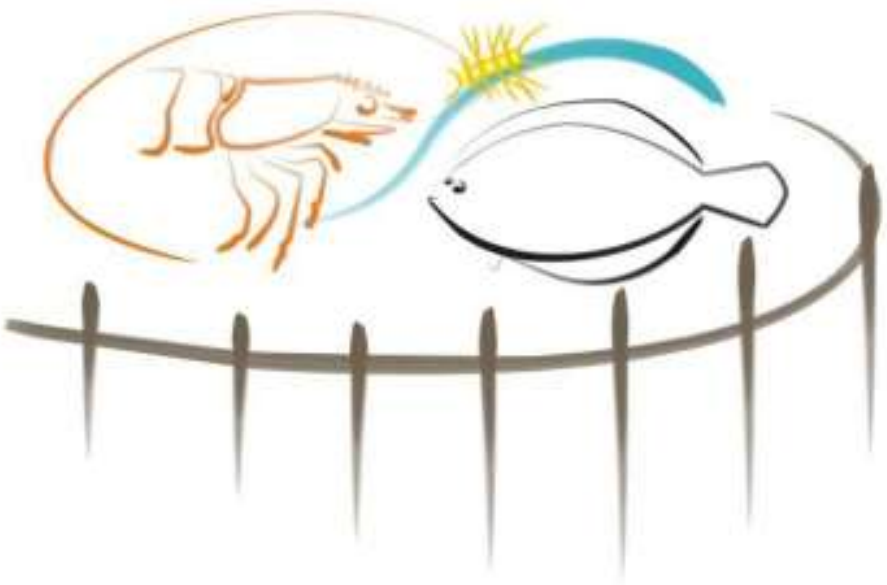
UNIVERSIDADE FEDERAL DO RIO GRANDE - FURG

INSTITUTO DE OCEANOGRAFIA

PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA

TESE DE DOUTORADO



PRODUÇÃO DE MACROALGAS EM CULTIVO MULTITRÓFICO INTEGRADO COM CAMARÃO E PEIXE: ABSORÇÃO DE NUTRIENTES E APLICAÇÃO DA BIOMASSA PRODUZIDA

ANDREZZA CARVALHO CHAGAS

Rio Grande, RS

2025

UNIVERSIDADE FEDERAL DO RIO GRANDE - FURG
INSTITUTO DE OCEANOGRAFIA

PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA

PRODUÇÃO DE MACROALGAS NO CULTIVO MULTITRÓFICO INTEGRADO COM CAMARÃO E PEIXE: ABSORÇÃO DE NUTRIENTES E APLICAÇÃO DA BIOMASSA PRODUZIDA

ANDREZZA CARVALHO CHAGAS

Tese apresentada como parte dos requisitos para obtenção do grau de Doutor em Aquicultura no Programa de Pós-Graduação em Aquicultura da Universidade Federal do Rio Grande-FURG.

Orientadora: Luís Henrique da Silva Poersch

Co-orientadora: Gamze Turan

RIO GRANDE-RS

Fevereiro de 2025

Pós Graduação em AQUICULTURA
Universidade Federal do Rio Grande - FURG

ATA 05/2025

ATA DE DEFESA DA 91 ª TESE DE DOUTORADO EM AQUICULTURA

No dia vinte e quatro de fevereiro de dois mil e vinte e cinco, as quatorze horas, reuniu-se a Banca Examinadora de Tese de Doutorado em Aquicultura, de **ANDREZZA CARVALHO CHAGAS**, orientada pelo Prof. Dr. Luis Henrique Poersch (IO/FURG), composta pelos seguintes membros: Prof. Dr. Luis Henrique Poersch (Orientador – IO/FURG), Profa. DRa. Gamze Turan (Coorientadora – EGE UNIVERSITY), Prof. Dr. Geraldo Kipper Fóes (IO/FURG), Prof. Dr. Wagner Cotroni Valenti (UNESP) e Profa. Dra. Gabriele Rodrigues de Lara (Pontificia Universidad Catolica de Valparaiso/CHILE). Título da Tese: **"PRODUÇÃO DE MACROALGAS EM CULTIVO MULTITRÓFICO INTEGRADO COM CAMARÃO E PEIXE: ABSORÇÃO DE NUTRIENTES E APLICAÇÃO DA BIOMASSA PRODUZIDA".** Dando início à defesa, o Coordenador do PPGAq Prof. Dr. Ricardo Vieira Rodrigues, passou a presidência da sessão ao Prof. Dr. Luis Henrique Poersch, que na qualidade de orientador, passou a palavra para a candidata apresentar a Tese. Após ampla discussão entre os membros da Banca e a candidata, a Banca se reuniu sob a presidência do Coordenador. Durante esse encontro ficou estabelecido que as sugestões dos membros da Banca Examinadora devem ser incorporadas na versão final da Tese, ficando a cargo do Orientador o cumprimento desta decisão. A candidata **ANDREZZA CARVALHO CHAGAS** foi considerada **APROVADA**, devendo a versão definitiva da Tese ser entregue a Secretaria do PPGAq, no prazo estabelecido nas Normas Complementares do Programa. Nada mais havendo a tratar, foi lavrada a presente ata, que após lida e aprovada, será assinada pela Banca Examinadora, pela candidata e pelo Coordenador do PPGAq.

gov.br Documento assinado digitalmente LUIS HENRIQUE DA SILVA POERSCH Data: 17/03/2025 09:36:14-0300 Verifique em https://validar.iti.gov.br

PROF. DR. LUIS HENRIQUE POERSCH (ORIENTADOR – IO/FURG)

Gamze Turan

PROFa. DRa. GAMZE TURAN (COORIENTADORA - EGE UNIVERSITY/TURQUIA)

PROF. DR. GERALDO KIPPER FÓES (IO/FURG)

gov.br Documento assinado digitalmente GERALDO KIPPER FOES Data: 14/03/2025 14:26:09-0300 Verifique em https://validar.iti.gov.br

PROFa. DRa. GABRIELE RODRIGUES DE LARA (PUCV/CHILE)

Firma electrónica avanzada GABRIELE RODRIGUES DE LARA 2025.03.18 17:02:51 -0300

gov.br Documento assinado digitalmente WAGNER COTRONI VALENTI Data: 18/03/2025 13:46:38-0300 Verifique em https://validar.iti.gov.br

PROF. DR. WAGNER COTRONI VALENTI (UNESP)

gov.br Documento assinado digitalmente ANDREZZA CARVALHO CHAGAS Data: 07/03/2025 13:10:12-0300 Verifique em https://validar.iti.gov.br

ANDREZZA CARVALHO CHAGAS

gov.br Documento assinado digitalmente RICARDO VIEIRA RODRIGUES Data: 07/03/2025 14:47:07-0300 Verifique em https://validar.iti.gov.br

PROF. DR. RICARDO VIEIRA RODRIGUES (COORDENADOR DO PPGAq)

Ficha Catalográfica

C433p Chagas, Andrezza Carvalho.
Produção de macroalgas no cultivo multitrófico integrado com camarão e peixe: absorção de nutrientes e aplicação da biomassa produzida / Andrezza Carvalho Chagas. – 2025.
196 f.

Tese (doutorado) – Universidade Federal do Rio Grande – FURG, Programa de Pós-Graduação em Aquicultura, Rio Grande/RS, 2025.
Orientador: Dr. Luís Henrique da Silva Poersch.
Coorientadora: Dra. Gamze Turan.

1. IMTA 2. Macroalgas 3. Camarão 4. Ostra 5. Peixe 6. Nutrientes 7. Sólidos 8. Bioflocos 9. Biorremediador 10. Compostos Bioativos 11. Economia circular 12. Ração 13. Estresse oxidativo I. Poersch, Luís Henrique da Silva II. Turan, Gamze III. Título.

CDU 639.512

Catalogação na Fonte: Bibliotecário José Paulo dos Santos CRB 10/2344

Sumário

AGRADECIMENTOS

Gostaria de agradecer em primeiro toda a minha família, que tanto me apoiou e me incentivou durantes todos esses anos. Em especial ao meu pai que sempre entendeu meus caminhos e a minha afilhada que me amou mesmo à distância.

Quero agradecer ao meu orientador Mineiro, que me incentivou a ter boas ideias e coragem para realiza-las. Muito obrigada a todas as oportunidades que me proporcionou, por todos os ensinamentos, e pelas conversas me incentivando a continuar e ser melhor.

Também agradeço a minha co-orientadora Gamze pelas correções e ajuda durante o doutorado. Obrigada ao Alessandro pela ajuda técnica nos experimentos, pelas correções dos manuscritos, mas também por todas as conversas sobre o futuro.

Agradeço aos professores do PPGAqui, em especial ao Ricardo e Marcelo Tesser. E aos professores do projeto Camarão por todo ensinamento e oportunidade de trabalhar em equipe. Agradecer a Virginia Tech pela experiência do estágio, ter fornecido a estrutura para realização do experimento e ter me dado a oportunidade de trabalhar com outras áreas. Muito obrigada ao Fernando pelas conversas e ao Ethan por toda motivação e apoio, eu que tenho orgulho de ter trabalhado com você!

Obrigada ao ASTRAL Project – H2020 pelo financiamento nas pesquisas e a CAPES pela bolsa de doutorado.

Obrigada a equipe Multitrófico por tanta produção e amizade durante os seis anos de EMA. Queria agradecer a Mariana, Léa, Mayra, Jorge e Navinésia pela troca de ideias, pela ajuda nos experimentos, aos conselhos e principalmente as risadas.

Obrigada a minha família do cassino, Cynthia, Rafa, Lucas, Mayra, Helly, Pedro, Italo, Navinésia, Bianca, a companhia de vocês foi muito importante, amo vocês de verdade!

Aos meus amigos da graduação, Mariana, Nathan, Victor e Gabriel, que mesmo na distância estavam comigo nas pequenas ou grandes decisões. E aos meus amigos de Camocim, que ja se fazem presente a tanto tempo. E obrigada ao João Paulo por todo apoio nessa reta final de doutorado.

RESUMO

A utilização de sistemas com zero renovação de água, como o sistema Bioflocos (BFT), apesar de maior biossegurança tem como consequência o acúmulo de nitrato e fosfato no decorrer da produção. A possibilidade de inserção de espécies de diferentes níveis tróficos no sistema promove o aumento da produtividade com a reutilização dos resíduos para conversão em biomassa de interesse econômico, podendo ser trabalhada a economia circular. Portanto, a presente tese teve como objetivo determinar planos de manejos viáveis para a macroalga *Ulva lactuca* em cultivo integrado, com enfoque na produção e aplicação da biomassa de macroalgas e absorção de nutrientes. O primeiro experimento avaliou o efeito do sistema simbiótico no crescimento da macroalga, ostra e camarão. Foi constatado que não houve um aumento de biomassa da macroalga durante o experimento, no entanto ainda houve uma menor concentração de nitrato no tratamento simbiótico quando a macroalga estava inserida no sistema. Para a ostra, o uso do sistema simbiótico causou mortalidade nos animais, em contraste com o camarão que apresentou maior peso final no sistema integrado com simbiótico. O segundo capítulo avaliou o efeito das diferentes concentrações de sólidos suspensos totais e nutrientes do sistema em bioflocos no crescimento, absorção de nutrientes e composição nutricional da macroalga em sistema integrado com camarão. O tratamento com concentração de sólidos suspensos totais de 246 mg L^{-1} mostrou melhor desempenho em absorção de nitrato e fosfato com 55 e 31% de taxa de remoção, respectivamente. Além de apresentar maior teor de proteína e clorofila-a comparado ao tratamento controle (sem biofloco). O terceiro capítulo buscou avaliar diferentes profundidades de estrutura de cultivo para a macroalga quando cultivada em sistema integrado com camarão e peixe. Foram testadas duas profundidades, sendo elas, rasa (até 10 cm de profundidade) e funda (até 25 cm de profundidade). As macroalgas cultivadas em até 10 cm de profundidade mostraram melhor crescimento ao longo do cultivo comparado ao tratamento fundo. O quarto capítulo buscou avaliar o efeito da inserção da macroalga na comunidade microbiana quando cultivada em conjunto com o camarão. Ao final do experimento, a adição da macroalga *Ulva* ao cultivo proporcionou o decréscimo de diatomáceas, cianobactérias e ciliados comparado ao monocultivo de camarão, podendo estar associado a competição por nutrientes com a macroalga, mostrando vantagem ao uso do sistema integrado. O quinto capítulo da tese avaliou o crescimento, a absorção dos nutrientes e composição nutricional da macroalga em sistema heterotrófico e sistema quimioautotrófico. Os resultados mostraram uma taxa de crescimento da macroalga maior no tratamento quimioautotrófico ao final do experimento. Entretanto, o tratamento heterotrófico apresentou maior absorção de fosfato e nitrato com 57 e 56% respectivamente, além do incremento do

valor proteico da macroalga. O desempenho do camarão não foi afetado pelo tratamento, e houve maior peso final da tilápia no tratamento heterotrófico. O sexto capítulo buscou avaliar a inserção da macroalga como biorremediador para absorção de nutrientes do efluente de um cultivo de camarão com bioflocos. Para isso, foram coletados os efluentes de um sistema quimioautotrófico e heterotrófico (experimento anterior), o uso de aeração constante e a inserção de 1g/L de macroalga por 15 dias. Como resultado, uma maior taxa de remoção de nitrato (28%) foi encontrada no sistema heterotrófico, com uma taxa de crescimento relativa de 3.53 % dia $^{-1}$, mostrando melhor desempenho da macroalga. E o sétimo capítulo consistiu na aplicação da biomassa de macroalga produzida como aditivo na ração. Foram testadas diferentes inclusões da macroalga produzida em sistema integrado com bioflocos na ração da tilápia, sendo elas: 5, 10 e 15 % de inclusão da macroalga na ração e um tratamento controle sem macroalga, tendo como objetivo avaliar o desempenho zootécnico, hematológico e atividade antioxidante. Ao final do experimento foi realizado um teste de estresse salino. Não houve diferença significativa entre os tratamentos no desempenho zootécnico e composição proximal. A contagem de granulócitos foi superior no tratamento com 10% de inclusão de macroalga na dieta. Após o estresse salino, o tratamento com 5% de inclusão apresentou maior capacidade antioxidante no musculo, com uma menor oxidação proteica e lipídica. Com isso, a inserção da macroalga no cultivo integrado com camarão e peixe apresentou viabilidade e sustentabilidade com absorção de nutrientes, produção de biomassa e inclusão na ração.

Palavras-chaves: IMTA, macroalgas, camarão, ostra, peixe, nutrientes, sólidos, bioflocos, biorremediador, compostos bioativos, economia circular, ração, estresse oxidativo.

ABSTRACT

The use of systems with zero water renewal, such as the Biofloc system (BFT), despite being more biosecure, results in the accumulation of nitrate and phosphate over the course of production. The possibility of including species from different trophic levels in the system promotes an increase in yields by reusing waste to convert it into biomass of economic interest, which could contribute to the circular economy. Therefore, this thesis aimed to determine viable management plans for the macroalga *Ulva lactuca* in integrated cultivation, focusing on the production and application of macroalgae biomass and nutrient absorption. The first experiment evaluated the effect of the synbiotic system on the growth of macroalgae, oysters and shrimp. It was found that there was no increase in macroalgae biomass during the experiment, however there was a lower nitrate concentration in the synbiotic treatment when the macroalgae was inserted into the system. For the oyster, the use of the synbiotic system caused mortality in the animals, in contrast to the shrimp, which had a higher final weight in the system integrated with the synbiotic. The second chapter evaluated the effect of different concentrations of total suspended solids and nutrients in the biofloc system on the growth, nutrient absorption and nutritional composition of the macroalgae in an integrated system with shrimp. The treatment with a total suspended solids concentration of 246 mg L^{-1} showed the best performance in terms of nitrate and phosphate absorption, with a 55% and 31% removal rate, respectively. It also had a higher protein and chlorophyll-a content compared to the control treatment (without biofloc). The third chapter evaluated different depths of cultivation structure for the macroalgae when cultivated in an integrated system with shrimp and fish. Two depths were tested: shallow (up to 10 cm deep) and bottom (up to 25 cm deep). Macroalgae cultivated at depths of up to 10 cm showed better growth throughout cultivation compared to the deep treatment. The fourth chapter sought to evaluate the effect of inserting macroalgae into the microbial community when cultivated together with shrimp. At the end of the experiment, the addition of the macroalga *Ulva* to the cultivation provided a decrease in diatoms, cyanobacteria and ciliates compared to shrimp monoculture, which may be associated with competition for nutrients with the macroalga, showing an advantage to the use of the integrated system. The fifth chapter of the thesis evaluated the growth, nutrient absorption and nutritional composition of the macroalgae in heterotrophic and chemoautotrophic systems. The results showed a higher growth rate of the macroalgae in the chemoautotrophic treatment at the end of the experiment. However, the heterotrophic treatment showed greater absorption of phosphate and nitrate with 57 and 56% respectively, as well as an increase in the protein value of the macroalgae. The performance of the shrimp was not affected by the treatment, and the final weight of the tilapia

was higher in the heterotrophic treatment. The sixth chapter aims to evaluate the use of macroalgae as a bioremediator to absorb nutrients from the effluent of a biofloc shrimp cultivation. To do this, effluents were collected from a chemoautotrophic and heterotrophic system (previous experiment), using constant aeration and the insertion of 1g/L of macroalgae for 15 days. As a result, a higher nitrate removal rate (28%) was found in the heterotrophic system, with a relative growth rate of 3.53 % day $^{-1}$, showing better macroalgae performance. The seventh chapter consisted of the application of the macroalgae biomass produced as a feed additive. Different inclusions of macroalgae produced in an integrated system with bioflocs were tested in tilapia feed: 5, 10 and 15% inclusion of macroalgae in the feed and a control treatment without macroalgae, with the aim of evaluating zootechnical performance, hematology and antioxidant activity. A salt stress test was carried out at the end of the experiment. There was no significant difference between the treatments in zootechnical performance and proximal composition. The granulocyte count was higher in the treatment with 10% inclusion of macroalgae in the diet. After salt stress, the treatment with 5% inclusion showed greater antioxidant capacity in the muscle, with lower protein and lipid oxidation. As a result, the inclusion of macroalgae in integrated cultivation with shrimp and fish showed viability and sustainability in terms of nutrient absorption, biomass production and inclusion in the feed.

Keywords: IMTA, macroalgae, shrimp, oyster, fish, nutrients, solids, bioflocculation, bioremediator, bioactive compounds, circular economy, feed, oxidative stress.

INTRODUÇÃO GERAL

No ano de 2022 a produção de organismos aquáticos na aquicultura ultrapassou os valores de captura com uma diferença de 39.9 milhões de toneladas e um crescimento de 7.6% comparado a 2020 (FAO, 2024). O crescimento da aquicultura sobre a pesca, diminui a pressão sobre os estoques pesqueiros e possibilita a restauração da população de peixes em ambientes aquáticos. O ranking de produção aquícola consiste na produção de peixe tendo a carpa em primeiro lugar com 31 mil toneladas, seguido dos crustáceos com predominância dos camarões peneídeos com 7 mil toneladas, e as ostras no grupo dos moluscos com 7 mil toneladas (FAO, 2024). Dentro os produtores, a China ocupa a primeira posição com 53 milhões de toneladas, seguido com Índia, Indonésia e Vietnã (FAO, 2024). Com isso, o crescimento da atividade aquícola está associado com o avanço das formas de manejo, animais mais resistentes e sistemas mais biosseguros.

Apesar dos números de produção terem aumentado desde 1970 e ultrapassado os valores da pesca, o crescimento heterogêneo da aquicultura, dominado pela Ásia, representa um fator negativo, podendo ser um problema sócio-econômico para países costeiros de baixa renda (Sumaila et al., 2022). No entanto, como prática de incentivar países costeiros e que possuem recursos para atividade aquícola, o movimento "Revolução Azul" foi criado, e de acordo com Garlock et al., (2020) apesar do início tardio da atividade em muitos países, a contribuição e rápido crescimento de países não asiáticos tem sido crucial nos últimos anos, mostrando a expansão da atividade em outros lugares.

Esse avanço, além de buscar o aumento da produtividade dos sistemas, também requer o desenvolvimento e aplicação de práticas sustentáveis. Tal requisito tem se tornando mais frequente e valorizam o produto proveniente de sistemas aquícolas sustentáveis. A sustentabilidade aquícola é definida na produção de organismos aquáticos para atender a demanda necessária, sem prejudicar o ecossistema ao redor ou esgotar recursos naturais necessário para manutenção da atividade (Boyd et al., 2020). De acordo com Campanati et al., (2021) uma maior eficiência no uso dos recursos e menor produção de resíduos são fatores chaves na intensificação da aquicultura de forma sustentável.

Apesar da aquicultura se sobressair a produções de gado ou porco em conversão de alimento em biomassa, quando refere-se a uso de água os sistemas convencionais podem ser um fator negativo em sistemas aquícolas (Verdegem et al., 2023). Grande parte do nitrogênio da ração fornecido para o animal é lixiviado na água, e somado com o nitrogênio proveniente da excreção dos animais (Da Silva et al., 2013). Já a matéria orgânica é formada devido ao acumulo de fezes, resto de ração, e produção de biomassa bacteriana (Gaona et al., 2017). Com isso, o uso

de sistemas convencionais tem como característica a troca diária de água para a manutenção da qualidade de água (Amin et al., 2021), e essa liberação da matéria orgânica e nutrientes no corpo de água receptor e sua degradação tem causado problemas de eutrofização e inutilização de corpos de água (Gowen, 1994). Portanto, o surgimento de novos sistemas de cultivo, o uso de espécies extrativistas orgânicas e inorgânicas e a aplicação e valorização do produto são objetivos para um avanço sustentável na aquicultura.

Sistema de bioflocos

Devido ao surgimento de novas doenças a busca por sistemas com mínimas trocas de água promoveram o aparecimento de sistemas mais biosseguros, como o sistema de bioflocos. O controle dos nitrogenados ocorre por meio de um conjunto de microrganismos presente na água, gerando menores quantidade de efluentes para o ecossistema e entrada de água no cultivo (Khanjani et al., 2023; Wasielesky et al., 2013). O sistema de bioflocos pode ser caracterizado devido sua predominância de bactérias no cultivo e fertilização utilizada (Brandão et al., 2021). Diferentes fertilizações iniciais irão proporcionar o crescimento de diferentes bactérias, podendo ser bactérias heterotróficas, bactérias quimioautotróficas ou um sistema misto/maduro com ambas as bactérias presentes no sistema (Ferreira et al., 2021).

O sistema quimioautotrófico é formado a partir de fertilizações químicas prévias a estocagem dos animais, com cloreto de amônio e nitrito de sódio, que tem como característica o aparecimento de bactérias amônio oxidantes primeiro, seguidas pelas bactérias nitrito oxidantes, sendo o nitrato o produto final da oxidação (Ferreira et al., 2021). Como vantagem, o sistema possui uma menor formação de sólidos suspensos totais e consequentemente um menor consumo de oxigênio, além do menor uso de clarificadores mecânicos para remoção dos sólidos em excesso. Devido a baixa produção de sólidos, o uso de substratos artificiais promove a fixação e proliferação de bactérias no sistema, melhorando a qualidade de água (Lara et al., 2021). Por outro lado, as bactérias quimioautotróficas consomem muito mais carbono inorgânico no sistema, sendo necessário manter o nível da alcalinidade acima de 150 mg L^{-1} de $CaCO_3$. Devido ao consumo constante do carbono, o sistema requer a adição de insumos como hidróxido de sódio ou carbonato de cálcio frequentemente no sistema. De acordo com (Furtado et al., 2011) quando ocorrem picos de nitrito no sistema, é aconselhável a manutenção do nível da alcalinidade acima de 300 mg L^{-1} de $CaCO_3$ para favorecer o aparecimento das bactérias nitrito oxidantes. Outra característica do sistema são as altas concentrações de nitrato, como produto final da oxidação o nitrato é acumulado diariamente no sistema (Furtado et al., 2015). Apesar das concentrações letais de nitrato serem elevadas, em altas concentrações são

necessárias o uso de renovações de água para diluir o nitrato no sistema, quando descartado sem tratamento em corpos de água pode causar doenças como metemoglobinemia em humanos (Macedo and Sipaúba-Tavares, 2010).

Diferente do sistema anterior, o sistema heterotrófico consiste na adição de uma fonte de carbono orgânico três dias antes da estocagem dos animais com uma relação de carbono/nitrogênio de 12:1, com a finalidade de aumentar a concentração de sólidos (Brandão et al., 2021). Após a estocagem dos animais, a adição de carbono orgânico pode ocorrer quando a concentração de amônia for superior a 1 mg L^{-1}, mantendo uma relação de 15 a 20 g de carboidrato para cada grama de nitrogênio disponível no sistema (Wasielesky et al., 2013). Essa adição de carbono favorece o crescimento de bactérias heterotróficas que utilizam a amônia disponível no sistema para a formação de biomassa microbiana (Krummenauer et al., 2011). Com isso, a formação de sólidos suspensos totais nesse sistema é muito superior ao sistema quimioautotrófico. Os sólidos suspensos totais na água que devem ser mantidos em níveis de 100 a 350 mg L^{-1} de acordo com Gaona et al., (2017) para que não afete negativamente a performance dos animais, sendo necessário o uso de clarificadores mecânicos para controle.

A utilização de um inóculo de um sistema em bioflocos em andamento também pode ser uma alternativa, sendo mais sustentável com o reuso de água e mais rápido no controle da qualidade de água. Esse sistema pode ser caracterizado como misto/maduro, onde ocorre a presença de ambos os grupos de bactérias, ocorrendo a produção de biomassa bacteriana por bactérias heterotróficas e acúmulo de nitrato por bactérias quimioautotróficas (Ferreira et al., 2021). De acordo com Krummenauer et al., (2014) deve-se realizar pelo menos um 25% de inóculo de um sistema de bioflocos em andamento, para que tenha a entrada de uma quantidade necessária de bactérias no sistema novo que seja eficiente no controle dos compostos nitrogenados. Nesse sistema, apesar do reuso de água de um sistema existente ser uma alternativa sustentável e fácil, o acumulo de matéria orgânica e nitrato ao final do cultivo pode gerar prejuízos ao ambiente quando ocorre o despejo do efluente sem tratamento prévio (Gowen, 1994).

Além dos benefícios no controle dos parâmetros de qualidade de água e biossegurança, o uso do sistema em bioflocos pode ser uma fonte suplementar para os organismos cultivados. A formação de biomassa bacteriana consiste em uma fonte de proteína, lipídio, vitaminas e micronutrientes, que irá variar de valor nutricional de acordo com variáveis do cultivo, como densidade, organismo cultivado e fertilização utilizados(Ahmad et al., 2017). Khanjani et al., (2023) mostraram que os valores de proteína encontrados podem variar de 25.54 a 39.10% do peso seco, no entanto devido a uma grande quantidade de minerais os valores de cinzas também

podem apresentar altas concentrações, variando de 25.02 – 41% do peso seco. Por possuir um atrativo valor nutricional, estudos como Jatobá et al., (2014) mostram que reduções no valor de proteína bruta nas dietas são possíveis em cultivos de semi-intensivos de camarão em viveiros, reduzindo o custo. A redução da porcentagem de ração ofertada para a tilápia também é possível quando cultivada em sistema de bioflocos, de acordo com Oliveira et al., (2021) juvenis de tilápia podem ser alimentados entre 4.3 e 6.1% do peso corporal por dia quando cultivados em bioflocos sem que seu desempenho zootécnico seja afetado, comparado a uma taxa de 10% do peso corporal por dia em tilápias cultivadas em água clara. Em sistema integrado essa taxa de arraçoamento pode ser mais baixa, sendo de 1% da biomassa do cultivo, como trabalhos de Holanda et al., (2022) e Poli et al., (2019), tendo como objetivo a indução do consumo dos flocos pelos peixes.

Outra forma de aproveitamento dos bioflocos é na fabricação de farinha para inclusão na ração, trabalhos como Gamboa-Delgado et al., (2011) e Bauer et al., (2012) mostram a possibilidade de substituição da farinha de peixe por farinha de bioflocos para o camarão *L. vannamei* e a tilápia *O. niloticus*. A produção constante de sólidos no sistema se torna favorável para a produção de farinha, no entanto as desvantagens do processo estão associadas com altas concentrações de cinzas e na coleta de grandes quantidades de floco para produção de ração. Portanto, apesar de ser um sistema novo, grandes são os avanços nas formas de manejo e aplicabilidade, podendo ser um sistema amplamente utilizado futuramente e com maior sustentabilidade ambiental.

Aquicultura Multitrófica Integrada (IMTA)

O avanço da aquicultura além de estar focado na produtividade do sistema, melhores crescimentos e aumento da densidade, também requer, biossegurança e sustentabilidade. A liberação de efluentes tem sido um dos grandes desafios de sustentabilidade em sistemas intensivos, causando problemas de eutrofização nos corpos de água (Sarà et al., 2018). O acúmulo de compostos inorgânicos e orgânicos no decorrer do cultivo constitui grande parte dos resíduos produzidos e não utilizados em sistemas de monocultivo, onde apenas uma espécie é cultivada e comercializada. Com isso, a Aquicultura Multitrófica integrada (*Integrated Multitrophic Aquaculture* – IMTA) surgiu como uma possibilidade do aproveitamento dos resíduos gerados através da inserção de diferentes espécies. O sistema é formado por uma espécie principal, de maior nível trófico, como peixes e crustáceos, e por espécies secundárias de nível trófico inferior que irão consumir os resíduos gerados pela espécie principal (Chopin, 2015; Troell et al., 2009).

A inserção de consumidores orgânicos, como peixes de baixo nível trófico e bivalves, promove a redução dos sólidos suspensos totais sem a necessidade do uso de clarificadores mecânicos, ou trocas de água. Holanda et al., (2020) mostrou que a integração da tainha ao cultivo do camarão em sistema de bioflocos quando dispostos em tanques diferentes, promoveu redução dos sólidos e aumento da produtividade. De acordo com Oliveira et al., (2021) quando cultivada em sistema de bioflocos a taxa de alimentação para a tilápia pode ser reduzida de 10% para 4 a 6% da biomassa. No entanto trabalhos como Holanda et al., (2022) e Poli et al., (2019) reduziram essa taxa para 1% quando cultivada em sistema integrado em bioflocos, com intuito de intensificar a filtração e consumo do floco pela tilápia, obtendo ainda crescimento e sobrevivência satisfatória. A tilápia tem estado em quarto lugar entre as espécies mais produzidas (FAO, 2024), sendo uma espécie secundária no sistema integrado que possui valor econômico agregado e procura no mercado consumidor. O crescimento da produção da tilápia deve-se a facilidade na obtenção de juvenis, o rápido crescimento e possibilidade do cultivo em diversos lugares, sendo uma espécie altamente produzida no Brasil (Santos et al., 2020).

Os bivalves também são organismos promissores para inserção no IMTA. Costa et al., (2021) mostraram que apesar da presença da ostra *Cassostrea gasar* no cultivo do camarão não influenciar na concentração de sólidos do cultivo, ocorreu uma predominância de flagelados no conteúdo estomacal da ostra, comprovando que a ostra conseguiu se beneficiar do sistema. Quando cultivada em sistema simbiótico com camarão, a ostra *Cassostrea* sp. mostrou melhor desempenho, reduzindo as concentrações de sólidos sedimentáveis comparado ao monocultivo de camarão (Lima et al., 2021). Apesar do uso de ostras ser vantajoso devido não ser necessária o aporte de ração, a alta carga de sólidos suspensos totais em sistemas intensivos se torna um desafio para o cultivo da espécie (Costa et al., 2023).

Além da matéria orgânica, o acúmulo de nitrogênio e fosforo também são frequentes em sistemas intensivos. Para controle desses compostos a inserção de consumidores inorgânicos se torna vantajoso para a sustentabilidade do sistema com aumento da produtividade. Custódio et al., (2017) explanam sobre o potencial da integração de halófitas em sistemas integrados para remoção de nitrato e fosfato do cultivo, além de possuírem potencial para alimentação humana e extração de metabólitos secundários para indústria farmacêutica.

O uso de macroalgas em sistema integrado tem crescido nos últimos anos devido a facilidade de manejo do grupo, absorção de nutrientes e valor econômico agregado. Segundo a FAO, (2022) produção de macroalgas pertence majoritariamente a aquicultura, tendo um crescimento de 1.4% na produção de 2019 para 2020. A ação de biorremediador da macroalga no sistema e diferentes formas de manejo tem sido avaliado em trabalhos como Alencar et al., (2010), que

testaram diferentes densidades da macroalga *Ulva lactuca* em escala laboratorial para melhor remoção dos nutrientes, apresentando uma remoção de 90% da amônia e 89% do ortofosfato do sistema na densidade de 3 g. L^{-1}. Alguns estudos em escala-piloto e offshore vem sendo realizado com macroalgas em sistema integrado. Resende et al., (2022) testaram a inserção da macroalga *Ulva* spp. em tanques com dourada e robalo em ambiente estuarino, constaram o aumento do nitrogênio no tecido da macroalga indicando a assimilação dos nutrientes. E Huo et al., (2012) mostraram que o cultivo da macroalga com *Pseudosciaena crocea* em meio off-shore foi eficaz na remoção dos nutrientes, estabelecendo uma proporção ótima de biomassa de macroalga para biomassa de peixe.

De acordo com Granada et al. (2018) o estabelecimento das espécies adequadas no sistema e práticas de manejo adequadas, o sistema IMTA oferece como vantagem além da bioremediação, o aumento do lucro a partir dos subprodutos vendidos das espécies secundárias, o aumento da economia local com geração de empregos, diversas colheitas no ano e controle de doenças. Mas apesar das vantagens, ainda existem desafios no sistema que dificultam o IMTA ser uma realidade na aquicultura mundial. A diversificação e escolha das espécies requer maior atenção e mão de obra qualificada, com o objetivo de manter a qualidade de água ideal para o cultivo de todas as espécies. Para a macroalga *Ulva lactuca* salinidades inferiores a 20 ppm podem afetar o crescimento da macroalga e seus bioprodutos (Bews et al., 2021), já para o camarão *Litopenaeus vannamei* por ser uma espécie eurihalina consegue se adaptar em amplitudes de salinidade de 5 a 40 ppm (Decamp et al., 2003), mostrando uma boa interação entre as espécies. No entanto, devido ao acumulo de matéria orgânica causado pelas excretas, restos de ração e biomassa microbiana (sistema de bioflocos) no cultivo de camarão, podem interferir no desempenho da macroalga devido a deposição dos sólidos sobre seu tecido (Carvalho et al., 2023b). Portanto, diversos estudos ainda devem ser realizados para implementar as práticas de manejo para maximizar o desempenho de todos os organismos no sistema. Assim também a realização dos resultados dos experimentos em escala comercial dá mais espaço ao avanço e aplicação do IMTA na aquicultura.

Produção de macroalgas

Segundo os dados de macroalga da FAO, (2024), aproximadamente 37 milhões de toneladas foi produzido no ano 2022 proveniente quase exclusivo da aquicultura. Tal crescimento com um aumento de 4% comparado aos dados de 2020, é decorrente da valorização da macroalga por compostos bioativos, alto valor de proteína e potencial como alimento saudável de baixa caloria (Mildenberger et al., 2022). Essa produção é dominada pela China

em primeiro lugar, seguido da Indonésia, Coreia do Sul e Filipinas, com a produção majoritariamente de algas vermelhas e pardas (FAO, 2024).

A produção em larga escala de macroalgas é predominantemente proveniente de áreas abertas e costeiras, tendo influência da disponibilidade de nutrientes, incidência de ondas e presença de herbívoros (FAO, 2024). Alguns desses fatores foram testados por Biancacci et al., (2022), como profundidade (1 – 5m), local (Okehampton Bay e Great Taylor Bay, na Tasmania) e estação do ano (Abril – Novembro) com efeito na produtividade, biofouling e composição bioquímica, e estabeleceram que as macroalgas deveriam ser colhidas entre Julho e Agosto para menor biofouling e maior produtividade na profundidade de 1 m, mostrando que a descrição do ambiente e formas de manejo são decisivos para produção da macroalga.

Além do maior crescimento das macroalgas, seu cultivo em um ambiente com diferentes fatores físicos e químicos pode proporcionar mudanças em sua composição bioquímica e nutricional (Duke et al., 1989). Em estudos pretéritos foi observado o aumento de proteína da macroalga *U. fasciata* quando cultivada em sistema integrado com bioflocos, com valores de 22,4% comparado ao cultivo em solução de laboratório de 12,40% de proteína, aumentando seu valor nutricional (Carvalho et al., 2023b). He et al. (2016) descreveram alguns fatores que influenciam no aumento da capacidade antioxidante e teor de fenóis, como salinidades inferiores a 25 aumentam a disponibilidade de enzimas antioxidantes, assim como a depleção ou altas concentrações de nutrientes podem influenciar na concentração dos aminoácidos e ácidos graxos na macroalga. Lourenço et al. (2002) descreveram aminoácidos importantes presentes na macroalga *U. fasciata* como, arginina, histidina, lisina, isoleucina e leucina. Também Queirós et al. (2021) avaliando a composição da macroalga *Ulva* no cultivo integrado em diferentes estações mostraram que altos valores de proteína foram encontrados no inverno, sendo vantajoso a realização de colheitas em um período em que a macroalga possuirá maior valor de mercado. Portanto, diferentes cultivos podem maximizar a concentração de bioprodutos extraídos da macroalga.

No entanto, além de fatores bióticos e abióticos como concentração de nutrientes e biofouling, Thomas et al. (2019), avaliando áreas para cultivo de macroalgas, estabeleceu que concentração de clorofila, descarte de óleo, transito de navios e áreas de pesca como fatores essenciais na implantação de cultivos, mostrando que recentemente a uso antrópico das áreas costeiras tem delimitado muito mais o cultivo do que a disponibilidade de nutrientes. Com isso, integrar a produção de macroalgas com outras atividades aquícolas proporciona melhor uso de área, aproveitamento de resíduos e aumento da produtividade.

O uso de macroalgas como agente biorremediador em cultivos de peixes e camarões tem aumentado cada vez mais como forma de mitigar impactos na produção e liberação de nutrientes. As macroalgas absorvem nitrogênio e fosforo como fonte de energia, e realização de processos fisiológicos e bioquímicos essenciais para crescimento e reprodução (Duke et al., 1989), portanto altas concentrações desses nutrientes podem causar uma proliferação exacerbada de macroalgas bentônicas (Zirino et al., 2016). Uma forma de avaliar o potencial do uso dos nitrogenados pela macroalga e sua viabilidade na inserção em cultivos é realizando a cinética de absorção, em um estudo realizado por Smart et al. (2022) mostraram que ambas as macroalgas pardas *Macrocystis pyrifera* e *Phyllospora comosa* obtiveram uma cinética máxima de absorção com 200 μmol gDW^{-1} h^{-1}, apresentando potencial na absorção de nutrientes. Em um estudo mais aplicado com a inserção da macroalga *Gracilaria* no cultivo com camarão em viveiros testando o uso de redes tubulares e gaiolas mostrou que a macroalga absorveu 49% do nitrato e 12% do fosfato produzido em 4 horas, supondo que 1 ha de macroalga teria o potencial de absorver 0.3 toneladas ha^{-1} ano^{-1} de nitrogênio e 0.02 toneladas ha^{-1} ano^{-1} de fosforo (Marinho-Soriano et al., 2009).

Apesar da produção de macroalgas e uso em sistemas integrados serem predominantemente de macroalgas vermelhas e pardas (FAO, 2024), o uso macroalgas verdes vem expandindo cada vez mais devido a diversificação de produtos extraído da macroalga, sendo aplicada desde industrias de biorefinaria, produção de celulose, bioplástico, e culinária moderna (Moreira et al., 2022). Com isso, a presença de características nutricionais e bioquímicas valorosas na macroalga faz com que seu cultivo e aplicação em cultivos integrados seja viável. Hernández et al. (2005) avaliaram a inserção da macroalga vermelha *Gracilariopsis longissima* e a macroalga verde *Ulva rotundata* como agente biorremediador em cultivo integrado com a dourada *Sparus aurata* e mostrou que a taxa de absorção de fosfato (8%) e nitrogênio (54%) foi superior com a macroalga verde em comparação a vermelha, além de uma maior produção de biomassa. Alguns trabalhos com o gênero *Ulva* como Alencar et al. (2010) e Copertino et al. (2009) mostraram crescimentos acima de 8,00% dia^{-1} e absorção de 90% da amônia disponível no cultivo em cultivos com efluente da Carcinocultura.

Além da inserção da macroalga em sistemas integrados abertos ou sistemas em terra extensivos, o uso de macroalgas em sistemas intensivos tem aumentado cada vez mais devido ao acumulo exacerbado da matéria orgânica e nutriente comparados a sistemas convencionais, como realizado por de Morais et al. (2023) e Legarda et al. (2021b) usando macroalgas do gênero *Ulva* em sistema de bioflocos. Para as macroalgas, a obtenção do sucesso na produção de biomassa em sistema bioflocos requer uma adaptação ao meio, no entanto, seu crescimento

pode sofrer interferências devido a deposição de sólidos suspensos totais no tecido fotossintetizante (Brito et al., 2014), a baixa entrada de luz na água e competição por espaço na estrutura (Alencar et al., 2010), ocasionando um mal desempenho. Assim como a integração da macroalga com o camarão também pode ocasionar eventos de herbivoria pelo camarão, causando uma diminuição na biomassa de macroalgas, Brito et al. (2014) tiveram como resultado uma melhora no desempenho zootécnico do camarão, como ganho de peso, em sistema integrado com a macroalga *Ulva lactuca*. Portanto, tais lacunas devem ser respondidas para a melhor produção de biomassa de macroalgas em sistema integrado com bioflocos.

