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CULTIVO INTEGRADO EM SISTEMA DE BIOFLOCOS DO CAMARÃO *Litopenaeus vannamei* (BOONE, 1931) COM AS MACROALGAS DOS GÊNEROS *Ulva* (LINNAEUS, 1753) E *Gracilaria* (GREVILLE, 1830)

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

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Luis Otavio Brito da Silva

Tese de doutorado apresentado ao Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura da Universidade Federal Rural de Pernambuco, como exigência para obtenção do título de Doutor.

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Resumo

Os problemas relacionados com as enfermidades têm ocasionado significativas perdas econômicas na indústria do cultivo de camarões. Neste sentido, os sistemas de cultivo mais eficientes no que se referem a maior produtividade e biossegurança, menor geração de efluentes são importantes para garantir a sustentabilidade da indústria. O objetivo foi avaliar o cultivo integrado de *Litopenaeus vannamei* com as macroalgas *Ulva* e *Gracilaria* em sistema de bioflocos, no que se refere à qualidade da água e crescimento dos camarões. No primeiro, foram utilizados camarões (4,5g, 566 camarões/m^3) e *Ulva lactuca* (2,0 Kg/m^3). O sistema de bioflocos integrado (camarões e macroalgas) reduziu a amônia total em 25,9%, nitrito em 72,8%, fosfato em 24,6% e sólidos suspensos totais em 12,9% e aumentou o peso final em 6,9% comparado ao bioflocos sem macroalgas. No segundo, foram utilizados camarões (2,6g, 425 camarões/m^3) e as macroalgas *Gracilaria birdiae* (2,0 Kg/m^3) e *Gracilaria domingensis* (2,0 Kg/m^3). O sistema de bioflocos integrado com *G. birdiae* aumentou o peso final em 21% e a produção em 7%, reduziu o FCA em 28% e a densidade de Cyanobacteria em 17% comparado ao bioflocos sem macroalgas. No terceiro, foram utilizados camarões (0,3g, 500 camarões/m^3) e *Gracilaria birdiae* em diferentes biomassas (2,5; 5,0 e 7,5 peso úmido Kg/m^3). O sistema de bioflocos integrado reduziu o nitrogênio inorgânico dissolvido entre 19 a 34%, *Vibrio* entre 8 a 83%, FCA entre 20 a 30%, e aumentou a concentração de proteína bruta no corpo do camarão entre 8 a 13%, peso final entre 25 a 32% e a produção entre 22 e 39% comparado ao bioflocos sem macroalgas. A utilização de macroalgas em sistema de bioflocos contribui para melhorar a qualidade da água e aumentar o crescimento dos camarões.

Palavras-chave: Bioflocos, sistema integrado, *Litopenaeus vannamei*, *Ulva*, *Gracilaria*.

Abstract

Problems related to disease have caused significant economic losses in the shrimp farming industry as well as decreased stocking densities and job opportunities. Therefore, the development of more efficient culture systems with higher yield and biosecurity and which generate less waste are important to ensure the sustainability of the industry. Three studies were conducted to evaluate the integrated culture of *Litopenaeus vannamei* with *Ulva* and *Gracilaria* seaweed in biofloc systems, in relation to water quality and shrimp growth. The first used shrimp (4.5g, 566 shrimp/m^3) and *Ulva lactuca* (2.0 Kg/m^3). The integrated biofloc system (shrimp and seaweed) reduced total ammonia nitrogen by 25.9%, nitrite-nitrogen by 72.8%, phosphate by 24.6% and total suspended solids by 12.9%, while increasing final shrimp weight by 6.9 % compared to a biofloc system without seaweed. The second used shrimp (2.6g, 425 shrimp/m^3) and *Gracilaria birdiae* (2.0 Kg/m^3) and *Gracilaria domingensis* (2.0 Kg/m^3). The integrated biofloc system with *G. birdiae* increased the final weight by 21% and yield by 7%, and decreased FCR by 28% and Cyanobacteria density by 17% as compared to biofloc without seaweed. The third study used shrimp (0.3g, 500 shrimp/m^3) and *Gracilaria birdiae* stocked at different biomasses (2.5; 5.0 and 7.5 fresh weight Kg/m^3). The integrated biofloc system reduced dissolved inorganic nitrogen by 19 to 34%, *Vibrio* density by 8 to 83%, and FCR by 20 to 30%, and increased the crude protein content of whole-body shrimp by 8 to 13%, final weight by 25 to 32% and yield by 22 to 39%. The use of seaweed in biofloc systems contributes to improved water quality and increased shrimp growth.

Key words: Biofloc, integrated system, *Litopenaeus vannamei*, *Ulva, Gracilaria.*

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1- Introdução

Sistemas tradicionais de cultivo de camarões marinhos utilizam altas taxas de renovação de água para manter a qualidade adequada, gerando desperdício dos recursos hídricos e podendo transforma-se em fonte de poluição ambiental, através das descargas de nitrogênio e fósforo provenientes dos fertilizantes utilizados, resíduos de rações e dos animais cultivados (HOPKINS et al., 1993).

Associado a isto, durante as últimas décadas existiram consideráveis surtos de doenças em sistemas tradicionais de cultivo, que afetaram significativamente a produção e a gestão operacional das fazendas (MISHRA et al., 2008). Neste sentido, a aquicultura está sempre em busca de técnicas de manejo que melhorem a eficiência da administração dos alimentos, da qualidade da água e dos solos (BRITO et al., 2011). Dentro destas estratégias pode-se destacar o manejo dos resíduos orgânicos e ciclagem dos nutrientes dentro dos próprios sistemas de cultivo (SAMOCHA et al., 2011), além dos sistemas de aquicultura integrada (TROELL et al., 2009; SIMÃO et al., 2013).

O sistema de bioflocos (flocos microbianos, sistema heterotrófico, zero ou mínima troca de água), vem sendo utilizando em diversas partes do mundo como estratégia de manejo que minimizar a utilização de água, redução da emissão de efluentes e aproveitamento de parte dos resíduos metabólicos para a nutrição dos camarões e peixes onívoros, através do consumo dos agregados microbianos (CRAB et al., 2012).

Entretanto, esta redução na taxa de renovação de água aumenta o acúmulo de resíduos, principalmente compostos nitrogenados (KRUMMENAUER et al., 2011). Estes resíduos são os principais problemas na produção de camarão, por causa da toxicidade aos animais cultivados (MONTOYA et al., 2002). Neste sentido, o balanço entre a produção de resíduos e a capacidade de assimilação dos mesmos pelo ambiente é de suma importância para o desenvolvimento dos cultivos intensivos (THAKUR e LIN, 2003).

Em sistemas tradicionais de cultivo de organismos aquáticos partes dos nutrientes acumulados nos sistemas podem ser assimilados pelas macroalgas e transformados em biomassa (CARTON-KAWAGOSHI et al., 2013). Além disso, as macroalgas podem ser utilizadas como fonte de alimento para os camarões (PORTILLO-CLARK et al., 2012).

Apesar da grande variedade de funções das macroalgas em sistemas tradicionais de cultivo, o papel das macroalgas em sistemas de bioflocos, principalmente no que se refere à qualidade da água e ao desempenho zootécnico dos camarões, ainda é desconhecido. Para a escolha das espécies de macroalgas para cultivos integrados com camarões, deve ser avaliar a sua eficiência na remoção de nutrientes e a habilidade de crescimento em condições hipertróficas (ABREU et al., 2011).

Neste sentido, o presente trabalho teve como objetivo avaliar a utilização de macroalgas *Ulva* e *Gracilaria* em sistema integrado com o camarão *Litopenaeus vannamei*, no que se refere à otimização da qualidade da água, aumento do crescimento e da sobrevivência dos camarões.

2- Revisão de literatura

2.1 Produção de camarão marinho no Brasil

A carcinicultura rapidamente expandiu-se em todas as partes do mundo, especialmente em área tropicais, devido ao alto valor comercial dos camarões (PÉREZ-LINARES et al., 2008). Atualmente *L. vannamei*, é a principal espécie de camarão cultivada no mundo, atingindo uma produção superior a 2,5 milhões de toneladas, representando 71,9% da produção mundial de crustáceos (FAO, 2012).

No Brasil os produtores e pesquisadores brasileiros concentraram mais esforços no cultivo da espécie *L. vannamei* (MAIA et al., 2011). Entretanto outras espécies apresentam potencial para produção comercial: *Litopenaeus schmitti* (MARQUÉZ et al., 2012); *Farfantepenaeus paulensis* (FÓES et al., 2011), *Farfantepenaeus brasiliensis* (LOPES et al., 2009) e *Farfantepenaeus subtilis* (SOUZA et al., 2009).

Em relação à produção de camarões marinhos no Brasil, o histórico é formado por diferentes situações, que vão desde altas rentabilidades até profundas crises (NUNES et al., 2011a). No ano de 2003 a produção de camarões foi de 90.196,5 toneladas (incremento de 50% em relação a 2002), com uma produtividade média de 6.084 kg/ha/ano (ROCHA et al., 2004). Em 2004, em decorrência do vírus da mionecrose infecciosa (IMNV), a atividade sofreu uma redução de 15% na produção em relação ao ano anterior (75.895 toneladas), baixando a produtividade para 4.573 Kg/ha/ano (RODRIGUES, 2005). Em 2005, surgiram os primeiros relatos do vírus da mancha branca (WSSV) em Santa Catarina, em 2008, no sul da Bahia e em 2011, em algumas áreas de Pernambuco, Paraíba e sul do Rio Grande do Norte (GUERRELHAS e TEIXEIRA, 2012), além de fungos, *Vibrios* e protozoários vem contribuindo de forma negativa no desempenho zootécnico dos camarões cultivados.

A produção brasileira de 2011, foi caracterizada pelas baixas densidades de estacagem nos viveiros (≤ 30 camarões/m^2), aumento da área inundada (19.845 ha de lâmina d’água), produtividade média de 3.510 kg/ha/ano e produção de 69.571 toneladas. Apesar das baixas densidades de estocagem mais de 42% das fazendas utilizam aeradores e 33% probióticos comerciais (ABCC, 2013).

Na região Nordeste, estão instaladas 1.429 (92% das fazendas em todo territorio Brasileiro) empreendimentos de cultivo de camarão, que produzem 92% do camarão cultivado. Dentre dos Estados do Nordeste, podemos destacar o Ceará (31.982 toneladas em 6.580 ha), Rio Grande do Norte (17.825 toneladas em 6.540 ha), Bahia (7.050 toneladas em 2.096 ha) e Pernambuco (4.309 toneladas em 1.541 ha) (ABCC, 2013).

Em Pernambuco podemos ressaltar os municípios de Recife (73 produtores, produção de 597 toneladas em 221 ha de lamina d água), Ilha de Itamaracá (36 produtores, produção de 240 toneladas em 113 ha de lamina d água) e Goiana (14 produtores, produção de 2.078 toneladas em 1.037 ha de lamina d água), que juntos são reponsáveis por 67,6% da produção Estadual. Esta produção também é caracterizada pelas baixas densidades de estocagem, onde 62% dos produtores utilizam densidades próximas a 10 camarões/m^2 (ABCC, 2013).

No entanto, para que a carcinicultura aumente a produção de forma sustentável, é indispensável à busca por sistemas de cultivos alternativos, sobretudo, que previnam a entrada de patógenos e que favoreçam melhores resultados zootécnicos. Tudo isso, porque a legislação ambiental brasileira (Lei 12.727 e Resoluções CONAMA) restringe o aumento das áreas de cultivo de camarão marinho. Por este motivo, para aumentar a produção e oferta do produto são necessários sistemas de cultivos mais eficientes e com menor geração de resíduos (GUERRELHAS et al., 2011; GUERRELHAS e TEIXEIRA, 2012), ambientalmente e economicamente sustentáveis (FUNGE-SMITH e BRIGGS, 1998; KUHN et al., 2010a).

Segundo Immanuel et al. (2010 e 2012), o forte impacto causado pelo WSSV, exige dos produtores novas estratégias de cultivo, que melhorem a atividade imune dos camarões. Esta incidência de enfermidades geralmente ocorre quando não são seguidas práticas de manejo sustentável, sendo altamente recomendado que esta atividade seja bem planejada e executada, objetivando manter uma boa condição de saúde dos animais cultivados (HERNÁNDEZ e NUNES, 2000).

2.2 Sistema de Bioflocos

A crescente demanda por produtos pesqueiros e a queda na captura dos mesmos tem resultado no aumento significativo da aquicultura nos últimos anos. Além disso, a população humana continua com crescimento acelerado, sendo necessário que a indústria alimentícia desenvolva ferramentas que maximizem a produção de alimentos.

A aquicultura não foge a esta regra, mas este crescimento deve ser influenciado por tecnologias economicamente e ambientalmente sustentáveis, neste sentido, o sistema de bioflocos pode ser uma alternativa viável (AVNIMELECH, 2009; CRAB et al., 2012; PÉREZ-FUENTES et al., 2013).

Entre os principais critérios para justificar a utilização do sistema de bioflocos estão: à produção de organismos aquáticos de forma sustentável, dentro dos padrões de biossegurança, redução de efluentes e utilização de pequenas áreas para a realização dos cultivos (AVNIMELECH, 2000; DE SCHRYVER et al., 2008; CRAB et al., 2012).

O sistema de bioflocos foi desenvolvido simultaneamente em Israel e nos Estados Unidos no inicio dos anos 90 (AVNIMELECH, 2005). Belize Aquaculture (Belize) foi a primeira fazenda comercial a utilizar este sistema de cultivo com sucesso. Na primeira tentativa a produção foi de 13,5 toneladas/camarões/ha, posteriormente, alcançando uma média de produção de 20 toneladas/camarão/ha (TAW, 2010). Outra referência na produção de camarões em bioflocos é o Waddell Mariculture Center (Estados Unidos), onde os camarões são cultivados em altas densidades (> 300 camarões/m^2), obtendo-se sobrevivência superior a 70% e crescimento semanal de aproximadamente 1,5 g (WASIELESKY et al., 2006; VENERO et al., 2009).

Em sistema de bioflocos são utilizadas elevadas densidades de estocagem, tanques revestidos com polietileno de alta densidade, controle da alimentação, intensa aeração, adição de carbono orgânico, pouca ou nenhuma troca de água durante a época de cultivo

(CHAMBERLAIN et al., 2001; EBELING e TIMMONS, 2008; GAO et al., 2012; POERSCH et al., 2012).

Os bioflocos são macroagregados formados por bactérias, microalgas, microflagelados, zooplâncton, nematoides, fungos, fezes e exoesqueleto de animais mortos, que podem contribuir substancialmente como uma fonte de suplementação alimentar, promovendo uma maior taxa de crescimento, aumento do peso final e redução do fator de conversão alimentar para camarões e peixes onívoros (MOSS, 1995; MOSS e PRUDER, 1995; BURFORD et al., 2003, 2004; JOHNSON et al., 2008; RAY et al., 2010a e 2012; FRÓES et al., 2012; ZHAO et al., 2012; PÉREZ-FUENTES et al., 2013).

Os camarões podem consumir bactérias (AVNIMELECH, 1999; MOSS et al., 2000), fitoplâncton (KENT et al., 2011; OTOSHI et al., 2011; GODOY et al., 2012) e zooplâncton (DECAMP et al., 2003 e 2007, LOUREIRO et al., 2012) agregados aos bioflocos, e estes têm aumentado significativamente as taxas de crescimento dos camarões (KUHN et al., 2010a; AUDELO-NARANJO et al., 2012). Além disso, melhorar a eficiência das enzimas protease, lipase, amilase, celulase, tripsina (XU et al., 2013; XU e PAN, 2012; YU et al., 2013), e a resposta imune dos camarões da espécie *L. vannamei* (XU e PAN, 2013a).

No sistema de bioflocos, a adição de carbono orgânico na coluna da água, serve de substrato para as bactérias heterotróficas transformarem os nutrientes dissolvidos provenientes da ração não consumida e do material fecal, em proteína microbiana. Proteína esta, que volta a ser disponibilizado para a alimentação dos camarões (AVNIMELECH, 1999, 2007, 2009; SAMOCHA et al., 2007; ASADUZZAMAN et al., 2010a; RAY et al., 2010b; CRAB et al., 2012).

Diversos tipos de elementos nutricionais foram observados nos bioflocos, como proteína bruta e lipídios (Tabela 1), incluindo os ácidos graxos poliinsaturados (PUFAs), minerais e vitaminas, são outros elementos nutritivos encontrados nos bioflocos (TACON et al., 2002). Apesar da presença dos ácidos graxos (PUFAs) na composição dos bioflocos, Crab et al. (2010) utilizando diferentes fontes de carbono observaram baixas concentrações de ácido linoleico, eicosapentaenóico (EPA) e docasahexanóico (DHA), porém foram altas as concentrações de ácido palmítico. Este valor nutricional pode ser influenciado pela composição da microbiota dos flocos (JU et al., 2008, 2009), salinidade da água (EKASARI et al., 2010; MAICA et al., 2012), temperatura, concentração de oxigênio dissolvido e fonte de carbono orgânico (PHULIA et al., 2012).

Além disso, é possível reduzir os níveis de proteína das rações para camarões (BALLESTER et al., 2010; MEGAHED, 2010; XU et al., 2012, 2013b), pois existe uma tendência da utilização de altos níveis de proteína, acreditando-se que esta prática acelera o crescimento (MARTINEZ-CÓRDOVA et al., 2003), consequentemente, aumentando os custos de produção, pois a ração é o item de maior custo na indústria de produção animal (NUNES et al., 2011b). Somando a isso, rações com altos níveis de proteína possuem baixa relação carbono: nitrogênio (C:N <10), que desfavorece a decomposição pelas bactérias dos compostos nitrogenados, resultando no acúmulo dos mesmos no ambiente de cultivo (AVNIMELECH, 2009; BALLESTER et al., 2010).

Outro importante fator é a possibilidade de redução da utilização de farinha de peixe e óleo de peixe nas rações (KUHN et al., 2010a; RAY et al., 2010b; SCOPEL et al., 2011; BAUER et al., 2012), pois o setor da aquicultura já no ano de 2006, consumiu 68,2% (3.724.000 toneladas) da farinha de peixe produzida no mundo e 88,5% (835.000 toneladas) de óleo de peixe (TACON e METIAN, 2008), pois estes componentes são onerosos e os estoques naturais de peixes estão sob explorados (NAYLOR et al., 2000; NONWACHAI et al., 2010).

Tabela 1. Composição centesimal dos bioflocos.

Referência	**% proteína matéria seca**	**% lipídio matéria seca**
Wasielesky et al. (2006)	31	0,4
Emerenciano et al. (2007)	30	0,4
Ballester et al. (2010)	30	4
Crab et al. (2010)	28 – 43	2- 5
Ekasari et al. (2010)	28 – 31	6 - 9
Kuhn et al. (2010b)	35 – 49	0 – 1
Megahed (2010)	19 – 20	11
Emerenciano et al. (2011)	30	0,5
Maica et al. (2011)	28 – 43	2 - 3
Xu et al. (2012)	25 – 31	2
Emerenciano et al. (2013)	24	0,6
Silva et al. (2013a)	23 – 25	2-7
Schveitzer et al. (2013)	18 – 28	1,6 – 3,2
Xu e Pan (2013b)	21 – 32	1,6 – 2,8

O manejo para formação dos bioflocos é através da manipulação da relação carbono-nitrogênio (C/N) (AVNIMELECH, 1999, 2009). Este processo de transformação ocorre com aumento da carga orgânica, seguindo os passos: água clara, bloom de algas, grande quantidade de espumas na superfície, acúmulo de material orgânico dissolvido, mudança na coloração da água para marrom, desaparecimento das espumas e surgimento dos flocos (CHAMBERLAIN et al., 2001; AVNIMELECH, 2009; KUBTIZA, 2011).

