 mortalidades em pH inferiores a 5,3 durante 96h de exposição (Allan & Maguire 1992).
1126 Conforme Hall & Van Ham (1998) incorporando o CO₂ se reduz o pH da água de 8,5
1127 para 5,9 gerando mudanças comportamentais e aumento nos níveis de glicose na
1128 hemolinfa de *Penaeus monodon*, quando comparado a mesma redução do pH pela
1129 adição de ácido sulfúrico. Os menores valores de pH mensurados nesse estudo foram
1130 superiores aos que geraram mortalidades nos estudos desenvolvidos pelos autores
1131 acima, no entanto as taxas de mortalidade foram de 100% para o pH de 6,03.

1132 A explicação para essa diferença se deve a maior capacidade de difusão do CO₂
1133 do que dos íons hidrogênio (H⁺), ou seja, o CO₂ molecular se difunde diretamente
1134 através das membranas celulares mais rapidamente do que prótons (Gutknecht et al.
1135 1977). Uma alteração no pH da hemolinfa gera condições favoráveis para a produção de
1136 espécies reativas de oxigênio (ERO) resultando em estresse oxidativo e danos as
1137 estruturas celulares como membranas lipídicas, proteínas e DNA (Lushchak 2011;
1138 Wang et al. 2012).

1139 Avaliando a tolerância dos juvenis de Kuruma prawn, *Marsupenaeus japonicus*,
1140 à exposição aguda ao CO₂ Kikkawa et al. (2008) calcularam a CL₅₀ 72h em 14.3% de
1141 CO₂. No presente estudo para o mesmo período de exposição encontramos um valor de
1142 CL₅₀ aproximadamente 50% inferior aos obtidos pelos autores acima, de modo que os
1143 juvenis de *L. vannamei* apresentam maior sensibilidade ao CO₂. Os resultados obtidos

1144 nesse estudo contradizem as recomendações da literatura (Van Wyk & Scarpa 1999;
1145 Boyd 2008), uma vez que camarões expostos a concentrações inferiores a 60 mg/L de
1146 CO₂, apresentaram mortalidade superior a 30%. Durante o presente estudo verificamos
1147 perda de equilíbrio nos camarões antes da morte, esse comportamento também foi
1148 verificado por Kikkawa et al. (2008).

1149 Um teste de toxicidade aguda fornece informações sobre a letalidade relativa de
1150 um tóxico, mas não pode prever a concentração que tem efeitos subletais e crônicos
1151 sobre os organismos (Buikema et al. 1982). No presente estudo utilizando o fator de 0,1
1152 sugerido por Sprague (1971) calculamos o nível de segurança em 5,9 mg/L de CO₂, de
1153 modo que juvenis de *L. vannamei* podem ser cultivados na presença desses níveis de
1154 CO₂ sem apresentar mortalidade dos camarões. No entanto, não recomendamos níveis
1155 como NOAEC de 23,8 mg/L de CO₂ para serem mantidos em sistemas de cultivo por
1156 períodos superiores ao testado no presente estudo.

1157 Wickins (1984) ao estudar os efeitos crônicos da hipercapnia verificou que os
1158 juvenis de *Penaeus occidentalis* expostos por 56 dias em pH 7,3 apresentaram redução
1159 no crescimento quando comparados aos camarões cultivados em água do mar sem
1160 adição de CO₂ (pH 7,6). O mesmo autor verificou diminuição do crescimento de juvenis
1161 de *Penaeus monodon* conforme a redução do pH, por meio de injeção de CO₂, e um
1162 maior intervalo entre mudas 8,2 e 6,6 dias para o pH 6,4 e 7,4, respectivamente.

1163 Monitoramentos circadianos de pH e OD ao longo do cultivo são importantes
1164 para tomadas de decisão que visem prevenir concentrações elevadas de CO₂ e
1165 condições de hipoxia, nas quais podem ocorrer mortalidades dos camarões prejudicando
1166 o sucesso do cultivo. Para a correção dos valores de pH e CO₂ podem ser aplicados uma
1167 série de compostos alcalinizantes como hidróxido de cálcio, carbonato e bicarbonato de

1168 sódio (Furtado et al. 2011). Baseado nas relações químicas, para remover 1 mg/L de
1169 CO₂ é necessário cerca de 0.84 mg/L de hidróxido de cálcio ou 0.64 mg/L de óxido de
1170 cálcio. Como o hidróxido de cálcio não dissolve rapidamente para reagir completamente
1171 com o CO₂, as dosagens acima indicadas devem ser dobradas (Boyd 2008). Outra
1172 estratégia que pode ser tomada para remoção de CO₂ é a utilização de colunas de
1173 desgaseificação (Moran 2010).

1174

1175 **Conclusão**

1176 No presente estudo de toxicidade aguda do CO₂ encontramos os valores de CL₅₀
1177 96 h foi 59,12 mg/L de CO₂ e o nível de segurança calculado em 5,9 mg/L de CO₂, já a
1178 maior concentração não letal foi de 23,8 mg/L de CO₂. Recomendamos que os níveis de
1179 CO₂ sejam mantidos dentro do limite de segurança calculado para espécie, a fim de
1180 evitar condições de hipercapnia que podem imunodeprimir os camarões tornando-os
1181 mais suscetíveis a enfermidades.

1182

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1188

1189 **Referências bibliográficas**

1190 ALLAN, GL & GB MAGUIRE. 1992. Effects of pH and salinity on survival, growth
1191 and osmoregulation in *Penaeus monodon* Fabricius. *Aquaculture*, 107: 33–47.

1192 APHA (American Public Health Association). 1998. Standard Methods for the
1193 Examination of Water and Wastewater, 20st edition. Washington, DC. 1193p.

1194 BIERBOWER, SM & RL COOPER. 2010. The effects of acute carbon dioxide on
1195 behavior and physiology in *Procambarus clarkii*. *J. Exp. Zool.*, 313(A): 484–497.

1196 BOYD, CE. 2008. Carbon Dioxide: Waste, Nutrient. *Glob. Aquac. Advoc.* July/August:
1197 62-65.

1198 BOYD, CE & CS TUCKER. 1998. Pond aquaculture water quality management.
1199 Kluwer Academic, Boston, 700p.

1200 BUIKEMA, AL, RR NIEDERLEHNER & JJ CAIRNS. 1982. Biological monitoring
1201 Part IV. Toxicity testing. *Water Res.*, 16: 239-262.

1202 CALDEIRA, K & ME WICKETT. 2003. Anthropogenic carbon and ocean pH. *Nature*,
1203 425: 365.

1204 CARTER, HA, L CEBALLOS-OSUNA, NA MILLER & JH STILLMAN. 2013.
1205 Impact of ocean acidification on metabolism and energetic during early life stages of
1206 the intertidal porcelain crab *Petrolisthes cinctipes*. *J. Exp. Biol.*, 216: 1412-1422.

1207 DISSANAYAKE, A & A ISHIMATSU. 2011. Synergistic effects of elevated CO₂ and
1208 temperature on the metabolic scope and activity in a shallow-water coastal decapod
1209 (*Metapenaeus joyneri*; Crustacea: Penaeidae). *J. Mar. Sci.*, 68(6): 1147-1154.

1210 ENVIRONMENTAL PROTECTION AGENCY (EPA), 2002. Methods for measuring
1211 the acute toxicity of effluents and receiving waters to freshwater and marine
1212 organisms, US EPA, Washington, DC.

1213 FEELY, RA, SC DONEY & SR COOLEY. 2009. Ocean acidification: present
1214 conditions and future changes in a high-CO₂ world. *Oceanography*, 22(4): 36–47.

1215 FURTADO, PS, LH POERSCH & W WASIELESKY. 2011. Effect of calcium
1216 hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical
1217 performance of shrimp *Litopenaeus vannamei* reared in bio-flocs technology (BFT)
1218 systems. Aquaculture, 321: 130-135.

1219 FURTADO, PS, FP SERRA, LH POERSCH & WJr WASIELESKY. 2014. Short
1220 communication: Acute toxicity of hydrogen peroxide in juvenile white shrimp
1221 *Litopenaeus vannamei* reared in biofloc technology systems. Aquacult Int., 22(2):
1222 653-659.

1223 GROTTUM, JA & T SIGHOLT. 1996. Acute toxicity of carbon dioxide on European
1224 Seabass (*Dicentrarchus labrax*): Mortality and effects on plasma ions. Comp.
1225 Biochem. Physiol. A, 115(4): 323-327.

1226 GUTKNECHT J, MA BISSON & FC TOSTESON. 1977. Diffusion of carbon dioxide
1227 through lipid bilayer membranes: effects of carbonic anhydrase, bicarbonate, and
1228 unstirred layers. J. Gen. Physiol., 69: 779-794.

1229 HALL, MR & EH VAN HAM. 1998. The Effects of Different Types of Stress on Blood
1230 Glucose in the Giant Tiger Prawn *Penaeus monodon*. J. World Aquacul. Soc., 29(3):
1231 290-299.

1232 HAMILTON, MA, RC RUSSO & RV THURSTON. 1977. Trimmed Spearman-Kaber
1233 Method for estimating Median Lethal Concentrations on Toxicity Bioassays.
1234 Environ. Sci. Technol., 11(7): 714-719.

1235 HENRY, R, G KORMANIK, N SMATRESK & J CAMERON. 1981. The role of
1236 CaCO₃ dissolution as a source of HCO₃⁻ for the buffering of hypercapnic acidosis in
1237 aquatic and terrestrial decapod crustaceans. J. Exp. Biol., 94: 269-274.

1238 JENSEN, L, PS FURTADO, MMS FUGIMURA, LO GARCIA, LH POERSCH, JR
1239 VERANI & WJr WASIELESKY. 2014. The effect of stocking density on the

1240 transport of pink shrimp *Farfantepenaeus brasiliensis* (Crustacea: Decapoda), as
1241 live bait for sport fishing in Brazil. Lat. Am. J. Aquat. Res., 42(1): 204-212.

1242 KIKKAWA, T, Y WATANABE, Y KATAYAMA, J KITA & A ISHIMATSU. 2008.
1243 Acute CO₂ tolerance limits of juveniles of three marine invertebrates, *Sepia lycidas*,
1244 *Sepioteuthis lessoniana*, and *Marsupenaeus japonicas*. Plankton and Benthos Res.,
1245 3(3): 184-187.

1246 KURIHARA, H. 2008. Effects of CO₂-driven ocean acidification on the early
1247 developmental stages of invertebrates. Mar. Ecol. Prog. Ser., 373: 275–284.

1248 LIN, YC & JC CHEN. 2001. Acute toxicity of ammonia on *Litopenaeus vannamei*
1249 (Boone) juveniles at different salinity levels. J. Exp. Mar. Biol. Ecol., 259: 109-119.

1250 LIN, YC & JC CHEN. 2003. Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone)
1251 juveniles at different salinity levels. Aquaculture, 224: 193-201.

1252 LUSHCHAK, VI. 2011. Environmentally induced oxidative stress in aquatic animals.
1253 Aquat. Toxicol., 101: 13–30.

1254 MORAN, D. 2010. Carbon dioxide degassing in fresh and saline water. I. Degassing
1255 performance of a cascade column. Aquacult. Eng., 43: 29–36.

1256 PONCE-PALAFIX, J, CA MARTINEZ-PALACIOS & LG ROSS. 1997. The effects
1257 of salinity and temperature on the growth and survival rates of juvenile white
1258 shrimp, *Penaeus vannamei*, Boone, 1931. Aquaculture, 157: 107-115.

1259 SANTOS, GA, JW SCHRAMA, J CAPELLE, JHWM ROMBOUT & JAJ VERRETH.
1260 2013. Effects of dissolved carbon dioxide on energy metabolism and stress
1261 responses in European seabass (*Dicentrarchus labrax*). Aquacult. Res., 44: 1370-
1262 1382.

1263 SILVA, AF, GR LARA, ELC BALLESTER, D KRUMMENAUER, PC ABREU &
1264 WJr WASIELESKY. 2013. Effect of high stocking densities on growth and survival

1265 of *Litopenaeus vannamei* in final growout phase, reared in biofloc technology (BFT)
1266 system. Ciênc. anim. bras., 14(3): 279-287.

1267 SPRAGUE, JB. 1971. Measurement of pollutant toxicity to fish — III. Sublethal effects
1268 and “safe” concentrations. Water Res., 5: 245–266.

1269 TAYLOR, EW & NM WHITELEY. 1989. Oxygen transport and acid–base balance in
1270 the haemolymph of the lobster, *Homarus gammarus*, during aerial exposure and
1271 resubmersion. J. Exp. Biol., 144: 417–436.

1272 TIMMONS, MB & JM EBELING. 2010. Recirculating Aquaculture, 2nd edition.
1273 Cayuga Aqua Ventures, Ithaca, NY. <http://www.bee.cornell.edu/aquain18/11/2010>.
1274 998p.

1275 UNESCO. 1983. Chemical methods for use in marine environmental monitoring.
1276 Manual and Guides 12, Intergovernmental Oceanographic Commission. Paris,
1277 France.

1278 VAN WYK, P & J SCARPA. 1999. Water quality and management. In: VAN WYK, P.
1279 et al. (Eds.). Farming marine shrimp in recirculating freshwater systems. Florida
1280 department of agriculture and consumer services, Tallahassee, Florida, 141-161.

1281 VINATEA, LA. 2004. Princípios químicos de qualidade da água em aquicultura: uma
1282 revisão para peixes e camarões. 2ª edição revisada e ampliada, Editora UFSC. 231p.

1283 VINATEA, L, AO GALVEZ, CL BROWDY, A STOKES, J VENERO, J HAVEMAN,
1284 BL LEWIS, A LAWSON, A SHULER & JW LEFFLER. 2010. Photosynthesis,
1285 water respiration and growth performance of *Litopenaeus vannamei* in a super-
1286 intensive raceway culture with zero water exchange: interaction of water quality
1287 variables. Aquacult. Eng., 42: 17–24.

1288 WANG, W-N, B-S LI, J-J LIU, L SHI, MJ ALAM, S-J SU, J WU, L WANG & A-L
1289 WANG. 2012. The respiratory burst activity and expression of catalase in white

1290 shrimp, *Litopenaeus vannamei*, during long-term exposure to pH stress.
1291 Ecotoxicology, 21: 1609–1616.

1292 WICKINS, JF. 1984. The effect of hypercapnic sea water on growth and mineralization
1293 in penaeid prawns. Aquaculture, 41: 37-48.

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CAPÍTULO III

1296

1297

1298 **AVALIAÇÃO DOS EFEITOS DA SUSPENSÃO DA AERAÇÃO ARTIFICIAL**
1299 **DURANTE CULTIVO DE *Litopenaeus vannamei* (Boone 1931) NO SISTEMA DE**
1300 **BIOFLOCOS SOBRE AS CONCENTRAÇÕES DE DIÓXIDO DE CARBONO**
1301 **DISSOLVIDO.**

1302

1303

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1314 **Resumo**

1315 O cultivo de camarões marinhos com tecnologia de bioflocos (BFT) tem como
1316 vantagens a utilização de elevadas densidades de estocagem (>150 camarões/m²) e
1317 ausência de renovação de água. Devido às fertilizações orgânicas, ocorre incremento
1318 dos sólidos suspensos totais (SST). E esse fato, associado à alta densidade de estocagem
1319 gera alta taxa de consumo de oxigênio e liberação de dióxido de carbono (CO₂). O
1320 objetivo deste estudo foi avaliar as concentrações de CO₂ em diferentes densidades de
1321 estocagem de camarões e bioflocos, na ausência de aeração artificial e com aplicação de
1322 peróxido de hidrogênio (H₂O₂). Foram testados oito tratamentos: quatro concentrações
1323 de sólidos suspensos totais (240, 500, 1000 e 2000 mg/L) e duas densidades de
1324 estocagem (250 e 500 camarões/m²) com três repetições cada (24 tanques com volume
1325 útil de 200L). Os camarões, com peso médio de $4,04 \pm 0,73$ g, foram aclimatados por 96
1326 horas, antes de simular a falta de energia elétrica por 36 horas. Quando a concentração
1327 de oxigênio dissolvido próxima a 4,0 mg/L foram aplicados dosagens de 14,3
1328 $\mu\text{LH}_2\text{O}_2/\text{L}$. O monitoramento do oxigênio dissolvido, temperatura, pH, alcalinidade e
1329 CO₂ foram realizados a cada 3 horas durante as 36 horas. Ao término do teste os
1330 camarões de cada unidade experimental foram contados para cálculo da sobrevivência.
1331 Os resultados dos parâmetros de qualidade da água estiveram dentro da faixa
1332 recomendada para o cultivo da espécie, com exceção dos valores de pH e CO₂. A
1333 sobrevivência final foi superior a 90% e não houve diferença estatística significativa
1334 entre os tratamentos ($p>0,05$). Foi verificado que as concentrações de CO₂ são
1335 incrementadas ao longo do tempo com aumento da densidade de estocagem e com o
1336 aumento de sólidos suspensos totais.

1337 **Palavras-chave:** dióxido de carbono, *Litopenaeus vannamei*, bioflocos, peróxido de
1338 hidrogênio.

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1344 **Abstract**

1345 The rearing of marine shrimps in biofloc technology system (BFT) has the advantages
1346 of using high stocking densities (>150 shrimps/m²) and zero water exchange. Due to the
1347 organic fertilizations there is an increase in the total suspended solids (TSS), that
1348 generates high oxygen consumption and production of carbon dioxide (CO₂). The aim
1349 of this study was to evaluate the concentrations of CO₂ in different stocking densities of
1350 shrimps and different amounts of bioflocs when the aeration system fails. Hydrogen
1351 peroxide (H₂O₂) was the oxygen source for the organisms. For such, different rearing
1352 conditions were tested, being eight treatments: four concentrations of total suspended
1353 solids (240, 500, 1000 and 2000 mg/L) and two stocking densities (250 and 500
1354 shrimps/m²) with three repetitions each (24 tanks with 200L of useful volume). Shrimps
1355 with average weight of 4.04 ± 0.73 g were acclimated for 96 hours, before simulating an
1356 electrical power failure during 36 hours. It was determined the minimum concentration
1357 of 4.0 mg/L of dissolved oxygen (DO) for the application of 14.3 μ LH₂O₂/L.
1358 Monitoring of dissolved oxygen, temperature, pH, alkalinity and CO₂ was carried out
1359 every 3 hours during 36 hours. At the end of the test, the shrimps of each experimental
1360 unit were counted to calculate the survival. The results of the water quality parameters
1361 were within the recommended ranges for the rearing of the species, with the exception
1362 of the pH and CO₂ levels. The final survival was above 90% and there was no
1363 significant statistical differences among the treatments ($p > 0.05$). It was verified that the
1364 levels of CO₂ rise over time with the increase of the density and total suspended solids.
1365 Therefore, it is suggested that the use of hydrogen peroxide should not exceed 36-hour
1366 time to avoid reaching lethal levels of CO₂.

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1368 **Keywords:** Carbon dioxide, *Litopenaeus vannamei*, bioflocs, hydrogen peroxide

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1373 **Introdução**

1374

1375 Os cultivos de organismos aquáticos em sistemas de bioflocos (BFT) exigem de
1376 monitoramento constante dos parâmetros de qualidade da água, uma vez que ao longo
1377 do cultivo geralmente ocorre declínio da alcalinidade e do pH, um incremento dos
1378 níveis de sólidos suspensos totais (SST), bem como, o aumento das concentrações de
1379 dióxido de carbono dissolvido (Furtado et al. 2011; Gaona et al. 2011).

1380 O dióxido de carbono dissolvido (CO₂) é tanto um resíduo como um nutriente na
1381 aquicultura. Como nutriente, o CO₂ é utilizado pelos organismos fotoautotróficos, e
1382 como resíduo, tendo em vista que os organismos aeróbicos liberam dióxido de carbono
1383 e altas concentrações deste composto podem ser tóxicas para os animais aquáticos
1384 (Boyd 2008). Os camarões peneídeos são particularmente sensíveis ao incremento de
1385 CO₂, de modo que viveiros de cultivo devem ser manejados para minimizá-los (Hall &
1386 Van Ham 1998). Em viveiros de cultivo as concentrações de CO₂ frequentemente
1387 variam entre 5 e 10 mg/L durante o amanhecer, mas podem exceder 20 mg/L em
1388 viveiros com muita matéria orgânica (Boyd 2008).

1389 O material particulado suspenso em sistemas BFT pode melhorar
1390 significativamente a taxa de conversão alimentar, reduzindo custos de produção
1391 (Wasielesky et al. 2006). No entanto, quando o número de microrganismos
1392 heterotróficos excede o de fotoautotróficos, a produção de dióxido de carbono excede a
1393 produção de oxigênio dissolvido, podendo gerar acúmulo de CO₂ no sistema, reduzindo
1394 o pH (Vinatea et al. 2010; Furtado et al. 2011). Conforme Schweitzer et al. (2013), as
1395 concentrações de SST entre 400 e 600 mg/L são considerados mais adequados para o
1396 cultivo superintensivo de *L. vannamei* em sistemas BFT. Além disso, no sistema BFT as

1397 densidades de cultivo usuais são elevadas (150 a 600 camarões/m²), proporcionando
1398 maior biomassa nos tanques de cultivo (Silva et al. 2013).

1399 De acordo com Vinatea et al. (2009), em cultivos de *L. vannamei* em sistemas
1400 BFT os níveis de oxigênio dissolvido na água, na ausência de aeração, tornam-se
1401 críticos em aproximadamente 30 minutos. Nessas condições o peróxido de hidrogênio
1402 (H₂O₂) pode ser uma fonte alternativa de oxigênio emergencial para os camarões
1403 (Furtado et al. 2014). No entanto, não há registro na literatura sobre as concentrações de
1404 CO₂ durante a ausência de aeração em regimes de cultivo superintensivos. O objetivo
1405 deste estudo foi avaliar as concentrações de CO₂ em diferentes densidades de camarões
1406 e bioflocos, quando ocorre ausência de aeração artificial e se aplica peróxido de
1407 hidrogênio (H₂O₂), como fonte alternativa de oxigênio para os camarões.

1408

1409 **Material e Métodos**

1410 *Local e instalações*

1411

1412 O estudo foi conduzido na Estação Marinha de Aquicultura Prof. Marcos
1413 Alberto Marchiori (EMA), do Instituto Oceanográfico, da Universidade Federal do Rio
1414 Grande, localizada na praia do Cassino, Rio Grande, Rio Grande do Sul, Brasil.

1415 *Camarões e delineamento experimental*

1416 Os camarões utilizados no presente estudo foram adquiridos no laboratório
1417 Aquatec Ltda (Canguaretama, Rio Grande do Norte, Brasil). Após a chegada dos
1418 náuplios de *L. vannamei* nas instalações da EMA, estes foram mantidos no setor de
1419 larvicultura de camarões marinhos até pós-larvas 15 (PLs15). Posteriormente, os

1420 mesmos foram cultivados em tanque de 35 m³ (400 PLs/m³) em sistema BFT até o peso
1421 médio de 4,04±0,73g. A seguir, os camarões foram transferidos para as unidades
1422 experimentais com volume útil de 200 L (tanque circular de polietileno com 0,36 m²) e
1423 com dois difusores de ar (aero-tubeTM) no fundo do tanque, para prover oxigenação da
1424 água no nível de saturação e manter os sólidos em suspensão durante as 96 h de
1425 aclimação. Durante aclimação os animais foram alimentados duas vezes ao dia
1426 (08:00 e 17:00 h), com ração comercial (Centro Oeste Rações S.A.- Brasil) com 38% de
1427 proteína bruta e 8% de lipídeos na formulação, com auxílio de bandejas de alimentação
1428 (Wasielesky et al. 2006). Durante a fase experimental sem aeração artificial os camarões
1429 permaneceram em jejum.

1430 Foram testados oito tratamentos: quatro concentrações de sólidos suspensos
1431 totais (240, 500, 1000 e 2000 mg/L) e duas densidades de estocagem (250 e 500
1432 camarões/m²) com três repetições cada (24 tanques). Após o período de aclimação foi
1433 simulado a falta de energia elétrica por 36 horas. Durante a simulação foram aplicadas
1434 dosagens de 14,3 µLH₂O₂/L proposto por Furtado et al. (2014), sempre que a
1435 concentração de oxigênio dissolvido (OD) estava próxima a 4,0 mg/L.

1436 Para alcançar as diferentes concentrações de sólidos suspensos totais (SST)
1437 foram coletados bioflocos de um clarificador (volume útil de 800L) acoplado ao tanque
1438 de cultivo de origem dos camarões utilizados nesse estudo. O tempo de clarificação foi
1439 de 3 horas. Após esse período, a bomba foi desligada e o material sedimentado no
1440 clarificador foi resuspendido com aeração intensa para homogenizar o material e
1441 manter-lo aeróbico. Foram tomadas amostras de bioflocos para filtração e cálculo de
1442 concentração de sólidos suspensos totais (SST). Então, com os resultados de SST, foram
1443 calculados os volumes de bioflocos necessários para cada concentração de SST nos
1444 tanques dos diferentes tratamentos.

1445 *Parâmetros físicos e químicos de qualidade da água*

1446 O fotoperíodo experimental foi de 14h luz/10h escuro com iluminação natural de
1447 intensidade (média) de 18000 lx, medida na superfície da água. A salinidade 33 foi
1448 mantida igual ao tanque de origem dos camarões e bioflocos. As determinações de
1449 temperatura e oxigênio dissolvido foram realizadas a cada 3 horas por meio de oxímetro
1450 YSI 55 (Yellow Springs, OH, USA). As determinações de alcalinidade, salinidade, pH e
1451 concentrações de dióxido de carbono dissolvido foram mensuradas a cada 6 horas
1452 seguindo a metodologia proposta por APHA (1998), com auxílio de refratômetro óptico
1453 (Atago®, Japan), de pH-metro YSI 100 (Yellow Springs, OH, USA) e pelo software
1454 CO₂ Analysis Salt® (Timmons & Ebeling 2010), respectivamente. As concentrações de
1455 amônia total (N-AT) (NH₃ + NH₄⁺), nitrito (N-NO₂⁻) e nitrato (N-NO₃⁻) foram
1456 mensurados a cada 6 h, conforme metodologias preconizadas pela UNESCO (1983),
1457 Bendschneider & Robinson (1952) e Aminot & Chaussepied (1983), respectivamente.
1458 Os sólidos suspensos totais (SST mg/L) foram determinados no início do período de
1459 aclimatação, no início e fim do teste por gravimetria mediante filtragem de alíquotas de
1460 20 mL de água em filtros de fibra de vidro GF 50-A, conforme Strickland & Parsons
1461 (1972).

1462 Ao final do período experimental, o sistema de aeração foi restaurado e todos os
1463 camarões vivos em cada uma das 24 unidades experimentais foram contados para
1464 avaliação da sobrevivência nos diferentes tratamentos.

1465

1466 *Análise Estatística*

1467

1468 Para a análise estatística dos dados foi utilizado o software STATISTICA 7.0[®]
1469 (StatSoft Inc. 2004, Tulsa, Oklahoma, USA). Depois de verificada a homocedasticidade
1470 das variâncias (Levene) e a normalidade de distribuição dos dados (Kolmogorov-
1471 smirnov), foi realizada uma análise de variância de duas vias (ANOVA, two-way), para
1472 verificação de diferença significativa entre os dados obtidos. Quando detectada
1473 diferença significativa entre os tratamentos ($p < 0,05$), o teste de Tukey de comparação
1474 de médias foi utilizado. Os valores percentuais foram previamente transformados
1475 ($\arcsin^{0.5}$) antes de analisados (Zar 1996).