Economia circular

Com recursos limitados e os grandes impactos ambientais, medidas de recuperação e aproveitamento de resíduos e produtos têm sido aplicadas na aquicultura como estímulo a novas práticas de gestão (Campanati et al., 2021). A transformação de produtos faz parte do conceito de "Economia circular", onde recursos produzidos no sistema são reutilizados várias vezes em um circuito fechado (Cornejo-Ponce et al., 2020). Portanto, emprega-se o reaproveitamento de materiais com a maximização da aplicabilidade dos produtos gerados no sistema.

Quando aplicada a aquicultura, o termo economia circular reflete na adoção de práticas relacionadas a uma melhor utilização dos nutrientes produzidos, o gerenciamento de resíduos e a utilização de novos ingredientes para substituição na ração (Cooney et al., 2023). Tal conceito tem sido promovido pela Comissão Europeia como uma economia verde aplicada em setores marítimos e costeiros, como forma de destacar opções potenciais de produtos e reciclagem de resíduos, com foco em fornecer uma aquicultura sustentável (Campanati et al., 2021). Um método de avaliação e quantificação da sustentabilidade de um sistema foi proposto por Valenti et al. (2018), que relacionam 14 indicadores de sustentabilidade econômica, 22 indicadores ambientais e 20 indicadores sociais com o objetivo de quantificar a eficiência no uso de recursos, viabilidade e resiliência da produção e a capacidade de gerar benefícios na geração de renda e empregos.

O sistema multitrófico integrado, baseando-se na reutilização de resíduos por diferentes espécies se enquadra nos princípios da circularidade, e como forma de quantificar esses atributos Checa et al. (2024) avaliaram o gerenciamento de nutrientes e a eficiência no uso de recursos em quatro sistemas e em três instalações na Irlanda, Brasil e África do sul e tiveram como resultado um aumento de 90% dos princípios da circularidade com uso de água e 80 a 90% em biorremediação. E em adição a essa melhora da circularidade, a aplicação da biomassa

produzida no sistema também fornece maior sustentabilidade e um circuito onde o produto volta novamente ao sistema.

Por apresentar algumas características de fins nutricionais, as macroalgas do gênero *Ulva* passaram a serem utilizadas como aditivos ou substitutos de alguns ingredientes na ração de camarões e peixes. Corral-Rosales et al. (2018) utilizaram a macroalga como aditivo em dietas para maturação, devido as suas concentrações de carotenóides, vitaminas e polissacarídeos, gerando resultados significativos de maiores desovas por dia pelas fêmeas alimentadas com a dieta de macroalgas. Em outro trabalho, Corral-Rosales et al. (2018a) descreveram que a inclusão da macroalga *Ulva clathrata* na dieta para maturação de camarões pode ser usada para diminuir a exaustão e mortalidade das fêmeas na desova, e proporcionar um maior número total de ovos, naúplios por desova e taxa de incubação. A adição de macroalgas também é utilizada em dietas para peixes, Saleh et al. (2014) observaram que altas inclusões (75 $g.kg^{-1}$ ou 100 $g.kg^{-1}$) melhorou significativamente o crescimento, o teor de proteína e ácidos graxos insaturados da tilápia vermelha. Portanto, a produção de biomassa de macroalgas com elevado teor nutricional representa um produto de qualidade que poderá voltar ao sistema de produção como um fitorremediador ou aditivo na ração de organismos aquáticos.

ESTRUTURA DA TESE

A tese esta composta de sete capítulos. O primeiro capítulo busca avaliar o desempenho da macroalga em sistema simbiótico em cultivo integrado com camarão e ostra (submetido na *Aquaculture International*). O segundo capítulo avalia o efeito das diferentes concentrações de sólidos suspensos totais e nutrientes do sistema em bioflocos no crescimento, absorção de nutrientes e composição nutricional da macroalga em sistema integrado com camarão (publicado na *Phycology*). O terceiro capítulo consiste em avaliar a deposição de sólidos na macroalga no decorrer do cultivo e definir uma profundidade de estrutura de cultivo para inserção da macroalga em cultivo integrado com camarão e peixe em sistema de bioflocos (publicado na *Phycology*). O quarto capítulo avaliou o efeito da inserção da macroalga *Ulva lactuca* na composição do bioflocos em cultivo integrado com camarão (publicado na *Crustaceana*). O quinto capítulo consiste em avaliar o crescimento, a absorção dos nutrientes e composição nutricional da macroalga *Ulva lactuca* em duas estratégias de bioflocos: sistema heterotrófico e sistema quimioautotrófico (publicado na *Fishes*). O sexto capítulo avaliou a ação da macroalga como biorremediador no tratamento de efluente de um sistema integrado com bioflocos. O sétimo capítulo promove a economia circular do sistema, avaliando a inclusão da macroalga produzida em sistema integrado em bioflocos na dieta do peixe *Oreochromis niloticus*.

OBJETIVOS

Objetivo geral

Determinar estratégias de manejo para a execução do cultivo integrado do camarão *Litopenaeus vannamei,* da tilápia *Oreochromis niloticus* e a macroalga *Ulva lactuca*, com enfoque na produção de biomassa, absorção de nutrientes e composição nutricional da macroalga para aplicação da biomassa.

Objetivos específicos

Avaliar o efeito do sistema simbiótico no crescimento da macroalga em sistema integrado.

Avaliar a adição da macroalga na absorção de nutrientes em sistema integrado com camarão e ostra em sistema simbiótico.

Avaliar o efeito de diferentes concentrações de sólidos suspensos totais e nutrientes provenientes do sistema em bioflocos no crescimento, absorção de nutrientes e composição proximal e bioquímica da macroalga *Ulva lactuca*.

Determinar a melhor profundidade da estrutura de cultivo para o crescimento das macroalgas em sistema integrado com o camarão e peixe.

Avaliar a deposição de sólidos suspensos totais e sólidos sedimentáveis no decorrer do cultivo na macroalga *U. lactuca* em sistema integrado com camarão e peixe.

Avaliar o efeito da inserção da macroalga na comunidade microbiana do sistema em bioflocos.

Avaliar a absorção de nutrientes, composição proximal e bioquímica da macroalga cultivada em diferentes estratégias de bioflocos: sistema misto e sistema quimioautotrófico.

Analisar o efeito da macroalga como biorremediador no efluente de um sistema integrado com camarão e peixe com diferentes abordagens de fertilização.

Avaliar o efeito no desempenho zootécnico e bioquímica no peixe *Oreochromis niloticus* quando alimentado com diferentes inclusões da macroalga *Ulva lactuca* produzida em sistema integrado com bioflocos.

Referências

Ahmad, I., Babitha Rani, A.M., Verma, A.K., Maqsood, M., 2017. Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition. Aquac. Int. 25, 1215–1226. https://doi.org/10.1007/s10499-016-0108-8

Alencar, J.R. de, Junior, P.A.H., Celino, J.J., 2010. Cultivo de Camarão Branco Litopenaeus Vannamei (Boone , 1931) com a Macro- alga Ulva Lacuata Linneaus (Chlorophyta) no Tratamento de Efluentes em Sistema Fechado de Recirculação Cultivation of White Shrimp Litopenaeus Vannamei (Boone , 1931) with th. Rev. Biol. e Ciências da Terra 10, 117–137.

American Public Health Association (APHA), 1989. Standard Methods for the Examination of Water and Waste Water, 16th ed. American Public Health Association.

Amin, M., Agustono, A., Prayugo, P., Ali, M., Hum, N.N.M.F., 2021. Comparison of total nutrient recovery in aquaponics and conventional aquaculture systems. Open Agric. 6, 682–688. https://doi.org/10.1515/opag-2021-0032

Aminot, A., Chaussepied, M., 1983. Manuel des analyses chimiques en milieu marin. Centre National pour l'exploitation des océans, França.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, (21st edn). ed. American Public Health Association, Washington, DC, USA.

Astorg, P., 1997. Food carotenoids and cancer prevention: An overview of current research. Trends Food Sci. Technol. 8, 406–413. https://doi.org/10.1016/S0924-2244(97)01092-3

Baethgen, W.E & Alley, M.., 1989. A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant Kjeldahl digests. Communications in Soil Science and Plant Analysis.

Barbarino, E., Lourenço, S.O., 2005. An evaluation of methods for extraction and quantification of protein from marine macro- and microalgae. J. Appl. Phycol. 17, 447–460. https://doi.org/10.1007/s10811-005-1641-4

Bauer, W., Prentice-Hernandez, C., Tesser, M.B., Wasielesky, W., Poersch, L.H.S., 2012. Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the pacific white shrimp Litopenaeus vannamei. Aquaculture 342–343, 112–116. https://doi.org/10.1016/j.aquaculture.2012.02.023

Baumgarten, M. D. G. Z., De Barros Rocha, J. M., & Niencheski, L.F.H., 1996. Manual de análises em oceanografia química.

Bendschneider, K., Robinson, R.J., 1952. New spectrophotometric method for the determination of nitrite in water. Fresenius Environ. Bull.

Bews, E., Booher, L., Polizzi, T., Long, C., Kim, J.H., Edwards, M.S., 2021. Effects of salinity and nutrients on metabolism and growth of Ulva lactuca: Implications for bioremediation of coastal watersheds. Mar. Pollut. Bull. 166, 112199. https://doi.org/10.1016/j.marpolbul.2021.112199

Biancacci, C., Visch, W., Callahan, D.L., Farrington, G., Francis, D.S., Lamb, P., McVilly, A., Nardelli, A., Sanderson, J.C., Schwoerbel, J., Hurd, C.L., Evans, B., Macleod, C., Bellgrove, A., 2022. Optimisation of at-sea culture and harvest conditions for cultivated Macrocystis pyrifera: yield, biofouling and biochemical composition of cultured biomass. Front. Mar. Sci. 9, 1–19. https://doi.org/10.3389/fmars.2022.951538

Bogue, J.P., Smith, L.F., Lipsett, L., 1957. A Practical Handbook. J. Higher Educ. 28, 405. https://doi.org/10.2307/1979241

Boyd, C.E., D'Abramo, L.R., Glencross, B.D., Huyben, D.C., Juarez, L.M., Lockwood, G.S., McNevin, A.A., Tacon, A.G.J., Teletchea, F., Tomasso, J.R., Tucker, C.S., Valenti, W.C., 2020. Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. J. World Aquac. Soc. 51, 578–633. https://doi.org/10.1111/jwas.12714

Brandão, H., Xavier, Í.V., Santana, G.K.K., Santana, H.J.K., Krummenauer, D., Wasielesky, W., 2021. Heterotrophic versus mixed BFT system: Impacts on water use, suspended solids production and growth performance of Litopenaeus vannamei. Aquac. Eng. 95. https://doi.org/10.1016/j.aquaeng.2021.102194

Brito, L.O., Arantes, R., Magnotti, C., Derner, R., Pchara, F., Olivera, A., Vinatea, L., 2014. Water quality and growth of Pacific white shrimp Litopenaeus vannamei (Boone) in co-culture with green seaweed Ulva lactuca (Linaeus) in intensive system. Aquac. Int. 22, 497–508. https://doi.org/10.1007/s10499-013-9659-0

Buck, B.H., Troell, M.F., Krause, G., Angel, D.L., Grote, B., Chopin, T., 2018. State of the art and challenges for offshore Integrated multi-trophic aquaculture (IMTA). Front. Mar. Sci. https://doi.org/10.3389/fmars.2018.00165

Campanati, C., Willer, D., Schubert, J., Aldridge, D.C., 2021. Sustainable Intensification of Aquaculture through Nutrient Recycling and Circular Economies: More Fish, Less Waste, Blue Growth. Rev. Fish. Sci. Aquac. https://doi.org/10.1080/23308249.2021.1897520

Carl, C., De Nys, R., Lawton, R.J., Paul, N.A., 2014. Methods for the induction of reproduction in a tropical species of filamentous Ulva. PLoS One 9, 2–11. https://doi.org/10.1371/journal.pone.0097396

Carneiro, M.A.D.A., 2007. Estudo do crescimento, eficiência de biofiltração e cinética de absorção de nutrientes (N-NH, N-NO e P-$PO4^{3}$) da macroalga Gracilaria cervicornis (Turner) J. Agardh 75.

Carvalho, A., Costa, L.C. de O., Holanda, M., Gonçalves, M., Santos, J., Costa, C.S.B., Turan, G., Poersch, L.H., 2023a. Growth of the Macroalgae Ulva lactuca Cultivated at Different Depths in a Biofloc Integrated System with Shrimp and Fish. Phycology 3, 280–293. https://doi.org/10.3390/phycology3020018

Carvalho, A., Costa, L.C. de O., Holanda, M., Poersch, L.H., Turan, G., 2023b. Influence of Total Suspended Solids on the Growth of the Sea Lettuce Ulva lactuca Integrated with the Pacific White Shrimp Litopenaeus vannamei in a Biofloc System. Fishes 8, 163. https://doi.org/10.3390/fishes8030163

Castelar, B., Reis, R.P., dos Santos Calheiros, A.C., 2014. Ulva lactuca and U. flexuosa (Chlorophyta, Ulvophyceae) cultivation in Brazilian tropical waters: Recruitment, growth, and ulvan yield. J. Appl. Phycol. 26, 1989–1999. https://doi.org/10.1007/s10811-014-0329-z

Chemists, A. of O.A., 2005. Official methods of analysis.

Chopin, T., 2015. Marine Aquaculture in Canada: Well-Established Monocultures of Finfish and Shellfish and an Emerging Integrated Multi-Trophic Aquaculture (IMTA) Approach Including Seaweeds, Other Invertebrates, and Microbial Communities. Fisheries 40, 28–31. https://doi.org/10.1080/03632415.2014.986571

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche-González, J.A., Yarish, C., Neefus, C., 2001. Integrating seaweeds into marine aquaculture systems: A key toward sustainability. J. Phycol. 37, 975–986. https://doi.org/10.1046/j.1529-8817.2001.01137.x

Cooney, R., de Sousa, D.B., Fernández-Ríos, A., Mellett, S., Rowan, N., Morse, A.P., Hayes, M., Laso, J., Regueiro, L., Wan, A.H., Clifford, E., 2023. A circular economy framework for seafood waste

valorisation to meet challenges and opportunities for intensive production and sustainability. J. Clean. Prod. 392. https://doi.org/10.1016/j.jclepro.2023.136283

Copertino, M.D.S., Tormena, T., Seeliger, U., 2009. Biofiltering efficiency, uptake and assimilation rates of Ulva clathrata (Roth) J. Agardh (Clorophyceae) cultivated in shrimp aquaculture waste water. J. Appl. Phycol. 21, 31–45. https://doi.org/10.1007/s10811-008-9357-x

Cornejo-Ponce, L., Vilca-Salinas, P., Lienqueo-Aburto, H., Arenas, M.J., Pepe-Victoriano, R., Carpio, E., Rodríguez, J., 2020. Integrated aquaculture recirculation system (Iars) supported by solar energy as a circular economy alternative for resilient communities in arid/semi-arid zones in southern south america: A case study in the camarones town. Water (Switzerland). https://doi.org/10.3390/w12123469

Corral-Rosales, D.C., Cruz-Suárez, L.E., Ricque-Marie, D., Rodríguez-Jaramillo, C., Palacios, E., 2018a. Modulation of reproductive exhaustion using Ulva clathrata in Pacific white shrimp Litopenaeus vannamei (Boone, 1931) broodstock during commercial maturation. Aquac. Res. 49, 3711–3722. https://doi.org/10.1111/are.13839

Corral-Rosales, D.C., Palacios, E., Ricque-Marie, D., Cruz-Suárez, L.E., 2018b. Enhancement of reproductive performance in shrimp Litopenaeus vannamei (Boone, 1931) by supplementation of Ulva clathrata meal in maturation diet in two commercial hatcheries. Aquac. Res. 49, 1053–1059. https://doi.org/10.1111/are.13554

Costa, L.C. de O., Carvalho, A., Holanda, M., Santos, J., Borges, L., Guterres, B., Nam Junior, J., Fonseca, V., Muller, L., Romano, L., Botelho, S., Pias, M., Ventura, J., Poersch, L.H., 2023. Biological Responses of Oyster Crassostrea gasar Exposed to Different Concentrations of Biofloc. Fishes 8. https://doi.org/10.3390/fishes8120586

Costa, L.C. de O., Poersch, L.H. da S., Abreu, P.C., 2021. Biofloc removal by the oyster Crassostrea gasar as a candidate species to an Integrated Multi-Trophic Aquaculture (IMTA) system with the marine shrimp Litopenaeus vannamei. Aquaculture 540. https://doi.org/10.1016/j.aquaculture.2021.736731

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., Verstraete, W., 2007. Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270, 1–14. https://doi.org/10.1016/j.aquaculture.2007.05.006

Custódio, M., Villasante, S., Cremades, J., Calado, R., Lillebø, A.I., 2017. Unravelling the potential of halophytes for marine integrated multi-trophic aquaculture (IMTA)- A perspective on performance, opportunities and challenges. Aquac. Environ. Interact. 9, 445–460. https://doi.org/10.3354/AEI00244

Da Silva, K.R., Wasielesky, W., Abreu, P.C., 2013. Nitrogen and Phosphorus Dynamics in the Biofloc Production of the Pacific White Shrimp, Litopenaeus vannamei. J. World Aquac. Soc. 44, 30–41. https://doi.org/10.1111/jwas.12009

de Morais, A.P.M., Santos, I.L., Carneiro, R.F.S., Routledge, E.A.B., Hayashi, L., de Lorenzo, M.A., do Nascimento Vieira, F., 2023. Integrated multitrophic aquaculture system applied to shrimp, tilapia, and seaweed (Ulva ohnoi) using biofloc technology. Aquaculture 572. https://doi.org/10.1016/j.aquaculture.2023.739492

de Souza, R.L., de Lima, E.C.R., de Melo, F.P., Ferreira, M.G.P., de Souza Correia, E., 2019. The culture of Nile tilapia at different salinities using a biofloc system. Rev. Cienc. Agron. 50, 267–275. https://doi.org/10.5935/1806-6690.20190031

Decamp, O., Cody, J., Conquest, L., Delanoy, G., Tacon, A.G.J., 2003. Effect of salinity on natural community and production of Litopenaeus vannamei (Boone), within experimental zero-water

exchange culture systems. Aquac. Res. 34, 345–355. https://doi.org/10.1046/j.1365-2109.2003.00842.x

Duke, C.S., Litaker W., Ramus J, 1989. Effects of the temperature, nitrogen supply and tissue nitrogen on ammonium uptake rates of the Chlorophyte seaweeds *Ulva curvata* and *Codium decorticatum*. J. Phycol.

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

Eismann, A.I., Perpetuo Reis, R., Ferreira da Silva, A., Negrão Cavalcanti, D., 2020. Ulva spp. carotenoids: Responses to environmental conditions. Algal Res. 48. https://doi.org/10.1016/j.algal.2020.101916

FAO, 2022. State of the World Fisheries and Aquaculture - 2022 (SOFIA).

FAO, 2024, 2024. World fisheries and aquaculture in review, State of the World Fisheries and Aquaculture.

Farahdiba, A.U., Hidayah, E.N., Asmar, G.A., Myint, Y.W., 2020. Growth and Removal of Nitrogen and Phosphorus by a Macroalgae Cladophora glomerata under Different Nitrate Concentrations. Nat. Environ. Pollut. Technol. 19, 809–813. https://doi.org/10.46488/NEPT.2020.V19I02.038

Farasat, M., Khavari-Nejad, R.-A., Nabavi, S.M.B., Namjooyan, F., 2013. Antioxidant Properties of two Edible Green Seaweeds From Northern Coasts of the Persian Gulf. Jundishapur J. Nat. Pharm. Prod. 8, 47–52. https://doi.org/10.17795/jjnpp-7736

Ferreira, G.S., Santos, D., Schmachtl, F., Machado, C., Fernandes, V., Bögner, M., Schleder, D.D., Seiffert, W.Q., Vieira, F.N., 2021. Heterotrophic, chemoautotrophic and mature approaches in biofloc system for Pacific white shrimp. Aquaculture 533. https://doi.org/10.1016/j.aquaculture.2020.736099

Fillit, M., 1995. Seasonal Changes in the Photosynthetic Capacities and Pigment Content of Ulva rigida in a Mediterranean Coastal Lagoon. Bot. Mar. 38, 271–280. https://doi.org/10.1515/botm.1995.38.1-6.271

Fleckenstein, L.J., Tierney, T.W., Fisk, J.C., Ray, A.J., 2020. The effects of different solids and biological filters in intensive pacific white shrimp (Litopenaeus vannamei) production systems. Aquac. Eng. 91. https://doi.org/10.1016/j.aquaeng.2020.102120

Furtado, P.S., Campos, B.R., Serra, F.P., Klosterhoff, M., Romano, L.A., Wasielesky, W., 2015. Effects of nitrate toxicity in the Pacific white shrimp, Litopenaeus vannamei, reared with biofloc technology (BFT). Aquac. Int. 23, 315–327. https://doi.org/10.1007/s10499-014-9817-z

Furtado, P.S., Poersch, L.H., Wasielesky, W., 2011. Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical performance of shrimp Litopenaeus vannamei reared in bio-flocs technology (BFT) systems. Aquaculture 321, 130–135. https://doi.org/10.1016/j.aquaculture.2011.08.034

Gabriele Rodrigues de Lara, Luís Henrique Poersch, W.W., 2021. The quantity of artificial substrates influences the nitrogen cycle in the biofloc culture system of Litopenaeus vannamei. Aquac. Eng. 94.

Gamboa-Delgado, J., Peña-Rodríguez, A., Ricque-Marie, D., Cruz-Sáurez, L.E., 2011. Assessment of nutrient allocation and metabolic turnover rate in pacific white shrimp litopenaeus vannamei co-fed live macroalgae ulva clathrata and inert feed: Dual stable isotope analysis. J. Shellfish Res. 30, 969–978. https://doi.org/10.2983/035.030.0340

Ganesan, M., Mairh, O.P., Eswaran, K., Subba Rao, P. V., 1999. Effect of salinity, light intensity and nitrogen source on growth and composition of Ulva fasciata Delile (Chlorophyta, Ulvales). Indian J. Mar. Sci. 28, 70–73.

Gaona, C.A.P., de Almeida, M.S., Viau, V., Poersch, L.H., Wasielesky, W., 2017. Effect of different total suspended solids levels on a Litopenaeus vannamei (Boone, 1931) BFT culture system during biofloc formation. Aquac. Res. 48, 1070–1079. https://doi.org/10.1111/are.12949

Garlock, T., Asche, F., Anderson, J., Bjørndal, T., Kumar, G., Lorenzen, K., Ropicki, A., Smith, M.D., Tveterås, R., 2020. A Global Blue Revolution: Aquaculture Growth Across Regions, Species, and Countries. Rev. Fish. Sci. Aquac. 28, 107–116. https://doi.org/10.1080/23308249.2019.1678111

Gordalina, M., Pinheiro, H.M., Mateus, M., da Fonseca, M.M.R., Cesário, M.T., 2021. Macroalgae as protein sources—a review on protein bioactivity, extraction, purification and characterization. Appl. Sci. 11. https://doi.org/10.3390/app11177969

Gowen, R.J., 1994. Managing eutrophication associated with aquaculture development. J. Appl. Ichthyol. 10, 242–257. https://doi.org/10.1111/j.1439-0426.1994.tb00164.x

Granada, L., Lopes, S., Novais, S.C., Lemos, M.F.L., 2018. Modelling integrated multi-trophic aquaculture: Optimizing a three trophic level system. Aquaculture 495, 90–97. https://doi.org/10.1016/j.aquaculture.2018.05.029

Hanisak, M.D., 1990. The use of Gracilaria tikvahiae (Gracilariales, Rhodophyta) as a model system to understand the nitrogen nutrition of cultured seaweeds. Hydrobiologia 204–205, 79–87. https://doi.org/10.1007/BF00040218

He, J., Xu, Y., Chen, H., Sun, P., 2016. Extraction, structural characterization, and potential antioxidant activity of the polysaccharides from four seaweeds. Int. J. Mol. Sci. 17. https://doi.org/10.3390/ijms17121988

Hernández, I., Fernández-Engo, M.A., Pérez-Lloréns, J.L., Vergara, J.J., 2005. Integrated outdoor culture of two estuarine macroalgae as biofilters for dissolved nutrients from Sparus aurata waste waters. J. Appl. Phycol. 17, 557–567. https://doi.org/10.1007/s10811-005-9006-6

Holanda, M., Santana, G., Furtado, P., Rodrigues, R.V., Cerqueira, V.R., Sampaio, L.A., Wasielesky, W., Poersch, L.H., 2020. Evidence of total suspended solids control by Mugil liza reared in an integrated system with pacific white shrimp Litopenaeus vannamei using biofloc technology. Aquac. Reports 18. https://doi.org/10.1016/j.aqrep.2020.100479

Holanda, M., Wasielesky, W., de Lara, G.R., Poersch, L.H., 2022. Production of Marine Shrimp Integrated with Tilapia at High Densities and in a Biofloc System: Choosing the Best Spatial Configuration. Fishes 7. https://doi.org/10.3390/fishes7050283

Holdt, S.L., Kraan, S., 2011. Bioactive compounds in seaweed: Functional food applications and legislation. J. Appl. Phycol. 23, 543–597. https://doi.org/10.1007/s10811-010-9632-5

Huo, Y., Wu, H., Chai, Z., Xu, S., Han, F., Dong, L., He, P., 2012. Bioremediation efficiency of Gracilaria verrucosa for an integrated multi-trophic aquaculture system with Pseudosciaena crocea in Xiangshan harbor, China. Aquaculture. https://doi.org/10.1016/j.aquaculture.2011.11.002

Jatobá, A., Da Silva, B.C., Da Silva, J.S., Vieira, F. do N., Mouriño, J.L.P., Seiffert, W.Q., Toledo, T.M., 2014. Protein levels for Litopenaeus vannamei in semi-intensive and biofloc systems. Aquaculture 432, 365–371. https://doi.org/10.1016/j.aquaculture.2014.05.005

Jory, D.E.; Cabrera, T.R.; Dugger, D.M.; Fegan, D.; Lee, P.G.; Lawrence, L.; Jackson, C.J.; Mcintosh, R.P.; Castañeda, J.. I., Al., B.. et, 2011. A Global Review of Shrimp Feed Management: Status and Perspectives. Aquaculture 104–152.

Khanjani, M.H., Mozanzadeh, M.T., Sharifinia, M., Emerenciano, M.G.C., 2023. Biofloc: A sustainable dietary supplement, nutritional value and functional properties. Aquaculture 562, 738757. https://doi.org/10.1016/j.aquaculture.2022.738757

Khanjani, M.H., Zahedi, S., Mohammadi, A., 2022. Integrated multitrophic aquaculture (IMTA) as an environmentally friendly system for sustainable aquaculture: functionality, species, and application of biofloc technology (BFT). Environ. Sci. Pollut. Res. 29, 67513–67531. https://doi.org/10.1007/s11356-022-22371-8

Krummenauer, D., Peixoto, S., Cavalli, R.O., Poersch, L.H., Wasielesky, W., 2011. Superintensive culture of white shrimp, Litopenaeus vannamei, in a biofloc technology system in Southern Brazil at different stocking densities. J. World Aquac. Soc. 42, 726–733. https://doi.org/10.1111/j.1749-7345.2011.00507.x

Krummenauer, D., Samocha, T., Poersch, L., Lara, G., Wasielesky, W., 2014. The reuse of water on the culture of pacific white shrimp, litopenaeus vannamei, in BFT system. J. World Aquac. Soc. 45, 3–14. https://doi.org/10.1111/jwas.12093

Legarda, E.C., da Silva, D., Miranda, C.S., Pereira, P.K.M., Martins, M.A., Machado, C., de Lorenzo, M.A., Hayashi, L., do Nascimento Vieira, F., 2021a. Sea lettuce integrated with Pacific white shrimp and mullet cultivation in biofloc impact system performance and the sea lettuce nutritional composition. Aquaculture 534. https://doi.org/10.1016/j.aquaculture.2020.736265

Legarda, E.C., da Silva, D., Miranda, C.S., Pereira, P.K.M., Martins, M.A., Machado, C., de Lorenzo, M.A., Hayashi, L., do Nascimento Vieira, F., 2021b. Sea lettuce integrated with Pacific white shrimp and mullet cultivation in biofloc impact system performance and the sea lettuce nutritional composition. Aquaculture 534, 736265. https://doi.org/10.1016/j.aquaculture.2020.736265

Levavasseur, G., 1989. Analyse comparée des complexes pigment-protéines de chlorophycophytes marines benthiques. Phycologia 28, 1–14. https://doi.org/10.2216/i0031-8884-28-1-1.1

Li, E., Chen, L., Zeng, C., Chen, X., Yu, N., Lai, Q., Qin, J.G., 2007. Growth, body composition, respiration and ambient ammonia nitrogen tolerance of the juvenile white shrimp, Litopenaeus vannamei, at different salinities. Aquaculture 265, 385–390. https://doi.org/10.1016/j.aquaculture.2007.02.018

LICHTENTHALER, H.K., WELLBURN, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents . Biochem. Soc. Trans. 11, 591–592. https://doi.org/10.1042/bst0110591

Lima, P.C.M., Silva, A.E.M., Silva, D.A., Silva, S.M.B.C., Brito, L.O., Gálvez, A.O., 2021. Effect of stocking density of Crassostrea sp. in a multitrophic biofloc system with Litopenaeus vannamei in nursery. Aquaculture 530, 735913. https://doi.org/10.1016/j.aquaculture.2020.735913

Lin, Y.C., Chen, J.C., 2003. Acute toxicity of nitrite on Litopenaeus vannamei (Boone) juveniles at different salinity levels. Aquaculture 224, 193–201. https://doi.org/10.1016/S0044-8486(03)00220-5

Lin, Y.C., Chen, J.C., 2001. Acute toxicity of ammonia on Litopenaeus vannamei boone juveniles at different salinity levels. J. Exp. Mar. Bio. Ecol. 259, 109–119. https://doi.org/10.1016/S0022-0981(01)00227-1

Loureiro, R.R., Reis, R.P., Critchley, A.T., 2010. In vitro cultivation of three Kappaphycus alvarezii (Rhodophyta, Areschougiaceae) variants (green, red and brown) exposed to a commercial extract of the brown alga Ascophyllum nodosum (Fucaceae, Ochrophyta). J. Appl. Phycol. 22, 101–104. https://doi.org/10.1007/s10811-009-9412-2

Lourenço, S.O., Barbarino, E., De-Paula, J.C., Pereira, L.O.D.S., Lanfer Marquez, U.M., 2002. Amino

acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. Phycol. Res. 50, 233–241. https://doi.org/10.1046/j.1440-1835.2002.00278.x

Mabeau, S., Fleurence, J., 1993. Seaweed in food products: biochemical and nutritional aspects. Trends Food Sci. Technol. 4, 103–107. https://doi.org/10.1016/0924-2244(93)90091-N

Macedo, C.F., Sipaúba-Tavares, L., 2010. Eutrofização E Qualidade Da Água Na Piscicultura : Consequências E Recomendações Eutrophication and Water Quality in Pisciculture : Consequences and Recommendations. Bol. Inst. Pesca SãoPaulo, 149–163.

Marinho-Soriano, E., Panucci, R.A., Carneiro, M.A.A., Pereira, D.C., 2009. Evaluation of Gracilaria caudata J. Agardh for bioremediation of nutrients from shrimp farming wastewater. Bioresour. Technol. 100, 6192–6198. https://doi.org/10.1016/j.biortech.2009.06.102

Marinho, G., Nunes, C., Sousa-Pinto, I., Pereira, R., Rema, P., Valente, L.M.P., 2013. The IMTA-cultivated Chlorophyta Ulva spp. as a sustainable ingredient in Nile tilapia (Oreochromis niloticus) diets. J. Appl. Phycol. 25, 1359–1367. https://doi.org/10.1007/s10811-012-9965-3

Martins, M.A., da SILVA, V.F., Tarapuez, P.R., Hayashi, L., Vieira, F.D.N., 2020. Cultivation of the seaweed ulva spp. With effluent from a shrimp biofloc rearing system: Different species and stocking density. Bol. do Inst. Pesca. https://doi.org/10.20950/1678-2305.2020.46.3.602

Massocato, T.F., Robles-Carnero, V., Moreira, B.R., Castro-Varela, P., Pinheiro-Silva, L., Oliveira, W. da S., Vega, J., Avilés, A., Bonomi-Barufi, J., Rörig, L.R., Figueroa, F.L., 2022. Growth, biofiltration and photosynthetic performance of Ulva spp. cultivated in fishpond effluents: An outdoor study. Front. Mar. Sci. 9, 1–19. https://doi.org/10.3389/fmars.2022.981468

Messyasz, B., Rybak, A., 2011. Abiotic factors affecting the development of Ulva sp. (Ulvophyceae; Chlorophyta) in freshwater ecosystems. Aquat. Ecol. 45, 75–87. https://doi.org/10.1007/s10452-010-9333-9

Mildenberger, J., Stangeland, J.K., Rebours, C., 2022. Antioxidative activities, phenolic compounds and marine food allergens in the macroalgae Saccharina latissima produced in integrated multi-trophic aquaculture systems. Aquaculture 546, 737386. https://doi.org/10.1016/j.aquaculture.2021.737386

Moreira, A., Cruz, S., Marques, R., Cartaxana, P., 2022. The underexplored potential of green macroalgae in aquaculture. Rev. Aquac. 14, 5–26. https://doi.org/10.1111/raq.12580

Msuya, F.E., Neori, A., 2008. Effect of water aeration and nutrient load level on biomass yield, N uptake and protein content of the seaweed Ulva lactuca cultured in seawater tanks. J. Appl. Phycol. 20, 1021–1031. https://doi.org/10.1007/s10811-007-9300-6

Natori, M. M., Sussel, F. R., Santos, E. D., Previero, T. D. C., Viegas, E. M. M., & Gameiro, A.H., 2011. Desenvolvimento da carcinicultura marinha no Brasil e no mundo: avanços tecnológicos e desafios. Informações econômicas 41(2), 61–73.

Nobre, A.M., Robertson-Andersson, D., Neori, A., Sankar, K., 2010. Ecological-economic assessment of aquaculture options: Comparison between abalone monoculture and integrated multi-trophic aquaculture of abalone and seaweeds. Aquaculture 306, 116–126. https://doi.org/10.1016/j.aquaculture.2010.06.002

Oliveira, L.K., Pilz, L., Furtado, P.S., Ballester, E.L.C., Bicudo, Á.J. de A., 2021. Growth, nutritional efficiency, and profitability of juvenile GIFT strain of Nile tilapia (Oreochromis niloticus) reared in biofloc system on graded feeding rates. Aquaculture 541. https://doi.org/10.1016/j.aquaculture.2021.736830

Øyvind Hammer; David A.T. Harper; Paul D. Ryan, 2013. National knowledge resource consortium -a

national gateway of S&T on-line resources for CSIR and DST laboratories. Curr. Sci. 105, 1352–1357.

Peñalver, R., Lorenzo, J.M., Ros, G., Amarowicz, R., Pateiro, M., Nieto, G., 2020. Seaweeds as a functional ingredient for a healthy diet. Mar. Drugs 18, 1–27. https://doi.org/10.3390/md18060301

Pires, C.M., Bazzo, G.C., Barreto, P.L.M., do Espírito Santo, C.M., Ventura, T.F.B., Pedra, A.G.L.M., Rover, T., McGovern, M., Hayashi, L., 2021. Cultivation of the red seaweed Kappaphycus alvarezii using biofloc effluent. J. Appl. Phycol. 33, 1047–1058. https://doi.org/10.1007/s10811-020-02335-6

Poli, M.A., Legarda, E.C., de Lorenzo, M.A., Pinheiro, I., Martins, M.A., Seiffert, W.Q., do Nascimento Vieira, F., 2019. Integrated multitrophic aquaculture applied to shrimp rearing in a biofloc system. Aquaculture. https://doi.org/10.1016/j.aquaculture.2019.734274

Queirós, A.S., Circuncisão, A.R., Pereira, E., Válega, M., Abreu, M.H., Silva, A.M.S., Cardoso, S.M., 2021. Valuable nutrients from ulva rigida: Modulation by seasonal and cultivation factors. Appl. Sci. 11. https://doi.org/10.3390/app11136137

Queiroz, H.M., Ferreira, T.O., Taniguchi, C.A.K., Barcellos, D., do Nascimento, J.C., Nóbrega, G.N., Otero, X.L., Artur, A.G., 2020. Nitrogen mineralization and eutrophication risks in mangroves receiving shrimp farming effluents. Environ. Sci. Pollut. Res. 27, 34941–34950. https://doi.org/10.1007/s11356-020-09720-1

Ramos, R., Vinatea, L., Santos, J., Da Costa, R., 2010. Tratamiento de efluentes del cultivo de Litopenaeus vannamei mediante procesos de sedimentación, filtración y absorción. Lat. Am. J. Aquat. Res. 38, 188–200. https://doi.org/10.3856/vol38-issue2-fulltext-3

Rathod, M., Mody, K., Basha, S., 2014. Efficient removal of phosphate from aqueous solutions by red seaweed, Kappaphycus alverezii. J. Clean. Prod. 84, 484–493. https://doi.org/10.1016/j.jclepro.2014.03.064

Reis, W.G., Wasielesky, W., Abreu, P.C., Brandão, H., Krummenauer, D., 2019. Rearing of the Pacific white shrimp Litopenaeus vannamei (Boone, 1931)in BFT system with different photoperiods: Effects on the microbial community, water quality and zootechnical performance. Aquaculture 508, 19–29. https://doi.org/10.1016/j.aquaculture.2019.04.067

Resende, L., Flores, J., Moreira, C., Pacheco, D., Baeta, A., Garcia, A.C., Rocha, A.C.S., 2022. Effective and Low-Maintenance IMTA System as Effluent Treatment Unit for Promoting Sustainability in Coastal Aquaculture. Appl. Sci. 12. https://doi.org/10.3390/app12010398

Saleh, N.E., Shalaby, S.M., Sakr, E.M., Elmonem, A.I.A., Michael, F.R., 2014. Effect of Dietary Inclusion of Ulva fasciata on Red Hybrid Tilapia Growth and Carcass Composition. J. Appl. Aquac. 26, 197–207. https://doi.org/10.1080/10454438.2014.909279

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

Sampath-Wiley, P., Neefus, C.D., Jahnke, L.S., 2008. Seasonal effects of sun exposure and emersion on intertidal seaweed physiology: Fluctuations in antioxidant contents, photosynthetic pigments and photosynthetic efficiency in the red alga Porphyra umbilicalis Kützing (Rhodophyta, Bangiales). J. Exp. Mar. Bio. Ecol. 361, 83–91. https://doi.org/10.1016/j.jembe.2008.05.001

Santos, I. L. M., Melatti, E., de Souza Correia, E., Ferreira, D. A., & da Silva, L.O.B. (2020)., 2020. Economic viability of tilapia cultivation (Oreochromis niloticus) in net tanks located in the municipality of Glória-BA.

Sarà, G., Mangano, M.C., Johnson, M., Mazzola, A., 2018. Integrating multiple stressors in aquaculture to build the blue growth in a changing sea. Hydrobiologia 809, 5–17. https://doi.org/10.1007/s10750-017-3469-8

Schiavon, M., Moro, I., Pilon-Smits, E.A.H., Matozzo, V., Malagoli, M., Dalla Vecchia, F., 2012. Accumulation of selenium in Ulva sp. and effects on morphology, ultrastructure and antioxidant enzymes and metabolites. Aquat. Toxicol. 122–123, 222–231. https://doi.org/10.1016/j.aquatox.2012.06.014

Silva, A.F.R., Abreu, H., Silva, A.M.S., Cardoso, S.M., 2019. Effect of oven-drying on the recovery of valuable compounds from Ulva rigida, Gracilaria sp. and fucus vesiculosus. Mar. Drugs 17. https://doi.org/10.3390/md17020090

Smart, J.N., Schmid, M., Paine, E.R., Britton, D., Revill, A., Hurd, C.L., 2022. Seasonal ammonium uptake kinetics of four brown macroalgae: Implications for use in integrated multi-trophic aquaculture. J. Appl. Phycol. 34, 1693–1708. https://doi.org/10.1007/s10811-022-02743-w

Sumaila, U.R., Pierruci, A., Oyinlola, M.A., Cannas, R., Froese, R., Glaser, S., Jacquet, J., Kaiser, B.A., Issifu, I., Micheli, F., Naylor, R., Pauly, D., 2022. Aquaculture over-optimism? Front. Mar. Sci. 9, 1–10. https://doi.org/10.3389/fmars.2022.984354

Systems, M.A.I., Checa, D., Macey, B.M., Bolton, J.J., Brink-hull, M., Donohoe, P.O., Cardozo, A., Poersch, L.H., Inmaculada, S., 2024. Circularity Assessment in Aquaculture : The Case of Integrated.

