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Descrição da qualidade microbiológica no cultivo do camarão *Litopenaeus vannamei* em sistemas de bioflocos e tradicional

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Qualidade microbiológica no cultivo do camarão *Litopenaeus vannamei* em sistemas de bioflocos e tradicional

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RESUMO

Problemas de biossegurança em viveiros de cultivo convencional demandam maior controle da entrada de microrganismos potencialmente patógenos. Dentre as novas tecnologias o sistema "BFT" (Bio-Floc Technology) destaca-se por permitir o cultivo com altas densidades de estocagem e reduzida troca de água. Contudo, é necessário avaliar se a adição de nutrientes para estimular o crescimento de bactérias para formação dos bioflocos possa também estimular o crescimento de bactérias indesejáveis e assim comprometer o produto final. O presente estudo teve como objetivo descrever a carga bacteriana de interesse sanitário no cultivo de *Litopenaeus vannamei* em larga escala, em sistemas com biofloco "BFT" (SB) e convencional (SC), os quais foram realizados no litoral sul do estado de Pernambuco. Para o SB foram utilizados dois tanques de 200m², estocados com 375 camarões/m². Em relação ao SC, foram utilizados dois viveiros (2,85 ha cada), estocados com 12 camarões/m^2. As coletas foram realizadas a cada 15 dias aproximadamente, em três pontos do viveiro, onde foram retiradas amostras tanto de água como de camarão. As variáveis da água foram acompanhadas diariamente e, não apresentaram diferença significativa entre os sistemas SB e SC. Todos os parâmetros da água medidos estavam dentro dos níveis adequados para a espécie em relação a oxigênio dissolvido, pH, temperatura e salinidade. Foi analisado a presença de Staphylococcus coagulase-positiva, Salmonella, Coliformes Totais (CT), Termotolerantes (CTT) e Víbrios, e se estavam em consonância com a legislação vigente para camarão *in natura*. Não observou-se a presença de Salmonella nem *Staphylococcus* coagulase positiva em nenhum dos sistemas, estando de acordo com a legislação. Embora não haja padrões para víbrios, as quantidades encontradas nos camarões foram baixas se comparadas a outros trabalhos; bem como não houve sintomas de vibrioses nos animais. Em relação aos coliformes, os valores encontrados também foram baixos e em apenas oito das 87 coletas apresentaram valores uma pouco mais elevados, como por exemplo 240 NMP/g na 8° semana do SC1. Apesar da variação da carga de bactérias ao longo do cultivo, o produto final (dia da despesca), estava de acordo com a legislação da Agência Nacional de Vigilância Sanitária (ANVISA), sendo assim adequados ao consumo. De acordo com os resultados obtidos, o sistema BFT, empregado em escala comercial no nordeste do Brasil, demonstrou

manter a sanidade do produto final, sendo uma boa alternativa para o aumento da produtividade e controle de efluentes, ratificando sua sustentabilidade.

Palavras-chave: Cultivo de camarão, Staphylococcus, Salmonella e Vibrio

ABSTRACT

Biosecurity problems in conventional farming require greater control of potentially pathogenic microorganism entry. Among the new technologies the "BFT" system (Bio-floc Technology) stands out for allowing cultivation with high stocking densities and reduced water exchange. However, it is necessary to evaluate whether the addition of nutrients to stimulate the growth of bacteria for biofloc formation could also stimulate the growth of undesirable bacteria and thereby compromise the final product. The present study aimed to describe the bacterial load of sanitary interest in the *Litopenaeus vannamei* large scale farming systems with biofloc formation (BS) and conventional system (CS), which was performed on the southern coast of the state of Pernambuco. For the BS two tanks of 200m², stocked with 375 shrimp/m² were used. Regarding the CS, two ponds (2.85 ha each), stocked with 12 camarões/m^2 were used. Samples of wather and shrimps were collected every 15 days at three points around the pond. The water parameters were monitored daily, and no differences were found between the BS and CS. All measured water parameters were within appropriate levels for the species regarding to dissolved oxygen, pH, temperature and salinity. In the present study was investigated the presence of coagulase-positive Staphylococcus, Salmonella, Total Coliforms (TC), thermotolerant (TTC) and Vibrios. It was also evaluated whether it was in line with current legislation (RDC N° 12) for fresh shrimp. There were no observations of Salmonella and Staphylococcus in samples of both systems, which is consistent with the law. Although there are no standards for Vibrios, the amounts found were lower thanin other studies and there were no symptoms of Vibrio Infections in animals. In relation to coliforms, the values were also low and only eigth of the 87 samples showed a slightly higher value, such as 240 MPN / g in the 8th week of SC1. Despite of the variation in bacterial load during farming, in the final product (the last day of culture), was in accordance with the legislation of the Brazilian Health Surveillance Agency (ANVISA), and soit is suitable for consumption. According to the results, the BFT system, employed on a commercial scale in northeastern Brazil, demonstrated maintain sanity of the final product and is a good alternative for increasing productivity and effluent control, confirming its sustainability.

Keywords: shrimp farming, coagulase-positive Staphylococcus, Salmonella and Vibrio

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PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQÜICULTURA

DESCRIÇÃO DA QUALIDADE MICROBIOLÓGICA NO CULTIVO DO CAMARÃO *Litopenaeus vannamei* EM SISTEMAS DE BIOFLOCOS E TRADICIONAL.

Camila Mayara Santos Brito

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1-INTRODUÇÃO

O rápido crescimento da carcinicultura mundial veio acompanhado por impactos ambientais, visto que para cultivar camarões em viveiros de forma convencional utiliza-se um grande volume de água em renovações (10 a 15% diariamente). Esta água em muitos casos acaba sendo despejada no ambiente sem tratamento prévio. Além disso, o uso de grandes áreas para implantação dos viveiros (uma vez que estes são trabalhados em baixas densidades) acabam causando o desequilíbrio dos ecossistemas próximos as áreas de cultivo, gerando críticas de algumas organizações não governamentais (HOROWITZ e HOROWITZ, 2001).

A contaminação da água do ambiente próximo as fazendas também pode resultar em prejuízos aos produtores, uma vez que essas águas contaminadas acabam voltando às fazendas de cultivo, chegando a provocar até contaminações cruzadas entre fazendas vizinhas, auxiliando desta forma na proliferação de algumas doenças que podem resultar em severas diminuições de produção.

Na tentativa de reduzir a entrada de organismos vetores nos viveiros, são necessárias cada vez mais estratégias contra a disseminação de organismos potencialmente patógenos (HOROWITZ e HOROWITZ, 2001), exigindo a aplicação de diversas tecnologias de cultivo para impedir a introdução de água potencialmente contaminada nas fazendas de cultivo (FAST e MENASVETA, 2000).

Os cultivos de camarões em sistemas superintensivos com bioflocos, podem ser considerados uma forma de diminuir a entrada de microrganismos nos cultivos, uma vez que este utiliza pouca ou nenhuma renovação de água, e vêm sendo apresentado como uma forma de aquicultura responsável e ambientalmente correta, reduzindo significativamente a poluição dos ambientes próximos aos cultivos. Os bioflocos formados nestes sistemas pode ainda incrementar a dieta dos animais através do consumo dos agregados bacterianos que se formam nos viveiros, reduzindo assim os gastos com a alimentação dos animais (McINTOSH et al., 2000; BRATVOLD e BROWDY, 2001). Por outro lado, a grande quantidade de nutrientes utilizada para estimular a comunidade bacteriana heterotrófica podem também estimular outros grupos de bactéria, através da oferta de um ambiente favorável ao desenvolvimento destas, gerando questionamentos sobre a qualidade sanitária do produto final oriundo deste sistema.

