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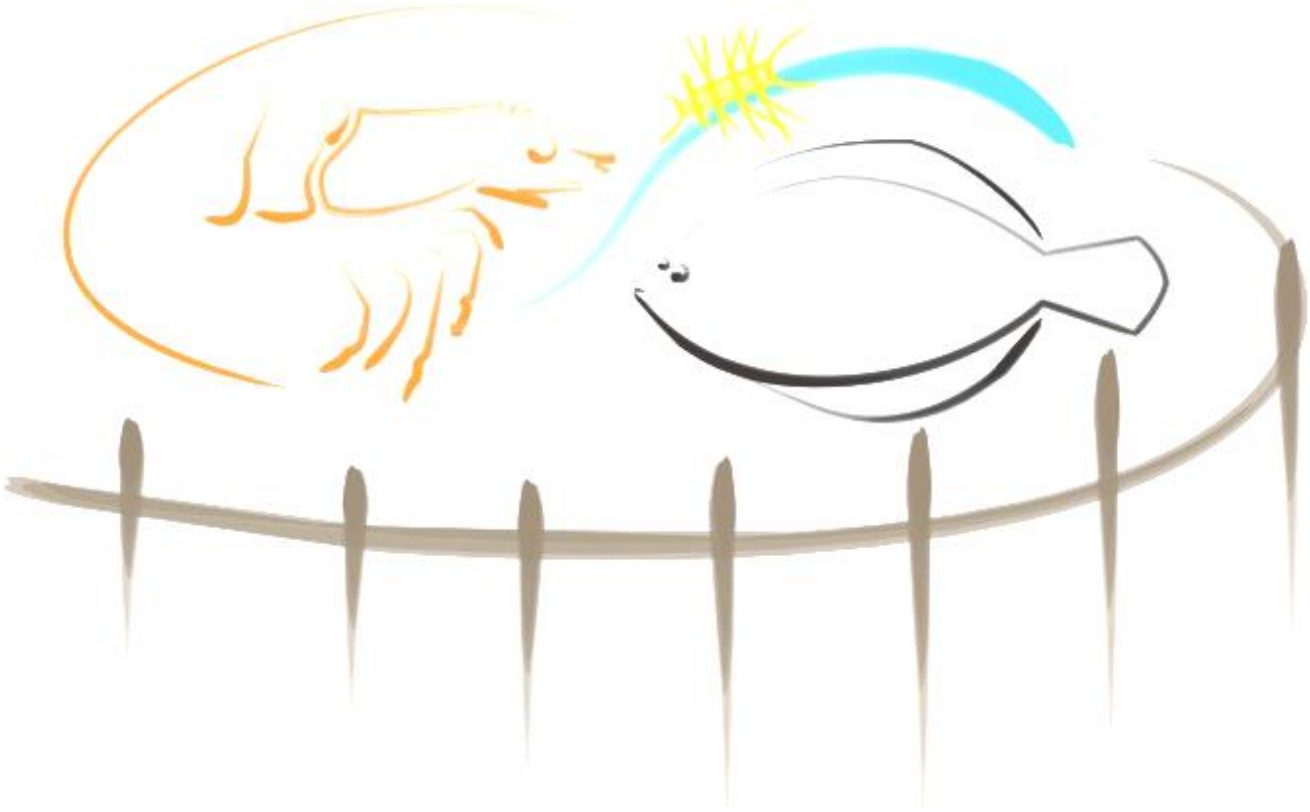

O EFEITO DO SISTEMA BFT NO DESEMPENHO ZOOTÉCNICO E FISIOLÓGICO DO CAMARÃO BRANCO DO ATLÂNTICO NORTE *Litopenaeus setiferus* CULTIVADO EM ÁGUA MARINHA E ÁGUA DE BAIXA SALINIDADE

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O efeito do sistema BFT no desempenho zootécnico e fisiológico do camarão branco do Atlântico Norte *Litopenaeus setiferus* cultivado em água marinha e água de baixa salinidade.

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EPÍGRAFE

Quando a excelência acadêmica é alcançada
é necessário apenas buscar a excelência humana
e isso é muito mais difícil ...

Saudoso professor Elpidio Beltrame...

RESUMO GERAL:

O camarão branco do Norte do Atlântico *Litopenaeus setiferus* é uma das espécies com maior valor comercial, no entanto a diminuição das suas populações naturais a coloca em uma situação ecologicamente sensível, não só para a sua produção, mas também para a sua conservação. *L. setiferus* é uma espécie que pode ser cultivada em diferentes ambientes, tanto em água marinha, ou de baixa salinidade, além disso, os avanços no desenvolvimento do Sistema BFT (*Biofloc Technology* – ou Sistema de Bioflocos) com camarões, permitem considerá-lo como uma alternativa para esta espécie. O sucesso deste sistema de cultivo está associado a uma intensa dinâmica bacteriana que participa no melhoramento da qualidade da água e na produção de proteína microbiana, isto tem consequência lógica sobre o estado fisiológico geral dos organismos. Esta espécie tem boa sobrevivência cultivada em água marinha ou de baixa salinidade, no entanto as taxas de crescimento ainda são baixas e a conversão alimentar é pouco eficiente. Por isso avaliar os parâmetros zootécnicos não é suficiente para avaliar o potencial de cultivo no sistema BFT. O objetivo de esta tese é utilizar alguns indicadores fisiológicos e bioquímicos para complementar a informação zootécnica.

Assim no primeiro capítulo objetivou fazer uma revisão do trabalho já feito com esta espécie para poder visualizar quais foram os resultados mais importantes obtidos até agora e em quais era importante continuar trabalhando. Encontrando que os principais problemas a resolver eram: Taxa de crescimento muito baixa, sobre todo durante as primeiras cinco gramas, conversão alimentar muito irregular (a ração comercial não considera os requerimentos nutricionais do *L. setiferus*) e o deterioro dos espermatóforos evitando o estabelecimento de um banco de reprodutores. Entretanto é importante considerar que as sobrevivências são em todas as pesquisas realizadas até agora altas e que a espécie gosta de um médio rico em alimento vivo, característica fundamental do BFT. Diante de essa situação, no capítulo dois, decidimos tentar experimentar com juvenis selvagens e coloca-los no sistema de cultivo com bioflocos em água marinha e de baixa salinidade. Os resultados não mostraram diferencias significativas, entretanto, mostraram animais totalmente estressados particularmente nos sistemas com fluxo contínuo. A interpretação de todos os indicadores foi complicada devido à altíssima atividade do sistema antioxidante e imune. Alta mortalidade e crescimento muito baixo

somente nos tratamentos com fluxo continuo de água. Segundo o IBR a maior afetação pelo estresse oxidativo foi o tratamento LSBFT, mais a maior mortalidade foi no tratamento LSCW.

Continuamos trabalhando com a coleta e reprodução dos adultos. Tentamos inseminação artificial, fizemos ablação do pedúnculo ocular e conseguimos produzir pós-larvas. Os protocolos de larvicultura que a espécie tem já publicados, funcionaram e conseguimos obter pós-larvas de qualidade para tentar novamente. No capítulo três, implementamos o mesmo experimento, a diferença foi que utilizamos camarões F0, é dizer camarões obtidos da reprodução dos adultos capturados no médio natural e reproduzidos em cativeiro. Colocamos as pós-larvas no dispositivo experimental e os resultados foram mais estáveis, porém, ainda com altos níveis de estresse oxidativo, mais com um sistema imune alerta e um desempenho zootécnico melhorado particularmente no sistema com bioflocos, independentemente da salinidade. O mesmo resultado negativo do experimento anterior, foi obtido com os tratamentos com fluxo contínuo novamente.

Assim e diante dos resultados obtidos nos dois primeiros experimentos, fomos para o quarto capítulo implementando um terceiro experimento decidimos retirar os tratamentos com fluxo contínuo e elevar a escala do cultivo. Utilizando tanques de 20 m^3 com sistema de bioflocos em água marinha e água de baixa salinidade. Os resultados obtidos são interessantes, a atividade antioxidante esteve presente mais foi a mais baixa dos três experimentos. Podemos detectar que os animais ficaram menos estressados, pelos valores obtidos nas enzimas de estresse oxidativo e a contagem de hemócitos. O sistema imune foi estimulado pela quantidade de hemócitos na hemolinfa dos dois tratamentos. Conseguimos avaliar o consumo de oxigênio no início do experimento, encontramos um alto consumo no tratamento de baixa salinidade, inclusive quando oferecemos alimento. Os camarões simplesmente não conseguiram comer. No final do experimento, após dos 90 dias de cultivo, o consumo baixou e quando a ração foi oferecida, passaram a comer. No tratamento com água marinha, os camarões tiveram do princípio ao fim do experimento um consumo baixo e se alimentaram normalmente. Estes resultados podem complementar ao resto dos resultados mostrando maiores requerimentos fisiológicos no tratamento de baixa salinidade o que deteriora o sistema imune e antioxidante.

Uma característica uniforme nos três experimentos é o sistema imune estimulado permanentemente, no sistema com bioflocos. Provavelmente as bactérias presentes nos bioflocos estimulam o sistema imune e eles ficaram melhor preparados para ataques patogénicos. O número de hemócitos presentes na hemolinfa foram maiores aos reportados para a mesma espécie por outros autores em sistemas convencionais de cultivo. Outra questão é que resultou em uma redução da atividade antioxidante no cultivo com tanques maiores, mas somente nos camarões expostos aos bioflocos, independentemente da salinidade. As sobrevivências foram baixas, assim como o crescimento. No entanto, com todo o bom desempenho fisiológico obtido é importante ressaltar que parte das mortalidades ocorridas nos três experimentos, tem relação direta com o manejo e podem ser resolvidas. podemos concluir, que a medida que o sistema com bioflocos madura e se estabiliza, e as condições de cultivo melhoram (infraestrutura, manejo) *Litopenaeus setiferus,* pode ser cultivada independentemente da salinidade. A espécie apresenta uma condição imunológica estimulada permanentemente. O estresse oxidativo parece diminuir, provavelmente com menor dano celular e o consumo de oxigênio e a energia são dirigidos para o crescimento da espécie.

Também foi confirmado que o sistema com bioflocos melhora o desempenho fisiológico, porém, para melhorar o desempenho zootécnico, ainda existe a necessidade de continuar pesquisando sobre questões nutricionais, de balanço iónico e de melhoramento do estado dos reprodutores. Questões que podem estar relacionadas com os benefícios que o sistema com bioflocos oferece, tais como: bactérias que controlam a qualidade da água, bactérias probióticas, abundância e diversidade de micro-organismos que servem como complemento alimentar, podem oferecer as condições adequadas para o melhor desempenho de *L. setiferus* em sistemas de cultivo, contribuindo para o estabelecimento de protocolos específicos para esta espécie em cultivo com bioflocos.

Palavras chave: Bioflocos, consumo de oxigênio, estresse oxidativo, imunologia.

INTRODUÇÃO GERAL:

Com o aumento do volume de produção, de comércio e de consumo de pescado, há uma demanda concorrente e crescente do setor aquícola para melhorar a sustentabilidade, a aceitabilidade social e a seguridade da saúde humana. A produção de produtos aquáticos em todo o mundo atingiu um máximo de aproximadamente 171 milhões de toneladas em 2016, das quais a aquicultura representou 47% do total e 53% excluindo as utilizações não alimentares (incluindo a redução para a preparação de farinha e óleo de peixe). Os camarões marinhos predominam na produção de crustáceos que são comumente criados na aquicultura costeira e é uma importante fonte de ganhos em divisas para vários países em desenvolvimento da América Latina e Ásia. O camarão é um produto amplamente comercializado e constitui o segundo grupo principal de espécies exportadas em termos de valor. O maior percentual de produção é registrado nos países da América Latina e no leste e sudeste da Ásia. Mas, grande parte do consumo ocorre nos países desenvolvidos da Europa e Estados Unidos. Embora as capturas de camarões selvagens contribuam com grandes volumes para a oferta total, a maioria dos camarões é atualmente cultivada. Na recente evolução da oferta, doenças e más condições climáticas têm sido um desafio contínuo para grandes produtores de camarões (FAO, 2018).

Camarão no sul do Golfo do México

A região sul do Golfo do México sustenta uma pescaria de camarões com base em três espécies de valor comercial significativo: o camarão marrom *Farfantepenaeus aztecus* (Ives, 1891), o camarão branco *Litopenaeus setiferus* (Linnaeus, 1767) e o camarão rosa *Farfantepenaeus duorarum* (Ives, 1891). Essas espécies possuem estratégias biológicas intimamente ligadas à Lagoa Terminos. Suas pós-larvas e pré-adultos usam esta laguna como refúgio e área de alimentação (Gracia & Soto, 1990). A Lagoa Términos, em Campeche, México, funciona como berçário de camarões brancos e a imigração de pós-larvas planctônicas ocorre através das enseadas de Puerto Real e El Carmen, ambas ligando a lagoa ao Golfo do México (Gierloff-Emdem, 1977; Mancilla-Peraza & Vargas-Flores, 1980). Como o camarão branco é a segunda espécie de maior importância comercial no Banco do Campeche, representando aproximadamente 13,5% da produção anual da pesca de camarão na região (CONAPESCA, 2012), é necessário

gerar um melhor conhecimento da biologia e ecologia da fase pós-larval e durante o recrutamento nas lagoas costeiras (Gómez-Ponce et al., 2018).

Espécies exóticas e Espécies nativas:

Nos últimos 10 anos têm se reportado a presença de espécies consideradas exóticas para o Golfo do México tais como camarão branco do Pacífico *L. vannamei* (Wakida-Kusunoki et al., 2011) e o camarão tigre gigante asiático *Penaeus monodon* (Wakida-kusunoki et al., 2013, Fuller et al., 2014). Até o momento não é possível concluir que estas duas espécies estejam estabelecidas na área de estudo ao longo da costa mexicana do Golfo do México. A baixa frequência dos espécimes encontrados até agora de ambas espécies no programa de monitoramento do camarão na pesca artesanal e industrial em lagoas e sistemas costeiros indica a ausência de uma população bem estabelecida de *P. monodon* e *L. vannamei*. Além disso, nenhum dos espécimes examinados estava com gônadas desenvolvidas. Amostragem adicional e monitoramento a longo prazo são necessários para avaliar os impactos potenciais da presença destes camarões nas espécies de camarões nativos, caso de estabelecer-se (Wakida-Kusunoki et al., 2013).

Como a maioria de nossos alimentos de origem vegetal e animal, a aquicultura também se baseia de forma significativa em espécies exóticas. A dependência de espécies exóticas na aquicultura na região asiática, a espinha dorsal da produção aquícola global, tem sido tratada anteriormente (De Silva et al., 2009), e na Europa por Turchini e De Silva (2008). Também foi demonstrado que na China, o principal produtor mundial de aquicultura, tem havido um aumento gradual da dependência de espécies exóticas (Liu e Li, 2010).

O caso da criação de camarões também fornece um exemplo adicional da importância de uma espécie exótica na aquicultura, particularmente na região Ásia-Pacífico, pois é um exemplo em que uma translocação foi efetuada para substituir um sistema de cultivo baseado em uma espécie nativa, *Penaeus monodon*, por *L. vannamei.*

A criação de camarões foi desenvolvida há mais de 50 anos e atualmente é responsável por quase 70% dos camarões vendidos globalmente (Benzie, 2009). Ao longo da história da produção de camarão, a produção dominada pela Ásia-Pacífico, e até

meados da década de 1990, a espécie predominantemente cultivada era o camarão *P. monodon*. No entanto, no início dos anos 90 começaram a ocorrer epidemias virais (focos virais de mancha branca e cabeça amarela) de *P. monodon* resultando em perdas severas de estoque e, consequentemente, perdas financeiras (Briggs et al. 2004; Kongkeo e Davy 2010). As principais nações produtoras de camarão da região, como a China, a Indonésia e a Tailândia, por exemplo, tomaram uma decisão política de transloucar camarões brancos ou camarões do Pacífico (*L. vannamei*) como uma solução para revitalizar o setor (Kongkeo e Davy, 2010). A disponibilidade de estoques de sementes de *L. vannamei* livre de patógenos específicos (SPF) favoreceu a escolha desta translocação (Benzie 2009).

L.vannamei é nativo da costa leste do Pacífico, se distribuindo desde o Golfo da Califórnia, México até tumbes, norte do Peru (Pérez-Farfante e Kensley, 1997). Hoje é a espécie de camarão peneído mais importante cultivada em todo o mundo (FAO, 2018) na atualidade, responsável por quase 70% da produção de camarão cultivada na Ásia, que por sua vez responde por 85% da produção mundial de camarão cultivado. Nos principais países produtores de camarão, como a Tailândia (Wyban 2007) e na China, as espécies transloucadas dominam quase completamente o setor (Kongkeo e Davy, 2010). No geral, do ponto de vista da produção e da economia, a translocação de *L. vannamei* foi bem-sucedida. Esta condição levou à introdução e presença deste camarão em águas naturais fora de sua distribuição geográfica natural.

O camarão *P. monodon* é a espécie de camarão maiormente explorada comercialmente no mundo. Sua distribuição natural é o Pacífico Indo-Ocidental, indo da costa oriental da África e da Península Arábica, até o sudeste da Ásia, o mar do Japão e o norte da Austrália (FAO, 1980). As introduções de *P. monodon* no sudeste dos EUA devem-se provavelmente ao escape de instalações de aquacultura após inundações por tempestades e furacões, ou pela migração de áreas onde os camarões tigre se estabeleceram anteriormente na natureza. Embora menos provável, outras vias de introdução (por exemplo, descarga de água de lastro) são possíveis (Altuve et al., 2008; Knott et al., 2012). A presença de camarão tigre em locais na costa mexicana do Golfo do México indica que esta espécie é agora distribuída quase por todo o Golfo. Os caminhos de introdução de camarão tigre gigante em águas mexicanas não são claros. A mais provável rota é a migração das águas do norte do Golfo do México, onde camarão tigre já se estabeleceu em estado selvagem (Knott et al. 2012).

Diana (2009) considerou o escape de espécies em cultivos aquáticos e seu perigo potencial como espécies invasoras, quase que inevitavelmente, o consenso geral é que as espécies exóticas cultivadas tiveram impacto na biodiversidade (Moyle e Leidy 1992; Naylor, 2001). Por outro lado, o conhecimento detalhado sobre esse assunto tende a mostrar que a evidência explícita de espécies exóticas impactando na biodiversidade ainda está incompleta.

Beverton (1992) revisou o estado global das introduções e concluiu que a maioria das espécies introduzidas se mostrou não viável ou ecologicamente neutra; uma pequena proporção tem sido benéfica, e alguns, principalmente os colonizadores gerais e os poderosos predadores, prejudicaram seriamente a fauna nativa de peixes. Na mesma linha, Gozlan (2008) sugeriu que a introdução de peixes de água doce não nativos para fins de produção de alimentos pode não ser tão ruim, sugerindo que a grande maioria das pesquisas sobre translocações concentra-se nos poucos impactos negativos e riscos associados.

Estas pesquisas demonstram que a maior parte das translocações de peixes de água doce não teve grandes impactos ecológicos, mas gerou grandes benefícios sociais. Impactos sobre a biodiversidade através da introdução de patógenos associados a translocações para fins de aquicultura são poucos e distantes.

Flegel (2006) alertou para a maior probabilidade da introdução de novos vírus patogênicos em associação com translocações de camarão. Tem sido sugerido que dois novos vírus, o Vírus da Síndrome de Taura (TSV) e o Vírus da Myonecrosis Infecciosa, foram introduzidos na região asiática através da translocação de estoque de sementes de *L. vannamei* (De Silva et al. 2007).

Os impactos gerais de esses novos patógenos sobre a fauna aquática ainda estão para ser vistos. O episódio também sugere que, apesar de todas as sementes e ninhadas transloucadas para a região asiática serem SPF, todos os riscos associados não podem, no entanto, ser completamente eliminados.

O camarão branco do Norte do Atlântico *L. setiferus* e a importância das espécies nativas:

L. setiferus é capturado ao longo da costa atlântica dos EUA, da Carolina do Norte até a Flórida e no Golfo do México. Esta espécie é de grande importância econômica para ambos os países. A espécie está distribuída na costa leste dos Estados Unidos desde Nova Jersey até a Florida, passando pelo Golfo do México, até o sul do México, desde Tamaulipas até Campeche. A distribuição da espécie é em profundidades que variam de dois até 90 m, em fundos lodosos, constituídos de matéria orgânica, areia e argila, sendo que os adultos são marinhos e os juvenis normalmente estuarinos (FAO, 2015).

Ao longo da sua história, a pesca do camarão branco foi sujeita a três tipos de pesca. Inicialmente, o camarão branco, como a maioria dos peneídos, apoiava uma pesca artesanal de juvenis no interior das lagoas costeiras e uma pesca sequencial no estágio adulto em alto mar com barcos de arrasto. A ação de ambas as pescarias colocou o recurso em seu nível máximo de exploração na década de 1970, atingiu seu nível máximo de exploração, cuja produção máxima registrada oscilou entre 1.200 e 2.200 toneladas por ano. O rendimento máximo sustentável para este período foi estimado em 1.650 toneladas (Gracia, 1989). Como no caso do camarão rosa, após 1980 houve uma tendência de declínio sustentado para níveis muito baixos de produção.

Além disso, durante a década de 1980, uma nova pesca artesanal de camarão branco apareceu no ambiente marinho com redes de deriva. Essas artes de pesca atuam principalmente sobre os camarões adultos em estágio reprodutivo. Isto se deve ao alto valor econômico e também têm alta eficácia e rentabilidade por baixo custo de investimento para o processo de captura (Gracia, 1996). Esta alta rentabilidade causou um crescimento explosivo da pesca artesanal paralela, particularmente em reprodutores de camarão branco, o que também aumentou o esforço de pesca total sobre a população desse camarão. Gracia (1996) aponta que esse fator causou uma sobre exploração do recrutamento que causou o colapso da pesca do camarão branco (Gracia, 2004).

Na zona costeira do Golfo do México, a destruição de manguezais também produz uma diminuição nas áreas que levam ao desenvolvimento das primeiras fases da vida dos peixes e camarões. A instalação de plataformas de petróleo e termoelétricas, bem como as atividades inerentes ao desenvolvimento do turismo, também são fontes de alteração ou perda do habitat marinho. A atividade marítima nos portos, a dragagem, os

derramamentos de combustíveis e óleos e a introdução de flora e fauna exóticas por meio de águas de lastro, contribuem para a longa lista de impactos antropogênicos sobre as populações naturais de camarões. Além de todos esses impactos naturais entre os quais se destacam: marés vermelhas, furacões, tempestades tropicais, ventos do norte, suradas ou ventos do sul, El Niño ou La Niña, ressurgências, etc., os recursos pesqueiros precisam continuamente adaptar suas populações, o que se traduz em declínio notável destas.

Diante deste panorama das populações naturais desta espécie, a aquicultura se apresenta como uma possibilidade para a produção e para a conservação da mesma. *L. setiferus* já tem sido cultivado, tentando produzi-lo principalmente como isca para a pesca esportiva nos Estados Unidos (Samocha et al., 1998; Gandy, 2007). Tem se observado que é uma espécie de crescimento menor que *L. vannamei*, mas a literatura mostra que *L. setiferus* é menos sensível a doenças virais (Sandifer, 1993; Chapman e Browdy, 2004).

É possível que as taxas de crescimento equivalentes de *L. vannamei* sejam atingidas, procurando melhores fontes de alimentação. Não devemos esquecer que todas as rações comerciais estão desenhadas para cobrir os requerimentos nutricionais de *L. vannamei*, espécie que assimila primeiro as proteínas durante a alimentação e que tolera diversas relações energia-proteína nos diferentes estágios do ciclo de vida e que *L. setiferus* parece preferir dietas com altas taxas de carboidratos porque os assimila primeiro, o que indica que é uma espécie menos carnívora que *L. vannamei*, já que usa uma mistura de proteínas e carboidratos para atender às demandas energéticas de crescimento e funções básicas (Rosas et al., 2001)

L. setiferus é uma espécie nativa do Atlântico Norte, que tolera baixas salinidades e é resistente a diferentes condições ambientais (Laramore et al., 2001). A mesma não apresentaria nenhum problema ambiental diante a ruptura dos viveiros de cultivo ou pela inundação de fazendas causadas pelos furacões e tormentas tropicais no Golfo do México (Valenzuela et al., 2002). Na atualidade o desenvolvimento de fazendas de camarão está baseado em espécies amplamente domesticadas e normalmente exóticas para muitos países e regiões tais como *L. vannamei* e *P. monodon*. Porém para manter a sustentabilidade, é preciso desenvolver programas de domesticação de espécies nativas (Gaxiola et al., 2008). Para isso é preciso realizar pesquisas que gerem tecnologias de cultivo funcionais para estimular a produção das espécies locais.