Tabarsa, M., Rezaei, M., Ramezanpour, Z., Waaland, J.R., 2012. Chemical compositions of the marine algae Gracilaria salicornia (Rhodophyta) and Ulva lactuca (Chlorophyta) as a potential food source. J. Sci. Food Agric. 92, 2500–2506. https://doi.org/10.1002/jsfa.5659

Thomas, J.B.E., Ramos, F.S., Gröndahl, F., 2019. Identifying Suitable Sites for Macroalgae Cultivation on the Swedish West Coast. Coast. Manag. 47, 88–106. https://doi.org/10.1080/08920753.2019.1540906

Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A.H., Fang, J.G., 2009. Ecological engineering in aquaculture - Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. Aquaculture 297, 1–9. https://doi.org/10.1016/j.aquaculture.2009.09.010

Tsagkamilis, P., Danielidis, D., Dring, M.J., Katsaros, C., 2010. Removal of phosphate by the green seaweed Ulva lactuca in a small-scale sewage treatment plant (Ios Island, Aegean Sea, Greece). J. Appl. Phycol. 22, 331–339. https://doi.org/10.1007/s10811-009-9463-4

Unesco, 1983. Chemical methods for use in marine environmental monitoring. Intergovernmental Oceanographic Commission.

Valente, L.M.P., Araújo, M., Batista, S., Peixoto, M.J., Sousa-Pinto, I., Brotas, V., Cunha, L.M., Rema, P., 2016. Carotenoid deposition, flesh quality and immunological response of Nile tilapia fed increasing levels of IMTA-cultivated Ulva spp. J. Appl. Phycol. 28, 691–701. https://doi.org/10.1007/s10811-015-0590-9

Valenti, W.C., Kimpara, J.M., Preto, B. de L., Moraes-Valenti, P., 2018. Indicators of sustainability to assess aquaculture systems. Ecol. Indic. 88, 402–413. https://doi.org/10.1016/j.ecolind.2017.12.068

Van Wyk, P., Davis-Hodgkins, M., Laramore, R., Main, K.L., Mountain, J., Scarpa, J., 1999. Farming Marine Shrimp in Recirculating Freshwater Systems Harbor Branch Oceanographic Institution. Farming Mar. shrimp Recirc. Freshw. Syst. 220, 125–140.

Verdegem, M., Buschmann, A.H., Latt, U.W., Dalsgaard, A.J.T., Lovatelli, A., 2023. The contribution

of aquaculture systems to global aquaculture production. J. World Aquac. Soc. 54, 206–250. https://doi.org/10.1111/jwas.12963

Verdian, A.H., Effendi, I., Budidardi, T., Diatin, I., 2020. Production performance improvement of white shrimp (Litopenaeus vannamei) culture with integrated multi trophic aquaculture system in Seribu Islands, Jakarta, Indonesia. Iran. J. Fish. Sci. https://doi.org/10.22092/ijfs.2019.120676

Wan, A.H.L., Davies, S.J., Soler-Vila, A., Fitzgerald, R., Johnson, M.P., 2019. Macroalgae as a sustainable aquafeed ingredient. Rev. Aquac. https://doi.org/10.1111/raq.12241

Wasielesky, W., Krummenauer, D., Lara, G., Fóes, G., 2013. Cultivo de camarões em sistema de biofl ocos : realidades e perspectivas 30–36.

Wyban, J., Walsh, W.A., Godin, D.M., 1995. Temperature effects on growth, feeding rate and feed conversion of the Pacific white shrimp (Penaeus vannamei). Aquaculture 138, 267–279. https://doi.org/10.1016/0044-8486(95)00032-1

Yildiz, G., Celikler, S., Vatan, O., Dere, S., 2012. Determination of the anti-oxidative capacity and bioactive compounds in green seaweed Ulva rigida C. Agardh. Int. J. Food Prop. 15, 1182–1189. https://doi.org/10.1080/10942912.2010.517341

Zimmermann, S., Kiessling, A., Zhang, J., 2023. The future of intensive tilapia production and the circular bioeconomy without effluents: Biofloc technology, recirculation aquaculture systems, bio-RAS, partitioned aquaculture systems and integrated multitrophic aquaculture. Rev. Aquac. 15, 22–31. https://doi.org/10.1111/raq.12744

Zirino, A., Elwany, H., Facca, C., Maicu', F., Neira, C., Mendoza, G., 2016. Nitrogen to phosphorus ratio in the Venice (Italy) Lagoon (2001-2010) and its relation to macroalgae. Mar. Chem. 180, 33–41. https://doi.org/10.1016/j.marchem.2016.01.002

CAPÍTULO 1: Efeito do sistema simbiótico no crescimento e absorção de nutrientes da macroalga *Ulva lactuca* em sistema integrado com camarão e ostra.

Artigo submetido na *Aquaculture International*.

Resumo

O sistema simbiótico, caracterizado pelo pré-tratamento das fontes de carbono através da degradação causada por microrganismos, tem como função a manutenção da qualidade de água do sistema além de fornecer uma fonte complementar de alimento para os organismos cultivados. Por ser um sistema intensivo, a produção e acumulo de resíduos orgânicos e inorgânicos é alta, podendo ocasionar problemas dentro do cultivo ou no descarte de efluente. No entanto, o uso de organismos de baixo nível trófico pode se beneficiar dos resíduos produzidos para geração de biomassa. Com isso, estre trabalho tem como objetivo avaliar o efeito do sistema simbiótico na produção do camarão *Penaeus vannamei*, da ostra *Crassostreia virginica* e da macroalga *Ulva lactuca*. Durante 35 dias foi realizado um berçário de camarão na densidade de 1.500 camarões m^{-3} em tanques de 0,06 m^3 com aeração constante e iluminação artificial. Foram realizados quatro tratamentos com três réplicas cada, sendo eles: Controle: cultivo de camarão em água clara com renovação de água, ostra e macroalgas; Monocultivo: cultivo de camarão em um sistema simbiótico; SO: cultivo de camarão integrado com ostra em um sistema simbiótico; SMO: cultivo de camarão integrado com ostra e macroalgas em um sistema simbiótico. Foram realizadas análises de qualidade de água e a performance dos organismos. Como resultado, o uso do sistema simbiótico resultou em um menor uso de água para renovações comparado ao tratamento controle, através da manutenção da qualidade de água por microrganismos. A inclusão de macroalgas no sistema simbiótico levou a uma menor concentração de nitrato ao final do experimento, mostrando que a macroalga teve um bom desempenho na absorção dos nutrientes gerados no sistema. No entanto, a concentração de sólidos suspensos totais no sistema simbiótico afetou negativamente o crescimento das macroalgas através da deposição nas laminas fotossintetizantes e causou a mortalidade das ostras. O tratamento SMO obteve ao final do berçário uma biomassa final mais alta e uma taxa de conversão alimentar mais baixa no camarão em comparação com a monocultivo, resultado da integração de outros organismos no sistema que auxiliam na qualidade de água. Portanto, o uso do sistema integrado com camarão, ostra e macroalgas em um sistema simbiótico proporcionou menor uso de água e concentrações de nitrato, bem como maior produtividade de camarão.

Effect of the synbiotic system on the performance of shrimp *Penaeus vannamei*, oyster *Crassostrea virginica*, and macroalgae *Ulva lactuca* in an integrated system.

Andrezza Carvalho [1]*, Otávio Augusto Lacerda Ferreira Pimentel[2], Dariano Krummenauer [2], Ethan McAlhaney [3], Stephen Urick [3], Jireh Clarington [3], Jonathan van Senten [3], Michael H. Schwarz [3] Gamze Turan[4], and Luís H. Poersch [1]

[1] Marine Aquaculture Station, Institute of Oceanography, Federal University of Rio Grande—FURG, Rua do Hotel, nº[2], Cassino, Rio Grande 96210030, RS, Brazil; andrezzachagas@hotmail.com, otavio.pimentel@yahoo.com, darianok@gmail.com, lpoersch@gmail.com

[2] Laboratory of Ecology of Microorganisms Applied to Aquaculture, Institute of Oceanography, Federal University of Rio Grande - FURG, Av. Itália, Km 08, Rio Grande, RS 96201-900, Brazil

[3] Virginia Seafood Agricultural Research and Extension Center, Virginia Polytechnic Institute and State University, Hampton, Virginia, USA. mschwarz@vt.edu, jonat86@vt.edu, surick@vt.edu, emcalha07@vt.edu, cjireh7@vt.edu.

[4] Aquaculture Department, Fisheries Faculty, Ege University, Izmir 35100, Turkey; gamzeturan2000@gmail.com

* Correspondence author: Andrezza Carvalho, Marine Aquaculture Station, Institute of Oceanography, Federal University of Rio Grande—FURG, Rua do Hotel, nº[2], Cassino, Rio Grande 96210030, RS, Brazil; Tel +55 88 99620-6137 andrezzachagas@hotmail.com (A.C).

Abstract

The synbiotic system improves the water quality and provides a complementary source of food for the shrimp. However, as it is an intensive system, there is an accumulation of organic matter and inorganic nitrogen, which can be used by species of different trophic levels. This work aims to evaluate the effect of the synbiotic system on the production of the shrimp *Penaeus vannamei*, the oyster *Crassostreia virginica*, and the macroalga *Ulva lactuca*. A *P. vannamei* nursery was carried out over 35 days at a density of 1500 shrimp m^{-3} in 0.06 m^3 tanks with constant aeration and artificial lighting. There were four treatments, Control: shrimp culture in clear water, oyster, and macroalgae; Monoculture: shrimp culture in a synbiotic system; SO: shrimp culture integrated with oyster in a synbiotic system; SMO: shrimp culture integrated with oyster and macroalgae in a synbiotic system. As a result, the use of the synbiotic system resulted in minimal water renewal compared to the control treatment. The inclusion of macroalgae in the synbiotic system led to a lower nitrate concentration at the end of the trial. However, the total suspended solids in the synbiotic system negatively affected the growth of the macroalgae, and caused oyster mortality. The SMO promoted a higher final biomass and a lower feed conversion ratio in the shrimp compared to the monoculture. Therefore, the use of the integrated system with shrimp, oyster, and macroalgae in a synbiotic system provided lower water use and nitrate concentrations, as well as higher shrimp productivity.

keywords: nutrient absorption; nitrate; survival; yield; IMTA.

1. Introduction

The intensification of aquaculture systems is necessary due to higher demand caused by population growth and the demand for consumption of aquatic organisms (FAO 2024). Intensive systems are designed to increase final productivity, with greater biosecurity, less water use, and control of physical and chemical parameters. These characteristics are a result of the predominance of heterotrophic and chemoautotrophic bacteria in the system, stimulated by the use of a carbon source, with the purpose of control ammonia and nitrite concentrations in the system (Krummenauer et al. 2011). The choice of carbon source is an important factor in the development of bacterial groups in the system, so if only inorganic carbon sources are chosen, the establishment of chemoautotrophic bacteria will prevail, while for organic carbon sources the growth and predominance of heterotrophic bacteria is increased (Ferreira et al. 2021).

Recently, as a way of improving the availability of carbon present in bran, carbon sources have been pre-processed using aerobic and anaerobic processes (Hussain et al. 2021). The use of complex carbohydrates such as rice bran to form the synbiotic system has the advantage of greater nutritional value and the availability and low cost of the product on the market (Oladosu et al. 2016). For this, the use of pre-treatment is necessary as a way of increasing the solubility of the bran in water, decreasing the fiber content and increasing the protein content, thus transforming complex carbon molecules into simple molecules through the action of microorganisms (Romano et al. 2018). The improved availability of the carbon source leads to the production of microbial flocs consisting of various microorganisms and bacteria that improve water quality (Khanjani et al. 2024). As a result, the application of symbiotic systems has increased considerably since 2012, initially being tested with the rainbow trout *Oncorhynchus mykiss* and the white shrimp *Penaeus vannamei* (Huynh et al. 2017). Some advantages of the synbiotic system are also associated with the proliferation of beneficial bacteria that help the immune system of the cultivated organisms (Hussain et al. 2021), in addition to conferring greater growth and weight gain to the shrimp compared to traditional systems (Crab et al. 2007).

The production of microbial aggregates through the proliferation of heterotrophic bacteria combined with minimal water exchange gives the system a high concentration of total suspended solids and dissolved nutrients that are accumulated and not used during production (Samocha et al. 2007). This waste can be used by other organisms instead of being discarded into the environment. This has been made possible by the implementation of integrated multitrophic aquaculture (IMTA), which consists of the culture of a main species, such as

shrimp and fish, and the insertion of secondary species of different trophic levels, such as organic and inorganic consumers, thus diversifying production with greater sustainability (Troell et al. 2009).

According to Khanjani et al. (2022) the choice of species is crucial to the functioning of the integrated system, as the species must complement each other, have a high growth rate, adapt to the system, and have market value. With this in mind, due to the ease of management and the increasing levels of production and consumption in the market, the category of crustaceans represents the target species most used in integrated systems (Khanjani et al. 2022). The Pacific white shrimp *Penaeus vannamei* is the most produced species and is of interest for inclusion in cultivation (FAO 2022). The role of the organic consumer in the system is to consume total suspended solids, and the species used include low trophic level fish such as tilapia and the use of bivalves. Holanda et al. (2022) showed the tilapia *Oreochromis niloticus* as a filtering organism for floc, resulting in a low feed conversion ratio and higher system productivity. However, unlike fish that still need an external food source with commercial feed, bivalves are characterized by their high filtration rate, added economic value and not needing to use inputs, making their insertion into the system more significant (Chopin 2015). Costa et al. (2021) showed that the oyster *Crassostrea gasar*, when produced in a biofloc system, performed a selected filtration of flagellates from the system, showing that the species is able to benefit from the biofloc system. The *Crassostrea virginica* oyster species is of great economic importance when it comes to cultivation and environmental importance when it comes to protecting reefs (Grabowski et al. 2012), with the *Crassostrea* genus being the most produced in the mollusc group (FAO 2024).

Among the inorganic consumers of interest in the integrated system is the use of macroalgae, due to their ease of management, not requiring the use of inputs, high growth rate and market value (Chopin and Fitzsimmons 2017). Korzen et al. (2016) show that the excess nutrients produced in fish farming are viable for the sustainable production of macroalgae, with maximum daily growth rates of 17 % day^{-1} for the macroalga *Ulva rigida*. This growth was associated with an increase in protein and carbohydrates in the macroalgae tissue. Hadley et al. (2015) show that adopting some forms of management such as partial harvesting and moderate water flows can maximize *Ulva* production in integrated systems.

In contrast to coastal environments, when inserted into systems with a high organic load, such as biofloc systems, its performance can be affected due to the low availability of light and the deposition of solids on the photosynthesizing laminae (Carvalho et al., 2023; Morais et al., 2023; Legarda et al., 2021). For oysters, Lima et al. (2021) carried out a pre-test with different

concentrations of settleable solids in a monoculture of oysters and found a negative and significant correlation between the increase in solids and the oysters survival. However, little is known about the effect of the solids and the synbiotic system on the performance of the oyster and macroalgae when cultivated when grown in association with other organisms. Therefore, this work aims to evaluate the effect of the synbiotic system on the performance of the shrimp *Penaeus vannamei*, the oyster *Crassostreia virginica*, and the macroalga *Ulva lactuca* when cultivated in an integrated system.

2. Materials and methods

2.1 Location and origin of the animals

The experiment was conducted at the Virginia Seafood Agricultural Research and Extension Center (VSAREC) at Virginia Polytechnic Institute and State University, located in Hampton, Virginia, USA.

The shrimp post-larvae (Homegrown Shrimp USA, LLC) were kept in the laboratory for 20 days and then were stocked in the experiment with an initial weight of 0.11 ± 0.01 g (mean ± standard deviation) at a density of 1500 shrimp m^{-3}. The oysters came from a commercial farm (H.M Terry company, VA, USA) and underwent a 20-day acclimatization period with a gradual increase in temperature to acclimatize to the conditions of the experimental units. They had a mean initial weight of 70.67 ± 2.38 g at the start of the experiment and were stocked at a density of 50 oysters m^{-2}. The macroalgae was kept in an acclimatization period for 20 days before the start of the experiment, stocked at a density of 0.7 g L^{-1} and with a mean initial weight of 43.12 ± 0.11 g in each tank.

2.2 Preparing the matrix tank

The water used in the experiment was made from chlorinated tap fresh water and dechlorinated and salted until the concentration of 28 mg L^{-1} using artificial salt (Instant Ocean Sea Salt). A matrix tank with a useful volume of 70 liters, a heater, and constant aeration was used to prepare the synbiotic system, with 15 fertilizations prior to the start of the experiment. During the trial, the experimental units received 20 daily fertilizations. The fertilizer consisted of rice bran (20 g m^{-3}), dextrose (2 g m^{-3}), calcium carbonate (2 g m^{-3}), powder probiotic (Sanolife Mic, INVE aquaculture) (0.4 g m^{-3}) composed of *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus pumilus* at a concentration of 5×10^{10} colony forming units (CFU) g^{-1}, and water at a salinity of 28 g L^{-1} in the proportion of 20 times the amount of rice bran. The fertilizer went through a 12-hour aerobic phase (microbial respiration) and a 12-hour anaerobic phase (fermentation) and was then disposed in the matrix tank. During the previous

fertilizations, ammonium chloride at a concentration of 1 mg L^{-1} was added to the tank every two days as a source of nitrogen to encourage the growth of the bacterial community. Nine pillows measuring 10 cm × 10 cm were added to the matrix tank as a source of substrate for the bacteria to adhere to. The pillows were made up of K1 Kaldnes Biological Media and at the start of the experiment one pillow was placed in each tank of the synbiotic system treatments.

2.3 Experimental design

The experiment lasted 35 days as a shrimp nursery period. There were twelve 0.06 m^{-3} glass tanks, constantly aerated by means of a blower that sent air to porous stones. A light blocking material was used on the sides and bottom of the tanks so that light only penetrated the surface. The tanks were artificially lighted with portable LED lights (Fluval) kept 40 cm from the surface of the tank and with a 12:12 light/dark photoperiod. The oysters were kept at the bottom of the tank, close to the aeration point. The macroalgae were kept in baskets with 28 × 28 ×20 cm (length×width×height), near the surface of the tank at a depth of 10 centimeters (Figure 1).

The experiment consisted of four treatments with three replicates each and in a completely randomized experimental design: Control: shrimp culture in clear water, oyster, and macroalgae; Monoculture: shrimp culture in a synbiotic system; SO: shrimp culture integrated integrated with oyster in a synbiotic system; SOM: shrimp culture integrated with oyster and macroalgae in a synbiotic system. For the control, water renewals of 20 to 30% of the tank volume were adopted when ammonia concentrations were higher than 2 mg L^{-1}. In the synbiotic treatments, inoculum from the mother tank was added to each experimental unit (12% of the tank volume).

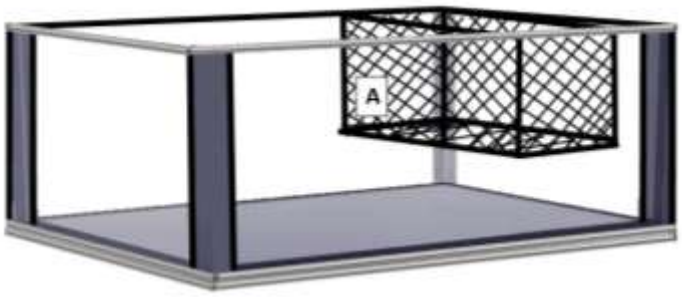


Figure 1. Design of the experimental system, consisting of a tank with shrimp, oysters, and macroalgae. (A) structure near the surface for the macroalgae culture.

2.4 Physical and chemical parameters

Temperature (°C), dissolved oxygen (mg L^{-1}), and salinity (g L^{-1}) were measured daily with a portable multiparameter (Multiparameter YSI, model Pro2030), and pH was measured three times a week (portable pH meter Hanna, model HI98107). Nitrogen compounds such as total

ammonia nitrogen (TAN), nitrite nitrogen (NO_2^--N, mg L^{-1}), and nitrate nitrogen (NO_3^--N, mg L^{-1}) were analyzed twice a week, following the Hach methods 8038, 8507 and 8039, respectively. Orthophosphate (PO_4^{3-}) was analyzed weekly (Hach method 8048). Total suspended solids (TSS, mg L^{-1}) and settleable solids (SS, ml L^{-1}) were analyzed twice a week using a multiparameter colorimeter (Hach, model DR900) and the methodology proposed by APHA, (2005), respectively. For the SMO treatment, two samples of total suspended solids were taken. The first sample was taken with the macroalgae in the tank, and the second sample was taken using the method of suspending the solids that had decanted in the macroalgae, removing the macroalgae from the tank, waiting 3 minutes for the solids to homogenize in the water and then sampling the water. Alkalinity (mg $CaCO_3$ L^{-1}) was measured twice a week according to the methodology proposed by APHA, (2012), and when necessary, applications of sodium bicarbonate were made to maintain alkalinity above 150 mg $CaCO_3$ L^{-1}, according to Furtado et al., (2011).

2.5 Organism performance

For the weekly weighing procedure, the macroalgae were moved inside the structure to remove any solids adhered to the surface, and then the structure was removed from the tank and kept in the open air for 10 minutes to remove excess water and then weighed. The following formula was used to calculate the Relative Growth Rate (RGR) (Loureiro et al. 2010):

RGR (% day^{-1}): 100 × [ln (final weight (g) / initial weight (g)) / (final time - initial time)]

To evaluate the performance of the shrimp, samplings were carried out every two weeks during the experimental period. The shrimp were fed twice a day with 50% protein commercial feed (Zeigler Bros), with weekly adjustments to the amount of feed based on the recommendations of Jory at al., (2011). The performance of the shrimp was assessed according to the following formulas:

Average final weight (g): final biomass of live animals (g) / total number of animals;
Weekly weight gain (WGW, g $week^{-1}$): weight gain (g) / number of weeks.
Specific growth rate (SGR, % day^{-1}) = 100 × [(LN final mean weight - LN initial mean weight)/ days of cultivation]
Final biomass (g): ∑ final weight of all live animals (g);
Feed conversion ratio (FCR) = ∑ feed offered (g) / (biomass gain (g));
Survival (%) = (final number of animals / initial number of animals) × 100;
Productivity (kg m^{-3}): (biomass gain (kg)) / tank volume (m^3);

The performance of the oysters was obtained by measuring their length (mm), width (mm), height (mm) using an analogical caliper (Galtsoff, 1964) and weight (g) at the beginning and end of the experiment. The survival of the oysters in the tank was monitored daily. The presence of completely open oysters indicated mortality and these oysters were counted and removed from the tank. Final biomass (g), survival (%) and productivity were calculated using the following formulas:

Final biomass (g): final average weight × number of individuals;

Survival (%) = (final number of animals / initial number of animals) × 100;

Productivity (kg m^{-3}): (biomass gain (kg)) / tank volume (m^3);

2.6 Statistical analysis

Data is presented as mean ± standard deviation. The normality and homoscedasticity of the data were checked using the Shapiro-Wilk and the Levene tests, respectively. Once the assumptions were met, a Student's t-test was carried out on the performance of the macroalgae in the control and SMO treatments and a one-way ANOVA followed by a Tukey post-hoc test for the other analyses. When the assumptions of the Student's t-test or ANOVA were not met, the non-parametric Kruskall Wallis test was used. A minimum significance level of 5% ($p \leq 0.05$) was applied to all analyses.

3. Results

3.1 Physical and chemical parameters

No differences were found in temperature, dissolved oxygen, and pH among the treatments (*p*-value > 0.05) (Table 1). Nitrate showed a significant difference among treatments (*p*-value ≤ 0.05), with higher values in the monoculture and SO (shrimp and oyster) treatments, followed by the SMO (shrimp, macroalgae, and oyster) treatment, and the control. Both solids parameters had a significant difference among the treatments (*p*-value ≤ 0.05) (Table 1). Settleable solids (SS) and total suspended solids (TSS) were higher in the treatments without macroalgae, namely Monoculture and SO. Regarding water renewal, larger volumes of water were used in the Control treatment compared to the treatments with the synbiotic system (Table 1).

Table 1. Water quality parameters during the 35 days of culture, in the Control, Monoculture, SO, and SMO treatments.

Parameters	**Control**	**Monoculture**	**SO**	**SMO**
Temperature (°C)	25.72 ± 0.26	25.42 ± 0.16	25.54 ± 0.31	25.32 ± 0.31
DO (mg L^{-1})	6.53 ± 3.33	6.59 ± 3.38	6.36 ± 3.26	6.54 ± 3.24

pH	8.14 ± 0.42	8.13 ± 0.42	8.12 ± 0.42	8.15 ± 0.41
Salinity (g L^{-1})	28.40 ± 0.36	28.71 ± 0.21	28.64 ± 0.18	28.61 ± 0.17
Alkalinity (mg $CaCO_3$ L^{-1})	169.33 ± 10.52	184.67 ± 21.09	169.33 ± 16.69	164.00 ± 13.03
TAN (mg L^{-1})	1.85 ± 0.73	1.84 ± 0.59	2.04 ± 0.65	1.60 ± 0.56
NO_2^-- N (mg L^{-1})	5.93 ± 4.18	7.42 ± 4.42	7.00 ± 4.67	6.04 ± 3.50
NO_3^-- N (mg L^{-1})	19.66 ± 12.68 a	47.45 ± 40.46 c	42.99 ± 36.81 c	27.54 ± 18.64 b
PO_4^{3-} (mg L^{-1})	3.85 ± 2.87	10.39 ± 8.60	10.49 ± 6.88	11.09 ± 7.78
SS (ml L^{-1})	0.08 ± 0.04 a	0.99 ± 0.50 b	0.80 ± 0.33 b	0.36 ± 0.40 a
TSS (mg L^{-1})	10.61 ± 8.18 a	73.12 ± 54.58 b	68.48 ± 49.04 b	37.67 ± 31.53 ab
Water renewal (L) #	308.33 ± 48.05 b	00.00 ± 00.00 a	20.00 ± 20.00 a	00.00 ± 00.00 a

Data are mean ± standard deviation. Different letters in the same line represent significant differences (p-value ≤ 0.05) among treatments after one way ANOVA. DO: dissolved oxygen; TAN: total ammonia nitrogen; NO_2^-N: nitrite nitrogen; NO_3^-- N: nitrate nitrogen; PO_4^{3-}**:** orthophosphate; SS: settleable solids; TSS: total suspended solids. # total volume of water used for renovations.

During the experimental time, the nitrate concentration showed a difference among the treatments (p-value ≤ 0.05) from day 29 onwards (Figure 2). The control and SMO treatments showed low nitrate concentrations compared to the Monoculture and SO treatments. At the end of the trial, the SMO treatment with the presence of macroalgae had a lower nitrate concentration compared to the other treatments with synbiotic system.

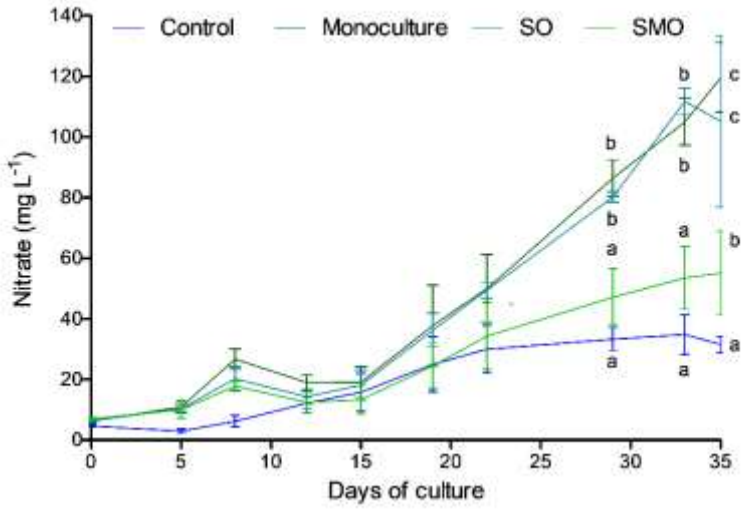


Figure 2. Nitrate concentration during the 35 days of culture, in the Control, Monoculture, SO, and SMO treatments. Different letters on the same day mean a significant difference among the treatments

The concentration of total suspended solids (TSS) increased during the experimental period (Figure 3). The lowest concentrations were found in the Control treatment, followed by

the SMO treatment. The SO and Monoculture treatments had higher concentrations of total suspended solids, which only differed at the beginning of the trial (Figure 3A). The concentration of solids with and without the macroalgae in the tank in the SMO treatment had a significant difference (p-value ≤ 0.05) on almost all sampling days, with a higher concentration of solids occurring after the macroalgae was removed from the tank (Figure 3B).

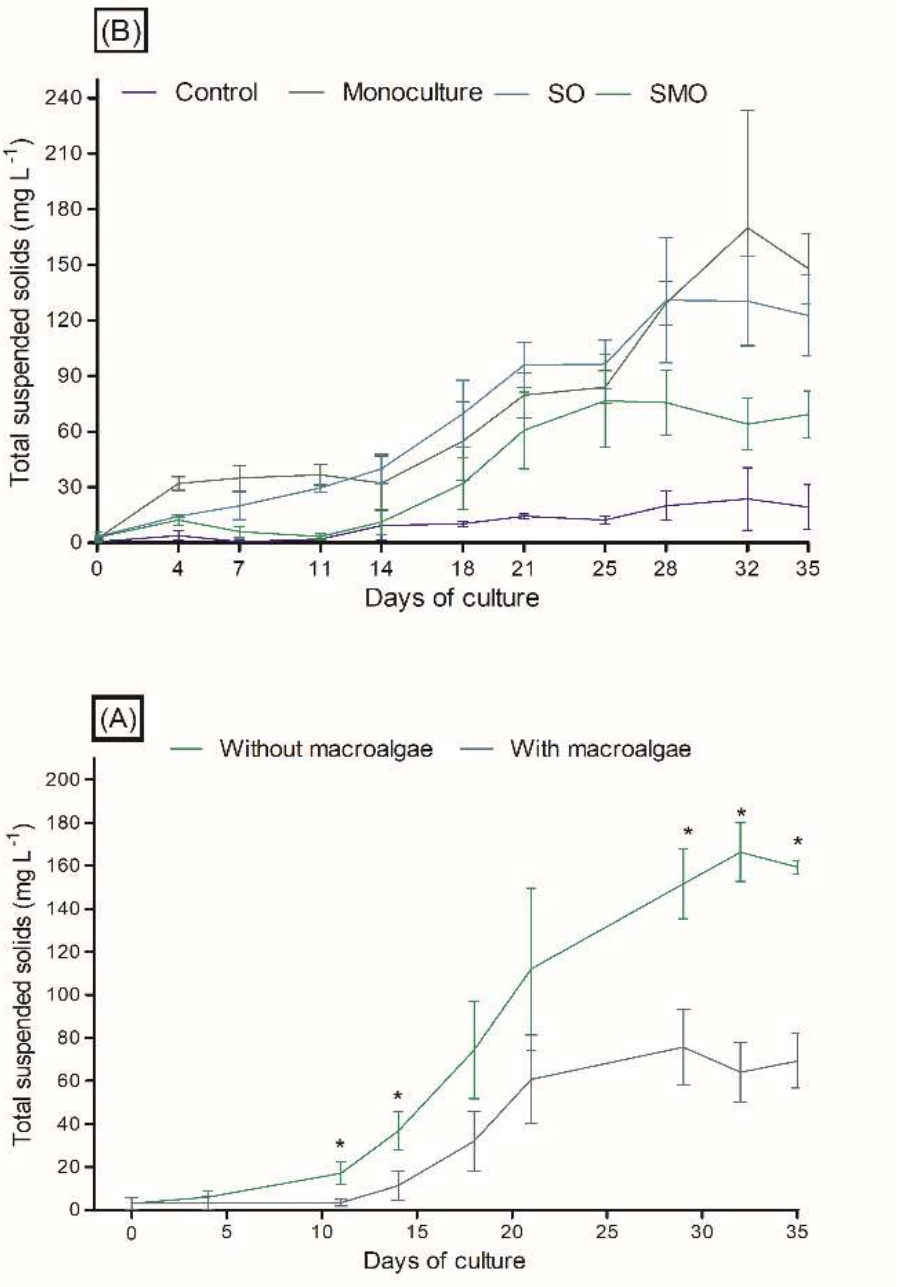


Figure 3. A) Total suspended solids (TSS) concentration during the 35 days of culture, in the Control, Monoculture, SO, and SMO treatments. B) Concentration of total suspended solids (TSS) before and after moving the macroalgae in the structure, in the SMO treatment.

Figure 3. A) Total suspended solids (TSS) concentration during the 35 days of culture, in the Control, Monoculture, SO, and SMO treatments. B) Concentration of total suspended solids (TSS) before and after moving the macroalgae in the structure, in the SMO treatment.

3.2 Organism performance

The final mean weight and relative growth rate of the macroalgae showed no significant difference between the treatments (Table 2). During the experimental time, on day 7 and day 21, the macroalgae in the Control treatment had higher biomass than the SMO treatment. In the Control treatment there was growth of the macroalgae until day 21, then at the end of the trial there was a decrease in biomass. For the SMO treatment, the biomass values did not differ among the days of culture (Figure 4).

Table 2. Macroalgae performance at the end of 35 days of culture, in the Control and SMO treatments.

Macroalgae	**Control**	**SMO**
Initial mean weight (g)	43.11 ± 0.10	43.12 ± 0.11
Final mean weight (g)	49.71 ± 4.82	45.86 ± 4.25
RGR (% day^{-1})	0.13 ± 0.30	0.00 ± 0.00

Data are mean ± standard deviation. RGR relative growth rate.

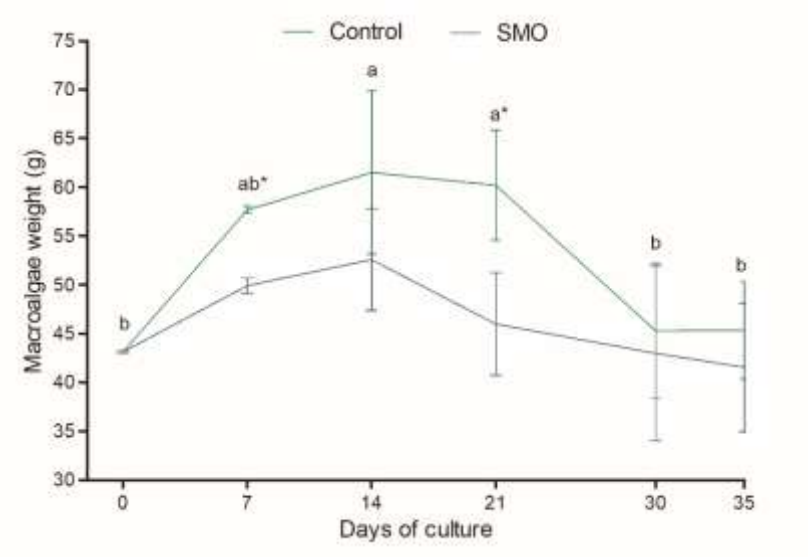


Figure 4. Weight of the macroalgae over the 35 days of culture. Asterisk (*) means significant difference between treatments on the same day. Lowercase letters represent differences between days of the same treatment.

The shrimp cultivated in the integrated system with the synbiotic had higher final biomass, productivity, and a lower FCR (Table 3). However, the Monoculture treatment with synbiotic had lower values for mean final weight and yield, associated with a high FCR. For the oysters, the synbiotic system affected their survival in cultivation, with overall mortality of

the oysters in the SO treatment, and lower survival in the SMO treatment compared to the Control treatment in clear water (Table 4). However, in both treatments there was no oyster weight gain at the end of the experimental period.

Table 3. Shrimp performance at the end of 35 days of culture in the Control, Monoculture, SO and SMO treatments.

Shrimp	**Control**	**Monoculture**	**SO**	**SMO**
Initial weight (g)	0.11 ± 0.00	0.11 ± 0.11	0.11 ± 0.11	0.11 ± 0.11
Final mean weight (g)	1.12 ± 0.09	1.09 ± 0.08	1.27 ± 0.10	1.26 ± 0.06
WGW (g week^{-1})	0.20 ± 0.02	0.20 ± 0.02	0.23 ± 0.02	0.23 ± 0.01
SGR (% day^{-1})	6.62 ± 0.22	6.54 ± 0.21	6.99 ± 0.23	6.97 ± 0.13
Final biomass (g)	90.69 ± 1.20 ab	84.66 ± 6.05 b	95.34 ± 12.34 ab	106.33 ± 7.89 a
Survival (%)	81.33 ± 7.02	78.00 ± 1.00	75.00 ± 7.00	84.33 ± 4.04
FCR	1.16 ± 0.03 ab	1.25 ± 0.10 b	1.10 ± 0.16 ab	0.97 ± 0.07 a
Yield (kg m^{-3})	1.51 ± 0.02 ab	1.41 ± 0.10 b	1.59 ± 0.21 ab	1.77 ± 0.13 a

Data are mean ± standard deviation. Different letters on the same line show a significant difference among the treatments. WGW: weekly weight gain; SGR: specific growth rate; FCR: feed conversion ratio.

Table 4. Oysters performance at the end of 35 days of culture in the Control, SO, and SMO treatments.

Oysters	**Control**	**SO**	**SMO**
Initial mean weight (g)	70.11 ± 1.89	70.12 ± 1.86	71.78 ± 3.59
Final mean weight (g)	70.05 ± 5.55	-	71.25 ± 5.95
Weight gain (g)	0.54 ± 2.87	-	-1.18 ± 0.80
Final Biomass (g)	493.12 ± 64.02	-	402.42 ± 284.66
Survival (%)	87.50 ± 12.50 a	0.00 ± 0.00 b	45.83 ± 50.52 ab
Yield (kg m^{-3})	9.02 ± 47.77	-	-19.64 ± 13.39
Length (cm)	51.04 ± 1.30	-	53.34 ± 2.23
Width (cm)	24.53 ± 0.63	-	24.36 ± 0.88
Height (cm)	87.76 ± 1.23	-	87.24 ± 2.97

Data are mean ± standard deviation. Different letters on the same line show a significant difference among the treatments.

4. Discussion

The choice of species for integrated multitrophic culture is essential for maintaining the water quality and increasing productivity (Granada et al. 2018). The management methods and system chosen should be compatible with the cultivation characteristics of the species chosen. According to the literature, the *C. virginica* oyster is able to tolerate a wide range of temperatures (Linhoss et al. 2016). However, the specimens in this experiment were produced in temperate waters and are used to low temperatures. Therefore, the temperature maintained in this experiment had a general average of 25.0 °C in order to maintain an acceptable level for all the organisms cultivated. For the shrimp, although the temperature maintained was below the optimum temperature for a nursery (Ren et al. 2021), it did not limit the growth and survival of the animals.

The maintenance of nitrogen compounds in the system is also crucial for the performance of the organisms. The conventional system (i.e., clear water) has the characteristic of increasing ammonia levels due to animal excretion and the absence of bacteria in the system to oxidize or consume the compound (Verdegem et al. 2023). Therefore, to maintain the ammonia concentration in the control treatment, it was necessary to change the water, resulting in a larger use of water in this treatment compared to the synbiotic treatments. The pre-treatment of bran in the synbiotic system increases the availability of carbon and accelerates bacterial growth, which will work to control nitrogenous compounds without the need to renew water (Oladosu et al. 2016), making production more sustainable and viable compared to conventional systems.

In the process of nitrogen oxidation by bacteria, nitrate represents the final compound that is accumulated over the production cycle (Furtado et al. 2015). In order to utilize the waste produced in the system, inorganic consumer organisms such as macroalgae are integrated (Chopin et al. 2001). In the experiment, the treatments with synbiotic system, without the macroalgae, showed an increase in the concentration of nitrate over the trial due to the oxidation of ammonia to nitrite and then to nitrate by the action of the nitrifying bacteria. Since there were no organisms to assimilate this compound, it accumulated in the water. In comparison, the synbiotic system with the presence of macroalgae showed lower nitrate concentrations, being similar to the control treatment over several days of culture, where there was frequent water renewal. Therefore, the addition of the macroalgae to the system integrated with the synbiotic was able to absorb the nitrate available for biomass growth. High nitrate concentrations of 30 and 40 mg L^{-1} associated with the availability of phosphate resulted in a high percentage of daily growth of the macroalgae (60.57%), showing the need for these nutrients for better performance (Shakouri and Balouch 2020). Jones et al., (2001) mention that the use of macroalgae in integrated systems resulted in lower nitrate concentrations compared to

monoculture systems and integrated shrimp and oyster cultivations. For this reason, the use of macroalgae as bioremediation organisms has increased due to more sustainable and highly productive aquaculture practices (Chopin et al. 2001). For the control treatment, despite the insertion of the macroalgae in the system, water renewal were constant, so nitrogen levels remained low, and only at the end of the trial did the nitrifying bacteria establish themselves and there was a small accumulation of nitrate. According to Ferreira et al., (2021), nitrifying bacteria take around 30 to 45 days to establish themselves in the system.

Unlike nitrogen compounds that can in some cases be lost into the atmosphere, phosphate is only decomposed by organisms or accumulated in sediment-free environments (Da Silva et al. 2013). For the biofloc system, Da Silva et al., (2013), evaluating the dynamics of nitrogen and phosphorus, showed that for 42 days in an intensive shrimp culture, the final phosphate concentration was 1.4 mg L^{-1}. For the synbiotic system, the pre-treatment of the bran assist in a greater production of phosphate in the water, reaching concentrations of 29 mg L^{-1} as found by Lima et al., (2021). Despite being a limiting compound in the growth of macroalgae, the phosphate levels required to maintain activity by the macroalgae are lower than the nitrogen concentration. The nitrogen:phosphorus ratio of 30:1 is the most ideal for maximizing absorption rates (Duke et al. 1989). Therefore, phosphate production in the synbiotic system was higher than the macroalgae's need for absorption, with no significant phosphate removal rates in this experiment.