Dois métodos primários são utilizados para formação dos bioflocos, adição de fontes de carbono orgânico diretamente na água ou uso de alimentos com especial taxa C/N (AVNIMELECH, 1999, 2009). Açúcar (XU et al., 2012; XU et al., 2013), melaço de cana de açúcar (SCHNEIDER et al., 2006; MAIA et al., 2012; BALOI et al., 2013), farelo de trigo (CAMPOS et al., 2007; MEGAHED, 2010), farelo de milho (AVNIMELECH e KOCHBA, 2009; ASADUZZAMAN et al., 2010a), acetato (CRAB et al., 2010; PHULIA et al., 2012) e glicerol (EKASARI et al., 2010; CRAB et al., 2010) são fontes de carbono orgânico que podem aumentar a relação C/N.

O controle da relação C/N através da entrada de alimento e/ou dos hidratos de carbono causam um grande impacto na comunidade microbiana. Uma maior quantidade de carbono orgânico no sistema (alta C/ N) conduz a uma maior taxa de assimilação de amônia nas proteínas celulares de bactérias heterotróficas (EBELING et al., 2006; PANJAITAN, 2010).

Os baixos níveis de carbono orgânico significam que o nitrogênio necessário para o crescimento bacteriano deve ser obtido a partir da coluna de água, o que ajuda a melhorar a qualidade da mesma (HARI et al., 2004, 2006; NEAL et al., 2010). No entanto, uma menor relação C/N faz com que não ocorra um número tão elevado de bactérias heterotróficas, assim, há uma maior disponibilização de nutrientes para as microalgas, que são capazes de estabelecer uma maior população (EBELING et al., 2006; HARGREAVES, 2006; ASADUZZAMAN et al., 2010b). Tem sido sugerida uma relação C/N acima de 10, para favorecer uma condição heterotrófica, porém abaixo desta relação tende-se a favorecer uma condição mais autotrófica (AVNIMELECH, 1999, 2009; PANJAITAN, 2010).

Os microrganismos formadores dos bioflocos atuam na ruptura de produtos de resíduos a compostos mais simples, menos tóxicos, além de servirem como alimento para os camarões (BURFORD et al., 2003, 2004). Entretanto, partes dos nutrientes (7,7% do nitrogênio inorgânico dissolvido, 31,25% do nitrogênio orgânico dissolvido, 17,3% do fósforo inorgânico dissolvido e 16,8% do fósforo orgânico dissolvido) podem ficar acumuladas no sistema de bioflocos (SILVA et al., 2013b). Além disso, já foram observados picos de amônia total superior 2,0 mg/L, altas concentrações de nitrito e nitrato, além do acúmulo de fosfato (COHEN et al., 2005; AZIM e LITTLE, 2008; EMERENCIANO et al., 2011; KRUMMENAUER et al., 2012).

Os compostos nitrogenados (amônia e nitrito) são potencialmente tóxicos para os animais aquáticos (RAY et al., 2011), sendo um dos fatores limitantes para o crescimento e sobrevivência dos camarões (CHEN e LIN, 1992; EBELING et al., 2006), afetando o consumo de oxigênio, muda e excreção da amônia (CHEN e LIN, 1992) e supressão da imunocompetência (HARGREAVES, 1998).

A máxima concentração da amônia para camarões do gênero *Litopenaeus* vária em função do tempo de exposição, estágio de vida, da salinidade, temperatura, pH é concentração de oxigênio dissolvido da água (KIR e KUMLU, 2006; BARBIERI, 2010; COBO et al., 2012). Existem três caminhos para remoção da amônia em sistema aquícola: algas, bactérias nitrificantes e bactérias heterotróficas (EBELING et al., 2006; BOYD, 2008; CASTILLO-SORIANO et al., 2013). Neste sentido, estes microorganismos tem fundamental importância para manter a boa qualidade do ambiente de cultivo (RAY et al., 2011).

Apesar dos avanços feitos nos últimos anos referentes ao sistema de bioflocos, são necessárias pesquisas nas áreas de seleção e posicionamento dos aeradores; avaliação dos sistemas integrados; identificação dos microrganismos com características benéficas que possam ser inoculados; manipulação das características nutricionais dos bioflocos e determinação do impacto das diferentes fontes de carbono na qualidade e característica do biofloco (CRAB et al., 2012).

2.3 Aquicultura Multitrófica Integrada

O conceito de aquicultura integrada surgiu em meados de 1970, como ferramenta para o tratamento dos resíduos dos efluentes, porém, no início de 1990, este conceito surgiu renovado, agora não apenas para reduzir o excesso de nutrientes e matéria orgânica gerada pelos sistemas intensivos de aquicultura, mas também para diversificar a produção, aumentar o retorno econômico e proporcionar a sustentabilidade dos empreendimentos (BARRINGTON et al., 2009, 2010; SOTO, 2009; TROEL, 2009). Segundo Angel e Freeman (2009) os cultivos integrados são definidos como o cultivo de duas os mais espécies de diferentes níveis tróficos em uma unidade de cultivo, ou em estreita proximidade, para que eles possam interagir em relação ao fluxo de energia.

Este conceito torna-se cada vez mais importante, principalmente pelo crescimento significativo da aquicultura mundial ao longo dos últimos anos, que está atrelada as práticas intensivas, resultando em muitos casos, no aumento da geração de resíduos, além dos desafios ambientais e sociais. Neste sentido, os sistemas integrados podem atenuar alguns destes problemas associados à monocultura das espécies (TROELL et al., 2009; LANDER et al., 2013).

O sistema integrado utilizar moluscos, macroalgas e outros organismos, sendo conhecido pela sigla IMTA ("Integrated multi-trophic aquaculture"). É uma estratégia de baixo custo para minimizar os resíduos de compostos nitrogenados e fosfatados, reduzindo a possibilidade de surtos de doenças, além de incrementar a renda e mitigar os problemas ambientais gerados pelas diversas atividades da aquicultura (ANGEL e FREEMAN, 2009; SOTO, 2009; TROELL et al., 2009; BARRINGTON et al., 2009, 2010; BUTTERWORTH, 2010; REN et al., 2012; LANDER et al., 2013). Porém, é necessária uma combinação apropriada das espécies para a transformação de resíduos em biomassa, proporcionando o equilíbrio ambiental, econômico e social (BARRINGTON et al., 2009; BUTTERWORTH, 2010). Para a escolha das espécies, devem ser levadas em consideração as funções que cada organismo desempenha no ecossistema, seu potencial econômico e a sua aceitação no mercado consumidor (TROELL et al., 2009; BARRINGTON et al., 2010). Além da sustentabilidade ambiental e econômica, os sistemas integrados podem aumentar a aceitabilidade dos produtos aquicolas.

Segundo Barrington et al. (2009) os requesitos necessários para garantir a sustenbilidade do sistema IMTA são a escolha de espécies nativas com tecnologia disponível, transformando resíduos em biomassa, melhorando com isso, a qualidade da água e o crescimento da espécie principal. Além disso, as espécies devem ser regulamentadas pelas agências ambientais para favorecer a sustentabilidade do sistema.

África do Sul, Canadá, Chile, China, Estados Unidos, Grã-Bretanha, Irlanda do Norte são exemplos de países que executam a aquicultura integrada em escala comercial. França, Portugal e Espanha têm projetos de pesquisa relacionados ao tema (BARRINGTON et al., 2009). Os principais grupos com potencial para os sistemas integrados são as macroalgas, moluscos bivalves, poliquetas, equinodermatas, crustáceos (camarões e lagostas) e os peixes marinhos de importância comercial (TROEL et al., 2009).

Nos últimos anos é possível observar um rápido crescimento em pesquisas relacionadas a cultivos integrados de macroalgas com peixes e camarões, em sistemas tradicionais (HUO et al., 2012), principalmente pela associação da degradação ambiental proporcionada pelos sistemas aquicolas (TROELL et al., 2009), aumentos dos custos com combustíveis, água e outros insumos, estão estimulando o interesse na produção eco-eficiente que minimizem o consumo de recursos naturais e redução da poluição (TROELL, 2009). Estes sistemas de cultivos integrados tem a vantagem de possuir características mais similares ao ambiente natural (MARINHO-SORIANO et al., 2009a), além de melhorar o crescimento e produção das espécies cultivadas (TROELL, 2009).

Este sistema integrado torna-se cada vez mais importante, pois segundo Henry-Silva e Camargo (2008), à medida que as normas ambientais se tornam mais rigorosas, a administração e a eliminação dos resíduos serão cada vez mais importantes nas atividades de aquicultura.

Os principais benefícios dos sistemas integrados são o equilíbrio da produção com sustentabilidade ambiental, através da redução de efluentes, prevenção de doenças, diferenciação de produto pela certificação e diversificação (BARRINGTON et al., 2009, 2010; TROELL, 2009; YOKOYAMA e ISHIHI, 2010).

2.4 Macroalgas na Aquicultura

A produção mundial de macroalgas foi de 19 milhões de toneladas em 2010, onde 95,5% desta produção foram provenientes de cultivos, gerando aproximadamente U$$ 57 bilhões. Os principais países produtores em 2010 foram: China (58,4%, 11,1 milhões de toneladas), Indonésia (20,6%, 3,9 milhões de toneladas), Filipinas (9,5%, 1,8 milhões de toneladas), República da Coreia (4,7%, 901.700 toneladas), República Popular Democrática da Coréia (2,3%, 444.300 toneladas), Japão (2,3%, 432.800 toneladas), Malásia (1,1%, 207.900 toneladas) e a República Unida da Tanzânia (0,7%, 132.000 toneladas), representando 99,6% da produção mundial. Os principais grupos cultivados foram: *Laminaria japonica*, *Kappaphycus alvarezii*, *Eucheuma* spp., *Gracilaria* spp., *Porphyra* spp., *Undaria pinnatifida*, *Fusiform sargassum* e *Caulerpa* spp. (FAO, 2012).

Os principais sistemas de cultivo são: balsas, long-line ou gaiolas, porém a escolha da estrutura de cultivo depende da correnteza, do fluxo e refluxo da água, do tipo de fundo, da profundiade do ambiente, do acesso ao local, dos fenomenos naturais e conflitos com outras atividades (SEAP, 2003).

As macroalgas podem ser utilizadas para a produção de ficocolóides e de substâncias bioactivas (MARINHO-SORIANO et. al., 2011), matéria prima para as indústrias de fármacos e cosméticos (NEORI et al., 2004), fabricação de papel, fertilizantes agrícolas, tratamento de vermes, aglutinantes de rações (PAGAND, 1999), consumo humano (SANTOPRETE e BERNI, 2011), alimentação animal, biomassa para combustivel, industria têxtil e remoção de metais tóxicos (MCHUGH, 2003).

Outra função das macroalgas é a contribuição da biorremediação de nutrientes da água, pois segundo Xu et al. (2008a) entre 52-95% do nitrogênio e 85% do fósforo que entram via ração nos sistemas de aquicultura tradicional são perdidos no ambiente. Já Avnimelech (2009) descreve que apenas 25% dos nutrientes adicionados como alimento peletizados são incorporados pelos animais.

Entretanto, esta incorporação pode variar com a espécie, sistema de cultivo e o nível de proteína das rações; Hari et al. (2006) encontrou em sistema extensivo de

Penaeus monodon com diferentes níveis de proteína e adição de carboidrato na água uma incorporação de 16 a 21 % de nitrogênio na biomassa despescada. Teichert-Coddington et al. (1999) e Briggs e Funge-Smith (1994) encontraram em sistema semi-intensivo com *L. vannamei* incorporação de 14 e 18% do nitrogênio do sistema, respectivamente. Em relação a sistemas intensivos, Jackson et al. (2003) no cultivo de *P. monodon* encontraram entre 21 e 22% e Thakur e Lin (2003) entre 23 a 31%, já para o cultivo de *L. vannamei* em sistema de bioflocos, Silva et al. (2013b) encontraram 39% nitrogênio e 35% fósforo retido na biomassa despescada do camarão.

O restante dos nutrientes não assimilados pelos camarões pode ser disponibilizado em forma de efluentes, gerando eutrofização dos corpos adjacentes. Para tratar ou minimizar as descargas de nutrientes, podemos utilizar macroalgas que podem transformar estes resíduos em biomassa, contribuindo significativamente para biorremediação da água de cultivo (TROELL et al., 2003; MATOS et al., 2006; NEORI, 2008; HE et al., 2008; ROCHA et al., 2008; RAMOS et al., 2008; MARINHO-SORIANO et al., 2009 a,b, 2011; ALENCAR et al., 2010; HUO et al., 2011, 2012; SHUKRI e SURIF, 2011; SKRIPTSOVA e MIROSHNIKOVA, 2011; NIRMALA et al., 2012; ROBLEDO et al., 2012; CARTON-KAWAGOSHI et al., 2013).

As macroalgas podem absorver entre 34% a 94% da amônia da água, 11 a 57% do nitrito, 7 a 100% do nitrato e entre 34 e 93% do fosfato (Tabela 2). Segundo Jones et al. (2001, 2002) a utilização de macroalgas e/ou moluscos bivalves como organismos biorremediadores é uma importante ferramenta para redução de nutrientes dos cultivos de camarão, contribuindo com a sustentabilidade da atividade. Esta biorremediação reduz os impactos aos ambientes adjacentes e não denegrir a imagem da atividade perante alguns ramos da sociedade civil, apesar da aqüicultura poluir menos que diversas outras atividades ligadas à produção de alimentos (BRITO et al., 2004). Alonso

– Rodrigues e Páez–Osuna (2003) relatam que os efluentes da carcinicultura apresentam melhores qualidades físicas e químicas da água comparada às descargas domésticas tratadas.

Esta remoção de compostos nitrogenados e fósforo pelas macroalgas melhoram a condição da água de cultivo, proporcionando incremento nos resultados de crescimento e produção dos camarões (XU et al., 2008b; KHOI e FOTEDAR, 2011). Porém ocorre redução da taxa de remoção de nutrientes da água durante o período de cultivo (MAI et al., 2010), pois fatores como concentração inicial de amônia e fosfato, salinidade, temperatura, turbidez, ciclo de vida, taxa de crescimento e biomassa de estocagem das macroalgas, interferem na eficiência de absorção (CHOPIN et al., 2001; THI et al., 2012; DU et al., 2013).

Outro fator importante da utilização das macroalgas é sua utilização como fonte de alimento, seja pela pastagem diretamente das macroalgas e/ou do biofilme que se forma na área superficial (LOMBARDI et al., 2006). O biofilme é definido como a comunidade de microrganismos compostos pelos organismos autotróficos e heterotróficos associados a uma matriz celular aderidas a um substrato submerso (VIAU et al., 2013), que além de contribuir como fonte de alimento para camarões, também melhora a qualidade da água de cultivo (AUDELO-NARARJO et al., 2011; ANAND et al., 2012). O aumento do crescimento dos camarões em sistemas tradicionais integrado com macroalgas já foram descritos para as espécies: *L. vannamei* (CRUZ-SUÁREZ et al., 2010; PEÑA-RODRIGUEZ et al., 2010; GAMBOA-DELGADO, 2011), *P. monodon* (TSUTSUI et al., 2010; IZZATI, 2011) e *Farfantepenaeus californiensis* (PORTILLO-CLARK et al., 2012).

Tabela 2. Eficiência na remoção de nutrientes das macroalgas dos gêneros *Ulva* e *Gracilaria*.

Referência	**Espécies**	**% remoção Amônia**	**% remoção Nitrito**	**% remoção Nitrato**	**% remoção Fosfato**
Ramos et al. (2008)	*U. fasciata*	38	11	27	49
Copertino et al. (2009)	*U. clathrata*	70 - 82	-	-	50
Marinho-Soriano et al. (2009a)	*G. caudata*	59	-	-	-
Marinho-Soriano et al. (2009b)	*G. birdiae*	34	-	100	93
Alencar et al. (2010)	*U. lactuca*	94	-	-	-
Ramos et al. (2010)	*U. fasciata*	49	31	-	-
Shukri e Surif (2011)	*G. manilaensis*	83	33	68	-
Skriptsova e Miroshnikova (2011)	*G.vermiculophyla*	73	-	-	34
Marinho-Soriano et al. (2011)	*G. caudata*	-	57	70	-
Khoi e Fotedar (2011)	*U. lactuca*	59 - 81	-	-	-
Abreu et al. (2011)	*G. vermiculophylla*	80	-	-	-
Huo et al. (2011)	*G. verrucosa*	54	49	75	49
Al-Hafedh et al. (2012)	*U. lactuca*	80	-	-	41
	G. arcuata	83	-	-	41
Robledo et al. (2012)	*G. córnea*	61 - 88	23	-	-

Além disso, a utilização das macroalgas em sistema integrado com camarão, favorecer maior transformação do nitrogênio do sistema de cultivo em biomassa de camarão (KHOI e FOTEDAR, 2011), aumentando os níveis do ácido docosahexaenoico (DHA), ácido eicosapentaenoico (EPA) e caratenóides nos tecidos dos camarões (CRUZ-SUAREZ et al., 2010). Além da prevenção do bloom do fitoplâncton, aumentando a diversidade das espécies (HUO et al., 2011; MARINHO-SORIANO et al., 2011).

As macroalgas do gênero *Ulva* (NUNES, 2002; TYLER et al., 2005; COPERTINO et al., 2009; YOKOYAMA e ISHIHI, 2010; AL-HAFEDH et al., 2012; SÁNCHEZ et al., 2012) e *Gracilaria* (NEORI et al., 2004; ABREU et al., 2011; SHUKRI e SURIF, 2011; SKRIPTSOVA e MIROSHNIKOVA, 2011; ROBLEDO et al., 2012) são as mais promissoras para o cultivo integrado com camarões, pois são eficientes na remoção de nutrientes e podem ser fonte de alimento suplementar para os camarões marinhos.

As macroalgas do gênero *Ulva*, são algas verdes da família Ulvaceae, possuem bom nivel de proteína bruta e lipídios (Tabela 3). A espécie *U. lactuca* possui 10,69g de proteína bruta e 0,99g de lipídios a cada 100g de peso seco (TABARSA et al., 2012). Já as macroalgas do gênero *Gracilaria* são algas vermelhas do filo Rhodophyta e também possuem bom nível de proteína bruta e lipídios (Tabela 3). A espécie *G. birdiae* possui 12,6g de proteína e 1,1g de lipídios totais e *G. domingensis* possui 16,6g de proteína e 1,6g de lipídios totais a cada 100g de peso seco (FRANÇA-PIRES et al., 2012a,b). Entretanto, a composição centesimal das macroalgas pode variar de acordo a espécie, habitat e condições ambientais, como salinidade, temperatura e concentração de nutrientes na água de cultivo (MARINHO-SORIANO et al., 2006; CRUZ-SUÁREZ et al., 2010).

