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Influência do sistema de cultivo na resistência de juvenis de *Macrobrachium rosenbergii* **(De Man, 1879) à exposição aguda de amônia**

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PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

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Ítalo Felipe Mascena Braga

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“Dedico este trabalho a Maria de Lourdes, meu principal alicerce”

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Resumo

Macrobrachium rosenbergii é uma das espécies aquícolas mais cultivadas em ambientes de água doce, sendo tradicionalmente produzidos em sistemas semi-intensivos, no entanto, estudos recentes demonstraram a capacidade de aumentar a densidade de estocagem de camarões de água doce com a utilização do sistema de bioflocos. O cultivo experimental foi realizado durante 58 dias na Estação de Aquicultura da Universidade Federal Rural de Pernambuco, Brasil. Foram estocados camarões de 0,15 ± 0,001 g (375 PL/m^3) em oito tanques circulares (400 L) em delineamento experimental inteiramente casualizado com BFT (sistema de bioflocos) e WES (sistema com troca de água), com quatro repetições cada. Ao final deste período, os animais foram mantidos até 2,77 ± 0,11g e foram submetidos a um teste de estresse agudo, envolvendo cinco concentrações de nitrogênio da amônia total (0, 5, 10, 20 e 40 mg/L) para cada sistema. O peso final, ganho de peso, taxa de crescimento específico, biomassa final e produtividade foram influenciados positivamente pelo sistema de BFT. A sobrevivência foi superior a 65% nos tratamentos BFT e WES, sem diferença significativa entre os tratamentos (P> 0,05). O consumo de água no tratamento biofloco (BFT) foi 10 vezes inferior ao controle (WES). Os juvenis cultivados em bioflocos e posteriormente submetidos à toxicidade da amônia foram mais resistentes quando comparados àqueles criados em um sistema de renovação de água, podendo suportar concentrações de até 40 mg/L de NAT, durante um período de 96 horas, sem interferir na sobrevivência dos camarões.

Palavras-chave: Camarão de água doce; qualidade água; desempenho zootécnico; consumo de água; resposta ao estresse.

Abstract

Macrobrachium rosenbergii is one of the most cultivated freshwater specie in the world, its cultivation is traditionally carried out in semi-intensive systems, however, recent studies have demonstrated the capacity to increase the stocking density of freshwater prawns using the biofloc system . The experiment was carried out for 58 days at the Aquaculture Station of the Federal Rural University of Pernambuco, Brazil. Juveniles of 0.15 ± 0.001 g (375 PL m^{-3}) were stored in eight circular tanks (400 L) in a completely randomized experimental design with BFT (biofloc system) and WES (water exchange system), with four replicates each. At the end of this period, the animals were maintained up to 2.77 ± 0.11 g and underwent an acute stress test, involving five concentrations of TAN (0, 5, 10, 20 and 40 mg L^{-1}) for each system. The final weight, weight gain, specific growth rate, final biomass and productivity were positively influenced by the BFT system. Survival was greater than 65% in BFT and WES treatments, with no statistical difference between treatments (P> 0.05). Water consumption in the biofloc was 10 times lower than the control (WES). Juveniles cultivated in biofloc and later submitted to ammonia toxicity were more resistant when compared to those raised in a water renewal system, being able to withstand concentrations of up to 40 mg L^{-1} of TAN during a 96-hour period without interfering on survival of prawns.

Keywords: Freshwater prawn; water quality; growth performance; water consumption; stress response.

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1- Introdução

1.1- Contextualização da pesquisa

A carcinicultura de água doce é um dos setores da aquicultura que mais cresce mundialmente (SANTOS et al. 2017), no ano de 2016 a sua produção foi de 507 mil toneladas, sendo o *Macrobrachium rosenbergii* responsável por aproximadamente 46% (234 mil toneladas) desta produção (FAO, 2018a). No Brasil esta é a principal espécie de camarão de água doce cultivada e estima-se que sua produção nos últimos anos seja de 100 toneladas/ano (FAO, 2018b), entretanto há uma ausência de dados oficiais desta produção no país.

O camarão *M. rosenbergii* apresenta diversas características que justificam o interesse na sua produção, tais como facilidade na reprodução em cativeiro, alta taxa de fecundidade, adaptabilidade à ração inerte, bom crescimento, grande porte ao final do ciclo de cultivo, podendo atingir 32 cm de comprimento e 500 g de peso, maior resistência a doenças quando comparados às espécies de camarões marinhos, carne de excelente qualidade (LOBÃO, 1996; NEW, 2002; GUPTA et al., 2007; MOHANTY, 2010), além de elevado valor de mercado.

O cultivo desta espécie é tradicionalmente realizado com renovações diárias de água, e em menores densidades de estocagem (até 20 camarões/m²), devido ao comportamento territorialista e agressivo dos camarões do gênero *Macrobrachium* (VALENTI et al., 2010; NEGRINI et al., 2017). Porém, estudos recentes demonstram a capacidade de incrementar as densidades de estocagem dos cultivos dos camarões de água doce (KIMPARA *et al*., 2013; BALLESTER et al., 2017; NEGRINI et al., 2017), possibilitando assim o aumento da produtividade dos cultivos. Entretanto, isto proporciona o acúmulo de substâncias tóxicas inorgânicas, tais como os compostos nitrogenados (amônia e nitrito), que são um dos principais problemas de qualidade de água em cultivos intensivos (COLT e ARMSTRONG, 1981; TIMMONS e EBELING, 2007).

A amônia é o principal dejeto metabólico excretado pelos crustáceos, assim como em outros organismos aquáticos (REGNAULT, 1987; EBELING et al., 2006), e se encontra disponível na água principalmente em duas formas, amônio (NH_4^+), menos tóxica, e na forma não ionizada (NH_3), mais tóxica devido à capacidade de penetrar as membranas celulares (TIMMONS e EBELING, 2007). O equilíbrio das formas da amônia está relacionado a variáveis físicas e químicas da água como temperatura, pH e salinidade (ALABASTER e LOYD, 1982; TOMASSO 1986).

Nos crustáceos, elevada concentração deste composto no sistema de cultivo pode causar alcalinização e um aumento de volume da hemolinfa (MOULLAC e HAFFNER, 2000), dificultando os processos de excreção pelas brânquias dos animais, consequentemente gerando um aumento na concentração interna desse composto, ocasionando disfunção generalizada no metabolismo celular (MUGNIER e JUSTOU, 2004; SÁ, 2012). Além disso, reduz o crescimento dos camarões e pode até mesmo causar mortalidade (OSTRENSKY e WASIELESKY, 1995; EBELING et al., 2006).

Estudos de toxicidade aguda podem fornecer informações sobre a letalidade das substâncias, entretanto não podem prever efeitos subletais e crônicos causados nos organismos (BUIKEMA et al., 1982). A concentração aguda da amônia varia de acordo com a fase de desenvolvimento do animal, assim como pelo tempo de exposição ao composto. Estudos mostram que o CL_{50} para larvas de *M. rosenbergii* varia de 8,34 a 15,03 mg de NAT/L por um período de 96 horas (FIGUEROA-LUCERO et al., 2012; GOMES et al., 2016), e para juvenis recém metamorfoseados entre 0,54 a 2,52 mg/L de N-NH_3 (STRAUS et al., 1991). Entretanto, não existem estudos disponíveis para indivíduos de aproximadamente 2g de peso médio em sistema de bioflocos.

Em sistemas tradicionais, para que sejam mantidas as condições adequadas da qualidade de água é necessário que sejam realizadas trocas frequentes, parciais ou até mesmo totais, do volume das unidades de cultivo, gerando assim efluentes tóxicos devido ao excesso de nutrientes e matéria orgânica que são liberados em ambientes naturais (MARTÍNEZ et al., 2012), além da necessidade de grande aporte de água para utilização na renovação (NAYLOR et al., 2001). Tais fatores resultam em degradações ambientais e estimulam a busca por técnicas de cultivo mais sustentáveis (FROÉS et al., 2013).

A tecnologia de bioflocos está associada a sistemas de cultivo com mínimas trocas de água, estimulando a formação de um agregado de bactérias (heterotróficas e nitrificantes), cianobactérias, microalgas, protozoários e matéria orgânica (detritos, excretas e sobras de alimentação) (SAMOCHA et al., 2017), a partir da fertilização da água com fontes de carbono orgânico (açúcar, melaço, amido, farelos vegetais, rações, etc.) e aeração constante do ambiente de cultivo (PÉREZ-RUESTRO et al., 2014). Estes microrganismos, principalmente as bactérias heterotróficas são capazes de utilizar o carbono orgânico e nitrogênio inorgânico da amônia para a produção de biomassa bacteriana (AZIM e LITTLE, 2008; PÉREZ-FUENTES et al., 2013).

Além disto, neste sistema de cultivo, a amônia pode ser convertida em nitrito pelas bactérias oxidantes da amônia (BOA), as quais pertencem principalmente aos gêneros

Nitrosomonas e *Nitrosococcus*, e convertido em nitrato pelas bactérias oxidantes do nitrito (BON), as quais geralmente pertencem ao gênero *Nitrobacter* e *Nitrospira* (VAN LOOSDRECHT e JETTEM, 1998; AVNIMELECH, 2009). A manutenção dos níveis de alcalinidade entre 100 e 150 mg/L $CaCO_3$ (EBELING et al., 2006), e pH entre 7,8 e 8,0 (SAMOCHA et al., 2017), são capazes de melhorar a eficiência da oxidação dos compostos nitrogenados, através da adição periódica de uma fonte de carbono inorgânico.