Total suspended solids are produced through the bacterial biomass growth and accumulated organic matter, with an increase in concentration over time (Gaona et al. 2017). Oysters are filter-feeding organisms, and work to remove fine particles from the aquatic environment. Costa et al., (2021) and Lima et al., (2022) showed that the addition of oysters in cultivation systems resulted in a decrease in the microbial community of the water due to the filtration, caused by filtration activity. However, despite being filtering organisms, large amounts of solids can interfere with their performance. In this experiment, the increase in solids concentration caused mortality in the oyster in the synbiotic system (SO treatment), generating 100% mortality in two replicates after day 19 of culture. Lima et al. (2021), testing different concentrations of settleable solids on the survival of the oyster *C. gasar*, resulted in survival remaining at 40 to 60% at 10 to 20 ml L^{-1} of SS. For the oyster *C. virginica*, modeling studies showed results of the limiting effect on the oyster's filtration capacity caused by the concentration of solids, with ideal levels between 5 and 25 mg L^{-1} (Ehrich and Harris 2015). In our study, after day 14, concentrations of total suspended solids were higher than 50 mg L^{-1}, causing oyster mortality as solids accumulated, and presenting solid concentrations similar to shrimp monoculture.

Although the accumulation of suspended particles also occurred in the treatment with oyster, shrimp, and macroalgae (SMO), the concentrations of total suspended solids were lower than in other treatments in a synbiotic system. This result can be explained by the insertion of macroalgae in the system. The use of macroalgae into the system promotes a barrier to water movement, causing the sedimentation of solids on its photosynthetic lamina. This result was also reported by Brito et al. (2014), who found a 12.9% reduction in total suspended solids with the insertion of the macroalgae *Ulva lactuca* in the shrimp culture with biofloc. However, this organic matter, despite not being in suspension in the water column, is still within the cultivation system. This can be proven by removing the macroalgae from the tank, which increased the concentration of total suspended solids by an average of 2 times. The same was presented in a biofloc system by Carvalho et al. (2023), who showed a 40% increase in the concentration of solids in the system when the macroalgae were removed from the system.

This deposition of solids is directly related to the performance of the macroalgae, resulting in a loss of biomass during the experimental time. The laminar structure of macroalgae is responsible for absorbing light (Summers et al. 2023). Therefore, environments with high turbidity or deposition of materials on the surface of the lamina reduce the ability to perform photosynthesis. In both treatments, macroalgae grew in the first two weeks of culture, with a higher biomass found in the control treatment. In the following weeks, solids accumulated in both treatments and the performance of the macroalgae was affected. The support capacity of the macroalgae culture compartment in the tank may also have influenced the loss of biomass from the third week onwards in the control treatment. The high density within the structure may have caused overlapping blades, low photosynthetic rate, and loss of biomass, as it showed a greater gain in biomass compared to the synbiotic system. Biancacci et al. (2022) show that high productivity and low epiphytism can be found when partial harvests are carried out.

In addition to the macroalgae as inorganic consumer organisms, the oyster *C. virginica* was added to filter the organic matter from the tank. In the natural environment, oysters preferentially select and filter small organic particles rich in carbon and nitrogen, between 1 and 3 µm in size (Haven and Morales-alamo, 1970). However, in aquaculture systems the increase in suspended material comes from the residue of unconsumed food, feces, and bacterial biomass, which comprises approximately 50% of particles smaller than 48 µm (Ekasari et al. 2014). This makes it difficult for oysters to filter them. As a result, in this experiment there was no weight gain due to the low filtration rate by the oysters in the control and SMO treatments, although there was a better survival rate compared to the SO treatment, which had a higher concentration of solids in the system and total mortality.

As the main species in the multitrophic system, the *P. vannamei* has a herbivorous habit, which favors the use and consumption of particles suspended in the water, complemented by the feed offered (Krummenauer et al. 2020). It is known that the synbiotic system not only provides better control over water quality, but also favors increased shrimp growth due to the intense availability of food suspended in the water, which results in a more sustainable system with higher final productivity (Moustafa et al ., 2020). Our results were similar to those found by Pimentel et al., (2024) comparing different vegetable brans in the synbiotic system fertilization.

In addition to the synbiotic system, the inclusion of other organisms in the cultivation system had a greater influence on the shrimp's performance, resulting in higher final biomass, yield, and a lower feed conversion ratio in this experiment. The same result was found by Lima et al. (2021) when they included the oyster *Crassostrea gasar* in a synbiotic system with the shrimp *L. vannamei*, showing higher final yield and final weight, but no difference in the feed conversion ratio. A biofilm may have been created on the surface of the shell, which was available to the shrimp because they were both at the bottom of the tank. Biofilm consists of an aggregate of microorganisms that are adhered to a surface (Wetzel 2012). This biofilm can be adhered to an artificial substrate or on the surface of organisms, increasing better results in maintaining water quality and animal performance (Ballester et al. 2007). Ferreira et al. (2016) showed that in addition to the suspended matter in the water column, the presence of biofilm allowed the shrimp to gain weight. In our results, the biofilm formed by the insertion of the oyster in the system provided an advantage in the availability of food for the shrimp in addition to the feed offered, showing good functionality in the use of the integrated system.

5. Conclusion

The integrated system with the shrimp *Penaeus vannamei*, the oyster *Crassostrea virginica*, and the macroalga *Ulva lactuca* in a synbiotic system allowed for better water quality with lower nitrate levels due to absorption by the macroalga. The use of the synbiotic system meant that water changes were not necessary, as it was able to keep ammonia and nitrite levels low. The shrimp performance in terms of feed conversion ratio and yield was superior in the integrated system with the two species, along with higher oyster survival. However, for the macroalgae, the use of the synbiotic system had a negative influence on their growth as a result of the accumulation of organic matter.

Acknowledgment

The authors are grateful for the financial support provided by Coordination for the Improvement of Higher-Level Personnel (CAPES), for the scholarships granted (process number: 88887.843154/2023-00, 88887.612993/2021-00, , 88887.852145/2023-00), National Council for Scientific and Technological Development (CNPq, process number: 313514/2023-2) and Foundation for Research Support of the State of Rio Grande do Sul - FAPERGS (process number: 21/2551-0002225-6)

Funding sources

This research was partially funded by the ASTRAL Project—H2020 grant agreement 863034. This study was funded by the Brazil's Coordination for the Improvement of Higher-Level Personnel (CAPES) (process number: 88887.843154/2023-00, 88887.612993/2021-00, 88887.852145/2023-00), National Council for Scientific and Technological Development (CNPq, process number: 313514/2023-2) and Foundation for Research Support of the State of Rio Grande do Sul - FAPERGS (process number: 21/2551-0002225-6).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Author Contributions

Andrezza Carvalho: Conceptualization, Investigation, Methodology, Data curation, Visualization, Formal analysis, Writing – original draft. **Otávio Augusto Lacerda Ferreira Pimentel:** Investigation, Data curation, Writing – review & editing. **Dariano Krummenauer:** Conceptualization, Resources, Project administration, Writing – review & editing. **Ethan McAlhaney:** Conceptualization, Supervision, Methodology, Writing – review & editing. **Stephen Urick:** Funding acquisition, Resources, Supervision, Project administration, Methodology, Writing – review & editing. **Jireh Clarington:** Investigation, Data curation, Writing – review & editing. **Jonathan van Senten:** Funding acquisition, Resources, Writing – review & editing. **Michael H. Schwarz:** Funding acquisition, Resources, Writing – review & editing. **Gamze Turan:** Supervision, Writing – review & editing. **Luis H. Poersch:** Funding acquisition, Resources, Supervision, Project administration, Methodology, Writing – review & editing.

Data availability

Data will be made available on request.

References

APHA (2005) Standard Methods for the Examination of Water and Wastewater, (21st edn). American Public Health Association, Washington, DC, USA.

Ballester ELC, Wasielesky W, Cavalli RO, Abreu PC (2007) Nursery of the pink shrimp *Farfantepenaeus paulensis* in cages with artificial substrates: Biofilm composition and shrimp performance. Aquaculture 269:355–362. https://doi.org/10.1016/j.aquaculture.2007.04.003

Biancacci C, Visch W, Callahan DL, et al (2022) Optimisation of at-sea culture and harvest conditions for cultivated *Macrocystis pyrifera*: yield, biofouling and biochemical composition of cultured biomass. Front Mar Sci 9:1–19. https://doi.org/10.3389/fmars.2022.951538

Brito LO, Arantes R, Magnotti C, et al (2014) Water quality and growth of Pacific white shrimp *Litopenaeus vannamei* (Boone) in co-culture with green seaweed *Ulva lactuca* (Linaeus) in intensive system. Aquac Int 22:497–508. https://doi.org/10.1007/s10499-013-9659-0

Galtsoff, P. S. 1964. The American Oyster *Crassostrea virginica* Gmelin. Fishery Bulletin, v. 64. United States Government Printing Office, Washington, D. C. iii 480 p.

Carvalho A, Costa LC de O, Holanda M, et al (2023) Growth of the Macroalgae *Ulva lactuca* Cultivated at Different Depths in a Biofloc Integrated System with Shrimp and Fish. Phycology 3:280–293. https://doi.org/10.3390/phycology3020018

Chopin T (2015) Marine Aquaculture in Canada: Well-Established Monocultures of Finfish and Shellfish and an Emerging Integrated Multi-Trophic Aquaculture (IMTA) Approach Including Seaweeds, Other Invertebrates, and Microbial Communities. Fisheries 40:28–31. https://doi.org/10.1080/03632415.2014.986571

Chopin T, Buschmann AH, Halling C, et al (2001) Integrating seaweeds into marine aquaculture systems: A key toward sustainability. J Phycol 37:975–986. https://doi.org/10.1046/j.1529-8817.2001.01137.x

Chopin T, Fitzsimmons KM (2017) Don't worry, kelp is on the way - Symbiotic aquaculture relationships

Costa LC de O, Poersch LH da S, Abreu PC (2021) Biofloc removal by the oyster *Crassostrea gasar* as a candidate species to an Integrated Multi-Trophic Aquaculture (IMTA) system with the marine shrimp Litopenaeus vannamei. Aquaculture 540:. https://doi.org/10.1016/j.aquaculture.2021.736731

Crab R, Avnimelech Y, Defoirdt T, et al (2007) Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270:1–14. https://doi.org/10.1016/j.aquaculture.2007.05.006

Da Silva KR, Wasielesky W, Abreu PC (2013) Nitrogen and Phosphorus Dynamics in the Biofloc Production of the Pacific White Shrimp, *Litopenaeus vannamei*. J World Aquac Soc 44:30–41. https://doi.org/10.1111/jwas.12009

Lima PCM, da Silva AEM, da Silva DA, et al (2022) Use of recirculation and settling chamber in synbiotic multi-trophic culture of *Crassostrea* sp. with *Litopenaeus vannamei*. Aquac Res 53:6626–6640. https://doi.org/10.1111/are.16132

Morais APM, Santos IL, Carneiro RFS, et al (2023) Integrated multitrophic aquaculture system applied to shrimp, tilapia, and seaweed (*Ulva ohnoi)* using biofloc technology. Aquaculture 572:. https://doi.org/10.1016/j.aquaculture.2023.739492

Duke CS, Litaker W., Ramus J (1989) Effects of the temperature, nitrogen supply and tissue nitrogen on ammonium uptake rates of the Chlorophyte seaweeds *Ulva curvata* and *Codium decorticatum*. J. Phycol. 25:113–120

Ehrich MK, Harris LA (2015) A review of existing eastern oyster filtration rate models. Ecol Modell 297:201–212. https://doi.org/10.1016/j.ecolmodel.2014.11.023

Ekasari J, Angela D, Waluyo SH, et al (2014) The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. Aquaculture 426–427:105–111. https://doi.org/10.1016/j.aquaculture.2014.01.023

FAO (2022) State of the World Fisheries and Aquaculture - 2022 (SOFIA)

FAO (2024) World fisheries and aquaculture in review

Ferreira GS, Santos D, Schmachtl F, et al (2021) Heterotrophic, chemoautotrophic and mature approaches in biofloc system for Pacific white shrimp. Aquaculture 533:. https://doi.org/10.1016/j.aquaculture.2020.736099

Ferreira LMH, Lara G, Wasielesky W, Abreu PC (2016) Biofilm versus biofloc: Are artificial substrates for biofilm production necessary in the BFT system? Aquac Int 24:921–930. https://doi.org/10.1007/s10499-015-9961-0

Furtado PS, Campos BR, Serra FP, et al (2015) Effects of nitrate toxicity in the Pacific white shrimp, *Litopenaeus vannamei*, reared with biofloc technology (BFT). Aquac Int 23:315–327. https://doi.org/10.1007/s10499-014-9817-z

Furtado PS, Poersch LH, Wasielesky W (2011) Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical performance of shrimp *Litopenaeus vannamei* reared in bio-flocs technology (BFT) systems. Aquaculture 321:130–135. https://doi.org/10.1016/j.aquaculture.2011.08.034

Gaona CAP, de Almeida MS, Viau V, et al (2017) Effect of different total suspended solids levels on a *Litopenaeus vannamei* (Boone, 1931) BFT culture system during biofloc formation. Aquac Res 48:1070–1079. https://doi.org/10.1111/are.12949

Grabowski JH, Brumbaugh RD, Conrad RF, et al (2012) Economic valuation of ecosystem services provided by oyster reefs. Bioscience 62:900–909. https://doi.org/10.1525/bio.2012.62.10.10

Granada L, Lopes S, Novais SC, Lemos MFL (2018) Modelling integrated multi-trophic aquaculture: Optimizing a three trophic level system. Aquaculture 495:90–97. https://doi.org/10.1016/j.aquaculture.2018.05.029

Hadley S, Wild-Allen K, Johnson C, Macleod C (2015) Modeling macroalgae growth and nutrient dynamics for integrated multi-trophic aquaculture. J Appl Phycol 27:901–916. https://doi.org/10.1007/s10811-014-0370-y

HAVEN DS, MORALES-ALAMO R (1970) Filtration of Particles From Suspension By the American Oyster *Crassostrea Virginica* . Biol Bull 139:248–264. https://doi.org/10.2307/1540081

Holanda M, Wasielesky W, de Lara GR, Poersch LH (2022) Production of Marine Shrimp Integrated with Tilapia at High Densities and in a Biofloc System: Choosing the Best Spatial Configuration. Fishes 7:. https://doi.org/10.3390/fishes7050283

Hussain AS, Mohammad DA, Sallam WS, et al (2021) Effects of culturing the Pacific white shrimp *Penaeus vannamei* in “biofloc” vs “synbiotic” systems on the growth and immune system. Aquaculture 542:736905. https://doi.org/10.1016/j.aquaculture.2021.736905

Huynh TG, Shiu YL, Nguyen TP, et al (2017) Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: A review. Fish Shellfish Immunol 64:367–382. https://doi.org/10.1016/j.fsi.2017.03.035

Jones AB, Dennison WC, Preston NP (2001) Integrated treatment of shrimp effluent by sedimentation, oyster filtration and macroalgal absorption: A laboratory scale study. Aquaculture 193:155–178. https://doi.org/10.1016/S0044-8486(00)00486-5

Jory, D.E.; Cabrera, T.R.; Dugger, D.M.; Fegan, D.; Lee, P.G.; Lawrence, L.; Jackson, C.J.; Mcintosh, R.P.; Castañeda J. I, Al. B. et (2011) A Global Review of Shrimp Feed Management: Status and Perspectives. Aquaculture 104–152

Khanjani MH, Mohammadi A, Emerenciano MGC (2024) Water quality in biofloc technology (BFT): an applied review for an evolving aquaculture. Springer International Publishing

Khanjani MH, Zahedi S, Mohammadi A (2022) Integrated multitrophic aquaculture (IMTA) as an environmentally friendly system for sustainable aquaculture: functionality, species, and application of biofloc technology (BFT). Environ Sci Pollut Res 29:67513–67531. https://doi.org/10.1007/s11356-022-22371-8

Korzen L, Abelson A, Israel A (2016) Growth, protein and carbohydrate contents in *Ulva rigida* and Gracilaria bursa-pastoris integrated with an offshore fish farm. J Appl Phycol 28:1835–1845. https://doi.org/10.1007/s10811-015-0691-5

Krummenauer D, Abreu PC, Poersch L, et al (2020) The relationship between shrimp (*Litopenaeus vannamei*) size and biofloc consumption determined by the stable isotope technique. Aquaculture 529:735635. https://doi.org/10.1016/j.aquaculture.2020.735635

Krummenauer D, Peixoto S, Cavalli RO, et al (2011) Superintensive culture of white shrimp, *Litopenaeus vannamei*, in a biofloc technology system in Southern Brazil at different stocking densities. J World Aquac Soc 42:726–733. https://doi.org/10.1111/j.1749-7345.2011.00507.x

Legarda EC, da Silva D, Miranda CS, et al (2021) Sea lettuce integrated with Pacific white shrimp and mullet cultivation in biofloc impact system performance and the sea lettuce nutritional composition. Aquaculture 534:736265. https://doi.org/10.1016/j.aquaculture.2020.736265

Lima PCM, Silva AEM, Silva DA, et al (2021) Effect of stocking density of *Crassostrea* sp. in a multitrophic biofloc system with *Litopenaeus vannamei* in nursery. Aquaculture 530:735913. https://doi.org/10.1016/j.aquaculture.2020.735913

Linhoss AC, Camacho R, Ashby S (2016) Oyster Habitat Suitability in the Northern Gulf of Mexico. J Shellfish Res 35:841–849. https://doi.org/10.2983/035.035.0412

Loureiro RR, Reis RP, Critchley AT (2010) In vitro cultivation of three *Kappaphycus alvarezii* (Rhodophyta, Areschougiaceae) variants (green, red and brown) exposed to a commercial extract of the brown alga *Ascophyllum nodosum* (Fucaceae, Ochrophyta). J Appl Phycol 22:101–104. https://doi.org/10.1007/s10811-009-9412-2

Moustafa EM, Saad TT, Khalil RH, et al (2020) The Ameliorative Role of Synbiotic Culture Techniques Application in White Shrimp (*Litopenaeus vannamei*) During Nursery Stage. Adv Anim Vet Sci 8:527–534. https://doi.org/10.17582/journal.aavs/2020/8.3.260.277

Oladosu Y, Rafii MY, Abdullah N, et al (2016) Fermentation Quality and Additives: A Case of Rice Straw Silage. Biomed Res Int 2016:. https://doi.org/10.1155/2016/7985167

Pimentel OALF, Wasielesky W, da Silva NP, et al (2024) Fertilizing synbiotic system with different vegetable brans: effects on nitrification, plankton composition, and growth of *Penaeus vannamei* in the nursery phase. Aquac Int. https://doi.org/10.1007/s10499-024-01471-x

Ren X, Wang Q, Shao H, et al (2021) Effects of Low Temperature on Shrimp and Crab Physiology, Behavior, and Growth: A Review. Front Mar Sci 8:. https://doi.org/10.3389/fmars.2021.746177

Romano N, Dauda AB, Ikhsan N, et al (2018) Fermenting rice bran as a carbon source for biofloc technology improved the water quality, growth, feeding efficiencies, and biochemical composition of African catfish *Clarias gariepinus* juveniles. Aquac Res 49:3691–3701. https://doi.org/10.1111/are.13837

Samocha TM, Patnaik S, Speed M, et al (2007) Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. Aquac Eng 36:184–191. https://doi.org/10.1016/j.aquaeng.2006.10.004

Shakouri A, Balouch GM (2020) The effects of nitrate and phosphate on growth of algae, *Ulva rigida*. Iran J Fish Sci 19:59–66. https://doi.org/10.22092/ijfs.2018.117843

Summers N, Fragoso GM, Johnsen G (2023) Photophysiologically active green, red, and brown

macroalgae living in the Arctic Polar Night. Sci Rep 13:1–16. https://doi.org/10.1038/s41598-023-44026-5

Troell M, Joyce A, Chopin T, et al (2009) Ecological engineering in aquaculture - Potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. Aquaculture 297:1–9. https://doi.org/10.1016/j.aquaculture.2009.09.010

Verdegem M, Buschmann AH, Latt UW, et al (2023) The contribution of aquaculture systems to global aquaculture production. J World Aquac Soc 54:206–250. https://doi.org/10.1111/jwas.12963

Wetzel RG (2012) Periphyton of Freshwater Ecosystems: Proceedings of the First International Workshop on Periphyton of Freshwater Ecosystems held in Växjö, Sweden, 14–17 September 1982 (Vol. 17). Springer Science & Business Media., 17th edn.

CAPÍTULO 2: Produção da macroalga *Ulva lactuca* integrada com o camarão *Penaeus vannamei* em sistema de bioflocos: efeito dos sólidos suspensos totais e da concentração de nutrientes.

Artigo publicado na *Phycology*.

https://doi.org/10.3390/phycology4010002

Resumo

De acordo com as características físico e químicas do ambiente de cultivo da macroalga podem ocorrer diferenças no seu desempenho em crescimento, absorção de nutrientes e perfil nutricional. O presente estudo avaliou o efeito de diferentes concentrações de nutrientes e sólidos suspensos totais sobre a taxa de remoção de nutrientes, crescimento e biocompostos da macroalga *U. lactuca* em um sistema integrado com o camarão *Penaeus vannamei*. O experimento teve duração de 45 dias, e foi realizado em tanques de 280 L de volume útil, com a densidade de 0,88 kg de macroalga m^{-2} e 200 camarões m^{-3}. Contou com cinco tratamentos com três repetições cada, com porcentagens de 0 (controle), 25, 50, 75 e 100% de inóculo de bioflocos (73,3 ± 5,7 e 325,0 ± 21,2 mg L^{-1} de nitrato e sólidos iniciais, respetivamente, no inóculo de 100%), provenientes de um cultivo de camarões, resultando em diferentes concentrações de sólidos e nutrientes. Foi utilizada uma estrutura de cultivo flutuante para as macroalgas. Os parâmetros de qualidade da água e a taxa de remoção de nutrientes foram mensurados. Não houve diferença no crescimento das macroalgas entre os tratamentos. Para a absorção de nutrientes, o tratamento com 75% de inóculo apresentou uma taxa de remoção de 55,0 ± 4,0 e 31,0 ± 10,0% de nitrato e fosfato, respetivamente. Além de apresentarem valores mais elevados de proteína, clorofila-a e valores mais baixos de cinzas em comparação com o controle (sem adição de bioflocos). O uso de macroalgas na produção integrada com camarão nas condições do tratamento com 75% de inóculo de bioflocos mostrou-se viável e sustentável.

Production of the Macroalgae *Ulva lactuca* Integrated with the Shrimp *Penaeus vannamei* in a Biofloc System: Effect of Total Suspended Solids and Nutrient Concentrations

Andrezza Carvalho [1]*, Ítalo Braga [2], Florencia Chaar [1], Alessandro Pereira Cardozo [1], José María Monserrat [1,3], Juan Rafael Buitrago Ramírez [1,3], Wilson Wasielesky, Jr. [1] and Luís H. Poersch [1]

[1] Marine Aquaculture Station, Institute of Oceanography, Federal University of Rio Grande—FURG, Rua do Hotel n° 2, Cassino, Rio Grande 96210030, RS, Brazil; chaar128@gmail.com (F.C.); ocalessandro@hotmail.com (A.P.C.); josemmonserrat@gmail.com (J.M.M.); juanrafaelb25@gmail.com (J.R.B.R.); manow@mikrus.com.br (W.W.J.); lpoersch@gmail.com (L.H.P.)
[2] Escola Agrícola de Jundiaí, Federal University of Rio Grande do Norte—UFRN, Jundiaí, Macaiba, Rio Grande do Norte 59280-000, RN, Brazil; italo_96felipe@hotmail.com
[3] Laboratory of Functional Biochemistry of Aquatic Organisms (BIFOA), Institute of Oceanography (IO), Federal University of Rio Grande—FURG, Rua do Hotel, n° 2, Cassino, Rio Grande 96210-030, RS, Brazil
* Correspondence: andrezzachagas@hotmail.com

Abstract: This study focused on evaluating the effect of different concentrations of nutrients and total suspended solids on the removal rate of nutrients and biocompounds from the macroalgae *U. lactuca* in an integrated system with the shrimp *Penaeus vannamei* in biofloc. The experiment lasted 45 days and included five treatments with three replicates each, with percentages of 0 (control), 25, 50, 75, and 100% biofloc inoculum (73.3 ± 5.7 and 325.0 ± 21.2 mg L^{-1} initial nitrate and solids, respectively, in the 100% inoculum), from a shrimp farm, resulting in different concentrations of solids and nutrients. The macroalgae were introduced into 280 L tanks at a density of 0.88 kg m^{-2}, along with 200 shrimp m^{-3}. The algae were separated by a floating structure. Water quality parameters were measured, and the nutrient removal rate was evaluated. The treatment with 75% inoculum showed a removal rate of 55.0 ± 4.0 and 31.0 ± 10.0% of nitrate and phosphate, respectively. There was no difference in macroalgae growth between the treatments; however, macroalgae grown in 75% inoculum had higher protein, chlorophyll-*a,* and lower ash values compared with the control. The use of macroalgae in integrated production with shrimp under the conditions of the treatment with 75% biofloc inoculum proved to be viable and sustainable.

Keywords: removal rate nutrients; nitrate; phosphate; bioremediator; chlorophyll; alkalinity

1. Introduction

Large-scale production of macroalgae comes from open and coastal areas, totaling 35.1 million tons, according to the FAO in 2022 [1]. However, these cultures are affected by the availability of nutrients, wave action, and the presence of herbivores, factors that can influence

the growth of macroalgae. To address these challenges, the integration of macroalgae cultivation with animal production systems that generate nutrient-rich effluents, such as shrimp farming, has gained prominence. This approach enables indoor cultivation of macroalgae while concurrently serving as a means of bioremediation for aquaculture wastewater. Studies by Alencar et al. [2] and Copertino et al. [3] have demonstrated the effectiveness of cultivating *Ulva* algae using effluents from shrimp farms. They achieved growth rates exceeding 8.0% per day and ammonia absorption rates of up to 90%, indicating enhanced macroalgae development while effectively removing nutrients from the effluents.

More sustainable farming has also gained importance over time, with the aim of producing animals with less environmental impact and effluent treatment systems. The integration of different species in cultivation represents an alternative for improving water quality and the use of waste. This approach is known as Integrated Multitrophic Aquaculture (IMTA), which stands out for its sustainability. IMTA involves the cultivation of both feeding species and consuming species that play a crucial role in recycling organic matter and residual nutrients [4]. Macroalgae participate in the absorption of nutrients, sequestration of carbon dioxide (CO_2), and maintenance of dissolved oxygen [5]. Integrating macroalgae into shrimp farming increases the system's productivity, and two or more species can be cultivated in the same area without negatively affecting their performance, thus increasing economic viability [6]. The advantages of integrated systems have been shown to be effective in studies such as Nobre et al. [7] with macroalgae and abalone, Holanda et al. [8] with shrimp and fish, Verdian et al. [9], which showed improvements in zootechnical performance when integrating shrimp, fish, and macroalgae, and Poli et al. [10] with shrimp, fish, and halophytes.

Biofloc technology is a production approach characterized by minimal water exchange, high productivity, and a reduced environmental impact. Central to this technology is the role of heterotrophic bacteria. These bacteria play a vital role in assimilating the ammonia produced by converting it into bacterial biomass, resulting in an increase in total suspended solids-TSS and oxygen consumption [11]. In parallel, chemoautotrophic bacteria within the system convert ammonia into nitrate while consuming inorganic carbon [11]. For shrimp farming, the solids level should be kept between 100 and 300 mg L^{-1}, with the excess being removed from the system using clarifiers [12] or partial water changes. The constant use of clarifiers to maintain a low concentration of solids can lead to greater energy expenditure. In addition, the low concentration of solids can affect water quality, causing nitrite spikes due to the low availability of bacteria in the system [13].

The minimal water exchange in cultures operated in biofloc systems favors the accumulation of nutrients and solids in the system, which, when released at the end of cultivation into the receiving body of water, can cause eutrophication [14]. Although the accumulation of nutrients is favorable to macroalgae growth, the successful production of macroalgae biomass in a biofloc system requires adaptation to the environment due to the distinct characteristics compared with the natural environment. Legarda et al. [15] showed that introducing macroalgae collected from the natural environment into the biofloc system can lead to stress and loss of biomass.

The production of solids in the water can harm the macroalgae since these solids block the entry of light into the photosynthetic tissues [16]. In addition, the presence of these solids limits the penetration of light into the water column, especially at greater depths [17]. Therefore, for the best development of macroalgae, the concentration of solids in the system must be optimized. The use of water exchanges can help to decrease the concentration of solids in the system, but it will increase energy costs and cause environmental problems with the disposal of effluents. According to Queiroz et al. [14], the disposal of effluent from shrimp farming in mangroves increases the nitrogen content in the soil and its mineralization, factors that trigger the eutrophication of mangrove areas. Water exchange in cultivation also leads to nutrient dilution, which can become a limiting factor or change the N:P ratio for macroalgae growth [18].

Thus, adjusting water quality parameters for integrated macroalgae cultivation can be used as a way of boosting macroalgae performance in biofloc systems. In addition to the growth of macroalgae, their cultivation in an environment with different physical and chemical factors can lead to changes in their biochemical and nutritional composition, which may be of interest to the pharmaceutical and food industries [18]. Previous studies have shown an increase in the protein content of the macroalgae *Ulva lactuca* when cultivated in an integrated biofloc system. The values reached 22.4%, in contrast to the 12.40% observed when cultivated in laboratory solution, highlighting its nutritional value [19]. He et al. [20] described some factors that influence the increase in antioxidant capacity and phenol content, such as salinities below 25, as well as the depletion or high concentrations of nutrients that can influence the concentration of amino acids and fatty acids in macroalgae. Therefore, the medium and conditions under which macroalgae are cultivated can enhance the biomass produced.

Therefore, the aim of this study is to evaluate nutrient absorption, nutritional composition, and bioactive compounds in the macroalgae *Ulva lactuca* when cultivated under varying

concentrations of nutrients and total suspended solids (biofloc) within an integrated system with the white shrimp *Penaeus vannamei*.

2. Materials and Methods

2.1. Experimental Design and Facilities

The experiment was carried out at the Marine Aquaculture Station, Institute of Oceanography of the Federal University of Rio Grande (IO-FURG), located on Cassino Beach, Rio Grande, Rio Grande do Sul. The macroalgae species used was *U. lactuca*, collected in a natural environment (32°17′52.30″ S–52°15′59.80″ W). After collection, the macroalgae were taken to the laboratory to remove the epiphyte species and identified using a molecular analysis and observing quadratic cells and a bilayer of cells characteristic of this species, as also identified by Alencar et al. [2]. After confirming the species, it was cultivated for 27 days before being stocked in the experimental units in a 1 m^3 circular tank with 10% biofloc inoculum, resulting in a concentration of 35.1 ± 2.74 mg L^{-1} of nitrate and 2.24 ± 1.2 mg L^{-1} of phosphate. The shrimp came from a biofloc system grow-out tank at the EMA/IO-FURG Carcinoculture Laboratory. The shrimp, with an initial weight of 3.85 ± 0.73 g, were stocked at a density of 200 shrimp m^{-3} to maintain the biofloc.

The experiment lasted 45 days and was carried out in an agricultural greenhouse. The experimental units were kept under constant aeration using a blower (4 HP), which injected air into the experimental units through two pieces of micro-perforated hose (each piece 20 cm long) per tank. During the experiment, the average light intensity was 28.68 ± 8.53 µmol m^{-2} s^{-1}, with a natural photoperiod of 14 h light and 10 h dark. Fifteen tanks were used, arranged randomly in the greenhouse, with a base diameter of 0.81 m, a height of 0.53 m, and a useful volume of 180 L (Figure 1). The shrimp and macroalgae were kept in the same tank, but to separate the animals, the macroalgae were cultivated inside a circular floating structure near the surface with a diameter of 0.60 m, with 5 mm mesh openings, with a depth of 10 cm to allow the macroalgae to move within the structure, at a density of 0.88 kg m^{-2} (Figure 1). The shrimp were fed with a commercial feed of 36% protein (Guabi Aqua QS 2–3 mm, Guabi Nutrition and Animal Health S.A., Campinas, São Paulo, Brazil), according to the methodology proposed by Jory et al. [21], twice a day, with the feed being adjusted weekly.

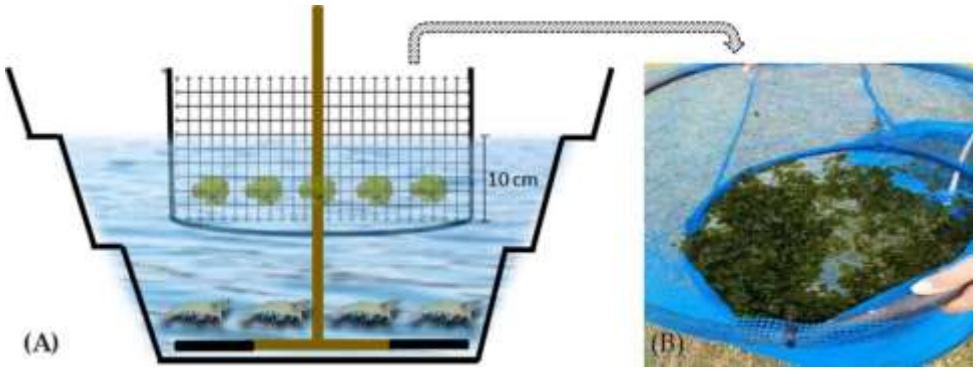


Figure 1. Diagram of the experimental units used for shrimp and macroalgae. (**A**) Tanks with a useful volume of 180 L, with constant aeration, and a circular structure for the macroalgae. (**B**) Circular structure with a diameter of 0.60 cm for the cultivation of macroalgae.

The experiment used a mature biofloc inoculum from a grow-out shrimp farm [11]. The nutrient concentrations of the inoculum were 0.09, 0.03, 73.33, and 1.60 mg L^{-1} of total ammoniacal nitrogen, nitrite, nitrate, and phosphate, respectively, 20 mL L^{-1} of settleable solids, 304 NTU for turbidity and 325 mg L^{-1} of total suspended solids. This inoculum was placed in different percentages in each treatment and diluted with seawater to complete the useful volume of the tank, arriving at different concentrations of nutrients and solids (Table 1).

The experiment consisted of five treatments with three replicates each. Presenting a control treatment CONT—cultivation in clear water, with the addition of organic carbon when ammonia reached concentrations of 1 mg L^{-1} [22]; and treatments with different percentages of biofloc inoculum, with 25, 50, 75 and 100% biofloc, resulting in: T100—concentration of approximately 100 mg L^{-1} of total suspended solids; T200—concentration of approximately 200 mg L^{-1} of total suspended solids; T250—concentration of approximately 250 mg L^{-1} of total suspended solids; T300—concentration of approximately 300 mg L^{-1} of total suspended solids (Table 1).

Table 1. Initial characteristics of the treatments CONT (cultivation in clear water), T100 (use of 25% biofloc inoculum, a concentration of approximately 100 mg L^{-1} of total suspended solids), T200 (use of 50% biofloc inoculum, a concentration of approximately 200 mg L^{-1} of total suspended solids), T250 (use of 75% biofloc inoculum, a concentration of approximately 250 mg L^{-1} of total suspended solids), T300 (use of 100% biofloc inoculum, a concentration of approximately 300 mg L^{-1} of total suspended solids).

Parameters	Treatments				
	CONT	**T100**	**T200**	**T250**	**T300**
Total ammonia N–NH_3 (mg L^{-1})	0.02 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.03	0.09 ± 0.01
Nitrite NO_2—N (mg L^{-1})	0.01 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.03	0.01 ± 0.03
Nitrate NO_3—N (mg L^{-1})	0.05 ± 0.04 [a]	28.33 ± 5.77 [b]	45.00 ± 0.00 [c]	56.67 ± 5.77 [d]	73.33 ± 5.77 [e]
Phosphate P–PO_4^{-3} (mg L^{-1})	0.14 ± 0.03 [a]	0.81 ± 0.12 [b]	1.25 ± 0.05 [c]	1.55 ± 0.14 [d]	1.60 ± 0.00 [e]

SS (mL L^{-1})	0.00 ± 0.00 [a]	1.97 ± 1.38 [b]	8.00 ± 1.00 [c]	11.67 ± 0.58 [d]	19.67 ± 0.58 [e]
TSS (mg L^{-1})	11.67 ± 2.89 [a]	111.67 ± 63.31 [b]	213.33 ± 50.08 [c]	246.67 ± 2.89 [d]	325.00 ± 21.21 [e]
Turbidity (NTU)	2.57 ± 0.37 [a]	26.23 ± 18.01 [b]	74.63 ± 62.35 [c]	176.10 ± 18.36 [d]	304.10 ± 12.77 [e]

SS (Settleable Solids); TSS (Total Suspended Solids). Different letters in the same line represent significant differences ($p \leq 0.05$) between treatments after one-way ANOVA with Tukey's post-hoc test.

2.2. Physical and Chemical Parameters

Temperature (°C), dissolved oxygen (DO, mg L^{-1}), and pH were measured daily in all tanks using a multi-parameter probe (model Pro-20, YSI Inc., Yellow Springs, OH, USA) and a bench pH meter (Seven2Go S7 Basic, Mettler Toledo, São Paulo, Brazil). Salinity was measured weekly using a multi-parameter probe (model Pro-20, YSI Inc., Yellow Springs, OH, USA). For the water quality analyses, the samples were collected in plastic containers and taken immediately for analysis. Total ammonia (N–NH_3, mg L^{-1}) and nitrite (NO_2-N, mg L^{-1}) were analyzed twice a week according to the methodology of UNESCO [23] and Bendschneider & Robinson [24], respectively. Nitrate (NO_3-N) and phosphate ($P–PO_4^{-3}$) (mg L^{-1}) were analyzed using the methodology described by Aminot & Chaussepied [25] and monitored twice a week. Turbidity and TSS were determined weekly. Turbidity (NTU) was measured using a portable turbidimeter (2100P, Hach™, Loveland, CO, USA), and total suspended solids (TSS) were quantified using filtration and gravimetry according to the methodology described by Strickland and Parsons [26]. Total alkalinity (mg $CaCO_3$ L^{-1}) was measured twice a week and monitored according to the methodology presented by APHA [27]. Calcium hydroxide was used to maintain alkalinity above 150 mg L^{-1} [28]. The settleable solids (SS) were measured weekly using the Imhoff cone [27].

2.3. Growth and Nutrient Absorption by Macroalgae

The biomass yield of the macroalgae was measured weekly by weighing the fresh biomass, where the macroalgae were removed from the water and left in the open air for 20 min to remove excess water. The following formula was used to calculate the Relative Growth Rate (RGR) [29]:

$$\text{RGR (\% } d^{-1}\text{): } 100 \times [\ln(\text{final weight (g)}/\text{initial weight (g)})/(\text{final time} - \text{initial time})] \quad (1)$$

The nutrient absorption efficiency (NRR) of macroalgae was calculated using the following formula [29]:

$$\text{NRR (\%): } 100 \times [(\text{nutrient concentration at initial time (mg } L^{-1}) - \text{nutrient concentration at final time (mg } L^{-1}))/\text{nutrient concentration at initial time (mg } L^{-1})] \quad (2)$$

2.4. Proximal Composition and Biocompounds of Macroalgae

Proximal composition analysis was carried out on the treatment with the biofloc inoculum that showed the best growth performance and nutrient absorption at the end of cultivation (T250) and on the CONT treatment for comparison. Random samples of algae were collected manually from each experimental unit, washed in running water, and then with distilled water. Excess water was removed using a manual centrifuge, followed by drying with paper towels. The samples were weighed to determine their wet weight and placed in an oven at 60 °C for 24 h, after which they were weighed again to obtain their dry weight. The samples were then ground for analysis.

The nitrogen content of the algae was determined using the Kjeldahl titration method according to AOAC [30] at the Laboratory of Aquatic Organism Nutrition-LANOA (EMA, FURG, Rio Grande, Brazil). The formula used to convert nitrogen into protein was:

$$\text{Protein (\% of dry weight)} = [(0.085 \times \text{Vol} \times 0.014/\text{sample}) \times 5.45] \times \frac{100}{} \quad (3)$$

where Vol is the volume spent on titration, and sample is the dry weight of the sample [31].

The crude fat content was determined using the Soxhlet method based on solvent extraction (petroleum ether), and the ash content was obtained using the gravimetric method in a muffle furnace at 600 °C. The crude fiber was obtained using washes in acidic and basic media.

The macroalgae extract was obtained using the methodology of Barbarino & Lourenço [32], with the addition of 1 mL of sodium hydroxide and centrifugation to prepare the crude extract for protein analysis. The macroalgae protein was analyzed using the Biuret method, following the methodology of Barbarino & Lourenço [32]. The extract and trichloroacetic acid (TCA) (25%) were added in a ratio of 2.5:1 (*v*/*v*) to precipitate the protein and kept in an ice-cold bath for 30 min. The solution was then centrifuged and washed with dilutions of TCA (10 and 5%), removing the supernatant until the protein pellet was formed. 0.5 mL of sodium hydroxide (0.1 N) was added to the pellet suspension, and 20 µL of the solution and 1 mL of the total protein kit were removed.