Regionalmente o cultivo de camarões é uma alternativa para todos os produtores da região sul do Golfo do México, A Península de Yucatán tem infraestruturas de cultivo construídas para o cultivo de tilápias e que foram abandonadas por produtores devido à baixa relação custo-benefício deste produto. A grande maioria tem abastecimento de água de baixa salinidade característica da região (2.0 a 5.0 ups), água com dureza e alcalinidade muito elevadas e outros próximos da zona costeira com água marinha entre 15- 35 ups. O camarão *L. vannamei* se apresenta como ideal para este tipo de cultivo. Entretanto, em muitos casos é impossível conseguir uma licença para cultivá-lo, já que pela grande quantidade de Áreas Naturais Protegidas na região o *L. vannamei* é considerado como uma espécie exótica. *L. setiferus* se apresenta então como uma alternativa viável para ser cultivada tanto em água marinha como de baixa salinidade, assim pode contribuir com a geração de renda para os produtores costeiros e terra adentro.

O sistema de cultivo com bioflocos (BFT):

O desenvolvimento de novas tecnologias para engorda de camarões em sistemas ambientalmente amigáveis é requisito para atender à crescente demanda de produtos alimentícios saudáveis e de alta qualidade. Para satisfazer estas demandas, os carcinicultores devem considerar a exclusão de doenças no sistema de produção e também proteger os ecossistemas aquáticos adjacentes das fazendas (Andreatta e Rosas, 2006). Novas tecnologias que ajudem a minimizar impactos ambientais, reduzir custos e aumentar a renda dos empreendimentos. Tal é o caso do Sistema BFT (Biofloc Technology) (Avnimelech, 2007; Azim e Little, 2008; Avnimelech e Kochba, 2009) ou Sistema com Bioflocos, que promove e potencializa o desenvolvimento de comunidades bacterianas heterotróficas nos sistemas de cultivo, que tem a habilidade de sintetizar proteínas que são aproveitadas pelos camarões, a partir de carbono orgânico e dos dejetos nitrogenados (Avnimelech, 1999, Ebeling et al., 2006). Para isso, esse sistema requer uma constante e abundante aeração da água nos tanques, com o objetivo de manter os sólidos suspensos (Krummenauer et al., 2011; Furtado et al., 2011).

As bactérias heterotróficas colonizam as partículas dos dejetos e absorvem nitrogênio, fósforo e outros nutrientes da água (Wasielesky et al., 2006; Serfling, 2006). Outra vantagem em relação ao controle dos efluentes em sistemas BFT, segundo Andreatta e Rosas (2006), é o aporte nutricional do meio, que também fortalece o sistema imunológico dos organismos. No entanto os micro-organismos presentes neste tipo de

cultivo proporcionam nutrientes adicionais para os camarões e contribuem para manter a boa qualidade de água (Otoshi et al., 2001; Burford, 2004; Ballester et al., 2007). Além dos benefícios diretos, têm se demonstrado que o alimento vivo aumenta a atividade das enzimas digestivas aumentando a assimilação da ração (Guzmán et al., 2001), que consequentemente pode afetar os metabólitos sanguíneos. Assim os resultados obtidos até agora em condições de laboratório com águas claras são evidentemente limitados já que estas condições diferem significativamente das que os camarões são submetidos em viveiros de cultivo, as quais afetam a fisiologia dos organismos de maneira importante (Rosas et al., 2002). Além disso, os nutrientes e o tipo de sistema de cultivo oferecem múltiplas possibilidades de favorecer o valor nutricional, sabor, cor, qualidade sanitária e textura dos camarões produzidos (Ezquerra-Brauer et al., 2004).

Parâmetros de avaliação da condição fisiológica dos camarões no sistema BFT:

Tradicionalmente, os indicadores de sucesso de um cultivo referem-se ao desempenho zootécnico, como crescimento ou sobrevivência e o fator de conversão alimentar. Entretanto todas essas respostas estão dadas pelos mecanismos fisiológicos, imunológicos e bioquímicos que os camarões sofrem no interior do corpo, dependendo das condições ambientais dos cultivos. Tem se demonstrado que os conhecimentos dos processos imunológicos associados à bioquímica fisiológica auxiliam na avaliação do estado de saúde dos camarões (Bachére et al., 1995; Le Moullac et al., 1998).

O consumo de oxigênio reflete a quantidade de energia utilizada para o metabolismo e para manter a homeostase (Rosas et al., 1999, Lignot et al., 2000). A taxa respiratória tem sido amplamente estudada em crustáceos com relação à salinidade, a temperatura, ao oxigênio dissolvido, a quantidade e qualidade do alimento, etc., já que esta é um bom indicador do estado fisiológico dos animais (Chen e Lin, 1998; Rosas et al., 2000). Da mesma forma, a quantidade e qualidade de hemócitos permite estabelecer uma parte do estado de saúde dos camarões, já que são considerados como a primeira linha de defesa, pois participam diretamente em processos de reconhecimento, processamento e amplificação da resposta imune (Destoumieux et al., 2000; Johansson *et al*., 2000). Ao mesmo tempo a atividade enzimática contribui com a qualidade e quantidade de assimilação do alimento disponível (Molina et al., 2000; Xu, et al., 2013).

Avaliar esse tipo de variáveis permite entender os mecanismos que os animais usam para se adaptar ao meio de cultivo e como essa informação pode ser usada para estabelecer uma proposta adequada para o cultivo de uma espécie. Por exemplo, alguns mecanismos de defesa celular em crustáceos dependem da produção controlada de radicais livres durante a fagocitose e o encapsulamento (Smith et al., 2003). Diante das mudanças nas condições ambientais o metabolismo aeróbico de crustáceos gera Espécies Reativas de Oxigênio (ROS). Certa concentração de ROS é necessário para a defesa contra as infecções microbianas, no entanto, a produção de ROS e os seus resíduos pode resultar em dano severo nas células. Para manter o equilíbrio, as ROS são eliminadas pelo sistema de defesa antioxidante que inclui, por exemplo, a superóxido dismutase (SOD), a catalase (CAT) e outras enzimas associadas com a desintoxicação de células, como a glutationa-S-transferase (GST) (Muñoz et al. , 2000, Ren et al., 2009, Quiu et al., 2011, Zhou et al., 2013, Song et al., 2015).

É por isso que o presente estudo pretende avaliar o potencial aquícola do camarão branco do Atlântico Norte *L. setiferus* em diferentes condições de cultivo, utilizando os parâmetros de desempenho zootécnico e os parâmetros que definem o estado fisiológico geral dos camarões nessas condições, com a finalidade de estabelecer um protocolo adequado para a produção comercial deste recurso.

OBJETIVO GERAL:

Avaliar o potencial de cultivo de *L. setiferus* em sistema BFT com água de baixa salinidade e marinha em comparação com um sistema de troca permanente de água para ambos os casos, através da avaliação dos parâmetros fisiológicos, zootécnicos e bioquímicos para estabelecer a condição dos animais na fase de engorda.

Objetivos específicos:

1. Avaliar o desempenho zootécnico do *L. setiferus* nos bioflocos através do monitoramento das taxas de crescimento, sobrevivência e o fator de conversão alimentar.
2. Diagnosticar o estado fisiológico de juvenis de *L. setiferus* cultivados em diferentes salinidades com o sistema BFT através da respirometría.

3. Examinar o estado de saúde de *L. setiferus* em sistema BFT em máxima e mínima salinidade através da contagem dos hemócitos.
4. Contrastar a atividade antioxidante de *L. setiferus* em sistema BFT em máxima e mínima salinidade através da avaliação das enzimas do sistema antioxidante no músculo.

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CAPÍTULO I

A BRIEF REVIEW ON THE USE OF NORTH ATLANTIC WHITE SHRIMP *Litopenaeus setiferus* AS AQUACULTURE TARGET SPECIES AND CULTIVATION PROSPECTS WITH THE SUPER INTENSIVE BIOFLOC SYSTEM.

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Observação: Tabelas e figuras foram incluídas no corpo do texto para facilitar a leitura da tese, além de um resumo estendido em português.

UMA BREVE REVISÃO SOBRE O USO DO CAMARÃO BRANCO DO ATLÂNTICO NORTE *Litopenaeus setiferus* COMO ESPÉCIE PARA A AQUICULTURA E PERSPECTIVAS DE CULTIVO COM O SISTEMA SUPER INTENSIVO DE BIOFLOCOS.

RESUMO

Em termos históricos, a exploração de camarão no Golfo do México tem sido a atividade pesqueira mais importante nesta região, tanto pelos volumes de captura, como pela geração de empregos diretos e indiretos na fase de extração, processamento e serviços associados. Porém o camarão branco do Norte do Atlântico *Litopenaeus setiferus*, tem sido estudado como recurso de importância para as pescarias. A atividade marítima nos portos, a dragagem, os derramamentos de combustíveis e óleos e a introdução de flora e fauna exóticas por meio de águas de lastro, contribuem para a longa lista de impactos antropogênicos sobre as populações naturais de camarões. Com todos esses impactos, além dos eventos naturais entre os quais se destacam: marés vermelhas, furacões, tempestades tropicais, ventos do norte, "suradas" ou ventos do sul, El Niño ou La Niña, ressurgências, etc., os recursos pesqueiros precisam continuamente adaptar suas populações, o que se traduz em declínio notável destas. Além do conhecimento gerado sobre a biologia e ecologia da espécie, na atualidade o desenvolvimento de fazendas de camarão está baseado em espécies amplamente domesticadas e normalmente exóticas para muitos países e regiões tais como o *L. vannamei* e *Penaeus monodon*, espécies que também estão aparecendo frequentemente em todo o Golfo de México, ainda sem saber o efeito do estabelecimento de estas. Porém para manter a sustentabilidade, é preciso desenvolver programas de domesticação de espécies nativas, não só para a produção comercial, mais também para a própria conservação.

Têm se realizado vários estudos para saber o comportamento da espécie em relação com as características das lagoas costeiras, alguns estudos sobre larvas de camarão em lagunas costeiras do Golfo do México incluíram análise da imigração de larvas de camarão, as larvas planctônicas se desenvolvem, movendo-se em direção à costa em busca de zonas de refúgio nas lagoas e estuários costeiros, aonde chegam como pós-larvas. Lá os camarões são recrutados nas áreas estuarinas com vegetação submersa, que são usadas como áreas de berçário. Sabe se que os camarões brancos são grandes consumidores de macro bentos, gostam de presas vivas. A partir dos trabalhos realizados

no sul dos Estados Unidos e em outras partes do Golfo do México, se sabe que as taxas de crescimento são menores que as do *L. vannamei*, é dizer, cresce mais devagar, embora possa alcançar o mesmo peso que *L. vannamei* em um tempo maior. Atualmente se sabe que as rações disponíveis comercialmente estão projetadas para o camarão branco do Pacífico, porém as conversões alimentares ainda são pouco eficientes. Portanto, há pesquisas que determinam que o aproveitamento das relações energia/proteína não são iguais nas duas espécies, *L. vannamei* assimila primeiro as proteínas e *L. setiferus* os carboidratos, isso é de vital importância para o crescimento e eficiência do consumo.

Entretanto, as sobrevivências em cultivo semi-intensivo são altas em todos os trabalhos publicados, porém, ainda está faltando pesquisas sobre o desenvolvimento de rações específicas para outras espécies que permita diversificar a carcinicultura. Outros pesquisadores têm aperfeiçoado as técnicas de reprodução desta espécie, mais ainda se tem o problema do deterioro dos espermatóforos dos machos em cultivo aos poucos dias de cativeiro. Ambientes como o sistema com Bioflocos ou BFT podem manter entre outras coisas, grande quantidade de alimento vivo, principalmente bactérias, entre elas as probióticas que além de melhorar a qualidade da água, melhoram a condição nutricional e imunológica dos camarões.

Segundo os artigos publicados, o cultivo larval de *L. setiferus*, não apresenta problemas básicos para a produção de pós-larvas, com um esquema baseado em alimento vivo utilizando alimento micro particulado ou micro encapsulado como complemento. Provavelmente as características do sistema com Bioflocos apresentem um ambiente que possa melhorar o desempenho fisiológico e zootécnico e faça do *L. setiferus* uma boa alternativa de cultivo para os produtores na costa norte do Atlântico.

É importante agregar outras variáveis de avaliação do potencial das espécies para serem cultivadas. Tradicionalmente os indicadores de sucesso de um cultivo referem-se ao desempenho zootécnico, como crescimento ou sobrevivência e o fator de conversão alimentar. Entretanto todas essas respostas estão dadas pelos mecanismos fisiológicos, imunológicos e bioquímicos que os camarões sofrem ao interior do corpo, dependendo das condições ambientais dos cultivos. Tem se demonstrado que os conhecimentos dos processos imunológicos associados à bioquímica fisiológica auxiliam na avaliação do estado de saúde dos camarões. Avaliar esse tipo de variáveis permite entender os mecanismos que os animais usam para se adaptar ao meio de cultivo e como essa

informação pode ser usada para estabelecer uma proposta adequada para o cultivo de uma espécie.

O intuito de este capítulo é fazer uma revisão do trabalho feito com *L. setiferus*, através dos diferentes trabalhos de pesquisa realizados até agora em diversas disciplinas relacionadas com a produção de esta espécie para poder estabelecer as linhas de pesquisa a seguir no futuro, no só para a produção comercial da espécie, mas também para a própria conservação.

Palavras chave: Aquacultura, *Litopenaeus setiferus*, espécies nativas, bioflocos

A BRIEF REVIEW OF THE USE OF NORTH ATLANTIC WHITE SHRIMP *Litopenaeus setiferus* AS AQUACULTURE TARGET SPECIES AND CULTURE PROSPECTS ONTHE SUPER INTENSIVE BIOFLOC SYSTEM.

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ABSTRACT

The Northern white shrimp, *Litopenaeus setiferus*, has been studied as an important resource for fisheries. In the las years research have been done to know the behavior of the species in relation to the characteristics in the coastal lagoons. It is a species that tolerates low salinities and is resistant to different extreme environmental conditions. At present, the development of shrimp farms is based on widely domesticated and normally exotic species for many countries and regions such as *Litopenaeus vannamei* and *Penaeus monodon*. But to maintain environmental sustainability, it is necessary to develop programs of domestication of native species, not only for production, but also for conservation itself. From the work done in different parts of the Gulf of México, it is known that growth rates are lower than those of *L. vannamei*, that commercially available feed are designed for Pacific white shrimp, and food conversions are still inefficient, high survivals in semi-intensive culture

Other researchers have been developing breeding techniques, but there is still a problem of spermatophore deterioration in males. The larval culture does not present basic problems in post-larvae production, meanly if use live food-based scheme including micro-particulate or micro-encapsulated food as a complement. Probably the characteristics of the biofloc system present an environment that can improve the physiological and zootechnical performance and make L. *setiferus* a good alternative of aquaculture in North Atlantic coast.

Keywords: Aquaculture, *Litopenaeus setiferus*, Native species, Biofloc

INTRODUCTION

Northern Atlantic white shrimp *Litopenaeus setiferus* is one of the species with the highest commercial value in all the Gulf of México. The decline in natural populations places the species in an ecologically sensitive situation, not only for its production but also for its conservation. *L. setiferus* is caught along the Atlantic coast of the USA, from North Carolina to Florida and the Gulf of Mexico. This species is economic important for both countries. The species is distributed on the East coast of the United States from New Jersey to Florida, through the Gulf of Mexico, to the South of Mexico, from Tamaulipas to Campeche. The distribution of the species is at depths ranging from two up to 90 m in muddy bottoms made up of organic matter, sand and clay. In general, adults are distributed in marine waters, while juveniles are present in estuarine waters (FAO, 2015).

L. setiferus is a euryhaline species that can be grown in different environments, either in seawater, brackish or low salinity waters. In the coastal zone of the Gulf of Mexico, the destruction of mangrove also produces a decrease in the areas that lead to the development of the first phases of the life of the fish and shrimps. The installation of oil platforms and thermoelectric plants, as well as the activities inherent to the development of tourism, are also sources of alteration or loss of the marine habitat. Maritime activity in ports, dredging, fuel and oil spills and the introduction of exotic flora and fauna through sewage contribute to the long list of anthropogenic impacts on natural shrimp populations. With all these impacts, in addition to natural events such as red tides, hurricanes, tropical storms, Northern and south winds, El Niño or La Niña, upwellings, etc., fish resources must continuously adapt their populations, which translates into a notable decline (Arenas-Fuentes and Jiménez-Badillo, 2004).

In the last 10 years, the presence of exotic species in Gulf of Mexico, such as *L. vannamei* (Wakida-Kusunoki et al., 2011 and *Penaeus monodon* (Wakida-Kusunoki et al., 2013; Fuller et al., 2014), it is becoming more frequent in time and space, since there are specimens, practically throughout the Gulf of Mexico and even in the Caribbean. It is not possible to conclude that the Asian giant tiger shrimp or the Pacific white shrimp are established in the study area along the coast of the Gulf of Mexico. The low frequency of the specimens found so far of both species in the shrimp monitoring program in the artisanal and industrial fishery in lagoons and coastal systems indicates the absence of a

well-established population of P. monodon or *L. vannamei*. In addition, none of the species examined were carrying eggs. To assess the potential impacts of the presence of these shrimps on native shrimp species, additional sampling and monitoring are needed (Wakida-Kusunoki et al., 2013).

In this panorama of the natural populations of this species, aquaculture presents itself as a possibility for the production and conservation of the same. At present the development of shrimp farms is based on widely domesticated and normally exotic species for many countries and regions such as *L. vannamei* and *Penaeus monodon*. However, to maintain sustainability, it is necessary to develop domestication programs of native species (Gaxiola et al., 2008). For this it is necessary to carry out researches that generate technologies of functional cultivation to stimulate the production of the local species.

SHRIMP FISHERIES:

Historically, shrimp fishing in the Gulf of Mexico has been the most important fishing activity in this region, both in terms of catch volumes and the generation of direct and indirect jobs in the extraction, processing and associated services. The most important species in which shrimp fishing is based in the Gulf of Mexico are common to the entire area, both in Mexican and US waters. However, many studies on local shrimp species including *L. setiferus* (Gracia, 2004) have been developed.

The life cycle of white shrimp in the Gulf of Mexico begins when adults reproduce on the continental shelf between four and 40 m (Williams, 1984). There, fertilized eggs are benthonic at the sea where, 12 to 14 hours later, a nauplii larva emerges feeding on its own reserves. For 12 to 15 days, planktonic larvae develop, moving toward the coastal waters in search of refuge areas in the lagoons and coastal estuaries, where they arrive as post-larvae. In these areas the shrimps are recruited into the estuarine areas with submerged vegetation, which are used as nursery areas. In these areas, shrimps spend between seven and nine months before migrating back to the sea, where they are recruited as adults (Renaud 1986; Zein-Eldin, 1986, Chow et al., 1993; Misamore and Browdy, 1996; Rosas et al., 1999; Rosas et al., 2004). The Northern Atlantic white shrimp *L. setiferus* has been extensively studied from the perspective of a resource of importance

for fisheries (Gracia, 1999), so Gracia (1991, 1996) established patterns of relations between populations and recruitment and among populations and over fishing of *L. setiferus*, Gracia and Soto, (1990) describe how post-larvae and pre-adults use Laguna de Terminos in the southern Gulf of Mexico as a nursery, refuge and feeding zone.

Some studies on shrimp larvae in coastal lagoons in the Gulf of Mexico have included analysis of current and migration of shrimp larvae at Carmen and Laguna Machona (Gierloff-Emdem, 1977; Flores-Coto et al., 2010). Even models and simulation programs have been developed, for example, Diop et al. (2007) have done an estimated model that demonstrated a significant and positive relationship between the abundance of the first stages of life of the shrimps and the temperature and salinity. At the same time, the loss of wetlands and the high discharges of the rivers were related to a lower abundance. Biological factors such as the number of shrimp juveniles in the early stages of life predicted late juvenile abundance, with a similar relationship between late juveniles and adult catch per unit of effort (CPUE).

Baker et al., (2014) used a model to determine the growth of juveniles and the effects of mortality on the natural populations of the species, finding modest changes in juvenile growth and mortality, it was projected that they would have an impact on the size of fish stocks, greater than all fishing mortality in recent decades. These results suggest that variability in juvenile survival can be a strong driver of adult population size and that processes that regulate juvenile growth and mortality need to be adequately understood for effective management of coastal nurseries and shrimp stocks, thus confirming results obtained by Minello and Zimmerman (1991), who established the role of coastal lagoons as regulators of growth and survival of shrimp juveniles.

ECOLOGY AND BEHAVIOR

At the same time and based on the importance of the species for the fisheries several researchers began to study the patterns of behavior and how these relate to the ecology of the whole ecosystem. Mc Tiegue and Zimmerman (1991) observed differences between the responses of two species of shrimp, brown shrimp *F. aztecus* and white shrimp *L. setiferus*, concluding that white and brown shrimp are omnivorous, but they showed differences of feeding based in the degree of carnivory and herbivory. These

differences may reflect resource partitioning strategies and life history linked to the temporal availability of food. Brown shrimps gain specialized advantage through being carnivorous during the spring months when white shrimp are not present. White shrimp gain advantage through herbivory during the summer months, feeding on unimportant sites and resources for shrimp. They suggest that vegetable debris may have a very limited growth value for shrimp, although this finding is inconclusive.

The relationship between the abrasive material in the gut and the ability to digest debris needs to be required. Beseres and Feller (2007) have shown that white shrimps are big consumers of macro benthos, but they prefer live preys. Because they decrease macrobenthos by predation, both in experimental devices in the laboratory and in the field, this suggests that predation takes precedence as a determinant in some seasonal cycles. As white shrimps are mobile and their densities fluctuate widely inter and intra-annually, their impact can also be expected to vary spatially and temporally.

Webb and Kneib (2004) measured growth and movements of juvenile white shrimp *L. setiferus* in the tidal stream subsystem of the Duplin River, Sapelo Island, Georgia. Over a two-year period, 15,974 juvenile shrimps (40-80 mm TL) were internally marked with uniquely encoded microfiber marks and released into the shallow upper reaches of four tidal streams or marshes. In conclusion, it can achieve a favorable balance between adequate habitat (space) at low tide, which tends to increase the residence time and density of juvenile shrimp, providing sufficient intertidal forage habitat and predatory refuge at high tide to promote high growth rates and survival of juvenile shrimps. Spatially explicit information on growth rates and the degree of movement of shrimp within their nurseries are initial steps needed to meet the challenge of maintaining a quality nursery for sustainable shrimp fisheries and meet other demands associated with human development in watersheds estuaries.

REPRODUCTION:

King (1948) described the general anatomy and histology of the reproductive organs of shrimp, *L. setiferus* and the principles on gross developmental changes in organ size and coloring relating histological and cytological changes, so that the field worker, without access to a microscopy laboratory, could more accurately determine and record

the stage of development. It laid the basis for shrimp reproduction and made observations on details of reproduction that are now fully known, for example indicating that a female "could" probably spawn more than once in a single breeding season. Studies that would later be confirmed and detailed with electronic microscopy by Chow et al. (1991) and Bauer et al. *(*1991), who particularly described the details of the spermatophore structure inside the male ejaculatory duct, immediately after ejaculation, completing the information provided by Ro et al. (1990) who suggested that the vas deferens works in the production of the support matrix and in the consolidation and orientation of the spermatozoid, as well as in the secretion of some of the acellular components of the spermatophore and storage of the developing spermatophore.

The female thelycum in the *L. setiferus* case is opened, so the fertilization of the eggs is closely related to the proper transfer and adhesion of the spermatophore, since the spermatozoa are transported and protected by this structure. The spermatophore is expelled from the terminal ampoule through the genital pore.

During the expulsion process, the two halfs are assembled outside the body forming a complete spermatophore, with structures that facilitate the anchoring on the surface of the female's thelycum (Ro et al., 1990; Chow et al., 1991). Castille and Lawrence (1989) evaluated the maturation of *F. aztecus* and *L. setiferus* by visual, histological, and biochemical observations. Changes in carbohydrate, lipid, and protein contents were described in gonads and digestive glands of both males and females, and in the terminal portion of the male reproductive tract.