1476

1477 **Resultados**

1478

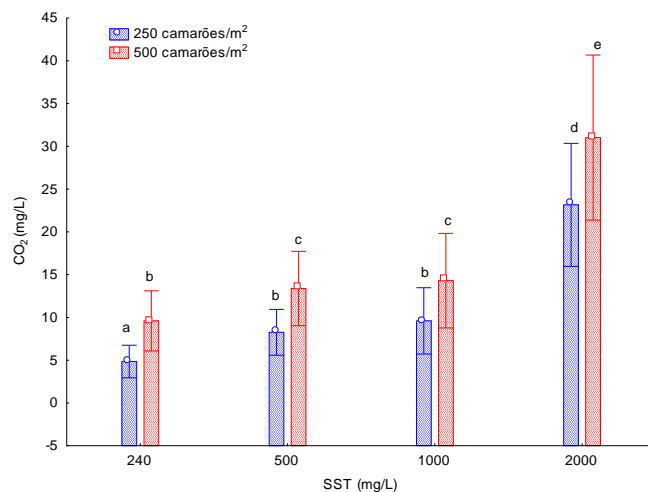
1479 Os dados mensurados de temperatura, salinidade, alcalinidade e nitrito não
1480 apresentaram diferenças significativas entre os tratamentos ($p > 0,05$). Os níveis de
1481 oxigênio dissolvido foram menores nos tratamentos com 2000 mg/L, havendo diferença
1482 significativa em relação aos demais tratamentos ($p < 0,05$). Nos tratamentos com maior
1483 biomassa foi necessário maior volume de peróxido de hidrogênio ao longo do estudo.
1484 As concentrações de N-AT foram significativamente ($p < 0,05$) maiores nos tratamentos
1485 com 500 camarões/m² nas concentrações de SST de 500, 1000 e 2000 mg/L, enquanto
1486 as concentrações de nitrato foram significativamente maiores nos tratamentos com
1487 concentrações de SST de 1000 e 2000 mg/L, independente da densidade de estocagem.
1488 Os resultados dos parâmetros de qualidade da água são apresentados na Tabela 1.

1489 Tabela 1 - Valores dos parâmetros de qualidade da água de cultivo de *L. vannamei* em
1490 sistema BFT nos tratamentos com diferentes concentrações de SST e densidades de
1491 estocagem, durante 36 horas sem aeração artificial.

Parâmetros	250 camarões/m ²				500 camarões/m ²			
	240	500	1000	2000	240	500	1000	2000
Temp (°C)	26,94±0,77	26,57±0,65	27,01±0,22	26,46±0,69	26,91±0,76	26,83±0,56	26,92±0,54	26,81±0,52
OD (mg/L)	9,49±2,01 ^A	8,65±2,80 ^A	8,49±1,40 ^A	6,79±1,61 ^B	8,52±2,61 ^A	8,05±1,54 ^A	8,12±2,53 ^A	6,48±1,66 ^B
Sal (g/L)	33,04±0,57	33,07±0,65	33,01±0,52	33,06±0,60	33,01±0,36	33,03±0,56	33,02±0,44	33,01±0,32
Alca	155,0±10,1	160,0±4,2	160,0±2,5	160,0±5,5	155,2±10,5	165,4±6,3	165,4±2,5	160,5±5,6
pH	7,53±0,37 ^A	7,38±0,29 ^A	7,33±0,43 ^{AB}	6,79±0,40 ^C	7,26±0,4 ^{AB}	7,16±0,29 ^{AB}	7,16±0,46 ^{AB}	6,68±0,45 ^C
CO ₂ (mg/L)	4,84±3,43 ^A	8,27±4,84 ^A	9,60±7,0 ^{AB}	23,16±12,9 ^C	9,59±6,3 ^{AB}	13,38±7,8 ^{AB}	14,31±9,9 ^{AB}	31,03±17,41 ^D
NAT (mg/L)	0,41±0,21 ^A	0,66±0,65 ^A	0,82±0,68 ^{AB}	0,48±0,45 ^A	0,34±0,18 ^A	1,61±1,28 ^{BC}	2,32±1,86 ^C	1,56±1,04 ^{BC}
N-NO ₂ ⁻ (mg/L)	0,05±0,03	0,05±0,03	0,07±0,04	0,09±0,06	0,05±0,04	0,04±0,02	0,09±0,14	0,08±0,06
N-NO ₃ ⁻ (mg/L)	32,83±1,72 ^A	46,51±1,93 ^A	96,5±9,35 ^B	95,17±5,91 ^B	35,33±2,2 ^A	48,40±3,36 ^A	99,50±13,1 ^B	98,0±12,71 ^B

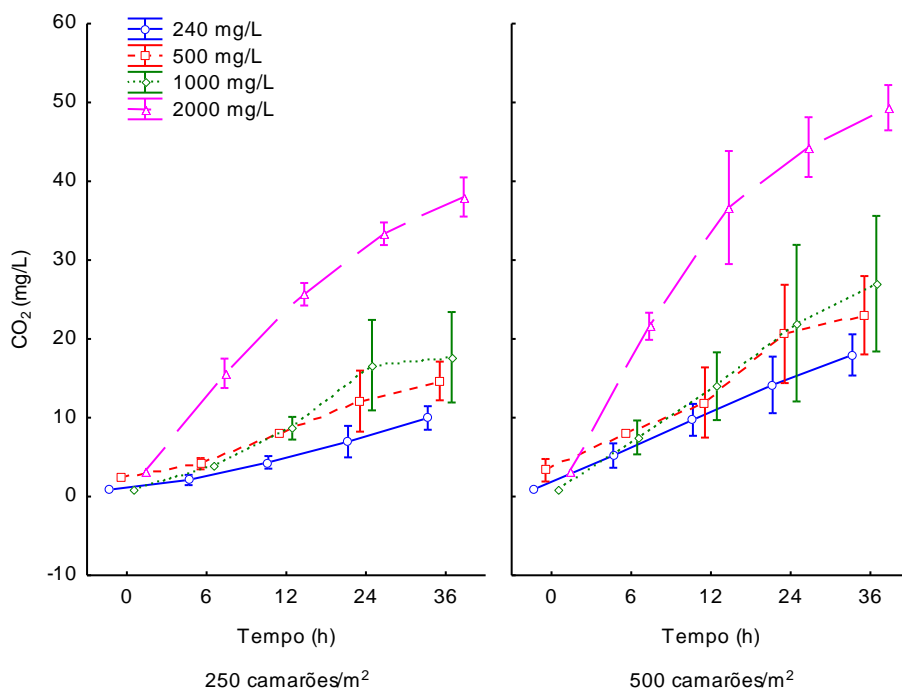
1492 ¹Os dados correspondem a media de 3 replicas ± desvio padrão. Letras diferentes
1493 sobrescritas na mesma linha indicam diferença significativa (p<0,05) entre os
1494 tratamentos. Temperatura (Temp), Oxigênio dissolvido (OD), Salinidade (Sal),
1495 Alcalinidade (Alca), nitrogênio amoniacal total (NAT), nitrito (N-NO₂⁻), nitrato (N-
1496 NO₃⁻).

1497 Foi observado aumento nas concentrações de CO₂, conforme incremento da
1498 densidade de estocagem, de SST e ao longo do tempo, havendo diferenças significativas
1499 (p<0,05) entre os tratamentos (figura 1 e 2). Simultaneamente, os valores de pH
1500 decaíram com aumento da densidade de estocagem, de SST e ao longo do tempo,
1501 havendo diferença significativa entre os tratamentos (p<0,05), como pode ser
1502 visualizado na figura 3. A concentração de SST foi mais impactante que a densidade de
1503 camarões estocados ao longo do tempo. A sobrevivência média final foi superior a 90%
1504 em todos os tratamentos, não houve diferença significativa entre os tratamentos
1505 (p>0,05).



1506

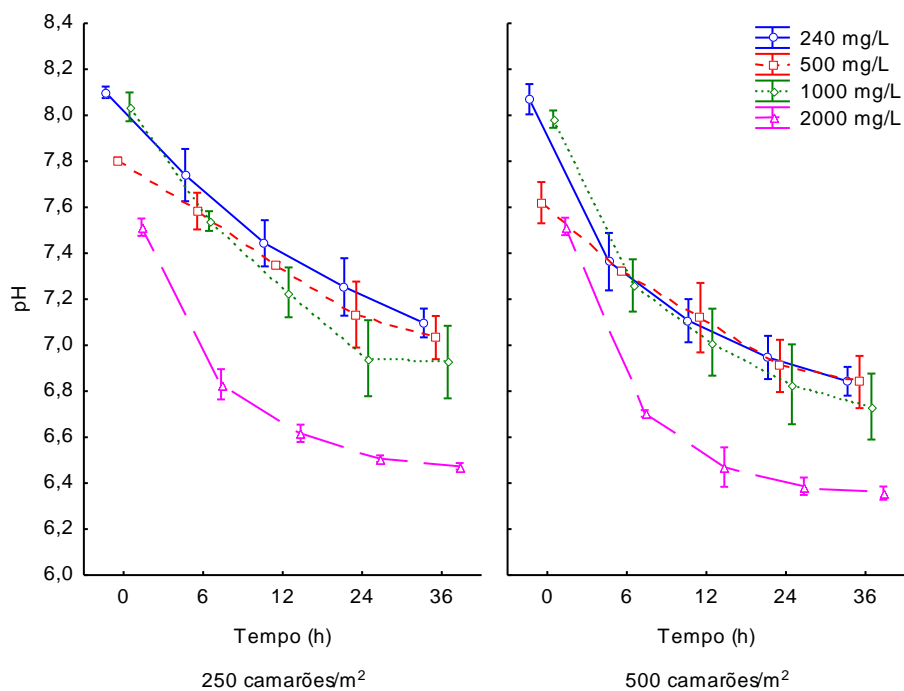
1507 Figura 1 - Variações das concentrações de CO₂ na água de cultivo de *L. vannamei* em
 1508 sistema BFT, nos tratamentos com 250 e 500 camarões/m², com quatro concentrações
 1509 de SST. Letras diferentes sobre as barras indicam diferença significativa (p < 0,05).



1510

1511 Figura 2 - Variações das concentrações de CO₂ ao longo de 36 horas, sem aeração na
 1512 água de cultivo de *L. vannamei* em sistema BFT, nos tratamentos com 250 e 500
 1513 camarões/m², com quatro concentrações de SST. As barras verticais indicam o desvio
 1514 padrão.

1515



1516

1517 Figura 3 - Variações dos valores de pH ao longo de 36 horas, sem aeração na água de
 1518 cultivo de *L. vannamei* em sistema BFT, nos tratamentos com 250 e 500 camarões/m²,
 1519 com quatro concentrações de SST. As barras verticais indicam o desvio padrão.

1520 Ao término das 36 horas sem aeração artificial, a mesma foi restaurada e as
 1521 concentrações de CO₂ em todos os tratamentos reduziram significativamente, para
 1522 valores inferiores a 8 mg/L de CO₂.

1523

1524 **Discussão**

1525

1526 Os parâmetros físicos e químicos de qualidade da água, mensurados ao longo do
 1527 período experimental mantiveram-se em níveis adequados para a sobrevivência dos
 1528 juvenis de *L. vannamei* (Ponce-Palafox et al. 1997; Van Wyk & Scarpa 1999; Lin &
 1529 Chen 2001, 2003; Khun et al. 2010).

1530 As menores concentrações de oxigênio dissolvido foram observadas nos
 1531 tratamentos com maior biomassa, devido ao maior consumo de oxigênio havendo

1532 necessidade de maior número de aplicações de H₂O₂ nesses tratamentos. No entanto, as
1533 concentrações de oxigênio dissolvido no estudo em média ficaram próximas a 6,0 mg/L
1534 sendo consideradas ideais para a sobrevivência de camarões peneídeos (Van Wyk &
1535 Scarpa 1999).

1536 Os valores médios de N-AT ficaram na faixa considerada tolerada para o cultivo
1537 destes juvenis (Lin & Chen 2001). No entanto houve diferença significativa entre os
1538 tratamentos ($p < 0,05$), uma vez que os tratamentos com maior SST exigiram aplicações
1539 de H₂O₂ mais frequentes do que os tratamentos com menor SST, e desse modo a ação
1540 desinfetante do H₂O₂ impactou negativamente a comunidade microbiana incrementando
1541 os níveis de N-AT nesses tratamentos. As concentrações de nitrito se mantiveram dentro
1542 dos níveis aceitáveis para o cultivo dos juvenis de *L. vannamei* (Lin & Chen 2003).

1543 Os valores iniciais de nitrato foram diferentes entre os tratamentos devido à
1544 diferença das concentrações de SST entre os mesmos, ou seja, os tratamentos com
1545 maior inóculo de bioflocos continham concentrações mais elevadas de nitrato, pois a
1546 fonte inicial dos bioflocos apresentava concentrações elevadas de nitrato. De acordo
1547 com Kuhn et al. (2010) ao avaliarem o efeito crônico do nitrato, reportaram impacto
1548 negativo sobre a sobrevivência e crescimento do *L. vannamei*, quando em concentrações
1549 maiores que 220 mg/L em médias salinidades (11‰). Por tanto, as concentrações dos
1550 diferentes compostos nitrogenados nos diferentes tratamentos ao longo do estudo não
1551 afetaram negativamente a sobrevivência dos camarões.

1552 Os parâmetros de qualidade de água analisados indicam que durante 36h o
1553 sistema pode ser manejado com segurança até níveis de 1000 mg/L de SST. No entanto
1554 Schweitzer et al. (2013) trabalhando com diferentes níveis de SST e por períodos
1555 prolongados verificaram maior grau de oclusão das brânquias dos camarões em sistemas
1556 com mais de 800 mg/L de SST. Para manejar as concentrações de SST pode ser

1557 utilizado o processo de clarificação, o qual auxilia na manutenção dos níveis de
1558 oxigênio dissolvido elevados ao longo do cultivo, redução das concentrações de fósforo,
1559 nitrato e aumento da alcalinidade (Ray et al. 2010; Gaona et al. 2011).

1560 Os valores de pH foram reduzindo ao longo do tempo em todos tratamentos,
1561 sendo que no tratamento SST 2000 mg/L, independente da densidade de estocagem, os
1562 valores ficaram abaixo do limite considerado ideal para o cultivo da espécie (Van Wyk
1563 & Scarpa 1999). Uma alteração no pH pode gerar a produção de espécies reativas de
1564 oxigênio (ERO) nas células do camarão, gerando estresse oxidativo e danos as
1565 estruturas celulares como membranas lipídicas, proteínas e DNA (Lushchak 2011;
1566 Wang et al. 2012). Contudo, os valores de pH mensurados ao longo do estudo estão
1567 distantes dos valores de pH letal para peneídeos (Allan & Maguire 1992; Capítulo I).

1568 Conforme Hall & Van Ham (1998), o CO₂ reduz o pH de 8,5 para 5,9 gerando
1569 um estresse maior no metabolismo de *Penaeus monodon* do que a mesma redução do
1570 pH pela adição de ácido sulfúrico. Segundo Van Wyk and Scarpa (1999), níveis de CO₂
1571 inferiores a 5 mg/L são ideais, até 20 mg/L na água são considerados aceitáveis para
1572 peneídeos, enquanto concentrações entre 20 e 60 mg/L causam interferências na troca
1573 de CO₂ nas brânquias. De acordo com os resultados obtidos no capítulo II dessa tese,
1574 concentrações de 59 mg/L de CO₂ são letais e o nível de segurança de CO₂ calculado
1575 para *L. vannamei* é de 5,9 mg/L. O CO₂ ingressa no organismo através do epitélio
1576 branquial resultando no declínio do pH da hemolinfa afetando negativamente a
1577 capacidade da mesma transportar oxigênio.

1578 As concentrações de CO₂ mensurados nos tratamentos com até 1000 mg/L foram
1579 considerados aceitáveis para os camarões nesse período de 36 horas, enquanto que os
1580 camarões dos tratamentos com níveis de SST de 2000 mg/L foram expostos a
1581 concentrações subletais de CO₂. Contudo, períodos de até 36 horas sem aeração em

1582 sistemas BFT com aplicação de H₂O₂ são possíveis para condições de SST de até 2000
1583 mg/L. Períodos de utilização do H₂O₂ como fonte alternativa de oxigênio, superiores as
1584 36 horas, talvez possam ser possíveis se aplicado algum tipo de dispositivo que rompa a
1585 tensão superficial da água, removendo CO₂.

1586 Ao término das 36 horas a aeração foi restaurada e os níveis de CO₂ em todos os
1587 tratamentos ficaram abaixo de 8 mg/L, de modo que o sistema de aeração bem
1588 dimensionado age como degaseificador eficiente, outra forma de remover CO₂ é
1589 através da aplicação de hidróxido de cálcio (Boyd 2008; Furtado et al. 2011). Apesar
1590 dos fatores estressantes como as alterações dos níveis de oxigênio dissolvido, alterações
1591 de pH e incremento das concentrações de CO₂ os valores encontrados de sobrevivência
1592 foram elevados no término desse estudo.

1593

1594 **Conclusão**

1595 Nas condições testadas o camarão marinho *L. vannamei* suportou um período de
1596 36 horas sem aeração com aplicação de H₂O₂ como fonte alternativa de oxigênio
1597 dissolvido. As concentrações de CO₂ se elevam com o incremento da biomassa de
1598 camarões e de sólidos suspensos totais, com o passar do tempo, alcançando níveis
1599 subletais para os camarões. O dióxido de carbono dissolvido acumulado pode ser
1600 removido pela restauração do sistema de aeração, promovendo a degaseificação.

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1605 são bolsistas de produtividade do CNPq.

1606

1607 **Referências bibliográficas**

1608 ALLAN, GL & GB MAGUIRE. 1992. Effects of pH and salinity on survival, growth
1609 and osmoregulation in *Penaeus monodon* Fabricius. *Aquaculture*, 107: 33–47.

1610 AMINOT, A & M CHAUSSEPIED. 1983. Manuel des analyses chimiques en milieu
1611 marin. Brest, CNEXO.

1612 APHA (American Public Health Association). 1998. Standard methods for the
1613 examination of water and wastewater. 20th edition. Washington, DC.

1614 BENDSCHNEIDER, K & RJ ROBINSON. 1952. A new spectrophotometric method
1615 for the determination of nitrite in sea water. *J. Mar. Res.*, 11: 87-96.

1616 BOYD, CE. 2008. Carbon Dioxide: Waste, Nutrient. *Glob. Aquac. Advoc.*, July/August
1617 62-65.

1618 FURTADO, PS, LH POERSCH & WJr WASIELESKY. 2011. Effect of Calcium
1619 Hydroxide, Carbonate and Sodium Bicarbonate on Water Quality and Zootechnical
1620 Performance of Shrimp *Litopenaeus vannamei* Reared in Bio-Flocs Technology
1621 (BFT) Systems. *Aquaculture*, 321: 130-135.

1622 FURTADO, PS, FP SERRA, LH POERSCH & WJr WASIELESKY. 2014. Short
1623 communication: Acute toxicity of hydrogen peroxide in juvenile white shrimp
1624 *Litopenaeus vannamei* reared in biofloc technology systems. *Aquacult Int.*, 22(2):
1625 653-659.

1626 GAONA, CAP, LH POERSCH, D KRUMMENAUER, GK FOES, & WJ
1627 WASIELESKY. 2011. The effect of solids removal on water quality, growth and
1628 survival of *Litopenaeus vannamei* in a biofloc technology culture system. *IJRA*, 12:
1629 54-73.

1630 HALL, MR & EH VAN HAM. 1998. The effects of different types of stress on blood
1631 glucose in the giant tiger prawn *Penaeus monodon*. J. World Aquac. Soc., 29(3):
1632 290-299.

1633 KUHN, DD, SA SMITH, GD BOARDMAN, MW ANGIER, LFJ MARSH & J
1634 GEORGE. 2010. Chronic toxicity of nitrate to Pacific white shrimp, *Litopenaeus*
1635 *vannamei*: Impacts on survival, growth, antennae length, and pathology.
1636 Aquaculture, 309: 109-114.

1637 LIN, YC & JC CHEN. 2001. Acute toxicity of ammonia on *Litopenaeus vannamei*
1638 (Boone) juveniles at different salinity levels. J. Exp. Mar. Biol. Ecol. 259:109-119.

1639 LIN, YC & JC CHEN. 2003. Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone)
1640 juveniles at different salinity levels. Aquaculture, 224: 193-201.

1641 LUSHCHAK, VI. 2011. Environmentally induced oxidative stress in aquatic animals.
1642 Aquatic. Toxicol., 101: 13–30.

1643 PONCE-PALAFIX J, CA MARTÍNEZ-PALACIOS & LG ROSS. 1997. The effect of
1644 salinity and temperature on the growth and survival rates of white shrimp, *Penaeus*
1645 *vannamei*, Boone, 1931. Aquaculture, 157: 107–115.

1646 RAY, JA, BL LEWIS, CL BROWDY & JW LEFFLER. 2010. Suspended solids
1647 removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of
1648 a plant-based feed in minimal-exchange, superintensive culture systems.
1649 Aquaculture, 299: 89-98.

1650 SCHVEITZER, R, R ARANTES, PFS COSTÓDIO, CME SANTO, LA VINATEA,
1651 WQ SEIFFERT & ER ANDREATTA. 2013. Effect of different biofloc levels on
1652 microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank
1653 system operated with no water exchange. Aquacult. Eng., 56: 59-70.

1654 SILVA, AF, GR LARA, ELC BALLESTER, D KRUMMENAUER, PC ABREU &
1655 WJr WASIELESKY. 2013. Effect of high stocking densities on growth and survival
1656 of *Litopenaeus vannamei* in final growout phase, reared in biofloc technology (BFT)
1657 system. Ciênc. anim. bras., 4(3): 279-287.

1658 STRICKLAND, JDH & TR PARSONS. 1972. A practical handbook of seawater
1659 analysis. Ottawa: Fishery Research Board Canada, 310p.

1660 TIMMONS, MB & JM EBELING. 2010. Recirculating Aquaculture, 2nd edition.
1661 Cayuga Aqua Ventures, Ithaca, NY. <http://www.bee.cornell.edu/aquain18/11/2010>.
1662 998p.

1663 UNESCO. 1983. Chemical methods for use in marine environmental monitoring.
1664 Manual and Guides 12, Intergovernmental Oceanographic Commission. Paris,
1665 France.

1666 VAN WYK, P & J SCARPA. 1999. Water quality and management. In: VAN WYK, P.
1667 et al. (Eds.). Farming marine shrimp in recirculating freshwater systems. Florida
1668 department of agriculture and consumer services, Tallahassee, Florida, 141-161.

1669 VINATEA, L, AO GÁLVEZ, J VENERO, J LEFFLER & C BROWDY. 2009. Oxygen
1670 consumption of *Litopenaeus vannamei* juveniles in heterotrophic medium with zero
1671 water exchange. Pesq. Agropec. Bras., 44(5): 534-538.

1672 VINATEA, L, AO GALVEZ, CL BROWDY, A STOKES, J VENERO, J HAVEMAN,
1673 BL LEWIS, A LAWSON, A SHULER & JW LEFFLER. 2010. Photosynthesis,
1674 water respiration and growth performance of *Litopenaeus vannamei* in a super-
1675 intensive raceway culture with zero water exchange: interaction of water quality
1676 variables. Aquacult. Eng., 42: 17-24.

1677 WANG, W-N, B-S LI, J-J LIU, L SHI, MJ ALAM, S-J SU, J WU, L WANG & A-L
1678 WANG. 2012. The respiratory burst activity and expression of catalase in white

1679 shrimp, *Litopenaeus vannamei*, during long-term exposure to pH stress.
1680 Ecotoxicology, 21: 1609–1616.

1681 WASIELESKY, W, H ATWOOD, A STOKES & CL BROWDY. 2006. Effect of
1682 natural production in a zero exchange suspended microbial floc based super-
1683 intensive culture system for white shrimp *Litopenaeus vannamei*. Aquaculture, 258:
1684 396-408.

1685 ZAR, JH. 1996. Biostatistical Analysis. Third Edition New Jersey: Prentice Hall, 662p.

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1703 **CAPÍTULO IV**

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1707

1708 **EFEITO DAS CONCENTRAÇÕES DE DIÓXIDO DE CARBONO DISSOLVIDO**

1709 **NO CONSUMO DE OXIGÊNIO DO CAMARÃO BRANCO DO PACÍFICO**

1710 *Litopenaeus vannamei* (Boone 1931).

1711

1712

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1726 **Resumo**

1727 Durante o cultivo do camarão branco do Pacífico *Litopenaeus vannamei* com tecnologia
1728 de bioflocos (BFT) ocorre o incremento dos níveis de sólidos suspensos totais, assim
1729 como da biomassa de camarões nos tanques, que pode chegar a 2-8 kg/m². Quando
1730 ocorre uma falha no sistema de aeração, as concentrações de oxigênio dissolvido
1731 diminuem rapidamente e as de dióxido de carbono (CO₂) incrementam-se. Durante esse
1732 proceso, os organismos são expostos a uma condição de hipoxia e de hipercapnia. Nesse
1733 estudo buscamos avaliar o efeito de diferentes concentrações de CO₂ sobre as taxas de
1734 consumo de oxigênio do camarão *L. vannamei*. Para tal, foi utilizado um respirômetro
1735 de fluxo contínuo em recirculação (5,3±0,7 L/h), com oximêtro digital de fibra óptica de
1736 10 canais. Foram utilizados oito juvenis de *L. vannamei* (12,1±1,4g) por tratamento,
1737 sendo que cada um foi colocado em uma câmara respirométrica de 0,6 L. Os camarões
1738 foram aclimatados as condições experimentais por 18 horas e então foi injetado o CO₂
1739 nas concentrações experimentais: 5, 30, 60, 95, 150 e 300 mgCO₂/L, por seis horas. Na
1740 concentração 60 mgCO₂/L houve um incremento do consumo de oxigênio. Porém nos
1741 tratamentos 95, 150 e 300 mgCO₂/L os camarões apresentaram redução do consumo de
1742 oxigênio e perda de equilíbrio. De acordo com os resultados obtidos recomenda-se que
1743 durante o cultivo os níveis de CO₂ sejam mantidos próximos a 5 mgCO₂/L.

1744 **Palavras-chave:** dióxido de carbono, hipercapnia, *Litopenaeus vannamei*, consumo de
1745 oxigênio.

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1750 **Abstract**

1751 During the cultivation of the Pacific white shrimp *Litopenaeus vannamei* with biofloc
1752 technology (BFT), an increase in the total suspended solids levels is observed, as well
1753 as the shrimp biomass in the tanks can reach 2-8 kg/m². If a failure occurs in the
1754 aeration system, oxygen levels decrease rapidly and carbon dioxide (CO₂) increases
1755 with the same quickness. In this situation, organisms experience a condition of hypoxia
1756 and hypercapnia. In this study we aimed to evaluate the effect of different
1757 concentrations of CO₂ on the oxygen consumption of *L. vannamei*. For this purpose, a
1758 continuous-flow respirometer with water recirculation (5.3 ± 0.7 L/h) equipped with a
1759 digital fiber-optic oxymeter of 10 channels was used. Eight juveniles of *L. vannamei*
1760 (12.1 ± 1, 4 g) were used in each treatment, one per respiratory chamber (0.6 L). The
1761 shrimp had been acclimated to the experimental conditions for 18 hours when CO₂ was
1762 injected in the concentrations of each treatment (5, 30, 60, 95, 150 and 300 mgCO₂/L)
1763 with an exposure time of six hours. In the treatments of up to 60 mgCO₂/L, an increase
1764 was observed in the consumption of oxygen. However, in the treatments of 95, 150 and
1765 300 mgCO₂/L, the consumption of oxygen by the shrimp decreased and they presented
1766 loss of equilibrium. We recommend that CO₂ should be maintained in 5 mgCO₂/L
1767 during the cultivation.