Desta forma, é importante que a comunidade microbiana seja formada por organismos naturais de ecossistemas marinhos e que a comunidade contaminante esteja em concentrações aceitáveis pela Agência Nacional de Vigilância Sanitária (Brasil, 2001) que regulariza a legislação dos alimentos, para garantir a boa qualidade e segurança do produto fornecido.

Coliformes, Salmonelas e Estafilococos fazem parte das bactérias que são introduzidas nos sistemas aquáticos por meio de contaminações e que, em quantidades elevadas, levam a inviabilidade do produto para consumo. Já as bactérias do gênero Vibrio pertencem aos ecossistemas marinhos, mas em quantidades acentuadas podem se tornar patógenos aos camarões e a humanos.

Desta forma, objetivou-se com o presente trabalho analisar a presença de enterobactérias contaminantes e do gênero Vibrio em amostras provenientes de água e camarão de dois sistemas: Convencional (SC) e Biofloco (SB).

2-REVISÃO DE LITERATURA

A crescente diferença entre a quantidade de pescado capturado e a demanda de consumo fazem da aquicultura uma excelente alternativa para o fornecimento de alimento. Dados da FAO (2012) demonstram que a produção de pescado mundial girou em torno de 128,3 milhões de toneladas para o consumo humano e a carcinicultura representou 15% deste total. Rocha (2009) concluíram que a produção do camarão por extrativismo teria atingido seu limite de exploração sustentável no mundo. Por essa razão, o fornecimento desse produto pela carcinicultura vem sendo considerado importante, visto que a demanda desse tipo de pescado é crescente. Dentre as espécies aquáticas mais produzidas mundialmente, o cultivo do camarão da espécie *Litopenaeus vannamei* gerou maior renda em relação a outros cultivos como o de salmão e carpa. A produção desta espécie vem sendo considerada a mais importante, correspondendo a 15,4% da renda total gerada pela produção de pescado (FAO, 2009). Para Bezerra et al. (2007), este aumento da quantidade de camarão produzido está relacionado a alguns fatores como: condições climáticas, hidrobiológicas e topográficas e, principalmente, à viabilidade técnica, incluindo os desenvolvimentos com tecnologias relacionadas à produção de pós-larvas, manejo e processamento.

Além da importância econômica do cultivo da espécie exótica *L. vannamei*, conhecido como "Camarão Branco do Pacífico" ou "Camarão Cinza" introduzido no Brasil no ano de 1980, que também demonstrou alta adaptabilidade às condições climáticas brasileiras, devido à sua rusticidade, rapidez no crescimento, ampla faixa de tolerância à salinidade, e sua capacidade em aproveitar dietas com níveis protéicos variando de 20% a 40%, melhorando assim as chances de sucesso da espécie para fins de cultivo (COSTA, 2004). Sua produção no Brasil, teve início baseando-se em tecnologias importadas, porém nos últimos anos vem se desenvolvendo de forma acelerada e com base em tecnologias próprias (MOLES e BUNGE, 2003).

O rápido crescimento da carcinicultura veio acompanhado por impactos ambientais, entre eles destacam-se o desmatamento das áreas de mangue (aumento da erosão, da temperatura da evaporação, e perda da biodiversidade), e a contaminação dos corpos hídricos pelo aumento de carga orgânica, substâncias químicas e geração de sedimentos, que resulta em aumento da turbidez, eutrofização e redução da biodiversidade causando desequilíbrio ambiental (CHO e COWEY, 1991; ORMOND,

2004). Uma vez que para produzir um quilo de camarão em viveiros de engorda, utiliza-se uma quantidade que varia de 39 a 199 toneladas de água (HOPKINS e VILLALÓN, 1992). A água despejada, normalmente rica em nutrientes, é quase sempre devolvida ao ambiente natural sem tratamento em bacias de sedimentações, causando desequilíbrio nos ecossistemas adjacentes às áreas de cultivo (HOPKINS et al, 1995; BAIRD, et al,1996; GAA, 2003).

Como o volume de água empregado nos cultivos com renovação é muito grande, não há como realizar o tratamento desta, e essas águas contaminadas acabam voltando às fazendas de cultivo e algumas vezes causam até contaminações cruzadas entre fazendas vizinhas. Segundo Samocha et al. (2007), baixa de produção relacionadas a doenças nos viveiros em fazendas de camarão tem como resultado severas diminuições de produção em todo o mundo.

O cultivo de camarões marinhos nas Américas pode ser dividido em três momentos, considerando as estratégias de sistemas utilizadas (SAMPAIO et al., 2010). O primeiro momento, foi destacado por grandes áreas de cultivo em viveiros com baixa densidade de estocagem. O segundo momento, foi marcado também pelo cultivo em viveiros, contudo com um aporte de tecnologias maior, que possibilitaram o aumento de produtividade. No terceiro momento, correspondente à atualidade, caracteriza-se por doenças como a Síndrome de Taura (TSV), Mancha Branca (WSSV), Mionecrose Infecciosa (IMNV) entre outras.

Com o surgimento cada vez mais frequente de microrganismos potencialmente patógenos, o controle do volume destes vetores para dentro dos viveiros passou a ser priorizado nas estratégias contra a disseminação de doenças (HOROWITZ e HOROWITZ, 2001). Sendo este o motivo para o desenvolvimento e para a aplicação de diversas tecnologias de cultivo, que reduzem ou eliminam a introdução de água potencialmente contaminada nas fazendas de cultivo (FAST e MANASVETA, 2000). Da mesma forma, problemas relacionados com a propagação de doenças e desequilíbrios nos ecossistemas ambientais como consequências pelas decorrentes introduções de águas oriundas de cultivos vêm gerando críticas de algumas organizações não governamentais (HOROWITZ e HOROWITZ, 2001).

Os cultivos de camarões em sistemas superintensivos com bioflocos, utiliza pouca ou nenhuma renovação de água, e vêm sendo apresentado como uma forma de aquicultura responsável e ambientalmente correta, uma vez que este tipo de sistema

reduz significativamente a poluição dos ambientes próximos aos cultivos, podendo-se reduzir também os riscos de introdução e disseminação de doenças. Ainda é possível, incrementar a dieta dos animais através do consumo dos agregados bacterianos que se formam nos viveiros, reduzindo assim os gastos com a alimentação dos animais (MCINTOSH et al., 2000; BRATVOLD e BROWDY, 2001).