Em adição, o sistema de bioflocos pode ser utilizado como fonte de alimentação suplementar aos organismos cultivados (ASADUZZAMAN et al., 2010) contribuindo para uma melhora no desempenho dos mesmos, devido ao seu elevado valor nutricional (EKASARI et al., 2014; XU e PAN, 2014), principalmente os níveis de proteína bruta variando de 35 a 38% e lipídeos de 1 a 5% (TACON et al., 2002; AZIM e LITTLE, 2008). A composição nutricional destes flocos varia de acordo com as condições de cada sistema de produção, tais como temperatura, salinidade, pH, fotoperíodo, intensidade da aeração e tipo de carbono orgânico disponível para o desenvolvimento bacteriano (SAMOCHA et al., 2017). O consumo do floco é capaz de melhorar a atividade enzimática, contribuindo para um maior aproveitamento das rações utilizadas (XU e PAN, 2012; XU et al., 2013), podendo reduzir uma das principais problemáticas em cultivos intensivos, que é o baixo aproveitamento da ração (MEGAHED, 2010; EMERENCIANO, 2013).

Além do papel do biofloco para o controle de compostos nitrogenados e nutrição dos organismos cultivados, Kim et al. (2014) sugerem que os bioflocos ou os microrganimos associados ao biofloco, podem aumentar a expressão de alguns genes relacionados com a resposta imune dos camarões. Melo et al. (2016) e Aguilera-Rivera et al. (2018), relatam que *Litopenaeus vannamei* cultivado previamente no sistema de bioflocos é mais resistente à exposição a compostos tóxicos (NO_2) e Vibrio *harveyi*, respectivamente. Entretanto, não se sabe o real efeito deste sistema de cultivo sobre os camarões de água doce, portanto, o objetivo deste trabalho é determinar a influência da tecnologia de bioflocos no crescimento e resistência de *M. rosenbergii* à exposição aguda de nitrogênio da amônia total.

1.2- Objetivos do trabalho

Objetivo geral

Avaliar a influência da tecnologia de bioflocos, no cultivo de juvenis do camarão de água doce *Macrobrachium rosenbergii*, na resistência a exposição por um período de 96 h, ao nitrogênio da amônia total.

Objetivos específicos

- Avaliar o efeito dos diferentes sistemas de cultivo no desempenho zootécnico de pós-larvas de *M. rosenbergii*;
- Determinar a influência do sistema de cultivo autotrófico (com troca de água) e heterotrófico (sistema de bioflocos) no consumo de água para a produção de *M. rosenbergii;*
- Determinar a influência do sistema de cultivo autotrófico (com troca de água) e heterotrófico (sistema de bioflocos) na resistência de *M. rosenbergii* expostos a NAT por 96 horas

ARTIGO

Parte dos resultados obtidos durante o trabalho experimental desta dissertação está apresentada no artigo intitulado

"Nursery phase performance of *Macrobrachium rosenbergii* in bioflocs and water exchange system" (manuscrito), que se encontra anexado.

Artigo científico a ser submetido à Revista: **Aquaculture Research - https://mc.manuscriptcentral.com/are - ISSN 1365-2109 (versão impressa),**

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Todas as normas de redação e citação, deste capítulo, atendem as normas estabelecidas pela referida revista (em anexo).

ARTIGO

Nursery phase performance of *Macrobrachium rosenbergii* in bioflocs and water exchange system

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Keywords: freshwater prawn, water quality, growth performance, stress response, water consumption

ABSTRACT

The aim of this work was to evaluate the effect of different systems on growth performance and TAN resistance of *M. rosenbergii* in nursery phase. The experiment was carried out 58 days and the prawns of 0.15 ± 0.001 g were stored (375 PL m^{-3}) in eight circular tanks (400 L) in a completely randomized experimental design with BFT (biofloc system) and WES (water exchange system), with four replicates. At the end of this period, the animals was raised until 2.77 ± 0.11g for a stress test involving five concentrations of TAN (0, 5, 10, 20 and 40 mg L^{-1}) for each system. The final weight, gain weight, SGR, final biomass and productive were improved by BFT system ($p<0.05$). Survival was greater than 65%, with no statistical difference between the treatments ($P>0.05$), therefore water consumption in the tanks with biofloc was 10 times lower than in the control (WES). The juveniles raised in biofloc (89.3%) submitted to ammonia toxicity were more resistant when compared to those raised in a water-exchanged system. The animals support concentrations of up to 40 mg L^{-1} to the biofloc system without affecting the survival of the prawns.

Introduction

Freshwater prawn agribusiness is one of the fastest growing aquaculture sector worldwide (Santos, Arruda, Azevedo & Pontes, 2017). In 2016, its production was 507,000 tons, being 46% (234,000 tons) of *Macrobrachium rosenbergii* (FAO, 2018a). In Brazil, this is the main cultivated specie of freshwater prawn, however in recent years the production is stagnant in 100 tons per year (FAO, 2018b).

This interest in the production of *Macrobrachium* is due to some of its features, such as captive breeding facility, high fertility rate, adaptability to inert ration, good growth, large enterprises at the end of the growing cycle, reaching 32 cm long and 500 g of weight. In addition, greater resistance to disease when compared to the marine shrimps, meat of excellent quality (Lobão, 1996; New, 2002; Gupta & Sehgal-Kaur, 2007; Mohanty, 2010), as well as high market value.

This specie is traditionally culture in semi-intensive systems, using fertilized ponds with inorganic and organic nutrients, daily exchange water and lower stocking densities, due to the behavior territorial and aggressive of individuals this genus *Macrobrachium* (Valenti, New, Salin & Ye, 2010; Negrini et al., 2017). However, recent studies demonstrate the ability to increase the stocking densities of freshwater prawns with the use of the biofloc system (Ballester et al., 2017; Negrini et al., 2017).

The biofloc system is based on stimulating the formation of an aggregate of bacteria (heterotrophic and nitrifying), cyanobacteria, microalgae, protozoa and organic matter (detritus, faeces and waste of feed) (Samocha et al., 2017), from the water fertilization with organic carbon sources (sugar, molasses, starch, vegetable brans, etc.) and constant aeration of the culture environment (Pérez-Ruestro, Perez-Fuentes & Hernández-Vergara, 2014). This biota is responsible for the conversion of inorganic nitrogenous compounds, providing a water quality

control (Emerenciano, Gaxiola & Cuzon, 2013; Samocha et al., 2017) and has high nutritional value (Ekasari et al., 2014; Xu & Pan, 2014), such as crude protein between 35 to 38% and lipids 1 to 5% (Azim & Little, 2008) . In addition, biofloc system is able to improve the enzymatic activity, contributing to a greater utilization of feed used (Xu & Pan, 2012; Xu, Pan, Sun & Huang, 2013), as well stimulate non-specific immune system of post larvae of marine shrimps (Kim et al., 2014).

Despite the benefits of this system for other species *Litopenaeus vannamei* and *Oreochromis niloticus*,(Lima, Melo, Ferreira & Correia, 2017; Marinho et al., 2017; Brito et al., 2018; Souza, Lima, Melo, Padilha, & Correia, 2019), few studies have evaluated the effect of this technology in the production of freshwater prawns (Pérez-Fuentes, Pérez-Ruestro, & Hernandez-Vergara, 2013, Ballester et al., 2017; Miao, Sun, Bu, Zhu, & Chen, 2017; Negrini et al., 2017). Therefore, the aim of the present study was to evaluate the influence of different culture systems (biofloc x water exchange) in the performance and TAN resistance of *Macrobrachium rosenbergii* during the nursery phase.

Materials and methods

Experimental facilities

An outdoor trial was conducted for 58 days at the Aquaculture Productions Systems Laboratory (LAPAq) of Fisheries and Aquaculture Department (DEPAq) of the Rural Federal University of Pernambuco (UFRPE), Recife, Brazil. The experimental design was completely randomized with two treatments: BFT (biofloc system) and WES (water exchange system), both with four replicates. Eight fiberglass tanks with a working volume of 400 L and bottom area of 1 m^2, covered with a black screen (70% UV protection) to avoid the escape of animals.

The aeration system was maintained by a radial blower of 2 CV, allowing an individual output for each experimental unit, using micro-perforated hose (0.7 m per unit).

Preparation of biofloc system

The preparation of the biofloc inoculum lasted 60 days, and was carried out in four fiberglass tanks that were supplied with 200 L of freshwater, filtered (200 μm) and chlorinated with 10 ppm active chlorine using sodium hypochlorite, and dechlorinated by constant aeration for 24 hours. An initial biomass (1 kg m^3) of tilapia (*O. niloticus*, weight mean 4.0g) was placed in each tank. The fish were fed twice daily with Pirá 36, extruded commercial feed (Guabi®, Brazil) with guaranteed levels of 10% moisture, 36% crude protein, 8% ether extract, 37% carbohydrate, 14% ash and 5% crude fiber, which was offered in an amount ranging from 15 to 7% of the biomass.

During the preparation of the biofloc, it was added molasses (30% of organic carbon) to maintain a C:N ratio of 6:1 (Samocha et al. 2007), assuming that the protein contained 16% nitrogen (Craig & Helfrich, 2002). Therefore, in 1000g of feed, contains 360g crude protein and there are 57.6 g of nitrogen, being necessary to apply 345.6 g of organic carbon, then 1.15 kg of molasses. After the maturation, the biofloc from the matrix tanks was homogenized, transferred to each experimental unit, and represented 50% of the total tank volume (200L). The remaining 200 L were filled with filtered fresh water and sterilized as described above.