Leung-Trujillo and Lawrence, (1987) tested the effect of captivity on the sperm quality of *L. setiferus* from the Gulf of Mexico. They found no significant decline in the quality or quantity of sperm until the third week of captivity. The average live sperm decreased to 20.7% in the fifth week in captivity. Bray et al. (1990) obtained females from the hybridization between *L. setiferus* and *Litopenaeus schmitti*. Their results are indicative of the phylogenetic relationships and the genomic compatibility of these species. Misamore and Browdy (1997) evaluated the potential for spontaneous hybridization between *L. setiferus* and *L. vannamei*. Prezygotic barriers to hybridization were evaluated utilizing quantitative analysis of behavioral interactions associated with mating, artificial insemination, and a novel in vitro fertilization technique. This study

documents in vitro fertilization for the first time in two *Litopenaeus* species. In vitro fertilization was achieved by upwelling a concentrated sperm suspension under a spawning female.

Misamore and Browdy, (1996) observed the mating behavior of *L. setiferus* and *L. vannamei* for comparison with other subgenres of *Litopenaeus.* According to video analysis and visual observations, the mating behavior of *L. setiferus* was divided into four sequential stages. During the Chase stage, the male closely follows the female, mirroring the changes in the female's direction. In the Probe stage, the male approaches the female ventrally and probes the region of the male of the female with its antennae. The Embrace stage is characterized by the inverting male juxtaposing the ventral surfaces with the female and wrapping its pereiopods around the female's shell. In the final stage, the Flex stage, the male collapses its uropods, holds the abdomen slightly and rotates perpendicular to the female's midline, forming a U-shape around the female. *L. vannamei* exhibited a mating behavior like that of *L. setiferus.* However, the reduction in the angle of rotation and in the abdominal curvature during the flexion stage differed from that of *L. setiferus.*

A generalized model of mating behavior within the genus *Litopenaeus* was proposed. All this knowledge has given a great deal of information; however, they have not yet solved the breeding problems of the species, but that are major advances in research to find the appropriate model of captive maintenance.

Thus, from the first report of the success of maturation and spawning of *L. setiferus* under laboratory conditions, the difficulty of males in transferring spermatophore, a problem associated with bacterial infections, was recorded for Brown et al. (1979) and Alfaro et al. (1993) found that the genital apparatus of male penaeid shrimp, *L. setiferus,* blackens with resulting detrimental effects on mating for production of larvae when animals are kept in controlled maturation/reproduction situations. A progressive, melanized condition of the male reproductive tract was shown to be associated with bacterial infection. It is suggested that the condition could be a progressive syndrome with bacterial invasion perhaps only in the advanced stages, or that more than one etiology may be involved in deterioration and blackening of the *L. setiferus* reproductive system.

Sandifer et al. (1984) trying to do artificial insemination tried the manual expulsion of the spermatophores. They found that shrimp with melanized terminal ampullae generally did not expel a spermatophore following electrical stimulation. And Rosas et al. (1993) found that it is necessary to look for a better method of insemination without manipulation for shrimps like *L. setiferus* that do not regenerate easily after electroejaculation.

Many studies have been carried out with the objective of elucidating the possible causes of the decrease of the reproductive quality in shrimp males. This phenomenon was defined as "degenerative syndrome of the male reproductive system" (SDARM) and is characterized by a progressive decrease in the number of spermatozoa and an increase in the percentage of abnormal and dead cells (Chamberlain et al., 1983, Talbot, et al., 1989). Melanization and low numbers of viable sperm cells have been frequent features in the *L. setiferus* population in the southern Gulf of Mexico (Pascual et al., 1998).

According to Rosas et al., (2004), changes in the structure of the benthic community in which the population feeds as a result of environmental disturbances caused by marine pollution and / or deleterious effects of high temperatures that increase year by year that affect reproductive quality and the genotoxic damage resulting from the accumulation of estuarine phase pollutants and the increase in crossbreeding as a consequence of population reduction due to overfishing are some of the most important hypotheses. Pascual et al., (2003b), found that there are differences in sperm quality between populations at the extremes of the distribution range and that are 20 times less spermatozoa for populations in the Southern Gulf of Mexico than those observed in populations North American Atlantic. Then, there is an inverse relationship between temperature and reproductive activity and sperm quality.

Laboratory results showed that there is a marked effect of temperature on the sperm quality of adult males of *L. setiferus.* Above 26 ° C, an increase in the proportion of abnormal cells was observed, indicating a reduction in the reproductive potential of organisms. Although shrimp presented a broad metabolic capacity to tolerate different experimental temperatures (Sánchez et al., 2002), the effect on sperm quality showed that the reproductive system is much more sensitive to temperature changes than energy metabolism. The results show that *L. setiferus* is a species sensitive to changes in

temperature and can be considered an indicator species of changes caused by global warming or the El Niño phenomenon in this region of the Gulf of Mexico (Rosas et al., 2004).

Goimier et al. (2006) evaluate the effect of amount of dietary protein levels on blood indicators of protein metabolism and immune condition (blood protein, hemocyanin, osmotic pressure, and hemocytes concentrations), and reproductive capacity of pre-adult *L. setiferus* males (*F*0). Finding that their results indicate that an immune reaction can occur in response to dietary protein (DP) of feed, with excess DP affecting several physiological functions included the sperm synthesis and sperm quality. Influence on hemocytes concentration in shrimp fed protein in excess could activate an attack on sperm cells in the vas deferens provoking loss of sperm quality in such conditions. Results indicate an optimal DP of 45 for maintaining the broodstock bank of *L. setiferus.* This would favor the thesis that by placing *L. setiferus* in a medium with abundant protein, of microbial origin in the case of the BFT system, a better immunological condition could be stimulated that would generate better growth and reproduction results for this species.

PHYSIOLOGY AND NUTRITION

Bottino et al. (1980) analyzed the fatty acids of three species of shrimp from the Gulf of Mexico, (*L. setiferus*), (*F. aztecus*) and (*F. duorarum*), periodically for one year. When variations in fatty acid levels were compared to water temperature, there was a lag period of one month between a change in water temperature and corresponding variation in fatty acid composition. This slow change in shrimp fatty acids suggests that changes are made through the food chain and not through endogenous adjustment to a change in water temperature. These experiments suggest that diet exerts a strong influence on the composition of shrimp body lipids and, on the contrary, the endogenous synthesis or modification occurs to a lesser degree. Lee and Lawrence (1985) investigated the relationships between protein level, size, apparent digestibility, digestive enzyme activities and *L. setiferus* growth. They found higher digestive enzyme activities in the diet with 22% protein and concluded that they may be evidence of an adaptation to a diet containing fewer digestible proteins.

McTigue and Feller (1989) measured whether the weight of the digestive tract content material relative to the body weight of the shrimp to determine whether bowel fullness remained constant over time or whether the variations were correlated with changes in the levels of light and tide. Factors that influence the eating pattern of an organism are complex and can inhibit the definition of a pattern even when there is one. *L. setiferus* juveniles did not exhibit any clear temporal trend in increasing or decreasing intestinal content weights relative to their body weights, even though maximum content was observed in the morning. Factors responsible for this maximum are unknown but may be associated with a change in activity levels between night and day.

Rosas et al. (1995) determined the role of the digestive gland in the respiratory metabolism of adult males of *L. setiferus* as a step in the direction of proposing a feeding scheme based on the cycle of activity of the digestive gland, finding that the metabolic activity of the digestive gland was higher six h after feeding. This could mean that the assimilation, started two hours after food intake, reached six hours after feeding. Eight hours after feeding, the rate of oxygen consumption of the digestive gland decreased and fell to values like those recorded for animals subjected to 72 hours of fasting. In other words, there was a high correlation between the rate of oxygen consumption of the animal and the concentration of glucose in the hemolymph and between the rate of oxygen consumption by the digestive gland and the concentration of glycogen in the digestive gland, in relation to the time after feeding. Gallardo et al. (1995) presents a basic feeding scheme for *L. setiferus* larvae based on diatoms (*Chaetoceros ceratosporum*), flagellates (*Tetraselmis chuii*) and Artemia nauplii.

These authors concluded that the increase in the concentration of algae and *Artemia* indicates a pattern of development of the digestive system associated with the development of the larvae and that this pattern should be considered to improve the production of larvae of this species under controlled conditions. García, et al. (1998) studied the dietary protein requirement of *L. setiferus* and pink shrimp post-larvae, *F. duorarum*, through growth, survival and yield, concluding that the protein requirement of post-larvae *L. setiferus* and *F. duorarum* are 50% protein, despite the evidence provided by studies on the dietary habits of these two species, which recorded important differences in the animal / vegetable ratio of their diet. Guzmán et al. (2001) investigated the effect

of protein and energy content on the activity of digestive enzymes and growth and survival of *L. setiferus* post-larvae under controlled conditions, the results suggest that dietary carbohydrates cannot spare protein because of the growth rates obtained with diets containing 200-300 g kg^{-1} protein were significantly lowered.

Renaud, (1986), Martinez et al. (1998), Rosas et al. (1998) and Rosas et al. (1999) stated that dissolved oxygen (OD), is an environmental regulator of shrimp metabolism. The regulatory role of OD is given by its direct intervention in the ability of organisms to obtain respiration energy through oxidative phosphorylation. These same authors also found that a relatively small reduction in the OD of 1.0 mg L^{-1} caused a reduction of up to 25% in the energy channeled to the biomass production and taking into account this behavior, it is possible to infer that the abundance of *L. setiferus* in tropical coastal environments along with dissolved oxygen levels could be of great help in establishing the health status of ecosystems.

Alcaraz et al. (1999a; 1999b) measured the combined effect of ammonium, nitrite and dissolved oxygen on post-larvae consumption of *L. setiferus* and reported that this species is more sensitive than *P. monodon* to the same pollutants. Taboada et al. (1998) conducted an experiment to study the effect of protein level on growth rate, survival, pre and postprandial oxygen uptake, and excretion of ammonia with juveniles of *L. setiferus*. The results indicate that juveniles of *L. setiferus* use protein when fed diets high in proteins (50%) and low (10%), and substrates lipoproteins with 30% protein. Under these conditions *L. setiferus* grows best with a 30% protein diet, where shrimps use food more efficiently.

Brito et al. (2000) studied the growth rate, soluble protein content, oxygen consumption, ammonia excretion and digestive enzymatic activity in *L. setiferus* post larvae in four feeding regimes that included combinations of *Artemia* nauplii fresh hatch, micro-particulate commercial diet and microalgae. They determined that the partial replacement of *Artemia* nauplii by artificial diet, with or without addition of algae in the initial post-larva stage, will benefit the growth and nutritional status of *L. setiferus* post-larvae. Gallardo et al. (2002) studied the adequate level of replacement of *Artemia* nauplii and microalgae by a micro-particulate diet for larvae of *L. setiferus*. Obtaining that in the

absence of algae, the replacement of *Artemia* nauplii resulted in slower development, lower resistance to salinity, lower growth and lower survival than in algae fed larvae.

Brito et al. (2004) quantified the amounts of energy channeled for growth (P), maintenance (R), excretion (U) and exudation (Ev) in six dietary treatments during the initial development of post-larvae in *L. setiferus* and *L. vannamei* grown at 28 ° C and salinity of 35 ppt. The results showed that the divergences between these two species during the early post-larval stages appear only when the animals were in a poor physiological condition due to an inefficient diet. *Artemia* nauplii, partially replaced by commercial micro-particulate diet with an algae supplement, integrated a high-quality diet for both species, producing high growth efficiency.

Arena et al. (2007) studied, the effect of different levels of carbohydrates (CBH) and proteins (P) (22.6% CBH-35% P, 11.6% CBH-45% P and 55% P-0% CBH) on the growth rate and activity of α-amylase and its phenotypic expression in cultured *L. setiferus*, finding that the specific α-amylase activity was inversely proportional to the carbohydrate concentration in the diet, indicating the protein-inducing effect on the shrimp diet. The phenotypic expression measured by allele frequency showed that the different alleles were affected by the level of dietary protein. While the allele *a* is reduced according to the protein increment, alleles *b* and *c* showed increase in their expression according to protein concentration, demonstrating that these alleles were responsible for the increase in amylase activity in shrimps fed high levels of protein in the diet. The modulating role of diet components in the enzymatic activity of shrimp can be discussed.

Various investigations have demonstrated the usefulness of blood parameters as tools for monitoring the physiological condition of wild and cultured shrimp exposed to very diverse environmental conditions. Sánchez et al. (2001) observed that a change in water temperature between 27 and 30 °C produces variations in the levels of proteins, lactate, glucose, triglycerides, cholesterol and the immune response in wild adults of *L. setiferus* and that under captive conditions the levels of proteins, triglycerides and cholesterol are lower than those observed in freshly caught animals.

The levels of hemocyanin (OxyHc) blood proteins, glucose, triacylglycerols, cholesterol and lactate of juveniles of *L. vannamei* have been investigated with the

objective of constructing a baseline to establish the nutritional status of shrimps kept under experimental or cultivated conditions in tanks that simulate commercial production conditions (Pascual et al., 2003a). From this and other studies, it was established that food quality is the factor controlling the levels of metabolites in the blood, which suggests that these parameters can be used to establish whether a wild population is well nourished or under some pressure associated with some serious environmental disturbance (Rosas et al., 2001; Rosas et al., 2002).

Rosas et al. (2002) showed that cultured shrimp are well adapted to use protein as a source of energy and for growth in order to maintain osmotic pressure and produce glycogen and glucose by the glyco-gene. In addition, the immune system, with a strong protein base, depends significantly on protein metabolism (Vargas-Albores and Yepiz-Plascencia 2000). It is well documented that *L. setiferus* juveniles are subject to sudden changes in salinity in the coastal lagoons where they live, where they are also exposed to daily variations in temperature or dissolved oxygen (Rosas et al., 1997, Rosas et al., 2001) In such circumstances, juveniles have the need to adjust osmotic pressure as a result of being in a more diluted environment. In the process of physiological adjustment at low salinity, the shrimp will use the pool of free amino acids like a form to reduce the increase of the cellular volume caused by the water intake, reducing the general concentration of proteins dissolved in the hemolymph (Rosas et al., 2001).

AQUACULTURE:

The knowledge generated since 1990s about *L. setiferus*, it started the interest to culture under controlled conditions. Thus, with growing concerns about viral diseases and the severe difficulties experienced by shrimp breeders in South Carolina to obtain *L. vannamei* post-larvae free from the infectious hypodermal hematopoietic necrosis virus (IHHN), they provoked a second assessment of potential culture of *L. setiferus* native (Sandifer et al 1993). According to Sandifer et al. (1993), Parker and Holcomb (1973), Parker et al. (1974), Charles and Conte (1978), the first who showed that *L. setiferus* was the best native shrimp species to aquaculture, cited by Sandifer. *et al.* (1993), demonstrated that the performance of *L. vannamei*, Pacific white shrimp, was superior to that of *L. setiferus* in semi-intensive ponds**.**

Thus, after these first experiments, the team of James M. Waddell, Jr. Mariculture Research and Development Center, carried out cycles of culture of this species in years 1985 and 1989. In 1985, a nursery of 0.l ha and one of 0.25 ha were stored with *L. setiferus* obtained from the Continental Fisheries hatchery, Ltd. in Panama City, Florida. Three tanks-0.1, 0.25 and 0.5 Ha were stocked with *L. vannamei* post larvae from Amorient Aquafarm Hatchery in Hawaii. *L. vannamei* were certified free of pathogens, and both species were stocked in post-larvae with 7-9 days of age at an estimated initial density of 12 animals m^2 for 147 days. For the 1989 experiment the post-larvae of *L. setiferus* were produced at the Waddell Mariculture Center from breeding farms and wild from South Carolina were stocked in two densities, 40 and 60 shrimp / m2, in nurseries of 0, 1 ha doubled. Duplicate 0.1 ha nurseries were stocked with *L. vannamei* post larvae from a private hatchery in Costa Rica at 60 m^2 for comparison for 145 days.

Yields for 1985 from the *L. setiferus* shrimp were slightly higher than the previous high levels for intensive farms. The 1989 experiment demonstrated for the first time that *L. setiferus* is possible to grow intensively. Overall, survival and yield levels reached with *L. setiferus* fell within the range of previous intensive culture results with *L. vannamei* at similar population densities. However, *L. setiferus* was typically 2.0 g lower than *L. vannamei* at harvest. These data reinforced the findings of other researchers (McKee et al., 1989, Browdy et al., 1991) that *L. vannamei* exhibited better growth and yield in culture than *L. setiferus* and concluded that in order to compete with others, producers of South Carolina had to work preferentially with *L. vannamei* culture and to consider *L. setiferus* as a good alternative, because this species had a slower growth rate, and that the culture methods developed were designed to *L. vannamei.*

However, it appears that the nursery culture system provided a suitable environment for the "normal" growth to *L. setiferus*, even at storage densities many times larger than those found in nature. However, the maximum growth rate recorded for this species in the wild was not reached in the environment. Sandifer et al. (1993) found that the main disadvantage of *L. setiferus* is the low growth rate, but no commercial hatchery is interested in producing post-larvae today and the advantages are that it is not a natural host for the IHHNV virus, the breeders are available in the environment, it is native to the region, and has no problems with the physical-chemical characteristics of the region.

Seed supply is likely to continue to be an important factor limiting the commercial use of *L. setiferus* for at least several years (to date). Meanwhile, additional research into nutrient requirements, responses to different commercial diets and temperature and population density effects on growth, as well as changes in nursery management procedures, are needed to better evaluate *L. setiferus* as an alternative species for aquaculture.

Williams et al. (1996) tested different storage densities in a recirculation system with *L. vannamei* and *L. setiferus*. Although the growth responses were similar, the growth of *L. setiferus* was lower than *L. vannamei*. There was an inverse relationship between storage density and survival for *L. setiferus*. The survival of *L. vannamei* was highly variable but was negatively correlated with density. Based on the results of the study, *L. setiferus* shows a high-density tolerance like that of *L. vannamei* and, therefore, may be suitable for intensive cropping systems. Concluding that the depressed growth rates of *L. setiferus*, which do not appear to be due to the effects of water density or quality, must be solved to achieve growth rates like *L. vannamei.*

A few years later and with the growing interest in live or frozen bait for recreational fishery, the interest in the intensive cultivation of *L. setiferus* returned, although economic profitability studies (Mc Kee., et al. 1989) had not been done yet opportunity to *L. setiferus*. A team from the University of Texas A & M conducted a study to evaluate whether intensive shrimp farming developed for *L. vannamei* could be used successfully to produce *L. setiferus* on bait size in outdoor nurseries in Southern Texas , evaluating the effect of two high densities, two feeding types and two water circulation methods on growth, survival and shrimp production for 120 days to determine if the data generated by the study could improve the economic viability of operating a *L. setiferus* shrimp bait production facility in Texas (Samocha et al., 1998).

The shrimp reached a suitable bait size (average weight of 5.1-7.4 g) within 90 to 120 days of culture. The mean survival value for the four nurseries stocked at 700 PL6 / m^2 was 58.1% ("high exchange"), while the mean survival value of the four ponds stored at 350 PL6 / m^2 ("low exchange") was of 64.5%. The difference in survival between "high" and "low" trading was -6.4%. The negative value shows reduced performance (for example, the high density negatively affected in shrimp survival in this study).

A positive survival value of 10.1% was obtained in nurseries fed a high protein ratio in the diet. Samocha et al. (1998) concluded that post-larvae 6 of *L. setiferus* can be stocked at a density of 700 PL/m^2 in a small outdoor nursery and can reach a bait size of 6.2 g in 94 days with 73, 6% survival and an extrapolated yield of 31,256 kg/ha when offered a commercial feed. The study also suggests that to support high shrimp biomass, production nurseries should be equipped with a 1.0 hp paddle aerator and a similar installation of a bottom aeration grid, air transport pumps and a nursery divider in the center.

Preliminary economic analysis and effect testing suggest that, under the conditions of this study, feed had the greatest effect on survival, growth and profitability. Efforts to develop a more complete and balanced food for *L. setiferus* may be the technological advance needed to make shrimp bait production more economically viable (Samocha., et al 1998).

Palomino et al. (2001) evaluated the effects of storage density and water exchange on the growth rate, survival and performance index of *L. setiferus* post-larvae under laboratory conditions. The experiment was carried out with post-larvae (PL10 to PL40) at the densities of 50, 150, 250 and 350 shrimp/m^2 and several water exchange rates per day (0, 6, 12 and 18%).

The maximum growth rate was obtained for shrimp with 12% water exchange per day at all densities. There was a reduction in the maximum growth rate in relation to the density with the highest values in shrimp stocked at a density of 50 and 150 shrimp/m^2 (mean value 0.53 mg/d) and lowest in shrimp stored at a density of 350 shrimp/m^2 (0.24 mg/d). Thus, reporting the lowest values in the growth rate of this species.

Valenzuela et al. (2002) stocked 0.5 g of 40 pl/m^2 in net tanks in marine water and low salinity water for 116 days of culture, finding a slight tendency to a higher growth in fresh water during the first 75 days of culture. It was obtained at the end of the experiment shrimps with 15,34 and 10,35 g in marine water and of low salinity respectively. It was obtained a growth rate of 0.92 g in seawater and 0.61 g without in low salinity water, showing that by improving the conditions of culture and favoring the development of live food inside the nursery (green water), one can have success in the creation of this species.

A recent review of new shrimp culture technologies has been carried out and it is suggested to use them for the culture of shrimp for live bait, particularly *L. setiferus*, *F. duorarum* and *F. aztecus*, using ready-made spawning females directly at sea, spawn them in the laboratory, use intensive nursery systems, without water exchange and using adequate feeds and all possible biosecurity measures (Gandy, 2007).

Pérez-Velazquez et al. (2013) have studied the effects of salinity (1.0-48 ups) on the biological performance, as measured by growth and survival, of *L. vannamei* and *L. setiferus*, grown at temperatures of 20, 24 or 28 C. The low growth and survival of *L. vannamei* was observed after 21-28 d of culture at low salinity (2.0 and 4.0 g/L) at 20 °C. the salinity for 8.0 and up to 32 ups significantly increased survival at this temperature, indicating that avoiding low temperatures is critical for the survival of this species when raised at low salinity. A large improvement in the growth rate of *L. vannamei* was observed at 24 °C but still not optimal compared to growth observed at 28 °C. Regardless of salinity, high survival rates were observed at 24 and 28 °C, but growth variable.

Unlike *L. vannamei*, *L. setiferus*, which was reared for 28 days at 24 °C, showed better growth performance at 8 ups compared to 2, 16 and 32 ups. Under similar experimental conditions, *L. setiferus* had considerably lower weight gain and survival than *L. vannamei*, according to previous literature (between 0.35 and 0.45 g/w).

PERSPECTIVES:

The current situation of the shrimp industry poses challenges that need to be overcome, replacing fishmeal, formulating feeds that will help mitigate the effects of diseases, appropriate use of natural foods, prebiotics, probiotics and reducing the use of antibiotics, as well as the management of microbial ecology, among others, will be the basis of research with cultivated shrimps. Research on nutrition and food will be particularly influenced by market variables, pressures on environmental policy and availability of inputs, among other factors**.** Specifically, the researches carried out with this species have generated important advances in the production of larvae with an already established and proven scheme, even more if there are problems to avoid the deterioration

of the captive males which limits the reproduction and the intensive production of quality post-larvae of this species. The formulation of special feeds for different types of shrimps is a basic necessity, however, perhaps the best option is to provide the prawns with an environment rich in microorganisms that provide excellent water quality, which generate an important ecological succession to maintain heterotrophic bacteria to nematodes that help to improve nutritional and immunological status in the natural way in shrimps, that is to say to continue working with the system with Biofloc (BFT) until finding the optimal conditions for the survival and the growth.

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CAPÍTULO II

ANTIOXIDANT AND IMMUNE RESPONSE OF WILD JUVENILE L*itopenaeus setiferus* FROM THE GULF OF MEXICO REARED IN DIFFERENT CULTURE SYSTEMS

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Observação: Tabelas e figuras foram incluídas no corpo do texto para facilitar a leitura da tese, além de um resumo estendido em português.

RESPOSTA ANTIOXIDANTE DE JUVENILES SELVAGENS DE *Litopenaeus setiferus* DO GOLFO DO MEXICO CULTIVADOS EM DIFERENTES CONDIÇÕES DE CULTIVO

RESUMO:

A determinação do estado fisiológico e de saúde dos organismos vivos em geral costuma ser um conceito controverso devido à falta de critérios específicos que permitam definir com precisão quais são os limites de tolerância às variações ambientais em um contexto multifatorial. Em organismos aquáticos numerosos testes foram realizados tentando determinar quais são os limites toleráveis de fatores ambientais e contaminantes que determinam o desempenho e sobrevivência de muitas espécies de peixes, crustáceos e moluscos. A interação entre as variáveis e a capacidade fisiológica dos organismos para manter a homeostase são elementos que devem ser considerados na avaliação do estado de saúde. Tradicionalmente os indicadores de sucesso de um cultivo referem-se ao desempenho zootécnico, como crescimento ou sobrevivência e o fator de conversão alimentar. Entretanto todas essas respostas estão dadas pelos mecanismos fisiológicos, imunológicos e bioquímicos que os animais sofrem ao interior do corpo, dependendo das condições ambientais dos cultivos.