Outra importante função das macroalgas e a presença dos componentes farmacológicos e bioativos como: β-glucano, carragenina, sulfatos, laminarina e polissacarídeos (galactose), que possuem atividade antibacteriana e antiviral. Estes componentes quando absorvida pelos camarões, aumenta a resposta imune dos mesmos (HUYNH et al., 2011; SIRIRUSTANANUN et al., 2011; CRUCES et al., 2012; PESO-ECHARRI et al., 2012;SILVA et al., 2013c), reduzindo as taxas de mortalidade por doenças bacteriana e viral, que causam grande mortalidades. Esta característica torna ainda mais importante, pois estes componentes podem ser uma alternativa ao uso indiscriminado de antibióticos que pode aumentar a resistência dos patógenos, além do alto custo do uso e da restrição pela legislação (KANDHASAMY e ARUNACHALAM, 2008).

Alguns estudos relatam que os extratos de macroalgas via alimentação ou banhos de imersão têm aumentado à sobrevivência dos camarões infectados por *Vibrio*: *Sargassum fusiforme* (HUANG et al., 2006); *Gelidium amnassi* (FU et al., 2007); *Sargassum hemiphyllum* (HUYNH et al., 2011); *Gracilaria fisheri* (KANJANA et al. 2011); *Ulva fasciata* (SELVIN et al., 2011); *Gracilaria tenuistipitata* (SIRIRUSTANANUN et al., 2011), por vírus: *Sargassum* spp. (IMMANUEL et al., 2010, 2012); *Sargassum hemiphyllum* (HUYNH et al., 2011); *Gracilaria tenuistipitata* (LIN et al., 2011); *Gracilaria tenuistipitata* (SIRIRUSTANANUN et al., 2011); *Sargassum wightii* (SIVAGNANAVELMURUGAN et al., 2012) (Tabela 4).

Segundo Portillo-Clark et al. (2012) os cultivos integrado podem melhorar a qualidade da água, aumentar o crescimento dos camarões e a sua resistência a doenças, no entanto, é necessário mais trabalhos para determinar as espécies que são mais adequadas, pois o exato papel na nutrição dos camarões não é conhecida e a sua utilização para aumentar a produção de cultivo ainda encontra-se em fase experimental.

Tabela 3. Composição centesimal das macroalgas dos gêneros *Ulva* e *Gracilaria*.

Referência	Espécies	Proteína[1]	Lipidios[1]	AE[2]	PUFA[3]	Cinzas[1]
Marinho-Soriano et al. (2006)	*G. cervicornis*	197	4	-	-	105
Marinho-Soriano et al. (2007)	*G. cervicornis*	230	4	-	-	80
Cruz-Suarez et al. (2009)	*U. clathrata*	234	10	-	-	160
Cruz-Suarez et al. (2010)	*U. clathrata*	207	15	-	24	384
Gressler et al. (2010)	*G.birdiae*	71	1,3	-	29,9	225
	G. domingensis	62	1,3	-	14,8	238
Peña-Rodriguez et al. (2011)	*U. clathrata*	210 - 250	25 – 35	316- 339	30-34	448-496
Tabarsa et al. (2012)	*G. cervicornis*	95	20	520	17	389
	U. lactuca	100	9	258	24	180
França Pires et al. (2012a)	*G. birdiae*	126	11	-	-	55
França Pires et al. (2012b)	*G. domingensis*	166	16	-	-	124

[1] g/Kg de peso seco;

[2] Aminoácidos essenciais – mg/g de proteína;

[3] % do lipídio total, PUFA- Ácidos graxos poliinsaturados.

Tabela 4. Utilização de extratos das macroalgas via alimentação ou banhos de imersão no aumento da sobrevivência dos camarões submetidos aos diferentes desafios.

Referência	Macroalgas	Via	Desafios	Dose
Huang et al. (2006)	*Sargassum fusiforme*	Alimentação	*Vibrio harveyi*	0,5 – 2,0 % ração
Barroso et al. (2007)	*Botryocladia occidentalis*	Imersão	Sobrevivência e crescimento	0,5 -1,0 µg/L
Fu et al. (2007)	*Gelidium amansii*	Imersão	*Vibrio alginolyticus*	200 – 600 mg/L
	Gelidium amansii	Alimentação	*Vibrio alginolyticus*	0,5 – 2,0 g/Kg ração
Lima et al. (2009)	*Spatoglossum schroederi*	Imersão	Estresse por hipóxia	0,5 - 2,0 mg/L,
Immanuel et al. (2010)	*Sargassum* spp.	*Artemia*	Vírus da mancha branca	250 – 750 mg/L
Yeh et al. (2010)	*Gracilaria tenuistipitata*	Imersão	Estresse por salinidade	200 – 600 mg/L
Huynh et al. (2011)	*Sargassum hemiphyllum*	Imersão	*Vibrio alginolyticus* e Vírus da mancha branca	100 – 500 mg/L
Kanjana et al. (2011)	*Gracilaria fisheri*	*Artemia*	*Vibrio harveyi*	0,5 e 1,0 mg/L
Lin et al. (2011)	*Gracilaria tenuistipitata*	Imersão	Vírus da mancha branca	400 e 600 mg/L
Sirirustananun et al. (2011)	*Gracilaria tenuistipitata*	Alimentação	*Vibrio alginolyticus* e Vírus da mancha branca	0,5 – 2,0 g/Kg ração
Selvin et al. (2011)	*Ulva fasciata*	Alimentação	*V. fischeri, alginolyticus, harveyi*	0,5 – 1,5 g/Kg ração
Chen et al. (2012)	*Gracilaria tenuistipitata*	Alimentação	Exposição a amônia (5mg/L)	0,5 – 2,0 g/Kg ração
Immanuel et al. (2012)	*Sargassum wightii*	Alimentação	Vírus da mancha branca	0,1 – 0,3 % ração
Sivagnanavelmurugan et al. (2012)	*Sargassum wightii*	*A.franciscana*	Vírus da mancha branca	100 – 400 mg/L

* Os resultados obtidos nos diferentes desafios citados aumentaram a sobrevivência e a resposta imune dos camarões.

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4- Artigos Científicos

4. 1 - Artigo Científico I

Water quality and growth of juvenile Pacific white shrimp *Litopenaeus vannamei* (Boone) in co-culture with green alga *Ulva lactuca* (Linaeus) in intensive system

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estabelecidas pela referida revista (em anexo).

Water quality and growth of Pacific white shrimp *Litopenaeus vannamei* (Boone) in co-culture with green seaweed *Ulva lactuca* (Linaeus) in intensive system.

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Abstract An indoor trial was conducted for 28 days to evaluate the effects and interactions of biofloc and seaweed *Ulva lactuca* in water quality and growth of Pacific white shrimp *Litopenaeus vannamei* in intensive system. *L. vannamei* (4.54 ± 0.09 g) were stocked in experimental tanks at a density 132 shrimp m^{-2} (566 shrimp m^{-3}) and the *Ulva lactuca* was stocked at a density 0.46 kg m^{-2} (2.0 Kg m^{-3}). Biofloc with seaweed (BF-S) significantly reduced ($P < 0.05$) total ammonia nitrogen (TAN) by 25.9 %, nitrite-nitrogen (NO_2-N) by 72.8%, phosphate (PO_4^3-P) by 24.6% and total suspended solids by 12.9% in the water and significantly increased ($P < 0.05$) settleable solids by 34.2% and final weight of shrimp by 6.9 % as compared to biofloc without seaweed (BF-WS). The BF-S can contribute by reducing nitrogen compounds (TAN and NO_2-N), phosphate (PO_4^3-P) and total suspended solids in water and increased final weight of shrimp.

Keywords: Biofloc • Seaweed • Shrimp • Water quality • Growth

Abbreviations

LCM - Marine Shrimp Laboratory

TAN – Total ammonia nitrogen

NO_2-N – Nitrite-nitrogen

PO_4^{3}–P – Phosphate

TSS – Total suspended solids

SGR - Specific growth rate

FCR - Feed conversion ratio

BF-S - Biofloc with seaweed

BF-WS - Biofloc without seaweed

WBF-S – Without biofloc with seaweed

WBF-WS - Without biofloc and without seaweed

SS – Settleable solids

Introduction

Studies about nitrogen intake by shrimp through feed report rates of 16 to 31 %, depending on the amount of protein in the feed, the species of shrimp and the culture system (Jackson et al. 2003; Thakur and Lin 2003). In biofloc tank, only 35 % of phosphorus and 39.1% of nitrogen added as pelletized feed and organic carbon are incorporated by shrimp and the rest remain can lead to eutrophication of the system (Silva et al. 2013).

Inefficient nitrogen use by organisms implies an increase in the nitrogen content in the water, which can have a negative impact on the quality and growth of shrimp (Hargreaves 1998). According to Ebeling et al. (2006), total ammonia nitrogen (TAN) concentration from feed is a major limiting factor to production in intensive systems. A reduced water renewal rate and the recycling of nutrients within the tank water are strategies used to minimize the loss of nutrients and the discharge of effluents from shrimp farms (Samocha et al. 2011). In biofloc, nutrients can be incorporated into bacterial biomass, which serves as feed for shrimp (Wasielesky Jr et al. 2006; Avnimelech 2009; Ray et al. 2010; Emerenciano et al. 2011; Xu et al. 2012).

One option for the removal of these nutrients from water is the use of seaweed *Ulva* (Ramos et al. 2008, 2010; Copertino et al. 2009; Alencar et al. 2010; Yokoyama and Ishihi 2010; Khoi and Fotedar 2011; Al-Hafedh et al. 2012; Sánchez et al. 2012; Thi et al. 2012), which produce compounds that help to prevent disease outbreaks (*Vibrio*) in marine shrimp (Selvin et al. 2011). The seaweed can also serve as a food source for shrimp species, such as *Litopenaeus vannamei* (Cruz-Suárez et al. 2010), *Penaeus monodon* (Tsutsui et al. 2010; Izzati 2011) and *Farfantepenaeus californiensis* (Portillo-Clark et al. 2012). However, the determination of the exact role seaweed play in the enhancement of production in shrimp farms is still in the experimental phase (Portillo-

Clark et al. 2012). The aim of this study was to evaluate the effects and interactions of biofloc and seaweed *Ulva lactuca* on the water quality and growth of Pacific white shrimp *Litopenaeus vannamei* in intensive system.

Materials and methods

The experiment was carried out for a period 28 days during November to December 2011 at Marine Shrimp Laboratory (LCM) of the Federal University of Santa Catarina (UFSC) (Brazil) (27º 34’ 56”S, 048º 26’ 27”W). A 2 x 2 factorial design was employed analyzing Factor 1 (with/without biofloc) and Factor 2 (with/without seaweed) in the following treatments: biofloc with seaweed (BF-S); biofloc without seaweed (BF-WS); without biofloc with seaweed (WBF-S); and without biofloc and without seaweed (WBF-WS).

Juvenile Pacific white shrimp *L. vannamei* (4.54 ± 0.09 g) were obtained from LCM and stocked at a density of 132 shrimp m^{-2} (566 shrimp m^{-3}). *Ulva lactuca* biomass was collected from the Barra da Lagoa Beach (27º 34’ 26.16¨S, 048º 25’ 21.46¨W), and stocked at a density 0.46 kg m^{-2} (2.0 Kg m^{-3}). The seaweed was washed at the laboratory with filtered seawater. The water was drained and the material was carefully inspected to eliminate encrusted organisms and then weighed (Tsutsui et al. 2010).

Twelve black-plastic 0.64 m^{-2} (0.15 m^{-3}) tanks were filled with seawater (35 ppt) filtered through a 0.5-µm sieve and maintained under constant aeration by perforated 20 cm PVC pipes. The light intensity was kept at ~ 2000 lux using a metal halide lamp with a 12-h light/dark photoperiod. The temperature was maintained around 27 °C with a 100W heater for each experimental tank.

Beginning with day 8 of culture, water renewal was performed at a daily rate of 12 % in the WBF-WS and WBF-S treatments. Hydrated lime ($Ca(OH)_2$) was used to maintain alkalinity and pH above 100 mg L^{-1} and 7.5, respectively in WBF-WS and WBF-S (Furtado et al. 2011). The shrimp were given a commercial feed (composition: 35% crude protein, 7.5 % ether extract, 10 % moisture, 5.0 % crude fiber, 13.0 % mineral matter, 3.0 % calcium, 1.45 % phosphorus). The feed was provided initially at a ratio of 4% of the biomass of the shrimp (Van Wyk, 1999) and adjusted daily based on the estimated shrimp consumption, mortality rate and feed leftover. The feed was offered three times a day (8:00, 12:00 and 16:00 h). Additional molasses (55% carbon, 3% crude protein, 5% moisture, 0.3 % ether extract, 0.08 % crude fiber, 20 % mineral matter, 0.1 % phosphorus, 5% calcium) was used in BF-WS-and BF-S three times a day as an organic carbon source to maintain the C:N ratio at 20:1 (Avnimelech 2009).

Shrimp weight were monitored on a weekly to determine shrimp growth and adjust the amount of feed and organic carbon offered. At the end of the experiment, the gain in biomass, specific growth rate (SGR), mean final weight, weekly growth, feed conversion ratio (FCR), survival and yield were determined based on the following equations: Biomass gain (g) = final biomass (g) – initial biomass (g); SGR (% day^{-1}) = 100 x [ln final weight (g) – ln initial weight (g)] / time; Final weight (g) = final biomass (g) / survival; Weekly growth (g $week^{-1}$) = biomass gain (g) / times (weeks) of culture; FCR = consumed feed (dry weight)/ biomass gain; Survival (%) = (number of individuals at end of evaluation period / initial number of individuals stocked) x 100; Yield (Kg m^{-3}) = final biomass (kg) / volume of experimental unit (m^3).

Dissolved oxygen and temperature were determined (YSI model 55, Yellow Springs, Ohio, USA) twice a day (8:00 and 16:00 h). Salinity (YSI 30), pH (YSI model 100) and settleable solids (SS) (Imhoff cone) (Avnimelech 2009) were determined twice a week.

Total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2-N), total suspended solids (TSS), phosphate (PO_4^{3}–P) and alkalinity (mg L^{-1} $CaCO_3$) were determined twice a week, following the methods described by Koroleff (1969), Bendschneider and Robison (1952), A.P.H.A. (1995), Amino and Chaussepied (1983), and A.P.H.A. (1995) respectively.

The Cochran and Shapiro-Wilk tests were employed to determine the homogeneity of the variances and normality of the data. Two-way analysis of variance (ANOVA) was used to determine the effect biofloc (with and without) and seaweed (with and without), and their interaction. The survival of shrimp was analyzed using arcsine-transformed data and TAN log (x), although, non-transformed data are presented in the tables. Tukey's test was used when differences between factors and treatments were detected ($P < 0.05$). The Student's *t* test ($P < 0.05$) was used to SS and the Kruskal-Wallis test ($P < 0.05$) was used to alkalinity. All data were analyzed using the ASSISTAT program, version 7.6 (Campina Grande, Brazil)

Results

The water quality parameters for oxygen, temperature, salinity and pH were not affected by biofloc and seaweed ($P > 0.05$) (Table 1). TAN concentration was lower in the BF-WS and BF-S as compared to WBF-WS and WBF-S ($P < 0.05$) (Fig. 1), and had significant effects of biofloc and seaweed (Table 1). The mean TAN concentration in BF-S (0.749 mg L^{-1}) was 25.9% lower as compared to BF-WS (1.011 mg L^{-1}) and that of WBF-S (2.543 mg L^{-1}) was 14.5 % lower as compared to WBF-WS (2.976 mg L^{-1}).

Nitrite- nitrogen (NO_2-N) concentration was lower in the BF-WS and BF-S as compared to WBF-S and WBF-WS ($P < 0.05$) (Fig. 1), and had significant effects of biofloc and the interaction between biofloc and seaweed (Table 1). The mean NO_2-N concentration in BF-S (0.051 mg L^{-1}) was 72.8% lower as compared to BF-WS (0.187 mg L^{-1}). PO_4^{3}–P concentration was lower in the BF-S and BF-WS as compared to WBF-

S and WBF-WS ($P < 0.05$) (Fig. 1), and had significant effects of biofloc and the interaction between biofloc and seaweed (Table 1). The mean PO_4^{3}–P concentration in BF-S (0.416 mg L^{-1}) was 24.6% lower as compared to BF-WS (0.552 mg L^{-1}).

Significant differences in alkalinity were found in the BF-WS and BF-S as compared to WBF-WS and WBF-S (Table 1). A reduction in alkalinity occurred in the WBF-WS and WBF-S beginning with day 16 (Fig. 2), requiring the addition of inorganic carbon to maintain alkalinity at levels above 100 mg L^{-1}. Significantly, differences were found in TSS ($P < 0.05$), with the highest level in the BF-S and BF-WS (Fig. 2), and had significant effects of the biofloc and interaction between the factors ($P < 0.05$) (Table 1). The mean TSS concentration in BF-S (588.6 mg L^{-1}) was 12.9 % lower as compared to BF-WS (675.9 mg L^{-1}). The SS was significantly higher (34.2%) in the BF-S (19 mL L^{-1}) as compared to BF-WS (13.5 mL L^{-1}) ($P < 0.05$) (Table 1 and Fig 2).

Mean final weight of the shrimp was highest (6.96%) in the BF-S (7.04 ± 0.17 g) ($P < 0.05$), and had significant effect of the biofloc and seaweed (Table 2). Yield was higher in the BF-S and BF-WS as compared to WBF-S and WBF-WS ($P < 0.05$), and had significant effect of the biofloc. Weekly growth was significantly highest in the BF-S (0.61 ± 0.03 g $week^{-1}$) as compared to other treatments ($P < 0.05$). The SGR (% day^{-1}) significant difference ($P < 0.05$) between the tanks with and without biofloc ($P < 0.05$). Moreover, the FCR no significant difference ($P > 0.05$) between the treatments (Table 2).

Discussion

Concerning the mean water quality parameters, oxygen concentrations remained above 5 mg L^{-1}, temperature above 27° C, salinity above 29 – 32, and pH above 7.8 - 7.9 and thus did not limit the *L. vannamei* growth (Van Wyk and Scarpa 1999).

The interaction between biofloc and seaweed (BF-S) reduced TAN, NO_2-N, PO_4^{3}–P and TSS and increased SS and final shrimp weight (6.9%) as compared to BF-WS. The biofloc was also associated higher specific growth rates and yield.

The lower TAN absorption in WBF-S (14.5%) as compared to BF-S (25.9%) is related to the non application of molasses and the successive water changes, reducing the influence of seaweed. According to Mai et al (2010), the removal rate of TAN decreased with increasing TAN concentration. Using *Ulva*, Ramos et al. (2008) found a TAN concentration reduction ranging from 41 to 47 %; Copertino et al. (2009) report a 70 – 72 %; Alencar et al. (2010) report a 94 %; Ramos et al. (2010) report a 49 %; Khoi and Fotedar (2011) report a 59 to 81% and Al-Hafedh et al (2012) report 80%.

The addition of organic carbon to the water immobilizes inorganic nitrogen, transforming it into microbial protein (Avnimelech 2009). However, even with the application of organic carbon at a C:N ratio of 20:1, fluctuations occurred in TAN concentrations. Thakur and Lin (2003), Cohen et al. (2005), Azim and Little (2008), and Ray et al. (2010) report similar fluctuations in TAN concentration with an addition of organic carbon. This variation was probably related to the state of maturation of the system and the amount of these nutrients used by the microalgae and by nitrifying and heterotrophic bacteria (Hargreaves 1998 and 2006).