Biofloc and water exchange systems

In the biofloc treatment, there was no exchange water, just to replace the losses from evaporation. Furthermore, there was added molasses, as organic carbon source, when the concentration of TAN was higher than 0.8 mg L^{-1} (Samocha et al., 2017) and sodium bicarbonate ($NaHCO_3$) for keep the alkalinity level ≥ 100 mg L^{-1} (Samocha et al., 2017).

In the water exchange system, the tanks were filled with freshwater (400 L), how described previously. To management of the nitrogen compounds in the system were realized partial renewals of 50% every three days, modified from Pérez-Fuentes et al. (2013), or when the total ammonia nitrogen (TAN) was higher than 0.8 mg L^{-1}. The total volume of water used in the BFT and WES during the experiment was measured and calculated the water consumption by prawn biomass (consume of water. $biomass^{-1}$).

Prawn stocking, feeding and monitoring

Post-larvae of *M. rosenbergii* (PL_{30}, 0.15±0.001g), were obtained in commercial prawn hatchery (Aquamarão, Goiana, Pernambuco, Brazil), and acclimatized in fiberglass tanks (working volume 800L) at a stocking density 1 PL. L^{-1}, for a period of five days. During this period the animals were fed with commercial shrimp feed (Wean, 0.8 mm, M&M, In Vivo, Brazil), with guaranteed levels of 45% crude protein, 9.5% ether extract, 29.5% carbohydrate, 13% moisture, 12% ash and 4% crude fiber. When the levels of TAN or nitrite were higher than 1 mg L^{-1}, the water was renewed (50%). Afterward, the animals were counted, weighed and transferred to the culture tanks at a density of 375 PL m^{-3} (150 PL m^{-2}).

Prawns were initially fed pelletized commercial feed Wean (0.8 mm, M&M, In Vivo, Brazil) and, after 30 days, on Densisty 40 CR2 (1 to 1.7 mm, Presence®, Brazil), with guaranteed levels of 40% crude protein, 9% ether extract, 13% moisture, 12% ash and 4% crude fiber. The feed was offered three times a day, at 9:00 A.M., 1:00 P.M. and 5:00 P.M. The initial feeding rate was 30% of the biomass adjusted during the experiment according to consumption and biomass determined by biometrics that were taken weekly (Ballester et al., 2017).

At the end of the experiment all animals were weighed and counted, for determination of performance variables such as final weight, weight gain (WP = final weight – initial weight), feed conversion ratio (FCR = Quantity of feed provided . increased of biomass^{-1}), specific growth rate [SGR = 100 (ln final weight – ln initial weight). time of culture^{-1}], survival [S = 100 x (population final . population initial^{-1})] and productivity [Pr = final biomass (Kg) . volume^{-1} (m ^{3})].

Water quality

The temperature, dissolved oxygen and pH were measured in each tank, twice daily (8:00 A.M. and 4:00 P.M.), with the use of multi-parameter AK 88 (by AKSO, Brazil). Weekly collections were made of each tank water samples for the determination of concentrations of total ammonia nitrogen (TAN), nitrogen nitrite (N-NO_2) and alkalinity (mg $CaCO_3$ L^{-1}), and biweekly for nitrate (NO_3) and orthophosphate (PO_4^{-3}). The readings were held in spectrophotometer for Nessler methods, diazotization, and cadmium reduction method of the amino acid, respectively. The total alkalinity was determined by volumetric titration (APHA, 1995).

The total suspended solids (mg L^{-1}) was measured every two weeks, according to APHA (1995) and the settleable solids (mL L^{-1}), twice a week, using the Imhoff cones, when one-liter samples of water from each experimental unit were collected, and the corresponding volume of these solids were measured after settle period of 30 minutes (Avnimelech, 2009). Adopted as ideal level of solids sedimentation varying from five to 15 mL L^{-1} (Emerenciano, Martínez-Córdoba, Baeza & Porchas, 2017).

Ammonia stress test

At the end of the nursery, the animals were kept under the same experimental conditions until reaching the mean weight of 2.77 ± 0.11 g, when they were transferred to buckets of 10L and stocking density of one juvenile L^{-1}, for an acute exposure test to different (96hr) concentrations of TAN. Each culture system, BFT and WES, was submitted to the concentrations 0, 5, 10, 20, 40 mg L^{-1}, totaling ten treatments (BFT_0, BFT_5, BFT_{10}, BFT_{20}, BFT_{40}, WES_0, WES_5, WES_{10}, WES_{20}, WES_{40}), with three replicates each. The experimental units were supplied and renewed daily with previously treated water and biofloc with the following parameters of water quality: temperature 27.0 ° C, pH 7.7; dissolved oxygen 6.7 mg L^{-1}, and TAN stock (10,000 mg L^{-1}) prepared from the addition of ammonia chloride P.A.

Actual concentrations in the stress test for the treatments with biofloc system and partial renovations of water are expressed in Table 1, and were close to nominal concentrations. During the period of exposure to nitrogen compounds, the animals were fed commercial feed pelletized for marine shrimp, Camanutri 35 J (2.4 mm, Presence®, Brazil) once a day (9:00 A.M.) , with guaranteed levels of 35% crude protein, 8% ether extract, 13% moisture, 12% ash and 4% crude fiber. The feeding rate was 15% of the total biomass, as Valenti (2010). Every day, the dead animals were removed and counted.

Insert table 1

Statistical Analysis

Performance variables of prawns were initially tested for normality (Shapiro-Wilk) to the water quality variables was applied the test of normality of D'agostino-Pearson, and for both were applied homoscedasticity (Cochran), at the level of significance of 5%. When normality and the homogeneity of the sample were found, was applied T-student to analysis of means, when identified significant differences, t-test was applied ($\alpha < 0.05$). The nonparametric Kruskal-Wallis test was used on nitrite, nitrate, dissolved oxygen, pH and temperature, followed

with the post-hoc test of Wilcoxon-Mann-Whitney. All the data were analyzed using SysEAPRO software version 1.0.

Results

The average values of water temperature, dissolved oxygen, pH, alkalinity, total ammonia nitrogen, nitrite nitrogen, nitrate, orthophosphate, settable solids and total suspended solids, monitored during the trial period are presented in Table 2.

Insert table 2

Temperature and dissolved oxygen had an average of 27° C and 6.3 mg L^{-1}, respectively, showing no significant difference ($\alpha > 0.05$) among treatments, while the pH was significantly higher ($\alpha < 0.05$) in the BFT system (8.20) when compared with the WES system (7.64).

Total ammonia nitrogen (TAN) varied between 0.01 and 0.81 mg L^{-1}, showing lowest level in the WES when compared to the BFT. This variable means remained stable, at BFT treatment and, in the WES from the first and second week of culture, respectively (Figure 1A). Regarding the average concentrations of nitrite nitrogen showed no significant difference ($\alpha > 0.05$) (Figure 1B). Presented a trend of nitrate buildup from the 15th day of culture in BFT, while in the WES treatment occurred this nutrient reduction due to the constant exchanges of water (Figure 1C). The ranging of alkalinity was 10 to 140 mg $CaCO_3L^{-1}$ during the 58 days of experiment (Figure 1D), being significantly higher than in the treatment of biofloc.

Insert figure 1

The results of the productive performance of prawns *M. rosenbergii* grown in two intensive systems (BFT and WES), are presented in table 3. There was no significant difference ($\alpha > 0.05$) among treatments to survival. However, for the variables of final weight, weight gain, specific growth rate, feed conversion ratio, final biomass and productive BFT system was better ($\alpha < 0.05$).

Insert table 3

The water consumption during the trial was 3.12 m^3 and 0.426m^3 for WES and BFT, respectively (Figure 2A), the greater consumption in associate with the lower biomass production (Figure 2B) resulted in the higher ratio of Water:Biomass of animals, 32.32 m^3 Kg^{-1} (Figure 2C).

Insert figure 2

Daily survival rate of juveniles exposed to different concentrations of TAN for a period of 96 hours is expressed in table 4. There are no mortality in the experimental units on the first day. The culture systems did not show influence on survival rate until 72 hours of exposure to total ammonia nitrogen ($P>0.05$).

Insert table 4

Discussion

The water quality (temperature and oxygen dissolved) were of according to New et al. (2002) and Emerenciano et al. (2017) for a good growth prawn. Small variations of pH (7.62-8.60) may occur due to buffering capacity of the water, corroborating with the found by Suantika, Turendro & Situmorang (2017). In addition, the pH was within the range of great growth for the nitrite oxidizing bacteria, which require values between 7.2 and 8.2 (Ebeling, Timmons & Bisogni, 2006), and have contributed to the low concentrations of this compound in this study. The largest concentrations of pH in the treatment BFT are related mainly by the addition of sodium bicarbonate in the tanks of this system to maintain levels of alkalinity ~ 100 mg L^{-1}.

Alkalinity was similar to that found by Ballester et al. (2017), that when testing the nursery culture of *M. rosenbergii* in recirculation system and biofloc, found concentrations ranging between 58 and 68 mg $CaCO_3$ L^{-1}, respectively. The reduction in levels of alkalinity in

the system can reduce the processes of bacterial nitrification (Samocha et al., 2017) and the ecdysis of animals, since carbonates are used for growth of shrimps (New, Wagner, Tidwell, Abramo & Kutty, 2010), therefore the highest concentration of alkalinity in BFT treatment was due to bicarbonate replacement to nitrification processes of this system.