1768 **Keywords:** carbon dioxide, *Litopenaeus vannamei*, hypercapnia, consumption of
1769 oxygen.

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1776 **Introdução**

1777

1778 O consumo de oxigênio de uma determinada espécie é importante para o cálculo
1779 efetivo da potência de aeração a ser instalada nos tanques de cultivo de uma
1780 determinada espécie. Esse parâmetro serve ainda para prover dados sobre o
1781 metabolismo dos organismos, expostos a fatores estressantes que possam surgir durante
1782 o cultivo (Bett & Vinatea 2009; Campos et al. 2014). O aumento na taxa de respiração
1783 em condições de estresse ambiental é um indicativo do aumento no consumo energético
1784 para a manutenção da homeostase. Vários trabalhos têm sido realizados com camarões
1785 marinhos, visando determinar a taxa de consumo de oxigênio em função de uma série
1786 de variáveis ambientais. Tsuzuki (1995) estudando *Farfantepenaeus paulensis* cita que
1787 as taxas respiratórias podem ser influenciadas por fatores intrínsecos (tamanho, idade,
1788 fase de muda, sexo, aclimação, etc). Bett & Vinatea (2009) quantificaram o consumo
1789 de oxigênio de juvenis de *L. vannamei* em condições combinadas de temperaturas (20,
1790 25 e 30°C) e salinidades (1, 13, 25 e 37 ‰) para juvenis com três tamanhos diferentes
1791 (2, 6 e 12 g).

1792 O *Litopenaeus vannamei* tem sido a espécie de camarão mais utilizada em
1793 sistemas de cultivo superintensivos com tecnologia de bioflocos (BFT), nos quais se
1794 trabalham com densidades de estocagem entre 150 e 600 camarões/m² e biomassas de 2
1795 a 8 kg/m² (Silva et al. 2013). Durante a formação dos bioflocos ocorre incremento dos
1796 sólidos em suspensão e matéria orgânica a níveis superiores a 1000 mg/L, sendo
1797 necessária a remoção do excesso de bioflocos por meio de clarificação, mantendo-os
1798 próximos a 500 mg/L (Gaona et al. 2011, Schweitzer et al. 2013). Em condições
1799 particulares quando falta energia elétrica e o sistema de aeração artificial é desativado as
1800 concentrações de oxigênio dissolvido diminuem rapidamente alcançando níveis críticos
1801 (0,65 mg/L) para *L. vannamei* em cerca de 30 minutos (Vinatea et al. 2009). Nesses

1802 episódios pode ser aplicadas doses de peróxido de hidrogênio como fonte emergencial
1803 de oxigênio para os camarões de forma segura evitando a mortalidade massiva de
1804 camarões cultivados (Furtado et al. 2014). No capítulo III dessa tese foi verificado que
1805 ocorre uma elevação nas concentrações de dióxido de carbono dissolvido (CO₂) ao
1806 longo do tempo, e essas concentrações são ainda maiores de acordo com incremento da
1807 biomassa de camarões e bioflocos.

1808 Quando a concentração de CO₂ na água é superior a 20mg/L, dificulta a
1809 excreção do CO₂ pelo epitélio branquial resultando no declínio do pH afetando
1810 negativamente a capacidade da hemocianina em transportar oxigênio e assim, reduzindo
1811 a oxigenação nos tecidos e aumentando a taxa de ventilação (Taylor & whiteley 1989;
1812 Van Wyk & Scarpa 1999). Para Van Wyk & Scarpa (1999) níveis de CO₂ inferiores a 5
1813 mg/L são ideais, até 20 mg/L são considerados aceitáveis para peneídeos.

1814 Em temperaturas elevadas nos cultivos de camarões marinhos, a solubilidade do
1815 oxigênio é menor, o consumo de oxigênio dos camarões e dos microorganismos é
1816 elevado, podendo levar a uma condição de hipóxia. Em condições de hipóxia a
1817 toxicidade do CO₂ é potencializada, pois interfere na capacidade de absorção do
1818 oxigênio (Boyd & Tucker 1998). O objetivo deste estudo foi avaliar o efeito do CO₂
1819 dissolvido no consumo de oxigênio de juvenis de *L. vannamei* cultivados com
1820 tecnologia de bioflocos.

1821

1822 **Material e Métodos**

1823

1824 *Local e instalações*

1825

1826 O estudo foi conduzido na Unidad Multidisciplinaria de Docencia e
1827 Investigación da Facultad de Ciencias da Universidad Nacional Autónoma de México -
1828 UNAM, no porto de abrigo em Sisal (21°9'55. 22 N, 90°1'54. 93 W) Hunucmá,
1829 Yucatán, México.

1830

1831 *Água, camarões e delineamento experimental*

1832 Os juvenis de *L. vannamei* foram obtidos de um cultivo em regime intensivo
1833 (150 camarões/m³) com tecnologia de bioflocos realizado nas instalações da UMDI. Os
1834 camarões foram cultivados por 110 dias em um tanque circular de 5 m de diâmetro
1835 (15m³), com base de fibra de vidro cobertos com geomembrana de PVC Alkorplan® de
1836 1,0 mm, protegidos com sombrite (50%) para reduzir a intensidade luminosa e limitar
1837 predação por pássaros. Os tanques foram abastecidos com água do mar de salinidade 38
1838 ‰, filtrada em filtro de areia. A aeração intensiva foi fornecida por meio de um
1839 soprador de ar (5HP). A drenagem foi modificada para poder excluir diariamente os
1840 sedimentos do fundo concentrados no centro do tanque, totalizando uma taxa de
1841 recâmbio diário de 1% do volume do tanque.

1842 Os camarões foram alimentados quatro vezes ao dia com dietas comerciais
1843 contendo 35% proteína bruta e 8% lipídeos. A metodologia de alimentação foi realizada
1844 com uso de bandejas, conforme proposto por Seiffert & Andreatta (2004). Os camarões
1845 permaneceram no setor de engorda até atingir o peso médio de 12,1±1,4 gramas.

1846 Posteriormente, os mesmos foram transferidos para 10 unidades experimentais
1847 com volume útil de 30 L. Foram estocados 10 camarões em cada tanque retangular de
1848 polietileno (área de fundo de 0,20 m²), resultando em uma densidade de estocagem de
1849 100 camarões/m². No fundo de cada unidade experimental havia um difusor de ar para

1850 prover oxigenação da água no nível de saturação. Os camarões permaneceram em jejum
1851 por 12h antes de serem colocados nas câmaras respirométricas.

1852 O experimento consistiu de seis tratamentos: as concentrações de dióxido de
1853 carbono testadas foram de 5, 30, 60, 95, 150 e 300 mgCO₂/L, por seis horas na
1854 salinidade 33. Durante todo período experimental os camarões permaneceram em jejum.
1855

1856 *Parâmetros físicos e químicos de qualidade da água*

1857 O fotoperíodo experimental adotado foi de 12h luz/12h escuro com iluminação
1858 artificial. A sala experimental teve a temperatura controlada em 26°C, de modo que a
1859 temperatura da água foi de 25°C. As determinações de pH, temperatura e CO₂ foram
1860 realizadas a cada 15 minutos durante as seis horas de exposição ao CO₂, com auxílio de
1861 pH metro YSI 100 (Yellow Springs, OH, USA), dioxide carbon test kit Hach® (Hack
1862 Company, Loveland, Colorado, USA) e pelo software CO₂ Analysis Salt® (Timmons &
1863 Ebeling 2010), respectivamente. A salinidade foi verificada diariamente com
1864 refratômetro óptico (Atago®, Japan). A alcalinidade foi analisada diariamente seguindo
1865 a metodologia proposta por APHA (1998).

1866

1867 *Consumo de oxigênio*

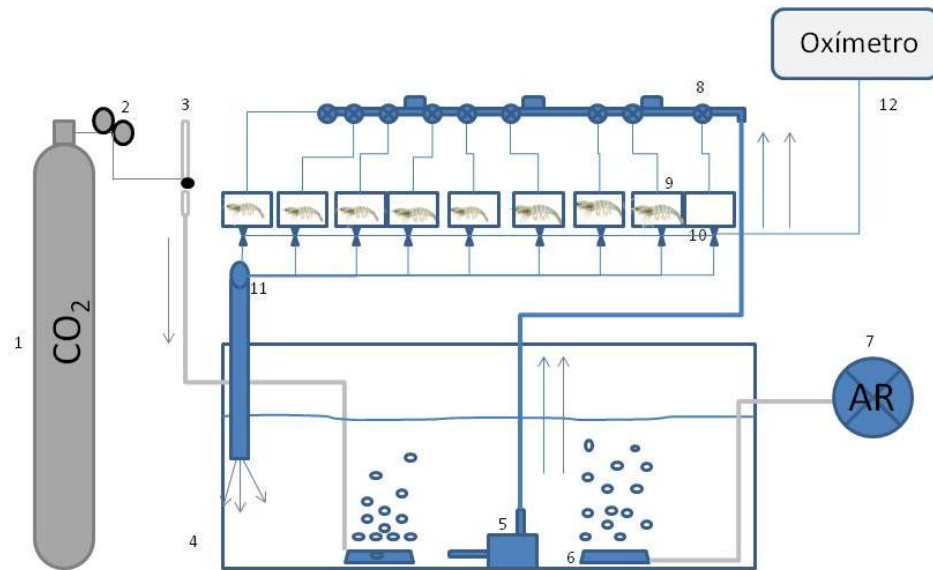
1868 A injeção do CO₂ nas câmaras respirométricas foi feita utilizado-se um cilindro
1869 de gás pressurizado de 30 kg com manômetro, válvula reguladora e conjunto de
1870 fluxômetros especiais para dióxido de carbono (escala de 0 a 15 L/min). Foi montado
1871 um sistema de recirculação composto por 10 câmaras respirométricas de 0,6L, um
1872 reservatório retangular com 250L, no qual havia uma bomba submersa (1750L/h,
1873 Boyu®) (Figura 1). A bomba transferia água do reservatório para uma tubulação com
1874 registros para controle de fluxo de água na entrada das câmaras respirométricas, das

1875 quais retornavam por gravidade para o reservatório. No reservatório de 250L havia três
1876 difusores de ar para prover aeração e um para prover CO₂ proveniente do cilindro de gás
1877 pressurizado.

1878 Utilizou-se um Oximêtro Digital de Fibra Óptica de 10 Canais Modelo OXY
1879 10, da Precision Sensing GmbH (Pre Sens[®], Germany) com sensores ópticos de
1880 oxigênio, conectado através de uma interfase a um computador com Software OXY 10.
1881 Foi programado para fazer registros de oxigênio dissolvido (OD) a cada minuto. Foi
1882 colocado um sensor para registrar os valores de OD antes de entrar nas câmaras
1883 respirométricas. Sensores individuais foram colocados na saída de cada câmara com e
1884 sem camarão, controle, para medir os níveis de OD de saída da câmara que indicava ao
1885 mesmo tempo a concentração de OD na câmara. O fluxo de água de saída foi regulado
1886 em $5,3 \pm 0,7$ L/h. Ajustou-se o mesmo fluxo para todas as câmaras.

1887 Os camarões foram mantidos em jejum 12 horas antes da estocagem nas
1888 câmaras respirométricas. Utilizaram-se oito camarões por tratamento. Os camarões
1889 foram colocados individualmente em oito câmaras respirométricas preenchidas com
1890 água do respectivo tratamento e conectadas ao sistema de recirculação. As câmaras
1891 foram lacradas cuidando para não deixar bolhas de ar dentro da câmara.

1892



1893

1894

1895 Figura 1 - Esquema ilustrativo do sistema experimental, onde 1: tanque de dióxido de
 1896 carbono (CO₂); 2: manômetro e válvula reguladora; 3: fluxômetro; 4: reservatório com
 1897 volume útil de 250L; 5: bomba submersa; 6: difusor de ar para oxigenação da água e
 1898 injeção CO₂; 7: soprador de ar; 8: registro para ajuste de fluxo de água; 9: câmaras
 1899 respirométricas com 0,6L de volume útil onde os camarões foram estocados; 10:
 1900 sensores ópticos; 11: tubulação de retorno das câmaras respirométricas para o
 1901 reservatório; 12: oxímetro digital acoplado ao computador.

1902

1903 Os camarões permaneceram um período total de 24 h no interior das câmaras
 1904 respirométricas. Bett & Vinatea (2009) verificaram que em 1h os camarões já estavam
 1905 aclimatados as condições experimentais, no entanto padronizaram em 2h de aclimação
 1906 para iniciar as medições de consumo de oxigênio, no presente estudo padronizamos em
 1907 18h de aclimação. Após aclimação houve uma hora de ajuste da concentração de
 1908 dióxido de carbono de acordo com cada tratamento e 5 horas de consumo de oxigênio
 para cada concentração de CO₂. Uma vez terminadas as medições, os camarões foram

1909 retirados das câmaras, secados e pesados (peso vivo) em uma balança digital modelo
1910 Scout (Ohaus[®]) com capacidade de 600±0,1g.

1911 Os dados de consumo de oxigênio foram expressos em mgO₂/g/h, segundo
1912 método de Lomholt & Johansen (1979):

$$1913 \quad VO_2 = ([O_{2e} - O_{2s}] \times F \text{ camarão}) - ([O_{2e} - O_{2s}] \times F \text{ controle}) / P_c$$

1914 Onde,

1915 VO₂ = consumo de oxigênio em mgO₂/g/h,

1916 O_{2e} = concentração de oxigênio em mg/L obtida na entrada da câmara,

1917 O_{2s} = concentração de oxigênio em mg/L obtida na saída da câmara,

1918 F= fluxo em L/h,

1919 P_c = peso corporal úmido (g).

1920

1921 *Análise Estatística*

1922 Para a análise estatística dos dados foi utilizado o software STATISTICA 7.0[®]
1923 (StatSoft Inc. 2004, Tulsa, Oklahoma, USA). Depois de verificada a homocedasticidade
1924 das variâncias (Levene) e a normalidade da distribuição dos dados (Kolmogorov-
1925 Smirnov), foi realizada uma análise de variância de uma via (ANOVA), para verificar
1926 possíveis diferenças significativas entre os dados obtidos. Quando detectada diferença
1927 significativa entre os tratamentos (p<0,05), foi aplicado o teste de Tukey para a
1928 comparação de médias.

1929

1930

1931 **Resultados**

1932

1933 *Parâmetros físicos e químicos de qualidade da água*

1934

1935 Os parâmetros físicos e químicos de qualidade da água monitorados ao longo do
 1936 experimento estão apresentados na Tabela 1. Os valores de dióxido de carbono e pH
 1937 foram significativamente diferentes ($p < 0,05$) entre os tratamentos, conforme planejado
 1938 no delineamento experimental. A concentração de oxigênio dissolvido foi menor nos
 1939 tratamentos com maior concentração de CO_2 ($p < 0,05$). A temperatura e a salinidade não
 1940 apresentaram diferenças significativas entre os tratamentos ($p > 0,05$) ao longo do estudo.
 1941 A alcalinidade não variou mantendo-se em $290 \text{ mgCaCO}_3/\text{L}$.
 1942 Tabela 1 - Valor médio \pm desvio padrão dos parâmetros físicos e químicos de qualidade
 1943 da água mensurados durante o período de 6 horas em que juvenis de *L. vannamei* foram
 1944 submetidos a diferentes concentrações de dióxido de carbono (CO_2).

Tratamentos	Parâmetros				
	T°C	OD (mg/L)	CO ₂ (mg/L)	pH	Salinidade (g/L)
5 mg/L	25,03 \pm 0,17	6,60 \pm 0,08 ^a	5,04 \pm 1,98 ^a	7,65 \pm 0,10 ^a	33,62 \pm 0,37
30 mg/L	25,00 \pm 0,24	6,57 \pm 0,09 ^a	30,01 \pm 3,53 ^b	7,18 \pm 0,05 ^b	33,67 \pm 0,36
60 mg/L	25,14 \pm 0,13	6,48 \pm 0,10 ^{ab}	59,29 \pm 4,31 ^c	6,88 \pm 0,04 ^c	33,72 \pm 0,34
95 mg/L	25,10 \pm 0,11	6,34 \pm 0,15 ^b	95,16 \pm 4,14 ^d	6,36 \pm 0,05 ^d	33,80 \pm 0,23
150 mg/L	25,04 \pm 0,12	5,96 \pm 0,29 ^{bc}	152,02 \pm 8,67 ^e	6,14 \pm 0,05 ^e	33,84 \pm 0,30
300 mg/L	25,08 \pm 0,15	5,80 \pm 0,24 ^c	300,04 \pm 14,10 ^f	5,90 \pm 0,03 ^f	33,90 \pm 0,28

1945 ¹Os dados correspondem a média de 8 réplicas \pm desvio padrão. Letras diferentes
 1946 sobrescritas na mesma linha indicam que as médias diferem significativamente
 1947 ($p < 0,05$). Temperatura (T°C), oxigênio dissolvido (OD), dióxido de carbono dissolvido
 1948 (CO_2).

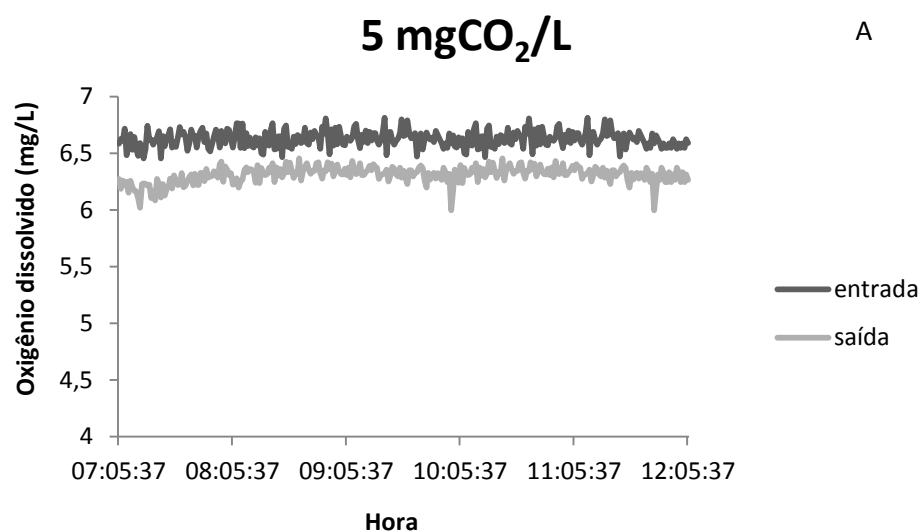
1949

1950 *Consumo de oxigênio*

1951 Os valores médios de OD na entrada e saída das câmaras respirométricas dos
1952 tratamentos 5, 30, 60, 95, 150 e 300 mgCO₂/L são apresentados nas Figuras 2A, B, C,
1953 D, E, F respectivamente. Na figura 2F os dados de OD são interrompidos cerca de duas
1954 horas de exposição ao CO₂ devido a mortalidade dos camarões. Os consumo de
1955 oxigênio nos diferentes tratamentos apresentaram diferenças significativas (p<0,05)
1956 (Figura 3). O resultado de consumo de oxigênio nos tratamentos 5, 30, 60, 95, 150 e 300
1957 mgCO₂/L foi 0,233±0,129, 0,33±0,072, 0,521±0,098, 0,46±0,12, 0,36±0,118, 0,3±0,049
1958 mgO₂/g/h, respectivamente.

1959 Até o tratamento 60 mgCO₂/L houve um incremento do consumo de oxigênio.
1960 Porém, nos tratamentos 95, 150 e 300 mgCO₂/L os camarões diminuíram o consumo de
1961 oxigênio e apresentaram alterações comportamentais típicas de narcose, com a
1962 consequente perda de equilíbrio. Os camarões com sintomas de anestesia nos
1963 tratamentos 95 e 150 mgCO₂/L apresentaram 100% de sobrevivência após o período de
1964 exposição ao CO₂. Enquanto que no tratamento 300 mgCO₂/L houve 62,5% de
1965 mortalidade (Figura 2F).

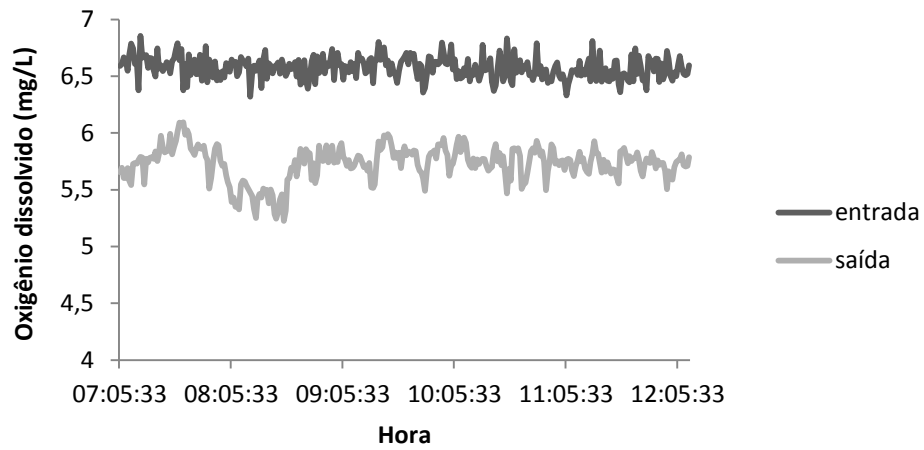
1966



1967

30 mgCO₂/L

B

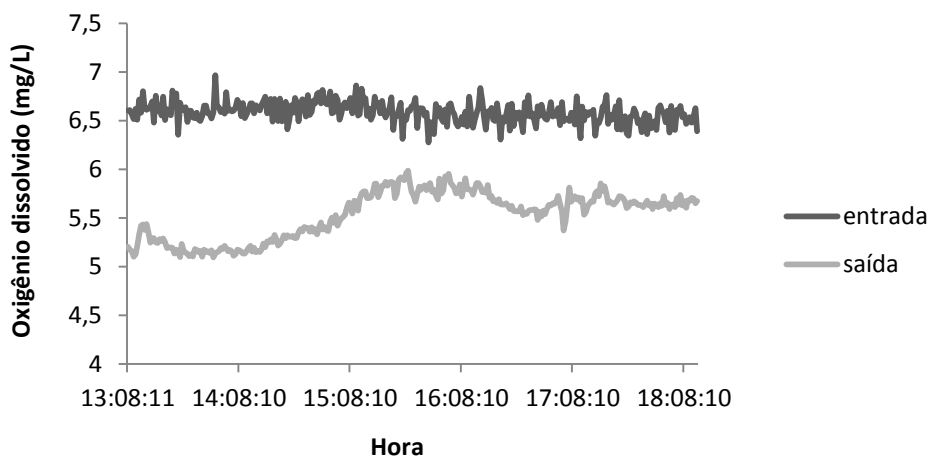


1968

1969

60 mgCO₂/L

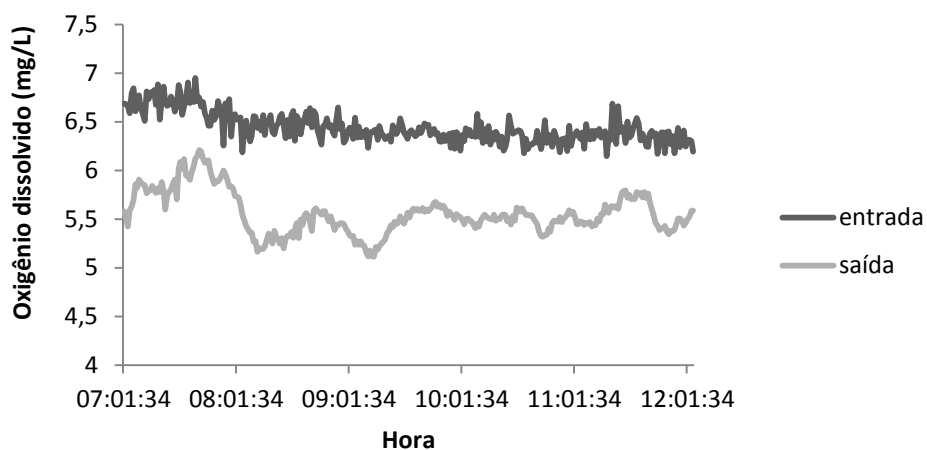
C



1970

95 mgCO₂/L

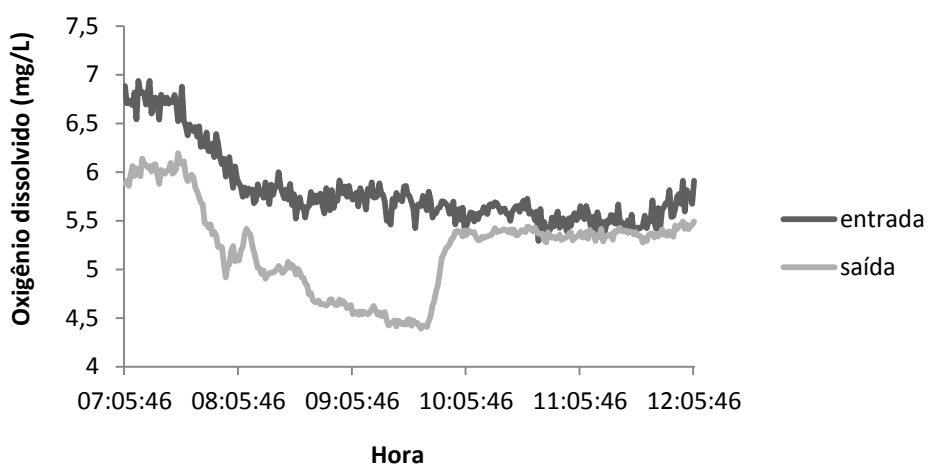
D



1971

150 mgCO₂/L

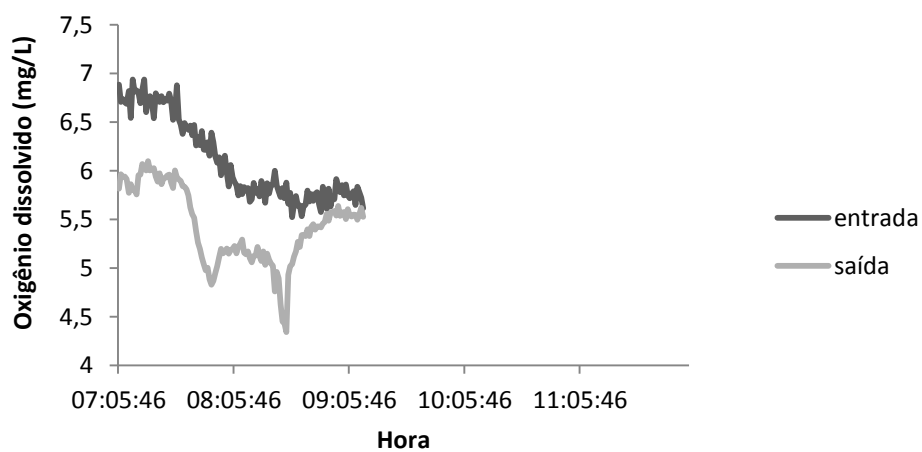
E



1972

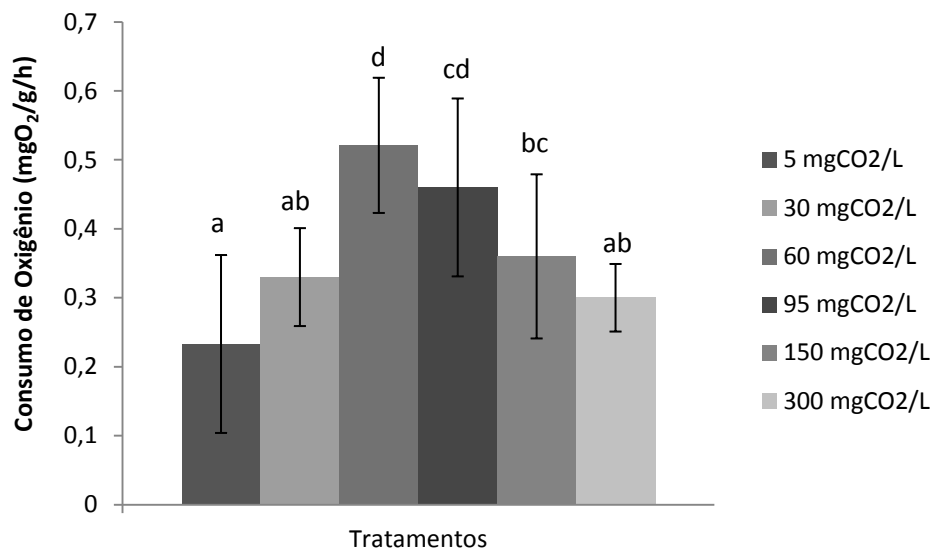
300 mgCO₂/L

F



1973

1974 Figura 2 - Valor médio da concentração de oxigênio dissolvido (mg/L) na entrada (linha
1975 superior) e saída (linha inferior) das câmaras respirométricas (n=8 por tratamento) ao
1976 longo de 5 horas de exposição as diferentes concentrações de CO₂: A- 5 mg/L; B- 30
1977 mg/L; C- 60mg/L; D- 95 mg/L; E- 150 mg/L; F- 300 mg/L.