Considerando os avanços nas tecnologias de cultivo particularmente o sistema de cultivo com bioflocos (BFT), decidiu-se desafiar o camarão branco do Atlântico Norte *Litopenaeus setiferus* para realizar pesquisas visando avaliar o potencial de cultivo desta espécie. Desenhou-se uma estratégia com três experimentos que inclui começar experimentando com animais selvagens para saber a resposta no cultivo, dos camarões após de serem capturados do meio natural e passar por um período de aclimatação. Foram coletados juvenis de *L. setiferus* com rede de arrasto na laguna de Términos 18°36′28″N 91°33′21″O, no estado de Campeche, México. Os camarões foram capturados em 24 ups e colocados em tanques de 20 m^3 para sua aclimatação em dois tratamentos Água Marinha 35 ups e água de baixa salinidade 5.0 ups.

Posteriormente quatro tratamentos foram implementados: água do mar (35 ± 0,42 ups) com bioflocos (MBFT) e água do mar clara (MCW); água de baixa salinidade (4,33 ± 3,92 ups) com bioflocos (LSBFT) e água clara de baixa salinidade (LSCW)

Considerando que diante das mudanças nas condições ambientais o metabolismo aeróbico de crustáceos gera substâncias reativas de oxigénio (ROS), certa concentração de ROS é necessária para a defesa contra as infecções microbianas, no entanto, a produção de ROS e os seus resíduos pode resultar em dano severo nas células. Para manter o equilíbrio, são eliminadas pelo sistema de defesa antioxidante que inclui por exemplo, a superóxido dismutase (SOD), a catalase (CAT) e outras enzimas associadas com a desintoxicação de células. Assim avaliou-se a resposta antioxidante de juvenis selvagens de *L. setiferus* submetidos a diferentes condições de cultivo durante de 45 dias. A quantidade de hemócitos totais, atividade de enzimas antioxidantes, peroxidação de lipídeos e proteínas carboniladas foram avaliadas em hepatopáncreas e músculo do camarão. Os resultados indicaram que em geral, os tratamentos com baixa salinidade apresentaram alta atividade redox, embora não tenham sido encontradas diferenças significativas ($p \leq 0,05$) entre a atividade enzimática antioxidante dos animais submetidos às diferentes condições de cultivo. A Análise de Componentes Principais (PCA) mostrou que a variabilidade foi distribuída entre todos os biomarcadores, e não houve um que, por si só, representasse o maior efeito. Diante destes resultados utilizou-se um "Índice de estresse" que é um método que combina e integra a resposta de vários biomarcadores em uma a resposta só, a Resposta Integrada dos Biomarcadores ou IBR pelas siglas em inglês. O IBR indicou uma maior atividade enzimática antioxidante nos tratamentos com baixa salinidade, principalmente LSBFT. Entretanto, o tratamento LSCW parece ter uma atividade menor, no entanto, mostrou uma alta mortalidade no meio do experimento, de tal forma que não conseguimos mensurar a real atividade enzimática nos momentos mais difíceis do cultivo, atividade que talvez fosse mais intensa de todos os tratamentos. O resultado mais importante foi constatar que há uma grande quantidade de estresse oxidativo em animais cultivados em água de baixa salinidade, talvez a idade dos camarões capturados fez com que eles precisassem de um meio mais salino em preparação para seu retorno da lagoa para o mar e, por outro lado, o fluxo permanente de água os manteve permanentemente estressados.

O maior número de hemócitos foi registrado nos tratamentos com bioflocos, mostrando provavelmente, que os animais nos tratamentos BFT estavam mantendo um alerta imunológico que talvez, permitiu uma redução no estresse oxidativo. Em relação ao desempenho zootécnico, os maiores incrementos em peso, sobrevivência e índice

hepatosomático foram também nos tratamentos com BFT, tanto em água marinha como de baixa salinidade.

Baseado nos resultados obtidos, podemos concluir então que houve estresse oxidativo em todos os tratamentos, no entanto os tratamentos BFT mostraram uma melhor condição imunológica e um ganho de peso constante e maior que nos tratamentos com água de baixa salinidade. Provavelmente as condições de cultivo, particularmente baixa salinidade e fluxo contínuo para manter a água limpa, conseguiram induzir estresse e dano oxidativo a proteínas e lipídios nas células musculares e hepatopáncreas de juvenis selvagens de *L. setiferus*.

Os bioflocos, independentemente da salinidade poderia ter contribuído para diminuir o estresse, estimulando o sistema antioxidante a manter o equilíbrio redox através de uma maior atividade das enzimas antioxidantes, e o sistema imunológico através da formação de um maior número de células na hemolinfa.

ANTIOXIDANT RESPONSE OF WILD JUVENILE *Litopenaeus setiferus* FROM THE GULF OF MEXICO REARED IN DIFFERENT CULTURE SYSTEMS

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ABSTRACT

The antioxidant response of wild juveniles of Atlantic white shrimp *Litopenaeus setiferus* subjected to different culture conditions was evaluated. Four treatments were implemented: seawater (35 ± 0.42 ppm) with biofloc (SBFT) and clear seawater (SCW) and low-salinity water (4.33 ± 3.92 ppm) with biofloc (LSBFT) and clear low-salinity water (LSCW). After 45 days, the amount of total hemocytes, the activity of antioxidant enzymes, the peroxidation of lipids and carbonylated proteins were evaluated in the hepatopancreas and muscle of shrimp. In general, the low-salinity treatments had a high redox activity, although no significant differences ($p \leq 0.05$) were found between the enzymes in the animals subjected to the different culture conditions. The principal component analysis (PCA) showed that the variability was distributed among all the biomarkers, and there was not a single biomarker that independently represented the greatest effect. The integrated response of the biomarkers (IBR) indicated greater activity and concentration in the treatments with low-salinity water, particularly in the LSBT. We conclude that culture conditions, particularly low salinity and continuous flow to maintain clear water, can induce stress and oxidative damage to proteins and lipids in the muscle and hepatopancreas cells of wild juveniles of *L. setiferus*. The biofloc, independent from salinity, could have contributed to diminishing the stress by stimulating the antioxidant system to maintain the redox balance through a greater activity of the enzymes and by

stimulating the immune system through the formation of a greater number of cells in the hemolymph.

Keywords: biofloc, low salinity, immunity, antioxidant enzymes
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INTRODUCTION:

In the southern Gulf of Mexico, the species *Litopenaeus setiferus* is exposed to a wide range of environmental pressures, including overfishing, pollution of coastal bays, estuaries and lagoons with pesticides associated with agriculture, sewage discharge from coastal cities or the petroleum industry that operates in the Campeche area (Arreguín et al., 1997; Gracia and Vázquez, 1998; Rosas et al., 2007). *L. setiferus* also presents serious deterioration of the reproductive quality of males (Rosas et al., 2004) in captivity and the natural environment. The global indiscriminate transfer of aquatic animals has been responsible for introducing, establishing and disseminating many pathogens to new geographic areas (Briggs et al., 2005). In addition, the development of shrimp farms is based on widely domesticated species that are usually exotic to many geographic regions, so it is necessary to develop domestication programs for native species to maintain their sustainability (Gaxiola et al., 2008).

Litopenaeus setiferus culture is an alternative not only for production purposes but also for conservation purposes for itself and other native species through sustainable biotechnologies (Valenzuela, 2009). Several experiments have been carried out in the northern Gulf of Mexico, mainly focused on the production of bait for sport fishing (Sandifer et al., 1993; Samocha et al., 1998; Gandy, 2001). In these studies, the growth was slightly less than that of the commonly harvested *L. vannamei;* however, *L. setiferus* is also a species that is much less sensitive to viral diseases (Sandifer et al., 1993; Chapman et al., 2004), tolerates low salinities and is resistant to some types of extreme conditions. Moreover, *L. setiferus* does not represent an escape risk in the region associated with natural disasters such as floods caused by hurricanes or tropical storms in the Gulf of Mexico (Laramore et al. 2001; Valenzuela et al., 2002). The survival and growth of *L. setiferus* may improve with an alternative culture that offers a greater amount of live food, such as biofloc technology (BFT). The objective of a BFT system is to

maintain water quality by providing constant and abundant aeration in the pond to keep organic matter in suspension. Heterotrophic bacteria colonize the waste particles and absorb nitrogen, phosphorus, and other soluble nutrients, maintaining the water quality (Otoshi et al., 2001; Wasielesky et al., 2006). An additional benefit is the presence of microbial protein, filtered by juveniles along with organic carbon and nitrogenous waste (Avnimelech, 2007; Ballester et al., 2007; Azim and Little, 2008; Avnimelech and Kochba, 2009). The nutritional contribution of microorganisms strengthens the immune response and antioxidant status of cultured shrimp (Ju et al., 2008; Xu and Pan, 2013; Da Silva et al. 2015).

Traditionally, the indicators of success of a culture refer to zootechnical performance, i.e., growth, survival or FCR. However, all these responses and the physiology, immunology and biochemical mechanisms depend on environmental conditions. For example, the immunological processes associated with the physiological biochemistry will assist in evaluating the health status (Bachère et al., 1995; Le Moullac et al., 1998). Evaluating these types of variables would reveal the mechanisms that shrimp use to adapt to the culture medium and identify how such information can be used to achieve optimal results for the species. Some cellular defense mechanisms in crustaceans depend on free radicals during phagocytosis and encapsulation in response to oxidative stress (Smith, 2003). Due to changes in environmental conditions, the aerobic metabolism of crustaceans generates oxygen-reactive substances (ORS) (Muñoz et al., 2000). A certain concentration of ORS is necessary for defense against microbial infections. ORS residues can result in severe cell damage and affect a crustacean's ability to maintain balance. One response mechanism is a lipid soluble antioxidant system (LSAS), which includes glutathione peroxidase, superoxide dismutase (SOD), and catalase (CAT) (Ren et al., 2009; Quiu et al., 2011; Zhou et al., 2013; Song et al, 2015). The present study aimed to evaluate the antioxidant response of wild juvenile shrimp *L. setiferus* that were acclimated and subjected to four different culture conditions.

MATERIAL AND METHODS

The experiment was carried out in the Unidad Multidisciplinaria de Docencia e Investigación (UMDI) belonging to Facultad de Ciencias de la Universidad Nacional Autónoma de México, Campus Sisal, located in northeastern coastal region of the state

of Yucatan (21º9'55.22 N, 90º1'54.93 W) in Puerto de Abrigo, S/N en Sisal, municipio de Hunucmá (Yucatan, Mexico).

Capture and transportation to the laboratory

A total of 350 *L. setiferus* juveniles (5.75±1.96 g) were collected at Laguna de Términos in Campeche, Mexico, and transported at a density of 2.5 juveniles L^{-1} in 50 L transparent plastic bags with one third water and two parts O_2, to the Port of Sisal, Yucatan. They were acclimatized in a 20 m^3 tank with seawater at 25 ppt for 10 days.

To continue acclimation, shrimp were separated into two groups. The first group was acclimated to the marine seawater treatments (35 ppt) and the second to low-salinity treatments (5.0 ppt). To reach the two salinity conditions, the salinity was adjusted in increments or decrements of 2.0 ppt over 10 days. At the end of salinity acclimation, the shrimp were distributed in 100 L fiberglass tanks at a density of 10 shrimp per tank (100 shrimp m^{-3}). The initial individual wet weight was recorded using an OHAUS balance (0.1 g of precision).

Experimental design

The experimental design consisted of four treatments with three replicates per treatment: seawater biofloc (MBFT), seawater clear water (MCW), low salinity biofloc (LSBFT) and low salinity clear water (LSCW). Treatments with clear water were adjusted to a daily renewal of 35%.

To obtain the BFT in each tank, 50 L (50%) of biofloc inoculum water was added (from the grow out system) at a C:N ratio of 20:1 (Avnimelech, 1999). The tanks were filled to their full capacity (100 L) with filtered clear water. During the experimental period, cane molasses was added as a source of organic carbon at a rate of 6.0 g of carbon (C) per gram of total ammonia nitrogen (TAN) when the ammonium reached 1.0 mg L^{-1}, as described in Avnimelech (2009), Ebeling et al. (2006) and Samocha et al. (2007).

At day 0, salinity was stabilized at 35 and 5 ups for seawater and low-salinity water, respectively. The experiment lasted 45 days.

Both seawater and low-salinity water (from well water) were filtered using a sand filter. A commercial feed (35% CP, Malta Clayton SA de CV) was supplied twice a day at a rate of 3.0% of biomass. Daily temperature, dissolved oxygen (DO) and pH were measured in the morning and afternoon, using a Hach®model SensION6-Hq40 multiparameter calibrated for each salinity level. Salinity was measured daily with a Fisherbrand® self-compensated refractometer. Ammonia, nitrite and nitrate concentrations were evaluated every third day with a Hach® colorimetric kit for diluted and full seawater. Solids were sedimented using Imhoff® cones (Aquatic Ecosystems Inc.®) every third day (Avnimelech, 2008).

To evaluate the shrimp zootechnical performance at the end of experiment, all shrimp were counted and the final individual live wet weight per tank was determined using an Ohaus balance (0.1 g readability).

Biochemical analysis

At the end of experiment, shrimp at the intermolt stage (Corteel et al., 2012) were placed in freezing water at 5.0˚C below the initial temperature to decrease their metabolic activity (Pascual et al., 2003) and were transported to the laboratory for analysis. For each shrimp, 100 µL of hemolymph was collected from the ventral sinus using a 30 G x 13 mm syringe preloaded with heparin. Hemolymph from animals in the intermolt stage C placed in a hematocytometer glass plate provided a total hemocyte count (THC). A portion of hepatopancreas (HP) and muscle tissue were stored at -80 ˚C for further analysis.

Homogenate preparation

In total, 100 mg of muscle samples previously stored at -80 ˚C were cold macerated using 1000 µL of 0.05 M Tris buffer (pH 7.4). Then, 500 µL of the obtained homogenate was separated and centrifuged at an RCF of 1000 x *g* for five min at 4.0 ˚C to determinate superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) enzymes, whereas 500 µL of the non-centrifuged homogenate was used to evaluate lipid and protein oxidation (LPO and PO). All analyses were evaluated using

10 µL of homogenized preparations by triplicate, reading the absorbance in 96-well plates using a spectrophotometer (PerkinElmer©, Waltham, Massachusetts, USA).

Protein determination in shrimp muscle

Protein was determined by the Bradford assay (1976) adapted to a microplate method using a dye reagent concentrate (Bio-Rad, Philadelphia, PA, USA) with bovine serum albumin as a standard (EMD Biosciences, Inc., La Jolla, CA, USA) and reading the absorbance at 595 nm.

Antioxidant enzymes in shrimp muscle

SOD activity was quantified using a commercial kit (Sigma-Aldrich, St. Louis, MO, USA). One unit of superoxide dismutase was defined as the amount of enzyme required to inhibit the activity of xanthine to 50% in 20 min. Absorbance was measured at 450 nm.

For CAT activity, an adaptation was made in a microplate using the technique reported by Hadwan and Abed (2016). Samples were incubated in two 96-well plates with 100 µL of phosphate buffer (pH 7.4) or 100 µL of the same buffer with hydrogen peroxide (H_2O_2). Both microplates were incubated for three min, and the reaction was stopped by addition of 100 µL of ammonium molybdate. Then, absorbance was measured at 450 nm. For both enzymes, the results were expressed in U mg protein^{-1}.

GST activity was evaluated with a microplate method using 190 µL of sodium phosphate buffer, 200 mM reduced glutathione and 100 mM CDNB (final concentrations) and reading the absorbance at 412 nm for five min. The activity was calculated using the extinction coefficient E=9.6 mM^{-1} cm^{-1} (Contreras-Vergara et al., 2007). The results were expressed in nmol mg^{-1} protein min^{-1}.

Lipid and protein oxidation in shrimp muscle

LPO was quantified according to Fox method and using the PeroxiDetect© kit (Sigma-Aldrich, St. Louis, MO, USA). The uncentrifuged extract was homogenized with methanol at a ratio of 1:1 (v/v) and then centrifuged at an RCF of 10,000 x g for five min at 27 ˚C. Then, 10 µL of extract were placed in two 96-well plates, and 160 µL of working

solution was added to incubate samples for 60 min. Absorbance was measured at 595 nm. A standard curve was prepared using serial dilutions with 129 µL of 1 M tertbutyl hydroperoxide (TBOOH) plus 871 µL of methanol. The results were expressed in nmol peroxide mL^{-1}. The PO level was evaluated as previously described (Mesquita *et al.*, 2014) where 100 µL of dinitrophenyl hydrazine (DNPH) plus 2 N HCl were combined with 100 µL of Tris buffer (pH 7.4) (blank) or 100 µL of uncentrifuged homogenate using Eppendorf© tubes. Tubes were incubated for 10 min, then 50 µL of 6 M NaOH was added to the samples and the blank. All tubes were centrifuged at an RCF of 10,000 x g for five min at 27˚C. Finally, 150 µL of samples and blank were added to a 96-well plate to read the absorbance at 450 nm. The results were expressed in nmol mg $protein^{-1}$.

Statistical analysis

For the statistical analysis, the Statistica 7.0 program (StatSoft, Tulsa, Oklahoma, USA) was used. The normality of the data was verified by the Kolmogorov-Smirnov test and the homoscedasticity by Levene's test. Subsequently, an ANOVA test was performed to confirm the existence of significant differences between treatments ($p < 0.05$). A principal component analysis (PCA) was also applied to detect any correlation between the variables. The integrated response of the biomarkers IBR was calculated by adding the calculated areas of star plots, after standardization of the data, according to Jia-Xie (2016).

RESULTS

All water quality variables were maintained at the recommended levels for shrimp culture throughout the experiment (Van Wyk and Scarpa, 1999, Table 1).

Table 1. Mean value ± SD of physicochemical parameters of the water after 45 days for *L. setiferus* wild juveniles in different culture conditions. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water

Treatment	OD mg/L	Temperature °C	pH	Salinity ups	NH^+_4 (mg/l)	NO_2 (mg/l)	NO_3 (mg/l)
MBFT	7.1 ± 0.5	26.5 ±0.9	7.4 ± 0.6	34.7 ± 1.4	0.4± 0.5	1.4 ± 1.2	82.4 ± 25.2
MCW	7.2 ± 0.6	26.6 ±0.7	8.2 ± 0.6	35 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
LSBFT	7.3 ± 0.3	26.4 ± 1	8.6 ± 0.5	4.9 ± 2	0.7 ± 0.26	3.9 ± 2.2	53.5 ± 18.5
LSCW	7.25 ±0.3	26.8 ±0.7	7.8 ± 0.4	4.3 ± 3.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Both treatments with clear water were kept without nitrogenous residues and those with biofloc (BFT) maintained a controlled level of NH_3^+ and NO_2^-, without water exchange. The ionic content of the water from all treatments is reported in Table 2.

Table 2. Results of ionic analysis of the experimental seawater (MCW) and low salinity water (LSW) used for *L. setiferus* wild juveniles in different culture conditions.

	MCW	LSCW
pH	7.8	7.4
ORP mV	-207.7	-79.1
DO mg/L	3.93	3.64
T °C	22.5	23.2
Salinity (ppt)	36.7	5.82
Hardness mg/$CaCO_3$/L	6361.9	734.5
Alkalinity Mg/$CaCO_3$/L	114.8	333.7
Na mg/L	11490	693
K mg/L	432.4	24.7
Ca mg/L	380.4	141.8
Mg mg/L	1396	106.0
Chloride mg/L	15800	1200.0
Sulfate mg/L	1380.21	74
Cations	13698.8	965.39
Anions	17180.21	1274
Balance	-3481.41	-308.61

The results of the zootechnical performance of the wild juveniles of *L. setiferus* after 45 days of experiment are shown in Table 3. The final weight, the weight gain and the survival were significantly higher in the MBFT treatment than the rest of the treatments. The lowest survival and lowest weight were found in the LSCW treatment. The relationship between the size of the hepatopancreas and the body size (HI) was higher in shrimp grown in seawater than in low-salinity water. In relation to survival, cultures with low salinity, mainly LSCW, was where high mortalities occurred (Table 3).

Table 3. Mean value ± SD zootechnical data after 45 days for L. setiferus wild juveniles in different culture conditions. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water

Treatment	Initial weight (IW) (g)	Final weight (FW) (g)	Weight gain (FW-IW) (g)	HI %	Survival %
MBFT	5.75 ± 1.12	9.28 ± 2.48[a]	3.53 ± 0.53[a]	4.19 ± 1.17[a]	56.67[a]
MCW	5.23 ± 0.96	8.08 ± 3.1[a]	2.78 ± 0.97[a]	4.29 ± 0.36[a]	60[a]
LSBFT	5.78 ± 1.26	8.33 ±1.62[a]	2.55 ± 0.18[b]	3.35 ± 0.89[b]	40[b]
LSCW	5.96 ± 1.75	4.49 ± 0.41[b]	0.28 ± 0.41[b]	3.45 ± 0.55[b]	26.67[b]

With respect to hemocytes, shrimp from low-salinity water treatments showed the highest number of hemocytes (Figure 1).

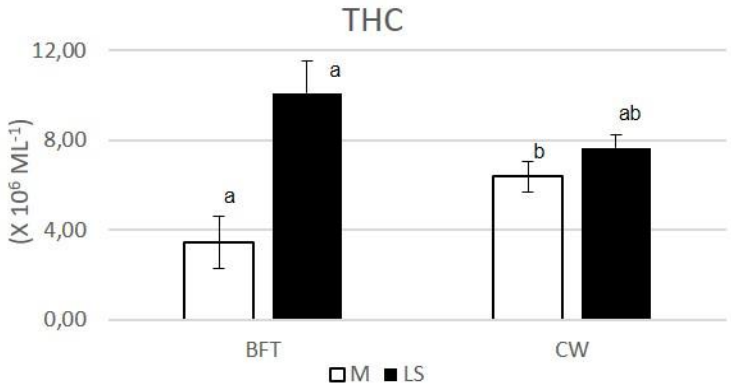

Figure 1. Mean values and SD for THC in *L. setiferus* wild juveniles in different culture conditions after 45d. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water

Antioxidant *enzymes*

The activity of the GST in muscle (Figure 2) showed no significant differences between treatments ($p < 0.05$).

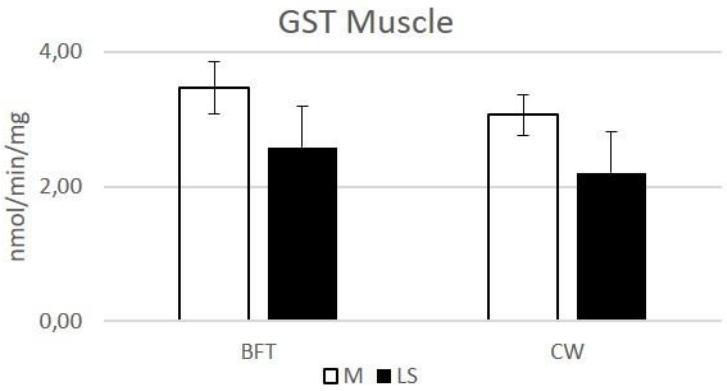

Figure 2. Mean values and SE for GST activity in muscle of *L. setiferus* wild juveniles in different culture conditions after 45d. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

CAT showed greater activity in the muscle than in the hepatopancreas (Figure 3), with the LSBFT treatment showing the highest activity of this enzyme. SOD showed greater activity in the hepatopancreas in all treatments (Figure 3) and showed greater activity in the LSBFT treatment in both tissues, however, there were no significant differences.

The LPO presented significant differences ($p <0.05$) in the samples of the hepatopancreas, but not in the muscle (Figure 4) among the treatments tested. The carboxylation of the proteins (PO) showed a higher activity in the hepatopancreas (Figure 4).