A prática da fertilização orgânica e inorgânica tem sido utilizada como uma importante ferramenta no cultivo de organismos aquáticos, adicionando-se nutrientes à água a fim de estimular a proliferação do fitoplâncton, da comunidade bentônica e a formação de agregados microbianos, aumentando a produtividade dos viveiros e incrementando o crescimento dos camarões (SCHROEDER et al., 1990; BOYD, 2001; HARI et al., 2004; WASIELESKY et al., 2006b; SILVA et al., 2010). Por outro lado, este estimulo pode acabar favorecendo grupos de bactérias indesejadas, desta maneira é importante que a comunidade microbiana seja formada por organismos naturais de ecossistemas marinhos e que a comunidade contaminante esteja em concentrações aceitáveis pela Agência Nacional de Vigilância Sanitária (BRASIL, 2001) que regulariza a legislação dos alimentos para garantir a boa qualidade e segurança do produto fornecido.

Coliformes, Salmonelas e Estafilococos fazem parte dos gêneros de bactérias que são introduzidas nos sistemas por meio de contaminações e que, em quantidades elevadas, levam a inviabilidade do produto. Já as bactérias do gênero Vibrio pertencem aos ecossistemas marinhos, mas podem se tornar patógenos aos camarões e a humanos.

O grupo dos coliformes totais incluem as bactérias com formato de bastonetes, capazes de fermentar lactose e produzir gás a 35ºC. Para os coliformes termotolerantes a definição é a mesma, porém a denominação termotolerante é utilizada para descrever coliformes que fermentam a lactose com produção de gás a 44,5ºC. *Escherichia coli* e algumas cepas de Klebsiellae Enterobacter apresentam esta característica de termotolerância, porém, as duas últimas podem apresentar origem não fecal, como vegetais e solo, ou seja, somente a *E. coli* tem como habitat primário o intestino humano e de animais, podendo ser muito mais significativa a quantificação direta desta do que a presença de coliformes totais como bioindicador de contaminação (DOYLE, 1996; GUIDELINES, 2014), A presença dos Coliformes em animais aquáticos, segundo

EPAGRI (1994), ocorre em todo mundo e é ocasionado pelos despejos de esgotos e rios contaminados no mar.

O *Staphylococcus aureus* é uma bactéria esférica, do grupo dos cocos Gram positivos, que se dividem em mais de um plano, formando aglomerados de células que lembram um cacho de uvas. São anaeróbicos facultativos, catalase e coagulase positivo, sendo este último um critério de diferenciação para o *Staphylococcus aureus* (FDA/CFSAN, 2005). A doença transmitida pela ingestão do alimento contaminado por toxinas, que são produzidas na multiplicação das células é considerada de risco III, que inclui doenças "de perigo moderado, usualmente de curta duração e sem ameaça de morte ou sequelas, com sintomas auto limitados, mas que causam severo desconforto" (ICMSF, 2002).Este microrganismo é inoculado nos alimentos através da contaminação no mal processamento dos alimentos.

A *Salmonella sp.* é um gênero da família Enterobacteriaceae, ao qual pertencem bactérias em formato de bastonetes. As bactérias deste gênero são os principais agentes de doenças de origem alimentar no mundo (WHO, 2005), A Food and Drug Administration relata uma estimativa de dois a quatro milhões de casos por ano (FDA/CFSAN, 2005). De acordo com Silva et al. (2007) as cepas mais frequentes envolvidas nas doenças humanas são as de *Salmonella entérica,* que respondem por 99% das salmoneloses.

As bactérias do gênero Vibrio possuem como habitat primário ecossistemas aquáticos. São os microrganismos pertencentes a este grupo considerados um dos mais importantes na aquicultura, pois infectam diversos organismos marinhos, como crustáceos, peixes e moluscos. Análises realizadas em camarões após sua despesca (*innatura*) já indicaram a presença de diversas bactérias do gênero Vibrio, entre elas *V. parahaemolyticus, V. dansela, V. alginolyticus, V. fluvialis* (HOSSEINI e CHERAGULLI, 2003) e *V. vulnificus* (NASCIMENTO et al., 2001). A infecção humana por víbrios através do consumo de frutos do mar contaminados pode trazer sérias consequências. O *V. parahaemolyticus* é relacionado a um grande número de intoxicações alimentares decorrente do consumo de frutos do mar. Sendo as três espécies que provocam doenças em humanos através de produtos de origem aquática: *V. parahaemolyticus, V. vulnificus, V. cholerae,* a última a que apresentam maior risco, uma vez que é classificada no grupo de risco IA, que inclui as doenças "de severo

perigo para a população em geral, com ameaça de morte, sequelas crônicas ou longa duração". No entanto, a bactéria *V. vulnificus* é um halófito obrigatório em pacientes que apresentam relatos de consumo de frutos do mar contaminados ou por exposição de feridas a água do mar (KLONTS, 1988).

A transmissão de microrganismos patogênicos ao homem pode ocorrer de diversas formas, dentre elas o consumo de produtos alimentícios de origem marinha, como peixes, moluscos e crustáceos oriundos da aquicultura (REGO, 2012). As infecções alimentares causadas por bactérias patogênicas são consideradas um dos perigos identificados no consumo dos organismos cultivados (BEIRÃO et al., 2003). Os bacilos Gram-negativos são os principais representantes da microbiota natural dos crustáceos marinhos de águas temperadas, já nos camarões tropicais a microbiota é composta por micrococos, corineformes e bacilos Gram-negativos (ICMSF, 1985). Contudo, esta composição pode ser alterada em função da carga microbiana da água onde se encontra o animal. Desta forma, a utilização de boas práticas de manejo ao longo da produção dos animais pode garantir um produto final de melhor qualidade também no aspecto sanitário.

Segundo Vandenberghe et al. (2003), algumas espécies do gênero *Aeromonas* spp., presentes naturalmente no meio aquático, como a *A. hydrophila*, podem desencadear sintomas de diarreia em humanos, indicando que moluscos, peixes e crustáceos podem ser veículos de intoxicação alimentar (HANNINEM et al., 1997). A transmissão de bactérias patogênicas de origem entérica, incluindo *Salmonella sp*, procedentes do consumo de produtos marinhos também têm sido relatada (HUSS et al., 2000). O texto do Código de Práticas para Produtos Pesqueiros, da Comissão do *Codex Alimentarius*, que inclui os produtos da aquicultura, recomenda uma atenção especial no controle de agentes patogênicos biológicos, como as bactérias (*Salmonella* spp., *Shigella* spp., *Vibrio* spp.) e parasitas (*Clonorchissinensis*, *Opisthorchis* spp.), contaminantes químicos (metais pesados, pesticidas, reagentes químicos industriais) e resíduos de medicamentos veterinários (antibióticos, parasiticidas). Segundo Associação Brasileira de Criadores de Camarão (2004), "a conquista da qualidade é, atualmente, um requisito essencial para se permanecer no mercado". Desta forma é necessário o cumprimento de especificações de qualidade, estabelecidas tanto pelas autoridades brasileiras como pelas autoridades sanitárias dos países para os quais o camarão é exportado. Além disso, o documento ressalta que "a adoção de um programa de

qualidade na fazenda não é um entrave à produção, e sim um sinônimo de produtividade e competitividade".