1978

1979 Figura 3 - Valores médios \pm desvio padrão do consumo de oxigênio (mg de oxigênio
1980 dissolvido/ gramas de camarão/hora) de juvenis de *L. vannamei* (n=8 por tratamento)
1981 expostos por 6 horas. Letras diferentes escritas sobre as barras indicam diferenças
1982 significativas entre os tratamentos ($p < 0,05$).

1983 **Discussão**

1984 A temperatura da água durante o experimento permaneceu dentro da faixa adequada
1985 de 24 a 32°C para o crescimento de camarões peneídeos (Van Wyk & Scarpa 1999). A
1986 salinidade média permaneceu dentro da faixa de 0,5 a 35 g/L indicada para o cultivo da
1987 espécie (Van Wyk & Scarpa 1999). As concentrações de oxigênio dissolvido no estudo
1988 foram próximas a 6,0 mg/L sendo consideradas ideais para o cultivo de camarões
1989 peneídeos (Van Wyk & Scarpa 1999). Deste modo os parâmetros de temperatura,
1990 salinidade e oxigênio dissolvido foram favoráveis para a sobrevivência dos camarões.

1991 Conforme Ponce-Palafox et al. (2013) o metabolismo respiratório do camarão
1992 branco do Pacífico pode ser influenciado pela temperatura, salinidade, tamanho e a
1993 interação entre esses fatores, sendo que a temperatura apresenta maior influência que a
1994 salinidade. Zhang et al. (2009) avaliando o efeito de diferentes salinidades (5, 15, 25, 35
1995 e 40g/L) no consumo de oxigênio de juvenis de *L. vannamei* com peso médio de 6
1996 gramas em 20°C, verificaram um menor consumo de oxigênio para os camarões na
1997 salinidade 5 (0,25 mgO₂/g/h) e maior consumo na salinidade 25 (0,35 mgO₂/g/h).

1998 Nos tratamentos 5 e 30 mgCO₂/L verificamos valores semelhantes de consumo de
1999 oxigênio aos encontrados por Zhang et al. (2009) na salinidade 35, mesmo com
2000 diferença entre o peso dos camarões e de temperatura da água. Bett & Vinatea (2009)
2001 verificaram a taxa de consumo de oxigênio para juvenis de *L. vannamei* com peso
2002 médio entre 10-14g em diferentes salinidades e temperaturas e encontraram a seguinte
2003 equação: consumo específico de oxigênio (mgO₂/g/h) = -0.0281 + 0.0135*T – 0.0019*S
2004 – 0.0007*W. Assim quando comparamos a média do tratamento com menor nível de
2005 CO₂ (0,233 mgO₂/g/h) com a equação de Bett & Vinatea (2009) alimentada com nossos
2006 dados de temperatura, peso médio e salinidade (0,238 mgO₂/g/h), verificamos que são
2007 similares e que 5 mgCO₂/L não afeta negativamente a taxa respiratória de *L. vannamei*.
2008 Esses dados corroboram o nível de segurança calculado em 5.9 mgCO₂/L por Furtado
2009 no capítulo II dessa tese.

2010 Nos tratamentos com maior nível de CO₂ verificou-se uma redução significativa nos
2011 níveis de oxigênio dissolvido, porém não suficiente para que os camarões
2012 experimentassem níveis de hipóxia (Diaz & Rosenberg 1995). Ao avaliar o consumo de
2013 oxigênio sob condições de hipóxia Ponce-Palafox et al. (2013) verificaram uma redução
2014 na taxa de consumo de oxigênio, sendo que os camarões (20g) adotaram
2015 comportamento de oxiconformadores. O consumo de oxigênio em condições de hipóxia

2016 torna-se extremamente dependente da concentração de oxigênio e a capacidade
2017 metabólica do camarão se reduz de 26-34% (Villarreal et al. 1994).

2018 Outro parâmetro que apresentou diferença significativa foi o pH, com o incremento
2019 dos níveis de CO₂ houve uma redução dos valores de pH. No entanto os menores
2020 valores de pH foram de 5,87 no tratamento 300 mgCO₂/L e não se aproximaram do pH
2021 4.5 a partir do qual juvenis de *L. vannamei* apresentaram mortalidades (Capítulo I).
2022 Zhang et al. (2006) concluíram que o peso corporal, temperatura, salinidade, pH e
2023 condições alimentares tem efeitos significativos na concentração letal de oxigênio
2024 dissolvido para *L. vannamei*. Nas condições utilizadas em seu estudo encontraram uma
2025 relação entre a concentração letal de oxigênio dissolvido (CLOD) e pH como:
2026 $CLOD=0.1487 \text{ pH}^2 -2.2488 \text{ pH}+8.8806$ ($r^2=0.99$, $P=0.0055$). Por tanto, utilizando o pH
2027 mais ácido obtido nesse estudo, calculamos que a concentração letal de oxigênio
2028 dissolvido seria de 0,804 mg/L. Deste modo é possível eliminar o pH e a concentração
2029 de oxigênio dissolvido como causa direta da mortalidade verificada no tratamento 300
2030 mgCO₂/L.

2031 Avaliando a toxicidade do CO₂ em juvenis de *L. vannamei* (Capítulo II)
2032 encontraram valores de CL₅₀ e limites de confiança 95% em 24, 48, 72 e 96 h de 130.05
2033 (104.2-162.1), 77.2 (73.8-80.02), 69.65 (65.47-74.32), 59.12 (53.08-66.07) mg/L de
2034 CO₂, respectivamente. Durante o presente estudo verificamos perda de equilíbrio nos
2035 camarões dos tratamentos 95, 150 e 300 mgCO₂/L, esse mesmo comportamento
2036 também foi verificado por Kikkawa et al. (2008). Porém os camarões do tratamento 300
2037 mgCO₂/L apresentaram sintomas de anestesia nos primeiros 115 minutos de exposição
2038 ao CO₂ e logo após iniciaram as mortalidades que ao final foram superior a 60%.

2039 O CO₂ molecular se difunde diretamente através das membranas celulares, ou
2040 seja, quando a concentração de CO₂ na água for elevada, dificultará a excreção do CO₂

2041 pelo epitélio branquial resultando no declínio do pH da hemolinfa (acidose respiratória)
2042 (Taylor & Whiteley 1989). O principal mecanismo de equilíbrio ácido-base está
2043 baseado na troca de íons através das brânquias, essas trocas são realizadas pela Na^+/K^+ -
2044 ATPase basolateral e possivelmente pela H^+ -ATPase apical (Henry et al., 1981). Um
2045 distúrbio no equilíbrio ácido-base ocasiona a redução do pH da hemolinfa podendo
2046 produzir espécies reativas de oxigênio (ERO) que por sua vez, podem gerar dano
2047 oxidativo as estruturas celulares como membranas lipídicas, proteínas e DNA (Wang et
2048 al. 2012).

2049 Conforme resultados obtidos nesse estudo e por Hall & Van Ham (1998) os
2050 camarões apresentam particular sensibilidade ao incremento de CO_2 , de modo que
2051 viveiros de cultivo devem ser manejados para manter as concentrações próximas ao
2052 nível de segurança 5,9 mgCO_2/L (Van Wyk & Scarpa 1999; Capítulo II) e minimizar
2053 flutuações diárias de CO_2 . Para controlar as concentrações de CO_2 dentro dos limites
2054 favoráveis para o cultivo de camarões e peixes podem ser instaladas colunas de
2055 degaseificação (Moran et al. 2010), sistemas de aeração corretamente dimensionados
2056 de acordo com a biomassa a ser cultivada e podem ser aplicados uma série de
2057 compostos alcalinizantes como hidróxido de cálcio, carbonato e bicarbonato de sódio
2058 (Furtado et al. 2011).

2059

2060 **Conclusão**

2061 No presente estudo foi verificado um incremento no consumo de oxigênio de
2062 juvenis de *L. vannamei* expostos a concentrações superiores a 30 mgCO_2/L .
2063 Recomendamos que durante o cultivo de *L. vannamei* os níveis de CO_2 sejam mantidos

2064 no nível de segurança de 5,9 mgCO₂/L, para reduzir o consumo de oxigênio e evitar
2065 condições de hipercapnia.

2066

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2076 **Referências bibliográficas**

2077 APHA (American Public Health Association). 1998. Standard methods for the
2078 examination of water and wastewater. 20st edition. Washington, DC.

2079 BETT, C & L VINATEA. 2009. Combined effect of body weight, temperature and
2080 salinity on shrimp *Litopenaeus vannamei* oxygen consumption rate. Braz. J.
2081 Oceanogr., 57(4): 305-314.

2082 BOYD, CE. 2008. Carbon Dioxide: Waste, Nutrient. Glob. Aquac. Advoc. July/August:
2083 62-65.

2084 BOYD, CE & CS TUCKER. 1998. Pond aquaculture water quality management.
2085 Kluwer Academic, Boston, 700p.

2086 CAMPOS, BR, PS FURTADO, F D'INCAO, LH POERSCH & WJr WASIELESKY.
2087 2014. The effect of ammonia, nitrite and nitrate on the oxygen consumption of
2088 juvenile pinkshrimp *Farfantepenaeus brasiliensis* (LATREILLE 1817)
2089 (CRUSTACEA: DECAPODA). J. Appl. Aquacult., 26(1): 94-101.

2090 DIAZ, RJ & R ROSENBERG. 1995. Marine benthic hypoxia: a review of its ecological
2091 effects and the behavioural responses of benthic macrofauna. Oceanogr. Mar. Biol.:
2092 An Annual Review, 33: 245-303.

2093 FURTADO, PS, LH POERSCH & WJr WASIELESKY. 2011. Effect of Calcium
2094 Hydroxide, Carbonate and Sodium Bicarbonate on Water Quality and Zootechnical
2095 Performance of Shrimp *Litopenaeus vannamei* Reared in Bio-Flocs Technology
2096 (BFT) Systems. Aquaculture, 321: 130-135.

2097 FURTADO, PS, FP SERRA, LH POERSCH, & WJr WASIELESKY. 2014. Short
2098 communication: Acute toxicity of hydrogen peroxide in juvenile white shrimp
2099 *Litopenaeus vannamei* reared in biofloc technology systems. Aquacult. Int., 22(2):
2100 653-659.

2101 GAONA, CAP, LH POERSCH, D KRUMMENAUER, GK FOES, & WJ
2102 WASIELESKY. 2011. The effect of solids removal on water quality, growth and
2103 survival of *Litopenaeus vannamei* in a biofloc technology culture system. IJRA, 12:
2104 54-73.

2105 HALL, MR & EH VAN HAM. 1998. The Effects of Different Types of Stress on Blood
2106 Glucose in the Giant Tiger Prawn *Penaeus monodon*. J. World Aquacult. Soc., 29
2107 (3): 290-299.

- 2108 HENRY, R, G KORMANIK, N SMATRESK & J CAMERON. 1981. The role of
2109 CaCO_3 dissolution as a source of HCO_3^- for the buffering of hypercapnic acidosis in
2110 aquatic and terrestrial decapod crustaceans. *J. Exp. Biol.*, 94: 269-274.
- 2111 KIKKAWA, T, Y WATANABE, Y KATAYAMA, J KITA & A ISHIMATSU. 2008.
2112 Acute CO_2 tolerance limits of juveniles of three marine invertebrates, *Sepia lycidas*,
2113 *Sepioteuthis lessoniana*, and *Marsupenaeus japonicus*. *Plankton and Benthos Res.*,
2114 3 (3): 184-187.
- 2115 LOMHOLT, JP & K JOHANSEN. 1979. Hypoxia acclimation in carp - how it affects
2116 O_2 uptake, ventilation, and O_2 extraction from water. *Physiol. Zool.*, 52: 38-49.
- 2117 MORAN, D. 2010. Carbon dioxide degassing in fresh and saline water. I. Degassing
2118 performance of a cascade column. *Aquacult. Eng.*, 43: 29–36.
- 2119 PONCE-PALAFOX, JT, A RUÍZ-LUNA, MG GARCÍA-ULLOA, HM ESPARZA-
2120 LEAL, JL ARREDONDO-FIGUEROA, CA MARTINEZ-PALACIOS & LG
2121 ROSS. 2013. A response-surface analysis of the relative importance of the
2122 temperature, salinity and body weight on the respiratory metabolism of the white
2123 shrimp *Litopenaeus vannamei* (Boone 1931), *Mar. Fres. Behav. Physiol.*, 46(6):
2124 399-417.
- 2125 SCHVEITZER, R, R ARANTES, PFS COSTÓDIO, CME SANTO, LA VINATEA,
2126 WQ SEIFFERT & ER ANDREATTA. 2013. Effect of different biofloc levels on
2127 microbial activity, water quality and performance of *Litopenaeus vannamei* in a
2128 tank system operated with no water exchange. *Aquacult. Eng.*, 56: 59– 70.
- 2129 SEIFFERT, WQ & E ANDREATTA. 2004. El manejo de la alimentación y la
2130 sostenibilidad em el cultivo de camarones en el Brasil. In L. Elizabeth Cruz Suárez,

2131 Denis Ricque Marie, Mireya Tapia Salazar, Martha G. Nieto López, David A.
2132 Villareal Cavazos, Scholz, U. y Gonzáles M. Avances en Nutrición Acuícola VII.
2133 VII Simposio Internacional de Nutrición Acuícola 16-19 Noviembre. Hermosillo,
2134 Sonora, México.

2135 SILVA, AF, GR LARA, ELC BALLESTER, D KRUMMENAUER, PC ABREU &
2136 WJr WASIELESKY. 2013. Effect of high stocking densities on growth and survival
2137 of *Litopenaeus vannamei* in final growout phase, reared in biofloc technology
2138 (BFT) system. Ciênc. anim. bras., 14(3): 279-287.

2139 TAYLOR, EW & NM WHITELEY. 1989. Oxygen transport and acid–base balance in
2140 the haemolymph of the lobster, *Homarus gammarus*, during aerial exposure and
2141 resubmersion. J. Exp. Biol., 144: 417–436.

2142 TIMMONS, MB & JM EBELING. 2010. Recirculating Aquaculture, 2nd edition.
2143 Cayuga Aqua Ventures, Ithaca, NY. <http://www.bee.cornell.edu/aquain18/11/2010>.
2144 998p.

2145 VAN WYK, P & J SCARPA. 1999. Water quality and management. In: VAN WYK, P.
2146 et al. (Eds.). Farming marine shrimp in recirculating freshwater systems. Florida
2147 department of agriculture and consumer services, Tallahassee, Florida, 141-161.

2148 VILLARREAL, H, P HINOJOSA & J NARANJO. 1994. Effect of temperature and
2149 salinity on the oxygen consumption of laboratory produced *Penaeus vannamei*
2150 postlarvae. Comp. Biochem. Physiol. A., 108: 331–336.

2151 VINATEA L, AG OLIVERA, J VENERO, J LEFFLER & C BROWDY. 2009. Oxygen
2152 consumption of *Litopenaeus vannamei* juveniles in heterotrophic medium with zero
2153 water exchange. Pesq. Agropec. Bras., 44: 534–538.

2154 WANG, W-N, B-S LI, J-J LIU, L SHI, MJ ALAM, S-J SU, J WU, L WANG & A-L
2155 WANG. 2012. The respiratory burst activity and expression of catalase in white
2156 shrimp, *Litopenaeus vannamei*, during long-term exposure to pH stress.
2157 Ecotoxicology, 21: 1609–1616.

2158 ZHANG, PD, XM ZHANG, J LI & GQ HUANG. 2006. The effects of body weight,
2159 temperature, salinity, pH, light intensity and feeding condition on lethal DO levels
2160 of whiteleg shrimp, *Litopenaeus vannamei* (Boone 1931). Aquaculture, 256: 579–
2161 587.

2162 ZHANG, P, X ZHANG, J LI & T GAO. 2009. Effect of salinity on survival, growth,
2163 oxygen consumption and ammonia-N excretion of juvenile whiteleg shrimp,
2164 *Litopenaeus vannamei*. Aquacult. Res., 40: 1419-1427.

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CAPÍTULO V

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2178 **The effect of different alkalinity levels on *Litopenaeus vannamei* reared with**
2179 **biofloc technology (BFT).**

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2181

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2199 **Resumo**

2200 No início do cultivo de camarões marinhos com tecnologia de bioflocos, há o processo
2201 de formação dos bioflocos e desenvolvimento da comunidade bacteriana autotrófica,
2202 concomitantemente há um incremento dos níveis de sólidos suspensos totais e um
2203 declínio dos níveis de alcalinidade e pH. Essa redução da alcalinidade e do pH ocorre
2204 devido ao consumo do carbono inorgânico pelas bactérias autotróficas presentes nos
2205 bioflocos e biofilmes. O objetivo desse estudo foi avaliar o efeito de diferentes
2206 alcalinidades na qualidade da água e desempenho zootécnico de camarões marinhos *L.*
2207 *vannamei* em sistema BFT. O experimento consistiu de quatro tratamentos com três
2208 repetições cada: 75, 150, 225, 300 mgCaCO₃/L. Para manter a alcalinidade nos níveis
2209 estabelecidos (± 10 mgCaCO₃/L) para cada tratamento foi aplicado bicarbonato de
2210 sódio (NaHCO₃). Para tal, doze unidades experimentais retangulares de polietileno
2211 (área=0,20 m²) com 50 L de volume útil foram estocadas com 30 juvenis de *L.*
2212 *vannamei* (0,20 ± 0,07g) correspondendo a uma densidade de estocagem de 150
2213 camarões/m² ou 375 camarões/m³ mantidos por um período experimental de 49 dias.
2214 Foram colocados substratos artificiais correspondentes a 74% da área das paredes do
2215 tanque. O tratamento 75 apresentou os maiores níveis de amônia e nitrito ao longo do
2216 estudo marcando diferença significativa (p<0,05) quando comparado aos demais
2217 tratamentos. Quanto ao desempenho zootécnico foi verificada diferença significativa
2218 (p<0,05) no peso final, ganho de peso semanal e taxa de crescimento específico, onde
2219 300 foi significativamente melhor (p<0,05) que 75. Quanto à sobrevivência não foi
2220 verificada diferença significativa (p>0,05), sendo os valores médios superiores a 88%.
2221 Os resultados obtidos indicam que alcalinidade mais elevada favorece a formação dos
2222 bioflocos e estabelecimento das bactérias nitrificantes, proporcionando menor emprego
2223 de carbono orgânico e ainda menor aplicação de compostos alcalinizantes no sistema de
2224 cultivo.

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2230 **Abstract**

2231 The initial stages of rearing marine shrimp using biofloc technology (BFT) involve the
2232 biofloc formation process. At the same time, there is an increase in the levels of total
2233 suspended solids and a decrease in alkalinity and pH. This reduction of alkalinity and
2234 pH occurs due to the consumption of inorganic carbon by the autotrophic bacteria
2235 present in the bioflocs and biofilms. The aim of this study was to evaluate the effects of
2236 different alkalinities on water quality and the zootechnical performance of the marine
2237 shrimp *Litopenaeus vannamei* in a BFT system. The experiment consisted of four
2238 treatments, with three replicates each: 75, 150, 225 and 300 mgCaCO₃/L. To maintain
2239 the alkalinity at the established level sodium bicarbonate was applied. For the
2240 experiments, twelve experimental units (area=0.20 m²) with an effective volume of 50 L
2241 were stocked with 30 juvenile *L. vannamei* (0.20 ± 0.07 g), to achieve a stocking
2242 density of 150 shrimps/m² and were maintained for an experimental period of 49 days.
2243 The 75 treatment presented the highest levels of ammonia and nitrite throughout the
2244 study, compared to the 150 and 300 treatments. The results showed that higher
2245 alkalinity favors biofloc formation and the establishment of nitrifying bacteria.

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2247 **Keywords:** Alkalinity, *Litopenaeus vannamei*, bioflocs, biofilm, nitrogenous
2248 compounds.

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2260 **Introduction**

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2262 To preserve the environment and natural resources, the expansion of aquaculture should
2263 occur in a sustainable manner. Biofloc technology (BFT) is a technique for rearing
2264 aquatic organisms that has recently been drawing attention due to its sustainability. This
2265 methodology results in improvements in water quality through the carbon:nitrogen
2266 balance, recycling of nutrients and increased natural productivity in the rearing system
2267 (Crab et al., 2012). The balance of carbon and nitrogen (C:N) at an aspect ratio of
2268 approximately 15-20:1 favors the assimilation of ammoniacal nitrogen by heterotrophic
2269 bacteria that exhibit the capacity to synthesize protein from organic carbon and
2270 ammonia (Avnimelech, 1999). According to Samocha et al. (2007), the addition of
2271 organic carbon sources, such as sugar cane molasses, can prevent increases in the
2272 concentration of total ammoniacal nitrogen during the rearing of *Litopenaeus vannamei*
2273 in BFT systems.

2274 Heterotrophic bacteria show growth rates and bacterial biomass production that
2275 are 10 times greater than nitrifying bacteria. Autotrophic bacteria perform nitrification,
2276 oxidizing ammonia to nitrite and, posteriorly, to nitrate. Due to the slow growth of these
2277 bacteria, nitrite tends to accumulate at significant concentrations in rearing
2278 environments without water renewal (Hargreaves, 2006). Because heterotrophic
2279 bacteria use organic carbon from molasses as an energy source and present faster
2280 growth than nitrifying bacteria, they eventually reduce the nitrification rate in the
2281 system (Chen et al., 2006). Zhu and Chen (2001) reported a reduction of greater than
2282 50% in the nitrification rate when the C/N ratio was increased from 0 to 1. However,
2283 they did not verify any difference in the nitrification rate between C/N ratios of 1 and 2,
2284 thus confirming the negative impact of organic matter on the nitrification rate.

2285 Bratvold and Browdy (2001) said that microorganisms fixed in artificial
2286 substrates increase the nitrification rate in rearing tanks, resulting in a decrease in the
2287 ammonia concentration. Biofilms formed on the surface of a submerged artificial
2288 substrate can be defined as a microbial union of autotrophic and heterotrophic bacteria
2289 associated with extracellular polymeric substances fixed to these submerged surfaces
2290 (Thompson et al., 2002). Such biofilms are composed of microorganisms that belong to
2291 the natural diets of many species of penaeid shrimps, serving as a food supplement for
2292 these animals (Ballester et al. 2007). Recently, Viau et al. (2013) demonstrated the
2293 contribution of biofilms formed on artificial substrates to the maintenance of water
2294 quality and the increased survival of juveniles of the pink shrimp *F. brasiliensis* during
2295 the nursery phase. Thus, to facilitate the development of nitrifying bacteria, it is
2296 important to add artificial substrates to rearing systems without water renewal.

2297 When rearing of *Litopenaeus vannamei* using biofloc technology, constant
2298 monitoring of the physical and chemical parameters of the water quality is necessary
2299 because during the process of biofloc formation, 3 important events occur: 1), elevation
2300 of the concentration of total ammoniacal nitrogen (TAN); 2), reduction of the
2301 concentration of TAN and elevation of the concentration of nitrite, through the action of
2302 ammonia-oxidizing autotrophic bacteria (AOB); and 3), reduction of the concentration
2303 of nitrite and elevation of the concentration of nitrate through the action of nitrite-
2304 oxidizing autotrophic bacteria (NOB). Throughout the rearing process, increases in the
2305 total suspended solids levels and decreases of alkalinity and pH levels have also been
2306 verified (Furtado et al., 2011, Gaona et al., 2011).

2307 This reduction of alkalinity occurs due to the consumption of inorganic carbon
2308 by the heterotrophic and nitrifying bacteria that form the bioflocs (Ebeling et al., 2006).
2309 For each gram of total ammoniacal nitrogen (TAN) oxidized to nitrate (N-NO_3^-),

2310 approximately 4.18 g of oxygen and 7.07 g of alkalinity are consumed and 0.17 g of
2311 bacterial biomass is produced (Chen et al., 2006). According to Ebeling et al. (2006), to
2312 each gram of ammoniacal nitrogen converted in microbial biomass, 4.71 g of dissolved
2313 oxygen, 3.57 g of alkalinity and 15.17 g of carbohydrates are consumed, with 8.07 g of
2314 microbial biomass and 9.65 g of carbon dioxide being produced. When the performance
2315 of nitrifying and heterotrophic bacteria is compared, it can be seen that nitrifying
2316 bacteria consume more alkalinity and produce much less microbial biomass, while
2317 heterotrophic bacteria consume approximately half the alkalinity consumed in the
2318 production of nitrifying bacteria and produce more biomass, with approximately the
2319 same level of dissolved oxygen being consumed (Ebeling et al., 2006).

2320 Considering the possible stratification of alkalinity and pH in biofilms, an
2321 alkalinity higher than 200 mgCaCO₃/L is recommended (Chen et al., 2006), especially
2322 when the water renewal rate is minimal or non-existent, as in BFT systems. Ebeling et
2323 al. (2006) indicate that, in systems with limited water exchange, alkalinity must be
2324 between 100-150 mg CaCO₃/L, while Boyd and Tucker (1998) suggest values above 75
2325 mg CaCO₃/L. Thus, there is no specific alkalinity level that is considered ideal and only
2326 recommended ranges of alkalinity values are available. However, Furtado et al. (2011)
2327 verified that alkalinity levels below 100 mg CaCO₃/L and pH levels below 7 negatively
2328 affect the nitrification rate in BFT systems.