To determine the concentration of chlorophyll-*a*, chlorophyll-*b*, carotenoids, phenolic compounds, and DPPH (2.2-diphenyl-1-picrylhydrazyl), 500 mg of sample and 5 mL of

methanol were used. The samples were macerated, then incubated in the dark for 60 min at 4 °C, and centrifuged (12,000× *g* × 10 min). Finally, the supernatant was collected for analysis. The absorbances at 664 and 647 ηm were measured to calculate chlorophyll-*a* (Chla = 11.75 × A664 − 2.35 × A647), chlorophyll-*b* (Chlb = 18.61 × A647 − 3.91 × A664) and carotenoids (Car = (1000 × A470 − 2.27 × Chla − 81.4 Chlb)/227) according to the methodology of Lichtenthaler & Wellburn [33].

Total phenolic compounds were determined by the Folin–Ciocalteau colorimetric method using the methodology described by Schiavon et al. [34] with modifications. A total volume of 2 mL of 2% Na_2CO_3 and 150 µL of Folin–Ciocalteau reagent (Sigma-Aldrich) were added to 200 µL of macroalgae extract. After 15 min of incubation in the dark, the samples were measured at 750 nm. The gallic acid standard curve was used (Sigma-Aldrich, St. Louis, MO, USA—100—1250 µg mL^{-1}, y = 0.0008x − 0.0156, R^2 = 0.999). The results were presented as mg of gallic acid equivalent per g of mass (mg GAE g^{-1}).

The DPPH radical scavenging effects of the samples were carried out using the methodology of Farasat et al. [35] with some modifications. A total volume of 400 µL of extract and 400 µL of 0.16 mM DPPH methanolic solution were used. The mixture was vortexed for 1 min and then incubated for 30 min in the dark. The absorbance was read at 517 nm in a spectrophotometer, where the antioxidant capacity was calculated using the following equation:

$$\% \text{ Inhibition} = (\text{Acotrol} - (\text{Asample} - \text{Ablank})/\text{Acotrol}) \times 100 \quad (4)$$

where Acotrol is the absorbance of the control (DPPH without extract), Asample is the absorbance of the extract (extract plus DPPH solution), and Ablank is the absorbance of the extract (extract without DPPH solution).

2.5. Shrimp Performance

Shrimp growth data were obtained from weekly biometrics that included the following formulas:

1. Mean final weight (g): final biomass of live animals (g)/total number of animals;
2. Weekly weight gain (g week-1): weight gain (g)/number of weeks.
3. Final biomass (g): ∑ final weight of all live animals (g);
4. Survival (%) = (final number of animals/initial number of animals) × 100;
5. Feed conversion factor (FCR) = ∑ration offered (g)/(biomass gains (g);
6. Productivity (kg m^{-3}): (final biomass (kg)/tank volume (m^3);

2.6. Statistical Analysis

The normality and homoscedasticity of these data were checked using the Shapiro–Wilk and Levene tests, respectively. Once these assumptions had been met, ANOVA was carried out, followed by Tukey's post-hoc test for water quality, macroalgae, and shrimp performance. Spearman's correlation was carried out for each week of the experiment between the variables treatment, nitrate concentration, phosphate concentration, total suspended solids concentration, macroalgae weight gain, nitrate removal rate, and phosphate removal rate. A student's t-test was applied to the proximal and biochemical composition of only the two treatments, CONT and T250. When the assumptions of ANOVA and the student's *t*-test were not met, the non-parametric Kruskal–Wallis test was used. A minimum significance level of 5% ($p \leq 0.05$) was applied in all analyses.

3. Results

3.1. Physical and Chemical Parameters

There were no significant differences ($p > 0.05$) between the treatments in the parameters temperature, dissolved oxygen (D.O), and ammonia. The pH remained higher in the T250 and T300 treatments, along with the alkalinity, which was also higher ($p > 0.05$) in the same treatments. Salinity differed between treatments ($p < 0.05$), ranging from 28 to 30. The lowest nitrite concentrations were observed in the CONT treatment. Nitrate and settleable solids (SS) had higher concentrations as the inoculum concentration increased. Phosphate and turbidity were lower in the CONT and T100 treatments and remained the same in the other treatments. Total suspended solids (TSS) in the CONT treatment increased compared with the initial concentration but remained lower than in the other treatments. The concentration of solids increased in the treatments in relation to the initial inoculum concentrations (Table 2).

As shown in Table 2, the best nitrate and phosphate removal rates were found in the T250 treatment with 55.0 ± 4.0 and 31.0 ± 10.0%, respectively. In contrast, the CONT treatment had an increase in nitrate and phosphate throughout the experiment.

Table 2. Water quality during the 45 days of cultivation, in treatments CONT (cultivation in clear water), T100 (use of 25% biofloc inoculum, a concentration of approximately 100 mg L^{-1} of total suspended solids), T200 (use of 50% biofloc inoculum, a concentration of approximately 200 mg L^{-1} of total suspended solids), T250 (use of 75% biofloc inoculum, a concentration of approximately 250 mg L^{-1} of total suspended solids), T300 (use of 100% biofloc inoculum, a concentration of approximately 300 mg L^{-1} of total suspended solids).

	Treatments				
Parameters	**CONT**	**T100**	**T200**	**T250**	**T300**
Temperature (°C)	26.92 ± 2.30	26.55 ± 2.18	26.56 ± 2.18	26.79 ± 2.46	26.56 ± 2.19

DO (mg L^{-1})	5.89 ± 0.70	6.03 ± 0.71	6.00 ± 0.70	5.90 ± 0.74	5.98 ± 0.71
pH	8.15 ± 0.09 [c]	8.18 ± 0.07 [c]	8.16 ± 0.08 [b]	8.23 ± 0.07 [a]	8.22 ± 0.07 [a]
Salinity (‰)	30.43 ± 1.81 [a]	30.00 ± 0.51 [a]	29.43 ± 1.57 [a]	29.19 ± 1.40 [ab]	27.90 ± 0.66 [b]
Alkalinity (mg $CaCO_3$ L^{-1})	173.33 ± 23.93 [cb]	174.36 ± 21.40 [cb]	175.66 ± 24.69 [cb]	205.92 ± 34.15 [a]	183.08 ± 27.05 [b]
Total ammonia N–NH_3 (mg L^{-1})	0.12 ± 0.07	0.11 ± 0.03	0.09 ± 0.03	0.10 ± 0.02	0.10 ± 0.03
Nitrite NO_2—N (mg L^{-1})	0.26 ± 0.55 [a]	1.06 ± 1.36 [b]	1.30 ± 1.96 [b]	1.40 ± 2.43 [b]	0.35 ± 0.40 [b]
Nitrate NO_3—N (mg L^{-1})	1.56 ± 3.13 [a]	19.53 ± 3.53 [b]	32.25 ± 4.85 [c]	44.97 ± 9.01 [d]	59.38 ± 6.80 [e]
Phosphate P-PO_4^{-3} (mg L^{-1})	0.56 ± 0.36 [a]	0.79 ± 0.38 [ab]	1.21 ± 0.39 [b]	1.16 ± 0.30 [b]	1.67 ± 0.26 [c]
SS (mL L^{-1})	2.65 ± 2.34 [a]	5.93 ± 3.33 [b]	11.15 ± 3.17 [c]	15.08 ± 4.39 [d]	21.71 ± 4.98 [e]
TSS (mg L^{-1})	134.05 ± 81.36 [a]	232.62 ± 92.76 [b]	290.48 ± 65.36 [b]	354.05 ± 88.69 [c]	403.69 ± 79.03 [c]
Turbidity (NTU)	80.37 ± 62.71 [a]	106.26 ± 79.79 [a]	167.31 ± 95.65 [ab]	232.91 ± 101.9 [b]	287.48 ± 104.59 [b]
Calcium hydroxide (g L^{-1}) [#]	0.13 ± 0.03	0.10 ± 0.00	0.13 ± 0.06	0.07 ± 0.06	0.08 ± 0.03
Removal rate					
Nitrate (%)	00.00 ± 00.00 [c]	28.0 ± 13.0 [b]	17.0 ± 15.0 [bc]	55.0 ± 4.0 [a]	38.0 ± 11.0 [ab]
Phosphate (%)	00.00 ± 00.00 [b]	00.00 ± 00.00 [b]	00.00 ± 00.00 [b]	31.0 ± 10.0 [a]	00.00 ± 00.00 [b]

DO (dissolved oxygen); SS (Settleable Solids); TSS (Total Suspended Solids); # Use of calcium hydroxide per treatment. Different letters in the same line represent significant differences ($p \leq 0.05$) between treatments after one-way ANOVA with Tukey's post-hoc test.

When looking at the nitrate values, the initial concentrations of the T300, T250, T200, and T100 treatments were higher than the final concentrations, showing a removal of the nitrogenous compound (Figure 2). In the control treatment, there was an increase in nitrate concentration on the 32nd day of the trial period. At the end of cultivation, the nitrate concentrations between treatments T300, T250, T200, and T100 were statistically similar.

Figure 2. Nitrate concentrations in treatments CONT (cultivation in clear water), T100 (use of 25% biofloc inoculum, a concentration of approximately 100 mg L^{-1} of total suspended solids), T200 (use of 50% biofloc inoculum, a concentration of approximately 200 mg L^{-1} of total suspended solids), T250 (use of 75% biofloc inoculum, a concentration of approximately 250 mg L^{-1} of total suspended solids), T300 (use of 100% biofloc inoculum, a concentration of approximately 300 mg L^{-1} of total suspended solids). Different lowercase letters on the same line represent a significant difference ($p \leq 0.05$) in the same treatment at the beginning and end. Different capital letters represent a significant difference ($p \leq 0.05$) between treatments on the same day after performing a one-way ANOVA followed by Tukey's test.

In general, there was a positive correlation between treatment and the concentration of total suspended solids, nitrate, and phosphate in all weeks of cultivation. In the first week, there was a positive correlation between the weight gain of the macroalgae and nitrate removal rate (correlation coefficient = 0.575; *p* valor = 0.025) and phosphate removal rate (correlation coefficient = 0.627; *p* valor = 0.012). The second week showed a positive correlation between the weight gain of the macroalgae and nitrate removal rate (correlation coefficient = 0.596; *p* valor = 0.019). The third week showed a positive correlation between treatment and nitrate removal rate (correlation coefficient = 0.542; *p* valor = 0.037). The fourth week showed a positive correlation between nitrate removal rate, treatment (correlation coefficient = 0.676; *p* valor = 0.006), nitrate concentration (correlation coefficient = 0.657; *p* valor = 0.008), and phosphate concentration (correlation coefficient = 0.738; *p* valor = 0.002). The fifth week showed a positive correlation between the nitrate removal rate with treatment (correlation coefficient = 0.873; *p* valor = 0.000), nitrate concentration (correlation coefficient = 0.864; *p* valor = 0.000), phosphate concentration (correlation coefficient = 0.555; *p* valor = 0.032) and total suspended solids (correlation coefficient = 0.717; *p* valor = 0.003). In the last week, there was a positive correlation between the treatment and the phosphate removal rate (correlation

coefficient = 0.683; *p* valor = 0.005) and a negative correlation between the weight gain of the macroalgae and the nitrate removal rate (correlation coefficient = −0.729; *p* valor = 0.002).

3.2. Macroalgae Performance

The average initial weight of the macroalgae in the treatments was 250.18 ± 0.28 g, with a gain in biomass throughout the experiment indicated by the growth rate (Table 3). There was no significant difference in final weight and relative growth rate (RGR) between treatments.

Table 3. Performance of macroalgae during 45 days of cultivation, in treatments CONT (cultivation in clear water), T100 (use of 25% biofloc inoculum, a concentration of approximately 100 mg L^{-1} of total suspended solids), T200 (use of 50% biofloc inoculum, a concentration of approximately 200 mg L^{-1} of total suspended solids), T250 (use of 75% biofloc inoculum, a concentration of approximately 250 mg L^{-1} of total suspended solids), T300 (use of 100% biofloc inoculum, a concentration of approximately 300 mg L^{-1} of total suspended solids).

	Treatments				
	CONT	**T100**	**T200**	**T250**	**T300**
Initial mean weight (g—FW)	250.31 ± 0.31	250.05 ± 0.02	250.0 ± 0.00	250.51 ± 0.41	250.06 ± 0.04
Final mean weight (g—FW)	387.33 ± 115.09	317.67 ± 60.86	289.0 ± 73.75	304.33 ± 76.20	275.0 ± 36.69
RGR (% dia^{-1})	0.89 ± 0.61	0.49 ± 0.41	0.27 ± 0.52	0.38 ± 0.52	0.19 ± 0.30

RGR (relative growth rate); FW (fresh weight).

3.3. Proximal Composition and Biocompounds of Macroalgae

The proximate and biochemical composition was carried out on the control treatment (CONT), and the treatment with the best performance in nutrient absorption, the T250 treatment, was used. Table 4 shows that the highest protein and chlorophyll-*a* values were found in the treatment with biofloc inoculum (T250), and the highest ash values were found in the CONT treatment.

Table 4. Proximal composition and biocompounds of the macroalgae (dry matter) at the end of cultivation in the CONT (cultivation in clear water) and T250 (use of 75% biofloc inoculum, a concentration of approximately 250 mg L^{-1} of total suspended solids) treatments.

	Treatments	
Proximal Composition	**CONT**	**T250**
Moisture (%) [#]	77.42 ± 0.09 [b]	75.50 ± 0.23 [a]
Protein content (%)	22.85 ± 0.37 [b]	24.26 ± 0.89 [a]
Lipids (%)	0.53 ± 0.19	0.48 ± 0.19
Ash (%)	30.17 ± 1.80 [b]	28.30 ± 0.64 [a]

Fiber (%)	10.65 ± 0.78	11.59 ± 3.41
Non-nitrogenous extract (%)	35.77 ± 1.30	35.58 ± 1.44
Biochemical Analysis		
Protein (%)	23.30 ± 1.59	25.85 ± 2.81
DPPH (%)	90.31± 5.38	92.97 ± 5.80
Chlorophyll-*a* (mg g^{-1})	2.18 ± 0.03 [b]	2.27 ± 0.03 [a]
Chlorophyll-*b* (mg g^{-1})	2.99 ± 0.28	3.31 ± 0.10
Carotenoids (mg g^{-1})	0.19 ± 0.10	0.10 ± 0.03
Total Polyphenols (mg GAE g^{-1})	0.38 ± 0.12	0.33 ± 0.09

NNE (non-nitrogenous extract); # humid matter. Different letters represent significant differences ($p \leq 0.05$) between treatments after Student's *t*-test.

3.4. Shrimp Performance

No significant differences were observed in shrimp performance parameters between the treatments during the experimental period (Table 5). The shrimp grew during the experimental period and survived successfully at the end of cultivation.

Table 5. Performance of the shrimp during the 45 days of cultivation, in the treatments CONT (cultivation in clear water), T100 (use of 25% biofloc inoculum, a concentration of approximately 100 mg L^{-1} of total suspended solids), T200 (use of 50% biofloc inoculum, a concentration of approximately 200 mg L^{-1} of total suspended solids), T250 (use of 75% biofloc inoculum, a concentration of approximately 250 mg L^{-1} of total suspended solids), T300 (use of 100% biofloc inoculum, a concentration of approximately 300 mg L^{-1} of total suspended solids).

	Treatments				
Shrimp	**CONT**	**T100**	**T200**	**T250**	**T300**
Final mean weight (g)	6.47 ± 0.27	6.60 ± 0.80	5.89 ± 0.25	6.72 ± 0.26	6.31 ± 0.16
WGW (g $week^{-1}$)	0.38 ± 0.04	0.40 ± 0.12	0.30 ± 0.04	0.42 ± 0.04	0.36 ± 0.03
Final biomass (g)	232.78 ± 9.84	227.72 ± 12.62	211.97 ± 8.96	235.07 ± 4.38	216.45 ± 17.65
Survival (%)	96.30 ± 6.41	96.30 ± 6.41	100.0 ± 0.0	97.22 ± 4.81	95.37 ± 8.02
FCR	1.90 ± 0.19	2.13 ± 0.30	2.18 ± 0.27	2.03 ± 0.09	2.41 ± 0.59
Productivity (kg m^{-3})	1.28 ± 0.04	1.26 ± 0.07	1.18 ± 0.05	1.31 ± 0.02	1.20 ± 0.10

FCR (Food Conversion Rate); WGW (Weekly Weight gain).

4. Discussion

Chopin [4] mentions that integrated systems increase the productivity of a system, with the production of two organisms in the same area with added economic value. The conditions of salinity [36], dissolved oxygen [37], pH [38], alkalinity [28], and TSS [12] were kept ideal for

the cultivation of *P. vannamei* shrimp. However, the temperature was below the optimum level, decreasing the animal's metabolism and affecting its growth [39], which explains the low weekly weight gain found in this study. Another factor that may have influenced shrimp growth was the movement of the feed into the macroalgae structure through aeration, making it impossible for the shrimp to consume all food. The FCR obtained in this study (overall FCR of 2.26 ± 0.55) was similar to that found by Samocha et al. [40] in a biofloc system and Morais et al. [41] with integrated cultivation of shrimp and macroalgae in biofloc.

The use of integrated culture with the biofloc system has been widely studied, but there are few studies using macroalgae due to the high organic load and different variables in the system. The difference in salinity between the treatments in this study is probably due to the lower salinity found in the biofloc inoculum used compared with the salinity found in seawater. However, the genus *Ulva* can tolerate wide ranges of salinity and can grow well in salinities above 20 [42]. As a result, the difference in salinity between the treatments did not represent a stressful situation for the macroalgae, as it was within the macroalgae optimum performance range.

In cultivation systems that work with biofloc, alkalinity decreases throughout cultivation, and the medium becomes more acidic. This is due to the nitrification process carried out by the systems chemoautotrophic bacteria, which consume 7.0 g of alkalinity for metabolism and produce 5.85 g of carbon dioxide [43]. According to Furtado et al. [28], it is recommended that alkalinity be kept above 150 mg $CaCO_3$ L^{-1}. To maintain it at this ideal level, calcium carbonate or calcium hydroxide can be used. Using a biofloc inoculum characterized as mature, Ferreira et al. [11] used 112.91 ± 2.99 g of calcium hydroxide in a useful volume of 300 L, resulting in 0.38 g L^{-1} of calcium hydroxide consumption over 35 days of cultivation. Data from this study showed reduced use of calcium hydroxide to correct alkalinity, with the lowest value in the BIO75 treatment being 0.07 ± 0.06 g L^{-1} of calcium hydroxide over 45 days of cultivation. The greater steadiness of alkalinity in our treatments is probably related to the absorption of CO_2 by the macroalgae. For macroalgae, carbon absorption is essential to maintain a balanced C:N:P (carbon:nitrogen:phosphorus) ratio, participating in the sequestration of carbon dioxide and reducing the acidity of the environment [4]. Another explanation could be the consumption of ammonia by the macroalgae, reducing the nitrification process in the system, and the use of inorganic carbon, which is economically advantageous to the system.

According to Silva et al. [44], only 22% of the nitrogen input is converted into shrimp biomass, 14% remains deposited in the sediment, and 57% is discarded into the environment, suggesting little efficiency in the use of available nitrogen. Ammonia is the most toxic

nitrogenous compound in cultivation, and its control methods include water renewal in conventional cultivation or organic fertilization in biofloc systems [38,45]. The CONT treatment, without the use of biofloc inoculum, aimed to use organic fertilization with molasses when concentrations exceeded 1 mg L^{-1} in order to encourage the growth of heterotrophic bacteria to convert the ammonia in the system into bacterial biomass [22]. However, our study showed that despite the high shrimp stocking (200 shrimp m^{-3}) and no water renewal in the control treatment, there was no increase in ammonia concentrations. The highest daily concentration in the control treatment was 0.54 mg L^{-1}, so it was not necessary to use molasses throughout the experimental period. Macroalgae have a greater affinity for absorbing ammonia because they require less energy during assimilation processes [46]. In this study, the macroalgae in the CONT treatment were probably responsible for the primary absorption of the ammonia produced in the culture, thus delaying the nitrification process and an increase in nitrate concentrations in the culture, which was only seen in the last week of cultivation. Only at the end of cultivation (day 32) was there an increase in nitrate concentration, showing a possible growth of chemoautotrophic bacteria. According to Ferreira et al. [11], the establishment of these bacteria is slow, requiring at least 30 days with the use of chemical fertilizer for them to occur. The use of macroalgae to absorb the compost is advantageous because of the use of residual nutrients to produce biomass with commercial value, minimizing the impacts of the toxicity of this compost on the animals.

In a biofloc system, nitrate is the most concentrated nitrogenous compound at the end of cultivation and is constantly accumulated throughout the experiment due to nitrification by bacteria, which convert ammonia into nitrite and then nitrate [38]. Improper disposal of high levels of nitrate in water bodies can cause diseases such as methemoglobinemia in the population [47]. Therefore, the use of macroalgae as a biological treatment can provide improvements in water quality and effluent treatment. In the absence of high concentrations of ammonia, the macroalgae tend to absorb the nitrate present in the water, which occurred in this experiment, showing an absorption rate of 55% of nitrate by the macroalgae in the T250 treatment. Testing variations from 5 to 400 mg L^{-1} of nitrate in the water, Farahdiba et al. [48] found an absorption rate of around 90% of nitrate in five days in a static system. Our system is considered to be continuous, where ammonia is produced daily from animal excretion and feed waste and converted into bacterial biomass and nitrate by the bacteria present in the biofloc system. Even though nitrate is produced in the system, there was a rate of removal by the macroalgae, where the final concentration was lower than the initial concentration of the culture.

Phosphate is produced through the leaching of feed and is accumulated throughout cultivation in a biofloc system, being important for macroalgae in the formation of tissues and the process of photosynthesis. As observed by Ramos et al. [49], the use of a form of biological phosphorus removal, such as macroalgae, can be more efficient than filtration and sedimentation. The better rate of nitrate and phosphate absorption may be related to the nitrogen:phosphorus (N:P) balance during the experimental period. According to Zirino et al. [50], the most appropriate N:P ratio for macroalgae would be 30:1, and when it is below 29, nitrogen can be limiting, and when it is above 29, phosphorus can be limiting. During the 12 nutrient samplings, six times the N:P ratio in the T250 treatment was close to ideal, unlike the other treatments which showed a phosphorus limitation in most of the samplings.

The production of solids can come from heterotrophic bacteria, feces production, and leaching from the feed [12]. As there was no addition of organic carbon in the control treatment, the increase in total suspended solids was due to zero water renewal and the accumulation of particulate organic matter, reaching an average of 134.05 ± 81.36 mg L^{-1} of total suspended solids. Therefore, at the end of the experiment, all treatments had a load of solids present in the system. Even without a significant difference in final weight between the treatments, the growth of the macroalgae may still have been affected due to the low availability of light caused by the biofloc. Macroalgae are sessile organisms in the system and can interfere with the movement of water and cause the accumulation of solids on its surface, as seen in studies such as Carvalho et al. [19,51]. The deposition of solids causes a "shading" effect on the macroalgae and limits their absorption of light. The use of a larger surface area of the cultivation structure adopted in this study compared with Carvalho et al. [19] may have minimized the effect of overlap due to greater movement of the macroalgae in the water column.

The different concentrations of nutrients and solids are directly linked to greater biomass production and nutrient uptake. In this study, the relative growth rate (RGR) of algae had an overall average of 0.45 ± 0.27% day^{-1}, showing an increase in biomass during cultivation. These data differed from those found by Morais et al. [41], who obtained a growth rate of 8.00 ± 0.01% day^{-1} with *U. ohnoi* at a density of 1 g L^{-1} in a biofloc system, where the shrimp water was filtered and pumped into the macroalgae tank once a week, presenting a lower solids load. This difference in growth can be explained by the fact that the macroalgae in this experiment were grown in the same experimental unit as the shrimp and were subject to the accumulation of solids, nutrients, and fluctuations in water quality parameters. This system was used as a way of evaluating the insertion of macroalgae into adapted shrimp farms. The low light incidence

for the macroalgae was also a key factor in the low growth rate caused by the greenhouse and the accumulation of solids.

It was also shown that the weight gain of the macroalgae was positively linked to the rate of nutrient removal, indicating that the macroalgae were absorbing nutrients for growth in the first and second weeks. However, in the last week, it was seen that a greater absorption of nitrate was related to weight loss of the macroalgae. Lower growth rates can also be caused by stress, which triggers reproductive events, with the loss of biomass for spore formation [3], so it is likely that the macroalgae were absorbing nutrients and converting this energy into reproductive events rather than growth. Drastic changes in temperature in agricultural greenhouses have been reported, where the lowest temperatures were recorded in the morning and the highest temperatures in the afternoon, which can cause intense spore release, as seen by Carl et al. [52]. In this experiment, the maximum temperature was 30.3, and the minimum was 21.7 °C. A reproduction event can be noticed due to the presence of "ghost tissues," which are transparent parts due to the release of spores [3], which was noticed in this study throughout the experiment. However, the performance of the macroalgae obtained in this study differed from that presented by Martins et al. [53], who obtained a decrease in biomass during the experiment, which may be associated with the natural environment where the macroalgae were collected and the acclimatization of the macroalgae to the system.

The composition of macroalgae can change according to the environment in which it is found, which can affect its nutritional composition [18]. Msuya & Neori [54] showed that the protein content increases significantly with increasing nutrient addition, reaching 44.3 ± 2.7% protein at the highest level of nutrient addition (38 g N m^{-2} day^{-1}). The macroalgae cultivated with a concentration of 56.67 ± 5.77 mg L^{-1} of nitrate (T250) showed higher protein concentrations, probably due to the greater availability of nitrogen in the water compared with the CONT treatment. Even so, the CONT treatment had a higher protein content than studies carried out in clear water, such as Tabarsa et al. [55], who found values of 10.69 ± 0.67 g 100 g^{-1} of protein in the macroalgae. This high concentration is probably due to the continuous absorption of ammonia excreted by the shrimp. As for the result found for ash, little is known about ash variations in the proximal composition of macroalgae; however, some factors can alter this concentration, such as high macroalgae densities and an inverse proportion of nitrogen concentration in the tissue [56]. Therefore, the higher the nitrogen concentration in the macroalgae tissue, the lower the ash concentration, both factors being verified in this study.

Chlorophylls are green pigments present in thylakoids and are essential in the reaction to capture light and carry out photosynthesis, and their increase is associated with the need to

increase the number of pigments to maximize photosynthesis [57]. Therefore, the higher concentrations of chlorophyll-*a* present in the treatment with inoculum (T250) are associated with the higher organic load present in this treatment, compared with the control, which started in clear water. The biofloc system contains microbial flocs that make up the total suspended solids. This organic load reduces the entry of light into the water and can negatively influence the growth of macroalgae.

The chlorophyll-*b* values found in this study were also higher than those found by Silva et al. [58], who showed maximum values of 1.21 ± 0.10 µg mg in the macroalgae *U. rigida* cultivated in a multitrophic system. According to Levavasseur [59], macroalgae increase their absorption spectrum to maximize the capture of light energy. Therefore, the macroalgae in this experiment increased the concentration of chlorophyll-*b* as a way of capturing light. Fillit. [60], evaluating the effect of seasonal changes on the pigment concentration of the macroalgae *U. rigida* showed that at times of the year with greater light intensity and photoperiod, the pigment content decreased and that shading effects caused by a greater density of algae also increased the pigment concentration.

Carotenoids are photosynthetic pigments responsible both for collecting light energy and for preventing the formation of reactive oxygen species caused by light and air stress [61]. Our values were similar to those found by Yildiz et al. [62] in the macroalgae *U. rigida* collected in the environment. However, Eismann et al. [63] show that the variation in total carotenoids is influenced by the growth rates of macroalgae. Therefore, high growth rates increase the carotenoid content, which can reach 0.92 ± 0.57 mg g^{-1}. The low RGR values found in this study may be due to the deposition of solids in the macroalgae because of their accumulation during cultivation and temperature variations, which may have influenced lower concentrations of total carotenoids compared with those found in the literature.

Sampath-Wiley et al. [64] showed that different stressors can affect antioxidant levels in macroalgae. Macroalgae cultivated in the lower intertidal zone that were protected from exposure to light and moisture loss showed a decrease in antioxidant levels. In contrast, the macroalgae cultivated in this study, which were subjected to temperature variations and high concentrations of solids and nutrients and consequently showed a high DPPH inhibition percentage, promoted an increase in antioxidant levels. For phenolic compounds, according to Mabeau & Fleurence [65], the concentrations vary according to the group of macroalgae studied. Green and red macroalgae have a higher protein content and lower levels of phenolic compounds compared with brown macroalgae. Our study showed values similar to those found

by Silva et al. [58] of 0.74 ± 0.13 mg GAE g^{-1} with the macroalgae *U. rigida* cultivated in an integrated system.

5. Conclusions

The utilization of macroalgae within an integrated system alongside shrimp proved to be feasible in the nitrate and total suspended solids concentrations at 56.67 ± 5.77 mgL^{-1} and 246.67 ± 2.89 mgL^{-1}, respectively. It also makes the system more sustainable by reusing water from existing biofloc cultivation. The macroalgae exhibited favorable nitrate and phosphate removal rates, indicating the assimilation of these compounds into biomass formation and an increase in economically valuable pigments within the macroalgae. Furthermore, in addition to nutrient absorption, the system effectively maintained alkalinity and pH levels, facilitating the efficient upkeep of biofloc cultivation.

Author Contributions: Conceptualization, A.C., A.P.C., and L.H.P.; Data curation, A.C., Í.B., F.C., and J.R.B.R.; Formal analysis, A.C., Í.B., F.C., J.M.M., and J.R.B.R.; Funding acquisition, L.H.P.; Investigation, A.C., Í.B., F.C., A.P.C., J.R.B.R., and L.H.P.; Methodology, A.C. and L.H.P.; Project administration, W.W.J. and L.H.P.; Resources, A.P.C., W.W.J. and L.H.P.; Supervision, A.P.C., J.M.M., W.W.J., and L.H.P.; Validation, A.C., Í.B., and F.C.; Visualization, J.M.M., J.R.B.R., W.W.J., and L.H.P.; Writing—original draft, A.C.; Writing—review & editing, Í.B., F.C., A.P.C., J.M.M., J.R.B.R., W.W.J., and L.H.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by ASTRAL Project—H2020 grant Agreement 863034.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The Authors are grateful to the ASTRAL project that has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 863034. Special thanks to the Brazilian Council of Research (CNPq) and Coordination for the Improvement of Higher Level or Education Personnel (CAPES). Luís H. Poersch and Wilson Wasielesky received a productivity research fellowship from CNPq.

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

References

1. FAO. *State of the World Fisheries and Aquaculture—2022 (SOFIA)*; FAO: Rome, Italy, 2022; ISBN 9789251072257.
2. de Alencar, J.R.; Junior, P.A.H.; Celino, J.J. Cultivo de Camarão Branco *Litopenaeus vannamei* (Boone, 1931) Com a Macroalga *Ulva lactuca* Linneaus (Chlorophyta) No Tratamento de Efluentes Em Sistema Fechado de Recirculação. *Rev. Biol. Ciências Terra* **2010**, *10*, 117–137.
3. Copertino, M.D.S.; Tormena, T.; Seeliger, U. Biofiltering Efficiency, Uptake and Assimilation Rates of *Ulva clathrata* (Roth) J. Agardh (Clorophyceae) Cultivated in Shrimp Aquaculture Waste Water. *J. Appl. Phycol.* **2009**, *21*, 31–45. https://doi.org/10.1007/s10811-008-9357-x.
4. Chopin, T. Marine Aquaculture in Canada: Well-Established Monocultures of Finfish and Shellfish and an Emerging Integrated Multi-Trophic Aquaculture (IMTA) Approach Including Seaweeds, Other Invertebrates, and Microbial Communities. *Fisheries* **2015**, *40*, 28–31. https://doi.org/10.1080/03632415.2014.986571.
5. Chopin, T.; Buschmann, A.H.; Halling, C.; Troell, M.; Kautsky, N.; Neori, A.; Kraemer, G.P.; Zertuche-González, J.A.; Yarish, C.; Neefus, C. Integrating Seaweeds into Marine Aquaculture Systems: A Key toward Sustainability. *J. Phycol.* **2001**, *37*, 975–986. https://doi.org/10.1046/j.1529-8817.2001.01137.x.
6. Troell, M.; Joyce, A.; Chopin, T.; Neori, A.; Buschmann, A.H.; Fang, J.G. Ecological Engineering in Aquaculture—Potential for Integrated Multi-Trophic Aquaculture (IMTA) in Marine Offshore Systems. *Aquaculture* **2009**, *297*, 1–9. https://doi.org/10.1016/j.aquaculture.2009.09.010.
7. Nobre, A.M.; Robertson-Andersson, D.; Neori, A.; Sankar, K. Ecological-Economic Assessment of Aquaculture Options: Comparison between Abalone Monoculture and Integrated Multi-Trophic Aquaculture of Abalone and Seaweeds. *Aquaculture* **2010**, *306*, 116–126. https://doi.org/10.1016/j.aquaculture.2010.06.002.
8. Holanda, M.; Wasielesky, W.; de Lara, G.R.; Poersch, L.H. Production of Marine Shrimp Integrated with Tilapia at High Densities and in a Biofloc System: Choosing the Best Spatial Configuration. *Fishes* **2022**, *7*, 283. https://doi.org/10.3390/fishes7050283.
9. Verdian, A.H.; Effendi, I.; Budidardi, T.; Diatin, I. Production Performance Improvement of White Shrimp (*Litopenaeus vannamei*) Culture with Integrated Multi Trophic Aquaculture System in Seribu Islands, Jakarta, Indonesia. *Iran. J. Fish. Sci.* **2020**, *19*, 1415–1427. https://doi.org/10.22092/ijfs.2019.120676.
10. Poli, M.A.; Legarda, E.C.; de Lorenzo, M.A.; Pinheiro, I.; Martins, M.A.; Seiffert, W.Q.; do Nascimento Vieira, F. Integrated Multitrophic Aquaculture Applied to Shrimp Rearing in a Biofloc System. *Aquaculture* **2019**, *511*, 734274. https://doi.org/10.1016/j.aquaculture.2019.734274.
11. Ferreira, G.S.; Santos, D.; Schmachtl, F.; Machado, C.; Fernandes, V.; Bögner, M.; Schleder, D.D.; Seiffert, W.Q.; Vieira, F.N. Heterotrophic, Chemoautotrophic and Mature Approaches in Biofloc System for Pacific White Shrimp. *Aquaculture* **2021**, *533*, 736099. https://doi.org/10.1016/j.aquaculture.2020.736099.
12. Gaona, C.A.P.; de Almeida, M.S.; Viau, V.; Poersch, L.H.; Wasielesky, W. Effect of Different Total Suspended Solids Levels on a *Litopenaeus vannamei* (Boone, 1931) BFT Culture System during Biofloc Formation. *Aquac. Res.* **2017**, *48*, 1070–1079. https://doi.org/10.1111/are.12949.
13. Fleckenstein, L.J.; Tierney, T.W.; Fisk, J.C.; Ray, A.J. The Effects of Different Solids and Biological Filters in Intensive Pacific White Shrimp (*Litopenaeus vannamei*) Production Systems. *Aquac. Eng.* **2020**, *91*, 102120. https://doi.org/10.1016/j.aquaeng.2020.102120.

14. Queiroz, H.M.; Ferreira, T.O.; Taniguchi, C.A.K.; Barcellos, D.; do Nascimento, J.C.; Nóbrega, G.N.; Otero, X.L.; Artur, A.G. Nitrogen Mineralization and Eutrophication Risks in Mangroves Receiving Shrimp Farming Effluents. *Environ. Sci. Pollut. Res.* **2020**, *27*, 34941–34950. https://doi.org/10.1007/s11356-020-09720-1.
15. Legarda, E.C.; da Silva, D.; Miranda, C.S.; Pereira, P.K.M.; Martins, M.A.; Machado, C.; de Lorenzo, M.A.; Hayashi, L.; do Nascimento Vieira, F. Sea Lettuce Integrated with Pacific White Shrimp and Mullet Cultivation in Biofloc Impact System Performance and the Sea Lettuce Nutritional Composition. *Aquaculture* **2021**, *534*, 736265. https://doi.org/10.1016/j.aquaculture.2020.736265.
16. Brito, L.O.; Arantes, R.; Magnotti, C.; Derner, R.; Pchara, F.; Olivera, A.; Vinatea, L. Water Quality and Growth of Pacific White Shrimp *Litopenaeus vannamei* (Boone) in Co-Culture with Green Seaweed *Ulva lactuca* (Linaeus) in Intensive System. *Aquac. Int.* **2014**, *22*, 497–508. https://doi.org/10.1007/s10499-013-9659-0.
17. Reis, W.G.; Wasielesky, W.; Abreu, P.C.; Brandão, H.; Krummenauer, D. Rearing of the Pacific White Shrimp *Litopenaeus vannamei* (Boone, 1931) in BFT System with Different Photoperiods: Effects on the Microbial Community, Water Quality and Zootechnical Performance. *Aquaculture* **2019**, *508*, 19–29. https://doi.org/10.1016/j.aquaculture.2019.04.067.
18. Duke, C.S.; Litaker, W.; Ramus, J. Effects of the Temperature, Nitrogen Supply and Tissue Nitrogen on Ammonium Uptake Rates of the Chlorophyte Seaweeds *Ulva curvata* and *Codium decorticatum*. *J. Phycol.* **1989**, *25*, 113–120.
19. Carvalho, A.; Costa, L.C.d.O.; Holanda, M.; Poersch, L.H.; Turan, G. Influence of Total Suspended Solids on the Growth of the Sea Lettuce *Ulva lactuca* Integrated with the Pacific White Shrimp *Litopenaeus vannamei* in a Biofloc System. *Fishes* **2023**, *8*, 163. https://doi.org/10.3390/fishes8030163.
20. He, J.; Xu, Y.; Chen, H.; Sun, P. Extraction, Structural Characterization, and Potential Antioxidant Activity of the Polysaccharides from Four Seaweeds. *Int. J. Mol. Sci.* **2016**, *17*, 1988. https://doi.org/10.3390/ijms17121988.
21. Jory, D.E.; Cabrera, T.R.; Dugger, D.M.; Fegan, D.; Lee, P.G.; Lawrence, L.; Jackson, C.J.; Mcintosh, R.P.; Castañeda, J.I.; McIntosh, R.; et al. A Global Review of Shrimp Feed Management: Status and Perspectives. *Aquaculture* **2011**, *318*, 104–152.
22. Wasielesky, W.; Krummenauer, D.; Lara, G.; Fóes, G. Cultivo de Camarões Em Sistema de Bioflocos: Realidades e Perspectivas. *Rev. ABCC* **2013**, *15*, 16–26.
23. Unesco. *Chemical Methods for Use in Marine Environmental Monitoring*; Intergovernmental Oceanographic Commission: Paris, France, 1983.
24. Bendschneider, K.; Robinson, R.J. New Spectrophotometric Method for the Determination of Nitrite in Water. *Fresenius Environ. Bull.* **1952**, *10*, 781–784.
25. Aminot, A.; Chaussepied, M. *Manuel Des Analyses Chimiques En Milieu Marin*; Centre National pour l'exploitation des océans: Paris, France, 1983.
26. Bogue, J.P.; Smith, L.F.; Lipsett, L. A Practical Handbook. *J. High. Educ.* **1957**, *28*, 405. https://doi.org/10.2307/1979241.
27. American Public Health Association APHA; American Water Works Association; Water Pollution Control Association. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association: Washington, DC, USA, 2005.
28. Furtado, P.S.; Poersch, L.H.; Wasielesky, W. Effect of Calcium Hydroxide, Carbonate and Sodium Bicarbonate on Water Quality and Zootechnical Performance of Shrimp *Litopenaeus vannamei* Reared in Biofloc Technology (BFT) Systems. *Aquaculture* **2011**, *321*, 130–135. https://doi.org/10.1016/j.aquaculture.2011.08.034.