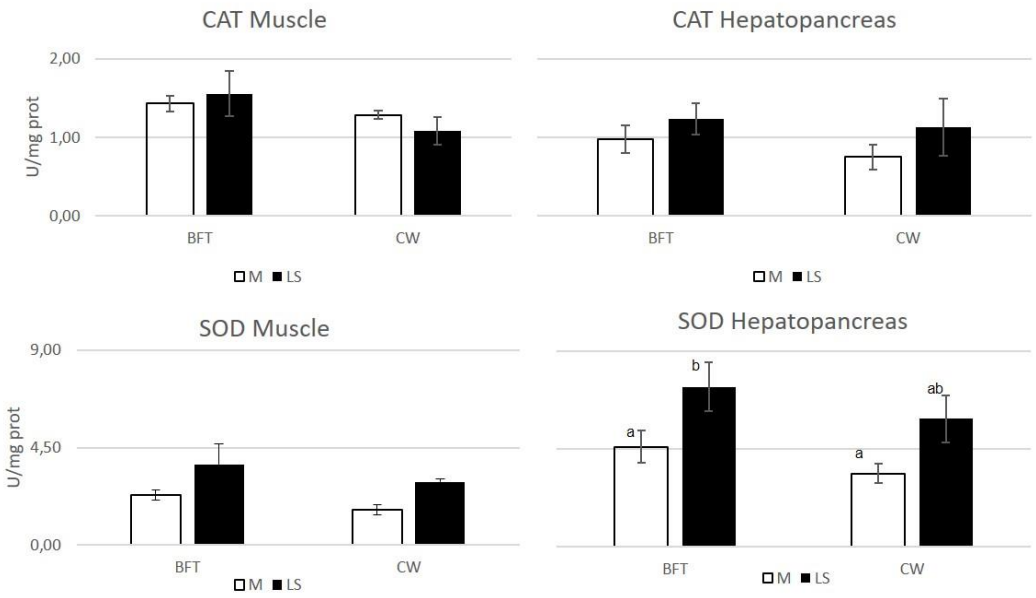

Figure 3. Mean values and SE for CAT and SOD activity in muscle and HP of *L. setiferus* wild juveniles in different culture conditions after 45d. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

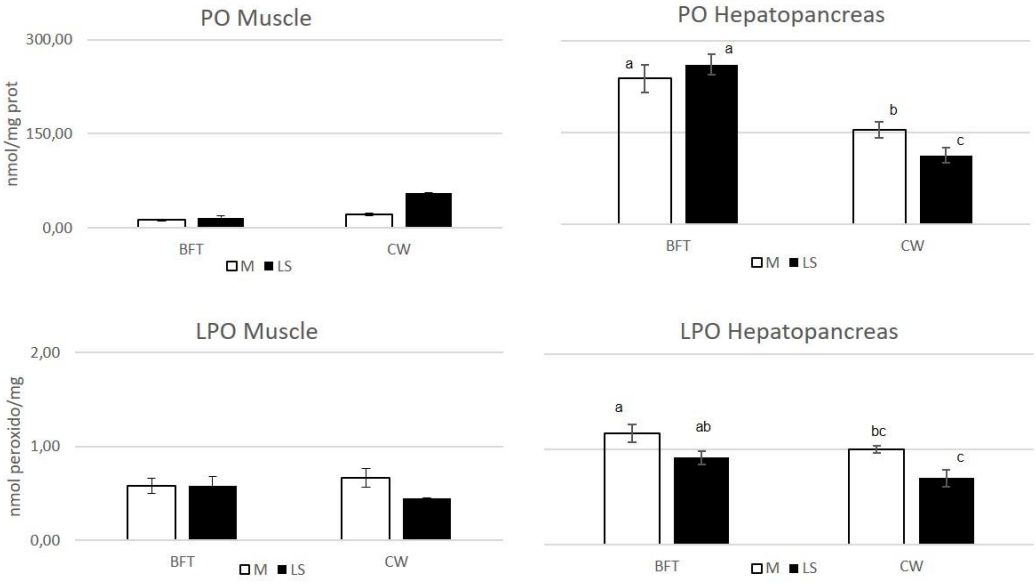

Figure 4. Mean values and SE for LPO AN PO activity in muscle and HP of *L. setiferus* wild juveniles in different culture conditions after 45d. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

Principal Component Analysis (PCA)

According to the results obtained (Figure 5), represents the percentage of variability explained by each component and we can see that component 1 that is the largest barely represents 20.1% which is not a good indicator that there is much correlation in the data. The poorly explained variance suggests that the variables are not correlated and can be considered independent of each other, thus we can confirm that the variability explained by the components is distributed among all the variables since the highest variance is only 20.1%.

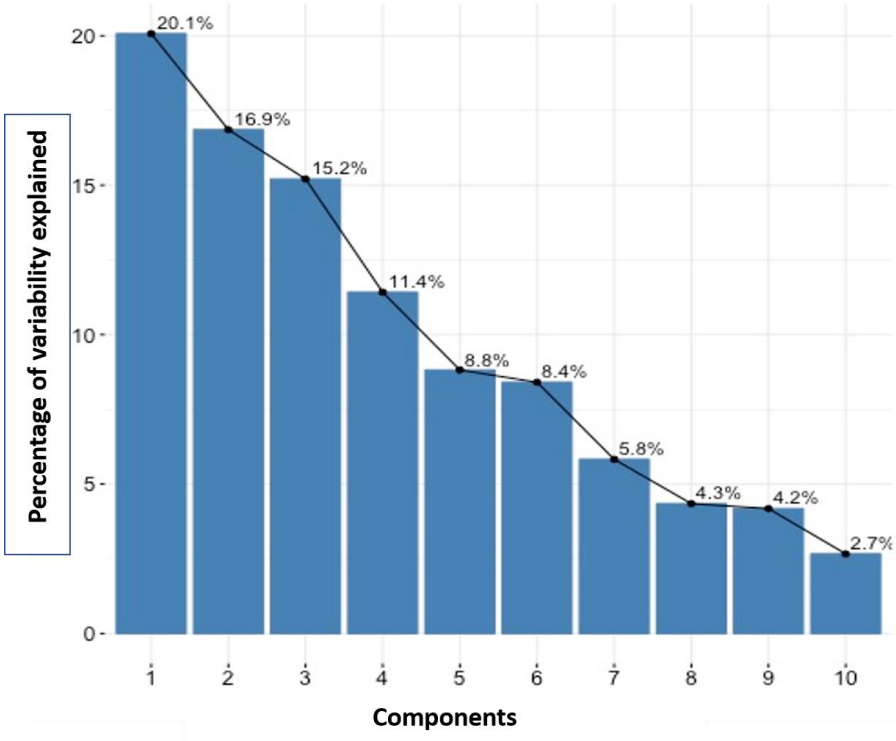

Figure. 5. Percentage of variability explained result of Principal Component Analysis, showing that there is no variable that has a greater effect on the treatments. of *L. setiferus* wild juveniles in different culture conditions after 45 d.

For the LSBFT treatment and the complete IBR (Figure 6) for each of the treatments, the lowest activity (4.8) was observed in the MCW treatment that behaved as a control and the highest activity (16.41) was observed in the LSBFT treatment.

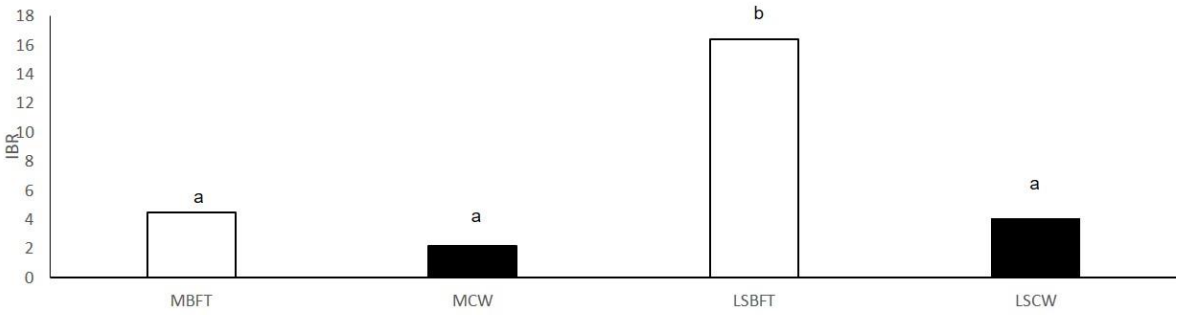

Figure. 6. Integrative biomarker response index (IBR) for *L. setiferus* wild juveniles in different culture conditions after 45 d. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

The star plot of the IBR (Figure 7) presents the response of the five biomarkers (SOD, CAT, GST, PO, LPO in the muscle and hepatopancreas tissue).

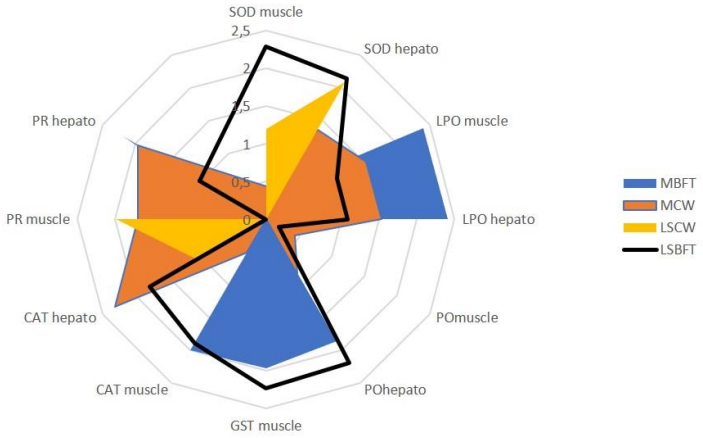

Figure. 7. Integrative biomarker response index (IBR) Star-plot for *L. setiferus* wild juveniles in different culture conditions after 45 d. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

DISCUSSION

The post larvae and juveniles of *L. setiferus* can develop much better in euryhaline environments since they have a better capacity for osmotic and ionic regulation than adults, which allows them to adapt to conditions of both high and very low salinity (Castille and Lawrence, 1980; Laramore et al., 2001). In this study, salinity was maintained at 5.0 and 35 mg L^{-1}, which is a known range for this species in the coastal lagoons where they live (Rozas and Minello, 2011). The water quality conditions in general were maintained at levels suitable for the culture of penaeid shrimp. The DO remained above 6.0 mL L^{-1}, the temperature ranged between 26 and 27°C, and the pH values were maintained in the upper limits but within what is established for these animals, due to the natural chemical composition of well water in the Yucatan Peninsula, which also has a high level of hardness and alkalinity (Van Wyk and Scarpa, 1999; Laramore et al., 2001; Rozas and Minello, 2011). Ammonium, nitrite and nitrate in BFT treatments were maintained within the normal limits for this type of culture (Lin and Chen, 2001, Campos et al., 2012).

With respect to growth, shrimp from marine water treatments showed the greatest increases in weight during the experiment (MBFT 3.53 g and MCW 2.78 g), which could indicate that animals in low salinity spent a greater amount of energy to stay alive (routine metabolism), this corroborated with the low consumption of food; as they have the lowest survival rate (40 and 26.6% for LSBFT and LSCW, respectively). Valenzuela (2009) found through the measurement of DO consumption that juveniles of *Farfantepenaeus duorarum* in BFT conditions presented a lower metabolic rate than animals in clear water and were able to grow in the face of environmental stress such as low temperature conditions.

The use of BFT can increase the growth, survival and reproductive capacity of animals in culture, according to Emerenciano et al. (2012) and Ekasari et al. (2013), since it contains a wide range of nutrients, particularly proteins, of microbial origin (Tacon 2002; Wasielesky et al., 2006; Kuhn, 2009;), which stimulates the immune and antioxidant system of shrimp (Xu and Pan, 2013; Cardona et al., 2015; Zhao et al., 2016) and even functions as a natural probiotic (Castex et al., 2009, Ferreira et al., 2015).

The lowest growth occurred in the LSCW treatment, along with the lowest survival. The difficulty of the wild juveniles to acclimatize to the low salinity conditions with constant water change was notable; perhaps the constant flow of water prevented them from adapting early on to captivity conditions since according to Castille and Lawrence (1980) and Rozas and Minello (2011), animals move continuously within different salinity gradients and do not remain in only one. Due the low survival in the clear water treatment, the ionic content of the water was tested, showing a very low concentration of potassium and magnesium (Table 2) which may have affected the metabolism of shrimp, since for example magnesium stimulates the activation of some enzymes, cellular regeneration and carbohydrate assimilation and potassium is involved in the activation of Na + -K + -ATPase enzyme, which acts on the regulation of extracellular volume. Inadequate levels of K + in water could seriously affect the osmoregulatory capacity of cells (Roy et al., 2007).

The HI values were significantly higher ($p < 0.05$) in the treatments with seawater, attributed to a better capacity to store and assimilate nutrients, which can be reflected in better nutritional and physiological condition and, therefore, a greater increase in weight. It is most likely that in the low-salinity water treatments, the nutrients were being used by the shrimp to maintain their daily metabolism and survival requirements rather than being used for growth.

The active response of the immune system of crustaceans usually implies a rapid change in the number of circulatory cells (Destoumieux et al., 2000; Johansson et al., 2000). In this study, the total number of hemocytes (THC) did show significant differences ($p < 0.05$) between the treatments, with the lowest number of total cells recorded for the SBFT treatment ($3.44x10^6$ mL^{-1}) and the highest for the LSBFT treatment (10.9 x106 mL^{-1}).

The values of THC, in concentrations of 106 cells mL^{-1}, obtained at the end of the 45 days of culture in the present work, were higher than those obtained in wild juveniles of *L. setiferus* extracted directly from the lagoon (Rosas et al., 2004), with reported concentrations of 20.07x103 cells mL^{-1}; these latter values could be interpreted as a "normal" stress condition in captured organisms. In this way, the values found for each of the treatments in this experiment can then be considered as a response of the shrimp

exposed to different culture conditions, to biofloc and particularly to continuous water flows and low salinity. It should be mentioned that a greater number of hemocytes does not necessarily imply a better immunological condition; however, during the respiratory outbreak of these cells, high amounts of ROS are produced, probably causing a greater amount of oxidative stress in the low-salinity treatments. and at the same time unchaining.

CONCLUSION:

In the present work, changes were documented in the amount of hemocytes and in the activity of antioxidant enzymes (CAT, GST, SOD) as well as lipid peroxidation and protein oxidation in the hepatopancreas and muscle tissue of wild juveniles of *L. setiferus* maintained in seawater with and without Biofloc and low-salinity water with and without Biofloc for 45 days. Wild shrimp presented difficulties in acclimatizing to a fixed change of salinity and a constant flow of water in the culture system (LSCW). Shrimp in the treatments with Biofloc (SBFT and LSBFT), independent from the salinity, showed a greater efficiency in terms of growth and adaptability to the culture, which could have contributed to a reduction in stress, stimulating the antioxidant system through a greater activity of the enzymes and stimulating the immune system through the formation of a greater number of cells in the hemolymph.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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CAPÍTULO III

OXIDATIVE STRESS IN EARLY F0 JUVENILES OF *Litopenaeus setiferus* REARED IN DIFFERENT CULTURE CONDITIONS.

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Observação: Tabelas e figuras foram incluídas no corpo do texto para facilitar a leitura da tese, além de um resumo estendido em português.

ESTRESSE OXIDATIVO EM JUVENIS F0 DE *Litopenaeus setiferus* CRIADOS EM DIFERENTES SISTEMAS DE CULTIVO.

RESUMO:

Nos sistemas de cultivo, busca-se que a energia máxima seja canalizada para o crescimento dos animais a partir de um ambiente que favoreça que a quantidade de energia destinada às atividades de manutenção da vida seja a menor possível. No caso da carcinicultura, os altos volumes de água utilizados durante a fase de engorda dificultam o controle do ambiente aquático, razão pela qual os organismos enfrentam importantes mudanças ambientais durante o crescimento. No entanto, em condições selvagens, os juvenis de camarões peneídeos se desenvolvem em estuários, o que significa que eles são adaptados para compensar mudanças súbitas e frequentes na salinidade, temperatura e concentrações de oxigênio dissolvido. Sob tais condições flutuantes, as reservas de energia e a sustentação do consumo de oxigênio permitem que os organismos mantenham a atividade metabólica para compensar outras mudanças ambientais.

Os limites de tolerância de uma espécie diante uma determinada condição ambiental, não são fixos, uma vez que estão sujeitos à história de vida dos indivíduos, revelando assim uma profunda associação entre o grau de compensação homeostática e a condição fisiológica dos organismos. Nesse sentido, os marcadores da condição fisiológica imunológica são importantes para aprofundar a compreensão dos mecanismos compensatórios que os organismos implantam diante de mudanças ambientais, deficiências nutricionais e presença de patógenos.

Com o intuito de encontrar condições de cultivo adequadas para juvenis de *L. setiferus* têm sido realizados diferentes esforços de coleta de camarões adultos para formar um banco de reprodutores que permita contar com pós-larvas em quantidade e de qualidade para a produção industrial incluindo a própria conservação da espécie.

Assim, após trabalhar com camarões juvenis selvagens e concluir que o sistema BFT proporciona ligeiras vantagens sobre os animais cultivados, o presente trabalho objetivou avaliar o grau de estresse oxidativo em animais produzidos em cativeiro F0, a partir de reprodutores selvagens em diferentes condições de cultivo. Foram obtidas desovas e desenvolvido a larvicultura, quando se atingiu o estágio de PL 10, estas foram aclimatadas a alta (35 ups) e baixa salinidade (5.0 ups).

Utilizaram-se quatro tratamentos que foram implementados da seguinte maneira: água do mar com bioflocos (MBFT) e água do mar (MCW); água de baixa salinidade com bioflocos (LSBFT) e água de baixa salinidade (LSCW). Os tratamentos com bioflocos (BFT) foram realizados sem troca de água e com fertilização orgânica. Os tratamentos com água clara foram realizados com fluxo continuo para garantir no mínimo 100% de troca de água por dia. O estudo teve duração de 45 dias. A qualidade da água não apresentou diferenças significativas. Os valores de nitrito para o tratamento LSBFT se mantiveram controlados, provavelmente pelo fato da ter utilizado inóculo de BFT de outro tanque previamente preparado.

Os parâmetros zootécnicos, não mostraram diferenças significativas ($p > 0.05$). O incremento em peso, sobrevivência e o índice hepatosomático foram ligeiramente menores nos tratamentos com água clara. No que se refere à primeira linha de defesa do sistema imunológico, a contagem total de hemócitos (TCH) mostrou diferença significativa entre os tratamentos BFT e CW, apresentando maior número de hemócitos nos BFTs e, isso provavelmente sugere uma melhor condição imunológica.

A atividade antioxidante (CAT, SOD, GST), a concentração reduzida de glutationa e a peroxidação lipídica e proteica foram avaliadas no músculo dos camarões. Em geral, todos os tratamentos apresentaram alta atividade redox, embora não tenham sido encontradas diferenças significativas ($p<0,05$) entre a atividade oxidativa dos animais submetidos às diferentes condições de cultivo.

Os resultados sugerem que a condição de cultivo (sistema de criação e salinidade) não diferenciou os níveis de estresse. Todos os tratamentos apresentaram níveis elevados, provavelmente, esta seja uma condição típica dos animais em cativeiro, mas os maiores valores de oxidação de proteínas e lipídeos demonstraram maior dano celular nos tratamentos de água clara. Também foi verificado que o desempenho zootécnico ainda é difícil estimar o consumo de ração para esta espécie e o manejo desta em tanques experimentais. Isto pode se converter em mais uma variável que causa estresse oxidativo, devido ao fato de que a espécie não é domesticada, foi complicado avaliar o consumo real de ração. O tratamento MCW apresentou a pior relação entre o peso do animal e o peso do hepatopáncreas, sendo maior nos tratamentos BFT.

De maneira geral podemos concluir que após dois experimentos com juvenis que o fluxo contínuo de água tanto marinha como de baixa salinidade parece ser outro fator causante

de estresse oxidativo. Há uma marcada diferença positiva quando se usa o sistema BFT. Tanto no estado imunológico como no uso de energia, os animais dos tratamentos CW estão investindo uma maior energia para sobreviver, diferentemente dos animais no sistema BFT que tem um crescimento ligeiramente melhor. Entretanto, ainda faltam muitos estudos para fazer com esta espécie seja utilizada em cultivos com bioflocos.

Palavras-chave: bioflocos, resposta antioxidante, imunidade, baixa salinidade

OXIDATIVE STRESS IN EARLY F_0 JUVENILES OF *Litopenaeus setiferus* REARED IN DIFFERENT CULTURE SYSTEMS.

Valenzuela-Jiménez, M.[ab]; Durruty-Lagunes, C.[a]; Rodríguez-Fuentes, G.[c]; Aguilera-Rivera, D.[a];Cuzon, G.[a]; Wasielesky Jr. W.[b]; and Gaxiola G.*[a]

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ABSTRACT:

To determine the suitable culture conditions for juveniles of Atlantic white shrimp, *Litopenaeus setiferus*, produced in captivity (F_0), four treatments were designed: marine biofloc (MBFT), marine clear water (MCW), low salinity biofloc (LSBFT), and low salinity clear water (LSCW). After 45 days, the treatments in the clear water had the largest increase in weight and survival, as well as the shrimp with the smallest hepatosomatic index. The amount of total hemocytes, antioxidant activity, glutathione S-transferase, reduced glutathione concentration, and lipid and protein peroxidation was evaluated in the muscle of the shrimp. In general, all the treatments had a high redox activity; however, no significant differences (p0.05) were found between the oxidative activity in the animals subjected to the different culture conditions. The total count of hemocytes (TCH) showed a noticeable difference between the biofloc (BFT) and clear water (CW) treatments in both salinities, showing a greater number of hemocytes ($p < 0.05$), which implies a better immune condition, in the BFT's. The results suggest that different culture conditions (rearing system, salinity) did not produce different levels of stress, because in all the treatments this was high. However, both the growth and immunological condition of the shrimp reared in BFT were significantly improved.

Keywords: biofloc, antioxidant response, low salinity

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INTRODUCTION

The Atlantic white shrimp, *Litopenaeus setiferus*, is distributed from Southern New York, USA to the Yucatan Peninsula, which is on the continental shelf adjacent to the Laguna de Términos (Ciudad del Carmen, Campeche, Mexico) (FAO, 1980). *L. setiferus* is an economically important species in the coastal zone of the Gulf of Mexico. Commercial catches are directed towards adults at sea and juveniles in estuaries and coastal lagoons (Arreguín-Sánchez et al., 1997; Gracia and Vázquez, 1998; DOF, 2014). The white shrimp has been exposed to different environmental pressures, which include excessive overfishing, pollution in estuaries and coastal lagoons due to pesticides used in intensive-agriculture lands, and effects of the oil industry and marine operations developed along the Gulf of Mexico and American Atlantic coast (Rosas et al., 2004).

Several efforts have been made to culture this species (Hopkins et al., 1993; Sandifer et al., 1993, Samocha et al., 1998). While this research has provided important information, there are still many doubts about the details required for its intensive production and domestication, such as its reproduction, mass production, grow-out, and adequate environmental parameters, and therefore its culture yield is still lower than *L. vannamei.* Advances in technologies as recirculation systems or BFT are presented as having high potential as alternative for shrimp culture, even in low salinity water considering that it is a euryhaline species.

The BFT was developed for the maintenance of good water quality (Avnimelech, 1999, Emerenciano et al., 2012). It consists of the formation of microbial aggregates formed by bacteria, protozoa, and organic remains. BFT provides a source of food which is permanently available (Avnimelech, 2007). In addition, it increases the health status, through the stimulation of the immune system and antioxidant status, of shrimp (Ju et al., 2008; Xu and Pan, 2013; Cardona et al., 2015; Liu et al., 2017; Aguilera-Rivera et al., 2018). Furthermore, it contributes to a large part of the shrimp protein requirements and improves growth (Burford et al., 2004; Crab et al., 2010, Wasielesky et al., 2006; Xu et al., 2012).

A variety of environmental factors, such as the oxygen availability, salinity, or pH, can affect the physiology of aquatic organisms and produce stresses, affecting the

organism's health status (Castille and Lawrence, 1981; Charmantier, 1998; Laramore et al., 2001; Chang et al., 2017), which in turn can affect growth and survival.

Among the immune parameters, the most commonly used biomarkers of immunity are a respiratory burst, the prophenoloxidase cascade, or lysozyme activity. Antioxidant enzymes are directly involved in the neutralization of reactive oxygen species (ROS) and play a key role in the prevention of damage caused by oxidative stress. ROS have been identified as key initiators of tissue damage and can increase enzymatic activity, transcription signals, and gene expression. The antioxidant and immune systems are closely linked to the responses to pathogens and other stress-related problems that could lead to specific diseases. The measurement of the activity of antioxidant enzymes, has proven to be an ideal tool for determining the response of aquatic invertebrates to diverse stressors (Cardona et al., 2015; Duan et al., 2015; Yuhu et al., 2016; Zhao et al., 2016).

The aim of this study was to identify the effects of rearing system conditions (BFT and clear water) and salinity levels (low and high) on the zootechnical performance and antioxidant response of early juvenile *L. setiferus* reared in controlled conditions.