2329 The aim of this study was to evaluate the effect of different levels of alkalinity
2330 on water quality parameters, biofloc formation and the zootechnical performance of *L.*
2331 *vannamei* cultivated in an intensive system without water renewal.

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2333 **Material and Methods**

2334 Infrastructure, biological material and experimental design

2335 The water used for the experiment was filtered through a sand filter (grain size = 1 mm)
2336 and posteriorly through a CUNO[®] filter (5 µm pore). It was chlorinated with 10 ppm
2337 and dechlorinated using 1 ppm ascorbic acid and intense aeration. During the study
2338 there was no renewal of water, only replacement of water that evaporated by adding
2339 dechlorinated fresh water to maintain the salinity at 26‰. In each experimental unit,
2340 there were two air diffusers (porous stone, 10 cm each) and a 2-Hp aerator (Blower) to
2341 provide water oxygenation and to keep the solids suspended. To maintain the water
2342 temperature close to 28°C, a heater with a thermostat (VisiTherm, Italy) was placed in
2343 each tank. The photoperiod in the experimental room was 12L/12D, with a 500 lx
2344 intensity provided by artificial lighting at the surface of the water. To allow fixation of
2345 microorganisms and development of a biofilm, an artificial Needlona[®] substrate (70 x
2346 28 cm, length x height) was placed in each tank with a total submerged area of 0.40 m²,
2347 equivalent to 74% of the total submerged area of the walls of the experimental unit.

2348 The biological material used in this study was acquired at the Aquatec Ltda
2349 laboratory (Canguaretama, Rio Grande do Norte, Brazil). After the *L. vannamei* nauplii
2350 arrived at the laboratory, they were maintained in the hatchery sector for marine
2351 shrimps until they reached the post-larval stage (PLs15). Subsequently, they were
2352 cultivated in a nursery (1,500 PL/m²) under a BFT system until they reached an average
2353 weight of 0.20±0.07 g. Then, the shrimps were transferred to twelve experimental units
2354 with an effective volume of 50 L, with 30 shrimps being placed in each rectangular
2355 polyethylene tank (bottom area of 0.20 m²), resulting in a stocking density of 150
2356 shrimps/m².

2357 Four levels of alkalinity were tested, with three replicates each: 75 mgCaCO₃/L;
2358 150 mgCaCO₃/L; 225 mgCaCO₃/L; and 300 mgCaCO₃/L. Before the shrimps were
2359 stocked, the alkalinity of the water in each experimental unit was adjusted according to

2360 the specified treatment. For the 75 mgCaCO₃/L treatment, 1 M hydrochloric acid
2361 (Synth[®]) was added, while for the 225 and 300 mgCaCO₃/L treatments, different
2362 concentrations of sodium bicarbonate (NaHCO₃) with a 99.0% purity (Carbonor[®]) were
2363 added (<0.15g/L per applications). The 150 mgCaCO₃/L treatment was equivalent to the
2364 alkalinity of the sea water, without the addition of acids or bases. Throughout the study
2365 period, different doses of sodium bicarbonate were added to maintain the alkalinity at
2366 the specified level for each treatment.

2367 The animals were fed a commercial diet (Centro Oeste Rações S.A.- Brazil)
2368 containing 40% crude protein and 8% lipid in its formulation, using feeding trays with a
2369 15 cm diameter (Wasiolesky et al., 2006), twice a day (08:00 and 17:00) for 49 days.
2370 Initially, the feeding rate (30% of the tank biomass/day) was established according to
2371 Jory et al. (2001). This value was adjusted posteriorly according to the consumption
2372 observed in the trays within each interval between feedings. The percentage of leaching
2373 of the dry matter in the feed was determined for the subsequent quantification of food
2374 consumption by the shrimps. For this purpose, a known amount of feed was placed in
2375 the feeding trays and deposited in the tanks, in duplicate, under the same experimental
2376 conditions, but without the shrimps. After 24 hours, the feed was removed from the
2377 trays and dried in a oven at 105 °C until reaching constant weight, and the percentage of
2378 leaching was calculated based on the difference in weight before and after the feed was
2379 placed in the tanks.

2380 Organic fertilization

2381 The sugar cane molasses that was used as carbon source was analyzed in the
2382 Hydrochemistry Laboratory of the Universidade Federal do Rio Grande to determine its

2383 carbon content (%) using a CHN Analyser (PerkinElmer PE 2400). A carbon content of
2384 37.46% was determined.

2385 To initiate the formation of bioflocs in both experiments, the amount of the
2386 organic carbon source to be added was based on the methodology proposed by
2387 Avnimelech (1999) and Ebeling et al. (2006), considering the C:N ratio (15:1) in the
2388 first five days of the experiment, with the intention of providing the initial substrate for
2389 the growth of heterotrophic bacteria. When the total ammoniacal nitrogen concentration
2390 reached 1 mg/L, corrections were made based on the C:N ratio (6:1) for the conversion
2391 of nitrogen in bacterial biomass, where for each 1 g of total ammoniacal nitrogen, 6 g of
2392 carbon organic are necessary.

2393

2394 Physical and chemical water quality parameters

2395 Determinations of pH, temperature and oxygen contents were conducted twice a day
2396 using a YSI 100 pH meter (Yellow Springs, OH, USA) and a YSI 55 oximeter (Yellow
2397 Springs, OH, USA), respectively. The salinity was verified every four days with an
2398 optical refractometer (Atago, model 103, Japan). The concentrations of total ammonia
2399 ($\text{TAN}=\text{NH}_3 + \text{NH}_4^+$) and nitrite (N-NO_2^-) were measured three times per week,
2400 according to methods recommended by UNESCO (1983) and Bendschneider and
2401 Robinson (1952), respectively. Alkalinity was also analyzed three times per week,
2402 following the methodology proposed by APHA (1998), and orthophosphate (P-PO_4^{-3})
2403 and the nitrate (N-NO_3^-) were measured once per week according to Aminot and
2404 Chaussepied (1983). Total suspended solids (TSS mg/L) were determined every 14 days
2405 through gravimetry by filtering aliquots of 20 mL of water through GF 50-A glass fiber
2406 filters, according to Strickland and Parsons (1972).

2407

2408 Zootechnical performance parameters

2409 Biometrics analyses were performed weekly, weighing 10 shrimps from each tank
2410 individually using a precision balance (± 0.01 g SD Marte[®]). At the end of the
2411 experiment, all of the living shrimps in each experimental unit were weighed and
2412 counted to evaluate their growth and survival in the different treatments. The following
2413 parameters were determined: Survival (%): [(final no. of shrimps / initial no. of
2414 shrimps) x 100]; Final average weight (g); Weight gain (g) = Final weight - Initial
2415 weight; Weekly weight gain (g/week) = [(Final weight - Initial weight) / no. of weeks of
2416 rearing] (Weekly weight gain was calculated from the moment the animals reached an
2417 average weight of 1 g, i.e., after the third week of rearing); and Apparent feed
2418 conversion (AFC) = Consumed feed / weight gain; specific growth rate (SGR; %/day) =
2419 [(ln final weight - ln initial weight) / days of experiment] x 100.

2420 Statistical analysis

2421 For statistical analysis of the data, the software STATISTICA 7.0[®] (StatSoft Inc. 2004,
2422 Tulsa, Oklahoma, USA) was used. After the homoscedasticity and the normality of the
2423 data were verified, they were subjected to analysis of variance with plots that were split
2424 in time (unifactorial ANOVA) to verify whether there was significant difference in the
2425 obtained data. When a significant difference was detected in the treatments ($p < 0.05$),
2426 the mean separation Tukey test (Sokal and Rohlf, 1969) was used. The percentage
2427 values were transformed ($\arcsin^{0.5}$) before being analyzed (Zar, 1996).

2428

2429 **Results**

2430

2431 Water quality

2432 The physical and chemical water quality parameters monitored throughout the 49-day

2433 experimental period are presented in **Table 1**. The average values of pH and alkalinity

2434 were significantly different ($p < 0.05$) according to the experimental design, with the 75

2435 treatment presenting the lowest values of pH and alkalinity; the 150 and 225 treatments

2436 showing intermediate values that differed from each other; and the 300 treatment

2437 presenting the highest values of pH and alkalinity ($p < 0.05$). The pH and alkalinity data

2438 obtained throughout the study are presented in **Fig. 1**.

2439 **Table 1** Average values \pm standard deviation of the physical and chemical water

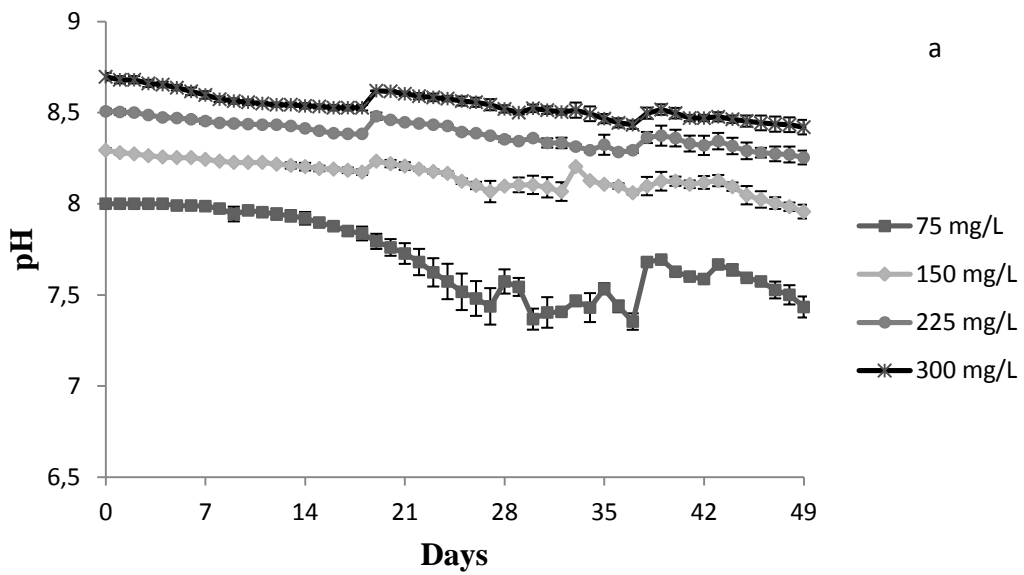
2440 parameters obtained during *L. vannamei* rearing (49 days) at different alkalinities¹. The

2441 maximum and minimum values of each parameter are shown in parentheses.

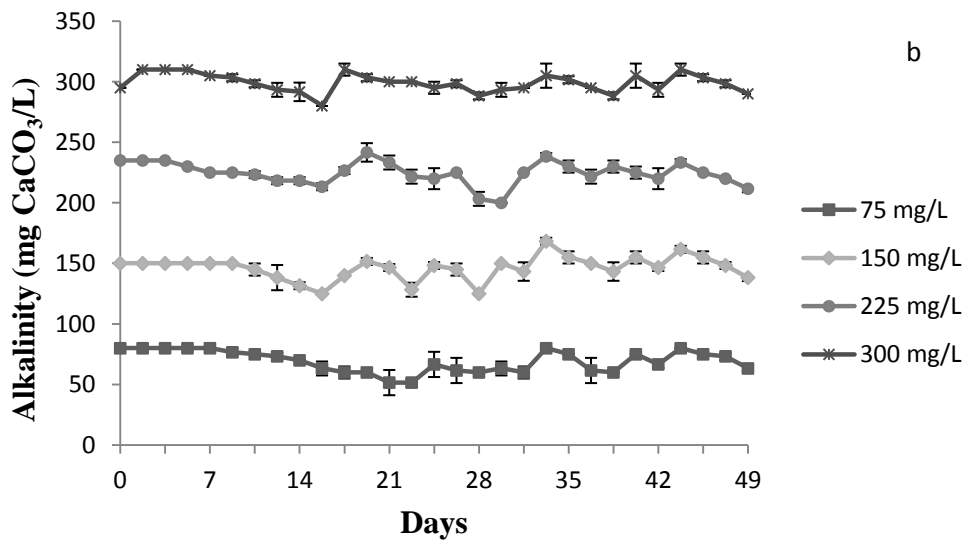
Parameters	Treatments			
	75	150	225	300
Temperature (°C)	28.12 \pm 0.61 (27.3 – 28.91)	28.05 \pm 0.71 (27.15 – 28.9)	28.00 \pm 0.75 (27.2 – 28.93)	28.16 \pm 0.81 (27.2 – 29.1)
Salinity	26.50 \pm 0.50 (26.0 – 27.0)	26.66 \pm 0.54 (26.0 – 28.0)	26.62 \pm 0.70 (26.0 – 28.0)	26.78 \pm 0.75 (26.0 – 28.0)
DO (mg/L)	6.08 \pm 0.16 (5.89 – 6.30)	6.08 \pm 0.17 (5.87 – 6.30)	6.10 \pm 0.13 (5.93 – 6.30)	6.14 \pm 0.14 (5.96 – 6.32)
pH	7.70 \pm 0.20 ^A (7.38 – 8.0)	8.05 \pm 0.10 ^B (7.95 – 8.28)	8.35 \pm 0.08 ^C (8.27 – 8.5)	8.53 \pm 0.09 ^D (8.37 – 8.68)
Alkalinity (mg CaCO ₃ /L)	70.26 \pm 9.92 ^A (50.0 – 80.0)	145.50 \pm 9.95 ^B (130.0 – 155.0)	224.33 \pm 9.87 ^C (210.0 – 235.0)	299.13 \pm 8.40 ^D (288.0 – 310.0)
TSS (mg/L)	255.25 \pm 231.69 (0 – 610.0)	290.58 \pm 263.43 (0 – 735.0)	300.53 \pm 269.66 (0 – 790.0)	295.25 \pm 233.95 (0 – 700.0)
TAN (mg/L)	0.52 \pm 0.88 ^A (0 – 4.0)	0.19 \pm 0.31 ^B (0 – 1.4)	0.32 \pm 0.55 ^{AB} (0 – 2.3)	0.20 \pm 0.36 ^B (0 – 1.7)
N-NO ₂ ⁻ (mg/L)	9.34 \pm 9.38 ^A (0 – 30.8)	1.86 \pm 2.31 ^B (0 – 9.6)	5.72 \pm 7.30 ^{AB} (0 – 19.5)	1.01 \pm 1.20 ^B (0 – 6.2)

N-NO ₃ ⁻ (mg/L)	24.26 ± 20.83 ^A (0 – 61.0)	30.27±25.80 ^{AB} (0 – 58.0)	24.46±22.20 ^{AB} (0 – 68.0)	35.18 ± 22.77 ^B (0 – 76.0)
P-PO ₄ ⁻³ (mg/L)	1.56 ± 1.06 (0 – 3.86)	1.57 ± 1.13 (0 – 3.84)	1.53 ± 1.10 (0 – 4.0)	1.43 ± 1.09 (0 – 3.6)

2442 ¹The data correspond to the average of 3 replicates ± standard deviation. Different
 2443 superscripted letters in the same line indicate that the averages differ significantly
 2444 ($p < 0.05$). Dissolved oxygen (DO), total ammoniacal nitrogen (TAN), nitrite (N-NO₂⁻),
 2445 nitrate (N-NO₃⁻) and orthophosphate (P-PO₄⁻³).



2446



2447

2448

2449 **Fig. 1** pH values in rearing water with different alkalinities throughout the 49 days of
2450 the experiment (a); alkalinity values measured in the rearing water in the different
2451 treatments during the 49 days of the experiment (b).

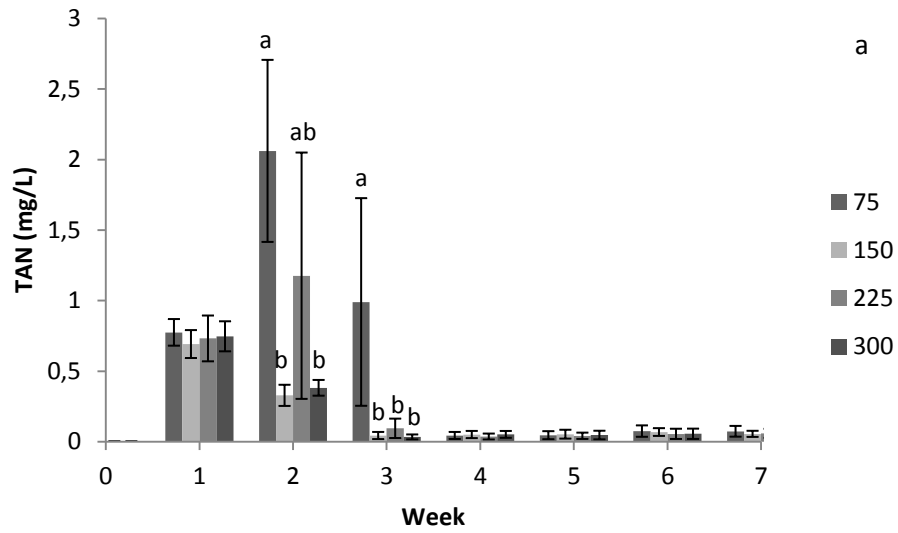
2452

2453 The average concentrations of ammonia (TAN) were significantly higher
2454 ($p<0.05$) in the 75 treatment compared to the 150 and 300 treatments, whereas there was
2455 no significant difference among the other treatments ($p>0.05$). During the study, an
2456 increase in the ammonia concentration was verified in the first several days after
2457 stocking that was similar for all treatments. In the second week of the study, the
2458 ammonia levels differed significantly among the treatments ($p<0.05$), with the 75
2459 treatment showing an increase, while the other treatments presented a reduction in the
2460 ammonia concentration. In the third week, the ammonia concentrations were reduced in
2461 all of the treatments, though the 75 treatment continued to present the highest ammonia
2462 concentration ($p<0.05$), while the other treatments presented concentrations close to 0.1
2463 mg/L. The ammonia concentration in the 75 treatment was reduced, nearly reaching 0.1
2464 mg/L between the third and fourth week, which was similar to the other treatments
2465 ($p>0.05$) (**Fig. 2a**).

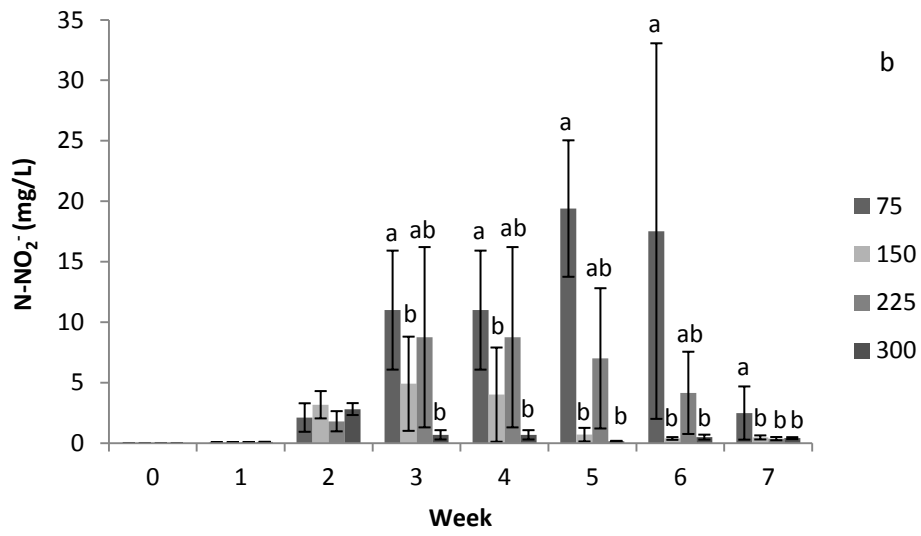
2466 The average concentrations of nitrite ($N-NO_2^-$) were significantly higher
2467 ($p<0.05$) in the 75 treatment compared to the 150 and 300 treatments, and there was no
2468 significant difference among the other treatments ($p>0.05$). After the first week of
2469 rearing, an increase of the nitrite concentration was verified that was similar for all of
2470 the treatments ($p>0.05$). In the third week, the nitrite concentrations increased in the 75,
2471 150 and 225 treatments, whereas in the 300 treatment, a reduction was observed in **Fig.**
2472 **2b**. In the fourth and fifth week of rearing, the nitrite concentrations continued to
2473 increase in the 75, 150 and 225 treatments, whereas in the 300 treatment, the nitrite

2474 values remained close to 1 mg/L. In the sixth week, there was a significant reduction
2475 observed in all treatments, though the 75 treatment continued to show the highest nitrite
2476 concentrations (>15 mg/L) ($p<0.05$); in contrast, the other treatments presented average
2477 concentrations below 5 mg/L. In the last week of the study, the nitrite concentrations
2478 were dramatically reduced in the 75 treatment (<5.0 mg/L), while in the other
2479 treatments, the concentrations were lower than 1 mg/L, differing significantly from the
2480 75 treatment ($p<0.05$) (**Fig. 2b**).

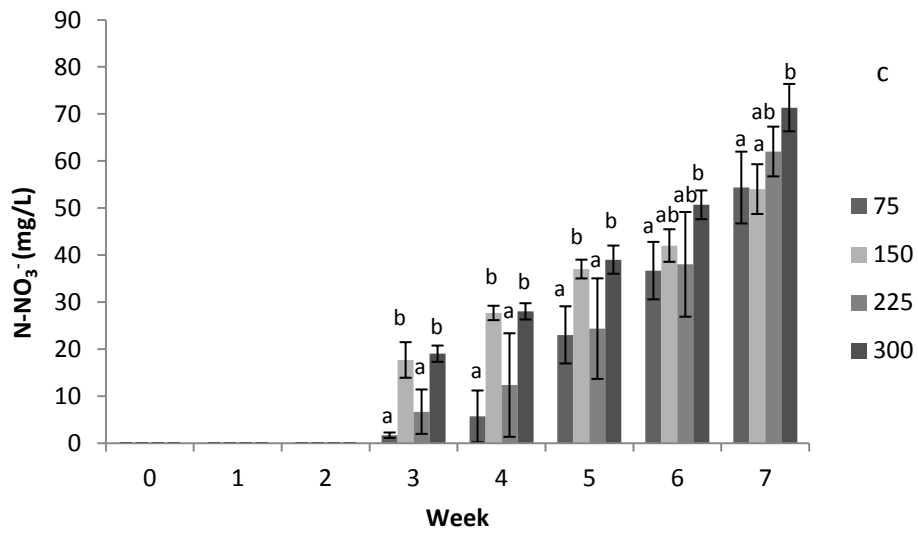
2481 The average concentrations of nitrate (N-NO_3^-) were significantly higher
2482 ($p<0.05$) in the 300 treatment compared to the 75 treatment, and there was no
2483 significant difference among the other treatments ($p>0.05$). An increase in the nitrate
2484 concentration was verified from the third week of rearing onward, with the highest
2485 concentrations being observed in the 150 and 300 treatments compared to the other
2486 treatments ($p<0.05$). From the third to the last week of rearing, the nitrate
2487 concentrations increased in all treatments. At the end of the study, there was a
2488 significant difference detected among the treatments ($p<0.05$), with the 75 and 150
2489 treatments presenting concentrations that were lower than those found in the 225 and
2490 300 treatments. The average nitrate concentrations recorded throughout the rearing are
2491 presented in **Fig. 2c**.



2492



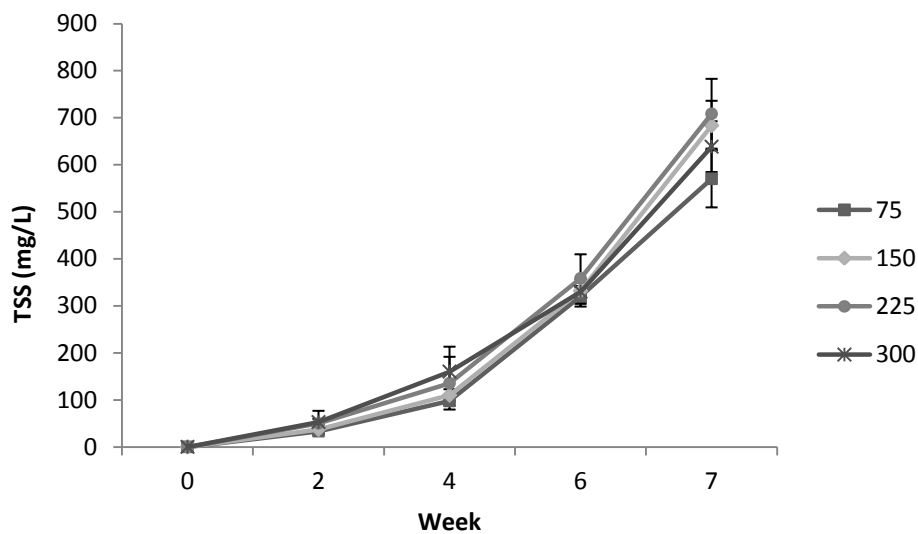
2493



2494

2495 **Fig. 2** Average concentrations \pm standard deviation of (a) total ammonia (TAN) in
2496 rearing water with different alkalinities throughout the 49 days of the experiment; (b)
2497 nitrite (N-NO₂⁻) measured in the rearing water in the different treatments over 49 days;
2498 (c) nitrate (N-NO₃⁻) measured in the rearing water of the different treatments over 49
2499 days.

2500 The average concentration of total suspended solids (TSS) was approximately
2501 290 mg/L. The TSS exhibited an increase over time in all of the treatments, such that at
2502 the end of the study, the concentration was approximately 700 mg/L. With regard to
2503 TSS, no significant difference was verified among the treatments ($p>0.05$). The average
2504 concentrations and standard deviations that were observed in the different treatments are
2505 presented in **Fig. 3**.



2506
2507 **Fig. 3** Average concentration \pm standard deviation of total suspended solids (TSS mg/L)
2508 in rearing water with different alkalinities over 49 days.

2509

2510 Zootechnical performance

2511

2512 The results for the zootechnical performance parameters of the juvenile *L. vannamei*
 2513 obtained at end of the 49 experimental days are shown in **Table 2**. The average survival
 2514 values were above 88%, and there was no significant difference among the treatments
 2515 ($p>0.05$). The final average weight (g), weight gain (g), weekly weight gain (g/week)
 2516 and specific growth rate (%/day) were significantly lower ($p<0.05$) in the 75 treatment
 2517 compared to the 300 treatment, whereas no significant difference was detected between
 2518 the 150 and 225 treatments ($p>0.05$). For the apparent feed conversion (AFC), the
 2519 results were similar for all treatments ($p>0.05$).

2520 **Table 2** Zootechnical performance parameters of juvenile *L. vannamei* cultivated for 49
 2521 days at different alkalinities.