29. Loureiro, R.R.; Reis, R.P.; Critchley, A.T. In Vitro Cultivation of Three *Kappaphycus alvarezii* (Rhodophyta, Areschougiaceae) Variants (Green, Red and Brown) Exposed to a Commercial Extract of the Brown Alga *Ascophyllum nodosum* (Fucaceae, Ochrophyta). *J. Appl. Phycol.* **2010**, *22*, 101–104. https://doi.org/10.1007/s10811-009-9412-2.
30. AOAC. *Official Methods of Analysis*; Association of Official Analytical Chemists: Arlington, VA, USA, 2005; p. 245.
31. Baethgen, W.E.; Alley, M. *A Manual Colorimetric Procedure for Measuring Ammonium Nitrogen in Soil and Plant Kjeldahl Digests*; Communications in Soil Science and Plant Analysis: v. 20, n. 9-10, p. 961-969, 1989.
32. Barbarino, E.; Lourenço, S.O. An Evaluation of Methods for Extraction and Quantification of Protein from Marine Macro and Microalgae. *J. Appl. Phycol.* **2005**, *17*, 447–460. https://doi.org/10.1007/s10811-005-1641-4.
33. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of Total Carotenoids and Chlorophylls *a* and *b* of Leaf Extracts in Different Solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592. https://doi.org/10.1042/bst0110591.
34. Schiavon, M.; Moro, I.; Pilon-Smits, E.A.H.; Matozzo, V.; Malagoli, M.; Dalla Vecchia, F. Accumulation of Selenium in *Ulva* Sp. and Effects on Morphology, Ultrastructure and Antioxidant Enzymes and Metabolites. *Aquat. Toxicol.* **2012**, *122–123*, 222–231. https://doi.org/10.1016/j.aquatox.2012.06.014.
35. Farasat, M.; Khavari-Nejad, R.-A.; Nabavi, S.M.B.; Namjooyan, F. Antioxidant Properties of Two Edible Green Seaweeds From Northern Coasts of the Persian Gulf. *Jundishapur J. Nat. Pharm. Prod.* **2013**, *8*, 47–52. https://doi.org/10.17795/jjnpp-7736.
36. Decamp, O.; Cody, J.; Conquest, L.; Delanoy, G.; Tacon, A.G.J. Effect of Salinity on Natural Community and Production of *Litopenaeus vannamei* (Boone), within Experimental Zero-Water Exchange Culture Systems. *Aquac. Res.* **2003**, *34*, 345–355. https://doi.org/10.1046/j.1365-2109.2003.00842.x.
37. Van Wyk, P.; Davis-Hodgkins, M.; Laramore, R.; Main, K.L.; Mountain, J.; Scarpa, J. Farming Marine Shrimp in Recirculating Freshwater Systems Harbor Branch Oceanographic Institution. *Farming Mar. Shrimp. Recirc. Freshw. Syst.* **1999**, *220*, 125–140.
38. Krummenauer, D.; Peixoto, S.; Cavalli, R.O.; Poersch, L.H.; Wasielesky, W. Superintensive Culture of White Shrimp, *Litopenaeus vannamei*, in a Biofloc Technology System in Southern Brazil at Different Stocking Densities. *J. World Aquac. Soc.* **2011**, *42*, 726–733. https://doi.org/10.1111/j.1749-7345.2011.00507.x.
39. Wyban, J.; Walsh, W.A.; Godin, D.M. Temperature Effects on Growth, Feeding Rate and Feed Conversion of the Pacific White Shrimp (*Penaeus vannamei*). *Aquaculture* **1995**, *138*, 267–279. https://doi.org/10.1016/0044-8486(95)00032-1.
40. Samocha, T.M.; Patnaik, S.; Speed, M.; Ali, A.M.; Burger, J.M.; Almeida, R.V.; Ayub, Z.; Harisanto, M.; Horowitz, A.; Brock, D.L. Use of Molasses as Carbon Source in Limited Discharge Nursery and Grow-out Systems for *Litopenaeus vannamei*. *Aquac. Eng.* **2007**, *36*, 184–191. https://doi.org/10.1016/j.aquaeng.2006.10.004.
41. de Morais, A.P.M.; Santos, I.L.; Carneiro, R.F.S.; Routledge, E.A.B.; Hayashi, L.; de Lorenzo, M.A.; do Nascimento Vieira, F. Integrated Multitrophic Aquaculture System Applied to Shrimp, Tilapia, and Seaweed (*Ulva ohnoi*) Using Biofloc Technology. *Aquaculture* **2023**, *572*, 739492. https://doi.org/10.1016/j.aquaculture.2023.739492.
42. Bews, E.; Booher, L.; Polizzi, T.; Long, C.; Kim, J.H.; Edwards, M.S. Effects of Salinity and Nutrients on Metabolism and Growth of *Ulva lactuca*: Implications for Bioremediation of Coastal Watersheds. *Mar. Pollut. Bull.* **2021**, *166*, 112199. https://doi.org/10.1016/j.marpolbul.2021.112199.

43. Ebeling, J.M.; Timmons, M.B.; Bisogni, J.J. Engineering Analysis of the Stoichiometry of Photoautotrophic, Autotrophic, and Heterotrophic Removal of Ammonia-Nitrogen in Aquaculture Systems. *Aquaculture* **2006**, *257*, 346–358. https://doi.org/10.1016/j.aquaculture.2006.03.019.
44. Da Silva, K.R.; Wasielesky, W.; Abreu, P.C. Nitrogen and Phosphorus Dynamics in the Biofloc Production of the Pacific White Shrimp, *Litopenaeus vannamei*. *J. World Aquac. Soc.* **2013**, *44*, 30–41. https://doi.org/10.1111/jwas.12009.
45. Lin, Y.C.; Chen, J.C. Acute Toxicity of Ammonia on *Litopenaeus vannamei* Boone Juveniles at Different Salinity Levels. *J. Exp. Mar. Biol. Ecol.* **2001**, *259*, 109–119. https://doi.org/10.1016/S0022-0981(01)00227-1.
46. Castelar, B.; Reis, R.P.; dos Santos Calheiros, A.C. *Ulva lactuca* and *U. flexuosa* (Chlorophyta, Ulvophyceae) Cultivation in Brazilian Tropical Waters: Recruitment, Growth, and Ulvan Yield. *J. Appl. Phycol.* **2014**, *26*, 1989–1999. https://doi.org/10.1007/s10811-014-0329-z.
47. Macedo, C.F.; Sipaúba-Tavares, L. Eutrofização E Qualidade Da Água Na Piscicultura : Consequências E Recomendações Eutrophication and Water Quality in Pisciculture: Consequences and Recommendations. *Bol. Inst. Pesca* **2010**, *36*, 149–163.
48. Farahdiba, A.U.; Hidayah, E.N.; Asmar, G.A.; Myint, Y.W. Growth and Removal of Nitrogen and Phosphorus by a Macroalgae *Cladophora glomerata* under Different Nitrate Concentrations. *Nat. Environ. Pollut. Technol.* **2020**, *19*, 809–813. https://doi.org/10.46488/NEPT.2020.V19I02.038.
49. Ramos, R.; Vinatea, L.; Santos, J.; Da Costa, R. Tratamiento de Efluentes Del Cultivo de *Litopenaeus vannamei* Mediante Procesos de Sedimentación, Filtración y Absorción. *Lat. Am. J. Aquat. Res.* **2010**, *38*, 188–200. https://doi.org/10.3856/vol38-issue2-fulltext-3.
50. Zirino, A.; Elwany, H.; Facca, C.; Maicu', F.; Neira, C.; Mendoza, G. Nitrogen to Phosphorus Ratio in the Venice (Italy) Lagoon (2001–2010) and Its Relation to Macroalgae. *Mar. Chem.* **2016**, *180*, 33–41. https://doi.org/10.1016/j.marchem.2016.01.002.
51. Carvalho, A.; Costa, L.C.d.O.; Holanda, M.; Gonçalves, M.; Santos, J.; Costa, C.S.B.; Turan, G.; Poersch, L.H. Growth of the Macroalgae *Ulva lactuca* Cultivated at Different Depths in a Biofloc Integrated System with Shrimp and Fish. *Phycology* **2023**, *3*, 280–293. https://doi.org/10.3390/phycology3020018.
52. Carl, C.; De Nys, R.; Lawton, R.J.; Paul, N.A. Methods for the Induction of Reproduction in a Tropical Species of Filamentous *Ulva*. *PLoS ONE* **2014**, *9*, 2–11. https://doi.org/10.1371/journal.pone.0097396.
53. Martins, M.A.; da SILVA, V.F.; Tarapuez, P.R.; Hayashi, L.; Vieira, F.D.N. Cultivation of the Seaweed *Ulva* Spp. with Effluent from a Shrimp Biofloc Rearing System: Different Species and Stocking Density. *Bol. Inst. Pesca* **2020**, v. 46, n. 3. https://doi.org/10.20950/1678-2305.2020.46.3.602.
54. Msuya, F.E.; Neori, A. Effect of Water Aeration and Nutrient Load Level on Biomass Yield, N Uptake and Protein Content of the Seaweed *Ulva lactuca* Cultured in Seawater Tanks. *J. Appl. Phycol.* **2008**, *20*, 1021–1031. https://doi.org/10.1007/s10811-007-9300-6.
55. Tabarsa, M.; Rezaei, M.; Ramezanpour, Z.; Waaland, J.R. Chemical Compositions of the Marine Algae *Gracilaria salicornia* (Rhodophyta) and *Ulva lactuca* (Chlorophyta) as a Potential Food Source. *J. Sci. Food Agric.* **2012**, *92*, 2500–2506. https://doi.org/10.1002/jsfa.5659.
56. Queirós, A.S.; Circuncisão, A.R.; Pereira, E.; Válega, M.; Abreu, M.H.; Silva, A.M.S.; Cardoso, S.M. Valuable Nutrients from *Ulva rigida*: Modulation by Seasonal and Cultivation Factors. *Appl. Sci.* **2021**, *11*, 6137. https://doi.org/10.3390/app11136137.
57. Holdt, S.L.; Kraan, S. Bioactive Compounds in Seaweed: Functional Food Applications and Legislation. *J. Appl. Phycol.* **2011**, *23*, 543–597. https://doi.org/10.1007/s10811-010-9632-5.

58. Silva, A.F.R.; Abreu, H.; Silva, A.M.S.; Cardoso, S.M. Effect of Oven-Drying on the Recovery of Valuable Compounds from *Ulva rigida*, *Gracilaria* Sp. and *Fucus vesiculosus*. *Mar. Drugs* **2019**, *17*, 90. https://doi.org/10.3390/md17020090.
59. Levavasseur, G. Analyse Comparée Des Complexes Pigment-Protéines de Chlorophycophytes Marines Benthiques. *Phycologia* **1989**, *28*, 1–14. https://doi.org/10.2216/i0031-8884-28-1-1.1.
60. Fillit, M. Seasonal Changes in the Photosynthetic Capacities and Pigment Content of *Ulva rigida* in a Mediterranean Coastal Lagoon. *Bot. Mar.* **1995**, *38*, 271–280. https://doi.org/10.1515/botm.1995.38.1-6.271.
61. Astorg, P. Food Carotenoids and Cancer Prevention: An Overview of Current Research. *Trends Food Sci. Technol.* **1997**, *8*, 406–413. https://doi.org/10.1016/S0924-2244(97)01092-3.
62. Yildiz, G.; Celikler, S.; Vatan, O.; Dere, S. Determination of the Anti-Oxidative Capacity and Bioactive Compounds in Green Seaweed *Ulva rigida* C. Agardh. *Int. J. Food Prop.* **2012**, *15*, 1182–1189. https://doi.org/10.1080/10942912.2010.517341.
63. Eismann, A.I.; Perpetuo Reis, R.; Ferreira da Silva, A.; Negrão Cavalcanti, D. *Ulva* Spp. Carotenoids: Responses to Environmental Conditions. *Algal Res.* **2020**, *48*, 101916. https://doi.org/10.1016/j.algal.2020.101916.
64. Sampath-Wiley, P.; Neefus, C.D.; Jahnke, L.S. Seasonal Effects of Sun Exposure and Emersion on Intertidal Seaweed Physiology: Fluctuations in Antioxidant Contents, Photosynthetic Pigments and Photosynthetic Efficiency in the Red Alga *Porphyra umbilicalis* Kützing (Rhodophyta, Bangiales). *J. Exp. Mar. Biol. Ecol.* **2008**, *361*, 83–91. https://doi.org/10.1016/j.jembe.2008.05.001.
65. Mabeau, S.; Fleurence, J. Seaweed in Food Products: Biochemical and Nutritional Aspects. *Trends Food Sci. Technol.* **1993**, *4*, 103–107. https://doi.org/10.1016/0924-2244(93)90091-N.

CAPÍTULO 3: Avaliação de diferentes profundidades de estruturas para as macroalgas em cultivo integrado com camarão *L. vannamei* e a tilápia O. *niloticus*.

Artigo publicado na *Phycology*.

https://doi.org/10.3390/phycology3020018

Resumo

Em um sistema de cultivo integrado com camarão e tilápia a formação de sólidos é constante, devido a formação de biomassa bacteriana e resíduos dos animais, aumentando a turbidez da água. Devido a necessidade da realização de fotossíntese, sistemas com alta carga orgânica podem afetar o desempenho das macroalgas, e pouco se sabe sobre estruturas de cultivo que melhorem o seu desempenho em sistemas de bioflocos. Para isso, foram realizados dois tratamentos em triplicata, sendo eles: flutuador raso, com 10 cm de profundidade a partir da superfície da água; e flutuador fundo com 30 cm de profundidade a partir da superfície da água. O experimento teve duração de 70 dias, com seis sistemas compostos por: um tanque de camarão de 16 m^3, um tanque de tilápia de 3 m^3 e um tanque de macroalgas de 3 m^3, com recirculação de água entre os tanques. O flutuador raso resultou em uma taxa de crescimento de até 0,95 ± 0,54% dia^{-1}, sendo superiores as encontradas no flutuador fundo. No flutuador raso, houve uma perda de biomassa apenas no final do cultivo devido à alta densidade de macroalgas, diminuição da temperatura e aumento da concentração de sólidos. O flutuador fundo apresentou perda de biomassa durante todo o ciclo de cultivo. O cultivo integrado de camarões, peixes e macroalgas se mostrou viável, com aumento da biomassa de macroalgas, com o uso de flutuadores rasos a 10 cm da superfície.

Growth of the Macroalgae *Ulva lactuca* Cultivated at Different Depths in a Biofloc Integrated System with Shrimp and Fish

Andrezza Carvalho [1,*], Léa Carolina de Oliveira Costa [2], Mariana Holanda [3], Mayra Gonçalves [1], Jorge Santos [1], César S. B. Costa [1], Gamze Turan [4] and Luís H. Poersch [1]

[1] Marine Aquaculture Station, Institute of Oceanography, Federal University of Rio Grande—FURG, Rua do Hotel nº2, Cassino, Rio Grande 96210030, RS, Brazil; mayragoncalves.gs@gmail.com (M.G.); jorgerenato_82@hotmail.com (J.S); costacsb@gmail.com (C.S.B.C.); lpoersch@gmail.com (L.H.P.).
[2] Instituto Federal de Educação Ciência e Tecnologia do Pará, Rodovia BR 316, KM 61, Castanhal 68740970, PA, Brasil; leacarolinacosta@yahoo.com.br (L.C.d.O.C).
[3] Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Valparaíso 2373223, Chile; mariana.holandalima@gmail.com (M.H).
[4] Aquaculture Department, Fisheries Faculty, Ege University, Bornova 35100, Izmir, Turkey; gamzeturan2000@gmail.com (G.T).

Abstract: The constant production of solids in intensive shrimp and tilapia culture can affect the performance of macroalgae when cultivated in an integrated system, and little is known about culture structures that enhance the performance of macroalgae in biofloc systems. The objective of this work was to evaluate different depths of culture structure for the macroalgae *Ulva lactuca* in an integrated system with *Litopenaeus vannamei* and *Oreochromis niloticus* in a biofloc system. The experiment lasted 70 days, with six systems composed of: a 16 m^3 shrimp tank, a 3 m^3 tilapia tank, and a 3 m^3 macroalgae tank, with water recirculation between tanks. Two treatments were carried out, shallow float, with a structural depth of 10 cm, and bottom float, where the depth was kept at 30 cm from the surface. The shallow float resulted in a growth rate of up to 0.95 ± 0.54% day^{-1}, with biomass loss only at the end of the culture due to the high density of macroalgae, decreasing temperature, and increasing solids concentration. The bottom float had biomass loss throughout the culture cycle. The integrated culture of shrimp, fish, and macroalgae is feasible with the use of shallow floats within 10 cm from the surface.

Keywords: total suspended solids; cultivation structure; temperature; density

1. Introduction

The shrimp *Litopenaeus vannamei* (Boone, 1931) is the most cultivated crustacean in the world, with current production of approximately 5.8 million tons [1]. Its advancement in production is due to the consequence of better adaptation in the area, the knowledge established about its reproductive cycle, and the simplicity in management practices [2], thus being the main species in an integrated culture system. The tilapia, *Oreochromis niloticus,* also represents an enhancement in integrated culture due to the possibility of a low feed supply (1% of biomass) to induce the intake behavior of the solids, increasing the productivity of the culture with lower

costs, as demonstrated by Poli et al. [3] with the integrated culture of shrimp, tilapia, and halophytes. The growth of tilapia production is due to the easiness in obtaining juveniles, the rapid growth, and the possibility of cultivation in several places, being a highly produced species in Brazil and with great economic interest.

In shrimp farming, only 25 to 30% of nitrogen and phosphorus from feed and fertilizers are used by the shrimp, and most of it is leached and lost in the water [4]. Choosing species that can reuse such waste and maximize production is essential for more sustainable systems. The use of organic and inorganic consuming species to take advantage of system waste characterizes Integrated Multi-Trophic Aquaculture (IMTA), which has the advantage of greater sustainability and final productivity [5]. A target species of higher trophic level is chosen, such as fish or shrimp, and organic and inorganic consuming species are integrated into the system with the objective to, respectively, reuse the organic matter and nutrients available in the water for the growth of new biomass with added economic value [6].

The cultivation of macroalgae associated with other aquatic organisms in aquaculture has gained momentum due to the increasing focus on sustainable systems with nutrient recycling. As inorganic consumers, macroalgae use nitrogen and phosphate compounds in the water for their growth [7]. Several studies have shown a better performance of macroalgae when cultivated in shrimp farm effluent and/or in integrated systems. Nardelli et al. [8] showed that macroalgae growth, oxygen production, and nutrient uptake were directly proportional to increasing trophic levels and species insertions in a system. The production of macroalgae is currently approximately 35 million tons in 2020 compared to 10 million in the 2000s [1]. The rise in economic interest in macroalgae cultivation is attributed to the extraction of by-products such as agar and carrageenan for the food and pharmaceutical industries [2] and the consumption of fresh macroalgae [9].

The use of the integrated system in conjunction with biofloc technology (BFT) has recently advanced to reuse waste and minimal water exchange through the control of nitrogenous compounds performed by a diversity of organisms present in the system [10]. This control in water quality occurs by heterotrophic bacteria, which transform ammonia in the presence of carbon and intense aeration into microbial biomass, and chemoautotrophic bacteria that convert ammonia to nitrite and subsequently to nitrate [11]. The transformation of ammonia to nitrate allows a low-toxicity nitrogen compound to accumulate without reducing water quality [12]. The presence of high concentrations of nutrients in the system can be advantageous for inorganic consuming species that reuse the waste from another species for biomass growth.

The control of total suspended solids in the integrated production of organisms in biofloc is advantageous to maintain water quality and complement the diet of cultured organisms. However, according to Gaona et al. [13], levels of 100 to 300 mg L^{-1} are optimal for the culture system. Organic consumers can also benefit from this production of solids and use them as complementary food sources. In an integrated system of *Litopenaeus vannamei* shrimp and *Mugil liza* mullet, Holanda et al. [14] found reduced solids concentrations in the integrated system compared to shrimp monoculture. Azim and Little [15] also showed that the protein level of tilapia diets can be reduced when tilapia is grown in biofloc due to the supplementation of the diet by microbial flocs. However, high concentrations of solids can negatively impact the performance of cultured organisms, such as shrimp and fish, for which gill obstruction may occur [13].

For macroalgae, the characteristics of the biofloc system impose many challenges to their use and active optimal culture conditions. High concentrations of solids can affect macroalgae growth due to the deposition of total suspended solids in the photosynthesizing tissue [16]. In addition to this deposition, the low transparency of the water can reduce light uptake by the macroalgae in the tanks. Reis et al. [17] show that with increasing depth, the light intensity decreases in a biofloc system due to the bacterial aggregates. Therefore, cultures of lower depth may limit macroalgae to the surface where there is greater light availability.

A shallow depth can limit the movement of macroalgae. The biofloc system has as a characteristic for its maintenance the use of intense aeration for the movement of particles in the water column [10]. This intense aeration can favor the movement of macroalgae in deeper structures, causing the algae to reach the bottom of the structure and then return to the surface, so there is no overlap between them. The overlapping of macroalgae by having a high density in a limited space can cause biomass loss during culture procedures [18]. Better management procedures are needed for the suitability of different organisms in biofloc-integrated cultures.

Adding different species into the culture can also alter the characteristics of the system. As previously mentioned, the insertion of a sessile organism can influence the movement of water and cause the removal of solids that should be suspended [16]. An inorganic consumer can also decrease the concentration of nutrients in the culture, being a bioremediator. Studies by Alencar et al. [18] and Copertino et al. [19] showed macroalgae of the genus *Ulva* with growth rates above 8.0% day^{-1} using shrimp culture effluent. The feasibility of using water from biofloc-rearing macroalgae cultivation still needs to be addressed.

Therefore, the aim of this work was to evaluate the cultivation of the macroalgae *Ulva lactuca* in different water depths in a biofloc-integrated system with shrimp and fish and to determine how macroalgae influence total suspended solids.

2. Materials and Methods

2.1. Experimental Design and Facilities

The experiment was conducted in a greenhouse at the Marine Aquaculture Station (Estação Marinha de Aquicultura—EMA), Institute of Oceanography, Federal University of Rio Grande (FURG), located in Cassino Bach, Rio Grande, Rio Grande do Sul. The macroalgae species used was *U. lactuca*, collected in a natural environment at Cassino Beach (32°17′52.30″ S–52°15′59.80″ W), Rio Grande, RS, Brazil. The macroalgae were taken to the macroalgae laboratory, the epiphytes removed, and then taken to a greenhouse. The algae were kept in a 1 m^3 circular culture structure inside the greenhouse, with 10% biofloc inoculum for adaptation, for 15 days. Mean culture concentrations were 27.7 ± 2.45 mg L^{-1} nitrate and 1.02 ± 0.48 mg L^{-1} phosphate. The identification of the macroalgae was performed using a microscope, observing quadratic cells characteristic of this species and a bilayer of cells, as also identified by Alencar et al. (2010). The shrimp came from a grow-out culture in the Carcinoculture laboratory at EMA. The juveniles of *O. niloticus* were obtained from a commercial fish farm. The experiment was approved by the Ethics and Animal Welfare Committee of FURG (Case number 23116.005895/2016-42).

To conduct the experiment (70 days of culture), six production systems were used, with constant aeration supplied by a 4CV blower, and daily light intensity of 28.68 ± 8.53 µmol m^{-2} s^{-1}. Each system consisted of three tanks. The first tank had 16 m^3 of usable volume, where 400 shrimp m^{-2} [20] with an initial weight of 4.6 ± 0.01 g were stocked, and water was circulated to a second tank of 3 m^3 of usable volume with the aid of a Boyu submersible pump 75 w (SPA 4000 L/h, BOYU©, Guangdong, China) where 35 fish m^{-3} were stocked, with an initial weight of 177.67 ± 32.06 g. The water passed to the third tank by gravity, where macroalgae were stocked at a density of 2.4 kg per 3 m^3 (or 0.8 kg per m^3), equivalent to 0.1 kg m^{-3} (considering 22 m^3 of the entire system), and the water returned to the shrimp tank by gravity (Figure 1).

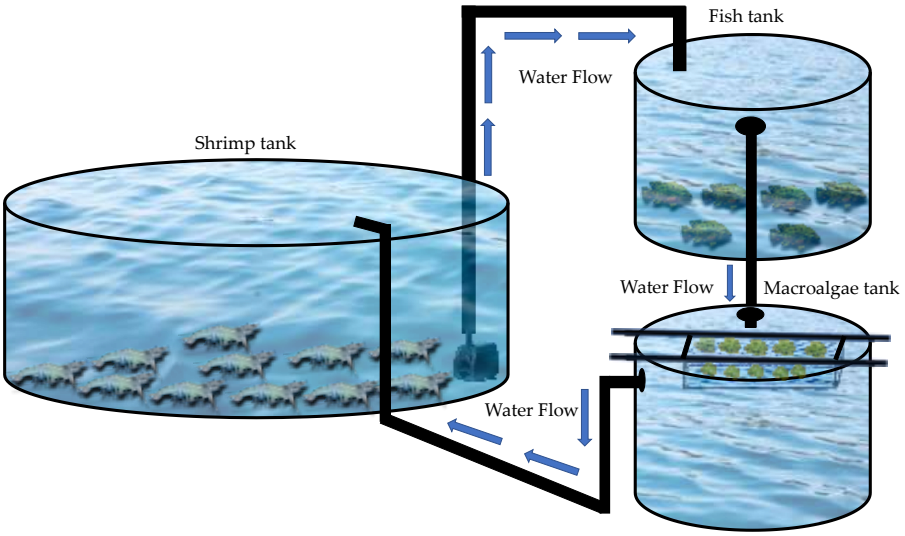


Figure 1. Scheme with a recirculation system, composed of a tank with shrimp of 16 m^3 useful volume, a tank with fish of 3 m^3 useful volume, and a tank with macroalgae of 3 m^3 useful volume, with a float used for the accommodation of the macroalgae.

At the beginning of the experiment, a biofloc inoculum from a grow-out shrimp culture was used with concentrations of 0.1, 0.2, 90.0, 4.7, and 600.0 mg L^{-1} of total ammoniacal nitrogen, nitrite, nitrate, phosphate, and total suspended solids, respectively. When excess solids occurred in the system, a clarification system was used in the tank. The clarifier consisted of a conical tank, with a usable volume of 150 L, made of fiberglass. The water from the shrimp tank was pumped to the top of the clarifier, and by decanting, the denser particles of solids were deposited at the bottom of the clarifier, and the lighter ones on the surface; water with lower solid concentration in the middle of the clarifier returned to the shrimp tank. When the value of total suspended solids exceeded the limit of 300 mg L^{-1} [21], the clarifier was added to the system.

The macroalgae were placed in floats made of PVC pipe and a 5 mm polyethylene mesh, forming a rectangular structure with length, width, and depth of 120 cm, 60 cm, and 40 cm, respectively. Each float with macroalgae was kept in the third tank, with a volume of 3 m^3 and 2.20 m diameter (Figure 1). There was one float for each tank, with macroalgae thallus varying from 10 to 20 cm in length. The experimental design was defined by two treatments (with three repetitions): shallow float, where the depth of the structure was placed within 10 cm from the surface; bottom float, where the depth was kept at 30 cm from the surface. The macroalgae were stirred inside the float, and solids decanted on their blades shaken off twice per day.

The shrimp and fish were fed twice per day (9 a.m. and 5 p.m.). The feed for the shrimp was supplied according to the daily feeding rate proposed by Jory et al. [22]. The fish were fed at a rate of 1% of the biomass to induce them to consume the biofloc and the supplied

commercial feed of 36% protein (Guabi Aqua QS 2–3 mm, Guabi Nutrition and Animal Health S.A., Campinas, São Paulo, Brazil).

2.2. Physical and Chemical Parameters

For the routine water quality analyses, due to the recirculation of water between the tanks, the water was collected only from the shrimp tank. For water quality monitoring, temperature (°C), dissolved oxygen (DO, mg L^{-1}), and pH were measured daily in the shrimp tanks using a multiparameter probe (model Pro-20, YSI Inc., Ohio, USA) and a benchtop pH meter (Seven2Go S7 Básico, Mettler Toledo, São Paulo, Brazil). Salinity (‰) was measured twice per week using a multiparameter probe (model Pro-20, YSI Inc., Ohio, USA). Light intensity was measured using an underwater luxmeter (PROTOMATIC Model 0824861, PCE Instruments, Júpiter, Florida, USA). For the water quality analyses, samples were collected from the shrimp tanks in plastic containers and taken immediately for analysis. Total alkalinity (mg $CaCO_3$ L^{-1}) was monitored according to the methodology presented by APHA [23] and was measured twice per week. Calcium hydroxide was used to maintain total alkalinity above 150 mg L^{-1} [24]. Total ammoniacal nitrogen (or TAN, mg L^{-1}) and nitrite (mg L^{-1}) were analyzed according to the methods of UNESCO [25] and Bendschneider and Robinson [26] twice per week. When the concentration of total ammoniacal nitrogen (TAN) was higher than 1 mg L^{-1}, molasses was applied for water quality control [10]. Nitrate (mg L^{-1}) and phosphate (mg L^{-1}) were analyzed using the methodology described by Aminot and Chaussepied [27] and monitored twice per week. Solids analyses in the shrimp tanks were performed twice per week. Turbidity (NTU) was measured by a portable turbidimeter (2100P, Hach®, Loveland, Colorado, EUA), and total suspended solids (or TSS, mg L^{-1}) were quantified by filtration and gravimetry according to the methodology described by Baumgarten et al. [28]. Settleable solids (or SS, ml L^{-1}) were measured using Imhoff cone according to the method proposed by APHA [23].

The solids that were decanted on the macroalgae were quantified every 15 days during the daily stirring process of shaking off solids decanted on macroalgae blades. For that quantification, an additional water collection was performed. There were two situations in each treatment, with macroalgae and without macroalgae. This collection was performed in the 3 m^3 tank where the macroalgae were located (Figure 1) for better quantification of the solids that were decanted. Following the procedure, first, the water was collected with the macroalgae in the tank. For the second collection, we stirred the macroalgae in the tank so that the solids would again become suspended in the system, and then we removed only the structure with the

macroalgae. There was a 10 min waiting time to homogenize the water, and then another water sample was collected in the same tank. Total suspended solids and settleable solids analyses were performed every 15 days.

2.3. Performance of Macroalgae

The biomass yield of the macroalgae was measured biweekly by weighing the fresh biomass, where the macroalgae were removed from the water and left outdoors for 20 min to reduce the humidity. The following equation was used to calculate the Relative Growth Rate (RGR) [29]:

$$\text{RGR (\% d}^{-1}\text{): } 100 \times [\ln(\text{final weight (g)/initial weight (g)})/(\text{final time} - \text{initial time})] \quad (1)$$

2.4. Shrimp and Fish Performance

Weekly and biweekly biometric measurements were performed for shrimp and fish, respectively, with the aid of a digital scale (BL3200H, MARTE®, Santa Rita do Sapucaí, Minas Gerais, Brazil). For the shrimp, 50 shrimp were collected and weighed. For fish, sampling was performed with 25 tilapia; the animals were anesthetized in clear water with 50 mg L^{-1} with benzocaine hydrochloride [30], and individual weighing was performed. The fish were then taken to a recovery tank and then to their tanks. The performance was analyzed with the following equations:

1. Final average weight (g): final biomass of live animals (g)/total number of animals;
2. Specific growth rate (g $week^{-1}$): weight gain (g)/number of weeks;
3. Final biomass yield (g): sum of final weight of all live animals (g);
4. Feed conversion rate (FCR) = feed offered (g)/(final biomass (g) − initial biomass (g));
5. Productivity (kg m^{-3}): [(final biomass (kg) − initial biomass (kg)) × 100]/tank volume (m^{-3}).
6. Survival (%) = (final number of animals/initial number of animals) × 100;

2.5. Statistical Analysis

The normality and homoscedasticity of the data were verified by the Shapiro–Wilk and Levene tests, respectively. Once the assumptions were met, the *t*-test was performed. The non-parametric Kruskall–Wallis test was used otherwise. A minimum significance level of 5% ($p \leq 0.05$) was applied in all analyses.

3. Results

3.1. Water Quality

The water quality results indicate that there was no significant difference between the two treatments throughout the 70 days of the experiment (Table 1). Figure 2 shows the temperature variation in the tanks during the days of culture, showing a higher temperature at the beginning and decreasing over the weeks. The experiment started at the end of April and ended in May, being the autumn period, close to winter, affecting the temperature of the tanks throughout the culture cycle.

Table 1. Mean values (± standard deviation) of water quality parameters for the treatments shallow float (5 to 10 cm depth) and bottom float (25 to 30 cm depth) during 70 days of experimentation.

	Treatments	
Parameters	Shallow Float	Bottom Float
Temperature (°C)	22.30 ± 1.59	22.28 ± 1.58
DO (mg L^{-1})	6.70 ± 0.56	6.72 ± 0.59
pH	8.00 ± 0.21	8.06 ± 0.20
Salinity(‰)	19.92 ± 0.98	19.00 ± 1.55
Alkalinity (mg$CaCO_3$ L^{-1})	218.14 ± 20.06	219.90 ± 22.30
TAN (mg L^{-1})	0.18 ± 0.20	0.15 ± 0.19
Nitrite (mg L^{-1})	2.16 ± 2.38	1.29 ± 1.30
Nitrate (mg L^{-1})	64.40 ± 28.39	61.71 ± 24.17
Phosphate (mg L^{-1})	5.99 ± 4.24	5.43 ± 3.78
Turbidity (NTU)	219.49 ± 86.66	196.02 ± 68.72
SS (ml L^{-1})	3.88 ± 2.09	5.01 ± 2.27
TSS (mg L^{-1})	307.92 ± 87.48	303.43 ± 91.60

DO = dissolved oxygen; TAN = total ammoniacal nitrogen; TSS = total suspended solids; SS = settleable solids. Temperature and DO (n = 140); pH (n = 70); salinity, alkalinity, TAN, nitrite, nitrate, phosphate, turbidity, SS, and TSS (n = 35).

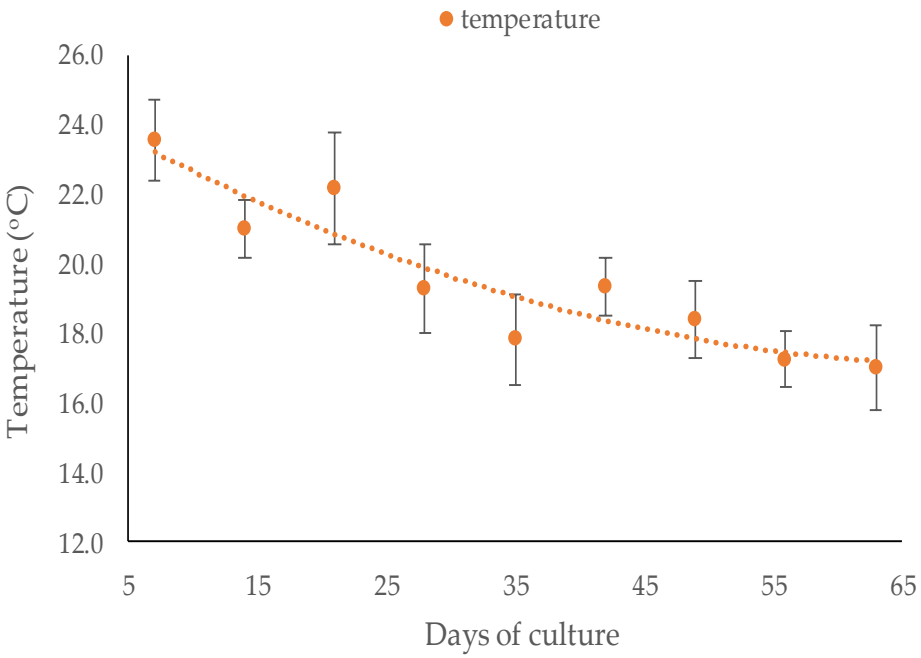


Figure 2. Mean ± standard deviation of the weekly temperature of the shrimp tanks during the 70 days of rearing (n = 6).

The accumulation of total suspended solids was constant throughout the production cycle due to the production of bacterial biomass and waste from feed and feces, showing that high concentrations were found on days 40 to 48 (Figure 3). The reduction in solid values after these days is due to the use of clarifiers in the system.

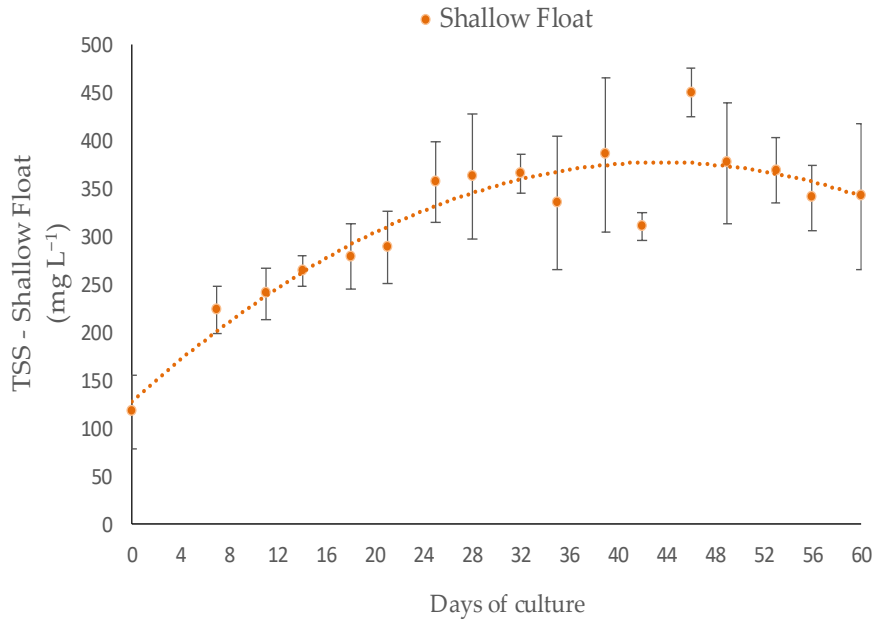


Figure 3. Mean ± standard deviation of total suspended solids (TSS) over the course of the cultivation days (n = 3).

The amount of solids decanted on macroalgae laminas, estimated by solid resuspension in the water after being shaken off from macroalgae laminas, significantly ($p < 0.05$) increased the mean concentration of settleable solids (SS) and total suspended solids (TSS) (Table 2). The SS and TSS means were higher when the macroalgae were stirred and removed from the tank and water was collected. There was a difference between tanks with and without macroalgae in total suspended solids of 198.7 ± 113.4 mg L^{-1} for the shallow treatment and 212.5 ± 102.6 mg L^{-1} in the bottom treatment. After removing the macroalgae from the system or moving them in the structures, the solids previously deposited were again suspended in the water, with an overall average increase of 39.4% and 40.1% in total suspended solids in the Shallow and Bottom float treatments, respectively.

Table 2. Concentrations of settleable solids (SS) and total suspended solids (TSS) (mean ± standard deviation) of the treatments shallow float (5 to 10 cm depth) and bottom float (25 to 30 cm depth), with and without macroalgae in the tank, during 70 days of experiment.

	Treatments			
	Shallow Float		**Bottom Float**	
Parameters	**with Macroalgae**	**without Macroalgae**	**with Macroalgae**	**without Macroalgae**
SS (ml L^{-1})	6.50 ± 3.50 [a]	14.50 ± 2.90 [b]	8.40 ± 3.50 [a]	16.40 ± 1.90 [b]
TSS (mg L^{-1})	305.10 ± 84.10 [a]	503.80 ± 40.10 [b]	317.00 ± 71.30 [a]	530.30 ± 40.10 [b]

Different letters represent significant differences ($p \leq 0.05$) with and without macroalgae in the same treatment after Student's *t*-test ($n = 12$).

In both depth treatments, mean values of SS and TSS were lower in tanks with macroalgae than without macroalgae throughout the experiment. However, differences between TSS values decreased towards the last days of cultivation, when the highest solid concentration in the water was observed (Figure 4).

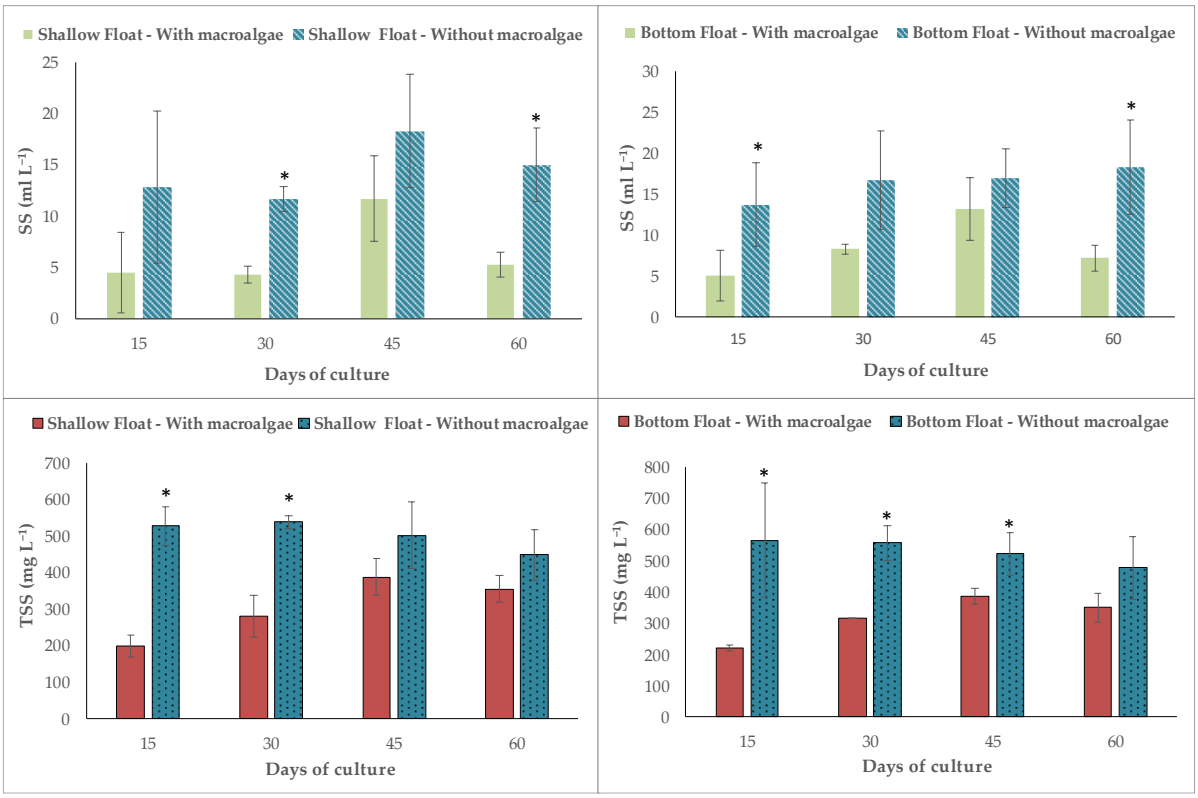


Figure 4. (**a**) Concentration of settleable solids (mean ± standard deviation) in the shallow float treatment (between 5 to 10 cm depth) with and without macroalgae. (**b**) Concentration of settleable solids (mean ± standard deviation) in the bottom float treatment (between 25 to 30 cm depth), with and without macroalgae. (**c**) Concentration of total suspended solids (mean ± standard deviation) in the shallow float treatment (between 5 to 10 cm depth) with and without macroalgae in the pond. (**d**) Total suspended solids concentration (mean ± standard deviation) in the bottom float treatment (between 25 and 30 cm depth) with and without macroalgae. Asterisks (*) represent significant difference ($p \leq 0.05$) with and without macroalgae after Student's *t*-test ($n = 3$).