MATERIAL AND METHODS

The experiment was carried out at Unidad Multidisciplinaria de Docencia e Investigación (UMDI) - Facultad de Ciencias - Universidad Nacional Autónoma de México, Campus Sisal, located in the coastal region NE of the Yucatan state (21°9′55.22N, 90°1′54.93W) in Puerto de Abrigo, S/N en Sisal, Hunucmá (Yucatan, Mexico).

Experimental shrimps and acclimation

Post-larvae of *L. setiferus* (PL10) were reared at the UMDI hatchery. Subsequently, post-larvae were stocked in a nursery tank (100 PL m^{-2}) in clear water conditions. Shrimp (0.98 ± 0.6 g) were acclimated to the experimental conditions for five days to reduce the stress caused by handling. The shrimp were fed twice per day with a commercial feed containing 35% crude protein (Api-Camarón, Malta Cleyton, Culiacan,

Sinaloa, Mexico). The feeding rate was set at 8.0% of the total biomass of each experimental unit.

After the first acclimation phase, shrimp were separated into two groups for a second acclimation. These groups were: the marine seawater (35 ppt) and low salinity treatments (5 ppt). The salinities were increased or decreased across five days to get both the required salinity conditions. At the end of the salinity acclimation, the shrimp were distributed into 100 L fiberglass tanks at the rate of 25 shrimp per tank (250 shrimp m^{-3}). The initial individual wet weight was recorded, using an OHAUS balance (0.1 g of precision).

Experimental design

Four treatments were established for the experimental design: marine biofloc (MBFT), marine clear water (MCW), low salinity biofloc (LSBFT), and low salinity clear water (LSCW). Three replicates for every treatment were conducted at nearly 100 g biomass per m^3. The trial lasted 45 days.

For both BFT treatments, 50 L of biofloc inoculum (from the grow-out system) was added into the tanks, using a C:N ratio of 20:1 (Avnimelech, 1999). The tanks were then filled to full capacity (100 L) with filtered clear water. During the experimental phase, cane molasses was added as a source of organic carbon at a rate of 6.0 g of carbon (C) per gram of total ammonia nitrogen (TAN), until the ammonia reached 1.0 mg L^{-1}, as described in Avnimelech (2009), Ebeling et al. (2006), and Samocha et al. (2007).

At day 0, the salinity was adjusted to 35 and 5 ppt for the seawater and low salinity water, respectively. Both the seawater and low salinity water (from well water origin), were filtered using a sand filter. A commercial feed (35% crude protein, Malta Cleyton, Culiacan, Sinaloa, Mexico) was supplied twice a day at a rate of 3% biomass. The daily temperature, dissolved oxygen (DO), and pH were measured at 08:00, 16:00, 20:00, 24:00, and 04:00, using a multiparameter Hach™model SensION6-Hq40 (Loveland, Colorado, USA), which was calibrated for each salinity level. Salinity was measured daily with a Fisherbrand™ (Fisher Scientific Company Ottawa, Canada) self-compensated refractometer. Ammonia, nitrite, and nitrate concentrations were evaluated every third

day with a Hach™ colorimetric kit for diluted and full seawater (Loveland, Colorado, USA). For both the clear water treatments, a 35% water exchange was adjusted daily, whereas for both the biofloc treatments, the 2% of the water volume that was lost by evaporation was replaced.

Sampling

At the end of experiment, the shrimp at the intermolt stage (Corteel et al., 2012) were placed in freezing water at 5°C below the initial temperature, to decrease the metabolism of the organisms (Pascual *et al.*, 2003). They were then transported to the laboratory for analysis. A portion of the muscle tissue was collected and stored at -80°C for further analysis.

Zootechnical performance

At the end of the experiment, all the shrimp were counted (survival), individual live wet weight per tank was determined on an Ohaus balance (0.1 g readability).
The final weight (FW) was calculated
FW (g)= FW – IW
Where
FW=Final weight
IW= initial weight

The hepatosomatic index was determined according to the methodology of Molina (2000) with the following formula:
HSI = hepatopancreas weight / shrimp weight (100)

Biochemical analysis
Protein determination in shrimp muscle

Protein was determined by the Bradford assay (1976), adapted to a microplate method using a dye reagent concentrate (Bio-Rad, Philadelphia, PA, USA). Bovine serum albumin was used as a standard (EMD Biosciences, Inc., La Jolla, CA, USA). Absorbance was read at 595 nm.

Antioxidant activity

Superoxide dismutase (SOD) activity was quantified with a commercial kit (Sigma-Aldrich, St. Louis, MO, USA). One unit of SOD was defined as the number of enzymes required to inhibit the activity of xanthine to 50% in 20 min. Absorbance was measured at 450 nm. For catalase (CAT) activity, adapted to microplate was measured using the technique reported by Hadwan and Abed (2016). Samples were incubated in two 96-well plates with 100 µL of phosphate buffer (pH 7.4) or 100 µL of the same buffer with hydrogen peroxide (H_2O_2). Both microplates were incubated for 3 min and then the reaction was stopped by an addition of 100 µL of ammonium molybdate. The absorbance was measured at 450 nm. For both enzymes, results were expressed in U mg protein^{-1}.

Glutathione S-transferase and reduced glutathione concentration

Glutathione S-transferase (GST) activity was evaluated with a microplate method using 190 µL of sodium phosphate buffer, 200 mM of reduced glutathione, and 1-Chloro-2,4-dinitrobenzene 100 mM (final concentrations). Absorbance was read at 412 nm for 5 mins. The activity was calculated using the extinction coefficient E=9.6 mM^{-1} cm^{-1} (Contreras-Vergara et al., 2007). All results were expressed in nmol mg^{-1} protein min^{-1}.

Total glutathione (GSH) was measured with a Sigma-Aldrich Glutathione Assay Kit (CS0260). This kit utilizes an enzymatic recycling method with glutathione reductase (Baker et al., 1990). The sulfhydryl group of GSH reacts with Ellman's reagent and produces a yellow colored compound that is read at 405 nm.

Lipid and protein oxidation in shrimp muscle

Lipid peroxidation (LPO) was quantified according to the Fox method, using the PeroxiDetect© kit (Sigma-Aldrich, St. Louis, MO, USA). Extracted without centrifugation, it was homogenized with methanol at a ratio of 1:1 (v/v) and then centrifugated at an RCF of 10,000 × g for five min at 27°C. Then, 10 µL of the extract was placed in two 96-well plates and 160 µL of the working solution was added to incubate samples during 60 min. Absorbance was measured at 595 nm. A standard curve was prepared using serial dilutions with 129 µL of 1 M tert-butyl hydroperoxide (TBOOH) plus 871 µL of methanol. Results were expressed in nmol peroxide mL^{-1}. The

protein oxidation (PO) level was evaluated as previously described (Mesquita et al., 2014), where 100 µL of dinitrophenyl hydrazine (DNPH) plus 2 N HCl was combined with 100 µL of Tris buffer (pH 7.4) (blank) or 100 µL of homogenized solution without centrifuging, using Eppendorf© tubes. Tubes were incubated for 10 min and then, 50 µL of 6 M NaOH was added to the samples and blank. All tubes were centrifugated at an RCF of 10,000 × *g* for 5 min at 27°C. Finally, 150 µL of the samples and blank were added into a 96-well plate to read the absorbance at 450 nm. Results were expressed in nmol mg protein^{-1}.

Total count of hemocytes

For each shrimp, 100 µL of hemolymph was collected from the ventral sinus using a syringe, 30G × 13 mm, preloaded with heparin. The hemolymph from the animals in the intermolt stage were placed in an hematocytometer glass plate. The total hemocyte count (TCH) was calculated with a TC10 Automated Cell Counter (Bio-Rad, Canada).

Statistical analysis

The normality of the data was verified by the Kolmogorov-Smirnov test and the homoscedasticity with the Levene Test. Subsequently, an ANOVA was performed to confirm the existence of significant differences between the treatments ($p < 0.05$). When significant differences were found, a Tukey post-hoc analysis was performed. For all statistical analyses the software package STATISTICA v7 for Windows (StatSoft Inc., 2011) was used.

RESULTS

Water quality

All water quality variables were maintained within the recommended levels for shrimp culture throughout the experiment (Van Wyk and Scarpa, 1999; Laramore et al., 2001; Rozas and Minello, 2011). Both treatments with clear water (CW) were kept without nitrogenous residues. Those of biofloc (BFT) maintained with cane molasses as source of organic carbon, without water exchange (Table 1).

Table 1. Mean value ± SD of the physicochemical parameters of the water after 45 days, for *L. setiferus* F0 early juveniles in different culture conditions. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

TREATMENT	OD mg/L	Temperature °C	pH	Salinity ppt	Ammonia (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)
MBFT	6.02 ± 0.43	28.47 ± 0.14	8.90 ± 0.30	33.70 ± 0.30	0.21 ± 0.18	0.75 ± 1.31	96.23 ± 11.17
MCW	6.12 ± 0.23	28.46 ± 0.17	8.74 ± 0.13	35.51 ± 0.36	0.24 ± 0.0	0.21 ± 0.06	0.0 ± 0.0
LSBFT	6.84 ± 1.63	28.80 ± 0.21	9.14 ± 0.24	3.44 ± 0.85	0.37 ± 0.35	0.41 ± 1.17	72.25 ± 13.6
LSCW	5.89 ± 0.34	28.90 ± 0.28	8.84 ± 0.15	3.12 ± 0.0	0.04 ± 0.06	0.04 ± 0.16	0.0 ± 0.0

Zootechnical performance

The results of the zootechnical performance of *L. setiferus* juveniles after 45 days of the experiment are shown in Table 2. The final weight and weight gain were significantly higher in the BFT treatments than in the CW treatments ($p<0.05$). The relationship between the size of the hepatopancreas and body (HI) was higher in shrimp grown in BFT than in CW ($p<0.05$). In relation to survival, only the LSCW treatment was significantly low ($p<0.05$).

Table 2. Mean value ± SD of the zootechnical data after 45 days, for *L. setiferus* F0 early juveniles in different culture conditions. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

Treatment	Initial weight (IW) (g)	Final weight (FW) (g)	Weight gain (FW-IW) (g)	Hepatosomatic Index (HI)	Survival (%)
MBFT	0.98 ± 0.6	1.70 ± 0.43	0.72 ± 0.23^{a}	5.14 ± 0.83^{a}	84± 16.0^{a}
MCW	0.98 ± 0.6	1.64 ± 0.51	0.66 ± 0.31^{b}	4.13 ± 0.69^{a}	89.3±8.3^{a}
LSBFT	0.98 ± 0.6	1.69 ± 0.70	0.71 ± 0.28^{a}	5.28 ± 0.87^{b}	71± 8.49^{b}
LSCW	0.98 ± 0.6	1.57 ± 0.41	0.59 ± 0.21^{b}	5.22 ± 0.91^{b}	68± 0^{b}

Antioxidant activity

The activities of SOD and CAT, measured in the muscle of *L. setiferus*, are shown in Figure 1. Both enzymes did not present significant differences ($p>0.05$).

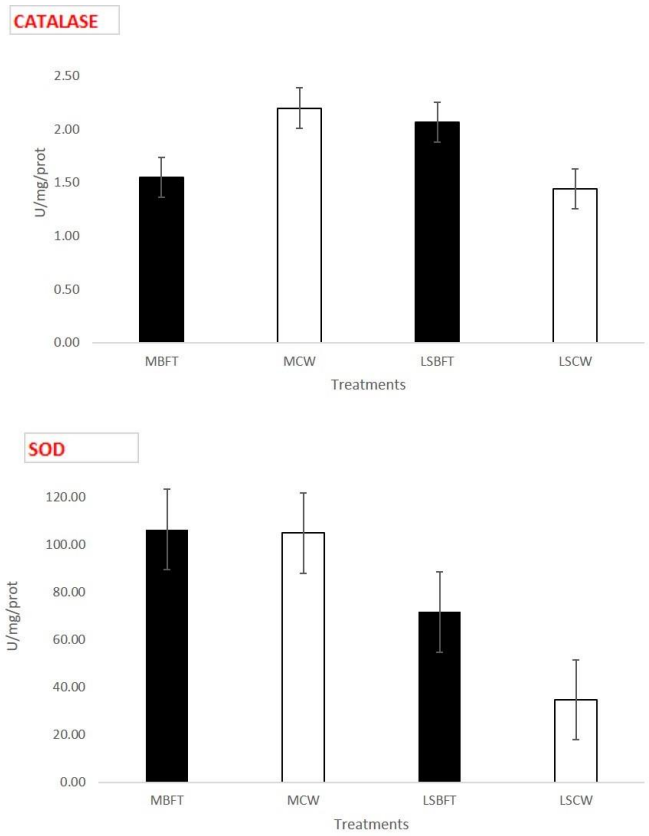

Figure 1. Oxidative enzyme activity of Catalase (CAT) and sodium dismutase (SOD) in the muscle of *L. setiferus* F_0 early juveniles in different culture conditions after 45 days. Mean values and SE. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

Glutathione S-transferase activity

The GST and GSH enzymes in the muscle showed high levels of activity. However, they did not show significant differences ($p>0.05$) between the treatments (Fig. 2).

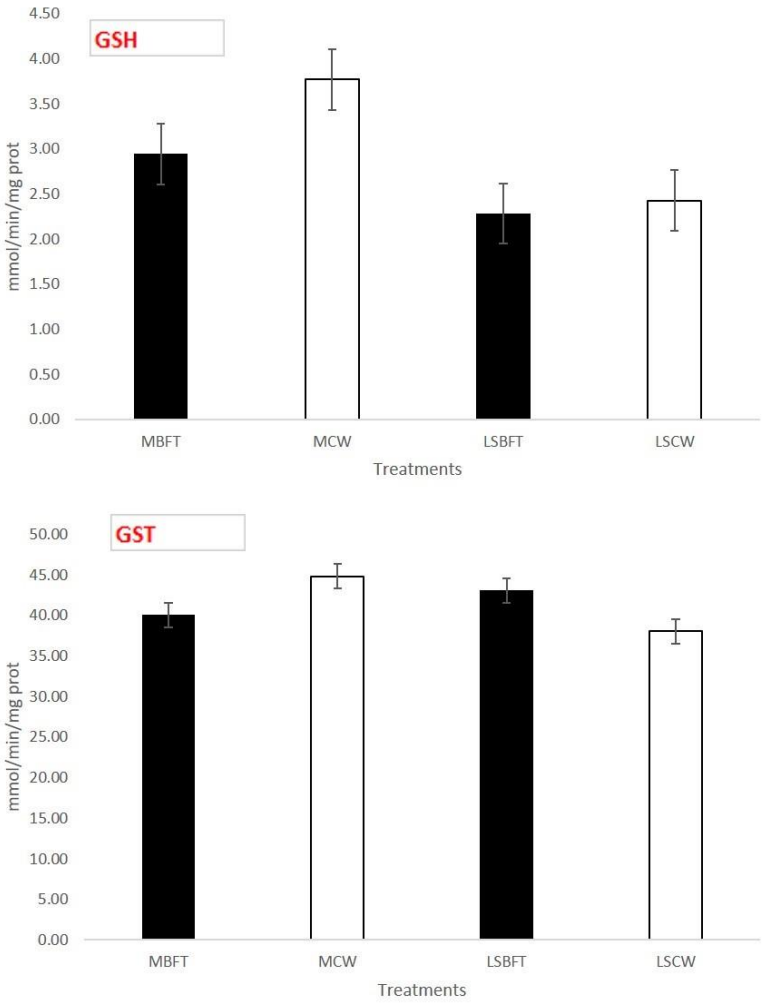

Figure 2. Mean values and SE for the total glutathione (GSH) and Glutathione S-transferase (GST) activity in the muscle of *L. setiferus* F_0 early juveniles in different culture conditions after 45 days. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

Lipid and protein oxidation in shrimp muscle

The LPO and PO did not show significant differences ($p>0.05$) between the treatments (Figure 3).

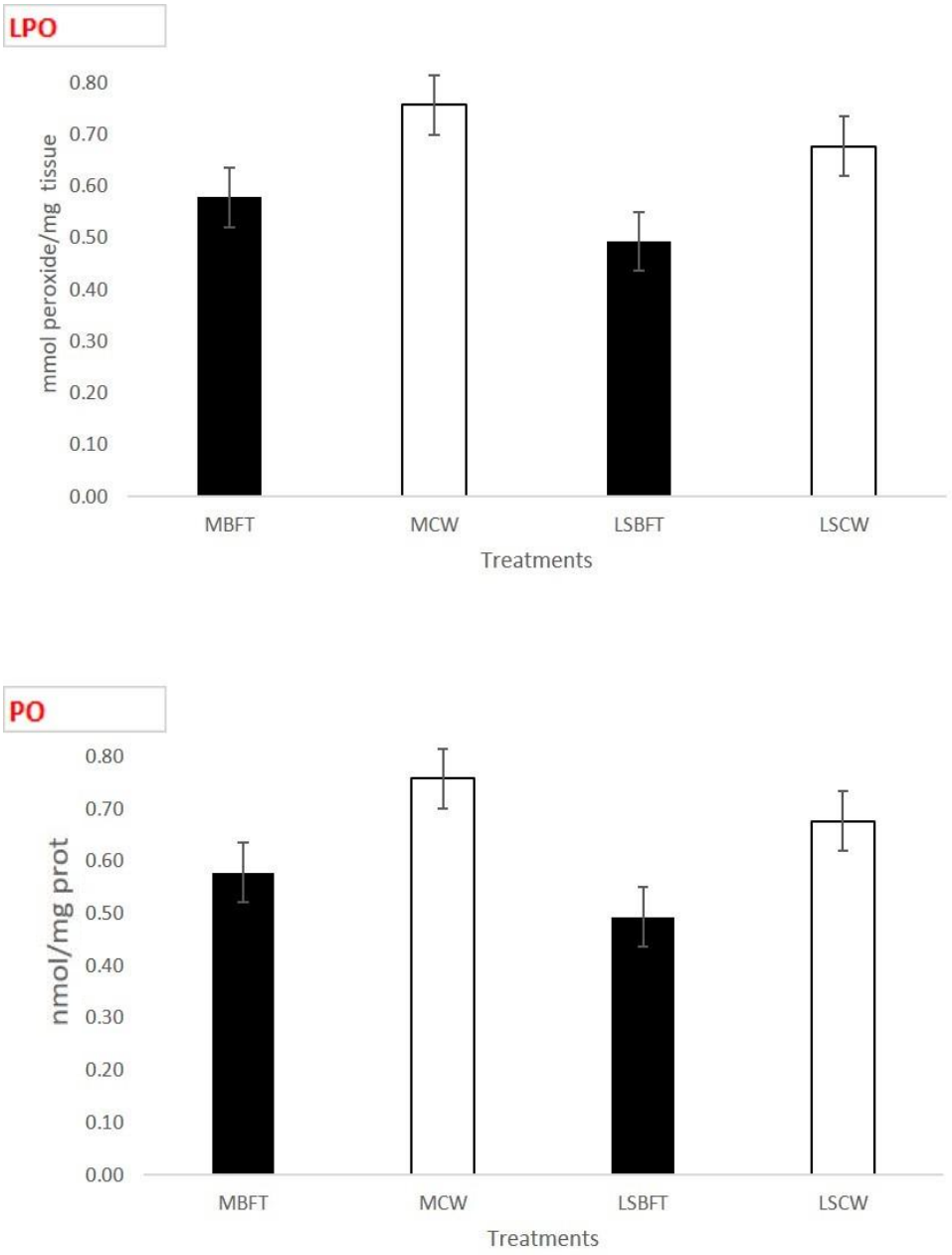

Figure 3. Mean values and SE for the Lipid peroxidation (LPO) and protein oxidation (PO) in the muscle of *L. setiferus* F_0 early juveniles in different culture conditions after 45 days. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

Immune response

The TCH showed a significant difference between the BFT and CW treatments, with a greater number of hemocytes ($p < 0.05$) in BFT either low or high salinity (Figure 4).

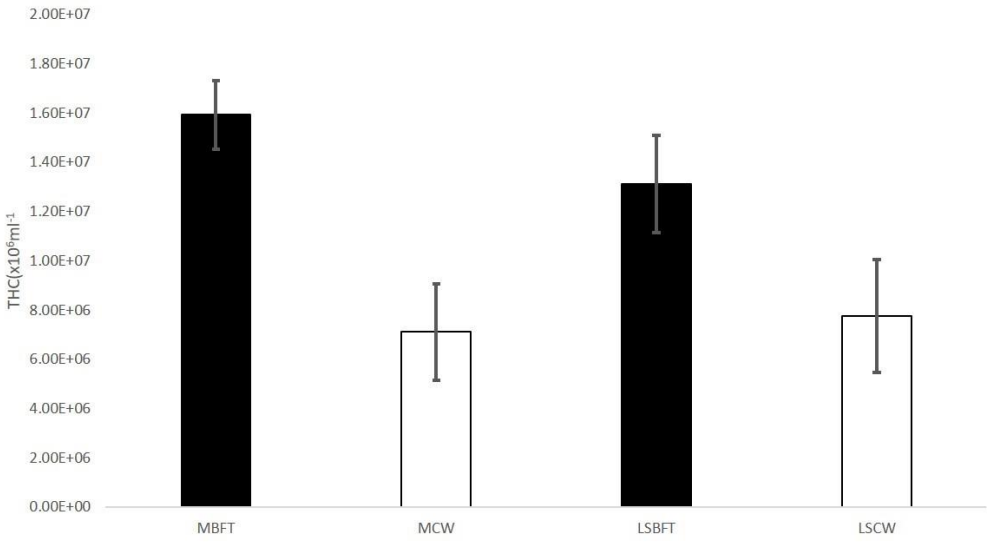

Fig 4. Mean values and SE for Total Count of Hemocytes (TCH) for *L. setiferus* F_0 early juveniles in different culture conditions after 45 days. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

DISCUSSION

Water quality

In super-intensive culture systems, such as BFT, water quality experiences constant variations relating to the performance of macro- and micro-organisms. These oscillations increase if you also work with low salinity conditions. For *L. setiferus* the intensive cultivation conditions are novel as it is not a domesticated species. In the present work, the physicochemical parameters were maintained within the recommended ranges for shrimp, even in the BFT (Van Wyk and Scarpa, 1999). The permanent flow of water in the CW treatments seems to have placed all the animals in environmental stress conditions. That is, there was stress due to a continuous flow of water and high nitrite levels, particularly in treatments with low salinity.

Zootechnical performance

The growth was better in BFT treatments, with results like those obtained by Rosas et al. (2001). Additionally, our results improved upon those obtained by Palomino et al. (2001). Both studies carried out their research in the same geographical region as the present work. Several authors have obtained very similar growth rates. However, it continues to be a very low weekly rate if we compare it with *L. vannamei*. This could be associated with the design of commercial diets that are focused on the production of *L. vannamei*. According to Rosas et al. (2001), the growth of *L. setiferus* improves with a lower protein/energy ratio, considering the ability of *L. setiferus* to metabolize carbohydrates before proteins. Commercial diets usually contain a high protein/energy ratio, considering the ability of *L. vannamei* to assimilate proteins first. Thus, it is necessary to work on the elaboration of a specific diet for *L. setiferus* to improve growth, which could even be a low-cost diet considering the protein contribution of BFT. The lowest survival was in the LSCW treatment, where the highest antioxidant activity was found. According to the TCH data this treatment had an exhausted immune system, which could explain the lower survival.

Antioxidant activity

This same condition is reflected in the results of the activity of antioxidant enzymes, which exhibit high levels. Generally, the oxidant capability of an organism under certain conditions can reflect its health status. Therefore, an enhanced antioxidant status can facilitate shrimp immune defense functions (Wu-Jie and Lu-Qing, 2013).

The increase in the levels of antioxidant activity in the cells is related to a rapid detoxifying response; thus, reflecting the importance of the SOD and CAT enzymes for removing the excess of ROS from the cells. In Figure 1 both enzymes, superoxide dismutase and catalase, showed greater activity in the MCW treatment, followed by the BFT treatments. In the MBFT treatment there was also high activity, which could be in relation to the high oxidative and immune activity in BFT treatments, caused by the improvement and stimulation of the immune system by the biofloc (Luschak, 2011; Xu and Pan, 2013). At a low salinity, *L. vannamei* usually exhibits slow growth, compared to shrimp in high salinity levels, and are more sensitive to water toxicity that includes ammonia, nitrite, and some water pollutants, including heavy metals and pesticides (Qui et al., 2011). Shrimp reared at low salinity had a lower immunity than those from high salinity and were more susceptible to any stress factor. In low salinity water increase the shrimp's oxygen consumption, due to hyper osmoregulation; therefore, more dietary energy is needed to offset the extra energy cost for osmoregulation, to improve the growth performance (Li et al., 2017).