The interaction between biofloc and seaweed (BF-S) to reduce NO_2-N concentration (72.8%), which was attributed to the addition of organic carbon, as a substrate for the growth of microbial biomass and also the consumption of TAN by seaweed. The higher initial TAN concentrations in treatments without biofloc (WBF-S and WBF-WS) caused an acceleration of the nitrification process, with a consequent pH and alkalinity reduction. Using *Ulva,* Ramos et al. (2008) found a NO_2-N concentration reduction ranging from 7 to 28%; Ramos et al. (2010) report a 31 % and Khoi and Fotedar (2011) report a 2 to 21%. However, the removal ability can decline during the life cycle, growth rate and biomass of seaweed stocked, increased concentration of nutrients, salinity and temperature and water renewal rate (Marinho-Soriano et al. 2009; Mai et al. 2010; Thi et al. 2012; Du et al. 2013).

The interaction between biofloc and seaweed (BF-S) reduce PO_4^3–P concentration (24.6%), was attributed to the addition of organic carbon, as a substrate for microbial biomass growth and also consumption by seaweed. Silva et al (2013) report the used phosphorus in the feed and molasses by microbial biomass. According to Hari et al. (2006), the addition of organic carbon alone was not sufficient to influence the phosphate concentration in the water because a large portion of the phosphorus (38.8 to 66.7 %) that enters a pond's system is deposited in the sediment (Thakur and Lin 2003). However, Emerenciano et al. (2011) report that this absorption does not occur in biofloc tanks, because the bottom is covered by a geomembrane and phosphate is available in the water column.

In relation *Ulva,* Ramos et al. (2008) found a PO_4^3–P concentration reduction ranging from 46 to 55 %; Copertino et al. (2009) report a 50 %; Ramos et al. (2010) report a 39 %; Khoi and Fotedar (2011) report a 50 to 55% and Al-Hafedh et al (2012) report 41%.

In the biofloc tank, a pH reduction generally occurs (Wasielesky Jr et al. 2006; Emerenciano et al. 2011) due to alkalinity consumption during ammonia-nitrogen conversion processes (Ebeling et al. 2006). According to Furtado et al. (2011), levels under 100 mg L^{-1}, of $CaCO_3$ and pH 7 for prolonged periods of time can affect the growth performance of shrimp in biofloc. In BF-WS and BF-S, pH and alkalinity were not significantly reduced due to lower alkalinity consumption in the nitrogen incorporation process by heterotrophic microbial biomass (Ebeling et al. 2006). In the

WBF-WS and WBF-S tanks, however, the addition of inorganic carbon was required to maintain desirable levels during the nitrite production process, which consumed calcium carbonate, releasing CO_2 and hydrogen into the water (Hargreaves 1998).

The TSS and SS concentrations are important tools for increasing the growth of shrimp in biofloc (Taw 2010; Ray et al. 2010). The addition organic carbon had a significant effect on the increase of the TSS concentration throughout the culture period and its values remained above the recommended levels by Samocha et al. (2007) ≤ 500 mg L^{-1} and Ray et al. (2010) ≤ 460 mg L^{-1}. However, in BF-S treatments, a TSS concentration was 12.91% lower as compared to BF-WS, probably uptake organic carbon by seaweed. Lobban et al. (1985) reported that an *Ulva* can use organic carbon for Krebs cycle activity, competing with microbial flora.

The higher SS concentration in BF-S and BF-WS is probably the increase of microbial biomass due to the addition of organic carbon into the tanks. Hari et al. (2006) reported that the addition organic carbon to the water column led to a significant increase in the biomass of the microbial community. However, in BF-S the SS concentration was 34.2% higher than BF-WS, probably seaweed fragments, which became aggregated to the SS concentration, were most likely consumed by the shrimp through their direct grazing of the seaweed along with the biofilm that forms on the surface of the seaweed (Lombardi et al. 2006; Butterworth, 2010; Tsutsui et al. 2010; Portillo-Clark et al. 2012).

The biofloc with seaweed (BF-S) was significantly higher on final weight and weekly growth than other treatments. These results are in agreement with those reported by Cruz-Suárez et al. (2010) for *L. vannamei*, Tsutsui et al. (2010) and Izzati (2011) for *P. monodon* and by Portillo-Clark et al. (2012) for *F. californiensis*, who describe a greater daily growth rate for shrimp culture in integrated systems with seaweed.

The addition of organic carbon had a positive effect on the shrimp's growth rate and this effect was amplified with seaweed. The positive effect of the use of organic carbon on shrimp growth has been described in previous studies (Hari et al. 2006; Campos et al. 2007; Samocha et al. 2007; Silva et al. 2009; Ekasari et al 2010; Neal et al. 2010; Gao et al. 2012; Maia et al. 2012; Baloi et al. 2013), but the presence of seaweed probably contributed to an increase in the nutritional value of biofloc. Higher growth rates among shrimp culture with the addition of seaweed have been attributed to more balanced levels of essential amino acids and sources of fatty acids ($\omega 6/\omega 3$) (Peña Rodríguez et al. 2011; Tabarsa et al. 2012). The integrated systems of shrimp with *Ulva* is a sustainable option for reducing the need for commercial shrimp feed, because the shrimp can use fresh *Ulva* as complementary food (Peña Rodríguez et al. 2010; Sánchez et al. 2012).

The biofloc with seaweed (BF-S) increased the mean final weight of the shrimp by 6.9 %, probably due to the incorporation of fragments into SS concentration, and because microorganisms adhered to the seaweed provide supplemental food for the shrimps (Lombardi et al. 2006; Butterworth, 2010; Tsutsui et al. 2010; Portillo-Clark et al. 2012). The biofloc with seaweed also improves the water quality, by reducing inorganic nitrogen compounds (TAN by 25.9%; NO_2-N by 72.8%), phosphate (PO_4^{3}-P 24.6% and TSS (12.9%) as compared to BF-WS. The inclusion of *Ulva* in biofloc promotes the uptake of waste nutrients and shrimp growth. However, further research should be conducted with other seaweed species and stocking densities to increase the uptake of waste and shrimp growth.

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Table 1. Effect of biofloc and seaweed on the water quality parameters of *L. vannamei* in co-culture with *U. lactuca* in intensive system during the 28-day experimental period.

Parameters	**Treatmensts**^{1}				**Significance (P-value)**$^{¥}$		
	BF-WS	**BF-S**	**WBF-WS**	**WBF-S**	**BF**	**S**	**BFxS**
Dissolved oxygen (mg L^{-1})	5.37 ± 0.11	5.34 ± 0.03	5.50 ± 0.29	5.48 ± 0.26	ns	ns	Ns
Temperature (°C)	27.7 ± 0.08	27.4 ± 0.25	27.2 ± 0.43	27.5 ± 0.10	ns	ns	Ns
Salinity (ppt)	32.3 ± 0.54	29.9 ± 1.05	30.8 ± 0.72	31.0 ± 0.27	ns	ns	Ns
pH	7.93 ± 0.04	7.89 ± 0.07	7.98 ± 0.03	7.93 ± 0.10	ns	ns	Ns
TAN (mg L^{-1})	1.011 ± 0.10^{b}	0.749 ± 0.15^{c}	2.976 ± 0.39^{a}	2.543 ± 0.23^{a}	*	*	Ns
NO_2-N (mg L^{-1})	0.188 ± 0.06^{c}	0.051 ± 0.05^{d}	0.303 ± 0.02^{b}	0.412 ± 0.03^{a}	*	ns	*
PO_4^{3}–P (mg L^{-1})	0.552 ± 0.03^{a}	0.416 ± 0.19^{b}	0.571 ± 0.04^{a}	0.739 ± 0.05^{a}	*	ns	*
Alkalinity (mg L^{-1} $CaCO_3$) $^{\#}$	169.5 ± 1.76^{a}	160.1 ± 13.72^{a}	123.8 ± 2.47^{b}	124.0 ± 4.37^{b}	-	-	-
TSS (mg L^{-1})	675.9 ± 32.67^{a}	588.6 ± 77.6^{b}	350.5 ± 15.36^{c}	386.5 ± 21.40^{c}	*	ns	*
SS (mL L^{-1}) $^{\#\#}$	13.5 ± 1.14^{b}	19.0 ± 2.43^{a}	-	-	-	-	-

Mean values in same row with different superscript letters differ significantly ($P < 0.05$).

BF= biofloc and S= seaweed; BF x S = biofloc x seaweed interaction. TAN = total ammonia nitrogen, NO_2–N = nitrite-nitrogen, PO_4^{3}–P = phosphate, TSS = total suspended solids, SS = settleable solids, ns - not significant ($P > 0.05$)

[1] The data correspond to the mean of three replicates ± standard deviation

* ($P < 0.05$)

[¥] Results from split-plot two way ANOVA and Tukey test; biofloc with seaweed (BF-S); biofloc without seaweed (BF-WS); without biofloc with seaweed (WBF-S); and without biofloc and without seaweed (WBF-WS).

[#] Kruskal-Wallis test (p < 0.05)

[##] Student’s t-test (p < 0.05).

Table 2. Effect of biofloc and seaweed on the shrimp production parameters of *L. vannamei* in co-culture with *U. lactuca* in intensive system during the 28-day experimental period.

Parameters	**Treatmensts**[1]				**Significance (P-value)**[¥]		
	BF-WS	**BF-S**	**WBF-WS**	**WBF-S**	**BF**	**S**	**BFxS**
Final weight (g)	6.55 ± 0.20^{b}	7.04 ± 0.17^{a}	5.85 ± 0.15^{c}	6.01 ± 0.24^{c}	*	*	ns
Yield (Kg m^{-3})	3.56 ± 0.05^{a}	3.72 ± 0.18^{a}	3.21 ± 0.19^{b}	3.21 ± 0.13^{b}	*	ns	ns
Survival (%)[#]	96 ± 0.04	93 ± 0.03	97 ± 0.05	94 ± 0	ns	ns	ns
FCR	2.11 ± 0.15	1.76 ± 0.20	2.27 ± 0.56	2.43 ± 0.74	ns	ns	ns
Weigth gain week^{-1}	0.48 ± 0.08^{b}	0.61 ± 0.03^{a}	0.35 ± 0.03^{c}	0.38 ± 0.09^{c}	*	ns	ns
SGR (% day^{-1})	0.98 ± 0.26^{a}	1.38 ± 0.06^{a}	0.52 ± 0.13^{b}	0.61 ± 0.36^{b}	*	ns	ns

Mean values in same row with different superscript letters differ significantly ($P < 0.05$).
BF= biofloc and S= seaweed; BF x S = biofloc x seaweed interaction. SGR (% day^{-1}) = 100 x [ln final weight (g) – ln initial weight (g)] / time and FCR = amount of feed consumed / biomass gain, ns - not significant ($P > 0.05$)

[1] The data correspond to the mean of three replicates ± standard deviation

*($P < 0.05$)

[¥] Results from split-plot two way ANOVA and Tukey’s test; biofloc with seaweed (BF-S); biofloc without seaweed (BF-WS); without biofloc with seaweed (WBF-S); and without biofloc and without seaweed (WBF-WS).

[#] The survival of shrimp was analyzed using arcsine-transformed data, although, non-transformed data are presented in the tables

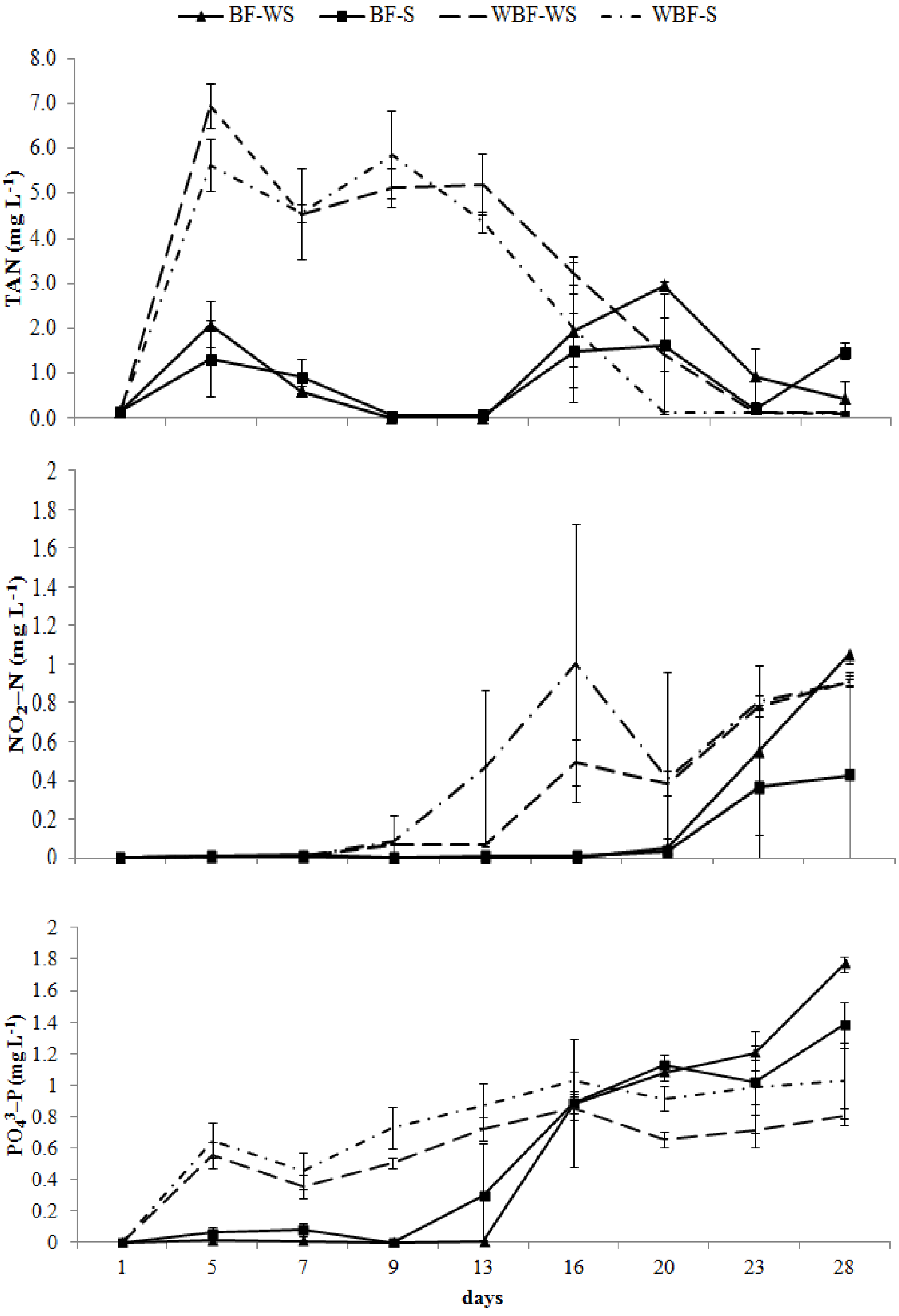

Fig. 1 Fluctuations of total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2–N) and phosphate (PO_4^{3}-P) concentrations on the tanks during 28 days experimental period. Values are means (±SD) of three replicate tanks per sampling time in each treatment.

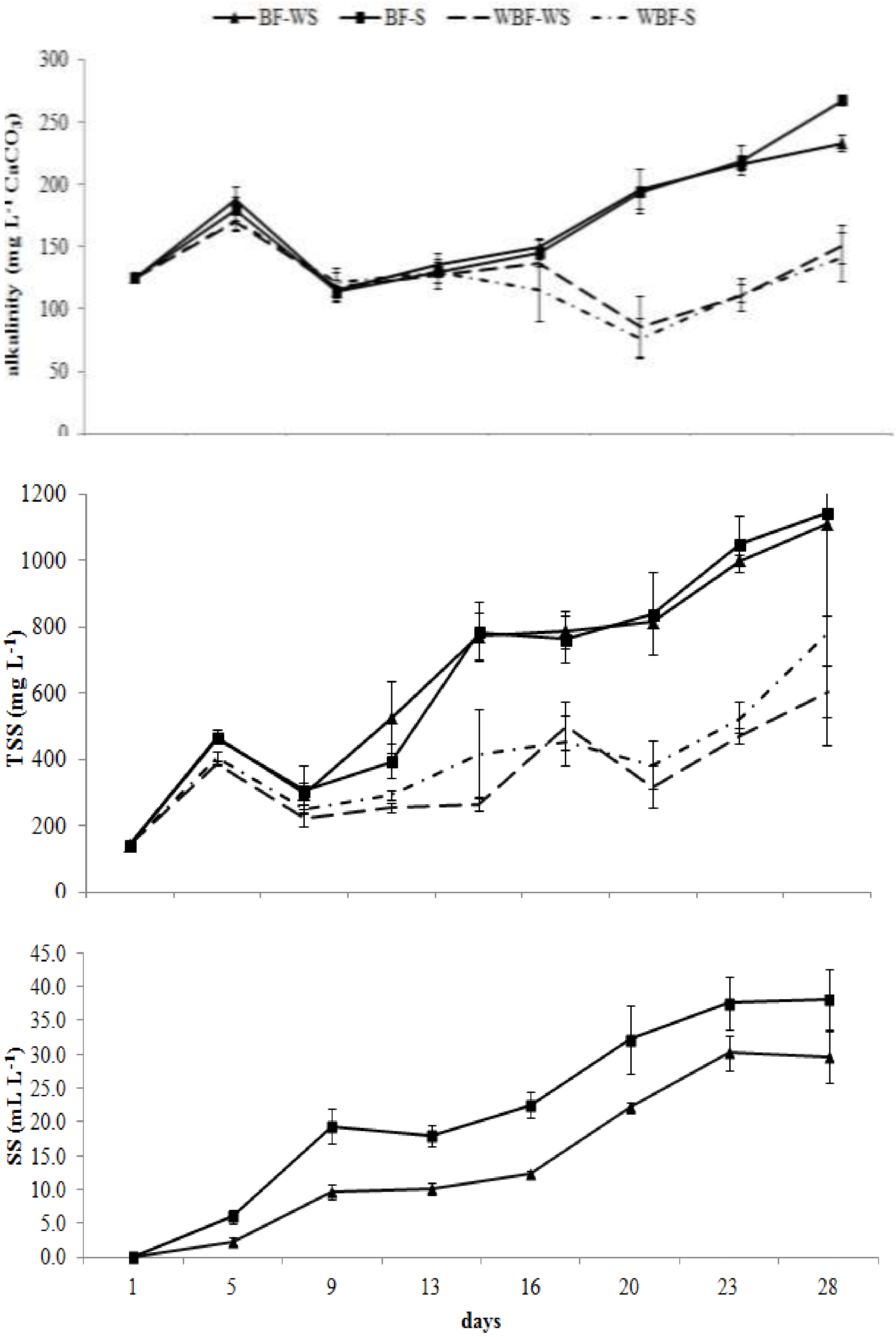

Fig. 2 Fluctuations of alkalinity (mg L^{-1} $CaCO_3$), total suspended solids (TSS) and settleable solids (SS) concentrations on the tanks during 28 days experimental period. Values are means (±SD) of three replicate tanks per sampling time in each treatment.

4.2 - Artigo científico II

Water quality, phytoplankton composition and growth of the Pacific white shrimp *Litopenaeus vannamei* (Boone) in biofloc integrated system with two red seaweed *Gracilaria* genera (Greville)

Luis Otavio Brito, Luis Vinatea, Roberta Borda Soares, William Severi, Rayzza Helena Miranda, Suzianny Maria Bezerra Cabral da Silva, Maria Raquel Moura Coimbra, Alfredo Olivera Gálvez

Artigo científico a ser encaminhado ao Aquaculture International
Todas as normas de redação e citação, deste capítulo, atendem as
estabelecidas pela referida revista (em anexo).