No intuito de proteger a saúde pública, padrões microbiológicos foram estabelecidos, o CONAMA através da resolução n° 357, estabelece para águas que podem ser destinadas a aquicultura e atividades de pesca o limite microbiológico apenas para coliformes de 2500 células bacterianas por 100 mililitros de água. Já a Resolução da Diretoria Colegiada n. 12 da Agência Nacional de Vigilância Sanitária (BRASIL, 2001) estabelece para pescado *in natura*, resfriado ou congelado, não consumido cru, o seguinte padrão microbiológico: ausência de Salmonella em 25g e tolerância de até 10^3 UFC/g de *Staphylococcus* coagulase positivo. Embora esta resolução não apresente padrões a respeito dos Coliformes e Vibrios para esta categoria, a importância dos coliformes na indicação da qualidade sanitária é inquestionável já que inseridos neste grupo se encontram os "termotolerantes", como por exemplo a *Escherichia coli*, que tem como habitat primário o intestino humano e de animais, sendo portanto um indicador de contaminação fecal (GUIDELINES, 2012).Ao tratar de alimentos de origem marinha os víbrios destacam-se por sua presença, uma vez que estes organismos habitam ambientes marinhos, e possuem capacidade de causar infecções alimentares através de alimentos. O gênero víbrio consiste de 28 espécies, e quatro são geralmente associadas a quadros de gastroenterites: *V. parahaemolyticus, V. vulnificus, V. alginolyticus* e *V. cholerae* (JAY et al., 2005)

Existem também outras espécies de víbrio que afetam os camarões, causando mortalidade dos estoques, como por exemplo: *V. harveyi e V. alginolyticus* que podem infectar larvas, juvenis e adultos de camarões peneídeos (LAVILLA-PITOGO et al., 1998).As bactérias deste gênero podem ainda colonizar e infectar: apêndices, intestino anterior, intestino médio, hepatopâncreas e até infecções letais como, por exemplo, septicemia (AJITHA et al., 2004).

3- OBJETIVOS

3.1- Geral

Gerar informações a respeito da qualidade microbiológica dos cultivos de camarões realizados em sistemas convencional e de bioflocos.

3.2- Específico(s)

1. Quantificar e identificar as bactérias contaminantes presentes nos camarões e a na água de cultivo de *L.vannamei* em sistemas de bioflocos e convencional.
2. Avaliar se os microrganismos analisados estão dentro dos padrões estabelecidos pela legislação alimentar.

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Microbiological quality of *Litopenaeus vannamei* culture using conventional and biofloc systems.

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Shrimp farming is a fast-expanding activity that has supported the growth in the supply of these crustaceans to consumers around the world. However, the end product is vulnerable to contamination at all stages of the process, including the rearing tanks, where current practices prioritize to raise stocking densities and the minimization of water renewal. It is thus important to evaluate the potential of these systems for the proliferation of undesirable micro-organisms, which may render the product unfit for human consumption. In the present study, the presence of coagulase-positive Staphylococcus, Salmonella, Vibrio, and total and thermotolerant coliforms was verified in biofloc tank and conventional pond systems used for the rearing of *Litopenaeus vannamei* in Pernambuco, Brazil, and the results were compared with the legislation regulating the marketing of fresh shrimp. Samples were collected from two biofloc tanks with a density of 375 shrimp/m², and two conventional ponds with 12 shrimp/m². None of the samples tested positive for either Salmonella or Staphylococcus, which is consistent with the legislation. While no standards are defined legally for Vibrio or Coliforms, very low concentrations were recorded in both systems, in comparison with other studies. While some variation in bacterial contamination was observed over the rearing process, the end product of both systems was fit for human consumption. The results of the study indicate that, while the water is not renewed in the biofloc system, the development of undesirable micro-organismscan be controlled, with no adverse effects for the end product.

Key words: Coliformes, coagulase-positive *Staphylococcus*, *Salmonella* and *Vibrio*

INTRODUCTION

In recent years, shrimp farming has expanded constantly around the world, reaching a total production of 5.7 million tons in 2010. More than two-thirds (70.6%) of this yield resulted from the raising of saltwater species, of which the Pacific white shrimp, *Litopenaeus vannamei*, is the species most commonly farmed, worldwide (FAO 2012).

Shrimp farming demands the maintenance of adequate conditions in order to guarantee the survival and rapid development of the animals. The adequacy of the conditions in which the shrimp is reared will also contribute to the quality of the end product destined for human consumption. Shrimp are a rich source of nutrients, but demand strict sanitary controls. These crustaceans are highly perishable foodstuff because it has pH (close to neutral), high water activity, chemical composition and unsaturated fat content vulnerable to oxidation, as well large amounts of free amino-acids (Lira, Silva, Silva, Cavalcanti, Oliveira & Albuquerque 2013).

Some of the micro-organisms found in fresh shrimp present a risk to human health. Resolution number 12 of the Executive Council of the Brazilian National Public Health Agency (Brasil 2001) establishes legal limits for the contamination of refrigerated or frozen fishery products (not destined for raw consumption) by Salmonella and coagulase-positive Staphylococcus. Salmonella are enteric bacteria with the potential to cause severe intestinal intoxications, given that the majority of the serotypes of the genus are pathogenic to humans (Maijala, Ranta & Seuna 2005). Staphylococcus also causes intoxications related to the ingestion of pre-formed enterotoxins (Alcarãz, Satorres, Sepúldeva & Centorbi 1997).

While the legislation does not establish parameters for the levels of Coliforms or Vibrio found in fishery products, these micro-organisms undoubtedly represent a potential public health problem. The coliforms include the thermotolerant strains, such as *Escherichia coli*, which is found predominantly in the intestines of humans and animals, and is thus an indicator of fecal contamination (GCWQ 2012). Resolution 357 of the Brazilian National Environment Council, CONAMA (2005) establishes a limit of 2500 coliforms per 100 mL of water for brackish waters used for aquaculture or fishing.

Enterobacteria may be present in fishery products as a result of the contamination of aquatic environments by fecal matter, and may contribute to the

deterioration of the product (Huss, Reilly & Embarek 1997, 2000; Lira *et al.* 2013). The identification of the microbiota found in the habitat from which the product has been harvested is essential for the implementation of measures to control the concentrations of harmful bacteria and reduce the risks to the health of the consumer (Gonçalves 2004).

The bacteria of the genus Vibrio, most of which inhabit aquatic ecosystems, are also relevant here, given that some species cause diseases in humans. *Vibrio vulnificus*, for example, can cause symptoms such as primary septicemia, and gastrointestinal and dermal infections following the consumption of contaminated shellfish (Moreno & Landgraf 1997). In addition, farmed shrimp infected with this micro-organism may present symptoms such as septicemia and internal infections accompanied by a number of clinical symptoms, such as opaque abdominal musculature and expanded chromatophores, which may be fatal, causing a decline in productivity (Salnier, Haffner, Goarant, Peva & Ansquer 2000).

Conventional shrimp farming systems require the daily renewal of large quantities of water in order to maintain the quality of the aquatic environment of the ponds. The accumulation of unconsumed feed, dead animals, and excreta tend to raise the concentrations of nitrogenous compounds to toxic levels, as well as provoking excessive phytoplankton growth. The water used to top up the ponds is not always treated, however, facilitating the transfer of potential pathogens, which may provoke diseases in the stock. There is also a major risk of contamination with enteric bacteriathat are pathogenic to humans, and which can seriously affect the quality of the end product.