The main sources of ammonia in the system are from excretion of organisms and the decomposition of organic matter (Emerenciano et al., 2017), in this study, concentrations of TAN agreed with the recommended by Perez-Fuentes et al. (2013), which suggest that ammonia levels should be less than 1 mg L^{-1}, for the control of water quality in biofloc system. Coyle, Alston & Sampaio (2010) report that mortalities can occur in nursery crops of *M. rosenbergii* when levels exceed 3 mg L^{-1}.

It should be noted that the lowest concentrations of TAN to treat WES are directly related with periodic renewals of the water. Despite the significant difference in the concentrations of TAN, there was no influence on survival of cultured prawns.

Nitrite is toxic to cultured organisms (Samocha et al., 2017), and its main source is through the oxidation of unionized ammonia, by oxidizing ammonia bacteria (OAB) such as *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus*, and *Nitrosovibrio* (Ebeling et al, 2007). The low concentrations of this compound in BFT might have occurred due to the presence of oxidizing nitrite bacteria (ONB), which convert nitrite to nitrate (Timmons & Ebeling, 2007). Since there was a reduction in the concentrations of Nitrite-N from the second week of culture, and consequently the increase of concentrations of nitrate, which tends to accumulate in intensive culture systems (Kuhn et al., 2010), besides the need for replacement of alkalinity during the culture cycle.

The maintenance of water quality in the WES treatment was performed through partial exchanged of water, in addition to the possible installation of biofilm on the walls and bottom

of the tanks, which may contribute to the reduction of the concentration of nitrogen compounds and phosphate (Shilta, Chadha & Pandey, 2016), and with the nutrition of raised organisms (Abreu et al., 2007; Zhang et al., 2016).

Studies show that biomass of marine shrimps is able to absorb only 10 to 35% of the phosphorus from feeding in semi-intensive and intensive culture systems (Thakur & Lin, 2003; Casillas-Hernández, Magallón-Barajas, Portillo-Clark & Páez-Osuna, 2006; Da Silva, Wasielesky & Abreu, 2013). Then on systems without renovations or with limited water exchange, such as BFT, this compound tends to increase during culture, as found by Correia et al. (2014).

The means found in relation to settable solids (SS) and total suspended solids (TSS) were in according to the cited by Emerenciano et al. (2017), which states that these levels should be kept between 5 and 15 mL L^{-1} and below 500 mg L^{-1}, respectively. There was a trend of accumulation along the culture for these variables, in BFT treatment, due to the addition of organic carbon source, generating an increase in the production of SS, which are composed mainly by heterotrophic and autotrophic bacteria (Luo, Avnimelech, Pan & Tan, 2013) and the increased bacterial biomass present in the system causes, as a result, an increase in total suspended solids (TSS).

For both treatments the final weight of the animals was similar to that found by Seenivasan, Radhakrishnan, Muralisankar & Bhavan (2014), that when assessing the use of different probiotics on the growth of post-larvae of *M. rosenbergii* (0.15 g) for a period of 60 days, they found the final weight ranging from 0.84 to 1.20 g. However the animals grown in BFT treatment reached the highest final weight, as well as a higher specific growth rate, which is higher than found by Negrini et al. (2017), corroborating with the tendency of this system, since it has a greater availability of food (floc) to farmed animals (Ballester et al., 2017).

Survival in this study were between 65 and 69%, no differing between culture systems, being the superior to that found by Negrini et al. (2017) to the same stocking density (150 individuals m^{-2}), which found 51% survival, may because. The biofloc achieved the higher productivity 0.34 kg m^{-3}, directly related to the greater weight of the animals on that system.

The BFT system presented the best result of FCR (2.5), standing next to found by Ballester et al. (2017), when compared with the WES, possibly due to less stress suffered by the animals, since they do not have undergone periodic renewals of water, in addition to the nutritional value of biofloc, especially its high level of crude protein (Samocha et al., 2017). However, this value is still very high, and can be minimized through the use of artificial substrates in the tanks, which are able to increase the areas for accommodation of post-larvae, in addition to possibly serve as a power source capable of aggregating food for the animals, improving the survival, reducing use of artificial feeding, and consequently, reduction in the feed conversion ratio (Zhang et al., 2016).

Water consumption in WES was 32.32 m^3 kg^{-1} of prawn produced, similar to that found in other intensive crops for the species *L. vannamei*, 32-40 m^3 Kg^{-1} (Boyd, 2005; Tucker & Hargreaves, 2008). While the BFT had a consumption of approximately ten times less than water (3.2 m^3 Kg^{-1}), since that system did not need partial renewals of water for the control of nitrogen compounds dissolved. Perez-Fuentes et al. (2014) found similar results, as well as for Nile tilapia (Lima et al., 2018) therefore; this confirms the potential of this system in reducing water consumption.

In crustaceans, high concentrations of ammonia in water can cause an alkalinization of the hemolymph and consequently an increase in the internal concentrations of this compound in animal (Mugnier & Justou, 2004). Acute toxicity studies can provide information about the lethality of substances, however cannot predict chronic and sublethal effects caused on

organisms (Buikema, Niederlehner & Cairns, 1982). To the conditions of temperature and pH on testing of acute stress, 27.0° C and 7.7 respectively, approximately 6.1% of the total ammonia dissolved in water is on your non-ionized (NH_3), therefore more toxic.

No mortalities were found in the treatment BFT_0, while for the treatment WES_0 a single individual died in one of the replicates, possibly due to stress caused in the renewal of water. The same treatment, for concentrations 5, 10 and 20 mg L^{-1} mortalities occurred from 24 hours of exposure of TAN, not differing significantly by control (WES_0), however the concentration of 40 mg L^{-1}, presented the lowest 20% survival along the 96 hours.

There was interaction between culture systems and the survival of prawns after 96 hours of exposure to concentrations of total ammonia nitrogen, where BFT treatments had higher survival, approximately 90%, when compared with WES. Juveniles grown in this system supported concentrations up to 40 mg L^{-1} of TAN. Melo, Ferreira, Braga & Correia (2016) found similar effects for *L. vannamei* exposed to different concentrations of nitrite.

Kim et al. (2014), suggest that biofloc or the microorganism associated with the biofloc can increase the expression of some genes related to the immune response of shrimps. Aguilera-Rivera et al. (2018), report that *L. vannamei* cultured in biofloc system previously developed effective resistance mechanisms related to hemocyanin of animals.

The mean lethal concentration (LC_{50}) for juvenile *M. rosenbergii* varies between 0.54 to 2.52 mg L^{-1} of $N\text{-}NH_3$ (Straus, Robinette & Heinen, 1991), being this species more resistant than others are of the same genus, as *M. amazonicum* (Dutra, Forneck, Brazão, Freire & Ballester, 2016). In this study, even if the concentrations of 10, 20 and 40 mg L^{-1} of NAT are within the average range for the LC_{50} of the species have not been reported high mortalities of animals, except in WES_{40} treatment, this may be related with the nursery stage performed prior to these animals.

Conclusions

The biofloc system influenced positively in the growth of *M. rosenbergii* in the nursery phase. Juveniles from this treatment exposed to the toxicity of ammonia, were more resistant when compared to those created in water-exchanged system. Animals support concentrations up to 40 mg L^{-1} for the biofloc without affecting the survival of animals. In addition, the biofloc reduces water consumption in the production of *Macrobrachium rosenbergii* up to 10 times.

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References

Abreu, P. C., Ballester, E. L. C., Odebrecht, C., Wasielesky, W., Cavalli, R. O., Granéli, W., & Anesio, A. M. (2007). Importance of biofilm as food source for shrimp (*Farfantepenaeus paulensis*) evaluated by stable isotopes (δ13C and δ15N). Journal of Experimental Marine Biology and Ecology, 347 (1-2), 88-96. https://doi.org/10.1016/j.jembe.2007.03.012

Aguilera-Rivera, D., Escalante-Herrera, K., Gaxiola, G., Prieto-Davó, A., Rodríguez-Fuentes, G., Guerra-Castro, E., Hernández-López, J., Chávez-Sánchez, M. C., & Rodríguez-Canul, R. (2018). Immune response of the Pacific white shrimp, *Litopenaeus vannamei*, previously reared in biofloc and after an infection assay with Vibrio *harveyi*. World Aquaculture Society, 50, 119-136. https://doi.org/10.1111/jwas.12543

APHA, Water Environment Federation, & American Water Works Association. (1999). Standard Methods for the Examination of Water and Wastewater (Part 4000-6000).

Standard Methods for the Examination of Water and Wastewater. https://doi.org/ISBN 9780875532356

Avnimelech, Y. (2009). *Biofloc Technology – A Practical Guide Book.* The World Aquaculture Society (1st ed.). Baton Rouge, TX:World Aquaculture Society.

Azim, M. E., & Little, D. C. (2008). The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture, 283, 29-35. https://doi.org/10.1016/j.aquaculture.2008.06.036

Ballester, E. L. C., Marzarotto, S. A., Castro, C. S., Frozza, A., Pastore, I., & Abreu, P. C. (2017). Productive performance of juvenile freshwater prawns *Macrobrachium rosenbergii* in biofloc system. Aquaculture Research, 48 (9), 4748-4755. https://doi.org/10.1111/are.13296

Boyd, C. E. (2005). Water use in aquaculture. World Aquaculture, 36 (3), 12–15, 70.