Parameters	75	150	225	300
Survival (%)	90.00 ± 3.33	88.00 ± 3.85	92.12 ± 5.30	91.22 ± 2.92
Initial weight (g)	0.20 ± 0.07	0.20 ± 0.07	0.20 ± 0.07	0.20 ± 0.07
Final weight (g)	4.78 ± 0.12 ^A	5.34 ± 0.31 ^{AB}	4.95 ± 0.23 ^{AB}	5.44 ± 0.42 ^B
Weight gain (g)	4.57 ± 0.13 ^A	5.13 ± 0.34 ^{AB}	4.74 ± 0.30 ^{AB}	5.24 ± 0.42 ^B
WWG (g/week)	0.91 ± 0.04 ^A	1.02 ± 0.07 ^{AB}	0.94 ± 0.05 ^{AB}	1.05 ± 0.08 ^B
SGR (%/day)	6.41 ± 0.05 ^A	6.64 ± 0.13 ^B	6.49 ± 0.09 ^{AB}	6.68 ± 0.16 ^B
AFC	1.15 ± 0.08 ^A	1.10 ± 0.08 ^A	1.11 ± 0.05 ^A	1.08 ± 0.07 ^A

2522 The data correspond to the average values from 3 replicates ± standard deviation.
 2523 Different superscripted letters in the same line indicate that the averages differ
 2524 significantly ($p<0.05$). Weekly weight gain (WWG), apparent feed conversion (AFC),
 2525 specific growth rate (SGR).

2526

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2530 **Discussion**

2531

2532 Water quality

2533

2534 In this study, the temperature, salinity and dissolved oxygen levels measured in this
2535 study were favorable for the growth and survival of the cultivated shrimps (Ponce-
2536 Palafox et al., 1997; Zhang et al., 2006; Maicá et al., 2012).

2537 According to Furtado et al. (2011), the alkalinity and pH levels decrease during
2538 rearing in BFT systems, and alkalinity levels below 100 mg CaCO₃/L and pH levels
2539 below 7 negatively affect the nitrification rate in such systems. The addition of sodium
2540 bicarbonate (NaHCO₃) provided physical and chemical water quality conditions that
2541 were favorable for biofloc development and the growth of juvenile *Litopenaeus*
2542 *vannamei*. The initial alkalinity levels determined in this study were corrected with
2543 sodium bicarbonate to maintain them within the specified level for each treatment, and
2544 the pH was therefore corrected through the addition of sodium bicarbonate. During this
2545 study, the greatest number of additions of sodium bicarbonate were necessary in the 75
2546 treatment and the fewest in the 300 treatment. There was a greater amount of pH
2547 oscillation in the 75 treatment than in the other treatments, with a higher pH stability
2548 being recorded at higher levels of alkalinity.

2549 According to Villaverde et al. (1997), the optimum pH range for nitrification can
2550 be determined based on three different effects of pH on nitrifying bacteria: activation-
2551 deactivation of the nitrifying bacteria; nutritional effects related to alkalinity (inorganic
2552 carbon); and inhibition of bacteria through free ammonia and free nitrous acid.
2553 Regarding the nitrification process, the optimum pH should be maintained in a range
2554 that varies according to the genera of the nitrifying bacteria and the requirements for

2555 their growth. For example, *Nitrossomonas*, which oxidize ammonia to nitrite (AOB),
2556 present an optimum pH between 7.8 – 8.8, while for *Nitrobacter*, which oxidize nitrite
2557 to nitrate (NOB), it varies between 7.2 – 9.0 (Chen et al. 2006).

2558 One of the most significant problems in rearing systems for aquatic organisms is
2559 the deterioration of water quality due to the accumulation of nitrogenous compounds,
2560 such as total ammonia (TAN= $\text{NH}_3^- + \text{NH}_4^+$), nitrite (N- NO_2^-) and nitrate (N- NO_3^-) (Colt
2561 and Armstrong 1981). Total ammonia (TAN) is generated through the excretion and
2562 decomposition of organic matter, and when TAN shows higher concentrations, it can
2563 negatively affect the performance of organisms during rearing, potentially leading to
2564 mortality (Lin and Chen, 2001). Nitrite is an intermediate in the oxidation of ammonia
2565 into nitrate by nitrifying bacteria. Nitrite, therefore, accumulates in the culture medium,
2566 reaching concentrations higher than safe levels (15.2 mg/L in salinity 25), reducing the
2567 growth and survival of the shrimps of this species (Lin and Chen, 2003). In contrast to
2568 nitrate, the final product of the nitrification of ammonia, is the least harmful nitrogenous
2569 compound for penaeids, as it needs to be present at concentrations above 60 mg/L to
2570 have deleterious effects (Van Wyk and Scarpa, 1999).

2571 In the present study, the average values of ammonia were in the range
2572 considered appropriate for the rearing of juvenile *L. vannamei* (Lin and Chen, 2001).
2573 However, there was a significant difference among the treatments ($p < 0.05$), with the 75
2574 and 225 treatment presenting the highest ammonia concentrations, requiring a greater
2575 load of organic carbon than the other treatments for the development of heterotrophic
2576 bacteria and the assimilation of ammoniacal nitrogen by these microorganisms.

2577 In contrast, the concentrations of nitrite remained within the accepted levels for
2578 the rearing of juvenile *L. vannamei* (Lin and Chen, 2003). According to Vinatea et al.
2579 2010, there is a significant reduction in the growth rate of shrimps at nitrite

2580 concentrations close to 9.0 mg/L. Therefore, the zootechnical performance of the study
2581 organisms during rearing in the 75 and 225 treatments could have been negatively
2582 affected by the nitrite levels measured throughout the study. The effect of alkalinity on
2583 the development of nitrite-oxidizing bacteria (NOB) was also verified in this study, as
2584 in the 75 treatment, which had the lowest alkalinity, the highest levels and longest
2585 period of high nitrite concentrations, while in the 150, 225 and 300 treatments, the
2586 nitrite levels decreased quickly.

2587 Heterotrophic bacteria can increase their biomass in a matter of hours, while
2588 autotrophic bacteria may require days for such an increase to be detected (Macgraw,
2589 2002). To encourage the development of autotrophic bacteria, artificial substrates
2590 indicated for fixing autotrophic bacteria are added (Bratvold and Browdy, 2001). The
2591 microorganisms fixed in artificial substrates increase the nitrification rate in rearing
2592 tanks, resulting in decreased ammoniacal nitrogen and nitrite concentrations. Several
2593 authors have reported the benefits of biofilms formed on artificial substrates for penaeid
2594 shrimp rearing (Bratvold and Browdy, 2001; Ballester et al., 2007; Viau et al., 2013).

2595 Regarding nitrate, accumulation was observed throughout the experiment in all
2596 treatments, such that the average values remained close to 30 mg/L, and all treatments
2597 presented maximum values above 50 mg/L, similar to data obtained by Scopel et al.
2598 (2011) and to the recommendations of Kuhn et al. (2010).

2599 Phosphorus levels increased throughout the rearing period, reaching a maximum
2600 level of 4.0 mg/L. These phosphorus concentrations in the rearing environment did not
2601 affect the development of the shrimps. The measured concentrations of total suspended
2602 solids (TSS) were approximately 290 mg/L and presented no significant difference
2603 ($p>0.05$) among the treatments. The TSS levels of up to 500 mg/L are considered
2604 adequate for rearing (Samocho et al., 2007). The TSS values remained within

2605 recommended levels until the sixth week of rearing, whereas in the last week of the
2606 experiment, the TSS values were above 700 mg/L. The low light intensity in the
2607 experimental room (500 lx) may have influenced the increases in TSS because in
2608 contrast to microalgae, microbial populations are more stable and are not highly
2609 dependent on light conditions (Avnimelech, 2006).

2610

2611 Zootechnical performance

2612

2613 In rearing marine shrimps using biofloc technology, survival is generally relatively
2614 high. Krummenauer et al. (2011) worked with stocking densities of 150 and 300
2615 shrimps/m² and obtained average survival rates of 92% and 81%, respectively.
2616 Additionally, Baloi et al. (2013) reported average survival rates above 86%, which is
2617 similar to those found in this study (>88%), without any significant difference among
2618 the treatments. Regarding the final weight and weight gain recorded in the shrimps,
2619 there was a significant difference, with the major results being obtained in the 300
2620 treatment and the poor in the 75 treatment. Concerning the specific growth rate (SGR),
2621 the lowest values were obtained in the 75 treatment (6.41 %/day), showing a significant
2622 difference from the 150 (6.64 %/day) and 300 (6.68 %/day) treatments, which presented
2623 suitable SGR values. The SGR values obtained in this study were superior to those
2624 obtained by Maicá et al. (2012), who cultivated juvenile *L. vannamei* at a salinity of 25
2625 using biofloc technology and verified an average growth rate of 5.37%/day.

2626 The weekly weight gain (WWG) was calculated from the time the animals
2627 reached an average weight of 1 g, i.e., at the end of exponential growth phase and the
2628 beginning of linear growth. Although there were significant differences among the
2629 treatments, we verified that the lowest WWG was obtained in the 75 treatment and the

2630 highest was obtained in the 300 treatment. The WWG results observed in this study are
2631 similar to the rate of 1.1 ± 0.1 g/week reported by Baloi et al. (2013), working with a
2632 density of 300 shrimps/m³, and superior to those of 0.85 and 0.92 g/week obtained by
2633 Krummenauer et al. (2011), working with densities of 150 and 300 shrimps/m².
2634 Therefore, even the WWG values of the shrimps in the 75 treatment are considered
2635 adequate for the commercial rearing of *L. vannamei* in a BFT system.

2636 The apparent feed conversion (AFC) values obtained, close to 1.11, are superior
2637 to those found by Baloi et al. (2013) (AFC of 2.6) and Krummenauer et al. (2011), who
2638 verified AFC values of 1.4 and 1.29 at densities of 150 and 300 shrimps/m²,
2639 respectively. These AFC values were possible due to controlling food consumption
2640 through the use of feeding trays and to the natural productivity of bioflocs and biofilms,
2641 which serve as a supplementary food source for penaeid shrimps (Wasiolesky et al.,
2642 2006; Ballester et al., 2007).

2643 The zootechnical performance of the shrimps cultivated in the 75 treatment was
2644 similar to those obtained in the 150 and 225 treatments, though it was inferior compared
2645 to the 300 treatment due to the negative effect of the prolonged period of nitrite
2646 exposure. This prolonged period of elevated nitrite levels occurred due to the retarded
2647 growth of nitrite-oxidizing bacteria (NOB). The conditions for the development of these
2648 bacteria were therefore appropriate in treatments with a higher alkalinity, particularly at
2649 300 mgCaCO₃/L. It is clear from the data obtained in several studies on *Litopenaeus*
2650 *vannamei* in BFT systems (Krummenauer et al., 2011; Maicá et al., 2012; Baloi et al.,
2651 2013) that the zootechnical performance indexes found in this study, even in the 75
2652 treatment, are considered adequate for the rearing of shrimps using biofloc technology.

2653

2654

2655 **Conclusion**

2656 Under the experimental conditions applied in this study, it was possible to form bioflocs
2657 and biofilms during the rearing of *Litopenaeus vannamei* without renewal of water at
2658 alkalinity levels above 70 mgCaCO₃/L and pH levels above 7.38. Nevertheless, the
2659 suitable nitrification rates and the suitable zootechnical performance occurred in
2660 treatments with higher alkalinity levels, particularly at an alkalinity of 300 mgCaCO₃/L.
2661 At higher alkalinities, an even higher stability of pH values was obtained.

2662

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2670

2671 **References**

- 2672 Aminot A, Chaussepied M (1983) Manuel des analyses chimiques en milieu marin.
2673 Brest, CNEXO.
- 2674 APHA (American Public Health Association) (1998). Standard methods for the
2675 examination of water and wastewater. 20st edition. Washington, DC.
- 2676 Avnimelech Y (1999) Carbon/nitrogen ratio as a control element in aquaculture
2677 systems. Aquaculture 176: 227-235

- 2678 Avnimelech Y (2006) Bio-filters: the need for an new comprehensive approach.
2679 Aquacult Eng 34:172–178
- 2680 Ballester ELC, Wasielesky W, Cavalli RO, Abreu PC (2007) Nursery of the pink
2681 shrimp *Farfantepenaeus paulensis* in cages with artificial substrates: Biofilm
2682 composition and shrimp performance. Aquaculture 269:355-362
- 2683 Baloi M, Arantes R, Schweitzer R, Magnotti C, Vinatea L (2013) Performance of Pacific
2684 white shrimp *Litopenaeus vannamei* raised in biofloc systems with varying levels
2685 of light exposure. Aquacult Eng 52:39– 44
- 2686 Bendschneider K, Robinson, RJ (1952) A new spectrophotometric method for the
2687 determination of nitrite in sea water. J Mar Res 11: 87-96.
- 2688 Boyd CE, Tucker CS (1998) Pond aquaculture water quality management. Boston:
2689 Kluwer Academic, 700 p.
- 2690 Bratvold D, Browdy CL (2001) Effects of sand sediment and vertical surfaces
2691 (Aquamats) on production, water quality, and microbial ecology in an intensive
2692 *Litopenaeus vannamei* culture system. Aquaculture 195:81-94
- 2693 Chen S, Ling J, Blancheton JP (2006) Nitrification kinetics of biofilm as affected by
2694 water quality factors. Aquacult Eng 34: 179–197
- 2695 Colt JE, Armstrong DA (1981) Nitrogen toxicity to crustaceans, fish and molluscs. In:
2696 Allen, L.J., Kinney, E.C. (Eds.), Proceedings of the Bioengineering Symposium
2697 for Fish Culture Section. American Fisheries Society, Bethesda, Maryland, pp.
2698 34-47.
- 2699 Crab R, Defoirdt T, Bossier P, Verstraete W (2012) Biofloc technology in aquaculture:
2700 Beneficial effects and future challenges. Aquaculture 356-357: 351-356

2701 Ebeling JM, Timmons MB, Bisogni JJ (2006) Engineering analysis of the stoichiometry
2702 of photoautotrophic, autotrophic, and heterotrophic control of ammonia-nitrogen
2703 in aquaculture in aquaculture production systems. *Aquaculture* 257:346-358

2704 Furtado PS, Poersch LH, Wasielesky WJr (2011) Effect of Calcium Hydroxide,
2705 Carbonate and Sodium Bicarbonate on Water Quality and Zootechnical
2706 Performance of Shrimp *Litopenaeus vannamei* Reared in Bio-Flocs Technology
2707 (BFT) Systems. *Aquaculture* 321:130-135

2708 Gaona CAP, Poersch LH, Krummenauer D, Foes GK, Wasielesky WJ (2011) The effect
2709 of solids removal on water quality, growth and survival of *Litopenaeus vannamei*
2710 in a biofloc technology culture system. *IJRA* 12: 54-73

2711 Hargreaves JA (2006) Photosynthetic suspended-growth systems in aquaculture.
2712 *Aquacult Eng* 34:344–363

2713 Jory DE, Cabrerias RT, Durwood MD, Fegan D, Lee GP, Lawrence AL, Jackson JC,
2714 McIntosh PR, Castañeda AJ (2001) A global review of shrimp feed management:
2715 status and perspectives. *Aquaculture the World* Aquaculture Society, Baton
2716 Rouge, LA USA.

2717 Krummenauer D, Cavalli RO, Poersch LH, Wasielesky W (2011) Superintensive
2718 Culture of White Shrimp, *Litopenaeus vannamei*, in a Biofloc Technology System
2719 in Southern Brazil at Different Stocking Densities. *J World Aquacult Soc* 42:726-
2720 733

2721 Kuhn DD, Smith SA, Boardman GD, Angier MW, Marsh LFJ, George J (2010) Chronic
2722 toxicity of nitrate to Pacific white shrimp, *Litopenaeus vannamei*: Impacts on
2723 survival, growth, antennae length, and pathology. *Aquaculture* 309: 109-114

2724 Lin YC, Chen JC (2001) Acute toxicity of ammonia on *Litopenaeus vannamei* (Boone)
2725 juveniles at different salinity levels. *J Exp Mar Biol Ecol* 259:109-119

- 2726 Lin YC, Chen JC (2003) Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone)
2727 juveniles at different salinity levels. *Aquaculture* 224:193-201
- 2728 Maicá PF, Borba MR, Wasielesky W (2012) Effect of low salinity on microbial floc
2729 composition and performance of *Litopenaeus vannamei* (Boone) juveniles reared
2730 in a zero-water-exchange super-intensive system. *Aquacult Res* 43:361–370
- 2731 MacGraw WJ (2002) Utilization of Heterotrophic and Autotrophic Bacteria in
2732 Aquaculture. *Global Aquacult Advoc* December: 82-83
- 2733 Ponce-Palafox J, Martinez-Palacios CA, Ross LG (1997) The effects of salinity and
2734 temperature on the growth and survival rates of juvenile white shrimp, *Penaeus*
2735 *vannamei*, Boone, 1931. *Aquaculture* 157:107-115
- 2736 Samocha TM, Patnaik S, Speed M, Ali AM, Burger JM, Almeida RV, Ayub Z,
2737 Harisanto M, Horowitz A, Brook DL (2007) Use of molasses as carbon source in
2738 limited discharge nursery and grow-out systems for *Litopenaeus vannamei*.
2739 *Aquacult. Eng.* 36:184-191
- 2740 Scopel BR, Schweitzer R, Seiffert WQ, Pierri V, Arantes RF, Vieira FN, Vinatea LA
2741 (2011) Substituição da farinha de peixe em dietas para camarões marinhos
2742 cultivados em sistema bioflocos. *Pesq Agropec Bras* 46(8):928-934
- 2743 Sokal RR, Rohlf FJ 1969. *Biometry. Principle and practices of statistics in biological*
2744 *research.* W. H. Freeman & Company. 776p.
- 2745 Strickland JDH, Parsons TR (1972) *A practical handbook of seawater analysis.* Ottawa:
2746 *Fishery Research Board Canada,* 310p.
- 2747 Thompson FL, Abreu PC, Wasielesky W (2002) Importance of biofilm for water quality
2748 and nourishment in intensive shrimp culture. *Aquaculture* 203:263–278

2749 UNESCO (1983) Chemical methods for use in marine environmental monitoring.
2750 Manual and Guides 12, Intergovernmental Oceanographic Commission. Paris,
2751 France.

2752 Van Wyk P, Scarpa J (1999) Water quality and management. In: Van Wyk, P. et al.
2753 (Eds.). Farming marine shrimp in recirculating freshwater systems. Florida
2754 department of agriculture and consumer services, Tallahassee, pp. 141-161.

2755 Viau VE, Souza DM, Rodríguez EM, Wasielesky WJr, Abreu PC, Ballester ELC (2013)
2756 Biofilm feeding by postlarvae of the pink shrimp *Farfantepenaeus brasiliensis*
2757 (Decapoda, Penaidae). *Aquacult Res*, 44: 783–794

2758 Villaverde S, Garcia-encina PA, Fdz-Polanco F (1997) Influence of pH over nitrifying
2759 biofilm activity in submerged biofilters. *Water Res* 31:1180–1186

2760 Vinatea L, Galvez AO, Browdy CL, Stokes A, Venero J, Haveman J, Lewis BL,
2761 Lawson A, Shuler A, Leffler JW (2010) Photosynthesis, water respiration and
2762 growth performance of *Litopenaeus vannamei* in a super-intensive raceway
2763 culture with zero water exchange: interaction of water quality variables. *Aquacult*
2764 *Eng* 42:17–24

2765 Wasielesky W, Atwood H, Stokes A, Browdy CL (2006) Effect of natural production in
2766 a zero exchange suspended microbial floc based super-intensive culture system
2767 for white shrimp *Litopenaeus vannamei*. *Aquaculture* 258:396-408

2768 Zar JH (1996) *Biostatistical Analysis*. Third Edition New Jersey: Prentice Hall, 662p.

2769 Zhang P, Zhang X, Li J, Huang G (2006) The effects of body weight, temperature,
2770 salinity, pH, light intensity and feeding condition on lethal DO levels of white leg
2771 shrimp, *Litopenaeus vannamei* (Boone, 1931). *Aquaculture* 256:579–587

2772 Zhu S, Chen S (2001) Impacts of Reynolds number on nitrification biofilm kinetics.
2773 *Aquacult Eng* 24:213–229

CAPÍTULO VI

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2777 **Application of different doses of calcium hydroxide in the farming shrimp**

2778 ***Litopenaeus vannamei* with the biofloc technology (BFT).**

2779

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2797 **Resumo**

2798 O cultivo de organismos aquáticos com tecnologia de bioflocos (BFT) necessita
2799 aplicações de compostos alcalinizantes para correção da alcalinidade e pH, já que ao
2800 longo do cultivo de *Litopenaeus vannamei* há um incremento dos níveis de sólidos
2801 suspensos totais e um declínio dos níveis de alcalinidade e pH. Essa redução da
2802 alcalinidade e do pH ocorre devido ao consumo da alcalinidade (carbono inorgânico)
2803 pelas bactérias heterotróficas e nitrificantes presentes nos bioflocos. O objetivo do
2804 presente estudo foi avaliar o efeito de diferentes dosagens de hidróxido de cálcio
2805 (Ca(OH)_2) na qualidade da água e desempenho zootécnico de camarões marinhos *L.*
2806 *vannamei* em sistema BFT. O experimento consistiu de quatro tratamentos com três
2807 repetições cada: tratamento controle (TC) teve o nível de pH mantido $>7,2$ através da
2808 aplicação de $0,05 \text{ g L}^{-1}$ de hidróxido de cálcio (Ca(OH)_2); Tratamento 10% (T10), foram
2809 aplicadas doses de hidróxido de cálcio equivalentes a 10% da quantidade de ração
2810 ofertada por dia aos camarões; Tratamento 20% (T20), foram aplicadas doses de
2811 hidróxido de cálcio equivalentes a 20% da quantidade de ração ofertada por dia aos
2812 camarões; Tratamento 40% (T40), foram aplicadas doses de hidróxido de cálcio
2813 equivalentes a 40% da quantidade de ração ofertada por dia aos camarões. Para tal, doze
2814 unidades experimentais com 150 L de volume útil foram estocadas com 85 juvenis de *L.*
2815 *vannamei* ($0,18 \pm 0,06 \text{ g}$) correspondendo a uma densidade de estocagem de 425
2816 camarões m^{-3} mantidos por um período experimental de 56 dias. O T40 apresentou os
2817 maiores níveis de amônia total (NAT) ao longo do estudo marcando diferença
2818 significativa ($p < 0,05$) quando comparado aos demais tratamentos. Os valores de
2819 alcalinidade e pH dos tratamentos TC e T10 foram similares e significativamente
2820 menores ($p < 0,05$) que os tratamentos T20 e T40, havendo, também, diferença
2821 significativa ($p < 0,05$) entre esses dois últimos tratamentos. Quanto ao desempenho
2822 zootécnico foi verificada diferença significativa ($p < 0,05$) no peso final, conversão
2823 alimentar aparente, taxa de crescimento específico e produtividade, onde TC, T10 e T20
2824 foram significativamente melhores ($p < 0,05$) que T40. Quanto a sobrevivência não foi
2825 verificada diferença significativa ($p > 0,05$), sendo os valores médios superiores a 85%.
2826 Os resultados obtidos no presente estudo indicam que para a correção da alcalinidade e
2827 pH podem ser aplicadas dosagens de $0,05 \text{ g L}^{-1}$ de hidróxido de cálcio ou aplicações
2828 diárias entre 10 e 20% da quantidade de ração ofertada aos camarões.

2829 **Abstract**

2830 The reduction of alkalinity and pH occurs due to the consumption of inorganic carbon
2831 by bacteria present in the biofloc. The objective of the study was to evaluate the effects
2832 of different doses of calcium hydroxide on the water quality and growth performance of
2833 the *L. vannamei* in a biofloc system. The experiment consisted of four treatments with
2834 three repetitions for each treatment: control treatment (TC), in which the pH maintained
2835 above 7.2 due to the application of 0.05 g L⁻¹ of calcium hydroxide, and treatments T10,
2836 T20 and T40, in which daily doses of calcium hydroxide equivalent to 10%, 20% and
2837 40% of the total amount of feed offered to the shrimp, respectively, were added to the
2838 environment. Twelve experimental units (150 L) were stocked with 85 juveniles of *L.*
2839 *vannamei* (0.18 ± 0.06 g), corresponding to a density of 425 shrimp m⁻³, and cultivated
2840 for a 56 days. The values of alkalinity and pH for treatments TC and T10 were similar
2841 and significantly lower (p<0.05) than those for T20 and T40, which differed (p<0.05)
2842 between each other. In terms of growth performance TC, T10 and T20 performed
2843 significantly better (p<0.05) than T40. There were no significant differences (p>0.05) in
2844 the survival rates. The results obtained indicate that doses of 0.05 g L⁻¹ of calcium
2845 hydroxide or daily applications between 10 and 20% of the total amount of feed offered
2846 to the shrimp can be used for the correction of alkalinity and pH.

2847

2848 **Keywords:** Alkalinity, Biofloc, Calcium hydroxide, *Litopenaeus vannamei*, pH

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2858 **Introduction**

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2860 The biofloc technology (BFT) has emerged as a sustainable tool for aquaculture that
2861 deals simultaneously with environmental, social and economic issues (Crab et al.,
2862 2012). Several benefits can be attributed to the biofloc technology, such as water quality
2863 control (of nitrogen compounds), natural food production, zero or minimal water
2864 renewal and high stocking densities, among others (De Schryver et al., 2008;
2865 Krummenauer et al., 2011). However, BFT systems require constant monitoring of
2866 water quality parameters, as observed in the farming of *Litopenaeus vannamei*, where
2867 the levels of alkalinity and pH decreased and the total suspended solids increased over
2868 time (Furtado et al., 2011; Gaona et al., 2011).

2869 A reduction in alkalinity and pH may occur due to the consumption of alkalinity
2870 (inorganic carbon) by heterotrophic and nitrifying bacteria that form the biofloc
2871 (Ebeling et al., 2006). According to Chen et al. (2006), for every gram of total
2872 ammoniacal nitrogen (TAN) oxidized to nitrate (NO_3^- -N), approximately 4.18 g of
2873 oxygen and 7.07 g of alkalinity are consumed and 0.17 g of bacterial biomass is
2874 produced. Ebeling et al. (2006), however, stated that for each gram of ammoniacal
2875 nitrogen converted to microbial biomass, 4.71 g of dissolved oxygen, 3.57 g of
2876 alkalinity and 15.17 g of carbohydrates are consumed, while 8.07 g of microbial
2877 biomass and 9.65 g of carbon dioxide are produced. If the performances of nitrifying
2878 and heterotrophic bacteria are compared, nitrifying bacteria consume more alkalinity
2879 and produce less microbial biomass than heterotrophic bacteria, which consume about
2880 half of the alkalinity and produce more biomass with approximately the same
2881 consumption of dissolved oxygen (Ebeling et al., 2006).

2882 The initial conditions of farming are predominantly photoautotrophic, and the
2883 pH levels can oscillate from 6.6 to 10.2 in a day as a result of phytoplankton
2884 photosynthesis, which removes carbon dioxide (CO₂) and elevates the pH. At night,
2885 CO₂ is released rather than removed by the animals and phytoplankton, reducing the pH
2886 of the water. The levels of total suspended solids increase and the heterotrophic
2887 bacterial community becomes dominant over time. This change helps attenuate daily
2888 oscillations in pH, and the process of photosynthesis is reduced because of high
2889 turbidity and a consequent reduction of brightness in the water column (Boyd, 1990;
2890 Vinatea et al., 2010).

2891 Ebeling et al. (2006) suggest that alkalinity be maintained between 100-150 mg
2892 CaCO₃ L⁻¹ in systems with limited water exchange. However, Boyd and Tucker (1998)
2893 suggest alkalinity levels higher than 75 mg CaCO₃L⁻¹. Thus, there is not a specific
2894 concentration of alkalinity that is considered ideal, but a range of alkalinity levels can
2895 work. Furtado et al. (2011) verified that alkalinity below 100 mg CaCO₃L⁻¹ and pH
2896 below 7.0 for long periods of time adversely affects the growth performance of *L.*
2897 *vannamei* being cultivated in the BFT system.