An alternative production system has been developed – known as biofloc technology (BFT) – which reduces or eliminates the need for the regular renewal of the water in the enclosures by re-using the effluents (Otoshi, Naguwa & Falesch 2008). The quality of the water in this system is maintained by stimulating the growth of bacteria through the addition of an extra source of carbon, such as molasses. The combination of these bacteria with the particles of organic residues and other micro-organisms results in the formation of microbial flakes. Together with the microalgae, these bacteria absorb phosphorus, nitrogen, and other excess nutrients in the water, recycling these compounds and contributing to the control of toxic compounds, such as ammonia (Goldman, Caron & Dennet 1987; 2001; Chamberlain, Avnmelech, Mcintosh & Velasco 2001; Avnmelech 2009). However, the stimuli provided by the addition of nutrients and the re-use of effluents derived from other production cycles have raised

doubts with the regard to the possibility of the accumulation of potentially pathogenic micro-organisms in these systems.

Given the lack of data on the behavior of potentially pathogenic micro-organisms in biofloc systems, the levels of bacterial contaminants found in tanks run using this system were described and analyzed in the present study. Comparisons were also made with samples from conventional production systems in order to confirm whether the management practices used to maintain water quality is effective in terms of the sanitary quality of the shrimp produced for human consumption.

MATERIAIS AND METHODS

The present study was conducted on a shrimp farm on the southern coast of the Brazilian state of Pernambuco (08°39'01.01"S, 035°06'52.31"W). The *L. vannamei* post-larvae (PL10-12), with a mean weight of 2.5±0.5 mg, were obtained from a commercial production laboratory. Following acclimatization, the larvae were transferred randomly to their respective experimental enclosures – (BS) biofloc system (two cement tanks each with 200 m² - BS1 and BS2), and (CS) traditional system (two earth bottoms ponds of 2.97 hectares – CS1 and CS2). The shrimp were stocked at a density of 375 individuals/m² in biofloc tanks and 12 individuals/m² in the traditional ponds.

Conventional farming system

Prior to the experiment, the ponds were prepared by drying and lime spreading the soil prior to the addition of the water. The water was also fertilized inorganically with nitrogen (calcium nitrate), phosphorus (simple super-phosphate), and silica (sodium metasilicate) at a proportion of 10:1:5, respectively, to encourage the growth of microalgae. Between 2% and 5% of the water was renewed daily, depending on its quality. These ponds were not aerated artificially.

Biofloc system

The tanks were filled with seawater and 12.5 ppm of calcium hypochlorite was added. After 24 hours of continuous aeration, the water was tested for chlorine residues. The tanks were then fertilized inorganically, with the nitrogen:phosphorus:silica at a proportion of 2.0:0.3:0.7 $mg.l^{-1}$ (Samocha, Patnaik, Speed, Ali, Burger, Almeida, Ayub, Harisanto, Horowitz & Brock 2007). After two hours, the water was inoculated with the

microalgal diatom *Thalassiosira fluviatilis*, at a concentration of 800 cells per milliliter. One day prior to shrimp stocking, and during the first three days of production, sugarcane molasses were applied as a source of carbon to stimulate the growth of heterotrophic bacteria and induce the formation of bioflocs. The proportion of molasses added to the water was determined based on a bromatological analysis of its composition and the quantity of feed provided, with the objective of maintaining the Carbon:Nitrogen ratio at approximately 20:1 (Avnimelech 1999).

The concentration of total ammoniacal nitrogen (TAN) was measured *in situ* in the biofloc tanks every two days using an ALFAKIT-AT10P photocolorimeter. The results determined whether additional organic fertilization was required, with the objective of maintaining the TAN levels below 1mg/L, using a proportion of 6g of carbon for each gram of ammoniacal nitrogen in the system (Samocha *et al*. 2007).

Culture management

During the 20 first days of the production process, a commercial feed (40% crude protein) was distributed into the enclosures. Animals were fed four times a day in the biofloc tanks (at 06:00 h, 12:00 h, 18:00 h, and 00:00h), and every two hours in the conventional ponds. Subsequently, a different commercial feed (35% crude protein) was offered twice a day, at 08:00 h and 16:00 h in both systems. The quantity of feed provided was based on a feeding table (Jory, Cabrera, Dugger, Fegan, Lee, Lawrence, Jackson, Mcintosh & Castañeda 2001), and supplied using feeding trays.

The physical-chemical parameters of the water – pH, temperature (°C), salinity, and dissolved oxygen (DO) concentrations ($mg.L^{-1}$) – were measured twice daily, at 08:00 h and 17:00 h in both types of enclosure, using a multiparameter YSI 556 MPS probe. In the biofloc systems, the volume of microbial flakes ($mL.L^{-1}$) was evaluated using one-liter water samples collected in a graduated Imhoff cone and decanted for 20 minutes. Three readings were taken in each enclosure every three days (Avnimelech 2009).

Bacteriological analyses

- Collection and preparation of samples

Samples of the water and shrimp were collected fortnightly, starting on the post-larvae stocking day (week 0) and ending when the shrimp were harvested, which

occurred when they had reached the standard marketing weight (10 g). The samples were sent to the Aquaculture Technology Laboratory at the Federal Rural University of Pernambucofor analysis.

The water samples were collected from three different points within each enclosure and stored in dark, sterile containers. The shrimp were collected using a hand net and placed in plastic bags with water and constant aeration.

For analysis, the water samples were homogenized and a 25 mL aliquot was transferred to an Erlenmeyer flask containing 225 mL of 1% sterile saline solution and Buffered Peptone Water (BPW) to achieve a dilution of 10^{-1}. For analysis, the shrimp were weighed, macerated, and added to a 1% sterile saline solution at a weight/volume ratio of 1:10, to achieve a dilution of 10^{-1}. The samples were then diluted serially to 1:10 (v:v) in test tubes containing 9 mL of 1% saline solution.

- Coagulase-positive *Staphylococcus*

Aliquots of 0.1 ml of the diluted samples of the macerated shrimp were plated in Mannitol Salt Agar Base, a selective medium for the cultivation of *Staphylococcus* spp., and incubated at 35ºC for 24h. The colony-forming units (CFUs) were then quantified and Gram stained, with a catalysis test being applied to the positive cocci. Gram-positive bacteria were tested for oxidation/fermentation in OF medium and incubated for 24h at 35ºC, under aerobiosis or anaerobiosis. The samples that presented a yellowish coloration under these conditions were classified as Staphylococcus. These isolates were then inoculated into Brain Heart Infusion (BHI) broth, incubated in a growth chamber for 24 h at 35ºC, and then tested for coagulase. For this, 0.5 ml of the suspension was mixed with 0.5ml of rabbit plasma, with the formation of a clot indicating the presence of coagulase-positive *Staphylococcus*.

- *Salmonella*

Aliquots of 1 mL of the 10^{-1} dilution of the shrimp samples incubated in BPW were added to Selenite broth. Following incubation for 24 h at 41ºC, Petri dishes containing Salmonella-Shigella agar were seeded with 0.1 mL of the culture, and then incubated for 24 h at 35ºC. The colonies with black centers were re-isolated in BHI agar and Gram stained. The Gram-negative bacilli were inoculated into tubes containing Triple Sugar Iron (TSI) agar and tested for oxidase to confirm the enterobacterial

family. The species contained in suspected samples were identified using commercial kits (API-20E, Biomerieux).