At a low salinity, organisms can increase the production of free radicals. The increase in the activity of SOD and CAT can increase the elimination of the free radicals and maintain the health of shrimp (Li et al., 2008). In the LSCW treatment, the activity of both enzymes was low, being significantly lowest for SOD (Figure 1) indicating an increase in the production of the superoxide anion ($O^{\cdot 2-}$) radical (Martin et al., 2012) or a depletion of the capacity of hemocytes to counteract it (Liu and Chen, 2004).

In the same way, GSH and GST both exhibited high levels (Figure 2). Neither GST or GSH showed significant differences ($p < 0.05$). However, a greater detoxifying activity probably indicates the enhancement of the immune system, activated probably by the system constantly changing water. GSH is the most abundant non-enzymatic antioxidant present in mammalian cells. Additionally, it is the main intracellular defense

mechanism against oxidative stress. The enzyme GST participates in the control of membrane permeability, sustains the redox balance, and protects against oxidative stress (Ren et al., 2009; Zhou et al., 2009; Xia and Wu, 2018). GSH is a substrate that maintains the proper level in the production of free radicals (Xu et al., 2012). A great proportion of the activity of GST, normally plays an important role in protecting the cells against the peroxidation system and maintaining the redox state of the cell. However, in conditions of high oxidative stress GST may be able to produce an increase of apoptotic bodies. On the other hand, the thiol group of GSH can combine with ROS and donate a hydrogen atom, thereby decreasing the number of oxygen free radicals, accelerating free radical scavenging, and reducing the occurrence of lipid peroxidation (Figure 3) (Xia and Wu, 2018).

The carboxylation of proteins and lipoperoxidation (Figure 3) indicates high levels of ROS and high levels of toxicity and deterioration of proteins and lipids, however, there were no significant differences, indicating probably that all experimental conditions are stress factors.

Excessive ROS will lead to lipid peroxidation and the accumulation of malondialdehyde (MDA), the main component of lipid peroxides, which has strong biotoxicity and damages cell structure and function (Song et al., 2015, Xu and Wu, 2018). Da Silva Martins (2015) shows that altered antioxidant responses and ameliorated lipid peroxidation in shrimps are maintained in BFT systems. This aligns with the results of the present study, where high activities of both GSH and GST were found, probably having to do with the lower activity of peroxidation in the treatments with BFT.

Immune response

The hemocyte count in the hemolymph has been considered a functional indicator of immune capability (Muñoz et al., 2000; Rodríguez and Le Moullac, 2000). Biofloc functions as an immunostimulant, enhancing the shrimp by innating immunity, thereby improving their resistance to pathogens (Kim et al., 2015). The TCH (Figure 4) showed significant differences between the biofloc and clear water treatments, corroborating the results of the antioxidant activity, confirming that biofloc functions as a stimulator of immunological activity.

CONCLUSION

The culture conditions did not differentiate in the levels of oxidative stress, as all the treatments had high stress levels. However, in the BFT treatments, the growth and immunological condition improved significantly. *L. setiferus* is a non-domesticated species and there is still more work to be done with the species in captivity to determine its true crop potential. In addition, in low salinity, it seems to contribute to diminish the effect of ionic imbalance by the results of survival and gained weight. In addition, in low salinity, it seems to contribute to diminish the effect of ionic imbalance by the results of survival and gained weight.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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CAPITULO IV

ZOOTECHNICAL AND PHYSIOLOGICAL PERFORMANCE OF THE WHITE SHRIMP OF THE NORTH ATLANTIC L*itopenaeus setiferus* REARED IN TO THE BFT SYSTEM IN MARINE AND LOW SALINITY WATER IN OUTDOOR TANKS.

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Observação: Tabelas e figuras foram incluídas no corpo do texto para facilitar a leitura da tese, além de um resumo estendido em português.

DESEMPENHO ZOOTÉCNICO E FISIOLÓGICO DO CAMARÃO BRANCO DO ATLÂNTICO NORTE *Litopenaeus setiferus* CRIADO NO SISTEMA BFT COM ÁGUA MARINHA E ÁGUA DE BAIXA SALINIDADE EM TANQUES EXTERNOS

RESUMO:

Há um interesse considerável no cultivo do camarão branco do Atlântico Norte *Litopenaeus setiferus* do na área costeira do Golfo do México, com água marinha, mas também no interior com água de baixa salinidade. Isto se deve ao fato de que existem riscos de usar uma espécie exótica, como *L. vannamei* e toda sua história de problemas com doenças.

Durante a década de 1990 foram realizados muitos trabalhos sobre como conseguir a produção de *L. setiferus* de 6-8 gramas, principalmente pela grande demanda da indústria da pescaria esportiva. Grandes avanços aconteceram, mas ao final o cultivo foi abandonado porque esta espécie apresentava uma taxa baixa de crescimento. Além disso, a espécie apresentava baixas sobrevivências e conversão alimentar ineficiente.

A espécie apresenta também algumas vantagens importantes, por exemplo, parece ser menos susceptível de ser atacada por doenças. Além disso, a produção de larvas está relativamente padronizada. Então, estas características fazem da espécie uma boa probabilidade de cultivo, porém, é necessário incrementar as pesquisas, na situação atual aonde o *L. vannamei* é cada vez mais atacado pelas doenças e a constante aparição de *L. vannamei* e do *P. monodon* em lugares aonde são considerados espécies exóticas. Ou seja, é necessário diversificar a Carcinicultura e desenvolver rações específicas para as exigências nutricionais das espécies nativas de importância comercial. Mas além destas questões, é necessário utilizar outros critérios de avaliação do desempenho dos camarões em cultivo. O estado fisiológico, imunológico e nutricional, assim como o grau de estresse ambiental no cultivo são indicadores que podem ajudar a fortalecer o desenvolvimento de novos pacotes tecnológicos específicos para espécies nativas.

Com o intuito de encontrar a base para um protocolo de manejo para produção intensiva de *L. setiferus* em um sistema com bioflocos, seja água marinha e de baixa salinidade, foram avaliados o desempenho zootécnico (Crescimento, sobrevivência, fator de

conversão alimentar e o índice hepatosomático) e fisiológico Estresse, oxidativo, estado imunológico em tanques circulares externos.

Dois tratamentos em triplicata, água do mar com bioflocos (MBFT) e água de baixa salinidade com bioflocos (LSBFT). O experimento teve a duração de 90 dias e foram avaliados indicadores de atividade antioxidante, as enzimas Super. óxido Dismutase (SOD), Catalase (CAT) e Glutamato-S-Transferase GST, a peroxidação de lipídeos (LPO) e a oxidação de proteínas (PO). Hemócitos totais na hemolinfa (THC) foram contados e o consumo de oxigênio foi medido em ambos os tratamentos com um oxímetro digital de 10 canais conectado a câmeras respirométricas de acrílico.

O desempenho zootécnico e a qualidade da água foram monitorados. Todos os dias os parâmetros físico-químicos e a alimentação foram registrados cinco vezes. Os resultados mostraram que a taxa de crescimento permaneceu baixa, a sobrevivência e o fator de conversão alimentar foram semelhantes em ambos os tratamentos. Os bioflocos foram inoculados de outros tanques de ambas as salinidades e logo foi mantido em relação às medições dos compostos nitrogenados utilizando melaço de cana.

De maneira geral podemos concluir que, teve atividade antioxidante e as enzimas mostraram atividade. No entanto, os baixos níveis destas, podem indicar que os bioflocos oferecem condições que causam baixos níveis de estresse. Assim, o grande número de hemócitos na hemolinfa, parece manifestar um estado de alerta imunológico permanente. Os resultados do consumo de oxigênio dos camarões nos dois tratamentos permitem concluir que os camarões estabilizaram seu consumo de oxigênio em poucas horas após serem colocados nas câmaras respirométricas. No início, os camarões apresentaram maior consumo. Ao final, reduziram o consumo de oxigênio, manifestando uma adaptação ao sistema em quanto ao consumo de energia. Os dados comprovam que é necessário continuar trabalhando com o manejo do cultivo, particularmente com o balanço iônico, por exemplo, em água com baixa salinidade. Ainda é necessário encontrar a combinação adequada dos íons para esta espécie e o manejo adequado dos compostos nitrogenados nos bioflocos em água marinha. Sabe-se que o nitrito é muito perigoso sobre tudo em baixa salinidade, mas também é importante o controle, da amônia, para aumentar a sobrevivência da espécie em sistema BFT.

Palavras chave: Bioflocos, atividade antioxidante, imunologia, consumo de oxigênio.

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ABSTRACT:

There is a considerable interest in the culture of the North Atlantic white shrimp *Litopenaeus setiferus* in the coastal area with marine water, but also inland with low-salinity water in the Gulf of México region, due to the risks for using an exotic species, such as *L. vannamei* and all its history of problems with diseases. With the intention of finding the basis for a management protocol for the intensive production of Litopenaeus setiferus in a BFT system with marine and low salinity water in outdoor tanks, were evaluated the zootechnical and physiological performance of this native species, which tolerates low salinities and is resistant to different extreme environmental conditions.

Two triplicate treatments, marine water with biofloc (MBFT) and low salinity water with biofloc (LSBFT) after 90 days were evaluated indicators of antioxidant activity, SOD, CAT and GST, peroxidation of lipids and carbonylated proteins. Total hemocytes were counted in the hemolymph and the oxygen consumption was measured for both treatments. Zootechnical performance and water quality were monitored. The growth rate remains low, the survival and the feed conversion factor were similar in both treatments. We can conclude then that, the low levels of antioxidant activity, the high number of hemocytes and the oxygen consumption, reflect an adaptation to the system and an excellent health condition, probably we must continue working a lot with the management of the crop, particularly, ion balance for example in low salinity water and adequate handling of biofloc and nitrogen compounds to increase survival in seawater.

Key Words: Biofloc, Immunology, antioxidant shrimp activity

INTRODUCTION:

From the work carried out with intensive culture to produce the Northern Atlantic white shrimp *Litopenaeus setiferus* in the Southern United States (Browdy et al., 1991, Sandifer et al., 1993, Samocha et al., 1998, Chapman e Browdy, 2004, Gandy, 2007) and southern Gulf of Mexico (Valenzuela et al., 2002, Rosas et al., 2004), it is known that the growth rates are lower than those of *L. vannamei*, and also grows more slowly, although it can reach the same weight as *L. vannamei* in a larger time. However, survivals in semi-intensive culture are higher in all published studies and there is also a great interest on the part of shrimp producers.

It is known that commercially available feeds are designed for Pacific white shrimp *L. vannamei* and *L. setiferus* feed conversions are still inefficient. Then, research has been done that determine that the energy / protein ratios are not the same in both species. *L. vannamei* assimilates first the proteins and *L. setiferus* the carbohydrates, this is vitally important for the growth and efficiency of consumption (Rosas et al., 2001, Guzmán et al., 2001),

The growth rate is reduced if the energy demand of increased metabolic rate exceeds the gain from increased food consumption (Abdelrahman, 2019). Researches are still lacking on the development of specific feeds for other species to allow the diversification of shrimp farming. Environments such as the system with biofloc or BFT, could maintain among other things, great amount of live food, mainly bacteria for improving the quality of the water, but overall to improve the nutritional and immunological condition, with the probiotic bacteria presence (Ekasari et al., 2014, Aguilera et al., 2018).

Moreover, *L. setiferus* is a native species of the North Atlantic, which tolerates low salinities and is resistant to different extreme environmental conditions (Laramore et al., 2001). There is considerable interest in the culture of *L. setiferus* in the coastal area with marine water, but also inland with low salinity waters near Gulf of México region. This is due to the risks represented in the coastal areas of Gulf of México using an exotic species, such as *L. vannamei* and all its history of problems with diseases.

Currently, in the Yucatan Peninsula, there is infrastructure for aquaculture, however, a technological package for this species, that satisfies the needs of the producers to have a hatchery where they can obtain post larvae of quality to obtain high rates of survival and a growth rate that makes it sustainable, has not been developed. As well as adequate technical assistance during breeding time. On the other hand, the shrimp production is the result of high survival and good growth rates. Although ponds were stocked with *L. setiferus* in the last few years, at similar rates and managed by similar procedures, shrimp survival varied greatly among ponds (Abdelrahman et al., 2019). One of the immediate characteristics of evaluation of the crop is the zootechnical performance. However, it is important to evaluate some other indicators that allow us to know if the animals are well in the ponds and if they are, that allows us to increase stocking densities, obtaining thus a greater quantity of biomass of shrimps of average size.

Many factors affect the shrimp growth, like other aquaculture species, including water temperature, water quality, feeding rates, diet composition, weight, stocking density and other variables. In general, the rate of physiological processes increases as the environmental factors are modified. Characteristics of the environmental culture media like adverse temperature, salinity and dissolved oxygen concentration are environmental stressors known to suppress crustacean immune responses. It has been demonstrated that the knowledge of the immunological processes associated with the physiological biochemistry helps to evaluate the state of health of the shrimp (Bachére et al., 1995; Le Moullac et al., 1998, Kim et al., 2015). Oxygen consumption reflects the amount of energy used for metabolism and to maintain homeostasis (Rosas et al., 1999, Lignot et al., 2000). The respiratory rate has been widely studied in crustaceans with respect to salinity, temperature, dissolved oxygen, quantity and quality of food, etc., since this is a good indicator of the physiological state of the animals (Chen and Lin, 1998; Rosas et al., 2000). Likewise, the quantity and quality of hemocytes allows establishing a part of the state of health of the shrimp, since they are considered as the first line of defense, since they participate directly in processes of recognition, processing and amplification of the immune response (Destoumieux et al., 2000, Johansson et al., 2000, Rodríguez and Le Moullac, 2000). At the same time antioxidant enzymatic activity contributes to the quality and quantity of assimilation of available food (Molina et al., 2000; Xu et al., 2013).

Evaluating this type of variables allows understanding the mechanisms that animals use to adapt to the culture medium and how this information can be used to establish a suitable proposal for the culture of a species. For example, some cellular defense mechanisms in crustaceans depend on the controlled production of free radicals during phagocytosis and encapsulation (Smith et al., 2003). Due to the changes in environmental conditions, the aerobic metabolism of crustaceans generates Oxygen Reactive Substances (ROS). A certain concentration of ROS is necessary for the defense against microbial infections. However, the production of ROS and its residues can result in severe damage in cells. To maintain balance, the ROS are eliminated by the antioxidant defense system which includes, for example, superoxide dismutase (SOD), catalase (CAT) and other enzymes associated with cell detoxification, such as glutathione-S-transferase (GST) (Muñoz et al., 2000, Ren et al., 2009, Quiu et al., 2011, Zhou et al., 2009, Song et al., 2015).

Therefore, the present study intended to evaluate the zootechnical and physiological performance of the North Atlantic white shrimp *L. setiferus* in a BFT system with marine and low salinity water in outdoor tanks. With the intention of finding the basis for a management protocol for the intensive production of this species

MATERIAL AND METHODS:

The experiment was carried out at Unidad Multidisciplinaria de Docencia e Investigación (UMDI) - Facultad de Ciencias - Universidad Nacional Autónoma de México, Campus Sisal, located in the coastal region NE of the Yucatan state (21°9′55.22N, 90°1′54.93W) in Puerto de Abrigo, S/N in Sisal, Hunucmá (Yucatan, Mexico).

Experimental shrimps and acclimation

Post-larvae of *L. setiferus* (PL10) were reared at the UMDI hatchery. Subsequently, post-larvae were stocked in a nursery tank 20 m^3 (250 PL m^{-3}) in clear water conditions, without water exchange, controlling ammonia concentrations with molasses (Avnimelech, 1999, Ebeling, 2006). The shrimp were fed five times per day with a commercial feed containing 35% crude protein (ApiCamarón, Malta Cleyton,

Culiacan, Sinaloa, Mexico). The feeding rate was set at 8.0% of the total biomass of each experimental unit per day.

After the first acclimation phase, shrimp were separated into two groups for a second acclimation. These groups were: the marine seawater (35 ppt) and low salinity treatments (5 ppt). The salinities were increased or decreased across five days to get both the required salinity conditions. At the end of the salinity acclimation, the shrimp were distributed into six 20 m^3 fiberglass tanks at the rate of 3,000 shrimp per tank (150 shrimp m^{-3}). The initial individual wet weight was recorded, using an OHAUS balance (0.1 g of precision). Juvenile shrimp (0.98 ± 0.6 g) were acclimated to the experimental conditions for five days to reduce the stress caused by handling.

Experimental design

Two treatments were established for the experimental design: marine biofloc (MBFT –salinity 35) and low salinity biofloc (LSBFT – salinity 5). Three replicates for every treatment were conducted at nearly 300 g biomass per m^3. The trial lasted 90 days.

For both BFT treatments, 1000 L of biofloc inoculum (from the grow-out system) was added into the tanks, using a C:N ratio of 20:1 (Avnimelech, 1999). The tanks were then filled to full capacity with filtered clear water. During the experimental phase, sugar cane molasses was added as a source of organic carbon at a rate of 6.0 g of carbon (C) per gram of total ammonia nitrogen (TAN), when ammonia reached 1.0 mg L^{-1}, as described in Avnimelech (2009), Ebeling et al. (2006), and Samocha et al. (2007).

Sampling

At the end of the experiment, the shrimp were caught and placed in water at a temperature 5°C lower than that of the culture tank to reduce the metabolism of the shrimp. (Pascual et al., 2003). Shrimp were then transported to the laboratory for analysis. A portion of the muscle tissue was collected and stored at -80°C freezer for further analysis.

Quality water

At day 0, the salinity was adjusted to 35 and 5 ppt for the seawater and low salinity water, respectively. Both the seawater and low salinity water (from well water source), were filtered using a sand filter. The daily temperature, dissolved oxygen (DO), and pH were measured at 08:00, 16:00, 20:00, 24:00, and 04:00, using a multiparameter Hach™model SensION6-Hq40 (Loveland, Colorado, USA), which was calibrated for each salinity level. Salinity was measured daily with a Fisherbrand™ (Fisher Scientific Company Ottawa, Canada) self-compensated refractometer. Water samples for the ammonia and nitrite analyses were collected every three days. The concentrations of ammonia and nitrite were determined according to the methodologies described in UNESCO (1983) and APHA (2012). Nitrate concentrations were evaluated every week with a Hach™ colorimetric kit for diluted and full seawater (Loveland, Colorado, USA). For both the biofloc treatments, the 2.0% of the water volume that was lost by evaporation was replaced with freshwater.

Zootechnical performance

Weekly samplings were taken from each tank, to record the increase in weight and adjust the amount of food supplied. At the end of the experiment, all the shrimp were counted (survival), individual live wet weight per tank was determined on an Ohaus balance (0.1 g readability).

The final weight (FW) was calculated
FW (g)= FW – IW
Where
FW=Final weight
IW= initial weight

The hepatosomatic index was determined according to the methodology of Molina et al., (2000) with the following formula:

HSI = hepatopancreas weight / shrimp weight (100)

A commercial feed (35% crude protein, Malta Clayton, Culiacan, Sinaloa, Mexico) feeding was offered 5 times a day at a rate of 3.0% biomass.

Biochemical analysis

Protein determination in shrimp muscle

Protein was determined by the Bradford assay (1976), adapted to a microplate method using a dye reagent concentrate (Bio-Rad, Philadelphia, PA, USA). Bovine serum albumin was used as a standard (EMD Biosciences, Inc., La Jolla, CA, USA). Absorbance was read at 595 nm.

Antioxidant activity

Superoxide dismutase (SOD) activity was quantified with a commercial kit (Sigma-Aldrich, St. Louis, MO, USA). One unit of SOD was defined as the number of enzymes required to inhibit the activity of xanthine to 50% in 20 min. Absorbance was measured at 450 nm. For catalase (CAT) activity, adapted to microplate was measured using the technique reported by Hadwan and Abed (2016). Samples were incubated in two 96-well plates with 100 µL of phosphate buffer (pH 7.4) or 100 µL of the same buffer with hydrogen peroxide (H_2O_2). Both microplates were incubated for three minutes and then the reaction was stopped by an addition of 100 µL of ammonium molybdate. The absorbance was measured at 450 nm. For both enzymes, results were expressed in U mg protein^{-1}.

Glutathione S-transferase (GST) activity was evaluated with a microplate method using 190 µL of sodium phosphate buffer, 200 mM of reduced glutathione, and 1-Chloro-2,4-dinitrobenzene 100 mM (final concentrations). Absorbance was read at 412 nm for 5 mins. The activity was calculated using the extinction coefficient E=9.6 mM^{-1} cm^{-1} (Contreras-Vergara et al., 2007). All results were expressed in nmol mg^{-1} protein min^{-1}.

Lipid and protein oxidation in shrimp muscle

Lipid peroxidation (LPO) was quantified according to the Fox method, using the PeroxiDetect© kit (Sigma-Aldrich, St. Louis, MO, USA). Extracted without centrifugation, it was homogenized with methanol at a ratio of 1:1 (v/v) and then centrifugated at an RCF of 10,000 × g for five minutes at 27 °C. Then, 10 µL of the extract was placed in two 96-well plates and 160 µL of the working solution was added to incubate samples during 60 min. Absorbance was measured at 595 nm. A standard curve was prepared using serial dilutions with 129 µL of 1 M tert-butyl hydroperoxide (TBOOH) plus 871 µL of methanol. Results were expressed in nmol peroxide mL^{-1}. The protein oxidation (PO) level was evaluated as previously described (Mesquita et al., 2014), where 100 µL of dinitrophenyl hydrazine (DNPH) plus 2 N HCl was combined with 100 µL of Tris buffer (pH 7.4) (blank) or 100 µL of homogenized solution without centrifuging, using Eppendorf© tubes. Tubes were incubated for 10 min and then, 50 µL of 6 M NaOH was added to the samples and blank. All tubes were centrifugated at an RCF of 10,000 × *g* for 5 min at 27 °C. Finally, 150 µL of the samples and blank were added into a 96-well plate to read the absorbance at 450 nm. Results were expressed in nmol mg $protein^{-1}$.

Total count of hemocytes

For each shrimp, 100 µL of hemolymph was collected from the ventral sinus using a syringe, 30G × 13 mm, preloaded with heparin. The hemolymph from the animals in the intermolt stage were placed in an hematocytometer glass plate. The total hemocyte count (TCH) was calculated with a TC10 Automated Cell Counter (Bio-Rad, Canada).

Oxygen consumption

A Precision Sensing (Pre Sens™) OXY 10 Model 10-Channel (PreSens Precision Sensing GmbH, Regensburg, Germany) Digital Optical Fiber Oximetry was used with optical oxygen sensors, connected through an interface to a computer with OXY 10 Software. Taking the account that routine metabolism (RM) defines as the energy invested in spontaneous activity in the absence of food, the animals remained fasted for 24 hours before taking the measurements, still considering that the BFT treatment shrimps always have availability of live food the ration was suspended 24 hours before the

evaluation. It was used nine shrimp per treatment for each sampling. All measurements were twice repeated. The shrimps were individually placed in nine acrylic glass chambers filled with water from the respective treatment and connected to the recirculation system. The chambers were sealed taking care not to leave air bubbles inside. The size and volume of the chambers depended on the size of the shrimp at the time of sampling; however, were the same size and volume for all shrimp from both treatments. One of the chambers ran out of shrimp and was considered as control for each treatment.

The shrimp remained a total period of 24 h inside the respirometric chamber. Data from the first 12 h were used to analyze RM. From 12 h, ration was provided in all the chambers including control. After the measurements were finished, the shrimp were removed from the chambers, dried and weighed (live weight) on an Ohaus® brand Scout Model Scale with a capacity of 600 g ± 0,1g.

Oxygen consumption was determined for each chamber with the input value minus the output value being multiplied by the flow (in ml hour $^{-1}$) of the water through the chambers. Finally, the value of the oxygen consumption was calculated by withdrawing the consumption value in the control chamber. Data were expressed as mg O2 g^1 h^{-1} according to Rosas, (2001).

$$VO_2 = ([(O_2e - O_2s)] \times F) / Pc$$

Where:

VO_2 = oxygen consumption in mgO_2 $g^{-1}h^{-1}$,
O_2e = oxygen concentration in mg L^{-1} obtained at the entrance of the chamber,
O_2s = oxygen concentration in mg L^{-1} obtained at the exit of the chamber,
F= flux in L h^{-1} e
Pc = wet body weight (g).

Statistical analysis

The normality of the data was analyzed by the Kolmogorov-Smirnov test and the homoscedasticity by the Levene test. Subsequently, an ANOVA was performed to confirm the existence of significant differences between the treatments ($p < 0.05$). When significant differences were found, a Tukey post-hoc analysis was performed. For all

statistical analyses the software package STATISTICA v7 for Windows (StatSoft Inc., 2011) was used. A Principal Components Analysis was also carried out to know, within each treatment, the variability of the indicators evaluated, using the program R Statistical Software.