Brito, L. O., Jr. Cardoso, L., Abreu, J. L., Severi, W., Moraes, L. B. S., & Galvez, A. O. (2018). Effects of two commercial feeds with high and low crude protein content on the performance of white shrimp *Litopenaeus vannamei* raised in an integrated biofloc system with the seaweed *Gracilaria birdiae.* Spanish Journal of Agricultural Research, 16 (1), 1-7. https://doi.org/10.5424/sjar/2018161-11451

Buikema, A. L., Niederlehner, B. R., & Cairns, J. (1982). Biological monitoring part IV-Toxicity testing. Water Research, 16 (3), 239-262. https://doi.org/10.1016/0043-1354(82)90188-9

Casillas-Hernández, R., Magallón-Barajas, F., Portillo-Clarck, G., & Páez-Osuna, F. (2006). Nutrient mass balances in semi-intensive shrimp ponds from Sonora, Mexico using two feeding strategies: Trays and mechanical dispersal. Aquaculture, 258 (1-4), 289-298. https://doi.org/10.1016/j.aquaculture.2006.03.027

Correia, E. S., Wilkenfeld, J. S., Morris, T. C., Wei, L., Prangnell, D. I., & Samocha, T. M. (2014). Intensive nursery production of the Pacific white shrimp *Litopenaeus vannamei* using two commercial feeds with high and low protein content in a biofloc-dominated system. Aquacultural Engineering, 59, 48-54. https://doi.org/10.1016/j.aquaeng.2014.02.002

Coyle, S. D., Alston, D. E., & Sampaio, C. M. S. (2010). Nursery Systems and Management. In Freshwater Prawns (pp. 108–126). Oxford, UK: Wiley-Blackwell. https://doi.org/10.1002/9781444314649.ch7

Craig, S., & Helfrich. L. A. (2002). Understanding Fish Nutrition. Feeds and Feeding (Publication 420–256). Virginia Cooperative Extension. Yorktown (Virginia). 4 pp.

Da Silva, K. R., Wasielesky, W., & Abreu, P. C. (2013). Nitrogen and Phosphorus Dynamics in the Biofloc Production of the Pacific White Shrimp, *Litopenaeus vannamei*. Journal of the World Aquaculture Society, 44 (1), 30-41. https://doi.org/10.1111/jwas.12009

Dutra. F. M., Forneck. S. C., Brazão. C. C., Freire. C. A., & Ballester. E. L. C. (2016). Acute toxicity of ammonia to various life stages of the Amazon River prawn, *Macrobrachium amazonicum*, Heller, 1862. Aquaculture, 453, 104-109. https://doi.org/10.1016/j.aquaculture.2015.11.038

Ebeling, J. M., Timmons, M. B., & Bisogni, J. J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture, 257 (1-4), 346-358. https://doi.org/10.1016/j.aquaculture.2006.03.019

Ekasari, J., Angela, D., Waluyo, S. H., Bachtiar, T., Surawidjaja, E. H., Bossier, P., & De Schryver, P. (2014). The size of biofloc determines the nutritional composition and the

nitrogen recovery by aquaculture animals. Aquaculture, 426-427, 105-111. https://doi.org/10.1016/j.aquaculture.2014.01.023

Emerenciano, M. G. C., Wasielesky, W., Soares, R. B., Ballester, E. C., Izeppi, E. M., & Cavalli, R. O. (2007). Crescimento e sobrevivência do camarão-rosa (*Farfantepenaeus paulensis*) na fase de berçário em meio heterotrófico. Acta Scientiarum – Biological Sciences, 29 (1), 1-7. https://doi.org/ 10.4025/actascibiolsci.v29i1.120

Emerenciano, M., Gaxiola, G., & Cuzo, G. (2013). Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry. Biomass Now - Cultivation and Utilization, 12, 301-328. https://doi.org/10.5772/53902

Emerenciano, M. G. C., Martínez-Córdova, L. R., Martínez-Porchas, M., & Miranda-Baeza, A. (2017). Biofloc Technology (BFT): A Tool for Water Quality Management in Aquaculture. In Water Quality. InTech, 5, 91-109. https://doi.org/10.5772/66416

FAO. (2018a). Fishery information: Data and Statistics Service. Aquaculture Production Statistics. FAO Fisheries Circular.

FAO. (2018b). Fishery and Aquaculture Statistics. Global aquaculture production 1950-2016 (FishstatJ). In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 2018. www.fao.org/fishery/statistics/software/fishstatj/en

Gupta, A., Sehgal, H. S., & Sehgal, G. K. (2007). Growth and carcass composition of giant freshwater prawn, *Macrobrachium rosenbergii* (De Man), fed different isonitrogenous and isocaloric diets. Aquaculture Research, 38 (13), 1355-1363. https://doi.org/10.1111/j.1365-2109.2007.01787.x

Kim, S. K., Pang, Z., Seo, H. C., Cho, Y. R., Samocha, T., & Jang, I. K. (2014). Effect of bioflocs on growth and immune activity of Pacific white shrimp, *Litopenaeus vannamei* postlarvae. Aquaculture Research, 45 (2), 362-371. https://doi.org/10.1111/are.12319

Kuhn. D., Smith. S. A., Boardman. G. D., Angier. M. W., Marsh. M., & Jr Flick. G. J. (2010). Chronic toxicity of nitrate to Pacific white shrimp. *Litopenaeus vannamei*: impacts on survival. Growth, antenna length, and pathology. Aquaculture, 309 (1-4), 109-114. https://doi.org/10.1016/j.aquaculture.2010.09.014

Lima, E. C. R., Souza, R. L., Girao, P. J. M., Braga, Í. F. M. & Correia, E. S. (2018). Culture of Nile tilapia in a biofloc system with different sources of carbon. Revista Ciência Agronômica, 49 (3), 458–466. https://doi.org/10.5935/1806-6690.20180052

Lobão, V. L., & Empresa Brasileira de Pesquisa Agropecuária. (1996). Camarão-da-malásia: cultivo. Brasília: EMBRAPA/SPI. Retrieved from https://www.worldcat.org/title/camarao-da-malasia-cultivo/oclc/45773873&referer=brief_results

Luo. G., Avnimelech. Y., Pan. Y. F., & Tan. H. X. (2013). Inorganic nitrogen dynamics in sequencing Batch reactors using biofloc technology to treat aquaculture sludge. Aquacultural Engineerin, 52, 73-79. https://doi.org/10.1016/j.aquaeng.2012.09.003

Marinho, Y. F.; Brito, L. O., Campos, C. V. F. S., Severi, W., Andrade, H. A., & Gálvez, A. O. (2017). Effect of the addition of *Chaetoceros calcitrans*, *Navicula* sp. and *Phaeodactylum tricornutum* (diatoms) on phytoplankton composition and growth of *Litopenaeus vannamei* (Boone) postlarvae reared in a biofloc system. Aquaculture Research, 48, 4155-4164. https://doi.org/10.1111/are.13235

Melo, F. P., Ferreira, M. G. P., Braga, Í. F. M., & Correia, E. de S. (2016). Toxicidade do nitrito para o camarão *Litopenaeus vannamei* cultivado em sistemas de água clara e bioflocos. Boletim do Instituto de Pesca, 42 (4), 855–865. https://doi.org/10.20950/1678-2305.2016v42n4p855

Miao, S., Sun, L., Bu, H., Zhu, J., & Chen, G. (2017). Effect of molasses addition at C:N ratio of 20:1 on the water quality and growth performance of giant freshwater prawn (*Macrobrachium rosenbergii*). Aquaculture International, 25 (4), 1409–1425. https://doi.org/10.1007/s10499-017-0124-3

Mohanthy, R. K. (2010). Impact of phased haversting population structure, feed intake pattern and growth performance of *Macrobrachium rosenbergii* De Man (giant freshwater prawn) in polyculture with carps in concurrent rice-fish culture. Aquaculture International, 18, 523-537. https://doi.org/10.1007/s10499-009-9263-5

Mugnier, C., & Justou, C. (2004). Combined effect of external ammonia and molt stage on the blue shrimp *Litopenaeus stylirostris* physiological response. Journal of Experimental Marine Biology and Ecology, 309, 35-46. https://doi.org/10.1016/j.jembe.2004.03.008

Negrini, C., Castro, C. S., Guimarães, A. T. B., Frozza, A., Kracizy, R. O., & Ballester, E. L. C. (2017). Stocking density for freshwater prawn *Macrobrachium rosenbergii* (Decapoda, Palaemonidae) in biofloc systems. Latin American Journal of Aquatic Research, 45 (5), 891-899. https://doi.org/10.3856/vol45-issue5-fulltext-3

New, M. B. (2002). Farming freshwater prawns: A manual for the culture of the giant river prawn (*Macrobrachium rosenbergii*). Rome, Italy: FAO Fisheries Technical Paper. n. 428. 212p.

New, M. B., Wagner, C. V., Tidwell. J. H., D'Abramo, L. R., & Kutty M. N. (2010). Freshwater Prawns Biology and Farming (1st ed.). Oxford, UK: Wiley-Blackwell.