- Coliforms

The Most Probable Number (NMP) of coliforms was determined using the 10^{-1}, 10^{-2} and 10^{-3} dilutions of the shrimp and water samples, with 1 mL of each solution being incubated in three inverted Durham tubes containing Lauryl Sulphate Triptose (LST) broth for presumptive testing of the bacteria of the coliform group. The tubes were incubated for 48 h at 35ºC. The subsequent formation of gas inside the Durham tubes was considered to be a positive reaction to the presence of coliforms. Positive tubes were examined for *Escherichia coli* in test tubes containing EC broth, which were incubated in a water bath for 24 h at 45ºC. The samples that produced gas were considered to be positive for fecal coliforms. Hoskins' table for multiple tubes (Silva, Junqueira, Silveira, Taniwaki, Santos & Gomes 2007; Calil, Ferreira, Brazão & Sovenhi 2013) was used to calculate the Most Probable Number (MPN) of coliforms per gram of shrimp or mL of water. This statistical approach usually returns higher values than those obtained by counting colonies on culture plates (Jay 1998).

- *Vibrio* spp.

For the identification and quantification of *Vibrio* spp., Petri dishes containing Tiosulphate Citrate Bile Sucrose (TCBS) agar were seeded with 0.1 mL aliquots of the dilutions of the shrimp and water samples. The dishes were incubated in a growth chamber for 24 h at 35ºC, after which, the number of CFUs between 30 and 300 were quantified. The colonies were then separated by morphology and isolated in Tryptone Soya Agar (TSA), (24 h at35ºC) and Gram stained. The colonies that presented straight or curved rods, Gram negative, were seeded in inclined TSI agar and then kept in a growth chamber for 24 h at 35ºC. The TSI agar tubes that presented a yellowish coloration in the bottom (indicating the fermentation of glucose) were evaluated for oxidation using a testing band. Following these tests, the bacteria were identified using API-20E kits (Biomerieux). The mean Vibrio concentrations found in the samples were compared between the two types of culture using Student's *t*-test ($P < 0.05$). The analyses were run in Oringi 8.1.

RESULTS AND DISCUSSION

Mean (± standard deviation) temperature in the biofloc tanks was 27.35±1.06°C, with a mean salinity of 11.96±2.60, pH of 7.46±0.34, and dissolved oxygen concentration of 7.04±0.93 $mg.L^{-1}$. In the conventional ponds, these values were 28.05±0.98°C, 12.75±2.23 (salinity), 8.01±0.36 (pH), and 8.06±1.50 $mg.L^{-1}$(DO). All these parameters were within the limits recommended for raising *L. vannamei* by Ponce-Palafox, Martinez-Palacios and Ross (2005).

The bacteriological analyses detected neither coagulase-positive *Staphylococcus* nor *Salmonella* spp. in the samples collected from either of the systems. This satisfies the norms established by the Executive Council of the Brazilian National Public Health Agency in resolution number 12 of January 2, 2001 (Brasil 2001). This resolution requires that unprocessed fishery products, refrigerated or frozen, destined for non-raw consumption must present no *Salmonella* spp. in a 25 g sample, and no more than 10^3CFU/g of coagulase-positive *Staphylococcus*. The detection of these micro-organisms in farmed shrimp is still rare, and their presence in the marketed product tends to depend on inadequate hygiene and sanitary standards during handling and transportation. A number of studies have recorded shrimp of substandard sanitary quality in different Brazilian regions. In the specific case of coagulase-positive *Staphylococcus*, for example, Nascimento, Vieira and Theophilo (2001) found that 40% of the shrimp being sold in São Luís, in the state of Maranhão, were unfit for human consumption, according to the standards set by the Brazilian legislation. Values of 43.4% were recorded in Niterói, Rio de Janeiro (Santos 2011) and 83.3% in Fortaleza, Ceará (Costa, Vieira, Vieira & Sampaio 2009).

Phan, Fitzgerald, Nathan, Moore, Uhde and Tancer (2005) found that 24.5% of the shrimp marketed in Vietnam were contaminated with Salmonella. In Brazil, however, Costa, Moreira, Carvalho, Menezes, Silva and Vieira (2011) did not detect this bacterium in fresh *L. vannamei* being sold in the city of Fortaleza, nor did Lira *et al.* (2013) detect it in the Atlantic seabob, *Xiphopenaeus kroyeri*, being sold fresh and smoked.

Salmonella does not appear to be common in farmed shrimp, as confirmed by Dalsgaard, Huss, H-Kittikun, and Larsen (1995), who analyzed 158 samples from a farm in Thailand, including the water, shrimp, sediments, feed, and fertilizers. The presence of this micro-organism in the shrimp-farming environment appears to be related to the contamination of the water by untreated fertilizers derived from animal by-products (Hatha & Hao 1998), which are no longer used in aquaculture operations.

Contamination of the end product by both Salmonella and Staphylococcus generally results from inadequate sanitary conditions during processing and storage, and in fact, contamination may occur at any stage in this process, from farm to consumer (Lira *et al.* 2013). However, few data are available on the sanitary quality of fresh shrimp produced by different farming systems.

Even so, the absence of specific pathogens, such as Salmonella and Staphylococcus, does not clarify the hygiene or sanitary quality of the enclosures. To understand these conditions, it is necessary to evaluate the occurrence of bioindicator micro-organisms. The results of the present study indicated low concentrations of both total (TC) and thermotolerant (TTC) coliforms in the water of both types of enclosure (Table 1), with only eight of the 87 samples exceeding the limit of 2500 CFU/100 ml for TTC established by resolution 357 of the Brazilian National Environment Council (CONAMA 2005). The contaminated water samples were obtained from three collections in the biofloc tanks and one in the conventional ponds.

The quantity of total coliforms present in the water appears to be related to the quantity of organic matter available in the production system (Vieira, Maia, Janebro, Fernandes & Ceballos 2000; Parente, Costa, Vieira, Reis, Hofer, Fonteles & Fernandes-Vieira 2011). In the conventional system, this concentration may vary according to the quality of the water used to refill the enclosures. During rainy periods, for example, supplies of standing water may contain higher concentrations of coliforms and other bacteria (Sinton, Hall, Lynch & Davis-Colley 2002), given that the precipitation may wash residues (including sewage) from the land into sea, rivers and lakes, as well as suspending bottom sediments.

In the biofloc system, organic matter is more abundant due to the presence of the bioflocs and the fact that the water is not renewed. In this case, the peaks in coliform density would be expected to correlate with those in biofloc concentrations, although this was only observed in the eighth week of the study period (Figure 1), when a peak was recorded in both concentrations. However, coliforms were unaffected by the second peak in biofloc concentrations (Table 1), indicating that the population dynamics of these bacteria in this closed system is regulated by other factors, such as competition with other groups of micro-organisms (Dolfingand & Gottschal 1997).

As suggested by Samocha *et al.* (2007), the volume of bioflocs in the water was maintained below 17 $ml.L^{-1}$ during the present study, although this required the partial refilling of the tanks in order to reduce the concentration in the tenth week (Figure 1).

According to Ebeling, Timmons and Bisogni (2006) the increase in carbon concentration in the biofloc system results in considerable growth of heterotrophic bacteria, with a consequent reduction in the availability of dissolved oxygen in the water.