3.2. Performance of Macroalgae

The biomass of macroalgae in the shallow treatment showed, throughout the experimental period, a significant difference ($p \leq 0.05$) from the bottom treatment. The bottom float treatment showed a decreasing trend in macroalgae biomass throughout the experimental period (Figure 5).

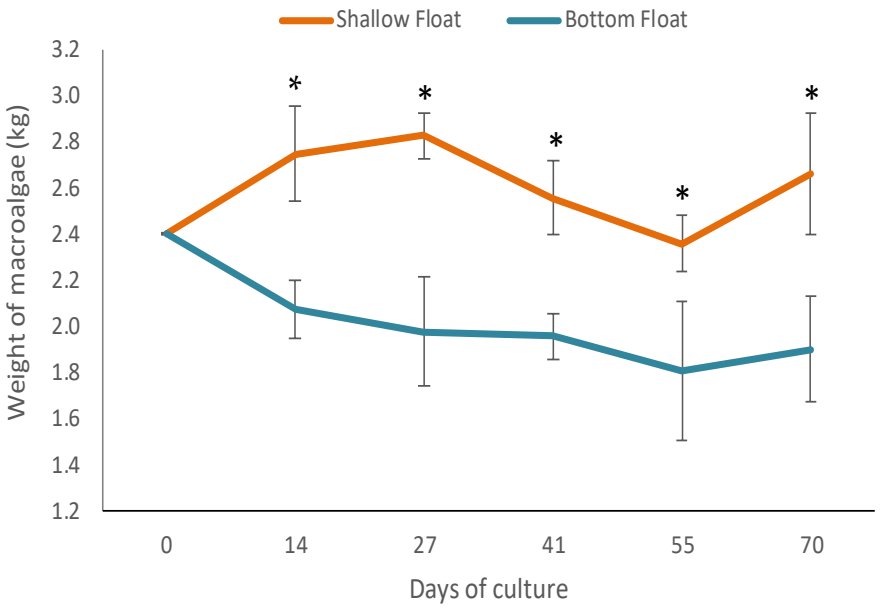


Figure 5. Mean macroalgae weight (kg—fresh weight) of the treatments, shallow float (between 5 to 10 cm depth) and bottom float (between 25 to 30 cm depth) during the 70 days of experiment. Asterisks (*) represent significant differences ($p \leq 0.05$) among treatments after Student's *t*-test ($n = 3$).

In 70 days of experimentation, the relative growth rate of macroalgae in the shallow float treatment was 0.14 ± 0.14% day^{-1} (Table 3), with an increase in biomass in the first weeks of culture (up to 0.95 ± 0.54% day^{-1}) and a decrease in biomass between sampling on days 41 and 55 of culture. At the end of the experiment, the shallow treatment showed a gain in macroalgae biomass.

Table 3. Macroalgae performance (mean ± standard deviation) of the treatments, shallow float (between 5 to 10 cm depth) and bottom float (between 25 to 30 cm depth) during 70 days of experiment.

	Treatments	
	Shallow Float	**Bottom Float**
SGR(% day^{-1})		
14 day	0.95 ± 0.54 [a]	−1.06 ± 0.43 [b]
27 day	0.23 ± 0.78	−0.39 ± 0.48
41 day	−0.72 ± 0.28	−0.05 ± 0.48
55 day	−0.58 ± 0.19	−0.63 ± 0.84
70 day	0.85 ± 0.58	0.38 ± 0.47
Initial mean weight (kg—FW)	2.40 ± 1.64	2.40 ± 0.88
Final mean weight (kg—FW)	2.63 ± 0.23 [a]	1.94 ± 0.20 [b]
RGR (% day^{-1})	0.14 ± 0.14 [a]	−0.35 ± 0.17 [b]
Biomass gain (kg)	0.26 ± 0.27 [a]	−0.50 ± 0.23 [b]

SGR, specific growth rate; RGR, relative growth rate; FW, fresh weight. Different lower-case letters represent statistical differences between treatments after Student's *t*-test ($n = 3$).

3.3. Shrimp Performance

There was no difference in the performance of fish and shrimp between the treatments during the 70 days of culture (Table 4). There was growth in the shrimp over the weeks of cultivation. Moreover, there was a weight gain of up to 9.93 ± 1.90 g week^{-1} for the fish, even though they were fed 1% of the biomass.

Table 4. Performance of shrimp and fish (mean ± standard deviation) of the treatments, shallow float (5 to 10 cm depth) and bottom float (25 to 30 cm depth) during 70 days of experiment.

	Treatments	
	Shallow Float	**Bottom Float**
Shrimp		
Final mean weight (g)	8.05 ± 0.52	8.92 ± 0.36
WWG (g week^{-1}) ##	0.38 ± 0.06	0.48 ± 0.04
Final biomass (kg)	27.90 ± 4.95	27.80 ± 2.94
FCR #	2.63 ± 0.47	2.99 ± 0.86
Yield (kg m^{-3})	1.33 ± 0.24	1.32 ± 0.24
Survival (%)	85.64 ± 19.84	76.24 ± 7.53
Fish		
Final mean weight (g)	289.74 ± 30.09	227.15 ± 48.97
WWG (g week^{-1}) ##	9.93 ± 1.90	8.02 ± 1.75
Final biomass (kg)	26.73 ± 1.73	16.94 ± 8.05
FCR #	0.39 ± 0.09	0.49 ± 0.16
Yield (kg m^{-3})	1.27 ± 0.08	0.81 ± 0.38
Survival (%)	88.77 ± 8.04	89.05 ± 6.07

#: FCR (food conversion rate); ##: WWG (weekly weight gain); n = 3.

4. Discussion

Integrated culture requires that water quality conditions meet the optimal levels for the cultivation of all species produced so that stress and poor development do not occur [5]. The use of a mature biofloc inoculum with the establishment of bacteria and the presence of nitrate [31] in this experiment provided high concentrations of total ammoniacal nitrogen and nitrite, which were controlled during culture, and no water quality problems were observed. As there was no control treatment without macroalgae, the uptake of nutrients by macroalgae was not verified. However, since there was no difference in the nitrogen content between the treatments, the loss of biomass in the bottom treatment did not cause nitrogen problems. The biomass of macroalgae used in the experiment was also low compared to the entire volume of the system, probably not causing significant nutrient uptake.

The biofloc system produces high concentrations of solids through heterotrophic bacteria, which use the ammonia produced in the system for their growth. According to Ebeling et al. [11], for each gram of ammonia transformed into bacterial biomass, 4.7 g of dissolved oxygen and 3.5 g of alkalinity are consumed, and 8 g of bacterial biomass is formed given their faster metabolism and establishment compared to chemoautotrophic bacteria. This bacterial biomass

produced is part of the total suspended solids, which, in high concentrations, can cause occlusion of the shrimp gills. Thus, a limit of 100 to 300 mg L^{-1} of total suspended solids should be determined [13]. The produced excess solids can be removed using clarifiers [21] and organic consumers in the system, such as tilapia [3].

Unlike shrimp and fish, macroalga is a sessile organism, and despite the movement of solids in the water column caused by vigorous aeration, macroalgae can act as a physical barrier to this movement and cause sedimentation of solids in the system. Brito et al. [16] observed the control of solids and turbidity of the water due to the deposition of particulate material on the macroalgae and increased settleable solids because of the incorporation of macroalgae fragments in the system.

In the present study, solids were deposited on the macroalgae and were not accounted for in the water quality analysis, with an overall average increase of 39.4% and 40.1% in total suspended solids in the shallow and bottom float treatments, respectively. Even when the total suspended solids concentrations were higher than 300 mg L^{-1}, and clarifiers were used, the solids that were decanted on the macroalgae were still there and were not removed from the system. The water going to the clarifier contained only the solids in suspension in the water column. However, the formation of this physical barrier of macroalgae may serve as a substrate for bacteria and aid in water quality; little is known about this relationship or the chemical effects between macroalgae and solids. Manual movement of the macroalgae in the culture structure was necessary at least twice per day to remove the solids from the top of the macroalgae and allow light to enter; however, the deposition of solids quickly occurred again. A sudden increase in the concentration of solids in the water column occurs, which, if for a prolonged time, can cause a drop in tank oxygen due to bacterial respiration [32] and obstruct the gills of cultured animals [13].

Besides the effects on water quality, the deposition of solids on the macroalgae can reduce their performance. The sedimentation of solids can obstruct photosynthetic laminae, reducing light absorption necessary for photosynthesis and hence, the growth of the macroalgae. Carvalho et al. [33] showed that a concentration of up to 400 mg L^{-1} of total suspended solids in culture does not negatively influence the macroalgae, even with deposition. That study was conducted in 3 L transparent carboys, with light entering from the surface and sides of the structure, promoting a wide surface area exposed to light so that the macroalgae had a greater availability of light to enhance performance. In this present experiment, on the other hand, the only light input to the macroalgae came through the surface of the tank. Therefore, it may have affected the growth of the macroalgae during the days of cultivation.

In addition to the deposition, the production of solids from feces, feed residues, and growth of bacterial biomass was continuous throughout the days of culture [13]. High concentrations of solids were found on days 40 to 48, exceeding 400 mg L^{-1} total suspended solids, requiring the permanent use of clarifiers. The accumulation of solids in this period may have influenced the decrease in biomass of the macroalgae between the weighing days 41 and 55. The high organic load may prevent light from entering the water, decreasing the macroalgae's photosynthetic efficiency and overall performance. Our results suggested that better *U. lactuca* performance integrated into a BFT system can be obtained from waters with TSS concentrations lower than 300 mg L^{-1} by using clarifiers.

The increase in depth of the bottom float structures (15 to 25 cm) provides more space for macroalgae movement and greater carrying capacity for the macroalgae biomass. However, according to Luo et al. [34], the biofloc system has great light limitations, with ammonia removal occurring predominantly by bacteria, as they do not need much light and can develop better in the system. Reis et al. [17] worked with different colors and wavelengths for the culture of shrimp *L. vannamei* and evaluated the penetration of each wavelength at the surface and at 20 and 40 cm of depth. These authors showed that the light penetration decreased with depth due to reflection or absorption by suspended particles in the water. Wavelengths of 79.05 ± 42.00 µmol m^{-2} m^{-1} and of 20.45 ± 23.40 µmol m^{-2} m^{-1} are absorbed, respectively, at the surface 20 and at 40 cm depth, in white light. This decrease of light in the water column may be a determinant in reducing macroalgae growth, causing the loss of biomass seen in the bottom float treatment.

Despite the deposition of solids on the macroalgae in both treatments, the shallow float treatment (5 to 10 cm) provided better conditions for macroalgae growth. The proximity to the surface probably allowed the macroalgae to capture more light for photosynthesis. The performance of the macroalgae was better than that shown by Legarda et al. [35], who observed a decrease in biomass of the macroalgae *U. fasciata* cultivated in an integrated biofloc system. The proximity to the surface and the adaptation of the macroalgae to the biofloc environment before the beginning of the experiment were possibly determining factors for better performance.

Despite the increase in biomass in this experiment in an integrated biofloc system, Resende et al. [36] showed a maximum specific growth rate of 3.91 ± 0.67% d^{-1} of the macroalgae *Ulva* spp. in integrated culture with clear water, during autumn with temperatures ranging from 9.6 to 14.9 °C. This shows that the biofloc system can interfere with the performance of the macroalgae, and that better management procedures can be adopted.

The high specific growth values observed by Resende et al. [36] in an autumn experiment are lower compared to the same experiment conducted in the spring months (specific growth rate of 14.48 ± 3.52% d^{-1}) with temperatures ranging from 19.57 to 28.5 °C and at 26.3 salinity. Culture temperature can be a key factor in macroalgae growth. Sudden changes in temperature can cause stress to the macroalgae, which usually initiate the release of spores for reproduction [19]. The loss of biomass in the last weeks of cultivation in this experiment may be associated with the sudden drop in temperature and consequent reproduction event as the release of spores was verified in the tanks.

In addition to temperature fluctuation, the salinity adopted in the culture also plays an important role in macroalgae physiology. The macroalgae of the genus *Ulva* are adapted to a wide range of salinity; however, Li et al. [37] and Bews et al. [38] point out that the minimum limit of salinity of the culture medium would be 20 ‰, as this is already a stress factor for the macroalgae and can affect their development. The average salinity in the experiment was 19.46 ± 0.65 ‰ and may have been a stressor for the macroalgae, causing lower growth rates. Mantri et al. [39] tested the best conditions for induction and spore release and growth of the macroalgae *U. fasciata*, and found that the highest spore release occurred at salinity 15 ‰, and at salinity 30 ‰, the highest growth rate (16.1 ± 0.28% day^{-1}) was observed at a mean temperature of 25 °C.

The carrying capacity of the algae culture structure could also have led to the decrease in biomass in the shallow float treatment. The increase in biomass at the beginning of the experiment decreased the free space in the structure, increasing the overlap of the macroalgae, causing shading and decreased light capture. After the biomass decreased and space was released, the macroalgae increased their biomass again in the shallow float treatment. This loss of biomass caused by the increased density was also verified by Alencar et al. [18], who showed that increasing the density of macroalgae in a given space caused decreased growth and loss of biomass throughout the culture cycle, probably due to interspecific competition for light and space.

Thus, better management and water quality parameters still need to be established for maximum macroalgae growth to occur in integrated biofloc culture. The use of clarifiers to remove solids and the maintenance of a concentration of 100 mg L^{-1} of total suspended solids would probably favor greater light input. The shallow float allowed the macroalgae to grow in culture, and the use of aeration within the macroalgae structure would probably be more efficient in moving the macroalgae in the structure, and less settling of solids would occur. An

improved management protocol could include partial harvests, which, according to Fernand et al. [40], can decrease the density, promoting greater light penetration and nutrient availability.

The shrimp, a euryhaline species, can tolerate large variations in salinity [37]. On the other hand, tilapia can be cultured in salinity from 0 to 16 ‰ with no loss of performance [41]. Therefore, the higher salinity used in this experiment—despite not causing mortality—may have promoted physiological damage to the fish. The low temperature during the weeks of culture influenced the performance of the cultured organisms. For shrimp, according to Ren et al. [42], low temperatures decreases responses to external events and locomotor activities, being a major limiting factor for growth, which might explain the low weight gain and poor apparent feed conversion. According to Nobrega et al. [43], the optimal temperature for tilapia culture would be 28 °C, and at 22 °C, there would be a reduction in food consumption and, consequently, in growth. That may justify the high apparent feed conversion factor. However, weekly weight gain was higher than that of Holanda et al. [44], rearing tilapia in an integrated system with shrimp.

The integrated multi-trophic culture using biofloc technology has shown advances with the objective of generating intensive cultures with a lower amount of waste generation. Brito et al. [16] showed that the integration of the macroalgae *U. lactuca* in shrimp culture resulted in the uptake of nitrogen and phosphate compounds in the system, improving water quality and, consequently, shrimp growth. In the experiment performed, the density of macroalgae used was low due to the large volume of the system, resulting in nutrient uptake. However, the production of biomass in the shallow treatment promoted the reuse of nutrients in the system to form a new product. Another inorganic consumer can be added to the system to maximize nutrient uptake. Poli et al. [3] reported better performance in productivity and nutrient uptake in the integrated system with *Sarcocornia ambigua* compared to monoculture.

Holanda et al. [14] showed a reduction in the organic load of biofloc culture when integrating mullet *Mugil liza* in shrimp culture compared to shrimp monoculture. In this experiment, tilapia were added to both treatments to consume the solids, but there was no quantification of the solids consumption. However, the addition of an organic consumer along with the macroalgae may help to reduce the solids and improve the light incidence in the water, favoring the growth of the macroalgae.

5. Conclusions

The integrated culture in a biofloc system presents distinct characteristics compared to conventional cultures in clear water due to the high load of solids and nutrients. The insertion

of the macroalgae in the integrated biofloc system showed deposition of solids on the macroalgae, decreasing the concentrations of total suspended solids and avoiding solids exiting the system by clarification. Even with this result, there was growth of the macroalgae *Ulva lactuca* in an integrated system with the shrimp *Litopenaeus vannamei* and tilapia *Oreochromis niloticus*, proving viability at depths of up to 10 cm in a biofloc system with an average TSS concentration of 300 mg L^{-1}.

Author Contributions: Conceptualization, A.C.; L.C; M.G; J.T; L.H.P; G.T and M.H.; methodology, A.C.; L.C; M.G; and M.H; software, A.C.; M.G and L.C..; validation, A.C.; L.C; M.G; J.T; M.H; G.T and L.H.P.; formal analysis, A.C.; M.G; J.T; M.H and L.C.; investigation, A.C.; L.C.; M.H.; M.G; J.T; G.T and L.H.P resources, C.C; G.T. and L.H.P.; data curation, A.C.; L.C. and M.G.; writing—original draft preparation, A.C. and L.C.; M.G. writing—review and editing, C.C; J.T; M.G; G.T; M.H. and L.H.P.; visualization, A.C.; L.C.; M.H.; L.H.P. and G.T.; supervision, C.C; G.T. and L.H.P.; project administration, L.C.; M.H.; L.H.P. and G.T.; funding acquisition, C.C; G.T. and L.H.P.. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by ASTRAL Project—H2020 grant Agreement 863034.

Institutional Review Board Statement: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors. The experiment was approved by the Ethics and Animal Welfare Committee of FURG (Case number 23116.005895/2016-42).

Acknowledgments: The authors are grateful for the financial support provided by the European Union (ASTRAL Project—H2020—Grant Agreement 863034). Special thanks to GUABI Nutrition and Animal Health S.A. for donating the commercial diets.

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

References

1. FAO. *The State of the World Fisheries and Aquaculture—2022 (SOFIA)*; FAO: Rome, Italy, 2022; ISBN 9789251072257.
2. Francavilla, M.; Pineda, A.; Lin, C.S.K.; Franchi, M.; Trotta, P.; Romero, A.A.; Luque, R. Natural Porous Agar Materials from Macroalgae. *Carbohydr. Polym.* **2013**, *92*, 1555–1560. https://doi.org/10.1016/j.carbpol.2012.11.005.
3. Poli, M.A.; Legarda, E.C.; de Lorenzo, M.A.; Pinheiro, I.; Martins, M.A.; Seiffert, W.Q.; do Nascimento Vieira, F. Integrated Multitrophic Aquaculture Applied to Shrimp Rearing in a Biofloc System. *Aquaculture* **2019**, *511*, 734274. https://doi.org/10.1016/j.aquaculture.2019.734274.
4. Da Silva, K.R.; Wasielesky, W.; Abreu, P.C. Nitrogen and Phosphorus Dynamics in the Biofloc Production of the Pacific White Shrimp, *Litopenaeus vannamei*. *J. World Aquac. Soc.* **2013**, *44*, 30–41. https://doi.org/10.1111/jwas.12009.
5. Chopin, T. Marine Aquaculture in Canada: Well-Established Monocultures of Finfish and Shellfish and an Emerging Integrated Multi-Trophic Aquaculture (IMTA) Approach Including Seaweeds, Other Invertebrates, and Microbial Communities. *Fisheries* **2015**, *40*, 28–31. https://doi.org/10.1080/03632415.2014.986571.
6. Troell, M.; Joyce, A.; Chopin, T.; Neori, A.; Buschmann, A.H.; Fang, J.G. Ecological Engineering in Aquaculture—Potential for Integrated Multi-Trophic Aquaculture (IMTA) in Marine Offshore Systems. *Aquaculture* **2009**, *297*, 1–9. https://doi.org/10.1016/j.aquaculture.2009.09.010.
7. Chopin, T.; Buschmann, A.H.; Halling, C.; Troell, M.; Kautsky, N.; Neori, A.; Kraemer, G.P.; Zertuche-González, J.A.; Yarish, C.; Neefus, C. Integrating Seaweeds into Marine Aquaculture Systems: A Key toward Sustainability. *J. Phycol.* **2001**, *37*, 975–986. https://doi.org/10.1046/j.1529-8817.2001.01137.x.
8. Nardelli, A.E.; Chiozzini, V.G.; Braga, E.S.; Chow, F. Integrated Multi-Trophic Farming System between the Green Seaweed *Ulva lactuca*, Mussel, and Fish: A Production and Bioremediation Solution. *J. Appl. Phycol.* **2019**, *31*, 847–856. https://doi.org/10.1007/s10811-018-1581-4.
9. Turan, G.; Tekogul, H. The Turkish Mezzes Formulated with Protein-Rich Green Sea Vegetable (Chlorophyta), *Ulva rigida*, Cultured in Onshore Tank System. *J. Aquat. Food Prod. Technol.* **2014**, *23*, 447–452. https://doi.org/10.1080/10498850.2012.723307.
10. Wasielesky, W.; Krummenauer, D.; Lara, G.; Fóes, G. *Cultivo de Camarões Em Sistema de Bioflocos: Realidades e Perspectivas*; Revista ABCC: Natal, Brazil, 2013; pp. 30–36.
11. Ebeling, J.M.; Timmons, M.B.; Bisogni, J.J. Engineering Analysis of the Stoichiometry of Photoautotrophic, Autotrophic, and Heterotrophic Removal of Ammonia-Nitrogen in Aquaculture Systems. *Aquaculture* **2006**, *257*, 346–358. https://doi.org/10.1016/j.aquaculture.2006.03.019.
12. Furtado, P.S.; Campos, B.R.; Serra, F.P.; Klosterhoff, M.; Romano, L.A.; Wasielesky, W. Effects of Nitrate Toxicity in the Pacific White Shrimp, *Litopenaeus vannamei*, Reared with Biofloc Technology (BFT). *Aquac. Int.* **2015**, *23*, 315–327. https://doi.org/10.1007/s10499-014-9817-z.
13. Gaona, C.A.P.; de Almeida, M.S.; Viau, V.; Poersch, L.H.; Wasielesky, W. Effect of Different Total Suspended Solids Levels on a *Litopenaeus vannamei* (Boone, 1931) BFT Culture System during Biofloc Formation. *Aquac. Res.* **2017**, *48*, 1070–1079. https://doi.org/10.1111/are.12949.
14. Holanda, M.; Santana, G.; Furtado, P.; Rodrigues, R.V.; Cerqueira, V.R.; Sampaio, L.A.; Wasielesky, W.; Poersch, L.H. Evidence of Total Suspended Solids Control by *Mugil liza* Reared in an Integrated System with Pacific White Shrimp *Litopenaeus vannamei* Using Biofloc Technology. *Aquac. Rep.* **2020, *18*, 100479**. https://doi.org/10.1016/j.aqrep.2020.100479.
15. Azim, M.E.; Little, D.C. The Biofloc Technology (BFT) in Indoor Tanks: Water Quality, Biofloc Composition, and Growth and Welfare of Nile Tilapia (*Oreochromis niloticus*). *Aquaculture* **2008**, *283*, 29–35. https://doi.org/10.1016/j.aquaculture.2008.06.036.

16. Brito, L.O.; Arantes, R.; Magnotti, C.; Derner, R.; Pchara, F.; Olivera, A.; Vinatea, L. Water Quality and Growth of Pacific White Shrimp *Litopenaeus vannamei* (Boone) in Co-Culture with Green Seaweed *Ulva lactuca* (Linaeus) in Intensive System. *Aquac. Int.* **2014**, *22*, 497–508. https://doi.org/10.1007/s10499-013-9659-0.
17. Reis, W.G.; Wasielesky, W.; Abreu, P.C.; Brandão, H.; Krummenauer, D. Rearing of the Pacific White Shrimp *Litopenaeus vannamei* (Boone, 1931) in BFT System with Different Photoperiods: Effects on the Microbial Community, Water Quality and Zootechnical Performance. *Aquaculture* **2019**, *508*, 19–29. https://doi.org/10.1016/j.aquaculture.2019.04.067.
18. de Alencar, J.R.; Junior, P.A.H.; Celino, J.J. Cultivo de Camarão Branco *Litopenaeus vannamei* (Boone, 1931) Com a Macroalga *Ulva lactuca* Linneaus (Chlorophyta) No Tratamento de Efluentes Em Sistema Fechado de Recirculação. *Rev. Biol. Ciências Terra* **2010**, *10*, 117–137.
19. Copertino, M.D.S.; Tormena, T.; Seeliger, U. Biofiltering Efficiency, Uptake and Assimilation Rates of *Ulva clathrata* (Roth) J. Agardh (Clorophyceae) Cultivated in Shrimp Aquaculture Waste Water. *J. Appl. Phycol.* **2009**, *21*, 31–45. https://doi.org/10.1007/s10811-008-9357-x.
20. Liu, G.; Zhu, S.; Liu, D.; Guo, X.; Ye, Z. Effects of Stocking Density of the White Shrimp *Litopenaeus vannamei* (Boone) on Immunities, Antioxidant Status, and Resistance against Vibrio Harveyi in a Biofloc System. *Fish Shellfish Immunol.* **2017**, *67*, 19–26. https://doi.org/10.1016/j.fsi.2017.05.038.
21. Gaona, C.A.P.; Poersch, L.H.; Krummenauer, D.; Foes, G.K.; Wasielesky, W.J. The effect of solids removal on water quality, growth and survival of *Litopenaeus vannamei* in a biofloc technology culture system. *Shrimp Int. J. Recirc. Aquac.* **2011**, *12*, 54–73.
22. Jory, D.E.; Cabrera, T.R.; Dugger, D.M.; Fegan, D.; Lee, P.G.; Lawrence, L.; Jackson, C.J.; Mcintosh, R.P.; Castañeda, J.I.; Al, B.; et al. A Global Review of Shrimp Feed Management: Status and Perspectives. *Aquaculture* **2011**, *318*, 104–152.
23. American Water Works Association, Water Pollution Control Association, APHA. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association: Washington, DC, USA, 2005.
24. Furtado, P.S.; Poersch, L.H.; Wasielesky, W. Effect of Calcium Hydroxide, Carbonate and Sodium Bicarbonate on Water Quality and Zootechnical Performance of Shrimp *Litopenaeus vannamei* Reared in Biofloc Technology (BFT) Systems. *Aquaculture* **2011**, *321*, 130–135. https://doi.org/10.1016/j.aquaculture.2011.08.034.
25. Unesco. *Chemical Methods for Use in Marine Environmental Monitoring*; Intergovernmental Oceanographic Commission: Paris, France, 1983.
26. Bendschneider, K.; Robinson, R.J. New Spectrophotometric Method for the Determination of Nitrite in Water. *Fresenius Environ. Bull.* **2001**, 10, 781–785.
27. Aminot, A.; Chaussepied, M. *Manuel des Analyses Chimiques en Milieu Marin*; Centre National pour l'exploitation des océans: Brest, France, 1983.
28. Baumgarten, M.D.G.Z.; De Barros Rocha, J.M.; Niencheski, L.F.H. *Manual de Análises em Oceanografia Química*; FURG: Rio Grande, Brazil, 1996.
29. Loureiro, R.R.; Reis, R.P.; Critchley, A.T. In Vitro Cultivation of Three *Kappaphycus alvarezii* (Rhodophyta, Areschougiaceae) Variants (Green, Red and Brown) Exposed to a Commercial Extract of the Brown Alga *Ascophyllum nodosum* (Fucaceae, Ochrophyta). *J. Appl. Phycol.* **2010**, *22*, 101–104. https://doi.org/10.1007/s10811-009-9412-2.
30. De Miranda Cabral Gontijo, Á.M.; Barreto, R.E.; Speit, G.; Valenzuela Reyes, V.A.; Volpato, G.L.; Favero Salvadori, D.M. Anesthesia of Fish with Benzocaine Does Not Interfere with Comet Assay Results. *Mutat. Res.-Genet. Toxicol. Environ. Mutagen.* **2003**, *534*, 165–172. https://doi.org/10.1016/S1383-5718(02)00276-0.

31. Ferreira, G.S.; Santos, D.; Schmachtl, F.; Machado, C.; Fernandes, V.; Bögner, M.; Schleder, D.D.; Seiffert, W.Q.; Vieira, F.N. Heterotrophic, Chemoautotrophic and Mature Approaches in Biofloc System for Pacific White Shrimp. *Aquaculture* **2021**, *533*, 736099. https://doi.org/10.1016/j.aquaculture.2020.736099.
32. Liang, W.; Luo, G.; Tan, H.; Ma, N.; Zhang, N.; Li, L. Efficiency of Biofloc Technology in Suspended Growth Reactors Treating Aquacultural Solid under Intermittent Aeration. *Aquac. Eng.* **2014**, *59*, 41–47. https://doi.org/10.1016/J.AQUAENG.2014.02.001.
33. Carvalho, A.; Costa, L.C.d.O.; Holanda, M.; Poersch, L.H.; Turan, G. Influence of Total Suspended Solids on the Growth of the Sea Lettuce *Ulva lactuca* Integrated with the Pacific White Shrimp *Litopenaeus vannamei* in a Biofloc System. *Fishes* **2023**, *8*, 163. https://doi.org/10.3390/fishes8030163.
34. Luo, M.B.; Liu, F.; Xu, Z.L. Growth and Nutrient Uptake Capacity of Two Co-Occurring Species, *Ulva prolifera* and *Ulva linza*. *Aquat. Bot.* **2012**, *100*, 18–24. https://doi.org/10.1016/j.aquabot.2012.03.006.
35. Legarda, E.C.; da Silva, D.; Miranda, C.S.; Pereira, P.K.M.; Martins, M.A.; Machado, C.; de Lorenzo, M.A.; Hayashi, L.; do Nascimento Vieira, F. Sea Lettuce Integrated with Pacific White Shrimp and Mullet Cultivation in Biofloc Impact System Performance and the Sea Lettuce Nutritional Composition. *Aquaculture* **2021**, *534*, 736265. https://doi.org/10.1016/j.aquaculture.2020.736265.
36. Resende, L.; Flores, J.; Moreira, C.; Pacheco, D.; Baeta, A.; Garcia, A.C.; Rocha, A.C.S. Effective and Low-Maintenance IMTA System as Effluent Treatment Unit for Promoting Sustainability in Coastal Aquaculture. *Appl. Sci.* **2022**, *12*, 398. https://doi.org/10.3390/app12010398.
37. Li, E.; Chen, L.; Zeng, C.; Chen, X.; Yu, N.; Lai, Q.; Qin, J.G. Growth, Body Composition, Respiration and Ambient Ammonia Nitrogen Tolerance of the Juvenile White Shrimp, *Litopenaeus vannamei*, at Different Salinities. *Aquaculture* **2007**, *265*, 385–390. https://doi.org/10.1016/j.aquaculture.2007.02.018.
38. Bews, E.; Booher, L.; Polizzi, T.; Long, C.; Kim, J.H.; Edwards, M.S. Effects of Salinity and Nutrients on Metabolism and Growth of *Ulva lactuca*: Implications for Bioremediation of Coastal Watersheds. *Mar. Pollut. Bull.* **2021**, *166*, 112199. https://doi.org/10.1016/j.marpolbul.2021.112199.
39. Mantri, V.A.; Singh, R.P.; Bijo, A.J.; Kumari, P.; Reddy, C.R.K.; Jha, B. Differential Response of Varying Salinity and Temperature on Zoospore Induction, Regeneration and Daily Growth Rate in *Ulva fasciata* (Chlorophyta, Ulvales). *J. Appl. Phycol.* **2011**, *23*, 243–250. https://doi.org/10.1007/s10811-010-9544-4.
40. Fernand, F.; Israel, A.; Skjermo, J.; Wichard, T.; Timmermans, K.R.; Golberg, A. Offshore Macroalgae Biomass for Bioenergy Production: Environmental Aspects, Technological Achievements and Challenges. *Renew. Sustain. Energy Rev.* **2017**, *75*, 35–45. https://doi.org/10.1016/j.rser.2016.10.046.
41. de Souza, R.L.; de Lima, E.C.R.; de Melo, F.P.; Ferreira, M.G.P.; de Souza Correia, E. The Culture of Nile Tilapia at Different Salinities Using a Biofloc System. *Rev. Cienc. Agron.* **2019**, *50*, 267–275. https://doi.org/10.5935/1806-6690.20190031.
42. Ren, X.; Wang, Q.; Shao, H.; Xu, Y.; Liu, P.; Li, J. Effects of Low Temperature on Shrimp and Crab Physiology, Behavior, and Growth: A Review. *Front. Mar. Sci.* **2021**, *8*, 746177. https://doi.org/10.3389/fmars.2021.746177.
43. Nobrega, R.O.; Banze, J.F.; Batista, R.O.; Fracalossi, D.M. Improving Winter Production of Nile Tilapia: What Can Be Done? *Aquac. Rep.* **2020**, *18*, 100453. https://doi.org/10.1016/j.aqrep.2020.100453.

44. Holanda, M.; Wasielesky, W.; de Lara, G.R.; Poersch, L.H. Production of Marine Shrimp Integrated with Tilapia at High Densities and in a Biofloc System: Choosing the Best Spatial Configuration. *Fishes* **2022**, *7*, 283. https://doi.org/10.3390/fishes7050283.

CAPÍTULO 4: Impacto do co-cultivo de macroalgas (*Ulva lactuca* f. fasciata) na composição do biofloco em um sistema de zero troca de água usado para a criação de camarão branco do pacífico (*Litopenaeus vannamei*) (decapoda, dendrobranchiata)

Artigo publicado na *Crustaceana*.

https://doi.org/10.1163/15685403-bja10363

Resumo

O sistema de bioflocos consiste na manipulação do carbono e nitrogênio na água para o desenvolvimento da comunidade microbiana, com a finalidade de manter os padrões de qualidade de água aceitáveis, necessitando de mínima ou nula renovação. A inserção de consumidor inorgânico, como a macroalga, associado ao cultivo de camarão pode interferir na comunidade microbiana devido a disponibilidade de compostos nitrogenados além de efeitos antioxidantes. Com isso, o trabalho buscou avaliar o efeito da inserção da macroalga *Ulva lactuca* na comunidade microbiana em um cultivo integrado com camarão em comparação ao monocultivo. O experimento contou com quatro tratamentos com três réplicas cada, sendo um monocultivo de camarão (300 indivíduos/m3) e três sistemas integrados de camarão com a macroalga *Ulva lactuca* em diferentes densidades de Ulva (1, 2 e 3 g/l). A pesquisa foi conduzida em condições de zero troca de água, e análises semanais de qualidade de água. Ao final do experimento foram coletadas amostras de água de todas as réplicas, levadas ao laboratório e feita a identificação de bactérias e zooplancton em microscópio. Como resultado, a inserção da macroalga no sistema proporcionou menores concentrações de nitrato ao final do cultivo, causando melhor manutenção da qualidade de água comparado ao monocultivo. Na comunidade microbiana, foi apresentado densidades reduzidas de cianófitas, que podem causar a proliferação de algas tóxicas em sistemas de aquicultura, e a presença de esporos com o aumento da densidade de macroalgas. A macroalga Ulva mostrou ser um agente de biorremediação de ocorrência natural quando inserida em sistema integrado com bioflocos.

Impact of the co-cultivation of macroalgae (*Ulva lactuca* f. *Fasciata*) on the composition of biofloc in a zero water exchange system used for rearing pacific white shrimp (*Litopenaeus vannamei*) (decapoda, dendrobranchiata)

BY

GAMZE TURAN[1,2,3)], ANDREZZA CARVALHO CHAGAS[1)], CÉSAR S.B. COSTA[1)], WILSON F.B. WASIELESKY JR.[1)] and LUÍS H. DA SILVA POERSCH[1)]

1) Marine Aquaculture Station, Laboratory of Multitrophic Cultivation, Federal University of Rio Grande, Rua do Hotel, 96210-000, Rio Grande — RS, Brazil

2) Aquaculture Department, Fisheries Faculty, Ege University, 35100, Bornova, Izmir, Turkey

ORCID iDs: Turan: 0000-0002-3610-6347; Carvalho Chagas: 0000-0002-1831-0096; Costa: 0000-0002-3948-6349; Wazielesky: 0000-0002-7267-4755; da Silva Poersch: 0000-0002-1663-6252

3) Corresponding author; e-mail: gamze.turan@ege.edu.tr

ABSTRACT

In a closed, or zero water exchange, greenhouse aquaculture system, this study examined the interaction between the aggregation flocs in the mono- and co-cultivation of Pacific white shrimp, *Litopenaeus vannamei* (300 individuals/m^3) with macroalgae, *Ulva lactuca* f. *fasciata* at different densities of *Ulva* (1, 2 and 3 g/l). The investigation was conducted under zero water exchange conditions. The accumulated flocs were compared between the various treatment groups: without *Ulva* (Treatment Control Group), *Ulva* at 1 g/l density (T1: Treatment 1), *Ulva* at 2 g/l (T2: Treatment, and *Ulva* at 3 g/l (T3: Treatment 3). Ultimately, it was determined that *Ulva* cultivation using the Biofloc system actually helped to ensure the water quality in the zero water exchange culture system during the 35-day experimental period with reduced cyanophyte densities, that can cause toxic algal blooms in aquaculture systems. This was interpreted to result from the fact that *Ulva* is a naturally occurring bioremediation agent.

Key words. — Biofloc composition, bioremediation, IMTA — Integrated multi-trophic aquacul- ture, shrimp culture, *Ulva*

1 Introduction

In order to remove toxins from a region or volume of water on which we focus and wish to control, and therefore to convert hazardous substances there present into less toxic or non-toxic ones, a method known as bioremediation involves stimulating microorganisms

with nutrients and other chemicals to fulfil such tasks (Das, 2014). Microorganisms are thought to be the earliest living things to have evolved, since they are able to adapt to changes in the environment. Thesedays, there is interest in using microorganisms like bacteria as biodegradation and bioremediation agents due to their capacity to reduce risk, degrade both natural and artificial materials, and accumulate harmful compounds (Karigar & Rao, 2011). Microorganisms regulate the biogeochemical cycle through fixing carbon, fixing nitrogen, metabolizing methane, and metabolizing sulfur, according to Das et al. (2006). Different metabolic enzymes produced by microorganisms can help remove pollutants safely by either destroying them directly or converting them toa safer or less toxic intermediate (Dash & Das, 2012).

Microorganisms utilized in bioremediation must have a resistant genotype for the specific pollutant; as a result, they have certain distinctive qualities that make them appropriate for bioremediation procedures (Stelting et al., 2010). According to Panigrahi et al. (2014), biofloc technology (BFT) is a promising technique thatencourages waste to be retained and converted into biofloc, which is a shrimp's natural meal in aquaculture systems. In order to maintain water quality, maintain biosecurity, support high densities of shrimp culture, and minimize water exchange in aquaculture systems, a variety of microorganisms, including heterotrophic bacteria, algae (dinoflagellates and diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans, and detritus, congregate and perform symbiotic processes. This collective activity is known as biofloc. When the heterotrophic microbe in the biofloc turns the nitrogenous waste in the culture tank from the uneaten meal into protein, protein is used as a feed for the shrimp in the biofloc technology (BFT) application. Through in situ bioremediation, the waste produced in the aquaculture system will be overcome by the development of a dense heterotrophic bacterial community as opposed to one that is dominated by algae (Panigrahi et al., 2014).Cheap carbohydrate sources, such tapioca or molasses, are frequently added to the water column in a ratio of roughly (12-15) : 1, which allows biofloc to transform the harmful elements into food sources that are good for the shrimp's ingestion. The presence of an extra carbon source promotes the growth of heterotrophic bacteriain ponds or tanks with high stocking density and little or no water exchange. Schneider et al. (2005) found that if the C: N ratio is balanced at a ratio of (10-15): 1, the addition of organic nitrogenous waste will cause ammonium to be transformed into bacterial biomass. Due to their greater rate of growth and microbial biomass yield per substrate, heterotrophic bacteria typically have a greater degree of dominance in BFT compared to nitrifying bacteria (Hargreaves, 2006). Determining the

microscopic makeup of biofloc can aid in improving comprehension of how biofloc is used. The interaction between the organisms in the biofloc system can be understood by identifying the function of each class of organisms that occurs in the zero water exchange culture system (e.g., bacteria and protozoa as organic matter decomposers, zooplankton as algae grazers, and phytoplankton as primary producers).

Both biologically and commercially, micro- and macro-algae, sometimes known as seaweeds, are significant primary producers in marine aquatic food webs. Marine algae are frequently regarded as important resources for the manufacturing sector. Growing microalgae for use as nutritional supplements, such as live feed and aquafeeds, is one of the biotechnology industry's most lucrative ventures (Masasa et al., 2021; Gencer, 2023a and 2023b; Gencer & Aguilar Vitorino, 2023). Algae have a great potential for bioremediation, just like bacteria do (Alazaiza etal., 2022).