Pérez-Fuentes, J. A., Pérez-Rostro, C. I., & Hernández-Vergara, M. P. (2013). Pond-reared Malaysian prawn *Macrobrachium rosenbergii* with the biofloc system. Aquaculture, 400-401, 105-110. https://doi.org/10.1016/j.aquaculture.2013.02.028

Pérez-Ruestro, I. C., Pérez-Fuentes, A. J., & Hernández-Vergara, M. P. (2014). Biofloc, a Technical Alternative for Culturing Malaysian Prawn *Macrobrachium rosenbergii*. In Sustainable Aquaculture Techniques. InTech. https://doi.org/10.5772/57501 ScienceDirect (Online service), F. (1972). Aquaculture. AQUACULTURE -AMSTERDAM- (Vol. 453). Elsevier Pub. Co. Retrieved from https://www.worldcat.org/title/acute-toxicity-of-ammonia-to-various-life-stages-of-the-amazon-river-prawn-macrobrachium-amazonicum-heller-1862/oclc/5983612265&referer=brief_results

Samocha, T. M., Patnaik, S., Speed, M., Ali, A. M., Burger, J. M., Almeida, R. V., Ayub, Z., Harisanto, M., Horowitz, A., & Brock, D. L. (2007). Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. Aquacultural Engineering, 36 (2), 184-191. https://doi.org/10.1016/j.aquaeng.2006.10.004

Samocha, T. M., Prangnell, D. I., Hanson, T. R., Treece, G. D., Morris, T. C., Castro, L. F. & Staresinic. N. (2017). Design and Operation of Super Intensive. Biofloc-Dominated Systems for Indoor Production of the Pacific White Shrimp *Litopenaeus vannamei* – The Texas A&M AgriLife Research Experience. (161th) Baton Rouge. Louisiana. USA, 368. TX: The World Aquaculture Society

Santos, D. B., Arruda, M. F., Azevedo, D. L. O., & Pontes, C. S. (2017). Behavioral responses of freshwater prawn according to feeding management in mixed and monosex populations. Boletim do Instituto de Pesca, 43 (4), 569-577. https://doi.org/10.20950/1678-2305.2017v43n4p569

Seenivasan. C., Radhakrishnan, S., Muralisankar, T., & Bhavan, P. S. (2014). Effects of probiotics on survival, growth and digestive enzymes activities in freshwater prawn *Macrobrachium rosenbergii* (De Man 1879). Proceedings of the Zoological Society, 69 (1), 52-60. https://doi.org/10.1007_s12595-014-0123-6

Shilta, M. T., Chadha, N. K., Pandey, P. K., & Sawant, P. B. (2016). Effect of biofilm on water quality and growth of *Etroplus suratensis* (Bloch, 1790). Aquaculture International, 24 (2), 661–674. https://doi.org/10.1007/s10499-015-9956-x

Souza, R. L., Lima, E. C. R., Melo, F. P., Padilha, M. G. F., & Correia, E. S. (2019). The culture of Nile tilapia at different salinities using a biofloc system. Revista Ciência Agronômica, 50 (2), 267-275. https://doi.org/10.5935/1806-6690.20190031

Straus, D. L., Robinette, H. R., & Heinen, J. N. (1991). Toxicity of un-ionized ammonia and high pH to post-larval and juvenile freshwater shrimp *Macrobrachium rosenbergii*. World Aquaculture Society, 22 (2), 128-133. https://doi.org/10.1111/j.1749-7345.1991.tb00725.x

Suantika, G., Turendro, O.R., & Situmorang, M. L. (2017). Use of Nitrifying Bacteria for Promoting Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man) Nursery Phase in Indoor System. Journal of Fisheries & Livestock Production, 05 (02), 1–5. https://doi.org/10.4172/2332-2608.1000228

Thakur, D. P., & Lin, C. K. (2003). Water quality and nutrient budget in closed shrimp (*Penaeus monodon*) culture systems. Aquacultural Egineneering, 27, 157 – 176. https://doi.org/10.1016/S0144-8609(02)00055-9

Timmons, M. B., & Ebeling, J. M. (2007). Water Quality. In: Recirculating aquaculture. (ed. by Timmons, M. B.; Ebeling, J. M.), pp. 39-78. Cayuga Aqua Ventures, Ithaca, New Yorque, USA.

Tucker, C. S., & Hargreaves, J. A. (2008). Environmental Best Management Practices for Aquaculture. (1st ed.). Ames, Iowa: Wiley-Blackwell.

Valenti, W.C., New, M. B., Salin, K. R., & Ye, J. (2010). Grow-out systems-monoculture. In: Freshwater prawns: biology and farming. (ed. by New, M. B., Valenti, W. C., Tidwell, J. H., D'Abramo, L. R., & Kutty, M. N.), pp. 154-179. Wiley-Blackwell, Oxford, England.

Xu, W. J., & Pan, L. Q. (2012). Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. Aquaculture, 356–357, 147–152. https://doi.org/10.1016/J.AQUACULTURE.2012.05.022

Xu, W. J., & Pan, L. Q. (2014). Dietary protein level and C/N ratio manipulation in zero-exchange culture of *Litopenaeus vannamei*: Evaluation of inorganic nitrogen control, biofloc composition and shrimp performance. Aquaculture Research, 45 (11), 1842-1851. https://doi.org/10.1111/are.12126

Xu, W. J., Pan, L. Q., Sun, X. H., & Huang, J. (2013). Effects of bioflocs on water quality, and survival, growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zero-water exchange culture tanks. Aquaculture Research, 44 (7), 1093-1102. https://doi.org/10.1111/j.1365-2109.2012.03115.x

Zhang, J., Chen, L., Dong, H., Duan, Y., Li, Z., Wen, G. & Xie, J. (2016). Artificial substrates in zero-water-exchange culture system regulate the rearing performance of Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) under the winter indoor condition. Aquaculture Research, 47 (1), 91-100. https://doi.org/10.1111/are.12473

TABLE 1- Actual and nominal concentrations ± standard deviance in exposure ammonia test during 96 hours of juveniles of *M. rosenbergii* in biofloc (BFT) and Water Exchange System (WES)

System	**Nominal concentration**	**Actual concentration**
BFT	0	0.76 ± 0.26
	5	5.81 ± 0.41
	10	10.73 ± 0.51
	20	21.21 ± 2.62
	40	40.28 ± 1.69
WES	0	0.19 ± 0.18
	5	5.53 ± 0.47
	10	11.05 ± 0.87
	20	22.13 ± 0.77
	40	40.69 ± 0.59

TABLE 2- Water quality parameters of *M. rosenbergii* juveniles culture in biofloc and water exchange system during a 58-day experimental period.

Variables	**Treatments**[1]	
	WES	**BFT**
Temperature (ºC)	27.4±1.0	27.3±0.9
Dissolved oxygen (mg L^{-1})	6.31±0.68	6.43±0.65
pH	7.64±0.38[a]	8.20±0.22[b]
Alkalinity (mg $CaCO_3$ L^{-1})	42.58±21.02[a]	93.36±25.25[b]
TAN (mg L^{-1})	0.23±0.18[a]	0.63±0.16[b]
N-NO_2(mg L^{-1})	0.13±0.19	0.12±0.19
NO_3 (mg L^{-1})	3.64±1.08[a]	20.70±7.58[b]
PO_4^{-3}(mg L^{-1})	98.96±1.08[a]	185.96±75.8[b]
SS (mL L^{-1})	-	5.05±3.01
TSS (mg L^{-1})	10.3±5.6[a]	129.30±47.8[b]

[1]The data correspond to the mean ± standard deviation; Different letters on the same line indicate a significant difference ($\alpha < 0.05$). TAN - Total ammonia nitrogen; N-NO_2 –Nitrite-nitrogen; NO_3–Nitrate, PO_4^{-3} – orthophosphate; SS – settable solids; TSS – total suspended solids.

TABLE 3- Performance of zootechnical parameters of *M. rosenbergii* juveniles culture in biofloc (BFT) and water exchange system (WES) during a 58-day experimental period.

Variables	**Treatments**[1]	
	WES	**BFT**
Final weight (g)	0.99±0.09[a]	1.31±0.07[b]
Gain weight (g)	0.84±0.09[a]	1.16±0.07[b]
SGR (%day^{-1})	3.25±0.16[a]	3.74±0.09[b]
Final biomass (g)	97.13±8.55[a]	137.74±26.59[b]
Survival (%)	65.65±7.97	69.83±12.23
Productive (kg m^{-3})	0.24±0.02[a]	0.34±0.07[b]
FCR	3.1±0.47[a]	2.50±0.71[b]

[1]The data correspond to the mean of four replicates ± standard deviation. Different letters on the same line indicate a significant difference ($\alpha < 0.05$). SGR – Specific growth rate; FCR – Feed conversion ratio.

TABLE 4- Survival rate ± standard deviation of *Macrobrachium rosenbergii* juveniles on ammonia stress test exposure for 96 hours.