Growth of *L. vannamei* in biofloc integrated system with *Gracilaria*

Water quality, phytoplankton composition and growth of the Pacific white shrimp *Litopenaeus vannamei* (Boone) in biofloc integrated system with two red seaweed *Gracilaria* genera (Greville)

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Abstract An indoor trial was conducted for 28 days to evaluate the water quality, phytoplankton composition and growth of Pacific white shrimp *Litopenaeus vannamei* in biofloc integrated system with two red seaweed *Gracilaria* genera. The experimental design was completely randomized with three treatments: Control (shrimp monoculture in biofloc); SB (shrimp and *Gracilaria birdiae* in biofloc integrated system) and SD (shrimp and *Gracilaria domingensis* in biofloc integrated system), with three replicates each. Random sampling was done (6% of total population per experimental unit) to confirm WSSV infection by nested-PCR analysis due to suspicion of virus presence in the experiment (treatment and control groups). Shrimp *L. vannamei* (2.63 ± 0.10 g) were stocked in experimental tanks at a density 425 shrimp m^{-3} and the *Gracilaria* was stocked at a biomass 2.0 Kg m^{-3}. Shrimp mortality starting for both experimental and control groups in 10 days of culture. Biofloc with seaweed increased settleable solids (by 26 – 52%); final weight (by 6 – 21%); weekly growth (by 17-43%); weight gain (by 17 – 43%); specific growth rate (by 16 – 36%), and yield (by 5 – 7%); and decreased feed conversion ratio (by 21 – 28%) and Cyanobacteria density about 17% as compared to biofloc without seaweed. The red seaweed *Gracilaria* in biofloc integrated system can enhance of shrimp growth and reduced Cyanobacteria density in WSSV presence.

Keywords: Integrated Aquaculture • Biofloc • Shrimp • Seaweed • Growth • Cyanobacteria • WSSV

Abbreviations

TAN – Total ammonia nitrogen

NO_2-N – Nitrite-nitrogen

NO_3-N - Nitrate-nitrogen

PO_4^{3}–P – Phosphate

TSS – Total suspended solids

SS – Settleable solids

SGR - Specific growth rate

FCR - Feed conversion ratio

SB - Shrimp and *Gracilaria birdiae* in biofloc integrated system

SD - Shrimp and *Gracilaria domingensis* in biofloc integrated system

WSSV – White spot syndrome virus

SRC – Sedgewick rafter chamber

Introduction

Shrimp farming in Brazil include a wide range of extensive, semi-intensive and intensive systems. In 2011, Brazilian shrimp production was 69,571 metric tons, from a culture area of 19,845 ha, with a mean yield of 3,510 kg ha year^{-1} (ABCC 2013). The infectious myonecrosis virus (IMNV) and white spot syndrome virus (WSSV) outbreaks have significantly losses in marine shrimp farming industry production (Muller et al. 2010; Guerrelhas and Teixeira 2012; Feijó et al. 2013). WSSV disease is a DNA virus withing the family Nimaviridae (Mayo 2002) and one of the most severe pathogen in the shrimp industry worldwide (Escobedo-Bonilla et al. 2008).

This biofloc system involves high stocking density, recycling nitrogen waste, zero or minimal water exchange, addition of organic carbon, artificial aeration encourage the development of a heterotrophic microbial community in the pond/tank (Avnimelech 2009; Ballester et al. 2010; Ray et al. 2010, 2011; Emerenciano et al. 2011; Crab et al. 2012; Gao et al. 2012). Bioflocs are formed of detritus, bacteria, microalgae, zooplankton, fungi, feces and exoskeletons of dead animals, all of which contribute to shrimp nutrition, increasing yield and survival, and decreasing FCR (Ju et al. 2008; Emerenciano et al. 2011; Audelo-Naranjo et al. 2012; Ray et al. 2012; Yu et al. 2013), as well as removal of nitrogen compounds from the system (Crab et al. 2012; Gao et al. 2012). However, Silva et al. (2013a) reported that 35% of the phosphorus and 39% of the nitrogen input into a biofloc system as feed and molasses may be lost to the environment.

The nitrogen and phosphorus wastes can be removed by red seaweed *Gracilaria* in traditional system (Xu et al. 2008a, b; Marinho-Soriano et al. 2009a, b; Abreu et al. 2011; Huo et al. 2011, 2012; Skriptsova and Miroshnikova 2011; Al-Hafedh et al. 2012; Du et al. 2013). Seaweed can also serve as a food source for shrimp (Cruz-Suarez et al.

2010; Tsutsui et al. 2010; Gamboa-Delgado et al., 2011; Izzati, 2011; Portillo-Clark et al. 2012; Sánchez et al. 2012; Brito et al. 2013). In addition, some studies have reported that extracts of seaweed help prevent bacteria and virus disease outbreaks in marine shrimp (Huynh et al. 2011; Kanjana et al. 2011; Lin et al. 2011; Selvin et al. 2011; Immanuel et al. 2012; Silva et al. 2013b; Sirirustananun et al. 2011).

Integrated aquaculture systems use seaweed and mollusks for bioremediation, an inexpensive strategy that can minimize wastes nitrogen and phosphorus, reduce risk of disease and increase the income of farmers while also mitigating the environmental problems caused by effluents (Troell et al. 2009; Barrington et al. 2010; Mai et al. 2010; Abreu et al. 2011; Khoi and Fotedar 2011). However, the use of seaweed in integrated systems depends on efficiency in nutrient removal and its ability to grow in hypereutrophic conditions (Abreu et al. 2011; Skriptsova and Miroshnikova 2011). Various species of red seaweed *Gracilaria* (Rhodophyta) occur naturally in coastal areas of the Pernambuco state in northeastern Brazil. Thus, the aim of this study was to evaluate water quality, phytoplankton composition and growth of Pacific white shrimp *Litopenaeus vannamei* in biofloc integrated system with two red seaweed *Gracilaria* genera.

Materials and Methods

An indoor trial was conducted for 28 days at the Sustainable Mariculture Laboratory (LAMARSU) of the Fisheries and Aquaculture Department (DEPAq) of the Rural Federal University at Pernambuco (UFRPE), Recife, Brazil (08°01’00.16¨S, 034°56’57.74”W). The experimental design was completely randomized with three treatments: Control (shrimp monoculture in biofloc); SB (shrimp and *Gracilaria birdiae* in biofloc integrated system) and SD (Shrimp and *Gracilaria domingensis* in biofloc integrated system), with three replicates each.

Five days prior to stocking shrimp and seaweed, water from a matrix tank (TAN 0.1 mg L^{-1}, NO_2-N 0.8 mg L^{-1}, NO_3-N 2.5 mg L^{-1}, PO_4^{3}-P 2.1 mg L^{-1}, alkalinity 106.5 mg $CaCO_3$ L^{-1} and TSS 102.8 mg L^{-1}) was mixed and equally distributed to fill twelve black-plastic tank (40 L, 0.20 m^2). The experimental units were maintained under constant aeration by three airstones per tank. No water exchange was carried out during the experimental period, except for the addition of dechlorinated freshwater to compensate for evaporation losses. The light intensity was kept at ~ 1000 lux using a fluorescent lamp with a natural photoperiod.

Juvenile Pacific white shrimp *L. vannamei* (2.63 ± 0.10 g) were obtained from a commercial shrimp farm (Ilha de Itamaracá beach, north coast of Pernambuco, Brazil, 07º 44’02.94¨S, 034º50’12.96¨W) and experimental units were stocked at a density of 425 shrimp m^{-3}. During the trial shrimp were fed three times per day (8:00, 12:00 and 16:00 h) with 32% crude protein and 7.5 % ether extract commercial feed (Evialis, Presence, Camanutri, Brazil). The feed was provided initially at a ratio of 8% of the biomass of the shrimp (Van Wyk 1999) and adjusted daily according to the estimated shrimp consumption, mortality rate and feed leftover. Molasses was used once time a day as an organic carbon source to maintain the C:N ratio at 12:1 (Samocha et al. 2007; Avnimelech 2009).

From each experimental unit, 6% of individuals were randomly selected and had hemolymph and gill tissues, collected. DNA was extracted using DNeasy® Blood and Tissue kit (Qiagen, USA), according to the manufacturer's protocol and a nested-PCR analysis was carried out for the confirmation of WSSV infection. For the first reaction, 100 pmoles of each primer specific to WSSV were used in 100 µl reaction solution containing 100 ηg of DNA, 1X PCR buffer, 2.5 mM of $MgCl_2$, 0.2 mM of each dNTP and 2U of *Taq* DNA polymerase. In the second reaction, 1 µl of the product of the 1st PCR and the specific primers of the nested-PCR were added, using the same conditions as in the first reaction. The thermal cycle conditions and primers were the same as described by Lo et al. (1996). Amplicons were visualized after electrophoresis in 1% agarose gel stained with ethidium bromide (Fig 1).

Samples of *Gracilaria* biomass were collected at the Pau Amarelo beach, Paulista, Pernambuco, Brazil (07°54’54.74¨S, 034°49’12.07¨W), and stored in plastic bags for laboratory analysis. Water was drained from all the samples, and the material was carefully inspected to eliminate encrusted organisms and then weighed. Seaweed with reproduction structures, signs of depigmentation and necrosis were discarded (Marinho-Soriano et al. 2011; Tsutsui et al. 2010) and stocked at a biomass 2.0 Kg m^{-3}.

Shrimp weight was monitored on a weekly basis to determine shrimp growth and adjust the amount of feed and organic carbon offered. At the end of the experiment, biomass gain, specific growth rate (SGR), mean final weight, weekly growth, feed conversion ratio (FCR), survival and yield were determined based on the following equations: Biomass gain (g) = final biomass (g) – initial biomass (g); SGR (% day^{-1}) = 100 x [ln final weight (g) – ln initial weight (g)] / time (days); Final weight (g) = final biomass (g) / survival; Weekly growth (g $week^{-1}$) = biomass gain (g) / times (weeks) of culture; FCR = feed supplied (dry weight)/ biomass gain; Survival (%) = (number of individuals at the end of evaluation period / initial number of individuals stocked) x 100; Yield (Kg m^{-3}) = final biomass (kg) / volume of experimental unit (m^3).

Dissolved oxygen and temperature were monitored (YSI model 55, Yellow Springs, Ohio, USA) twice a day (8:00 and 16:00 h). Salinity (YSI 30, Yellow Springs, Ohio, USA), pH (YSI model 100, Yellow Springs, Ohio, USA) and settleable solids (SS)

(Imhoff cone) (Avnimelech 2009) were monitored twice a week. Total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), total suspended solids (TSS), phosphate (PO_4^3–P) and alkalinity (mg L^{-1} $CaCO_3$) were monitored once a week, following the methods described by Koroleff (1976), Golterman et al. (1978), Mackereth et al. (1978), APHA (2005), and Felföldy et al. (1987), respectively.

Each week vertical sampling was performed in each tank using plastic bottles with a volume of 500 mL for phytoplankton collection. The water was filtered through a cylindrical-conical net (mesh: 15 µm) to 10 mL, to obtain a 50-fold more concentrated sample. The phytoplankton were fixed with formalin (4%), buffered with borax (1%) and stored in 10-mL plastic recipients. A Sedgewick rafter chamber (SRC) and stereomicroscope with magnification of 800 x were used for identification and quantification of the phytoplankton samples, respectively. The phytoplankton concentrations were expressed as cells per milliliter (cells mL^{-1}), and estimated according to the sample preparation methods described by Pereira-Neto et al. (2008), calculated according to the following formula

$$C = [(nm / nq) \times 1000] / F,$$

where C is phytoplankton concentration; *nm* is the number of organisms found in the quadrants analyzed in the chamber; *nq* is the number of quadrants analyzed in the chamber; 1000 – is the number of quadrants in the chamber and *F* is the dilution (50) correction factor.

A parametric one-way ANOVA was used to analyze production parameters, after confirming homocedasticity (Cochran $P < 0.05$) and normality (Shapiro-Wilk $P < 0.05$). Tukey’s test ($P < 0.05$) was performed to compare and rank means from the two treatments and the control. Water quality parameters and Cyanobacteria density were analyzed by performing repeated measures ANOVA. Shrimp survival were analyzed arcsine-transformed data, although non-transformed data are presented in the tables. Data analyses were performed using ASSISTAT Version 7.7 (Assistat Analytical Software, Campina Grande, Paraiba, Brazil).

Results

The addition seaweed in bioflocs system resulted in increased the tank surface area of 24% (*Gracilaria birdiae*) and 27% (*Gracilaria domingensis*). Shrimp mortality starting for both experimental and control groups in 10 days of culture and the confirmation of WSSV infection was obtained.

The water quality parameters for oxygen (6.13 to 6.37 mg L^{-1}), temperature (24.78 to 25.05 °C), salinity (35.61 to 36.22 ppt), pH (7.56 to 7.75), TAN (0.10 to 0.17 mg L^{-1}), NO_2-N (0.50 to 0.61 mg L^{-1}), NO_3-N (1.64 to 2.25 mg L^{-1}), PO_4^{3}–P (2.42 to 2.55 mg L^{-1}), TSS (251.59 to 283.67 mg L^{-1}) and alkalinity (82.26 to 91.30 mg L^{-1} $CaCO_3$) were not significantly different ($P > 0.05$) (Fig 2 and 3). However, SS were significantly higher ($P < 0.05$) in SB (14.5 mL L^{-1}) and SD (12 mL L^{-1}) as compared to control (9.5 mL L^{-1}) (Table 1).

The phytoplankton concentrations are summarized in Table 2. About 54 genera of phytoplankton belonging to Cyanobacteria (15 genera), Bacillariophyta (22 genera), Chlorophyta (11 genera), Dinophyta (4 genera) and Euglenophyta (2) were identified. Cyanobacteria (76 to 92%) were the most abundant organisms, followed by Chlorophyta, Bacillariophya, Dinophyta and Euglenophyta. The treatments with *Gracilaria* showed decreased ($P < 0.05$) about 17% in Cyanobacteria density as compared to control (Table 2).

The final weight (6.57 ± 0.02g), weight gain (3.92 ± 0.29g), SGR (2.12 ± 0.12%) and weekly growth (0.98 ± 0.07g) were significantly higher ($P < 0.05$) in SB as compared to

SD and control. The survival, FCR and yield were not significantly different ($P > 0.05$) (Table 3).

Discussion

The dissolved oxygen, salinity and nitrogen compounds (TAN, NO_2-N and NO_3-N) concentrations of the culture water were within the range recommended for marine shrimp culture. However, water temperature (24 to 25 °C) was lower than recommended (28 to 32°C) by Van Wyk and Scarpa (1999). The water temperature is important environmental factor for influence on the metabolic rate of the shrimp, growth and survival, rates of feed consumption, ammonia excretion, oxygen consumption and molt cycle (Van Wyk and Scarpa 1999). The WSSV presence combined with water temperature lower probably contributes for reduced survival rate. Vidal et al. (2001) demonstrated that WSSV does not kill *L. vannamei* when water temperatures are above 32 °C. Moreover, WSSV infected shrimp may be asymptomatic at temperatures above 27 º C, but the disease becomes evident if the water temperature decreases (Pantoja and Lightner 2008).

The alkalinity levels 106.5 mg $CaCO_3$ L^{-1} declined to near 60 mg $CaCO_3$ L^{-1}, likely due to the nitrification processes (autotrophic bacteria consumed 7.05 g alkalinity g^{-1} NH_4^+-N) and ammonium nitrogen converted into heterotrophic microbial biomass (heterotrophic bacteria consumed 3.57 g alkalinity g^{-1} NH_4^+-N) (Ebeling et al. 2006). pH is another important water quality parameter in biofloc system and it appears not to be influenced also by the presence of seaweed . The pH appears to decline due to the intense respiration of heterotrophic organisms, which increases the concentration of carbon dioxide in the culture tanks (Emerenciano et al. 2011; Yu et al. 2013).

In biofloc systems, high concentrations of total suspended solids can interfere shrimp growth, due to gill clogging, thus impairing respiration. Hopkins et al. (1993; 1994) and Marquez et al. (2012) reported increased mortality and heavy fouling (by epicommensal bacteria, feces and uneaten feed) of the gills of shrimp culture with increased stocking densities. Samocha et al. (2007) and Ray et al. (2010) recommend a TSS concentration ≤ 500 mg L^{-1}. The average TSS concentrations were lower than 500 mg L^{-1} in all treatments. The red seaweed *Gracilaria* not effectively using the organic carbon for metabolism, however, Brito et al. (2013) reported that an *Ulva lactuca* in biofloc systems reduced total suspended solids by 12.9%, well Lobban et al. (1985) reported use organic carbon for Krebs cycle activity. Biofloc with seaweed significantly increased settleable solids (SS) by 26 – 52%, influence on the development of the bioflocs, probably by colonization of the microorganisms that use seaweed as a substrate. Anand et al. (2012) observed increased the total heterotrophic bacterial in tanks with addition organic carbon and substrate artificial.

The stabilization of heterotrophic and nitrifying bacteria in the water (biofloc matrix tank TAN 0.6 mg L^{-1}, NO_2-N 0.1 mg L^{-1}, NO_3-N 2.5 mg L^{-1} and TSS 102.8 mg L^{-1}), probably limit the nitrogen and phosphorus seaweed uptake. However, sudden changes in TAN and NO_2-N and accumulate NO_3-N can occur in zero or minimal exchange water, probably due to the variation in the bacteria biomass and phytoplankton uptake (Cohen et al. 2005; Ray et al. 2010). In this situation, seaweed can TAN and NO_2-N uptake (Brito et al. 2013).

Phosphorus appear to accumulate during the cultivation period in tanks using the biofloc system (Emerenciano et al. 2011) and the use of red seaweed *Gracilaria* does not uptake its concentration. According to Xu et al. (2008b) and Khoi and Fotedar (2011), nutrient removal by seaweed improves the water quality and thus boosts shrimp growth and yield. However, uptake efficiency also decreased at higher environmental nutrient concentrations during the culture period, life cycle and growth rate of seaweed, seaweed stocked biomass and water renewal rate (Marinho-Soriano et al. 2009a; Mai et al. 2010; Du et al. 2013).

The phytoplankton richness found in this study (38,710 to 44,934 cells mL^{-1}) is higher as compared to Anand et al. (2012) (30 genera) and Asaduzzaman et al. (2010) (42 genera). However, the predominance of Cyanobacteria is similar to the results reported by Campos et al. (2009), Asaduzzaman et al. (2010), Ballester et al. (2010) and Neal et al. (2010) and Emerenciano et al. (2013) in zero exchange water with addition organic carbon. Predominance of Cyanobacteria density probably occurred due to the eutrophication process caused by the zero or minimal exchange water, causing a build-up of particles and an increase in the concentration of phosphorus, and the competitive advantages of these organisms over other plankton groups.

The treatments with *Gracilaria* showed decreased in Cyanobacteria density about 17% as compared to control. Cyanobacteria groups (*Shizothrix calcicola*, *Microcystis*, *Oscillatoria* and *Anabaena*), can negatively affect water quality by producing compounds that are toxic to some aquatic animals produce toxins (Jú et al. 2008; Yusoff et al. 2010). According to Zhou et al. (2009), this composition can directly affect the growth of penaeid shrimp. Even in intensive systems with zero exchange water, Bacilariophyta (diatoms) can contribute to shrimp nutrition, mainly by supplying highly unsaturated fatty acids (Ju et al. 2008). Results similar the lower phytoplankton density in co-culture systems (shrimp and seaweed), as compared to monoculture were observed by Cruz-Suárez et al. (2010) and Huo et al. (2011), probably related to decreased light.