The analysis of the samples of the post-larvae used to stock the enclosures (week 0) revealed that they were contaminated with coliforms, with TC concentrations of over 1000 MPN/g, and 240-460 MPN/g for TTCs (Table 2). This indicates that the post-larvae were already contaminated when they arrived on the farm. However, these concentrations decreased subsequently in both the production systems, with values for both groups of coliforms (TC and TTC), being reduced to below 150 MPN/g after three weeks (SB2).

The legislation of the European Union, one of the principal importers of Brazilian shrimp, limit the tolerable level of thermotolerant coliforms in cooked crustaceans to 100MPN/g (CEE 1992). This same limit is established by the Brazilian legislation on pre-cooked crustaceans. In the present study (Table 2), TTC concentrations were above this level in more samples from the conventional system – three samples from weeks 8 and 10 – probably due to the contact of the water with the soil, which may favor contamination by thermotolerant coliforms, such as *Klebsiella* and *Enterobacter*, which are found in plant roots (Doyle 1996; GCWQ 2012). By contrast, only one sample from the biofloc system (week 5) was contaminated.

The values used for comparison here refer to cooked shrimp, given the lack of official parameters in either the Brazilian legislation or that of the European Union for raw crustaceans. The lack of data for raw foodstuffs is related to the sensitivity of these micro-organisms to high temperatures, so that parameters are normally obtained only for processed or cooked foodstuffs, rather than raw ones (Sousa 2006).

Significant differences in the quantity of *Vibrio* spp. were found between almost all the water samples, with high mean values being recorded for the biofloc system (Table 3). The highest value (3.15×10^4 CFU/mL) was recorded during the third week for the samples from the biofloc tanks, whereas the highest value for the conventional ponds (0.64×10^3CFU/mL) was registered in the tenth week.

In the case of the shrimp samples, high values (1.54×10^7 CFU/g) were recorded in the post-larvae prior to stocking (week 0), although this contamination was reduced considerably by the third week (Table 3), although it rose again in the fifth week. These fluctuations were observed in both systems, and a significant difference – a higher value

in the conventional ponds – was recorded only in the last week. The availability of nutrients may lead to an excessive production of biomass, with bacterial proliferation being stimulated by the extracellular products of the phytoplankton, which may favor the development of Vibrio (Alam, Tomochika, Miyoshi & Shinoda 2001). This would be expected in the biofloc system, then, although the results of the present study indicate that, even with the increased availability of nutrients in this system, the growth of Vibrio was maintained relatively low by the standards of aquaculture systems. For example, Costa *et al.* (2009) recorded values of up to 11 x 10^{13} CFU/g in 24 samples of *L. vannamei* obtained from conventional ponds in the Brazilian state of Ceará, in contrast with the maximum value of 3.15x10^{4}CFU/g recorded in the present study (biofloc tanks, week 3).

As mentioned above, the Brazilian National Public Health Agency's resolution 12 establishes limits for contamination by some micro-organisms in different foodstuffs. Although there are still no data on Vibrio for the definition of contamination levels that would render the shrimp unfit for human consumption, given that these organisms are common in marine ecosystems and only some species affect humans. At least 12 of the 30 known Vibrio species are pathogenic in humans and/or have been associated with diseases transmitted by foodstuffs (Dalmasso, Civera & Bottero 2009; Pruzzo, Gallo & Canesi 2005). Vibrio species may cause diarrheal diseases in humans, as well as skin infections during immersion in estuarine or coastal waters, especially in persons with weakened immune systems, a phenomenon that has received increasing attention over the past ten years (Oliver, Belkin & Colwell 2005). One example is *Vibrio parahaemolyticus*, which has been identified as the principal cause of outbreaks of disease caused by shellfish in Japan and Korea (Lee, Lee, Kim & Park 2001). In the present study, *V. alginolyticus* was found in water samples, and *V. alginolyticus* and *V. cholera* were detected in shrimp samples collected from both types of enclosure (biofloc and conventional systems).

Vibrio cholerae is the most important species here (Dalmasso *et al.* 2009), representing a serious public health risk, with the O1 and O139 serogroups being linked to epidemics and pandemics of diarrhea in a number of different countries around the world (Pruzzo *et al.* 2005). Janda, Szalai, Tari and Paldi (1997) demonstrated that *V. parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio alginolyticus* were responsible for 86% of skin ulcers, in particular *V. alginolyticu*s, which was present in 71% of the lesions, while Rodrigues *et al*. (2001) observed this species in 66.6% of the lesions analyzed.

During the analysis of *Vibrio*, the bacterium *Aeromonas hydrophila* was found in the samples of shrimp from both systems. This pathogen was responsible for 114 (19.5%) of the cases of diarrhea analyzed by Hofer, Singer and Williams (2005) in São Bento do Una (Pernambuco), and while the patients presented symptoms of cholera (watery diarrhea and multiple daily evacuations), *V. cholera* was responsible for only two (0.3%) cases.

Vibrio spp. also an important group for the shrimp farming operations themselves, given that these bacteria may cause diseases in penaeid shrimp, including lesions in the soft tissue (with or without necrosis), or even retarding growth, resulting in problems for the transformation of the larvae and increased mortality (Costa *et al.* 2009). The presence of Vibrio in is frequently associated with low survival rates in larva production operations and shrimp farming systems. However, no evidence was found of infection by Vibrio in the shrimp analyzed in the present study.

All the shrimp samples analyzed in the present study were fit for human consumption according to the Brazilian legislation on Salmonella and Staphylococcus. While some samples presented relatively high concentrations of Coliforms and Vibrio, there are no official criteria for these micro-organisms, and all the samples can be considered to be adequate according to the standards of the Brazilian National Public Health Agency. Despite the greater quantities of nutrients available in the biofloc system, then, the observed concentrations of micro-organisms were relatively stable over time and within the limits set by public health agencies, guaranteeing that the end product was fit for human consumption.

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Table 1. Most Probable Number (MPN/mL and MPN/g) of total coliforms (TC) and thermophilic (TTC) present in the water samples from conventional (CS1 and CS2) and biofloc (BS1 and BS2) systems.

Weeks	Systems											
	BS1						BS2					
	TC	**TTC**	TC	**TTC**	TC	**TTC**	TC	**TTC**	TC	**TTC**	TC	**TTC**
0	23	**23**	< 3.0	**< 3.0**	29	**29**	36	**36**	240	**240**	93	**93**
1	< 3.0	**< 3.0**	< 3.0	**< 3.0**	< 3.0	**< 3,0**	3,6	**3,6**	< 3,0	**< 3,0**	< 3,0	**< 3,0**
3	< 3.0	**< 3.0**	< 3.0	**< 3.0**	< 3.0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**
5	< 3.0	**< 3.0**	< 3.0	**< 3.0**	< 3.0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**
8	23	**23**	43	**43**	< 3.0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**
10	23	**< 3.0**	43	**3.6**	< 3.0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**
12	< 3.0	**< 3.0**	< 3.0	**< 3.0**	< 3.0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**
17	7.4	**< 3.0**	< 3.0	**< 3.0**	3	**< 3,0**	3,6	**< 3,0**	9,4	**< 3,0**	3,6	**< 3,0**
	CS1						CS2					
	TC	**TTC**	TC	**TTC**	TC	**TTC**	TC	**TTC**	TC	**TTC**	TC	**TTC**
0	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**
1	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**
3	3,6	**3,6**	3,6	**3,6**	3,6	**3,6**	3,6	**3,6**	< 3,0	**< 3,0**	< 3,0	**< 3,0**
5	< 3,0	**< 3,0**	23	**23**	9,2	**9,2**	43	**43**	75	**75**	43	**43**
8	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**
10	< 3,0	**11**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**23**	< 3,0	**3**
12	--	--	--	--	--	--	< 3,0	**< 3,0**	< 3,0	**< 3,0**	< 3,0	**< 3,0**
17	--	--	--	--	--	--	--	--	--	--	--	--

-- No analyzes were made in this period.