Culture systems	**Nominal concentration TAN (mg/L)**	**Survival**				
		0h	**24h**	**48h**	**72h**	**96h**
Water Exchange System	0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	93.33 ± 11.6	93.33 ± 11.6
	5	100.00 ± 0.0	93.33 ± 11.6	93.33 ±11.6	86.67 ± 11.6	86.67 ± 11.6
	10	100.00 ± 0.0	86.67 ± 11.6	86.67 ± 11.6	86.67 ± 11.6	80.00 ± 20.0
	20	100.00 ± 0.0	93.33 ±11.6	93.33 ±11.6	93.33 ±11.6	93.33 ±11.6
	40	100.00 ± 0.0	100.00 ± 0.0	86.67 ± 23.1	60.00 ±20.0	20.00 ± 20.0
Biofloc	0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0
	5	100.00 ± 0.0	93.33 ± 11.6	93.33 ± 11.6	80.00 ± 0.0	80.00 ± 0.0
	10	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0
	20	100.00 ± 0.0	93.33 ± 11.6	86.67 ± 11.6	80.00 ±20.0	80.00 ± 20.0
	40	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	93.33 ±11.6	86.67 ± 23.1
Culture systems effect		NS	NS	NS	NS	*
WES		100.00 ± 0.0	94.66±9.1	92.00±12.6	81.33±18.3	74.67±31.6[a]
BFT		100.00 ± 0.0	97.33±7.0	96.00±8.3	90.67±12.8	89.33±12.8[b]
Concentrations Effect		NS	NS	NS	NS	*
	0	100.00 ± 0.0	100.00 ± 0.0	100.00 ± 0.0	96.67±8.2	96.67±8.2[a]
	5	100.00 ± 0.0	93.33±10.3	93.33±10.3	83.33±8.2	83.33±8.2[a]
	10	100.00 ± 0.0	93.33±10.3	93.33±10.3	90.00±16.7	90.00±16.7[a]
	20	100.00 ± 0.0	93.33±10.3	90.00±11.0	86.67±16.3	86.67±16.3[a]
	40	100.00 ± 0.0	100.00 ± 0.0	93.33±16.3	73.33±23.3	53.33±42.7[b]
Iteration System x Concentration			NS	NS	NS	*

* Significant at the 0.05 level

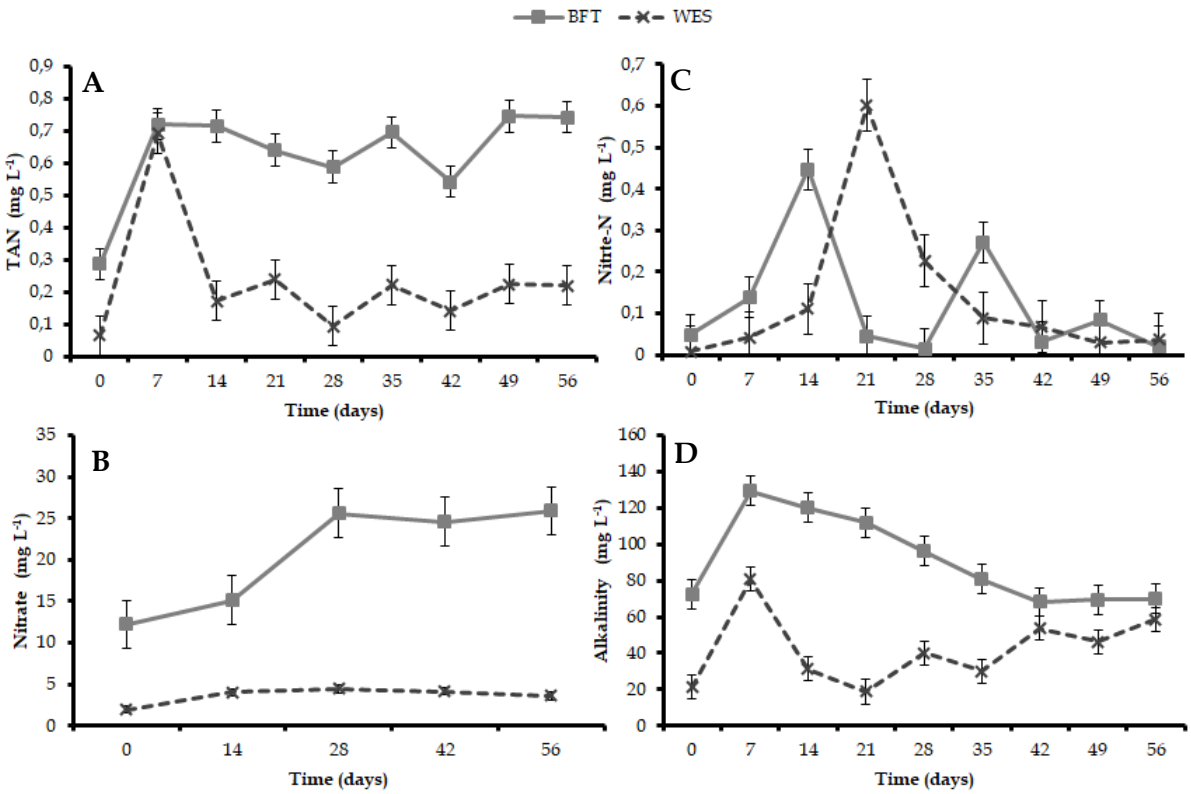

FIGURE 1 - Variation in nitrogen compounds (A- total ammonia nitrogen, B- nitrite nitrogen, C- nitrate) and alkalinity (D) over 58 days of *M. rosenbergii* in nursery phase in different systems of intensive culture.

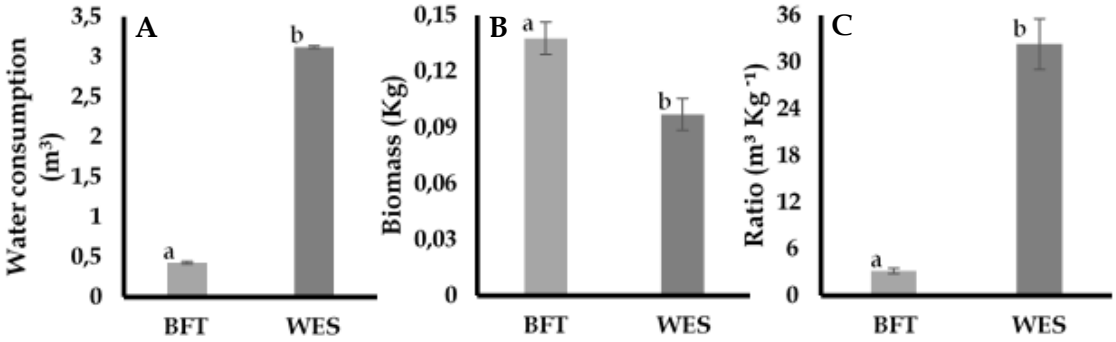

FIGURE 2 - Ratio of water consumption to biomass production for 58 days of *M. rosenbergii* culture in biofloc (BFT) and water exchange system (WES)

2- Considerações finais

O sistema de bioflocos influenciou positivamente no peso final, ganho de peso, taxa de crescimento especifico, produtividade de *M. rosenbergii* na fase de berçário. Os juvenis advindos deste tratamento submetidos à um teste de toxicidade aguda da amônia, foram mais resistentes quando comparados àqueles criados em sistema de renovação de água. Os animais suportam concentrações de até 40 mg L^{-1} para o sistema de bioflocos sem afetar na sobrevivência dos camarões. Além disso, o sistema de bioflocos reduz o consumo de água na produção de *M. rosenbergii* em até 10 vezes.

Fazem-se necessários estudos complementares que testem as concentrações letais medias (LC_{50}) para este sistema de cultivo, assim como o efeito da exposição prolongada dos animais a amônia (efeito crônico), associado a análises histológicas e de variáveis de bem-estar animal, como contagem total de hemócitos, neste sistema de cultivo.

3- Referências

AGUILERA-RIVERA, D.; Escalante-Herrera, K.; Gaxiola, G.; PRIETO-DAVÓ, A.; RODRÍGUEZ-FUENTES, G.; GUERRA-CASTRO, E.; HERNÁNDEZ-LÓPEZ, J.; CHÁVEZ-SÁNCHEZ, M. C.; RODRÍGUEZ-CANUL, R. Immune response of the Pacific white shrimp, *Litopenaeus vannamei*, previously reared in biofloc and after an infection assay with Vibrio *harveyi*. **World Aquaculture Society**, v.50, p.119-136, 2018.

ALABASTER, J. S.; LOYD, R. S. **Water Quality Criteria for Freshwater Fish**. Oxford: Elsevier Ltd. 1982, p. 343.

ASADUZZAMAN, M.; RAHMAN, S. M. S.; AZIM, M. E.; ISLAM, M. A.; WAHAB, M. A.; VERDEGEM, M. C. J.; VERRETH, J. A. J. Effects of C/N ratio and substrate addition on natural food communities in freshwater prawn monoculture ponds. **Aquaculture**, v. 306, p. 127- 136, 2010.

AVNIMELECH, Y. **Biofloc Thecnology- A practical guidebook**. Lousiana: The Word Aquaculture Society. 2009, p. 182.

AZIM, M. E.; LITTLE, D. C. The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). **Aquaculture**, v.283, p. 29-35, 2008.

BALLESTER, E. L. C.; MARZAROTTO, S. A.; CASTRO, C. S.; FROZZA, A.; PASTORE, I.; ABREU, P.C. Productive performance of juvenile freshwater prawns *Macrobrachium rosenbergii* in biofloc system. **Aquaculture Research**, v. 48, p. 4748-4755, 2017.

BUIKEMA, A. L.; NIEDERLEHNER, B. R.; CAIRNS, J. Biological monitoring part IV-Toxicity testing. Water Research, v.16 (3), p. 239-262,1982.

COLT, J.E.; ARMSTRONG, D. A. Nitrogen toxicity to fish, crustaceans and molluscs. In: ALLEN, L. J.; EC KINNEY. Proceedings of the Bioengeneering Symposium for Fish Culture Section, Bethesda, Maryland, Americam Fisheries Society, 1981, p.34-47.