2898 According to Van Wyk and Scarpa (1999), marine shrimp grow better with pH
2899 levels from 7.0 to 9.0. The pH of water influences almost all chemical reactions, as well
2900 as the balance of certain substances present in the water, such as ammonia, nitrite,
2901 hydrogen sulfide and some metals (Boyd and Tucker, 1998). Furthermore, pH also
2902 interferes with the physiological processes of the shrimp and the microorganisms that
2903 form the bioflocs. In bioflocs, the maintenance of pH within the range of 7.0 to 9.0
2904 promotes the development of nitrifying bacteria, which are responsible for the oxidation
2905 of ammonia into nitrite and nitrate (NH₄⁺ → NO₂⁻ → NO₃⁻). Ammonia is the end
2906 product of protein catabolism in most aquatic organisms, and it is present in the aquatic

2907 environment in both ionized (NH_4^+) and non-ionized forms (NH_3). Non-ionized
2908 ammonia diffuses more easily through cell membranes and is more toxic to aquatic
2909 organisms (Kinne, 1976; Fromm and Gillette, 1968). The ionization of ammonia is
2910 mediated primarily by pH; the percentage of NH_3 increases and the percentage of NH_4^+
2911 decreases with higher pH levels (>8.2). According to Colt and Armstrong (1981),
2912 animals can reduce or even cease food consumption to limit the internal accumulation
2913 of nitrogenous wastes when they are exposed to high concentrations of ammonia in the
2914 environment.

2915 Maintaining proper alkalinity in the farming environment helps reduce pH
2916 oscillations due to respiratory and photosynthetic processes (Van Wyk and Scarpa,
2917 1999). Queiroz et al. (2004) reported that the application of alkalizing substances is a
2918 common and efficient practice in the cultivation of aquatic organisms that enhances the
2919 production of fish and shrimp. Furtado et al. (2011) verified that the use of calcium
2920 hydroxide ($\text{Ca}(\text{OH})_2$) resulted in the best cost-effectiveness ratio when compared with
2921 sodium carbonate (Na_2CO_3) and sodium bicarbonate (NaHCO_3), as it provided adequate
2922 physical and chemical conditions for the development of biofloc and the growth of
2923 juvenile *Litopenaeus vannamei* at lower costs. Thus the objective of this study was to
2924 evaluate the effects of different doses of calcium hydroxide ($\text{Ca}(\text{OH})_2$) on water quality
2925 parameters and the performance of *L. vannamei* cultivated with the biofloc technology.
2926 The results of this research will assist the work of shrimp producers in maintaining
2927 water quality in BFT systems.

2928

2929 **Materials and Methods**

2930 Location and facilities

2931

2932 The study took place in the period from October to December of 2012 at the Marine
2933 Aquaculture Station Prof. Marcos Alberto Marchiori (EMA) of the Federal University
2934 of Rio Grande (FURG), located at Cassino beach, Southern Brazil.

2935 Water, biological material and experimental design

2936 The water used in the experiment was pumped from the beach and filtered through a
2937 sand filter and a cartridge with a pore size of 5 μm . The bioflocs were inoculated (30%)
2938 from the shrimp farming system, and there was no renewal of the water throughout the
2939 study. Dechlorinated fresh water was added to restore the volume lost through
2940 evaporation. During the experimental period, no applications of sugarcane molasses
2941 (organic carbon source) were required because the levels of TAN did not exceed 1 mg
2942 L^{-1} .

2943 The biological material used in this study was obtained from the laboratory
2944 Aquatec Ltda (Canguaretama, Rio Grande do Norte, Brazil). After the nauplii of *L.*
2945 *vannamei* had arrived at EMA, they were maintained in the larviculture sector until they
2946 reached the post-larvae 15 (PLs15) stage and then transferred to a nursery (1,500 PLs m^{-3})
2947 in a BFT system, where they reached a mean weight of 0.18 ± 0.06 g. The
2948 experimental units (150 L) were stocked with 85 shrimp in each tank (circular
2949 polyethylene tanks with a bottom area of 0.36 m^2), which resulted in a stocking density
2950 of 425 shrimp m^{-3} . Two air diffusers (aero-tubeTM) were placed at the bottom of each
2951 tank to oxygenate the water to saturation and to keep the solids in suspension.

2952 The experiment consisted of four treatments, each with three repetitions: control
2953 treatment (TC), in which the pH was maintained above 7.2 through the application of
2954 0.05 g L^{-1} of calcium hydroxide ($\text{Ca}(\text{OH})_2$), and treatments T10, T20 and T40, with

2955 daily doses of calcium hydroxide equivalent to 10%, 20% and 40% of the total amount
2956 of feed offered to the shrimp, respectively.

2957 The applications of calcium hydroxide in the control treatment (TC) started
2958 when the pH reached 7.2, in accordance with the procedures adopted after microscale
2959 tests with the cultivation of marine shrimp with biofloc technology at EMA. In the other
2960 treatments, calcium hydroxide was applied after the pH reached 7.7. When the pH
2961 levels reached 8.20, no additional calcium hydroxide was added until the pH returned to
2962 8.0. This protocol was used in all treatments to reduce the levels of non-ionized
2963 ammonia, which increase at pH levels higher than 8.20, and to improve the growth and
2964 survival of the shrimp (Colt and Armstrong, 1981).

2965 The animals were fed commercial rations (Centro Oeste Rações S.A. - Brazil)
2966 with 40% crude protein and 8% lipids (Guabi Potimar 40), offered in trays (Wasielesky
2967 et al., 2006) twice per day (08:00 and 17:00) for 56 days. The feeding rate was initially
2968 set as indicated by Jory et al. (2001), and subsequently adjusted to meet the
2969 consumption rate observed in the trays at the intervals between feedings. The leaching
2970 percentage of the feed dry matter was verified for further quantification of the food
2971 consumption by the shrimp. For this purpose, a known quantity of feed was placed in
2972 trays and deposited in the tanks in duplicates under the same experimental conditions,
2973 but without the presence of the shrimp. After 24 h, it was collected from the trays and
2974 dried in an oven at 105° C until it reached a constant weight, and the percentage of
2975 leaching was calculated as the difference of weight before and after exposure to water in
2976 the tanks.

2977 Correction of pH and alkalinity levels

2978 Calcium hydroxide ($\text{Ca}(\text{OH})_2$) is a water-soluble chemical compound with a molecular
2979 weight of 74 g with high neutralizing power (132%), reactivity of 62% and relative
2980 power of total neutralization of 81%. It is an effective alkalizer, although it may sharply
2981 raise the pH, depending on the dose that is applied (Whangchai et al., 2004). To correct
2982 the pH and alkalinity levels in treatments T10, T20 and T40, equations were adapted
2983 from Ebeling et al. (2006).

$$2984 \quad (1) P_{\text{TAN}} = F * \text{PC} * 0.144$$

2985 Where: P_{TAN} = Production rate of total ammoniacal nitrogen (TAN); F = Feeding (kg
2986 day^{-1}); PC = Protein concentration in the rations (decimal value). The use of the
2987 constant 0.144 means that 16% of the protein is nitrogen (Craig and Helfrich, 2002),
2988 and 90% of the nitrogen that is assimilated by marine shrimp is excreted as ammonia
2989 and urea nitrogen (14.4% or 0.144).

$$2990 \quad (2) Y = (7.05 * P_{\text{TAN}})$$

2991 Where: Y = Alkalinity (g CaCO_3); 7.05 g of alkalinity g TAN^{-1} (autotrophic bacteria);
2992 P_{TAN} = Production rate of total ammoniacal nitrogen (TAN);

$$2993 \quad (3) W = (Y * 100\%) / F$$

2994 Where: W = Percentage of F that must be supplemented with alkalinity; Y = Alkalinity
2995 (g CaCO_3); F = Feeding (kg day^{-1}).

2996 According to the calculations, P_{TAN} is 57.6 g and Y is 406.8 g for 1 kg of feed,
2997 resulting in a value of W equal to 40.68%. The percentages of supplementation with
2998 inorganic carbon (alkalinity as CaCO_3) were sought through applications of calcium
2999 hydroxide ($\text{Ca}(\text{OH})_2$), considering that heterotrophic bacteria consume 3.57 g of

3000 alkalinity for each gram of TAN and act in the same cultivation medium as the
3001 autotrophic bacteria.

$$3002 \quad (4) K = (F * (\%))/V$$

3003 Where: K = Concentration of $\text{Ca}(\text{OH})_2 \text{ L}^{-1}$; F = Feeding (kg day^{-1}); (%) = 10 (T10), 20
3004 (T20) or 40 (T40); V = Volume of the tank (L).

3005 It should be noted that the values of F were corrected every week according to
3006 the results of the biometrics and the daily feed intake observed at the feeding trays (with
3007 the feeding protocol proposed by Jory et al. (2001)).

3008

3009 Microscale test

3010 The microscale test was performed to establish the dosage to be applied to the control
3011 treatment (TC) (0.05 g L^{-1}). Plastic containers with a usable volume of 3 L were used,
3012 and each container had a porous stone to provide aeration. Five juveniles L^{-1} (mean
3013 weight of $0.18 \pm 0.06 \text{ g}$) were stocked in each unit at a density equivalent to 5,000
3014 shrimp m^{-3} . A super intensive nursery condition was simulated with two initial levels of
3015 pH and alkalinity and three repetitions. The temperature was maintained at $26 \text{ }^\circ\text{C}$,
3016 salinity at 30 and the total suspended solids at $1,300 \text{ mg L}^{-1}$. The shrimp were fed
3017 commercial rations once a day with 10% of the tank biomass (Guabi Potimar - 40%
3018 crude protein and 8% lipid).

3019 The pH, alkalinity, ammonia and nitrite levels were monitored before the
3020 application of calcium hydroxide and at 1, 24, 48 and 96 hours after its application. At
3021 the end of the test, the animals were counted to calculate the final survival.

3022

3023 Physical and chemical parameters of water quality

3024 The photoperiod of the experimental room was 12L/12D, provided through an artificial
3025 lighting intensity of 500 lx on the surface of the water. The levels of pH, temperature
3026 and oxygen were measured twice a day with a YSI 100 pH meter (Yellow Springs, OH,
3027 USA) and a YSI 55 oximeter (Yellow Springs, OH, USA), respectively. The salinity
3028 was measured every three days with an optical refractometer (Atago, Japan). The
3029 concentrations of total ammonia (TAN) ($\text{NH}_3 + \text{NH}_4^+$) and nitrite (NO_2^- -N) were
3030 measured three times per week, following the methodologies proposed by UNESCO
3031 (1983) and Bendschneider and Robinson (1952), respectively. The alkalinity was also
3032 analyzed three times per week, according to the protocol proposed by the APHA
3033 (1998); orthophosphate (PO_4 -P) and nitrate (NO_3^- -N) were measured once a week
3034 following Aminot and Chaussepied (1983). Total suspended solids (TSS mg L^{-1}) were
3035 determined once a week using gravimetry, which consisted of filtering 20 mL aliquots
3036 of water with GF 50-A glass fiber filters, as described by Strickland and Parsons (1972).

3037

3038 Zootechnical performance parameters

3039 The biometrics were assessed on a weekly basis by weighing 20 shrimp from each tank
3040 with a precision scale (± 0.01 g SD Marte[®]). At the end of the experiment, all of the live
3041 shrimp in each experimental unit were weighed and counted to determine growth and
3042 survival in the different treatments. The following parameters were determined:

3043 Survival (%): $[(\text{final } n / \text{initial } n) \times 100]$, where n = number of shrimp;

3044 Final mean weight (g) = Total biomass final / n final, where n = number of shrimp;

3045 Feed conversion rate (FCR) = (Feed applied – leaching) / weight gain;

3046 Specific growth rate (SGR; % day⁻¹) = [(ln final weight – ln initial weight) x days of
3047 experiment] x 100;

3048 Productivity (kg m⁻³) = final biomass - initial biomass (kg) / volume of the tank (m³).

3049

3050 Statistical analysis

3051

3052 The software STATISTICA 7.0[®] (StatSoft Inc. 2004, Tulsa, Oklahoma, USA) was used
3053 for statistical analyses of the data. An analysis of variance (ANOVA) was performed
3054 after the assumptions of the method were confirmed. To detect whether there were
3055 significant differences between treatments (p<0.05), Tukey's test (Sokal and Rohlf,
3056 1969) was applied to compare the means. The percentage values were transformed
3057 (arcsin^y^{0.5}) prior to the analyses (Zar, 1996).

3058

3059 **Results**

3060

3061 Water quality

3062 Microscale test

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3064 The levels of pH and alkalinity at 1, 24, 48 and 96 hours after the application of
3065 different concentrations of calcium hydroxide are shown in **Table 1**. The same dosage
3066 of calcium hydroxide caused a higher increase in pH level when the pH and alkalinity
3067 were initially lower. The application of calcium hydroxide in doses equal to 0.10 and
3068 0.20 g L⁻¹ raised the pH in more than one unit within the first hour after application,
3069 independent of the initial pH and alkalinity levels of the water. However, to correct the

3070 alkalinity and pH of the cultivation water, doses of calcium hydroxide were applied to
 3071 prevent the pH from suddenly reaching levels that might be lethal to the
 3072 microorganisms that form the biofloc.

3073 **Table 1** Increase of pH and alkalinity levels after the application of different doses of
 3074 calcium hydroxide in the water of *L. vannamei* raised with a BFT system in different
 3075 treatment groups.

Initial pH	Alkalinity	pH				Alkalinity (mg CaCO ₃ L ⁻¹)				
		[Ca(OH) ₂] (g L ⁻¹)	1 h	24 h	48 h	96 h	1 h	24 h	48 h	96 h
6.80	30	0.025	0.35 ± 0.07	0.40 ± 0.07	0.40 ± 0.05	0.33 ± 0.03	10.0 ± 5.0	15.0 ± 5.0	20.0 ± 5.0	17.0 ± 2.5
		0.05	0.70 ± 0.07	0.75 ± 0.11	0.71 ± 0.03	0.51 ± 0.02	25.0 ± 5.0	25.0 ± 2.5	30.0 ± 5.0	25 ± 2.5
		0.10	1.40 ± 0.05	1.35 ± 0.02	1.21 ± 0.02	1.01 ± 0.03	40.0 ± 5.0	65.0 ± 5.0	70.0 ± 2.5	70.0 ± 2.5
		0.20	2.60 ± 0.08	2.02 ± 0.02	1.81 ± 0.03	1.61 ± 0.02	45.0 ± 2.5	100.0 ± 5.0	140.0 ± 0	150.0 ± 0.0
7.50	100	0.025	0.30 ± 0.04	0.40 ± 0.03	0.35 ± 0.07	0.29 ± 0.03	10.0 ± 5.0	20.0 ± 5.0	25.0 ± 5.0	20.0 ± 0.0
		0.05	0.70 ± 0.08	0.65 ± 0.07	0.56 ± 0.08	0.45 ± 0.08	20.0 ± 0.0	25.0 ± 5.0	35.0 ± 2.5	32.5 ± 2.5
		0.10	1.16 ± 0.04	1.10 ± 0.05	1.05 ± 0.04	0.80 ± 0.04	45.0 ± 0.0	65.0 ± 5.0	87.5 ± 2.5	90.0 ± 2.5
		0.20	1.82 ± 0.03	1.67 ± 0.05	1.25 ± 0.05	1.00 ± 0.02	67.5 ± 2.5	120.0 ± 0.0	130 ± 0.0	130.0 ± 0.0

3076 The data correspond to the mean ± standard deviation.

3077 The concentrations of total ammoniacal nitrogen (TAN) and nitrite (NO₂⁻-N) at
 3078 1, 24, 48 and 96 hours after the application of different doses of calcium hydroxide are
 3079 shown in **Table 2**. In the test performed, doses of up to 0.20 g L⁻¹ of calcium hydroxide
 3080 were applied, and inhibition (or lack of nitrifying and heterotrophic bacteria) was
 3081 observed in the system only at the highest dose; ammonia and nitrite levels in the
 3082 treatment with 0.10 g L⁻¹ remained similar to the control group. There was no mortality
 3083 in the tanks where calcium hydroxide was applied. Finally, the doses of calcium
 3084 hydroxide adopted were lower than 0.10 g L⁻¹, although concentrations of 0.025 and

3085 0.05 g L⁻¹ have previously been considered more suitable to avoid possible problems in
 3086 the formation of the microbial community of biofloc.

3087 **Table 2** Concentrations of total ammoniacal nitrogen (TAN) and nitrite (NO₂⁻-N) after
 3088 the different doses of calcium hydroxide in the water of *L. vannamei* raised in a BFT
 3089 system in different treatment groups.

Initial pH	Alkalinity	[Ca(OH) ₂] (g L ⁻¹)	TAN (mg L ⁻¹)				NO ₂ ⁻ -N (mg L ⁻¹)			
			1 h	24 h	48 h	96 h	1 h	24 h	48 h	96 h
6.80	30	0.00	0.09 ± 0.01 ^a	0.16 ± 0.02	0.30 ± 0.03	0.18 ± 0.10	0.10 ± 0.02	0.08 ± 0.04	0.05 ± 0.03 ^a	0.10 ± 0.01 ^a
		0.025	0.08 ± 0.03 ^a	0.16 ± 0.04	0.22 ± 0.08	0.14 ± 0.04	0.10 ± 0.03	0.06 ± 0.02	0.10 ± 0.04 ^a	0.10 ± 0.03 ^a
		0.05	0.10 ± 0.05 ^a	0.17 ± 0.01	0.37 ± 0.04	0.16 ± 0.02	0.09 ± 0.02	0.08 ± 0.04	0.08 ± 0.03 ^a	0.10 ± 0.02 ^a
		0.10	0.12 ± 0.03 ^a	0.14 ± 0.05	0.23 ± 0.08	0.18 ± 0.04	0.09 ± 0.04	0.08 ± 0.0	0.12 ± 0.04 ^a	0.11 ± 0.03 ^a
		0.20	0.14 ± 0.05 ^b	0.14 ± 0.04	0.36 ± 0.11	0.25 ± 0.10	0.10 ± 0.02	0.11 ± 0.03	0.16 ± 0.04 ^b	0.16 ± 0.06 ^b
7.50	100	0.00	0.08 ± 0.02	0.05 ± 0.03 ^a	0.14 ± 0.02	0.10 ± 0.02 ^a	0.60 ± 0.12	0.70 ± 0.04	0.95 ± 0.15	1.35 ± 0.15
		0.025	0.08 ± 0.04	0.07 ± 0.02 ^a	0.15 ± 0.06	0.14 ± 0.04 ^a	0.62 ± 0.08	0.72 ± 0.04	0.91 ± 0.08	1.25 ± 0.13
		0.05	0.09 ± 0.01	0.08 ± 0.02 ^a	0.20 ± 0.10	0.14 ± 0.03 ^a	0.66 ± 0.10	0.78 ± 0.04	1.02 ± 0.05	1.50 ± 0.05
		0.10	0.07 ± 0.02	0.08 ± 0.01 ^a	0.14 ± 0.02	0.14 ± 0.05 ^a	0.64 ± 0.12	0.72 ± 0.02	1.25 ± 0.10	1.60 ± 0.05
		0.20	0.10 ± 0.03	0.16 ± 0.03 ^b	0.19 ± 0.05	0.32 ± 0.06 ^b	0.68 ± 0.11	0.72 ± 0.04	1.12 ± 0.08	1.60 ± 0.20

3090 The data correspond to the mean ± standard deviation. Superscript letters in the same
 3091 column indicate significantly different means (p<0.05).

3092

3093 Experiment

3094 The physical and chemical parameters of the water were monitored during the
 3095 experiment and are shown in **Table 3**. The mean values of pH and alkalinity were
 3096 significantly lower (p<0.05) in the treatments TC and T10 when compared to the other
 3097 treatments, and T20 was significantly lower than T40 (p<0.05). The mean values of
 3098 total ammoniacal nitrogen (TAN) were significantly higher (p<0.05) in group T40 when

3099 compared to the other treatments. The other water quality parameters did not show
 3100 significant differences ($p>0.05$) over the period of cultivation.

3101 **Table 3** Mean values \pm standard deviation of the physical and chemical parameters of
 3102 the water during the farming (56 days) of *L. vannamei* in different experimental
 3103 treatments¹.

Parameters	Treatments			
	TC	T10	T20	T40
Temperature (°C)	27.82 \pm 0.81	28.18 \pm 0.83	28.21 \pm 0.75	27.85 \pm 0.71
Salinity	25.84 \pm 1.42	25.78 \pm 1.24	25.84 \pm 1.36	25.75 \pm 1.30
DO (mg L ⁻¹)	6.08 \pm 0.10	6.01 \pm 0.15	6.02 \pm 0.13	6.09 \pm 0.11
pH	7.68 \pm 0.20 ^A	7.76 \pm 0.12 ^A	7.98 \pm 0.11 ^B	8.14 \pm 0.16 ^C
Alkalinity (mg CaCO ₃ L ⁻¹)	82.05 \pm 25.51 ^A	90.65 \pm 20.04 ^A	132.17 \pm 20.9 ^B	159.27 \pm 32.82 ^C
TSS (mg L ⁻¹)	963.60 \pm 383.96	995.72 \pm 333.43	998.53 \pm 397.16	924.37 \pm 365.91
TAN (mg L ⁻¹)	0.08 \pm 0.06 ^A	0.08 \pm 0.09 ^A	0.10 \pm 0.09 ^A	0.28 \pm 0.68 ^B
NO ₂ ⁻ -N (mg L ⁻¹)	0.07 \pm 0.03	0.07 \pm 0.05	0.08 \pm 0.05	0.09 \pm 0.08
NO ₃ ⁻ -N (mg L ⁻¹)	48.92 \pm 23.53	53.05 \pm 25.87	49.66 \pm 24.20	44.91 \pm 19.92
PO ₄ -P (mg L ⁻¹)	1.06 \pm 0.38	1.11 \pm 0.50	1.07 \pm 0.49	1.10 \pm 0.43

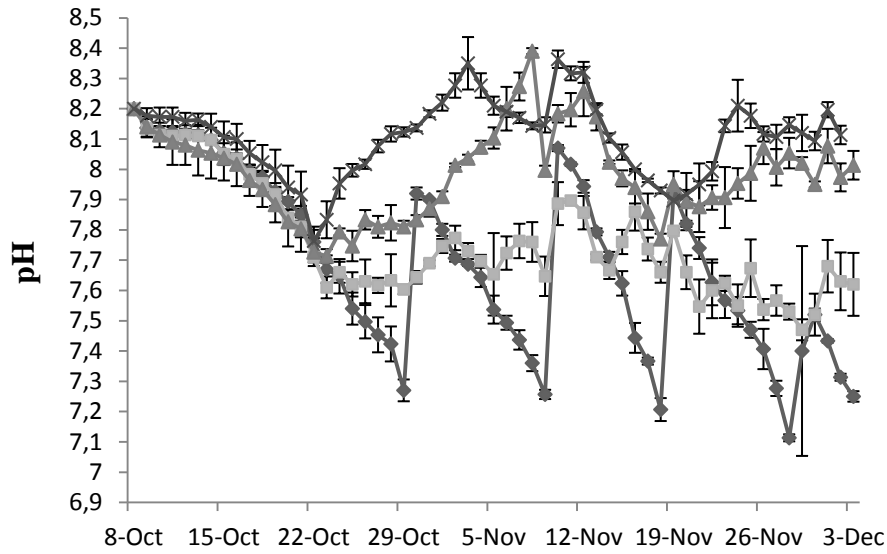
3104 ¹ The data correspond to the mean \pm standard deviation. Different superscript letters in
 3105 the same line indicate that the means differ significantly ($p<0.05$). Dissolved oxygen
 3106 (DO), total ammoniacal nitrogen (TAN), nitrite (NO₂⁻-N), nitrate (NO₃⁻-N) and
 3107 orthophosphate (PO₄-P).

3108 The first application of calcium hydroxide in the TC group (0.05 g L⁻¹) occurred
 3109 on the 20th day of the study, when pH was 7.2 and alkalinity 55 mg CaCO₃ L⁻¹. After
 3110 the application, the pH increased to 7.92 and the alkalinity increased to 100 mg CaCO₃
 3111 L⁻¹, though two more applications were necessary throughout the study. In the

3112 treatments T10, T20 and T40, the first dose was applied on the 14th day with an
3113 alkalinity of 100 mg CaCO₃ L⁻¹ and pH 7.7. The levels of pH and alkalinity in T40
3114 reached 8.30 and 195 mg CaCO₃ L⁻¹, respectively, after daily applications of calcium
3115 hydroxide for 14 days, while in T20, the levels of pH and alkalinity reached 8.3 and 160
3116 mg CaCO₃ L⁻¹, respectively, after 18 days of daily applications of calcium hydroxide.
3117 However, in the treatment T10, the pH ranged between 7.45 and 7.89 and the alkalinity
3118 between 65 and 93 mg CaCO₃ L⁻¹ after the initiation of applications.

3119 During the experiment, the highest dose of calcium hydroxide and the total
3120 applied in TC, T10, T20 and T40 were 0.05 g L⁻¹ and 30 g, 0.012 g L⁻¹ and 50 g, 0.022
3121 g L⁻¹ and 69.3 g and 0.04 g L⁻¹ and 70 g, respectively. Four applications of calcium
3122 hydroxide were performed in the TC group throughout the study, with the lower amount
3123 of this compound used for the correction of alkalinity and pH. Treatments T10, T20 and
3124 T40 received 33, 42 and 18 applications of calcium hydroxide, respectively. Only
3125 treatments T40 and T20 reached pH levels of up to 8.20; subsequent applications of
3126 calcium hydroxide were interrupted until the pH returned to 8.0, when they were
3127 resumed. **Fig. 1a** shows the pH data for the different treatments throughout the 56 days
3128 of the experiment, and **Fig. 1b** shows the alkalinity over the same period of time.

3129

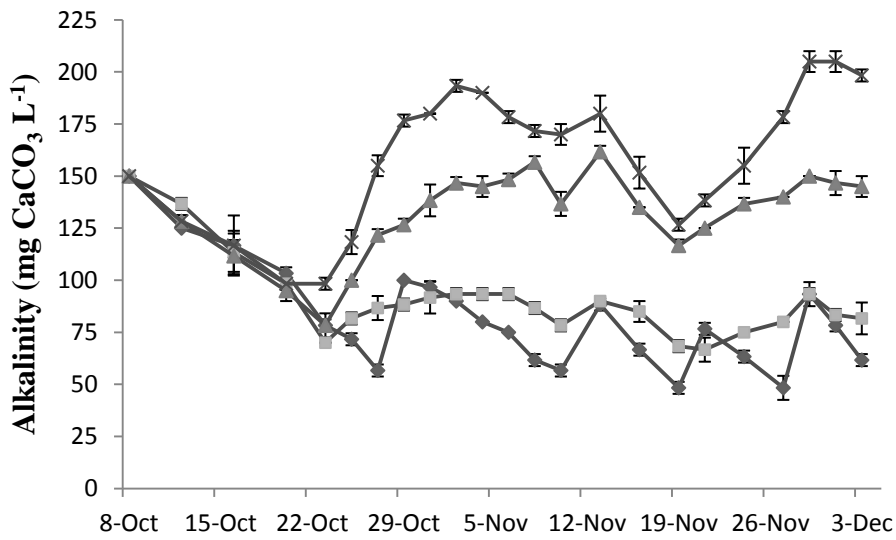


a

◆ TC ■ T10 ▲ T20 × T40

3130

3131



b

◆ TC ■ T10 ▲ T20 × T40

3132

3133 **Fig. 1** Values of pH (a) and alkalinity (b) in the farming water of the treatments TC,

3134 T10, T20 and T40 over 56 days. Deviation bar indicates \pm standard deviation.

3135

3136 Zootechnical performance parameters

3137

3138 The results of the zootechnical performance parameters of the *L. vannamei* juveniles at
 3139 the end of the experiment are presented in **Table 4**. There were no statistically
 3140 significant differences in the mean values of survival, which were higher than 85%
 3141 ($p>0.05$). Final weight, specific growth rate (SGR) and productivity (kg m^{-3}) were
 3142 significantly lower ($p<0.05$) in the treatment T40 than in the other treatments, and no
 3143 significant differences ($p>0.05$) occurred among the other treatments. The results of the
 3144 apparent feed conversion rate (FCR) were significantly better ($p<0.05$) for TC, T10 and
 3145 T20, while T40 showed the worst FCR.