The data for FCR (2.06 – 2.26) and weekly growth (0.81 – 0.98) in biofloc system with seaweed were similar to those observed by Ray et al. (2010, 2012); Coyle et al.

(2011) and Schveitzer et al. (2013), with densities near 460 shrimp m^{-3}. The increased results of final weight (6 – 21%), weight gain (17 – 43%); SGR (16 – 36%), and decreased FCR (21 – 28%) in the integrated treatments (shrimp and seaweed), indicating that the presence of seaweed improve shrimp performance in biofloc system. This fact is probably related to the shrimp grazing directly on the seaweed and on the biofilm formed on its surface (Lombardi et al. 2006; Tsutsui et al. 2010; Portillo-Clark et al. 2012). According to Brito et al. (2013), shrimp growth is higher in tanks containing seaweed (*Ulva lactuca*) than in those tanks without seaweed. The same was observed by Portillo-Clark et al. (2012), studying the integrated culture of *Caulerpa sertularioides* and *Farfantepenaeus californiensis*; by Sánchez et al. (2012) with *Ulva* sp. and *L. vannamei*; by Gamboa-Delgado et al. (2011) with *U. clathrata* and *L. vannamei*; by Izzati (2011) with *G. verrucosa* and *P. monodon*; by Tsutsui et al. (2010) with *Chaetomorpha ligustica* and *P. monodom* and by Cruz-Suarez et al. (2010) with *U. clathrata* and *L. vannamei*. Gamboa-Delgado *et al*. (2011) showed 31-79% of carbon and 73-98% of nitrogen in shrimp whole bodies from the seaweed consumption. The consumed seaweed may act as nutritional supplements and/or improve the utilization of nutrients from the artificial feed by shrimp (Cruz- Suárez et al. 2010).

WSSV causing cumulative mortality between 90 and 100% in *L. vannamei* culture within 3 to 10 days from the onset of clinical signs (Sánchez-Martínez et al. 2007). The survival rate (50 a 56%) in the biofloc during 28 days in WSSV presence was higher as compared to Pérez et al. (2005) with *L. vannamei* (PL_{20}, PL_{30} and PL_{40}) during 7 days at 25.8 ± 0.7°C, Gitterle et al. (2005) with *L. vannamei* (1.1 to 6.6g) during 15 to 29 days at 22 ± 2°C, although there are no studies on the evaluating survival rate of *L. vannamei* in biofloc system in WSSV presence.

The *Gracilaria* in biofloc system can improve SS concentration, decreased Cyanobacteria density, increased shrimp growth and decreased FCR. However, further research should be conducted to evaluate as the seaweed stocking biomass, for waste (nitrogen and phosphate) uptake, increase shrimp growth and survival. Moreover, available effect of extracts and fresh weight *Gracilaria* seaweed on growth and resistance to WSSV in *L. vannamei* in biofloc system.

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Table 1 Water quality parameters of Pacific white shrimp *Litopenaeus vannamei* in an integrated aquaculture with red seaweed *Gracilaria* in biofloc system in WSSV presence during the 28-day experiment period.

Parameters	Treatments[1]		
	Control	SB	SD
Dissolved oxygen (mg L^{-1})	6.37 ± 0.26^a	6.15 ± 0.30^a	6.20 ± 0.05^a
Temperature (°C)	25.04 ± 0.46^a	25.05 ± 0.47^a	24.78 ± 0.86^a
Salinity (ppt)	36.17 ± 2.47^a	35.61 ± 1.50^a	36.22 ± 0.84^a
Alkalinity (mg $CaCO_3$ L^{-1})	88.26 ± 12.06^a	91.30 ± 3.92^a	82.26 ± 12.60^a
pH	7.58 ± 0.13^a	7.75 ± 0.22^a	7.56 ± 0.08^a
TSS (mg L^{-1})	283.67 ± 25.91^a	275.21 ± 47.60^a	251.59 ± 41.79^a
SS (mg L^{-1})	9.5 ± 0.5^b	12.0 ± 2.0^{ab}	14.5 ± 0.5^a
TAN (mg L^{-1})	0.10 ± 0.03^a	0.17 ± 0.05^a	0.14 ± 0.09^a
NO_2-N (mg L^{-1})	0.53 ± 0.01^a	0.61 ± 0.04^a	0.50 ± 0.08^a
NO_3-N (mg L^{-1})	2.25 ± 0.22^a	1.64 ± 0.31^a	2.25 ± 0.68^a
PO_4^{3}–P (mg L^{-1})	2.42 ± 0.08^a	2.55 ± 0.14^a	2.53 ± 0.11^a

[1] The data correspond to the mean ± standard deviation.

Results from repeated measures ANOVA and Tukey's test. Mean values in the same row with different superscripts differ significantly ($P < 0.05$).

TAN= Total ammonia nitrogen, NO_2–N = nitrite-nitrogen, NO_3–N = nitrate-nirogen, PO_4^{3}–P = phosphate, TSS = total suspended solids and SS= settleable solids.

Control (shrimp monoculture in biofloc); SB (shrimp and *Gracilaria birdiae* in biofloc integrated system) and SD (shrimp and *Gracilaria domingensis* in biofloc integrated system)

Table 2 Phytoplankton density (cells mL^{-1}) of Pacific white shrimp *Litopenaeus vannamei* in an integrated aquaculture with red seaweed *Gracilaria* in biofloc system in WSSV presence during the 28-day experiment period.

Groups	Genera	Control	SB	SD
Cyanobacteria	*Anabaena*	216.74	185.64	245.41
	Anabaenopsis	11.54	0.00	0.00
	Aphanizomenon	0.00	0.00	18.75
	Aphanocapsa	12,088.62	3,163.19	569.12
	Dactylococcopsis	12.60	14.33	463.32
	Merismopedia	0.00	79.35	14.43
	Microcystis	834.39	877.67	531.41
	Oscillatoria	18,405.90	17,976.68	10,498.19
	Plectonema	54.97	85.23	8,139.43
	Pseudanabaena	220.69	234.83	165.29
	Raphidiopsis	4,210.92	2,123.92	1,284.63
	Schizothrix	5,337.60	5,268.93	4,956.72
	Synechocystis	136.29	123.02	2,841.74
	Synechococcus	0.00	0.00	16.45
	Spirulina	47.90	47.90	47.90
	cells mL^{-1}	**41,578.17**	**30,180.69**	**29,792.77**
	%	**92.53^{a}**	**76.71^{b}**	**76.96^{b}**
Bacillariophyta	*Acnanthes*	0.00	0.00	23.46
	Amphora	0.43	0.29	0.00
	Climacosphenia	0.00	0.87	0.00
	Cocconeis	0.29	0.00	3.17
	Coscinodiscus	0.00	0.00	0.58
	Cyclotela	1.59	1.15	0.00
	Cymbella	0.38	0.10	0.10
	Diatoma	44.34	60.50	35.97
	Diploneis	402.72	149.23	113.45
	Fragilaria	203.62	468.80	263.06
	Gyrosigma	0.29	0.00	61.17
	Navicula	24.05	43.67	203.23
	Nitzschia	1.73	0.29	1.15
	Orthoseira	154.23	399.21	775.34
	Penium	3.75	2.31	0.00
	Pinularia	0.29	1.44	0.77
	Rhabdonema	545.36	989.44	1,189.41
	Rhizosolenia	1.15	0.00	84.45
	Skeletonema	565.41	727.72	597.30
	Synedra	0.00	0.00	0.43

	Thalassiosira	0.58	0.00	0.60
	Chloridella	2.12	7.04	409.10
	cells mL^{-1}	**1,952.32**	**2,852.02**	**3,762.72**
	%	**4.34**^{a}	**7.25**^{a}	**9.72**^{a}
Chlorophyta	*Closterium*	7.79	4,051.25	0.29
	Phymathodocis	583.64	388.07	317.89
	Spirogyra	157.10	204.03	93.78
	Staurodesmus	0.00	0.00	51.94
	Botryococcus	38.48	18.28	8.47
	Cylindrocystis	0.00	432.83	3,462.60
	Mychonastes	264.50	654.05	343.05
	Schizomeris	7.79	109.51	132.73
	Planctonema	41.94	41.94	183.32
	Haematococcus	1.25	1.25	0.39
	Ulothrix	162.84	228.05	418.64
	cells mL^{-1}	**1,265.32**	**6,129.23**	**5,013.09**
	%	**2.82**^{b}	**15.58**^{a}	**12.95**^{a}
Euglenophyta	*Euglena*	0.87	0.58	0.00
	Trachelomonas	133.65	179.73	132.67
	cells mL^{-1}	**134.51**	**180.31**	**132.67**
	%	**0.30**^{a}	**0.46**^{a}	**0.34**^{a}
Dinophyta	*Peridinium*	1.41	1.06	4.52
	Pyrophacus	0.38	0.00	0.00
	Gimnodinium	2.09	1.30	4.33
	Scrippsiella	0.10	0.67	0.10
	cells mL^{-1}	**3.98**	**3.03**	**8.95**
	%	**0.01**^{a}	**0.01**^{a}	**0.02**^{a}
	total (cells mL^{-1})	**44,934.29**^{a}	**39,345.28**^{a}	**38,710.19**^{b}

[1] The data correspond to the mean ± standard deviation.

Results from repeated measures ANOVA and Tukey’s test. Mean values in the same row with different superscripts differ significantly ($P < 0.05$).

Control (shrimp monoculture in biofloc); SB (shrimp and *Gracilaria birdiae* in biofloc integrated system) and SD (shrimp and *Gracilaria domingensis* in biofloc integrated system)

Table 3 Performance parameters of Pacific white shrimp *Litopenaeus vannamei* in an integrated aquaculture with red seaweed *Gracilaria* in biofloc system in WSSV presence during the 28-day experiment period.

Parameters	Treatments[1]		
	Control	SB	SD
Final weight (g)	5.42 ± 0.19^c	6.57 ± 0.02^a	5.75 ± 0.08^b
Weight gain (g)	2.74 ± 0.12^c	3.92 ± 0.29^a	3.22 ± 0.07^b
Yield (Kg m^{-3})	1.30 ± 0.25^a	1.40 ± 0.09^a	1.37 ± 0.38^a
Survival (%)	56 ± 9^a	50 ± 3^a	56 ± 15^a
FCR	2.89 ± 0.53^a	2.06 ± 0.19^a	2.26 ± 0.45^a
growth $week^{-1}$	0.69 ± 0.03^c	0.98 ± 0.07^a	0.81 ± 0.02^b
SGR (% day^{-1})	1.56 ± 0.07^c	2.12 ± 0.12^a	1.81 ± 0.03^b

[1] The data correspond to the mean of three replicates ± standard deviation.

Results from one-way ANOVA and Tukey’s test. Mean values in the same row with different superscripts differ significantly ($P < 0.05$).

Control (shrimp monoculture in biofloc); SB (shrimp and *Gracilaria birdiae* in biofloc integrated system) and SD (shrimp and *Gracilaria domingensis* in biofloc integrated system)

SGR (% day^{-1}) = 100 x [ln final weight (g) – ln initial weight (g)] / time

FCR = feed supplied (dry weight)/ biomass gain.

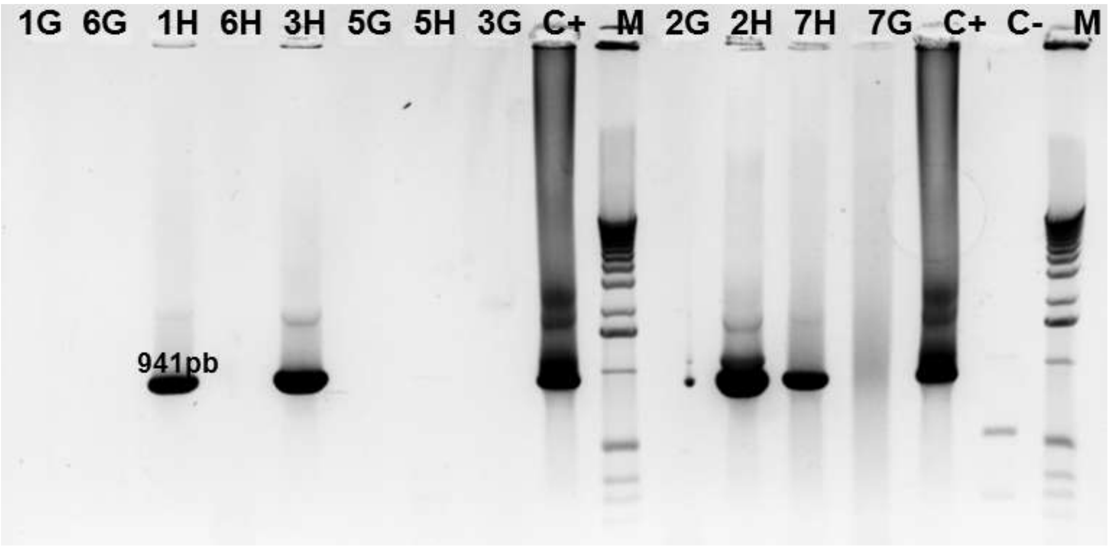

Fig.1 Amplicons after electrophoresis in 1% agarose gel stained with ethidium bromide. Samples of shrimp 1H, 3H, 2H and 7H are WSSV positive; C^+ = positive control; C^-= negative control (ultrapure water) M= 1 kb molecular weight marker (Invitrogen, USA), H= hemolymph and G= gill tissues.

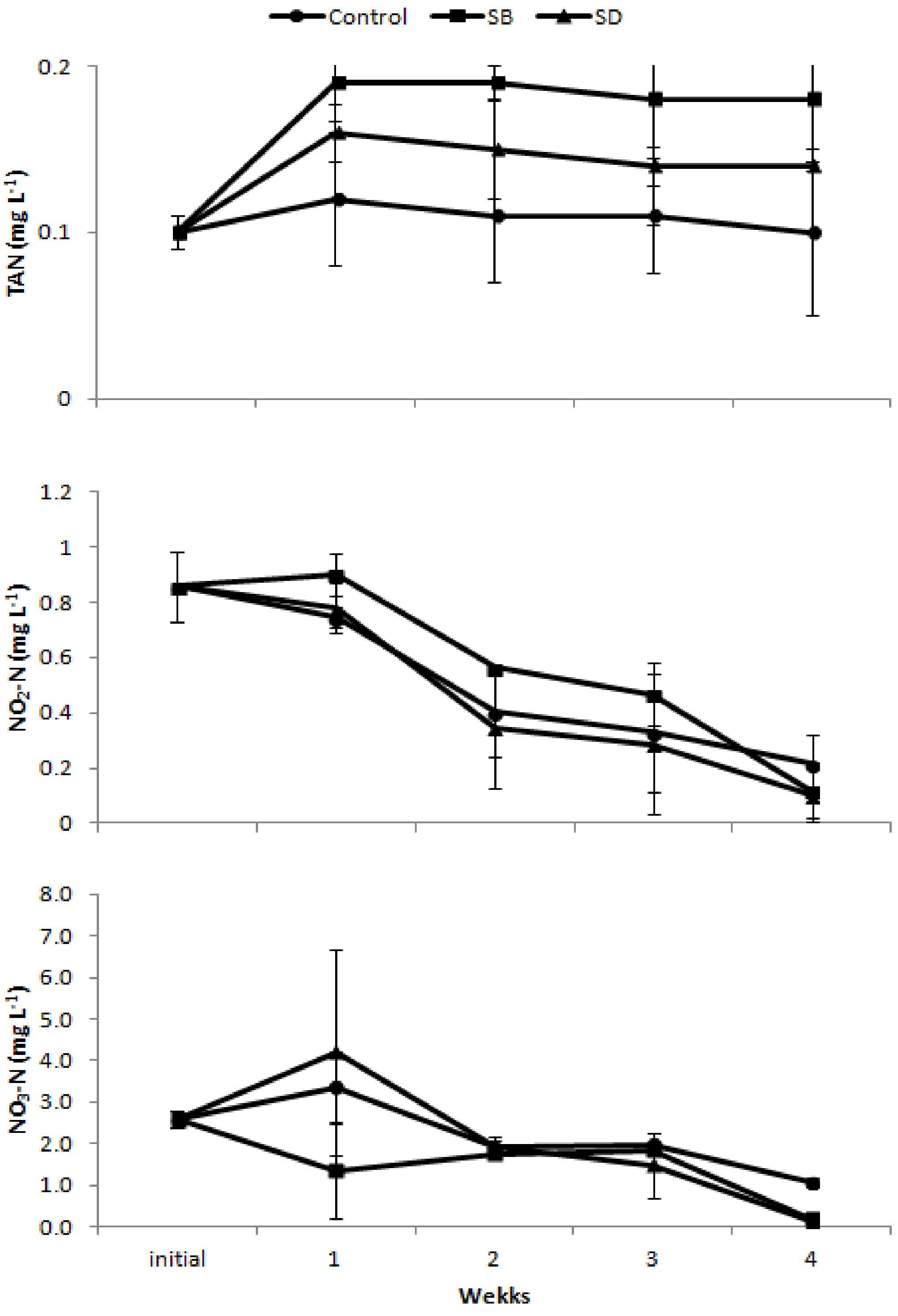

Fig. 2 Fluctuations of total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2–N) and nitrate-nitrogen (NO_3-N) concentrations on the tanks during 28 days experimental period.