EBELING, J. M; TIMMONS, M. B; BISOGNI, J. J. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic control of ammonia-nitrogen in aquaculture production systems. **Aquaculture**, v. 257, p. 346–358, 2006.

EKASARI, J.; ANGELA, D.; WALUYO, S. H.; BACHTIAR, T.; SURAWIDJAJA, E. H.; BOSSIER, P.; DE SCHRYVER, P.. The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. **Aquaculture**, v. 426-427, p.105-111, 2014.

EMERENCIANO, M.; GAXIOLA, G.; CUZO, G. Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry. In: MATOVIC, M. D. Biomass Now - Cultivation and Utilization, xx, Ecological Engineering, 2013, p. 301-328.

FAO. (2018a). Fishery information: Data and Statistics Service. Aquaculture Production Statistics. FAO Fisheries Circular.

FAO. (2018b). Fishery and Aquaculture Statistics. Global aquaculture production 1950-2016 (FishstatJ). In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 2018. www.fao.org/fishery/statistics/software/fishstatj/en

FIGUEROA-LUCERO, G.; HERNADEZ-RUBIO, M. C.; GUTIEREZ- LADRÓN DE GUEVARA, M. J. Acute toxicity of ammonia on *Macribrachium tenellum* (SMITH) larvae. **Revista internacional de contaminación ambiental,** v. 28 (2), p. 145-150, 2012.

FRÓES, C.; FÓES, G.; KRUMMENAUER, D.; POERSCH, L. H.; WASIELESKY JR., W.; Densidade de estocagem na engorda de camarão-branco cultivado em sistema de bioflocos. **Pesquisa Agropecuária Brasileira**, v.48 (8), p. 878-884, 2013.

GOMES JR, R. S.; LIMA, J. P. V.; CAVALLI, R. O.; CORREIA, E. C. Acute toxicity of ammonia and nitrite to painted river prawn, *Macrobrachium carcinus,* Larvae. **Journal of the World Aquaculture Society,** v. 47 (2), p. 239-247, 2016.

GUPTA, A.; SINGH, H. S.; KAUR, G. S. Growth and carcass composition of giant freshwater prawn, *Macrobrachium rosenbergii* (De Man), fed different isonitrogenous and isocaloric diets. **Aquaculture Research**, v. 38, p. 1355-1363, 2007.

KIM, S. K.; PANG, Z.; SEO, H.-C.; CHO, Y. R.; SAMOCHA, T.; JANG, I. K. Effect of bioflocs on growth and immune activity of Pacific white shrimp, *Litopenaeus vannamei* postlarvae. **Aquaculture Research,** v. 45, p. 362–371, 2014.

KIMPARA, J.M.; MORAES-VALENTI, P.; QUEIROZ, J. F.; NEW, M. B. Effects of intensification of the Amazon River prawn, *Macrobrachium amazonicum*, grow-out on effluent quality. **Journal of the World Aquaculture Society,** v. 44, p. 210-219, 2013.

LOBÃO, V. L. **Camarão-da-malásia: cultivo**. Brasilia: EMBRAPA – SPI,1996. 102 p.

MARTÍNEZ-PORCHAS, M.; MARTINEZ-CÓRDOVA, L. R. World aquaculture: environmental impacts and troubleshooting alternatives. **The Scientific World Journal**, v. 389623, p. 1–9, 2012.

MEGAHED, M. E. The Effect of microbial biofloc on water quality, survival and growth of the green tiger shrimp (*Penaeus semisulcatus*) fed with different crude protein levels. **Journal of the Arabian Aquaculture Society**, v. 5, p. 1-24, 2010.

MELO, F. P.; FERREIRA, M. G. P.; BRAGA, I. F. M.; CORREIA, E. S. Toxicidade do nitrito para o camarão *Litopenaeus vannamei* cultivado em sistemas de água clara e bioflocos. **Boletim do Instituto de Pesca**, v. 42 (4), p.855-865, 2016.

MOHANTHY, R. K. Impact of phased haversting population structure, feed intake pattern and growth performance of *Macrobrachium rosenbergii* De Man (giant freshwater prawn) in polyculture with carps in concurrent rice-fish culture. **Aquaculture International**, v. 18, p. 523-537, 2010.

MOULLAC, G.; HAFFNER, P. Environmental factors affecting immune responses in Crustacea. **Aquaculture,** v. 191, p.121-131, 2000.

NAYLOR, R. L; WILLIAMS, S. L.; STRONG, D. R. Aquaculture – A gateway for exotic species. **Science**, v. 294 (5547), p. 1655–1656, 2001.

NEGRINI, C.; CASTRO, C. S.; GUIMARÃES, A. T. B.; FROZZA, A.; KRACIZY, R. O.; BALLESTER, E. L. C. Stocking density for freshwater prawn *Macrobrachium rosenbergii* (Decapoda, Palaemonidae) in biofloc systems. **Latin American Journal of Aquatic Research,** v. 45 (5), p. 891-899, 2017.

NEW, M. B. **Farming freshwater prawns: A manual for the culture of the giant river prawn (*Macrobrachium rosenbergii*)**. Roma: FAO Fisheries Technical Paper, 2002. 212 p.

OSTRENSKY, A.; WASIELESKY, W. Acute toxicity of ammonia to various life stages of the São Paulo shrimp, *Penaeus paulensis* Pérez- Farfante, **Aquaculture**, v. 132, p.339-347, 1995.

PÉREZ-FUENTES, J. A.; PÉREZ-ROSTRO, C. I.; HERNANDEZ-VERGARA, M. P. Pond-reared Malaysian prawn *Macrobrachium rosenbergii* with the biofloc system. **Aquiculture,** v. 400-401, p. 105-110, 2013.

PÉREZ-ROSTRO, I. C.; PÉREZ-FUENTES, A. J.; HERNÁNDEZ-VERGARA, M. P. Biofloc, a Technical Alternative for Culturing Malaysian Prawn *Macrobrachium rosenbergii*. In: HERNANDEZ-VERGARA, M.; PEREZ-ROSTRO, C. Sustainable Aquaculture Techniques. Boca del Rio: InTech, 2014. p. 87-104.

REGNAULT, M. Nitrogen excretion in marine and fresh-water crustacea. **Biological Review,** v. 62, p. 1-24, 1987.

SÁ, M. V. C. **Limminocultura: limnologia para aquicultura.** Fortaleza, Brasil: Editora da Universidade Federal do Ceará, 2012. 218 p.

SAMOCHA, T. M.; PRANGNELL, D. I.; HANSON, T. R.; TREECE, G. D.; MORRIS, T. C.; CASTRO, L. F.; STARESINIC, N. **Design and Operation of Super Intensive, Biofloc-Dominated Systems for Indoor Production of the Pacific White Shrimp, *Litopenaeus vannamei*– The Texas A&M AgriLife Research Experience**. Baton Rouge, Louisiana: The World Aquaculture Society, 2017. 368p.

SANTOS, B. B.; ARRUDA, M. F.; AZEVEDO, D. L. O.; PONTES, C. S. Behavioral responses of freshwater prawn according to feeding management in mixed and monosex populations. **Boletim do Instituto de Pesca**, v.43 (4), p. 569-577, 2017.

STRAUS, D. L.; ROBINETTE, H. R.; HEINEN, J. N. Toxicity of un-ionized ammonia and high pH to post-larval and juvenile freshwater shrimp *Macrobrachium rosenbergii*. **World Aquaculture Society**, v. 22 (2), p. 128-133, 1991.

TIMMONS, M. B.; EBELING, J. M. Water Quality. In: TIMMONS, M. B.; EBELING, J. M. Recirculating aquaculture. Ithaca, Nova Iorque: Cayuga Aqua Ventures, 2007. p. 39-78.

TOMASSO, J.R.; GAMICHAEL, G.J. Acute toxicity of ammonia, nitrite and nitrate to the Guadalupe bass, *Micropterus treculi*. **Bulletin of Environmental Contamination and Toxicology**, v. 36(1), p. 866-870, 1986.

VALENTI, W. C.; NEW, M. B.; SALIN, K. R.; YE, J. Grow-out systems-monoculture. In: M. B. NEW; W.C VALENTI; J.H. TIDWELL; L.R. D'ABRAMO; M.N. KUTTY. Freshwater prawns: biology and farming. Oxford: Wiley-Blackwell, 2010. p. 154-179.

VAN LOOSDRECHT, M. C. M.; JETTEM, M. S. M. Microbiological conversions in nitrogen removal. **Water Science Technology**, v.38 (1), p. 1-7, 1998.

XU, W. J.; PAN, L. Q. Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. **Aquaculture**, v. 356–357, p. 147–152, 2012.

XU, W. J.; Pan, L. Q. Dietary protein level and C/N ratio manipulation in zero-exchange culture of *Litopenaeus vannamei*: Evaluation of inorganic nitrogen control, biofloc composition and shrimp performance. **Aquaculture Research**, v. 45 (11), p. 1842-1851, 2013.

XU, W. J.; PAN, L. Q.; SUN, X. H.; HUANG, J. Effects of bioflocs on water quality, and survival, growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zero-water exchange culture tanks. Aquaculture Research, v. 44 (7), p. 1093-1102, 2013.

ANEXO

Author Guidelines

Sections

Submission

Aims and Scope

Manuscript Categories and Requirements

Preparing Your Submission

Editorial Policies and Ethical Considerations

Author Licensing

Publication Process After Acceptance

Post Publication

Editorial Office Contact Details

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