3146 **Table 4** Zootechnical performance parameters of *L. vannamei* juveniles reared for 56
 3147 days in different experimental treatments.

Parameters	TC	T10	T20	T40
Survival (%)	92.15 ± 3.96	91.76 ± 4.07	93.72 ± 5.19	85.03 ± 3.13
Initial weight (g)	0.18 ± 0.06	0.18 ± 0.06	0.18 ± 0.06	0.18 ± 0.06
Final weight (g)	4.28 ± 1.34 ^A	4.38 ± 1.31 ^A	4.09 ± 1.30 ^A	3.08 ± 1.01 ^B
SGR (% day ⁻¹)	5.57 ± 0.02 ^A	5.61 ± 0.01 ^A	5.53 ± 0.09 ^A	5.07 ± 0.07 ^B
FCR	1.25 ± 0.04 ^A	1.24 ± 0.05 ^A	1.30 ± 0.13 ^A	1.79 ± 0.07 ^B
Productivity (kg m^{-3})	2.12 ± 0.12 ^A	2.15 ± 0.10 ^A	2.09 ± 0.2 ^A	1.45 ± 0.05 ^B

3148 The data correspond to the mean ± standard deviation. Different superscript letters in
 3149 the same line indicate significantly different means ($p<0.05$). Feed conversion rate
 3150 (FCR), specific growth rate (SGR).

3151

3152

3153 Discussion

3154

3155 Water quality

3156

3157 In the present study, the minimum temperature was 26.8 °C and the mean temperature
3158 was approximately 27.9 °C, which is within the range of temperatures considered ideal
3159 for the growth and survival of juveniles of the species studied (Wyban et al., 1995; Van
3160 Wyk and Scarpa, 1999).

3161 The shrimp *L. vannamei* shows hyperosmotic regulation in low salinity
3162 environments and hyposmotic regulation in high salinity environments, with an
3163 isosmotic point of 25 (Castille and Lawrence, 1981), and the mean salinity observed in
3164 the treatments was near this point. Maicá et al. (2012) studied the growth of *L.*
3165 *vannamei* juveniles (0.24 g) in salinities of 0, 2, 4 and 25 in the BFT system, and the
3166 percent survivals were 0, 22.5, 72.7 and 97.5%, respectively.

3167 The concentrations of dissolved oxygen in this study were near 6.0 mg L⁻¹ and
3168 considered ideal for the cultivation of penaeid shrimps (Van Wyk and Scarpa, 1999).
3169 The parameters of temperature, salinity and dissolved oxygen in this study were also
3170 favorable for the growth and survival of the shrimp.

3171 The nitrogen compounds important for the cultivation of aquatic organisms are
3172 total ammoniacal nitrogen (TAN), nitrite (NO₂⁻-N) and nitrate (NO₃⁻-N). TAN is
3173 generated from the decomposition and excretion of organic matter and can adversely
3174 affect the performance of cultured organisms or cause mortality if it is present in high
3175 concentrations (Lin and Chen, 2001). Nitrite is an intermediate product from the
3176 oxidation of ammonia into nitrate by nitrifying bacteria. This substance may accumulate
3177 in the growth medium, reach concentrations above safe levels (15.2 mg L⁻¹ in salinity
3178 25) and reduce the growth and survival of the shrimp (Lin and Chen, 2003). Nitrate is
3179 the final product of ammonia nitrification, and it is considered the least harmful
3180 compound to penaeids, with no deleterious effects observed in concentrations of up to
3181 60 mg L⁻¹ (Van Wyk and Scarpa, 1999).

3182 In this study, the mean values of TAN were within a range considered adequate
3183 for the cultivation of *L. vannamei* juveniles (Lin and Chen, 2001). However, there was a
3184 significant difference among the treatments ($p < 0.05$), with the highest concentrations of
3185 TAN observed in treatment T40. Despite this, the levels did not reach 1 mg TAN L⁻¹,
3186 and no organic fertilization was required. The concentrations of NO₂⁻-N remained
3187 within levels acceptable for the growth of *L. vannamei* juveniles (Lin and Chen, 2003),
3188 with no differences among treatments ($p > 0.05$). The initial values of nitrite were close
3189 to zero, due to the inoculum of biofloc that was used and the nitrifying and
3190 heterotrophic bacterial community that was established.

3191 The nitrate accumulated throughout the experiment in all treatments, and the
3192 mean values remained approximately 48 mg L⁻¹, with maximum values greater than 60
3193 mg L⁻¹, similar to those obtained by Scopel et al. (2011). Kuhn et al. (2010) assessed the
3194 chronic effect of nitrate and reported a negative impact on the survival and growth of *L.*
3195 *vannamei* when the compound was present in concentrations higher than 220 mg L⁻¹ at
3196 low salinities (11). Overall, the concentrations of the different nitrogen compounds in
3197 the treatment groups throughout the study did not negatively affect the zootechnical
3198 performance and survival of the shrimp.

3199 According to Ganguly et al. (1999), applications of 0.1 to 0.2 g L⁻¹ of calcium
3200 hydroxide show a bactericidal effect due to the stress caused by increased pH and major
3201 changes in the system of acid-base balance (CO₂-HCO₃⁻-CO₃⁻²). In the test we
3202 performed to choose the dose of calcium hydroxide to be applied in the TC, there was a
3203 significant increase of the total ammoniacal nitrogen levels at a dose of 0.20 g L⁻¹,
3204 although this increase was not observed at the other tested doses. Furtado et al. (2011)
3205 applied doses of up to 0.16 g L⁻¹ of calcium hydroxide in the cultivation of *L. vannamei*
3206 in a BFT system and did not report a TAN increment or an accumulation of nitrite in the

3207 system. The highest doses of calcium hydroxide applied during the study in the
3208 treatments TC, T10 and T20 were 0.05 g L⁻¹, 0.012 g L⁻¹ and 0.022 g L⁻¹, respectively.
3209 In these three treatments, no increases in the levels of ammonia or accumulations of
3210 nitrite could be observed, which is explained by inhibition or lack of nitrifying bacteria
3211 in the system. The nitrification occurred similarly in those three treatments. However,
3212 the highest dose applied in treatment T40 was 0.04 g L⁻¹, which was similar to the
3213 control group but applied with greater frequency. In TC, four applications led to a total
3214 of 30 g of calcium hydroxide, whereas the 28 applications in T40 resulted in 70 g of
3215 calcium hydroxide, which is more than twice the amount applied in TC. Thus, there was
3216 an increase in the levels of ammonia in T40, which correspond to an inhibition or
3217 reduction of nitrification in the system.

3218 An accumulation of phosphorus in the system does not directly affect the
3219 development of the shrimp, but it can generate conditions that are favorable for the
3220 proliferation of filamentous cyanobacteria, which can clog the gills of the shrimp and
3221 produce toxins that are harmful to penaeids (Wasielesky et al., 2006). The mean
3222 concentration of total suspended solids (TSS) was close to 1000 mg L⁻¹ in the different
3223 treatments. According to Samocha et al. (2007), TSS levels of up to 500 mg L⁻¹ are
3224 considered suitable for cultivation. The low luminous intensity in the experimental
3225 room (500 lx) may have influenced the increase in TSS, as microbial populations are
3226 more stable and less dependent on light conditions (Avnimelech, 2006). TSS levels can
3227 be reduced through the process of clarification, which assists in the maintenance of high
3228 dissolved oxygen levels, reduction of nitrate and phosphorus concentrations and
3229 increased alkalinity throughout farming (Gaona et al., 2011; Ray et al., 2010). However,
3230 the process of clarification was not necessary in the present study because dissolved

3231 oxygen levels remained above 5 mg L⁻¹ and the consumption of food was not reduced
3232 throughout the study.

3233 According to Queiroz et al. (2004), the alkalinity in farming ponds may be
3234 effectively corrected through the application of alkalizing compounds on the water
3235 layer. Either ponds that are waterproofed with geomembrane or tanks are used with
3236 biofloc technology, which reduces the cost of alkalizing compounds for the correction
3237 of soil pH and its disinfection before farming.

3238 In the present study, the initial levels of alkalinity and pH (150 mg CaCO₃ L⁻¹
3239 and 8.20, respectively) declined until the calcium hydroxide was applied in the different
3240 treatments. Krummenauer et al. (2011) analyzed the growth of shrimp in densities of
3241 150, 300 and 450 shrimp m⁻² and verified a decrease in the levels of pH, reaching
3242 minimum values of 6.1, 6.4 and 6.3, respectively, without applications of alkalizers.
3243 Gaona et al. (2011) applied doses of 0.02 g L⁻¹ of calcium hydroxide whenever the pH
3244 dropped to approximately 7.30, maintaining levels between 7.30 and 7.80 throughout
3245 the study. In treatments T20 and T40, the amount of calcium hydroxide that was added
3246 daily was responsible for raising the pH to 8.2, at which point application was stopped
3247 to avoid an increase in the fraction of unionized ammonia (NH₃) that is harmful to the
3248 growth and survival of the shrimp (Colt and Armstrong, 1981).

3249 The pH values among the treatments remained in a range (7.0 to 9.0) that is
3250 adequate for the cultivation of this species throughout the study (Van Wyk and Scarpa,
3251 1999). The lowest alkalinity values observed in treatments TC and T10 were below the
3252 recommended levels for *L. vannamei* (> 100 mg CaCO₃ L⁻¹), although alkalinity
3253 remained within the recommended range in treatments T20 and T40 (Van Wyk and
3254 Scarpa, 1999; Ebeling et al., 2006).

3255 We observed that the application of calcium hydroxide equivalent to 20 and 40%
3256 of the feed offered daily in the cultivation tanks exceeded the rate of inorganic carbon
3257 uptake by heterotrophic and nitrifying bacteria present in the BFT system, while a
3258 dosage of 10% kept the system near stability between the inorganic carbon consumption
3259 rates of bacteria and reserves of alkalinity in the water. The system in TC (0.05 g L^{-1})
3260 was maintained with the highest pH range and the lowest levels of alkalinity, although
3261 the other physical, chemical and biological parameters of water quality were not
3262 affected. Baloi et al. (2013) applied calcium hydroxide at 15% of the amount of feed
3263 daily offered when the alkalinity declined below $120 \text{ mg CaCO}_3 \text{ L}^{-1}$, and they observed
3264 a range of alkalinity from 112 to $230 \text{ mg CaCO}_3 \text{ L}^{-1}$ and pH levels from 7.0 to 8.3 in the
3265 treatment 12L:12D. Thus, alkalinity and pH can be corrected through daily applications
3266 between 10 and 20% of the feed offered in the farming tank or through doses of up to
3267 0.05 g L^{-1} when the pH is between 7.2 and 7.3. It is important to note, however, that the
3268 quality of calcium hydroxide varies depending on the manufacturer and its neutralizing
3269 power (NP) and rate of reactivity (RR) must be greater than 100% and 65%,
3270 respectively.

3271

3272 Zootechnical performance

3273

3274 The survival rates obtained in systems for farming marine shrimp with the biofloc
3275 technology are relatively high. Furtado et al. (2011) and Baloi et al. (2013) observed
3276 survival rates above 80% and 86%, respectively, which were similar to the rates in this
3277 study (>85%) and similar among treatments. The lowest values of final weight and
3278 specific growth rate (SGR) were obtained from treatment T40. Maicá et al. (2012)
3279 cultivated juveniles of *L. vannamei* in a salinity of 25 with the biofloc technology and

3280 verified a mean specific growth rate of $5.37\% \text{ day}^{-1}$, similar to the best results obtained
3281 in the present study (TC, T10 and T20).

3282 The best feed conversion rates (FCR) were recorded in treatments TC, T10 and
3283 T20, which are similar to those found by Furtado et al. (2011), who applied doses of up
3284 to 0.16 g L^{-1} of calcium hydroxide for alkalinity and pH correction (FCR of 1.4), and
3285 higher than those found by Baloi et al. (2013) (FCR of 2.6). Although the FCR obtained
3286 in T40 is lower than those from other treatments, this apparent food conversion value is
3287 not unfeasible for the commercial farming of *L. vannamei* with BFT systems. With
3288 regard to the productivity (kg m^{-3}), the mean values (~ 2.10) were greater in treatments
3289 TC, T10 and T20 than in treatment T40. These values are similar to those obtained by
3290 Scopel et al. (2011), in which a productivity of 2.10 kg m^{-3} with a density of 235
3291 shrimps m^{-2} was reached, and Baloi et al. (2013), who produced 2.3 kg m^{-2} of juveniles
3292 grown with a stocking density of $300 \text{ shrimp m}^{-3}$. However, Krummenauer et al. (2011)
3293 worked with densities of 150, 300 and $450 \text{ shrimp m}^{-2}$ and observed productivity means
3294 of 2.15, 4.09 and 3.04, respectively, which were higher than those obtained in the
3295 present study.

3296 Despite the fact that the worst growth performance was observed in treatment
3297 T40, differentiating it from other treatments, the conditions were not considered
3298 unsuitable for the cultivation of shrimp with the biofloc technology. New studies will be
3299 developed to evaluate the physiology and immunology of shrimp grown in the
3300 conditions of treatment T40, which showed the worst performance. In addition, further
3301 work can be performed to evaluate the effect of hydrated lime in the microorganisms of
3302 the bioflocs.

3303

3304

3305 **Conclusion**

3306 In these conditions we find that calcium hydroxide doses of 0.05 g L⁻¹ or daily
3307 applications between 10 and 20% of the amount of feed offered to the shrimp can be
3308 applied to correct alkalinity and pH without inhibition of the bacterial community
3309 present in biofloc systems. The results of this research will assist the work of shrimp
3310 producers in maintaining water quality in BFT systems that requires attention to the
3311 maintenance of alkalinity levels in order to minimize the daily fluctuation of pH.

3312

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3320 **References**

- 3321 Aminot A, Chaussepied, M (1983) Manuel des analyses chimiques en milieu marin.
3322 Brest, CNEXO.
- 3323 APHA (American Public Health Association) (1998) Standard methods for the
3324 examination of water and wastewater. 20st edition. Washington, DC.
- 3325 Avnimelech Y (2006). Bio-filters: the need for an new comprehensive approach.
3326 Aquacult Eng 34:172–178

- 3327 Baloi M, Arantes R, Schweitzer R, Magnotti C, Vinatea L (2013) Performance of Pacific
3328 white shrimp *Litopenaeus vannamei* raised in biofloc systems with varying levels
3329 of light exposure. *Aquacult Eng* 52:39-44
- 3330 Bendschneider K. Robinson RJ (1952) A new spectrophotometric method for the
3331 determination of nitrite in sea water. *J Mar Res* 11: 87-96
- 3332 Boyd CE (1990) Water quality in ponds for aquaculture. Birmingham, AL, USA:
3333 Birmingham Publishing, 482 p.
- 3334 Boyd CE, Tucker CS (1998) Pond aquaculture water quality management. Boston:
3335 Kluwer Academic, 700 p.
- 3336 Castille FL, Lawrence AL (1981) The effect of salinity on the osmotic, sodium and
3337 chloride concentrations in the hemolymph of euryhaline shrimps of the genus
3338 *Penaeus*. *Comp Biochem Physiol A* 68:75–80
- 3339 Chen S, Ling J, Blancheton JP (2006) Nitrification kinetics of biofilm as affected by
3340 water quality factors. *Aquacult Eng* 34:179–197
- 3341 Colt JE, Armstrong DA (1981) Nitrogen toxicity to crustaceans, fish, and molluscs. In:
3342 Allen, L.J., Kinney, E.C. (Eds.), *Proceedings of the Bio-Engineering Symposium*
3343 *for Fish Culture*. American Fisheries Society, Bethesda, pp34–47
- 3344 Crab R, Defoirdt T, Bossier P, Verstraete W (2012) Biofloc technology in aquaculture:
3345 Beneficial effects and future challenges. *Aquaculture* 356-357:351-356
- 3346 Craig S, Helfrich LA (2002) *Understanding Fish Nutrition, Feeds and Feeding*
3347 (Publication 420–256). Virginia Cooperative Extension, Yorktown (Virginia). 4
3348 pp.
- 3349 De Schryver P, Crab R, Defoirdt T, Boon N, Verstraete W (2008) The basics of bio-
3350 flocs technology: the added value for aquaculture. *Aquaculture* 277:125–137.

3351 Ebeling JM, Timmons MB, Bisogni JJ (2006) Engineering analysis of the stoichiometry
3352 of photoautotrophic, autotrophic, and heterotrophic control of ammonia-nitrogen
3353 in aquaculture in aquaculture production systems. *Aquaculture* 257:346-358

3354 Fromm PO, Gillette JR (1968) Effect of ambient ammonia on blood ammonia and
3355 nitrogen excretion of rainbow trout (*Salmo gairdneri*). *Comp Biochem Physiol*
3356 26:887-896

3357 Furtado PS, Poersch LH, Wasielesky WJr (2011) Effect of Calcium Hydroxide,
3358 Carbonate and Sodium Bicarbonate on Water Quality and Zootechnical
3359 Performance of Shrimp *Litopenaeus vannamei* Reared in Bio-Flocs Technology
3360 (BFT) Systems. *Aquaculture* 321:130-135

3361 Ganguly S, Chatterjee J, Jana BB (1999) Biogeochemical cycling bacterial activity in
3362 response to lime and fertilizer applications in pond systems. *Aquacult Int* 7:413–
3363 432.

3364 Gaona CAP, Poersch LH, Krummenauer D, Foes GK, Wasielesky WJ (2011) The effect
3365 of solids removal on water quality, growth and survival of *Litopenaeus vannamei*
3366 in a biofloc technology culture system. *IJRA* 12: 54-73

3367 Jory DE, Cabrerias RT, Durwood MD, Fegan D, Lee GP, Lawrence AL, Jackson JC,
3368 McIntosh PR, Castañeda AJ (2001) A global review of shrimp feed management:
3369 status and perspectives. *Aquaculture the World* Aquaculture Society, Baton
3370 Rouge, LA USA.

3371 Kinne O (1976) *Mar. Ecol.* Ed. John Wiley & Sons, NY, USA, Vol III, part 1, pp 577

3372 Krummenauer D, Cavalli RO, Poersch LH, Wasielesky WJr (2011) Superintensive
3373 Culture of White Shrimp, *Litopenaeus vannamei*, in a Biofloc Technology System
3374 in Southern Brazil at Different Stocking Densities. *J World Aquacult Soc* 42:726-
3375 733

- 3376 Kuhn D D, Smith SA, Boardman GD, Angier MW, Marsh LFJ, George J (2010)
3377 Chronic toxicity of nitrate to Pacific white shrimp, *Litopenaeus vannamei*:
3378 Impacts on survival, growth, antennae length, and pathology. *Aquaculture*
3379 309:109-114.
- 3380 Lin YC, Chen JC (2001) Acute toxicity of ammonia on *Litopenaeus vannamei* (Boone)
3381 juveniles at different salinity levels. *J Exp Mar Biol Ecol* 259:109-119
- 3382 Lin YC, Chen JC (2003) Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone)
3383 juveniles at different salinity levels. *Aquaculture* 224:193-201
- 3384 Maicá PF, Borba MR, Wasielesky W (2012) Effect of low salinity on microbial floc
3385 composition and performance of *Litopenaeus vannamei* (Boone) juveniles reared
3386 in a zero-water-exchange super-intensive system. *Aquacult Res* 43:361–370
- 3387 Queiroz JF, Nicolella G, Wood CW, Boyd CE (2004) Lime application methods, water
3388 and bottom soil acidity in fresh water fish ponds. *Sci Agric* 6 (5):469-475
- 3389 Ray JA, Lewis BL, Browdy CL, Leffler JW (2010) Suspended solids removal to
3390 improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-
3391 based feed in minimal-exchange, superintensive culture systems. *Aquaculture*
3392 299:89-98
- 3393 Samocha TM, Patnaik S, Speed M, Ali AM, Burger JM, Almeida RV, Ayub Z,
3394 Harisanto M, Horowitz A, Brook DL (2007) Use of molasses as carbon source in
3395 limited discharge nursery and grow-out systems for *Litopenaeus vannamei*.
3396 *Aquacult Eng* 36:184-191
- 3397 Scopel BR, Schweitzer R, Seiffert WQ, Pierri V, Arantes RF, Vieira FN, Vinatea LA
3398 (2011) Substituição da farinha de peixe em dietas para camarões marinhos
3399 cultivados em sistema bioflocos. *Pesq Agropec Bras* 46 (8):928-934

3400 Sokal RR, Rohlf FJ (1969) Biometry. Principle and practices of statistics in biological
3401 research. W. H. Freeman & Company. 776p.

3402 Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis. Ottawa:
3403 Fishery Research Board Canada, 310p.

3404 UNESCO (1983) Chemical methods for use in marine environmental monitoring.
3405 Manual and Guides 12, Intergovernmental Oceanographic Commission. Paris,
3406 France.

3407 Van Wyk P, Scarpa J (1999) Water quality and management. In: Van Wyk, P. et al.
3408 (Eds.). Farming marine shrimp in recirculating freshwater systems. Florida
3409 department of agriculture and consumer services, Tallahassee, pp141-161

3410 Vinatea L, Galvez AO, Browdy CL, Stokes A, Venero J, Haveman J, Lewis BL,
3411 Lawson A, Shuler A, Leffler JW (2010) Photosynthesis, water respiration and
3412 growth performance of *Litopenaeus vannamei* in a super-intensive raceway
3413 culture with zero water exchange: interaction of water quality variables. *Aquacult*
3414 *Eng* 42:17–24

3415 Wasielesky W, Atwood H, Stokes A, Browdy CL (2006) Effect of natural production in
3416 a zero exchange suspended microbial floc based super-intensive culture system
3417 for white shrimp *Litopenaeus vannamei*. *Aquaculture* 258:396-408

3418 Whangchai N, Veronica P, Migo P, Alfafara CG, Young KH, Nomura N, Matsumura M
3419 (2004) Strategies for alkalinity and pH control for ozonated shrimp pond water.
3420 *Aquac Eng* 30:1–13

3421 Wyban J, Walsh AW, Godi DM (1995) Temperature effects on growth, feeding rate and
3422 feed conversion of the Pacific white shrimp (*Penaeus vannamei*). *Aquaculture*
3423 138:267–279

3424 Zar JH (1996) Biostatistical Analysis. Third Edition New Jersey: Prentice Hall, 662p.

3425 **CONCLUSÃO GERAL**

3426 Os resultados obtidos no decorrer dessa tese, serão aplicados no dia a dia dos
3427 produtores de camarão que estão migrando dos sistemas de cultivo semi-intensivos,
3428 para os sistemas intensivos e superintensivos com bioflocos.

3429 1- Os resultados obtidos no ensaio de toxicidade aguda do pH evidenciam maior
3430 resistência dos camarões ao pH ácido do que ao pH básico. Nas condições
3431 experimentais descritas no presente estudo, a pH_{50} 96 h de pH ácido e básico foram de
3432 4.04 e 9.58 para juvenis de *L. vannamei*. Quanto ao ensaio de estresse oxidativo
3433 constatamos que os camarões expostos tanto ao pH 4.5 como ao pH 9.5 apresentaram
3434 desequilíbrio na atividade das enzimas antioxidantes. Portanto, o camarão branco do
3435 Pacífico *Litopenaeus vannamei* deve ser cultivado em sistemas com melhor controle de
3436 pH (menor amplitude diária e sem variações bruscas), para que o camarão possa ter seu
3437 melhor desempenho fisiológico refletindo em melhor crescimento, sobrevivência e
3438 produtividade ao longo do cultivo.

3439 2- No presente estudo de toxicidade aguda do CO_2 encontramos os valores de
3440 CL_{50} 96 h foi 59,12 mg/L de CO_2 e o nível de segurança calculado em 5,9 mg/L de CO_2 ,
3441 já a maior concentração não letal foi de 23,8 mg/L de CO_2 . Recomendamos que os
3442 níveis de CO_2 sejam mantidos dentro do limite de segurança calculado para espécie, a
3443 fim de evitar condições de hipercapnia que podem imunodeprimir os camarões
3444 tornando-os mais suscetíveis a enfermidades.

3445 3- Nas condições testadas o camarão marinho *L. vannamei* suportou um período
3446 de 36 horas sem aeração com aplicação de H_2O_2 como fonte alternativa de oxigênio
3447 dissolvido. As concentrações de CO_2 se elevam com o incremento da biomassa de
3448 camarões e de sólidos suspensos totais, com o passar do tempo, alcançando níveis

3449 subletais para os camarões. O dióxido de carbono dissolvido acumulado pode ser
3450 removido pela restauração do sistema de aeração, promovendo a desgaseificação.

3451 4- No presente estudo foi verificado um incremento no consumo de oxigênio de
3452 juvenis de *L. vannamei* expostos a concentrações superiores a 30 mgCO₂/L.
3453 Recomendamos que durante o cultivo de *L. vannamei* os níveis de CO₂ sejam mantidos
3454 no nível de segurança de 5,9 mgCO₂/L, para reduzir o consumo de oxigênio e evitar
3455 condições de hipercapnia.

3456 5-Nas condições experimentais empregadas nesse estudo foi possível formar os
3457 bioflocos e biofilme no cultivo de *Litopenaeus vannamei* sem renovação de água com
3458 níveis de alcalinidade superiores a 70 mgCaCO₃/L e pH superior a 7,38. Contudo, as
3459 melhores taxas de nitrificação e melhor desempenho zootécnico ocorreram nos
3460 tratamentos com maiores níveis de alcalinidade, principalmente na alcalinidade 300
3461 mgCaCO₃/L. Nas alcalinidades mais elevadas ainda se obteve maior estabilidade dos
3462 valores de pH.

3463 6- No cultivo de camarões marinhos em sistemas BFT deve-se atentar para a
3464 manutenção da alcalinidade em níveis que atenuem a flutuação diária do pH, mantendo
3465 o pH acima de 7,3. Para a correção da alcalinidade, pH e CO₂ pode ser aplicado
3466 dosagens de 0,05 g/L de hidróxido de cálcio ou aplicações diárias entre 10 e 20% da
3467 quantidade de ração ofertada aos camarões, isto sem inibir a comunidade bacteriana
3468 presentes nos bioflocos.

3469 Por fim, com este estudo esperamos ter mostrado a relevância da alcalinidade,
3470 pH e CO₂, na sobrevivência e crescimento, do camarão branco do Pacífico *Litopenaeus*
3471 *vannamei* em sistema de bioflocos.

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