Table 2. Most Probable Number (MPN/mL and MPN/g) of total coliforms (CS) and thermophilic (TTC) present in shrimp samples from conventional (CS1 and CS2) and biofloc (BS1 and BS2) systems.

Weeks	Systems											
	BS1						BS2					
	TC	**TTC**	TC	**TTC**	TC	**TTC**	TC	**TTC**	TC	**TTC**	TC	**TTC**
0	>1100	**460**	>1100	**240**	>1100	**7,4**	>1100	**460**	>1100	**240**	>1100	**7,4**
1	--	**--**	--	**--**	--	**--**	--	**--**	--	**--**	--	**--**
3	< 3,0	**< 3,0**	< 3,0	**<3,0**	43	**43**	93	**93**	75	**75**	150	**150**
5	3,6	**3,6**	23	**23**	3,6	**3,6**	43	**43**	3,6	**3,6**	< 3,0	**<3,0**
8	75	**75**	3,6	**3,6**	93	**93**	23	**23**	75	**75**	75	**75**
10	15	**9,2**	3	**<3,0**	23	**< 3,0**	150	**15**	150	**15**	290	**7,2**
12	120	**14**	43	**23**	460	**93**	23	**9,2**	23	**9,2**	21	**9,2**
17	< 3,0	**<3,0**	38	**3**	64	**7,2**	240	**7,4**	28	**15**	16	**6,1**
	CS1						CS2					
	TC	**TTC**	TC	**TTC**	TC	**TTC**	TC	**TTC**	TC	**TTC**	TC	**TTC**
0	>1100	**460**	>1100	**240**	>1100	**7,4**	>1100	**460**	>1100	**240**	>1100	**7,4**
1	--	**--**	--	**--**	--	**--**	--	**--**	--	**--**	--	**--**
3	14	**14**	23	**23**	23	**23**	9,2	**9,2**	3,6	**3,6**	9,2	**9,2**
5	3,6	**3,6**	9,2	**9,2**	15	**15**	240	**240**	93	**93**	460	**460**
8	240	**240**	43	**43**	15	**15**	15	**15**	43	**43**	21	**21**
10	93	**43**	93	**93**	460	**93**	460	**75**	460	**38**	1100	**9,2**
12	--	**--**	--	**--**	--	**--**	< 3,0	**<3,0**	< 3,0	**<3,0**	3,6	**3,6**
17	--	**--**	--	**--**	--	**--**	--	**--**	--	**--**	--	**--**

-- No analyzes were made in this period.

Table 3. Concentration of Vibrio spp. (Mean ± Standard Error) in water and shrimp samples from conventional (CS) and biofloc (BS) systems.

Sample	Weeks	Culture System	
		BS	CS
Water (10^3 UFC/mL)	0	5.11±1.46[a]	0±0.00[b]
	1	1.09±0.23[a]	0.53±0.20[a]
	3	31.5±7.63[a]	0.09±0.04[b]
	5	31.1±15.69[a]	0.36±0.08[b]
	8	2.84±0.82[a]	0.07±0.01[b]
	10	8.29±2.48[a]	0.64±0.17[b]
	12	2.68±0.67[a]	0.08±0.03[b]
	17	2.63±0.48	--
Shrimp (10^4 UFC/g)	0	1546±224.9[a]	1550±224.9[a]
	1	--	--
	3	1.04±0.47[a]	3.39±0.92[a]
	5	37.5±15.1[a]	6.87±1.35[a]
	8	3.15±1.09[a]	3.99±1.48[a]
	10	13.26±5.66[a]	16.06±6.14[a]
	12	0.05±0.01[a]	0.40±0.20[b]
	17	0.09±0.01	--

-- No analyzes were made in this period. Different letters in the same row indicate significant difference ($P < 0.05\%$).

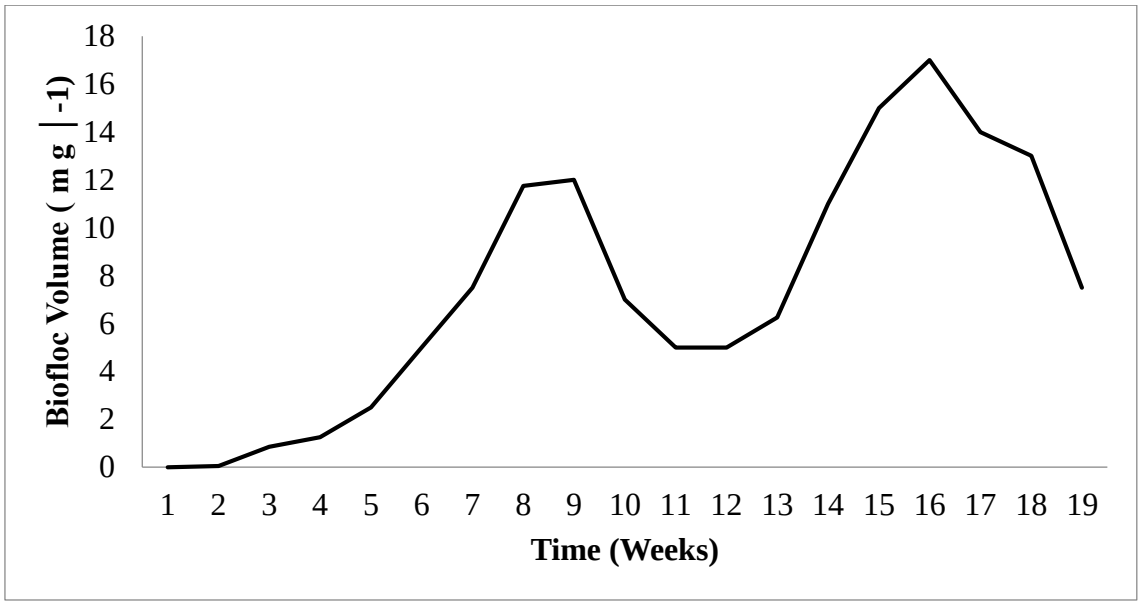

Figure 1: Mean biofloc volume during *Litopenaeus vannamei* culture in biofloc system.

6- NORMAS DA REVISTA

MANUSCRIPT FORMAT AND STRUCTURE

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All sections of the typescript should be on one side of A4 paper, double-spaced and with 30mm margins. A font size of 12pt should be used. Line numbering should be included, with numbering to continue from the first line to the end of the text (reference list). Line numbers should be continuous throughout the manuscript and NOT start over on each page**.**

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Carry out and describe all appropriate statistical analyses.

6.2. Structure

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