RESULTS:

All water quality parameters were maintained within the recommended levels for penaeid shrimp culture throughout the experiment (Van Wyk and Scarpa, 1999; Laramore et al., 2001; Rozas and Minello, 2011).

Table 1. Mean value ± SD of the physicochemical parameters of the water after 90 days, for *L. setiferus* F0 early juveniles in BFT system with marine (MBFT) and low salinity water (LSBFT) in outdoor ponds

TREATMENT	OD mg/L	Temperature °C	pH	Salinity ppt	Ammonia (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)
MBFT	5.33 ± 0.98	28.02 ± 0.58	8.03 ± 0.69	35.73 ± 1.01	0.29 ± 0.67	1.37 ± 1.85	92.31 ± 9.23
LSBFT	7.61 ± 1.20	27.49 ± 2.95	8.50 ± 1.15	5.25 ± 1.8	0.11 ± 0.18	1.47 ± 1.34	87.34 ± 10.1

The results of the zootechnical performance of the *L. setiferus* after 90 days of experiment are shown in Table 2. The final weight, the weight gain and the survival were significantly higher in the MBFT treatment than in the LSBFT. The relationship between the size of the hepatopancreas and the body size (HI) was higher in shrimp grown in low-salinity water (Table 2).

Table 2. Mean value ± SD of the zootechnical performance after 90 days, for *L. setiferus* F0 early juveniles in BFT system with marine (MBFT) and low salinity water (LSBFT) in outdoor ponds. Different letters indicate significant differences p <0.05.

Treat	**Initial weight (IW) (g)**	**Final weight (FW) (g)**	**Weight gain (FW-IW) (g)**	**Weekly Growth Rate (g)**	**Hepato somatic Index (HI)**	**Survival (%)**	**FCA**
MBFT	0.1 ± 0.3	2.47 ± 0.88[a]	2.46 ± 0.62[a]	0.2[a]	0.06 ± 0.0[a]	76.0 ± 6.0[a]	2,22 ± 0.24[a]
LSBF	0.1 ± 0.3	1.83 ± 0.47[b]	1.73 ± 0.43[b]	0.15[a]	0.13. ± 0.01[b]	67.0 ± 7.81[a]	3.48 ± 0.25[b]

Antioxidant activity

The activities of SOD and CAT, measured in the muscle of *L. setiferus*, are shown in Figure 1. CAT did not present significant differences (p>0.05), in comparison with SOD, which presented significant differences (p < 0.05). The lowest SOD activity was observed in MBFT treatment (p<0.05)

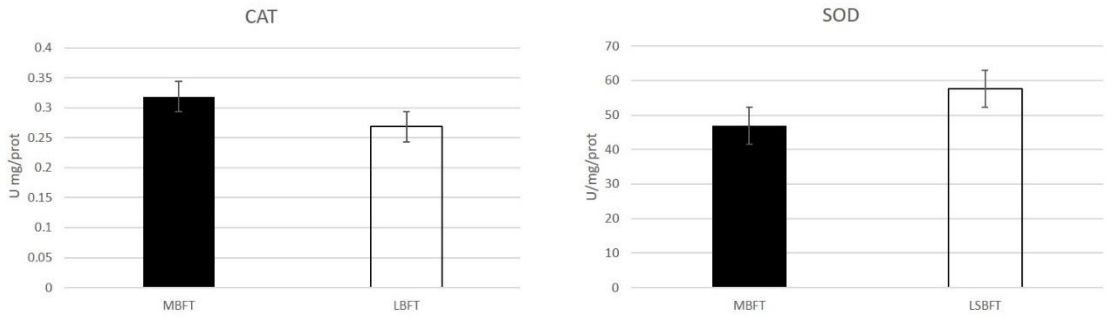

Figure 1. Mean values and SE for Oxidative enzyme activity of Catalase (CAT) and sodium dismutase (SOD) in the muscle of *L. setiferus* F_0 early juveniles in different culture conditions after 90 days. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

The LPO and PO did not show significant differences (p>0.05) between the treatments (Figure 2).

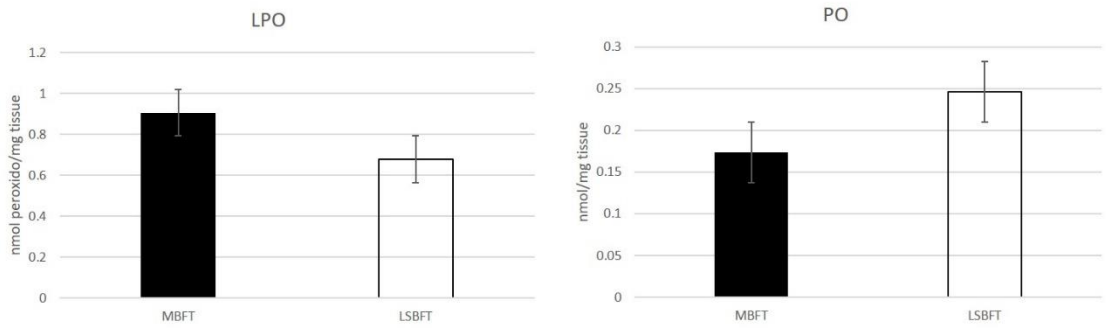

Figure 2. Mean values and SE for Lipid peroxidation (LPO) and protein oxidation (PO) in the muscle of *L. setiferus* F_0 early juveniles in different culture conditions after 90 days. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

Glutathione S-transferase activity

The GST enzyme in the muscle showed major levels of activity in LSBFT. However, they did not show significant differences ($p>0.05$) between the treatments (Figure 3).

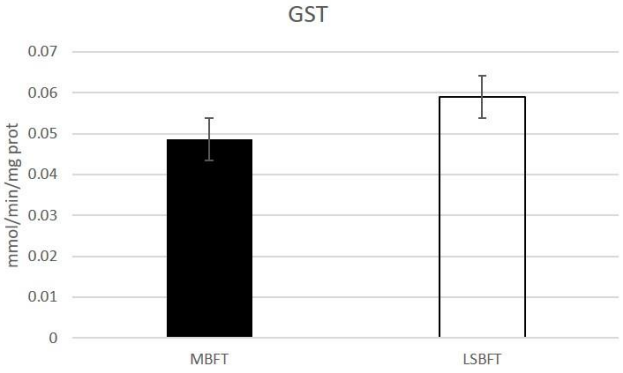

Figure 3. Mean values and SE for Glutathione S-transferase (GST) activity in the muscle of *L. setiferus* F_0 early juveniles in different culture conditions after 90 days. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

Immune response

The TCH did not showed a significant difference between the treatments, with a greater number of hemocytes ($p < 0.05$) in the both treatments (Figure 4.)

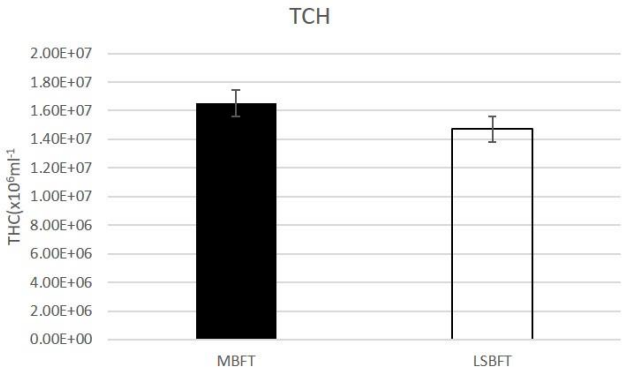

Figure 4. Mean values and SE Total Count of Hemocytes (TCH) for *L. setiferus* F_0 early juveniles in different culture conditions after 90 days. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

The measurements made in the respirometer showed the oxygen consumption of the shrimps in both treatments, at the beginning (30 days) and until the end (90 days) At the beginning the shrimp LSBFT treatment was very stressed, manifesting it in the increased oxygen consumption and lack of appetite. At the end of the experiment the shrimp lowered their oxygen consumption and fed normally (Figure 5).

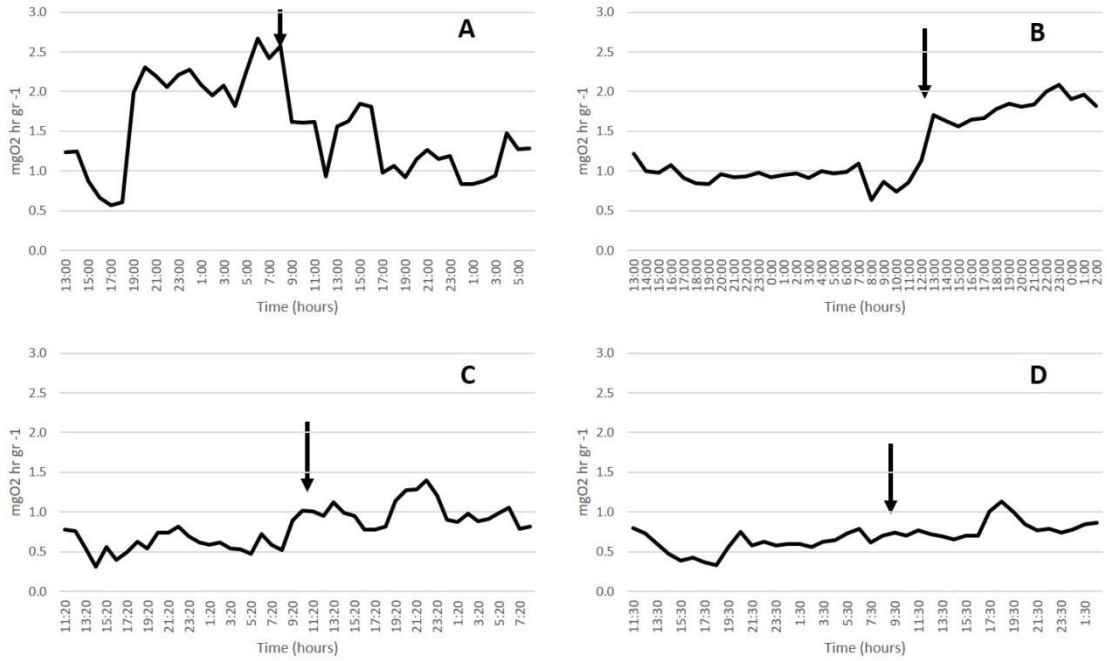

Figure 5. Average oxygen consumption and standard deviation for juveniles of *L. setiferus* at the beginning of the trial and after 90 days of culture. Black arrows point to the moment when food was offered A) Treatment LSBFT at the beginning, the shrimp did not feed, the oxygen consumption was more intense. B) LSBFT the consumption dropped at the end of the experiment and the animals were fed normally. C) In the MBFT treatment, from the beginning to the end D) oxygen consumption was lower and uniform and the shrimp were fed whenever they were offered feed. MBFT= Marine biofloc, MCW= Marine clear water, LSBFT= Low salinity biofloc, LSCW= Low salinity clear water.

DISCUSSION:

Quality water

The physicochemical parameters remained within what was expected for this culture system (Van Wyk et al., 1999, Decamp, 2003, Chen et al, 2006). It was not detected any significant differences. Total ammonia concentrations presented higher peaks in MBFT at the beginning of the experiment, but it was controlled with sugar cane molasses, the C:N ratio were maintained by adding cane molasses to stimulate ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), because probably the inoculated biofloc were immature. Probably, the problem was not the high value of nitrite, the problem was probably the exposure time (Lin and Chen, 2001, Li et al, 2007), added the extreme toxicity of nitrite in low salinity.

Zootechnical performance

The weekly growth rates were low but were better than results reported by other authors (Palomino et al., 2001 and Rosas et al., 2001) for the same Southern region of the Gulf of Mexico. *L. setiferus,* like all non-domesticated species, has a low growth rate as reported for *Farfantepenaeus paulensis* (0.14-.052 g w^{-1}) by Fróes et al., (2007) and Ballester et al., (2010), or for *Litopenaeus schmitti* (0.22 g w^{-1}) by Barbosa et al., (2014) and for *Farfantepenaeus duorarum* (0.023 g w^{-1}) by Parrak, (1979) and Gullian *et al.*, (2010).Highest values were in MBFT, however, one characteristic of this species is that up to 5.0 grams its growth is extremely slow, and older juveniles > 5.0 g grow at a higher rate (Sandifer et al., 1993, Samocha, et al., 1998, Perez-Velazquez et al., 2013). The relationship between the size of the animal and the size of the hepatopancreas don't have significant differences, this factor permits greater energy storage and greater production of enzymes for the osmoregulation and processing of nitrogenous compounds (Wang et al., 2004).

The feed conversion factor was also lower in the MBFT treatment. Greater survivals in MBFT treatment were detected probably due to the effect of nitrite levels and possibly due to the ionic imbalance of shrimps in LSBFT treatment.

Antioxidant activity

CAT and SOD did not show significant differences. In general values of enzymatic antioxidant activity are low (Liñan-Cabello et al., 2003, Vinagre et al., 2014). According to the principal components analysis (PCA), the CAT is the indicator that contributes most to the LSBFT treatment, not mean that the others physiology parameters are not important. Lower CAT and higher SOD are indicators of increased cell damage (Cardona et al., 2015), although the values were low in two units of LSBFT treatments. Another unit had the highest antioxidant activity and apparently the most cellular damage. The activity of the GST indicates high levels of detoxification in LSBFT. According to Cardona et al., (2015) and Xu et al., (2013) the BFT system stimulates the activity of the immune system and maintains the antioxidant and antimicrobial response during stress.

The values of lipoperoxidation and carbonylated proteins, show low activity, probably suggest low cell damage was. LPO produce cellular oxidative damage to membrane lipids ((Kwiecien et al., 2014), Oxidative stress can directly damage proteins or modify protein amino acids to promote protein carbonyl formation (Fedorova et al., 2014) Lipid peroxidation begins with the attack of any species capable of stealing an H^+ from the polyunsaturated fatty acids of the membranes with the formation of a peroxyl radical that can attack a fatty acid by propagating lipoperoxidation. The oxidized lipids accumulate in the membrane, destabilize it and cause an escape to occur (Halliwell, 1984)

There were no significant differences in the amount of hemocytes present in the shrimps of both treatments, it is important to note that the values reached are higher than those obtained by Rosas, et al., (2004), perhaps in response to the high bacterial activity in the biofloc. In low salinity shrimps have lower tolerance to stress, lower immunity also can be in many cases, exhaustion of hemocytes for higher energy demand (Liu and Chen, 2004), in addition, changes in environmental factors induces alterations in the immune response in crustaceans that are often immune suppressor (Le Moullac and Haffner, 2000).

Some physiological and immunological functions are activated with changes in salinity. In low salinity shrimps require protein as a source of amino acids to maintain osmotic pressure, so we find lower total protein in the muscle of LSBFT shrimp, use proteins to maintain osmolarity, shrimp are more protein-dependent Rosas et al. (2004).

Understanding the response of white shrimp to conditions that induce oxidative stress is of great importance in farming systems; knowing the levels of EROs and oxidative damage that occur during a given culture condition allow producers to suggest strategies to protect shrimp in culture.

Dissolved oxygen (DO) is one of the most important environmental stress factors in aquaculture and is affected by many environmental factors, such as the sudden change or death of the dominant population of phytoplankton, large reproduction of zooplankton in the pond, and decomposition of accumulated organic matter including unconsumed food and faeces, which will lead to sharp decreases of DO. Thus, decreases in DO are a common hazard in shrimp culture (Jiang *et al.*, 2005). The physiological responses of decapod crustaceans to the decrease of dissolved oxygen (DO) have been well documented especially in relation to DO effects on the respiratory response. McMahon (1988) carried out an examination of the processes involved in the regulation of the ventilating and cardiac mechanisms that contribute to the maintenance of the oxygen supply to tissues. L. *setiferus* juveniles can change their energy substrate in response to salinity and DO changes. This fact may be related to a possible strategy that allows them to obtain energy from proteins (Rosas et al., 1999)

In relation to this, the results obtained from oxygen consumption in the respirometry allow us to appreciate that the shrimp in both treatments were stressed at the beginning, however, the animals in LSBFT had a higher oxygen consumption at the beginning and at the end of the experimental period, even during the initial respirometry, they were not fed, showing a more stable oxygen consumption for the last sampling, where they already ate normally.

Meanwhile, those whom were in seawater, were in a better condition, since from the beginning they began to eat when they were offered food. In the end, both treatments have low and constant oxygen consumption, that is, they adapted to the culture system.

Based on achieved results it is possible to conclude that the biofloc system stabilizes and improve rear conditions for *L. setiferus* that it is a species with high potential to be cultivated, regardless of salinity. The shrimp have a permanently stimulated immune condition, the oxidative stress seems to decrease, which probably leads to less cell damage. Oxygen consumption could indicate that energy is directed toward growth. It seems that the system with biofloc improves physiological

performance, but now to improve zootechnical performance, it is still necessary to continue researching on nutritional (a diet specially designed for this species, for example), ion balance, and breeding status issues, things that may be related to the benefits that the system with biofloc offers, such as bacteria that control the quality of water and especially probiotic bacteria, as well as a great abundance and diversity of microorganisms to feed and all together offering the right conditions to grow new breeder already adapted to the crop in captivity.

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DISCUSSÃO GERAL:

A intenção geral de investigar o potencial para a aquacultura de outras espécies nativas de camarões na região sul do Golfo do México não é apenas uma questão de importância regional, mas também tem relação com a diversificação da Aquacultura. É impossível se pensar em trabalhar com as mesmas espécies e em todos os lugares. Também, tem a ver com o uso de recursos pesqueiros, com desenho e modificação de ração balanceada considerando novas necessidades nutricionais. Algo muito importante que tem relação com a domesticação de uma espécie é o melhoramento genético. Entretanto, poderíamos estar estimulando o maior crescimento através de maior consumo de proteínas e que conduz inevitavelmente a um maior consumo de farinha de peixe. Desde que não se encontre um substituto adequado como farinha de peixe, tudo o implica em aumento de consumo. Também tem a ver com a recuperação de populações de camarão que foram colocadas em risco pela sobre pesca. Em suma, é uma questão com muitas perspectivas e com muitas vantagens, se conseguirmos encontrar as opções certas.

A intenção do presente estudo foi pesquisar qual é a resposta *L. setiferus* em diferentes tipos de cultivo, com todos os avanços em biotecnologia aquícola que se tem, particularmente com o sistema com bioflocos. Assim, se ainda falta muito por conhecer sobre este sistema, os conhecimentos gerais e os princípios básicos publicados, assim como as experiencias aprendidas até agora, podem ser aplicados para cultivar camarões no sul do Golfo do México.

No primeiro capítulo deste trabalho conseguimos estabelecer as linhas de pesquisa a seguir no futuro, baseados na pesquisa realizada até agora, não só para a produção comercial da espécie, mais também para a própria conservação.

A partir destes conhecimentos, um dos primeiros desafios foi produzir as pós-larvas numa região fora da área de distribuição geográfica natural da espécie, assim começamos a viajar para coletar camarões adultos e tentar reproduzi-los em cativeiro. No entanto, embora que muitas vezes não conseguimos coletar o número suficiente de adultos e incluso alguma vez voltamos ao laboratório sem animais, conseguimos ter um pequeno banco de reprodutores, mais aos poucos dias começaram a mostrar problemas associados a vibriosis e a deterioro dos espermatóforos no caso dos machos. Um dos

grandes problemas desta espécie persiste, ainda temos que pesquisar sobre este problema para poder manter mais tempo os reprodutores, em boas condições.

Diante dessa situação, no segundo capítulo experimentamos com juvenis selvagens no sistema de cultivo com bioflocos em água marinha e de baixa salinidade. Os resultados mostraram animais totalmente estressados particularmente nos sistemas com fluxo contínuo. A interpretação de todos os indicadores foi complicada devido à altíssima atividade do sistema antioxidante e imune. Alta mortalidade e crescimento muito baixo somente nos tratamentos com fluxo continuo de água.

Continuamos trabalhando com a coleta e reprodução dos adultos. Tentamos inseminação artificial, fizemos ablação do pedúnculo ocular e conseguimos produzir pós-larvas. Os protocolos de larvicultura que a espécie tem já publicados, funcionam e conseguimos obter pós-larvas de qualidade para tentar novamente. No terceiro capítulo, repetimos o mesmo experimento, só que agora com pós-larvas F0. Colocamos as pós-larvas no dispositivo experimental e os dados foram mais estáveis, porém, ainda com altos níveis de estresse oxidativo, mais com um sistema imune alerta e um desempenho zootécnico melhorado particularmente no sistema com bioflocos, independentemente da salinidade. Novamente, alta mortalidade e crescimento muito baixo somente nos tratamentos com fluxo continuo de água.

Assim e diante dos resultados obtidos nos dois primeiros experimentos, fomos para o quarto capítulo implementando um terceiro experimento decidimos retirar os tratamentos com fluxo contínuo e elevar a escala do cultivo. Utilizando tanques de 20 m^3 com sistema de bioflocos em água marinha e água de baixa salinidade. Os resultados obtidos são interessantes, a atividade antioxidante esteve presente mais foi a mais baixa entre os três experimentos. Podemos detectar que os animais ficaram menos estressados. O sistema imune foi estimulado pela quantidade de hemócitos na hemolinfa dos dois tratamentos. Quando avaliamos o consumo de oxigênio no início do experimento, encontramos um alto consumo no tratamento de baixa salinidade, inclusive quando oferecemos alimento. Os camarões simplesmente não conseguiram comer. No final do experimento, após dos 90 dias de cultivo, o consumo baixou e quando a ração foi oferecida, passaram a comer. No tratamento com água marinha, os camarões tiveram do princípio ao fim do experimento um consumo baixo e se alimentaram normalmente. O

que pode ser interpretado como que os camarões em agua de baixa salinidade apresentam um maior consumo de oxigênio, principalmente ao começo do cultivo, o que complementado com o resto dos resultados, deixa animais com alta vulnerabilidade fisiológica e um maior requerimento de energia para manter o metabolismo de rotina, porém uma alta mortalidade e um menor desempenho zootécnico.

Uma característica uniforme nos três experimentos é o sistema imune estimulado permanentemente, no sistema com bioflocos. Provavelmente as bactérias presentes nos bioflocos estimulam o sistema imune e eles ficaram melhor preparados para o estresse ambiental e provavelmente para ataques patogénicos. O número de hemócitos presentes na hemolinfa foram maiores aos reportados para a mesma espécie por outros autores em sistemas convencionais de cultivo.

Outra característica é, que resultou em uma redução da atividade antioxidante no cultivo com tanques maiores, principalmente nos camarões expostos aos bioflocos, independentemente da salinidade. A terceira característica relevante é o consumo de oxigênio, é dizer, que uma vez adaptados ao sistema, sobretudo em baixa salinidade, os animais gastam energia não somente para manter o metabolismo de rotina (sobreviver), uma vez adaptados diminuem seu consumo de oxigénio, eficientizando o gasto metabólico, destinando uma boa quantidade de energia também para crescer.

As sobrevivências foram baixas, assim como o crescimento. No entanto, com todo o bom desempenho fisiológico obtido é importante ressaltar que parte das mortalidades ocorridas nos três experimentos, tem relação direta com o manejo. Por exemplo, nos processos de manejo dos nitrogenados, principalmente o nitrito e do balanço iónico, assim como de limpeza, alimentação ou de adição de melaço, praticas com maior cuidado devem ser aplicadas nos próximos experimentos e passar a outros desafios tais como melhorar as taxas de crescimento através de dietas especialmente desenhadas para *L. setiferus*. Além disso, as pesquisas devem determinar o balanço iónico adequado para a espécie, já que os resultados das análises da água indicam as carências de magnésio e potássio na região.

Através dos dados obtidos podemos concluir:

Que a medida que o sistema com bioflocos madura e se estabiliza, e as condições de cultivo melhoram (infraestrutura, manejo) *Litopenaeus setiferus*, pode ser cultivada independentemente da salinidade.

A espécie apresenta nos bioflocos uma condição imunológica estimulada permanentemente.

O estresse oxidativo parece diminuir, provavelmente com menor dano celular.

O consumo de oxigênio e a energia são dirigidos para o crescimento da espécie.

Também foi confirmado que o sistema com bioflocos melhora o desempenho fisiológico.

Assim, para melhorar o desempenho zootécnico, ainda existe a necessidade de continuar pesquisando sobre questões nutricionais, de balanço iónico e de melhoramento do estado dos reprodutores. Questões que podem estar relacionadas com os benefícios que o sistema com bioflocos oferece, tais como: bactérias que controlam a qualidade da água, bactérias probióticas, abundância e diversidade de micro-organismos que servem como complemento alimentar, podem oferecer as condições adequadas para o melhor desempenho de *L. setiferus* em sistemas de cultivo.