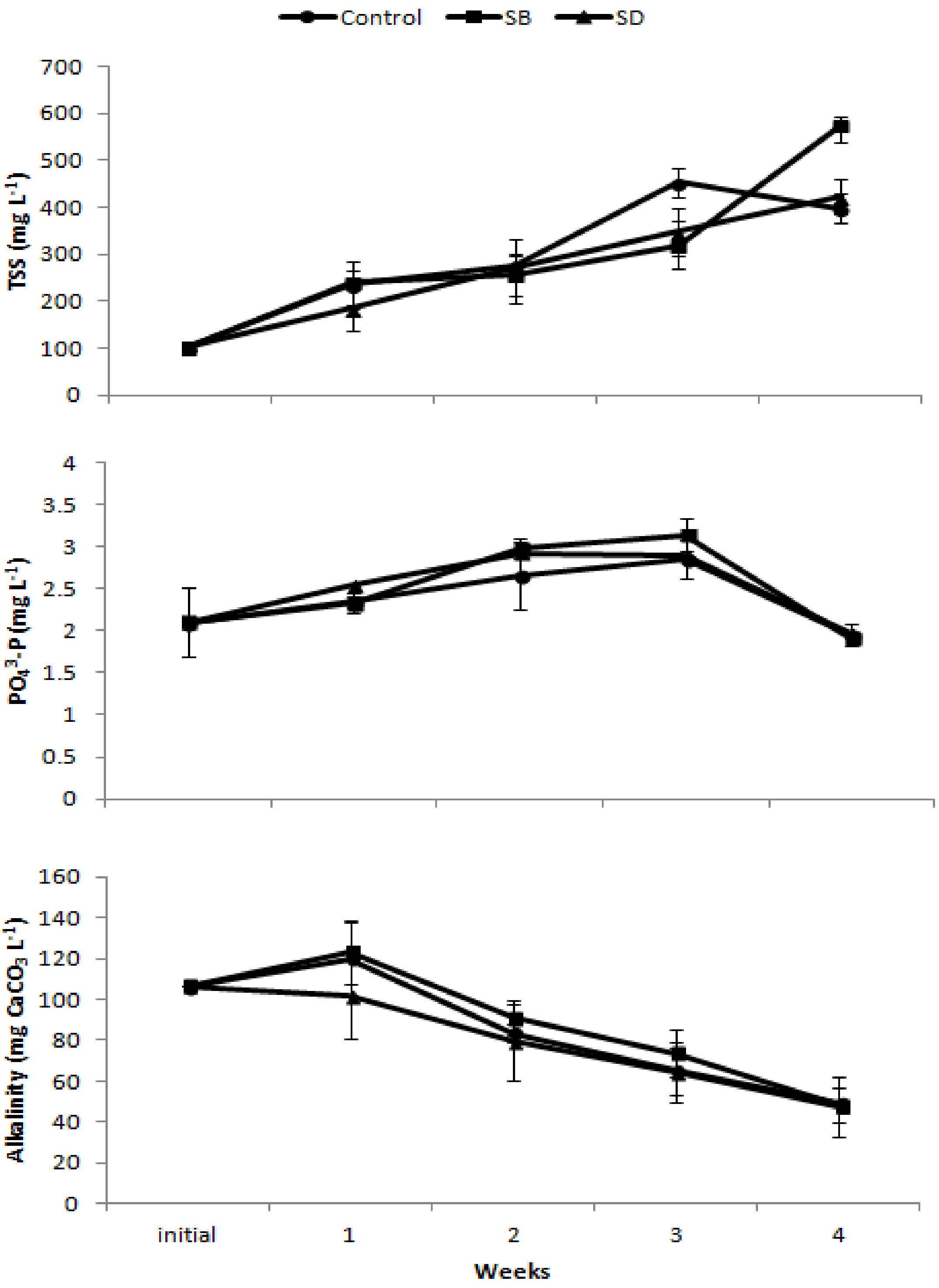

Fig. 3 Fluctuations of total suspend solids (TSS), phosphate (PO_4^{3}–P) and alkalinity (mg L^{-1} $CaCO_3$) concentrations on the tanks during 28 days experimental period.

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Luis Otavio Brito, Augusto Monteiro Chagas, Elizabeth Pereira da Silva, Roberta Borda Soares, William Severi & Alfredo Olivera Gálvez

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Water quality, *Vibrio* density and growth of the Pacific white shrimp *Litopenaeus vannamei* (Boone) in an integrated in biofloc system with red seaweed *Gracilaria birdiae* (Greville)

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Litopenaeus vannamei and *Gracilaria birdiae* in biofloc system

Keywords: Rodophyta, Penaeidae, nutrient uptake, bioremediation

Abstract

An indoor trial was conducted for 42 days to evaluate water quality, *Vibrio* density and growth of *Litopenaeus vannamei* in an integrated biofloc system with red seaweed *Gracilaria birdiae*. Shrimp individuals (0.34 ± 0.01 g) were stocked in experimental tanks at a density of 500 shrimp m^{-3} and *G. birdiae* was stocked at a biomass of 2.5, 5.0 and 7.5 fresh weight seaweed m^{-3}. No water exchange was carried out during the experimental period. Additional molasses was used once a day as an organic carbon source to maintain the C:N ratio at 12:1. The integrated biofloc system (shrimp and seaweed) significantly decreased ($P < 0.05$) dissolved inorganic nitrogen (DIN) concentration (by 19 - 34%), nitrate-nitrogen (NO_3-N) concentration (by 19 - 38%), *Vibrio* density (by 8 - 83%), and FCR (by 20 - 30%). Increases in crude protein in shrimp (by 8 - 13%), crude protein in seaweed (by 44 - 75%), shrimp yield (by 22 - 39%), final weight (by 25 - 32%), biomass weight (by 24 - 42%), weight gain (by 27 - 34%), and weekly growth (by 25 - 34%) were detected. *Gracilaria birdiae* in an integrated biofloc system with *L. vannamei* can contribute to water quality, lower *Vibrio* density, increased crude protein in shrimp and seaweed, and growth and yield parameters in shrimp culture.

Introduction

Zero or minimal water exchange can increase waste and nitrogen compound concentration (Krummenauer, Peixoto, Cavalli, Poersch & Wasielesky 2012). However, solid wastes, dissolved nutrients from uneaten feed and fecal material can be made available for shrimp nutrition because they add organic carbon to the water column, which acts as a substrate for heterotrophic bacteria transformation of waste into microbial protein (Avnimelech 2009; Gao, Shan, Zhang, Bao & Ma 2012; Zhao, Huang, Wang, Song, Yang, Zhang & Wang 2012; Pérez-Fuentes, Pérez-Rostro & Hernández-Vergara 2013; Xu & Pan 2013). About 39.1% of the nitrogen and 35% of the phosphorus input from shrimp feed and molasses in a biofloc system is incorporated into shrimp biomass (Silva, Wasielesky & Abreu 2013a). These values are higher than in a traditional system (about 25%) (Avnimelech 2009). However, large amounts of nitrogen and phosphorus (dissolved inorganic nitrogen - DIN 7.7%; dissolved organic nitrogen - DON 31.25%; dissolved inorganic phosphate - DIP 17.3%, and dissolved organic phosphate - DOP 16.8%) may not be assimilated by shrimp in a biofloc system (Silva *et al*. 2013a).

Integrated aquaculture systems have more balanced nutrient recycling than traditional aquaculture systems because they use species with different trophic levels, providing the opportunity to diversify aquaculture products and increase the growth and yield of crustaceans and fish (Troell, Joyce, Chopin, Neori, Buschmann & Fang, 2009; Barrington, Ridler, Chopin, Robinson & Robinson 2010; Ren, Stenton-Dozey, Plew, Fang & Gall 2012). Nevertheless, the efficiency of integrated aquaculture systems requires maintaining the optimal stock of biomass of the cultivated species (Ren *et al*. 2012).

Wastes are a major problem in shrimp yield because of their toxicity to cultured aquatic organisms. In intensive systems, shrimp can be exposed to high concentrations of nitrogen compounds, which interfere in shrimp immunity (Chen, Sim, Chiew, Yeh, Liou & Chen 2012). Thus, the balance between yield and environmental waste assimilation capacity is very important to the development of intensive systems (Thakur & Lin 2003).

The poor assimilation of nutrients (nitrogen and phosphorus) by shrimp can be compensated by their uptake by red seaweed *Gracilaria*, which can turn wastes into biomass, significantly improving water quality in traditional (Huo, Xu, Wang, Zhang, Zhang, Wu, Chen & He 2011; Marinho-Soriano, Azevedo, Trigueiro, Pereira & Carneiro 2011; Huo, Wu, Chai, Xu, Han, Dong & He 2012; Robledo, Navaro-Angulo, Lozano & Freile-Pelegrin 2012) and zero-exchange systems (Sánchez-Romero, Miranda-Baeza, López-Elías, Martínez-Córdova, Tejeda-Mansir & Márquez-Ríos, 2013). The green seaweed *Ulva* may also improve growth and yield of the shrimp in traditional (Gamboa-Delgado, Peña-Rodríguez, Ricque-Marie & Cruz-Suárez 2011) and intensive systems (Brito, Arantes, Magnotti, Derner, Pchara, Olivera & Vinatea, 2013).

In this context, this study evaluated water quality, *Vibrio* density and growth of *Litopenaeus vannamei* in an integrated biofloc system with red seaweed *Gracilaria birdiae*.

Materials and Methods

Experimental conditions

An indoor trial was conducted for 42 days at the Sustainable Mariculture Laboratory (LAMARSU) of the Fisheries and Aquaculture Department (DEPAq) of the Rural Federal University at Pernambuco (UFRPE), Recife, Brazil (08°01’00.16¨S, 034°56’57.74”W). The experimental design was completely randomized with four treatments: Control (monoculture *L. vannamei*); LG 2.5 (*L. vannamei* and 2.5 fresh weight m^{-3} *G. birdiae* in an integrated biofloc system); LG 5.0 (*L. vannamei* and 5.0 kg fresh weight m^{-3} *G. birdiae* in an integrated biofloc system) and LG 7.5 (*L. vannamei* and 7.5 fresh weight m^{-3} *G. birdiae* in an integrated biofloc system) with three replicates of each.

Five days prior to stocking shrimp and seaweed, water from a matrix tank (TAN 0.2 mg L^{-1}, NO_2-N 0.3 mg L^{-1}, NO_3-N 2.2 mg L^{-1}, alkalinity 133.9 mg $CaCO_3$ L^{-1} and TSS 133.6 mg L^{-1}) was mixed and equally distributed to fill twelve black-plastic tanks (40 L, 0.20 m^2) up to approximately 25% of the volume, and the remaining 75% of the tanks were completed with seawater. The experimental units were maintained under constant aeration by three airstones per tank. No water exchange was carried out during the experimental period, except for the addition of dechlorinated freshwater to compensate for evaporation losses. The light intensity was kept at ~ 1000 lux using a fluorescent lamp with a natural photoperiod. Additional molasses was used once a day as an organic carbon source to maintain the C:N ratio at 12:1 (Avnimelech 2009). Hydrated lime ($Ca(OH)_2$) was used to maintain alkalinity and pH > 100 mg L^{-1} and 7.5, respectively (Furtado, Poersch & Wasielesky 2011).

Shrimp and seaweed stocking, feeding, and monitoring

Specific pathogen-free post-larvae (PLs 10) of *L. vannamei* were obtained from a commercial laboratory (Potiporã, Barra de Sirinhaém, PE, Brazil). PLs were raised in two 400 L rectangular tanks until 25 days (average weight of 0.34 g), at a stocking density of 2500 PLs m^{-3}) in salinity of 35 g L^{-1}. The water for the culture was prepared with the addition of the microalgae *Navicula* sp (50 x 10^4 cells mL^{-1}). No water exchange was carried out during the experimental period, except for dechlorinated freshwater added to compensate for evaporation losses. Additional molasses was used once a day as an organic carbon source to maintain the C:N ratio at 12:1 (Avnimelech 2009). The post-larvae were fed five times a day (at 0800, 1100, 1300, 1500 and 1800h), with a commercial shrimp feed with 40% crude protein and 8% ether extract (Evialis, Presence, Camanutri, Brazil) based on the table of Van Wyk (1999).

Experimental units were stocked with Pacific white shrimp *L. vannamei* (0.34 ± 0.01 g initial weight) at a density of 500 shrimp m^{-3}. The shrimp were fed three times a day (at 0800, 1200 and 1600h), with a commercial shrimp feed with 40% crude protein and 8% ether extract (Evialis, Presence, Camanutri, Brazil) based on the table of Van Wyk (1999), and adjusted daily according to the estimated shrimp consumption, mortality rate and leftover feed.

Samples of *Gracilaria* biomass were collected at the Pau Amarelo beach, Paulista, Pernambuco, Brazil (07°54’54.74¨S, 034°49’12.07¨W), and stored in plastic bags for laboratory analysis. Water was drained from all the samples, and the material was carefully inspected to eliminate encrusted organisms and then weighed. Seaweed with reproduction structures, signs of depigmentation and necrosis were discarded (Marinho-Soriano *et al*. 2011; Tsutsui, Kanjanaworakul, Srisapoome, Aue-Umneoy & Hamano 2010). The seaweed was cultivated in rectangular PVC modules (20 x 6.5 x 2.2 cm, which increased surface area by 13%, placed horizontally into seaweed tanks. The rectangular PVC modules also were used in control tanks without seaweed.

Shrimp weight was monitored on a weekly basis to determine shrimp growth and adjust the amount of feed and organic carbon offered. At the end of the experiment, biomass gain, specific growth rate (SGR), mean final weight, weekly growth, feed conversion ratio (FCR), survival and yield were determined based on the following equations: Biomass gain (g) = final biomass (g) – initial biomass (g); SGR (% day^{-1}) = 100 x [ln final weight (g) – ln initial weight (g)] / time (days); Final weight (g) = final biomass (g) / survival; Weekly growth (g week^{-1}) = biomass gain (g) / times (weeks) of culture; FCR = feed supplied (dry weight)/ biomass gain; Survival (%) = (number of individuals at the end of evaluation period / initial number of individuals stocked) x 100; Yield (Kg m^{-3}) = final biomass (kg) / volume of experimental unit (m^3).

Water quality

Dissolved oxygen and temperature were monitored (YSI model 55, Yellow Springs, Ohio, USA) twice a day (8:00 and 16:00 h). Salinity (YSI 30, Yellow Springs, Ohio, USA), pH (YSI model 100, Yellow Springs, Ohio, USA) and settleable solids (SS) (Imhoff cone) (Avnimelech 2009) were monitored twice a week. Total ammonia nitrogen (TAN), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), total suspended solids (TSS), phosphate (PO_4^3–P) and alkalinity (mg L^{-1} $CaCO_3$) were monitored once a week, following the methods described by Koroleff (1976), Golterman, Clymo & Ohnstad (1978), Mackereth, Heron & Talling (1978), APHA (2005), and Felföldy, Szabo & Tothl (1987), respectively.

Vibrio monitoring

Presumptive analyses of *Vibrio* spp. were performed at the beginning and end of the trial, by sampling the water (500 mL). The preparation of sample dilutions and bacteriological assays were performed separately for the two samples and averaged by using the method described by APHA (2005). Bacteriological analyses of the water were conducted with appropriate sample dilutions (10^{-1} to 10^{-5}) with sterilized saline solution (2.5% NaCl). One milliliter of the homogenate was serially diluted (10^{-1} to 10^{-}

^{5}) and inoculated in thiosulphate–citrate–bile sucrose (TCBS) agar (Oxoid). Every analysis was duplicated using the spread plate method. All *Vibrio* inoculated plates were incubated at 24^{o}C for 30 h and colony forming units (CFU) were counted. Readings obtained with 25 and 250 colonies on a plate were used to calculate bacterial population numbers, recorded as CFU per unit of sample.

Proximate composition (Shrimp and Seaweed)

Proximate composition analysis of crude protein, crude lipid, moisture and ash contents of the shrimp (whole body) and seaweed samples were performed in triplicate using standard methods (AOAC 2000) at the Instituto Agronômico of Pernambuco (IPA), Recife, Brazil. Protein was determined by measuring nitrogen (N·x 6.25) using the Kjeldahl method; lipids determined by ether extraction using Soxhlet and ash by oven incineration at 550 °C. The moisture of the shrimp sample was determined by oven drying at 105 °C for 24 h.

Statistical Analysis

A parametric one-way ANOVA was used to analyze production parameters, after confirming homocedasticity (Cochran $P < 0.05$) and normality (Shapiro-Wilk $P < 0.05$). Tukey's test ($P < 0.05$) was performed to compare and rank means from the three treatments and the control. Water quality parameters were analyzed by performing repeated measures ANOVA. Data on *Vibrio* density and shrimp survival were analyzed using (log x) and arcsine-transformed data, respectively. Data analyses were performed using ASSISTAT Version 7.7 (Assistat Analytical Software, Campina Grande, Paraiba, Brazil).

Results

DIN uptake (%) of *G. birdiae* ranged from 19 to 34% as compared to control. However, no significant differences (*P* > 0.05) were detected in the stocked biomass of seaweed (Table 1). The principal DIN compounds were NO_3-N ranging from 73 to 82%, followed by NO_2-N ranging from 8 to 17%, and TAN ranging from 5 to 8%. No significant differences (*P* > 0.05) between treatments were detected in water quality regarding temperature, dissolved oxygen, pH, salinity, TAN, PO_4^{3}–P, TSS and SS. However, significant differences (*P* < 0.05) were recorded for DIN, NO_2-N, NO_3-N, and alkalinity between treatments (Table 1).

Insert Table 1

Vibrio density in water decreased in treatments with seaweed, ranging from 8 to 83% as compared to the control, and there were significant differences (*P* < 0.05) between treatments with different stocking biomass of seaweed (Figure 1).

Insert Figure 1

The shrimp (13.2 to 13.7% wet weight basis) in an integrated biofloc system had higher crude protein content (*P* < 0.05) than the control (12.1%). However, moisture (~80%), lipids content (~1.5 % wet weight basis) and ash (~2% wet weight basis) did not differ significantly (*P* > 0.05) compared to the control (Table 2). The seaweed (13.6 to 16.1% dry weight) in an integrated biofloc system, had higher crude protein content (*P* < 0.05) as compared to the initial value (9.2% dry weight). Lipids (0.4 to 0.5% dry weight) were lower (*P* < 0.05) than the initial amount (1.1% dry weight). However, moisture (80.6 to 81.8%) and ash (41.3 to 43.3% dry weight) did not differ significantly ($P > 0.05$) with their initial values (Table 3).

Insert Table 2 and 3

The shrimp survival rates were all above 89% during the 42-day experimental period. The shrimp FCR in integrated biofloc systems with seaweed was 20-30% significantly lower ($P < 0.05$) than the control. Performance parameters of shrimp in the integrated biofloc systems with seaweed were significantly higher ($P < 0.05$) than the control (final weight by 25-32%, yield by 22-39%, weight biomass by 24-42%, weight gain by 27-34% and weekly growth by 25-34%) (Table 4).

Insert Table 4

Discussion

The water quality parameter (dissolved oxygen, salinity, pH, TAN, NO_2-N, NO_3-N, alkalinity) concentrations in the culture water were within the range recommended for marine shrimp cultivation. However, water temperature (26 to 27 °C) was lower than recommended (28 to 32 °C) by Van Wyk and Scarpa (1999).

The red seaweed *G. birdiae* in an integrated biofloc system decreased DIN (19 - 34%) and NO_3-N (19 - 38%) concentration as compared to the control. Marinho-Soriano *et al.* (2011) using *G. caudata* recorded an uptake of 17% for DIN and 70% for NO_3-N. Other studies have reported a NO_3-N uptake of 47% (Huo *et al.* 2012) and 75% (Huo *et al.* 2011) using *G. verrucosa*, and Robledo *et al.* (2012) reported similar results (61 - 88.5%) with *Hydropuntia córnea* (*Gracilaria cornea*).

A positive effect was observed for DIN and NO_3-N uptake for increased seaweed stocking biomass. Similar results were observed for TAN and NO_3-N by Khoi & Fotedar (2011) with *Penaeus latisulcatus* and *Ulva lactuca,* although Robledo *et al.* (2012) observed a negative effect of seaweed stocking biomass for TAN and DIN uptake. In biofloc systems, heterotrophic and nitrifying bacteria are the main factors responsible for the transformation of TAN and NO_2-N (Castillo-Soriano, Ibarra-Junquera, Escalante-Minakata, Mendoza-Cano, Ornelas-Paz, Almanza-Ramírez & Meyer-Willerer 2013). In addition, NO_3-N (by 73 - 82%) was the main form of inorganic nitrogen in the tanks, followed by NO_2-N (by 8 - 17%) and TAN (by 5 - 8%), which facilitate their uptake, since NO_3-N and ammonium are the main form of inorganic nitrogen uptaken by red seaweed (Abreu, Pereira, Buschmann, Sousa-Pinto & Yarish 2011). Higher concentrations of NO_3-N in a biofloc system as compared to NO_2-N and TAN were also observed by Silva *et al*. (2013b) and Xu & Pan (2013) indicating that nitrifying bacteria were also present in the bioflocs, probably due to the lower utilization of the NO_3-N form by microbial communities.

The TAN and NO_2-N uptake by *Gracilaria* in zero exchange water depend on the photoperiod (16:8 light/dark for TAN and 14:10 light/dark NO_2-N) and a biomass ratio of shrimp:seaweed of 1:8 (Sánchez-Romero *et al*. 2013). In the experiments, the biomass ratios of shrimp: seaweed were 1.27 (LG 2.5), 2.74 (LG 5.0) and 4.3 (LG 7.5), light intensity was kept at ~ 1000 lux with a natural photoperiod. The lower values than those cited by Sánchez-Romero *et al*. (2013), probably decreased the uptake rate.

In biofloc systems, the phosphorus accumulated during the culture period (Silva *et al*. 2013b), and PO_4^{3}–P uptake was not recorded for the red seaweed *G. birdiae* in the integrated system. Two factors probably impede the uptake, an increase in the nutrient concentrations during the culture period (Carneiro, Freire & Marinho-Soriano 2011) and a decreased N:P ratio in the water (Du, Liu, Wang & Wang 2013).

In the monoculture (*L. vannamei*) and integrated system (*L. vannamei* and *G. birdiae*) the addition of inorganic carbon was necessary because of the nitrification processes and the conversion of ammonium nitrogen into heterotrophic microbial biomass (Ebeling, Timmons & Bisogni 2006 The use of carbon dioxide by photosynthesis increases alkalinity, because carbonate (CO_3^{2-}) accumulates in the water and hydrolyzes to form bicarbonate (HCO_3^-). For this to occur a greater number of H^+ from the water must dissociate to maintain a constant balance, resulting in more OH^- and less H^+ than when photosynthesis began (Cavalcante & Sá 2010).

In biofloc systems, high concentrations of TSS can interfere in shrimp growth, by causing gill occultation in cultured species (Ray, Lewis, Browdy & Leffler 2010). The TSS concentrations in our experiments were lower than those recommended in the literature, between 400 and 600 mg L^{-1} (Schveitzer, Arantes, Costódio, Santo, Arana, Seiffert & Andreatta (2013a), and did not interfere in shrimp growth. With respect to SS, the seaweed stocking biomass apparently had a favorable influence on the development of bioflocs, due to the colonization of microorganisms that use seaweed as a substrate. According to Lombardi, Almeida, Lima, Sale & Paula (2006) seaweed can play a role as a natural substrate, providing shade, shelter, and also bedding for other small organisms, which may improve the natural source of live food for shrimp. Increased tank surface area for shrimp in biofloc systems has been shown to improve water quality compared to tanks without substrate (Anand, Kumar, Panigrahi, Ghoshal, Dayal, Biswas, Sundaray, De, Ananda, Deo, Pillai & Ravichandran 2012; Arnold, Coman, Jackson & Groves 2009).

The zero or minimal exchange water increases the amount of organic matter in the water, favoring the development of *Vibrio*, because high concentrations of these species are related to large quantities of organic matter in the culture water (Ferreira, Bonetti & Seiffert 2011). However, the presence of seaweed (2.5 and 5.0 fresh weight Kg m^{-3}) in shrimp biofloc systems reduces *Vibrio* density (by 54 - 83%). Seaweed has bioactive compounds with: β-glucan, carotenoids, tocopherols, polyphenols and polysaccharides and other medicinal properties, serving as antioxidants and encouraging bioactivity against virulent and antibiotic resistant *Vibrio* (Peso-Echarri, Frontela-Saseta, González-Bermúdez, Ros-Berruezo & Martínez-Garciá 2012; Silva, Costa, Peixoto, Nascimento & Carneiro 2013b). These compounds enhance the shrimps' defense mechanisms by increasing the haemocyte and granulocyte counts, and by increasing the activity of phenoloxidase and superoxide dismutase, which decreased mortality rates from viral and *Vibrio* diseases (Huynh, Yeh, Lin, Shyn, Chen & Chen 2011; Kanjana, Radtanatip, Asuvapongratana, Withyachummarnkul & Wongprasert 2011; Sirirustananun, Chen, Lin, Yeh, Liou, Chen, Sim & Chiew 2011).

This decreased *Vibrio* density probably favored the shrimp growth, because *Vibrio* species are the most dangerous, causing morbidity, mortality, low growth and higher FCR (Vieira, Lima, Menezes, Costa, Sousa & Barreto 2009). However, there is a correlation between *Vibrio* density and increased seaweed stocking biomass. These results indicate the need to evaluate the dynamic changes of the composition of the *Vibrio* community and possibly harmful characteristics need to be examined by further research of the use of seaweed in biofloc systems.

The higher crude protein content of shrimp (13.2 – 13.7%) in an integrated system as compared to a monoculture was found by Cruz-Suárez, León, Penã - Rodríguez, Rodríguez-Penã, Moll & Ricque-Marie (2010). *Gracilaria* has balanced sources of *ω*3 and *ω*6 fatty acids, essential amino acids (valine, methionine, lysine and phenyl alanine), minerals (sodium, potassium and calcium) and vitamin C and E (Tabarsa, Rezaei, Ramezanpour & Waaland 2012; Syad, Shunmugiah & Kasi 2013). These components may act as nutritional supplements and/or improve the utilization of nutrients from the artificial feed by shrimp (Cruz- Suárez *et al*. 2010). Gamboa-Delgado *et al*. (2011) showed that whole body shrimp had high incorporation of carbon (31-79%) and nitrogen (73-98%) in integrated tanks (*L. vannamei* and *Ulva clathrata)*. The moisture, lipids and ash content found was similar to those identified by Wasielesky, Atwood, Stokes & Browdy (2006).

The integrated biofloc systems contributed to a higher crude protein content (13.6 – 16.1%) of seaweed, compared to the initial value (9.2%). This increase of crude protein content is associated to the higher concentration of nitrogen compounds (inorganic and organic) in biofloc systems, which metabolized protein by seaweed. Similar results were observed by Khoi & Fotedar (2011) in traditional system. The lipids content of seaweed in an integrated biofloc system (0.4 to 0.5% dry weight) was lower than the initial value (1.1% dry weight). The increased TSS and reduced light in the tanks probably affected the lipids content. Levy, Maxim & Friedlander (1992) showed an increase in unsaturated fatty acid with increasing photon flux density. However, there are many contradictory results in the literature about the influence of light on lipids content in seaweed (Khotimchenko & Yakovleva 2005). There are many factors that affect the lipids content in seaweed including nitrogen and phosphorus availability, temperature, salinity, pH, heavy metals and UV irradiance (Sharma, Schuhmann & Schenk 2012). The moisture and ash were similar to that observed by Tabarsa *et al*. (2012).

A positive effect of seaweed biomass on final weight, FCR and yield was recorded, similar to that reported by Brito *et al.* (2013) using *U. lactuca* and *L. vannamei* in a biofloc system; Portillo-Clark, Casillas-Hernández, Servín-Villegas, Magallón-Barajas (2012) with *Caulerpa sertularioides* and *Farfantepenaeus californiensis* at different temperatures; Gamboa-Delgado *et al*. (2011) with *Ulva clathrata* and *L. vannamei*; Izzati (2011) with *Gracilaria verrucosa* and *P. monodon*; Cruz-Suarez *et al.* (2010) with *U. clathrata* and *L. vannamei*; and Tsutsui *et al.* (2010) with *Chaetomorpha ligustica* in different growth phases of *Penaeus monodon.* Increased final weight in biofloc tanks with artificial substrate has been shown by Anand *et al*. (2012), Arnold *et al*. (2009), Audelo-Naranjo, Martínez-Córdova, Voltolina & Gómez-Jiménez (2011) and Schveitzer, Arantes, Baloi, Costódio, Arana, Seiffert & Andreatta (2013b).

In summary, the results of this experiment corroborate those of other studies in traditional system that reported the positive effect of the presence of seaweed on production parameters in shrimp culture. Our results indicate that *Gracilaria birdiae* (2.5 and 5.0 fresh weight m^{-3}) contributed to increased shrimp growth, because the red seaweed's DIN and NO_3-N uptake decreased *Vibrio* density, increased the crude protein content of shrimp and source supplement food for the shrimp in biofloc system. However, further research should be conducted to evaluate the potential use of other seaweed species associated to various stocking densities of shrimp.

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Table 1. Water quality parameters in an integrated biofloc system with *Litopenaeus vannamei* and *Gracilaria birdiae,* during the 42-day experiment period.

Variables	Treatment			
	Control	LG 2.5	LG 5.0	LG 7.5
Morning temperature (ºC)	26.08 ± 0.03^{a}	26.04 ± 0.06^{a}	26.07 ± 0.16^{a}	26.07 ± 0.08^{a}
Afternoon temperature (ºC)	27.48 ± 0.14^{a}	27.43 ± 0.16^{a}	27.60 ± 0.46^{a}	27.42 ± 0.04^{a}
Morning DO (mg L^{-1})	6.38 ± 0.04^{a}	6.41 ± 0.08^{a}	6.31 ± 0.16^{a}	6.32 ± 0.08^{a}
Afternoon DO (mg L^{-1})	6.62 ± 0.11^{a}	6.65 ± 0.01^{a}	6.57 ± 0.09^{a}	6.55 ± 0.09^{a}
pH	7.82 ± 0.04^{a}	7.88 ± 0.09^{a}	7.87 ± 0.03^{a}	7.89 ± 0.05^{a}
Salinity (g L^{-1})	36.01 ± 0.12^{a}	35.83 ± 0.22^{a}	35.95 ± 0.11^{a}	36.26 ± 0.41^{a}
DIN (mg L^{-1})	4.73 ± 0.61^{a}	3.83 ± 0.92^{ab}	3.23 ± 0.36^{b}	3.12 ± 0.70^{a}
TAN (mg L^{-1})	0.39 ± 0.14^{a}	0.21 ± 0.04^{a}	0.28 ± 0.30^{a}	0.15 ± 0.14^{a}
NO_2–N (mg L^{-1})	0.40 ± 0.02^{b}	0.45 ± 0.04^{ab}	0.54 ± 0.05^{a}	0.50 ± 0.03^{a}
NO_3-N (mg L^{-1})	3.93 ± 0.69^{a}	3.16 ± 0.89^{ab}	2.40 ± 0.34^{b}	2.46 ± 0.84^{b}
PO_4^{3}–P (mg L^{-1})	1.97 ± 0.09^{a}	1.98 ± 0.37^{a}	2.19 ± 0.13^{a}	2.20 ± 0.15^{a}
Alkalinity (mg $CaCO_3$ L^{-1})	126.1 ± 8.9^{b}	140.7 ± 17.0^{ab}	160.6 ± 13.3^{a}	160.5 ± 12.9^{a}
TSS (mg L^{-1})	213.3 ± 22.5^{a}	253.8 ± 42.9^{a}	223.1 ± 39.0^{a}	240.3 ± 70.1^{a}
SS (mg L^{-1})	4.59 ± 0.60^{a}	5.85 ± 0.76^{a}	6.58 ± 0.77^{a}	5.11 ± 1.03^{a}

The data correspond to the mean ± standard deviation. Results were analyzed by performing repeated ANOVA measures and the Tukey test. Mean values in the same row with different superscripts differ significantly ($P < 0.05$). Control (monoculture *L. vannamei*); LG 2.5 (*L. vannamei* and 2.5 kg m^{-3} *G. birdiae* in an integrated biofloc system); LG 5.0 (*L. vannamei* and 5.0 kg m^{-3} *G. birdiae* in an integrated biofloc system) and LG 7.5 (*L. vannamei* and 7.5 kg m^{-3} *G. birdiae* in an integrated biofloc system).. DO – dissolved oxygen, DIN (TAN+ NO_2–N + NO_3–N) dissolved inorganic nitrogen, Total ammonia nitrogen (TAN), nitrite (NO_2–N), nitrate (NO_3–N), phosphate (PO_4^{3}–P) and total suspended solids (TSS), settleable solids (SS).

Table 2. Proximate composition (% wet weight basis) of whole body *Litopenaeus vannamei* in an integrated biofloc system with *Gracilaria birdiae* during the 42-day experiment period.

	Control	LG 2.5	LG 5.0	LG7.5
Moisture (%)	81.51 ± 0.49^a	81.29 ± 0.38^a	82.16 ± 0.19^a	81.82 ± 0.70^a
Crude protein	12.17 ± 0.05^b	13.76 ± 0.08^a	13.50 ± 0.18^a	13.24 ± 0.40^a
Lipids	1.68 ± 0.20^a	1.59 ± 0.26^a	1.47 ± 0.14^a	1.69 ± 0.28^a
Ash	2.44 ± 0.06^a	2.60 ± 0.07^a	2.26 ± 0.09^a	2.19 ± 0.11^a

The data correspond to the mean of three replicates ± standard deviation. Results from one-way ANOVA and Tukey test. Mean values in the same column with different superscripts differ significantly ($P < 0.05$). Control (monoculture *L. vannamei*); LG 2.5 (*L. vannamei* and 2.5 kg m^{-3} *G. birdiae* in an integrated biofloc system); LG 5.0 (*L. vannamei* and 5.0 kg m^{-3} *G. birdiae* in an integrated biofloc system) and LG 7.5 (*L. vannamei* and 7.5 kg m^{-3} *G. birdiae* in an integrated biofloc system).

Table 3. Proximate composition (% wet weight basis) of *Gracilaria birdiae* in an integrated biofloc system with *Litopenaeus vannamei* during the 42-day experiment period.

	Initial	LG 2.5	LG 5.0	LG 7.5
Moisture (%)	8.34 ± 0.10^{a}	8.34 ± 0.44^{a}	8.38 ± 0.82^{a}	8.24 ± 0.96^{a}
Crude protein	9.2 ± 1.1^{b}	13.6 ± 2.0^{a}	16.1 ± 0.3^{a}	13.6 ± 0.8^{a}
Lipids	1.1 ± 0.3^{a}	0.5 ± 0.1^{b}	0.4 ± 0.1^{b}	0.4 ± 0.2^{b}
Ash	47.9 ± 4.5^{a}	43.3 ± 3.0^{a}	41.3 ± 4.1^{a}	41.8 ± 4.7^{a}

The data correspond to the mean of three replicates ± standard deviation. Results from one-way ANOVA and Tukey test. Mean values in the same column with different superscripts differ significantly ($P < 0.05$). Control (monoculture *L. vannamei*); LG 2.5 (*L. vannamei* and 2.5 kg m^{-3} *G. birdiae* in an integrated biofloc system); LG 5.0 (*L. vannamei* and 5.0 kg m^{-3} *G. birdiae* in an integrated biofloc system) and LG 7.5 (*L. vannamei* and 7.5 kg m^{-3} *G. birdiae* in an integrated biofloc system).

Table 4. Performance parameters of *Litopenaeus vannamei* reared in an integrated biofloc system with *Gracilaria birdiae* during the 42-day experiment period.

Performance parameters	Treatment			
	Control	LG 2.5	LG 5.0	LG 7.5
Final weight (g)	3.12 ± 0.25^b	4.12 ± 0.04^a	3.97 ± 0.24^a	3.90 ± 0.04^a
Survival (%)	90.0 ± 5.0^a	95.00 ± 5.00^a	91.67 ±5.77^a	89 ± 2.89^a
Yield (Kg m^3)	1.41 ± 0.18^b	1.96 ±0.09^a	1.82 ± 0.21^a	1.72 ± 0.06^a
Biomass Gain (g)	56.27 ± 7.35^b	78.22 ± 3.73^a	72.95 ± 8.49^a	68.93 ± 2.34^a
Weight Gain (g)	2.80 ± 0.23^b	3.77 ± 0.06^a	3.64 ±0.22^a	3.56 ± 0.01^a
g week^{-1}	0.47 ± 0.04^b	0.63 ± 0.01^a	0.61 ± 0.03^a	0.59 ± 0.01^a
FCR	1.74 ± 0.25^b	1.20 ± 0.06^a	1.30 ± 0.16^a	1.37 ± 0.05^a
SGR (% day^{-1})	5.42 ± 0.35^a	5.86 ± 0.18^a	5.90 ± 0.20^a	5.85 ± 0.27^a

The data correspond to the mean of three replicates ± standard deviation. Results from one-way ANOVA and Tukey test. Mean values in the same row with different superscripts differ significantly ($P < 0.05$). Control (monoculture *L. vannamei*); LG 2.5 (*L. vannamei* and 2.5 kg m^{-3} *G. birdiae* in an integrated biofloc system); LG 5.0 (*L. vannamei* and 5.0 kg m^{-3} *G. birdiae* in an integrated biofloc system) and LG 7.5 (*L. vannamei* and 7.5 kg m^{-3} *G. birdiae* in an integrated biofloc system).

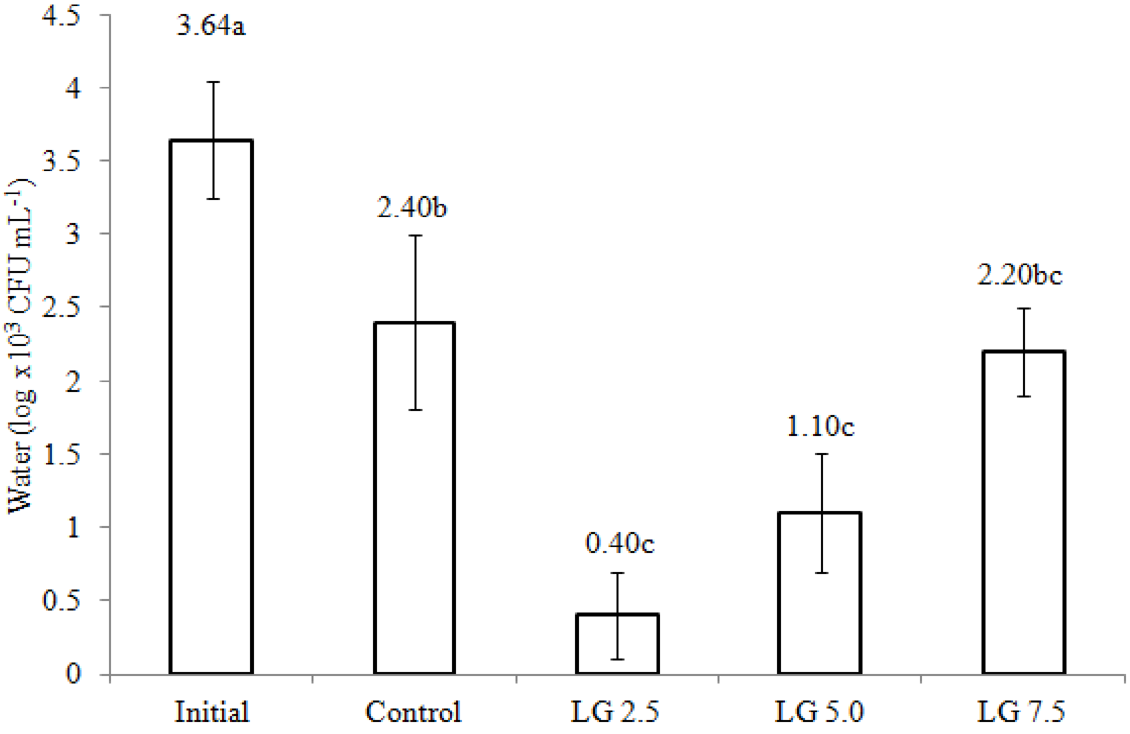

Figure 1. *Vibrio* density in an integrated biofloc system with *Litopenaeus vannamei* and *Gracilaria birdiae* during the 42-day experiment period. The data correspond to the mean of three replicates ± standard deviation. Results from one-way ANOVA and Tukey test. Mean values in the same row with different superscripts differ significantly ($P < 0.05$).

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