The goal of this study was to ascertain how the green macroalga *Ulva lactuca* f.*fasciata* (Delile) Hering, 1846, affected the microorganisms in the biofloc system,which was utilized to cultivate the marine shrimp, *Litopenaeus vannamei* (Boone,1931) [currently also again referred to as: *Penaeus vannamei* Boone, 1931] ina greenhouse system with nil water exchange. In order to accomplish this goal, three distinct *Ulva* densities were used to cultivate *L. vannamei*, with as a control group cultivating *L. vannamei* by itself. Additionally, a haemacytometer was usedto identify and count the microorganisms in the biofloc system under a microscope in each experimental treatment group. In order to ascertain the effectiveness of biofloc as a natural bioremediation agent for the removal of organic matter load inthe mono- and co-cultivation of *L. vannamei* with *Ulva* in a zero water exchange tank system, the analysis also included an examination of water quality parameters.

2 Material and methods

Experimental design

In this experiment, rounded tanks with a 300 litre capacity and 150 litres consumption volume were used. A density of 300 individuals/m^3 and an average weight of 2.26 0.64 g of juvenile Pacific white shrimp, *Litopenaeus vannamei*, were stocked in each tank. Next to the Treatment Control Group (in fact, "Treat- ment 0"), Treatment 1 (cultivation of *Ulva lactuca* f. *fasciata* at 1 g/l density with the shrimps), Treatment 2 (with *Ulva* at 2 g/l density), and Treatment 3 (with *Ulva*at 3 gr/l) were the three treatments carried out in the experiment, which involved twelve tanks in total (i.e., in three replicates of all of the four treatments).

After having been fermented for 24 hours, molasses — which serves as a carbohydrate or carbon source at a ratio of C N (10:1) was introduced into the biofloc stock tank to facilitate the breakdown process by the bacteria and other microorganisms for the formation of biofloc. For 35 days, the shrimp were cultivated until the water quality measurements reached the required thresholds. To determine the make-up of the microorganisms in the biofloc aggregation in thezero water exchange system, samples of the microorganisms in the culture tank were taken at the conclusion of the culture time. In all experimental tanks (YSI Pro-20, temperature, salinity, and dissolved oxygen were measured twice a day. ApH meter (Mettler Toledo FEP20) was used to assess the pH value once a day.

Total nitrogen and nitrite, as determined daily using UNESCO's (1983) method- ologies. The approach of Aminot & Chaussepied (1983) was used to quantify phosphate and nitrate three times a week. Every week, the amount of suspended solids (SST) was measured using a portable turbidimeter (Hach® 2100P) using the approach outlined by Baumgarten et al. (1996), with measurement via the Imhoffcone. Alkalinity was measured twice a week in accordance with the APHA (1998)approach.

Sample collection

A 50 ml water sample was extracted from each of the treatment tanks in order to identify tiny bacteria as well as phyto- and zooplankton. After being taken from the tanks, all of the water samples were transported back to the lab for additional qualitative and quantitative plankton analysis.

Microscopic identification and density determination

For biofloc microscopic identification, an advanced Nikon Eclipse E200 micro-scope was utilized. Haemacytometers were used to analyse bacteria and phyto- plankton both qualitatively and quantitatively. Additionally, zooplankton was analysed qualitatively and quantitatively by pouring 1 ml of material in drops onto aPetri dish cup and counting each drop under a microscope.

Statistical analysis

The Shapiro-Wilk and Levene tests were used to confirm the data's normality and homoscedasticity, respectively. An ANOVA was run once the assumptions were verified, and either the *t* -test, or Tukey's post-hoc analysis came next. When the assumptions of the ANOVA were not met, the nonparametric Kruskal-Wallistest was employed. In all analyses, a 5% ($p \leq$

0.05) minimum level of significancewas used.

3 Results

Through bioflocculation, several kinds of microscopic creatures were detected. The tiny organisms that have been identified are derived from many kinds of algae, bacteria, and numerous algae grazers, including copepods, rotifers, ciliates, and nematodes (fig. 1). Table I summarizes the results of the water parameters.

As the nutrients and nitrate values were lower in all three threatment groups (Treatments 1, 2 and 3) where macroalgae cultivation took place compared to thecontrol treatment where monoculture of the shrimp took place, the bioremediationprocess was successfully carried out by the microorganisms, with bacteria and macroalgae in the biofloc.

Nonetheless, compared to the other treatment groups, the nitrate content in Treatment 1, which had *Ulva lactuca* f. *fasciata* administered at a rate of 1 g/l, was noticeably lower. Phosphate, nitrite, and total ammonium nitrogen (TAN) levels did not significantly differ across treatment groups (table I). According to the results of the Imhoff cone measurement, there were less organic settleable solids in the tanks used for macroalgae production than in the control treatment groups used for shrimp monoculture. Through bioflocculation, numerous and diverse microscopic organism compositions were found in the experimental treatments groups (table II).

Microscopic organisms found in the biofloc are shown in fig. 1. The following identifications could be established, here enumerated according to the order in the figure: a, a rotifer; b, a ciliate; c, a copepod; d, a nematode; e, filamentous cyanophytes; f, unicellular cyanophytes; g, diatoms; h, unicellular non-motile green microalgae; and i, juvenile macroalgae. Each microorganism was observed and identified under the microscope at a magnification of 100×.

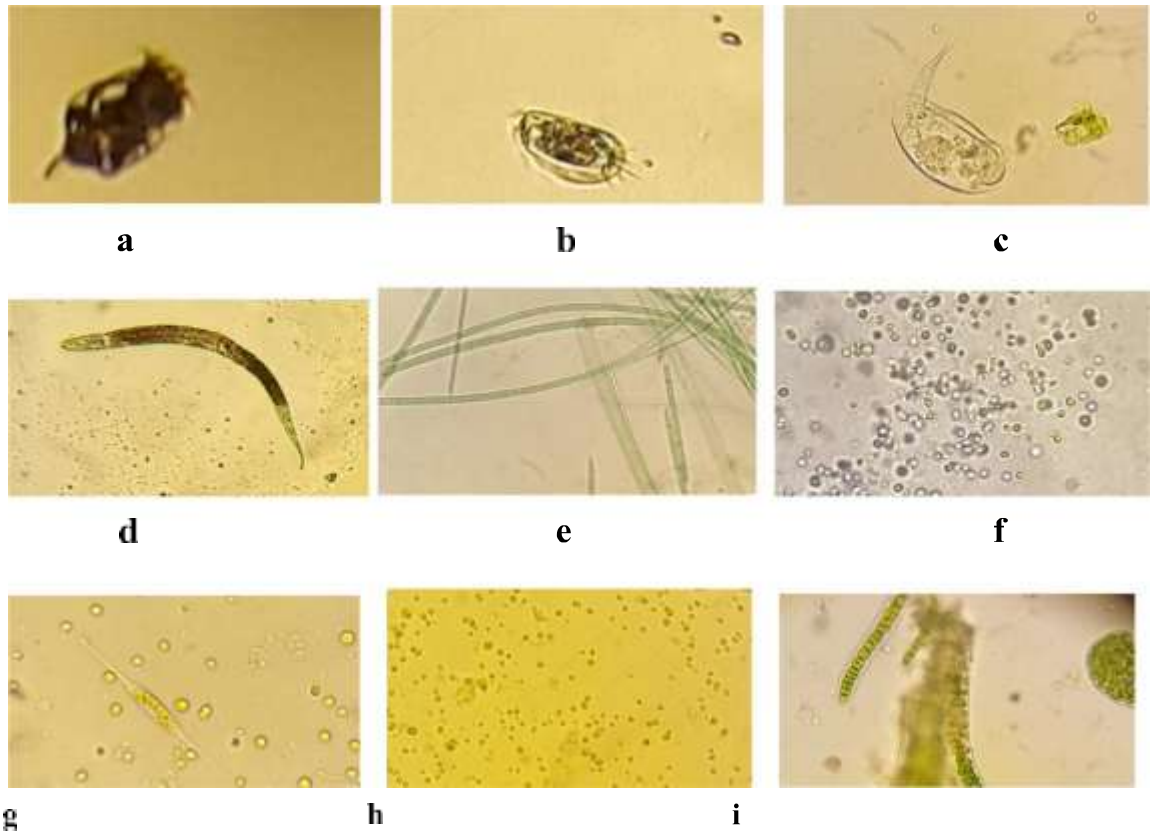


Fig. 1. Microscopic organisms identified in the biofloc resulting from adding *Ulva lactuca* f. *fasciata* (Delile) Hering, 1846 culture to zero water exchange cultures of *Litopenaeus vannamei* (Boone, 1931): a, rotifer; b, ciliate; c, copepod; d, nematode; e, filamentous cyanophytes; f, unicellular cyanophytes; g, diatoms; h, unicellular non-motile green microalgae; and, i, juvenile macroalgae. All originally observed under 100× magnification.

Parameter	Treatment			
	TR Control	TR 1	TR 2	TR 3
Temperature (ºC)	26.32 ± 1.75	26.46 ± 1.46	26.51 ± 1.80	26.69 ± 1.58
DO (mg/l)	7.31 ± 0.32	7.31 ± 0.32	7.28 ± 0.33	7.31 ± 0.34
pH	8.14 ± 0.14	8.16 ± 0.13	8.17 ± 0.13	8.15 ± 0.12
Salinity (ppt)	29.98 ± 0.56	29.82 ± 0.56	28.81 ± 1.01	29.61 ± 0.81
Turbidity (NTU)	246.60 ± 115.80 ^{b}	142.60 ± 74.40 ^{b}	117.50 ± 52.40 ^{a}	121.10 ± 78.60 ^{a}
Alkalinity (mg $CaCO_3$/l)	136.67 ± 21.49	151.97 ± 15.15	143.18 ± 17.04	148.79 ± 15.11
TSS (mg/l)	423.33 ± 13.50	295.33 ± 160.55	297.33 ± 159.55	302.00 ± 136.33
Imhoff Cone	13.93 ± 13.50 ^{b}	5.49 ± 4.72 ^{a}	4.57 ± 3.96 ^{a}	6.40 ± 6.01 ^{a}
TAN (mg/l)	0.28 ± 0.23	0.26 ± 0.17	0.24 ± 0.17	0.24 ± 0.15
Nitrite (mg/l)	1.35 ± 1.17	1.69 ± 1.47	1.30 ± 1.03	1.48 ± 1.21
Nitrate (mg/l)	62.00 ± 6.93 ^{b}	42.00 ± 3.46 ^{a}	51.30 ± 6.11 ^{ab}	50.70 ± 9.45 ^{ab}
Phosphate (mg/l)	3.40 ± 1.35	4.63 ± 1.90	2.67 ± 0.68	3.20 ± 0.00

Treatment Control Group (shrimp monoculture); Treatment 1 (TR 1, integrated shrimp and *Ulva lactuca* f. *fasciata* (Delile) Hering, 1846 cultivation at a density of *Ulva* of 1 g/l); Treatment 2 (TR 2, integrated shrimp culture with *Ulva* at 2 g/l); Treatment 3 (TR 3, integrated shrimp farming with *Ulva* at 3 g/l) during the 35 days of the experimental period. Different letters on the same line indicate significant differences ($p \leq 0.05$) between reatments after Tukey's test followed by one-way ANOVA. DO, dissolved oxygen; TSS, total suspended solids; TAN, total ammonia nitrogen.

TABLE II

Type and density (mean ± standard deviation) of microorganisms identified in the biofloc for the treatments for cultures of *Litopenaeus vannamei* (Boone, 1931).

Microorganisms	Phylum	Class (or family)	Genera	Density (mean ± SD) (number/ml)			
				Treatment control	Treatment 1	Treatment 2	Treatment 3
Bacteria				$7\,200\,000 \pm 800\,000^{a}$	$(36 \pm 2) \times 10^{6\ bc}$	$4\,700\,000 \pm 4\,000\,000^{bc}$	$57\,000\,000 \pm 6\,000\,000^{b}$
Phytoplankton	Ochro-phyta	Bacillario-phyceae	*Nitzschia*	6666.67 ± 5773.50^{b}	10.000 ± 10.000^{ab}	$13\,333.33 \pm 5773.50^{a}$	$13\,333.33 \pm 5773.50^{a}$
			Navicula	$0.00^{c} \pm 0.00$	0.00 ± 0.00^{c}	6666.67 ± 5773.50^{a}	3333.33 ± 5773.50^{b}
	Cyano-phyta	Cyanophyceae	Filamentous Cyanophyta *Oscillatoria*	$13\,333.33 \pm 5773.50^{a}$	0.00 ± 0.00^{c}	3333.33 ± 5773.50^{b}	3333.33 ± 5773.50^{b}
			Unicellular non-motile Cyanophyta	$13\,333.33 \pm 5773.50^{a}$	6666.67 ± 5773.50^{b}	6666.67 ± 5773.50^{b}	6666.67 ± 5773.50^{b}
	Chloro-phyta	Chlorophyceae	Unicellular non-motile *Chlorella*	6666.67 ± 5773.50^{b}	$16\,666.67 \pm 15\,275.25^{a}$	$16\,666.67 \pm 15\,275.25^{a}$	$13\,333.33 \pm 5773.50^{ab}$
			Unicellular motile *Chlamydomonas*	6666.67 ± 5773.50^{b}	$10\,000.00 \pm 17\,320.51^{a}$	6666.67 ± 5773.50^{ab}	0.00 ± 0.00^{b}
		Ulvaceae (Fam.)	*Ulva*	$0.00^{d} \pm 0.00$	1.67 ± 1.15^{c}	$13\,333.33 \pm 5773.50^{b}$	$20\,000.00 \pm 10\,000.00^{a}$
Zooplankton	Rotifera	Brachionidae	*Brachionus*	0.67 ± 0.58	1.00 ± 0.00	1.67 ± 0.58	0.67 ± 0.58
	Arthro-poda	Copepoda	Copepod	1.33 ± 0.58	1.00 ± 0.00	0.67 ± 0.58	1.00 ± 0.00
Protozoa	Cilio-phora	Ciliatae	Ciliate protozoon	1.67 ± 1.53^{a}	0.67 ± 0.58^{ab}	0.33 ± 0.58^{bc}	0.67 ± 0.58^{ab}
Nematode	Nema-toda		Nematode	1.00 ± 1.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Treatment Control Group (shrimp monoculture); Treatment 1 (TR 1, integrated shrimp and *Ulva lactuca* f. *fasciata* (Delile) Hering, 1846 cultivation at a density of *Ulva* of 1 g/l); Treatment 2 (TR 2, integrated shrimp culture with *Ulva* at 2 g/l); Treatment 3 (TR 3, integrated shrimp farming with *Ulva* at 3 g/l) during the 35 days of the experimental period. Different letters on the same line indicate significant differences ($p \leq 0.05$) between treatments after Tukey's test followed by one-way ANOVA.

4 Discussion

The microorganisms that may be present in the flocs can be classified into fivetypes, that overlap: (1) the ones that generate flocs; (2) the ones that feed on nitrate: bacteria; (3) microscopic unicellular algae; (4) the ones that feed on algae in the biofloc system; and (5) the saprophytes (organisms that get their nutrients from decaying organic materials). Each of these kinds of microorganisms performs a specific role in the biofloc system and interacts with the others to enable the successful completion of the bioremediation process. The detected floc-forming organisms are derived from algae biomass, which is utilized in the production of activated sludge. The organic matter in the tank provides the organic matter fromwhich sticky Extracellular Polymeric Substances (EPS) are secreted (Medina & Neis, 2007).

According to Sheng et al. (2010), these EPS are known to have a major impacton the physiochemical characteristics of the microbial aggregates, such as struc- ture, surface charge, flocculation, settling qualities, dewatering, and absorptive capacity. During bioflocculation, the floc acts as a bioremediation agent by attach-ing waste detritus to other organisms like zooplankton. Additionally, bacteria within the floc absorb ammonia from the water, primarily from shrimp metabolicwaste, and transform it into microbial protein.

According to Hargreaves (2013), bioflocs are collections of bacteria, algae, protozoa, and other particulate organic matter, including excrements and uneatenfeed, that are kept together by bacteria-secreted mucus that is bound by filamentous algae or retained by electrostatic attraction. At the conclusion of the trial, it was also observed that the waste in the tanks had gathered into small, rounded aggregates. This indicates that the biofloc microorganisms were actively workingto settle down the debris and were acting as a bioremediation agent to neutralize the pollutant in the tank's bottom and improve the water quality.

These tiny, rounded waste aggregates were also collected in the macroalgae cultivation units. It implies that the structures used for macroalgae growing servedas waste skimmers. This finding also clarifies the cause for the decreased amounts of organic settleable solids found in the tanks used for macroalgae growing. Heterotrophic bacteria belong to the saprophytes group, which is the group of organisms that get their nourishment from decomposing organic waste. In contrast to autotrophic species like phytoplankton and macroalgae, heterotrophic bacteria exploited the organic compounds from the organic matter left in the tanks as sources of food and energy.

Protozoa like ciliates and *Paramecium* that were found in the biofloc treatmenttank are

categorized as saprophytic protozoa because they use 40% of the nutrients for the formation of protozoan biomass and absorb organic substances via their cell walls (Lal, 2006). The faecal secretions of shrimp and the wastes of uneaten feed all contained nutrients (ammonia, nitrite, and nitrate) that needed to be neutralized by these saprophytic microbes in order to create the product ammonia.

According to Schramm et al. (1999), the denitrifying bacteria in the bioflocculation process converted the nitrates (NO_3^-) into gaseous nitrogen (N_2), which reduces the toxicity of the water and preserves its quality. In addition to consuming the provided pellet as food, this bacterium will also transform the nitrates in the water into protein that is good for shrimp eating. This is demonstrated by Hargreaves's (2013) research in the biofloc system, which showsthat although some nitrogen is integrated into the bacterial cells that make up themajority of the biofloc, shrimp intake of this microbial protein will have a secondeffect and aid in shrimp growth. Protozoa and zooplankton are categorized as algae grazers. Nematodes, protozoa, ciliates, rotifers, copepods, and other species witha dense composition that were available in the biofloc treatment tank as increasedfood from algae and phytoplankton kinds, were among the organisms detected inthe bioflocculation (Hargreaves, 2013).

By the end of the experiment, it was determined that algae from three distinct classes — Chlorophyceae (green algae), Bacillariophyceae (diatoms), Cyanophy- ceae (blue-green algae), and rarely Dinophyceae (dinoflagellates) — dominated the biofloc culture condition. This result is in line with research by Schrader et al. (2011) and Galvez (2015), which discovered that the cyanobacteria class was the most prevalent group of algae in the biofloc study, followed by Chlorophyta, Heterokontophyta, Euglenophyta, and Dinophyta.

The results of this study demonstrated a negative connection between juvenile*Ulva* and Cyanophyta species populations within the treatment groups. Compara- ble outcomes were also observed for the amount of bacteria, which decreased as *Ulva* densities rose in the treatment groups. The pathogenic bacteria *Vibrio* sp. that can infect shrimp, including *Vibrio alginolyticus* (Miyamoto, Nakamura & Tak- izawa, 1961) Sakazaki, 1968, were among the organisms categorized under the pathogenic group but were not detected. According to a study by Emerenciano etal. (2013), the natural probiotic in the biofloc could protect the shrimp against dam- aging ectoparasites and *Vibrio* sp. both inside and externally. Limiting the numbers of *Vibrio* sp. will also involve competition with the main heterotrophic bacteria and nitrifying bacteria for vital nutrients like nitrogen.

5 Conclusion

One possible technological advancement for a sustainable aquaculture environ- ment is bioflocculation. Furthermore, green macroalgae (*Ulva lactuca* f. *fasciata*)biofloc was found to be an effective and successful bioremediation and biodegra-dation agent for preserving the water quality in an aquaculture system with zero water exchange. In fact, it can provide the shrimp, *Litopenaeus vannamei,* with additional diet from the bioflocculant of a variety of microorganisms identified in the floc.

References

ALAZAIZA, M. Y. D., A. ALBAHNASAWI, Z. AHMAD, M. J. K. BASHIR, T. AL-WAHAIBI, M. S. S. ABUJAZAR, S. S. ABU AMR & D. E. NASSANI, 2022. Potential use of algae for the bioremediation of different types of wastewater and contaminants: production of bioproducts and biofuel for green circular economy. Journal of Environmental Management, **324**: 116415. (ISSN: 0301-4797). DOI:10.1016/j.jenvman.2022.116415.

AMINOT, A. & M. CHAUSSEPIED, 1983. Manuel des analyses chimiques en milieu marin: 1-395. CNEXO, Brest 395p. ISBN: 9782902721108, 2902721102, OCLC Number / Unique Identifier: 11179517.

APHA [AMERICAN PUBLIC HEALTH ASSOCIATION], 1989. Standard methods for the examination of water and waste water (16th ed.). (American Public Health Association, AWWA, WPFC, New York, NY).

BAUMGARTEN, M. D. G. Z., J. M. DE BARROS ROCHA & L. F. H. NIENCHESKI, 1996. Manual de
análises em oceanografia quimica: 1-132. (FURG, Rio Grande).

DAS, S., 2014. Microbial biodegradation and bioremediation: 1-643. (Laboratory of Environmental Microbiology and Ecology (LenME), National Institute of Technology, Rourkela, Odisha, India). DOI:10.1016/C2020-0-01871-2.

DASH, H. R.& S. DAS, 2012. Bioremediation of Mercury and importance of bacterial mergenes. Int. Biodeterior. Biodegrad., **75**: 207-213. DOI:10.1016/j.ibiod.2012.07.023.

EMERENCIANO, M., G. GAXIOLA & C. GERARD, 2013. Biofloc technology (BFT): a review for aquaculture application and animal food industry. In: M. D. MATOVIC (ed.), Biomass now — cultivation and utilization. (Intech Open Science Book Series, London). DOI:10.5772/53902.

GENCER, Ö., 2023a. Survival and moulting rates of larvae of *Callinectes sapidus* Rathbun, 1896 under different environmental circumstances (Brachyura, Portunidae). Crustaceana, **96**(11-12): 1135-1150. DOI:10.1163/15685403-bja10337.

GENCER, Ö., 2023b. The effects of using different stocking volumes on the development of broodstocks of the blue crab, *Callinectes sapidus* Rathbun, 1896 (Brachyura, Portunidae). Crustaceana, **96**(11-12): 1173-1181. DOI:10.1163/15685403-bja10348.

GENCER, Ö. & H. AGUILAR VITORINO, 2023. Effect of diet on growth performance of first crab stage *Callinectes sapidus* Rathbun, 1896 (Brachyura: Portunidae): a comparison of three different regimens. Animals, **13**: 1242. DOI:10.3390/ani13071242.

HARGREAVES, J. A., 2006. Photosynthetic suspended-growth systems in aquaculture. Aquacult. Engin., **34**(3): 344-363. DOI:10.1016/j.aquaeng.2005.08.009.

KARIGAR, C. S.& S. S. RAO, 2011. Role of microbial enzymes in the bioremediation of pollutants: a review. Enzym. Res., **2011**: 805187. DOI:10.4061/2011/805187.

LAL, R., 2006. Encyclopedia of soil science. In: B. S. GRIFFITHS (ed.), Nature. (Scottish Crop Research Institute, Invergowrie). DOI:10.1081/E-ESS-120042737.

MANAN, H., J. H. Z. MOH, N. A. KASAN, S. SURATMAN & M. IKHWANUDDIN, 2017. Identification
of biofloc microscopic composition as the natural bioremediation in zero water exchange of Pacific white shrimp, *Penaeus vannamei*, culture in closed hatchery system. Appl. Water Sci., **7**: 2437-2446. DOI:10.1007/s13201-016-0421-4.

MASASA, M., A. KUSHMARO, E. KRAMARSKY-WINTER, M. SHPIGEL, R. BARKAN, A. GOLBERG,
A. KRIBUS, N. SHASHAR & L. GUTTMAN, 2021. Mono-specific algal diets shape microbial networking in the gut of the sea urchin *Tripneustes gratilla elatensis*. Animal Microbiome, **3**(1): 1-21. DOI:10.1186/s42523-021-00140-1.

MEDINA, M. & U. NEIS, 2007. Symbiotic algal bacterial wastewater treatment: effect of food to microorganism ratio and hydraulic retention time on the process performance. Water Sci. Technol., **55**(11): 165-171. DOI:10.2166/wst.2007.351.

PANIGRAHI, A., M. SUNDARAM, P. RAVICHANDRAN & C. GOPAL, 2014. Microbial soup — eco

based approach for shrimp culture and management. ENVIS Centre Newsletter, **12**: 2. (CIBA, Indian Council of Agricultural Research, New Delhi). ISSN-0974-1550.

SCHRADER, K. K., B. W. GREEN & P. W. PERSCHBACHER, 2011. Development of phytoplankton communities and common off-flavors in a biofloctechnology system used for the culture of channel catfish (*Ictalurus punctatus*). Aquacult. Engin., **45**: 118-126. DOI:10.1016/j.aquaeng. 2011.08.004.

SCHRAMM, A., D. A. DE BEER, S. VAN DEN HEUVEL & S. OTTENGRAF, 1999. Microscale distribution of populations and activities of *Nitrosospira* and *Nitrospira* spp. along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridization and the use of microsensors. Appl. Environ. Microbiol., **65**(8): 3690-3696. DOI:10.1128/AEM.65.8.3690-3696.1999.

SHENG, G., H. YU & X. LI, 2010. Extracellular Polymeric Substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. Biotechnol. Adv., **28**(6): 882-894. DOI:10.1016/j.biotechadv.2010.08.001.

STELTING, S., R. G. BURNS, A. SUNNA, G. VISNOVSKY & C. BUNT, 2010. Immobilization of *Pseudomonas* sp. strain ADP: a stable inoculant for the bioremediation of atrazine. 19th World Congress of Soil Science, Soil Solutions for a Changing World, Brisbane, Australia. DOI:10. 1016/j.clay.2011.12.006.

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

CAPÍTULO 5: Efeito da fertilização orgânica e inorgânica na produção de flocos microbianos no cultivo multitrófico integrado da macroalga *Ulva lactuca* com a tilápia *Oreochromis niloticus* e o camarão *Penaeus vannamei*.

Artigo publicado na *Fishes*.

https://doi.org/10.3390/fishes9060191

Resumo

Diferentes fertilizações para a formação do sistema em bioflocos influenciam a predominância e estabelecimento de diferentes comunidades bacterianas, interferindo na qualidade de água e desempenho dos organismos. Este estudo avalia o crescimento e a absorção de nutrientes da macroalga *Ulva lactuca* quando cultivada em sistema integrado com o camarão *Penaeus vannamei* e a tilápia *Oreochromis niloticus* com duas abordagens de fertilização: orgânica (sistema heterotrófico) e inorgânica (sistema quimioautotrófico). O experimento teve duração de 45 dias e contou com dois tratamentos, cada um com três repetições: quimioautotrófico - utilizando fertilizantes químicos; Heterotrófico - utilizando inóculo proveniente de cultivo de camarões maduros em bioflocos, suplementado com carbono orgânico. Cada tratamento foi composto por três sistemas, cada um contendo um tanque de 4 m^3 para camarão, 0.7 m^3 para tilápia e 0.35 m^3 para macroalgas, com circulação contínua de água entre os tanques e aeração constante. Durante o período experimental foram realizadas as análises da qualidade da água, assim como o desempenho das macroalgas e dos animais. Os dados foram submetidos a análise estatística. Os resultados revelaram um aumento da biomassa de macroalgas e a remoção de nitrato (57%) e fosfato (47%) durante o cultivo, com uma maior taxa de crescimento específico observada no tratamento quimioautotrófico no final do experimento. No entanto, o tratamento heterotrófico apresentou níveis mais elevados de proteína nas macroalgas (18% de matéria seca) e taxas de remoção de fosfato (56%), juntamente com uma melhor manutenção dos compostos nitrogenados, não sendo necessário renovações de água. O desempenho das tilápias variou entre os tratamentos, com um peso final e um ganho de peso mais elevados registados no tratamento heterotrófico. O uso de um inóculo de um cultivo em andamento juntamente com a fertilização orgânica demonstrou a viabilidade do cultivo de macroalgas num sistema integrado com camarões e peixes.

Effect of Organic or Inorganic Fertilization on Microbial Flocs Production in Integrated Cultivation of *Ulva lactuca* with *Oreochromis niloticus* and *Penaeus vannamei*

Andrezza Carvalho [1]*, Hellyjúnyor Brandão [1], Julio C. Zemor [1], Alessandro Pereira Cardozo [1], Felipe N. Vieira [2], Marcelo H. Okamoto [1], Gamze Turan [3] and Luís H. Poersch [1]

[1] Marine Aquaculture Station, Institute of Oceanography, Federal University of Rio Grande—FURG, Rua do Hotel, no.2, Cassino, Rio Grande 96210030, RS, Brazil; hellyjunyor@gmail.com (H.B.); juliozemor@hotmail.com (J.C.Z.); ocalessandro@hotmail.com (A.P.C.); mar.okamoto@gmail.com (M.H.O.); lpoersch@gmail.com (L.H.P.)
[2] Laboratório de Camarões Marinhos, Departamento de Aquicultura, Centro de Ciências Agrárias, Univesidade Federal de Santa Catarina, Rua dos Coroas 503, CEP 88061-600, Barra da Lagoa, Florianópolis, Santa Catarina, Brazil; felipe.vieira@ufsc.br (F.N.V)
[3] Aquaculture Department, Fisheries Faculty, Ege University, Izmir 35100, Turkey; gamzeturan2000@gmail.com (G.T).
* Correspondence: andrezzachagas@hotmail.com

Abstract: Different fertilization regimes in biofloc systems influence the predominance of distinct bacterial populations, impacting water quality and organism performance. This study evaluates the growth and nutrient absorption of the macroalgae *Ulva lactuca* when cultivated in an integrated system with *Penaeus vannamei* and *Oreochromis niloticus* in chemoautotrophic and heterotrophic systems. The experiment lasted 45 days and comprised two treatments, each with three replicates: chemoautotrophic—utilizing chemical fertilizers; heterotrophic—employing inoculum from mature biofloc shrimp cultivation, supplemented with organic fertilizers. Each treatment consisted of three systems, each containing a 4 m^3 tank for shrimp, 0.7 m^3 for tilapia, and 0.35 m^3 for macroalgae, with continuous water circulation between tanks and constant aeration. Water quality analyses were carried out during the experiment, as were the performances of the macroalgae and animals. The data were subjected to a statistical analysis. Results revealed an increase in macroalgae biomass and the removal of nitrate (57%) and phosphate (47%) during cultivation, with a higher specific growth rate observed in the chemoautotrophic treatment. Nonetheless, the heterotrophic treatment exhibited higher levels of protein in the macroalgae (18% dry matter) and phosphate removal rates (56%), along with superior maintenance of water quality parameters. Tilapia performance varied across treatments, with a higher final weight and weight gain recorded in the heterotrophic treatment. The recycling of water from an ongoing biofloc cultivation with organic fertilization demonstrated viability for macroalgae cultivation within an integrated system involving shrimp and fish.

Keywords: nitrate; phosphate; growth; biocompounds; water renewal

Key Contribution: The use of macroalgae in an integrated system with different fertilizations showed a high rate of nitrate and phosphate removal. The use of partial harvests promoted high biomass production with a high protein content.

1. Introduction

Numerous recent studies have explored marine macroalgae as a source of human food, bioactive compounds, and supplements for marine organisms [1,2]. According to Chopin [3], macroalgae cultivation can be deemed sustainable due to their minimal need for feed, reduced land footprint, and bioremediation capabilities. The genus Ulva exhibits a global distribution, with the morphology and composition adapting to the environmental variables of the production site, rendering it a feasible and intriguing option for cultivation [4]. The cultivation of macroalgae integrated with other aquatic organisms has gained traction in aquaculture, referred to as Integrated Multitrophic Aquaculture (IMTA) [5]. Resende et al. [6] demonstrated the feasibility of incorporating macroalgae for nutrient absorption and biomass production in open cultivation systems with Sea bream *Sparus aurata* and European sea bass *Dicentrarchus labrax*. In addition to macroalgae serving as inorganic consumers, the IMTA system also includes organic consumers to consume the solids produced in the system. The tilapia *Oreochromis niloticus,* known for its ease of handling and feeding habits, has been utilized in integrated systems, showing positive results in solid filtration [7,8]. Due to its robustness, tilapia can also be produced in brackish water without negative effects on its zootechnical performance [9]. Both species could bring benefits when integrated into the cultivation of a main species, as in the farming of the Pacific white shrimp *Penaeus vannamei,* the most cultivated shrimp in the world, especially because it is an euryhaline [10], easy to manage and adapt [11], and holds significant economic interest [12], which has been employed as a primary species in integrated systems.

In conjunction with integrated systems, the utilization of biofloc technology has intensified production by improving the utilization of accumulated waste in cultivation. Studies by Brito et al. [13], Legarda et al. [14], and Morais et al. [15] have reported positive results from the production of shrimp, tilapia, and macroalgae in an integrated biofloc system. In general, biofloc technology offers enhanced biosecurity with reduced water exchange, facilitates water quality control through microbial activity, and serves as a supplementary food source for cultivated species [16]. Various fertilization approaches promote the growth and dominance of distinct bacterial groups within the system, including heterotrophic bacteria, chemoautotrophic bacteria, or a combination of both in mixed or mature systems [17]. The bacterial groups play a crucial role in nitrogen consumption and oxidation within the system, contributing to the maintenance of water quality for the organisms. Heterotrophic bacteria are favored by daily carbon source fertilization, typically at a ratio of 15 g of carbohydrate per gram of available nitrogen in the system [18]. Ammonia consumption by heterotrophic bacteria leads to bacterial

biomass production, increasing the total suspended solids concentration in the water, which should ideally be maintained within the range of 100 to 350 mg L^{-1}, as suggested by Gaona et al. [19] to avoid adverse effects on animal performance.

Another significant bacterial group comprises chemoautotrophs, which, according to Ebeling et al. [18], oxidize nitrogen within the system, converting ammonia to nitrite and eventually to nitrate, a less toxic final product for organisms. Chemical fertilization is utilized for system establishment, requiring approximately 30 to 45 days of chemical fertilization prior to cultivation initiation to maintain low concentrations of ammonia and nitrite [17]. Unlike heterotrophic bacteria, chemoautotrophic bacteria generate fewer solids in the system and consume less oxygen. However, they utilize more inorganic carbon, requiring alkalinity adjustments to maintain levels above 150 mg of $CaCO_3$ L^{-1} [20]. Nitrate accumulates as the final nitrogen product in this system's oxidation process, posing toxicity risks to cultivated organisms at high concentrations [21], and, when discharged untreated, can lead to diseases such as methemoglobinemia in humans [22]. Another viable option is to utilize a biofloc inoculum from an ongoing cultivation, providing enhanced stability in nitrogen control and greater sustainability through water reuse [17]. This approach results in a mixed system containing both heterotrophic and chemoautotrophic bacteria, aimed at regulating water quality by promoting bacterial biomass production and nitrification [23]. Organic fertilization is typically employed at the onset of cultivation to expedite the stabilization of ammonia until nitrifying bacteria become established [24].

The biofloc system is complex and subject to variations based on the fertilization strategy utilized, which can impact water quality, production costs, and animal performance. Brandão et al. [23] reported greater shrimp growth in mixed systems compared to heterotrophic systems. Conversely, tilapia performance was negatively impacted in chemoautotrophic systems due to low organic matter loads [8]. However, limited information exists regarding macroalgae performance within these systems. High concentrations of solids produced by heterotrophic bacteria may accumulate on macroalgae, hindering photosynthesis and affecting their performance [25]. Additionally, elevated nutrient concentrations present in the chemoautotrophic system can induce stress in macroalgae and trigger reproductive events [26]. Choosing the right cultivation system can provide better growing conditions and biomass production for the macroalgae. In addition to growth and nutrient absorption, the specific physical and chemical variables inherent to each cultivation system also influence the nutritional composition of macroalgae [27]. The production of biomass with enhanced nutritional value can offer economic advantages for the system through the generation of

valuable by-products. For instance, utilizing the biomass of macroalgae cultivated in the integrated system as a food source for shrimp and fish could prove beneficial for aquaculture [2]. Therefore, the objective of this study was to evaluate the growth performance, nutrient absorption, and bioactive compounds of the macroalga *Ulva lactuca* when cultivated in an integrated system with Pacific white shrimp *Penaeus vannamei* and tilapia *Oreochromis niloticus* using two biofloc fertilization strategies: a chemoautotrophic system and a heterotrophic system.

2. Materials and Methods

2.1. Location and Origin of the Animals

The experiment was conducted in an agricultural greenhouse situated at the Marine Station of Aquaculture (EMA), Institute of Oceanography, Federal University of Rio Grande (IO-FURG), located on Cassino Beach, Rio Grande, Rio Grande do Sul. The greenhouse was devoid of shading, and aeration within the tanks was provided by a blower through continuous air injection via micro-perforated hoses (aerotubes).

2.2. Animal Materials

The shrimp originated from a biofloc cultivation system within a grow-out greenhouse at EMA, with an initial weight of 7.13 ± 0.18 g. Tilapia were sourced from a recirculation system grow-out cultivation, starting with an initial weight of 412.33 ± 72.58 g. The macroalgae were cultivated in a greenhouse in a 1 m^3 tank containing water with 35.1 ± 2.74 mg L^{-1} of nitrate and 2.24 ± 1.2 mg L^{-1} of phosphate.

2.3. Experimental Design

The experiment, which spanned 45 days, was conducted on six experimental production systems. Each system comprised a 4 m^3 tank for shrimp (350 shrimp m^{-2}), a 0.7 m^3 tank for tilapia (10 fish per m^3), and a 0.35 m^3 tank for macroalgae cultivation (0.1 g m^3 of the useful volume of the entire system). A submerged pump circulated the system, transferring water into the macroalgae tank, which then flowed by gravity into the shrimp tank before returning to the tilapia tank (Figure 1). The macroalgae were contained within the tank using a circular structure with a diameter of 0.60 m positioned near the surface, constructed from polyethylene netting with 5 mm mesh openings.

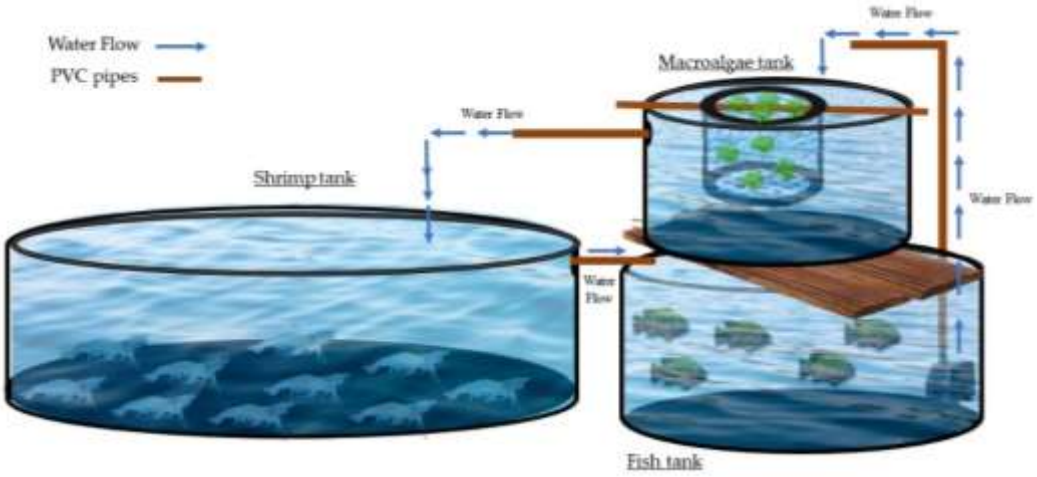


Figure 1. Design of the experimental system, consisting of a shrimp tank, a fish tank, and a macroalgae tank, with water recirculating between them.

2.4. Treatments

Two treatments were employed, each with three replicates: chemoautotrophic—a system utilizing chemical inorganic fertilization; heterotrophic—a system supplemented with organic fertilizer. Inoculum preparation for the chemoautotrophic system involved maintaining water with a salinity of 20 ppt in an 8 m^3 tank. Over 35 days, daily fertilization with sodium nitrite (Neon Comercial, São Paulo, SP, Brazil) and ammonium chloride (Neon Comercial, São Paulo, SP, Brazil) was conducted to achieve a concentration of 1 mg L^{-1} for each compound in the water. To establish bacterial populations in the system, six pillow-like structures containing biological media were placed in the main tank and then distributed among the replicates. The tank was continuously aerated and devoid of light, and no heaters were utilized to simulate greenhouse cultivation conditions.

Once the ammonia and nitrite concentrations stabilized and were converted into nitrate, the experiment started. Chemoautotrophic treatment replicates were prepared by blending 40% inoculum with water of salinity 20, up to a useful volume of 5 m^3. At the onset of the experiment, the water parameters were as follows: a temperature of 26.0 ± 0.4 °C, dissolved oxygen of 7.2 ± 0.5 mg L^{-1}, pH of 8.18 ± 0.3, alkalinity of 170.0 ± 2. 0 mg $CaCO_3$ L^{-1}, total ammonia nitrogen of 0.02 ± 0.02 mg L^{-1}, nitrite of 1.5 ± 0.2 mg L^{-1}, nitrate of 64.0 ± 1.7 mg L^{-1}, phosphate of 1.2 ± 0.4 mg L^{-1}, and total suspended solids of 160.0 ± 5.8 mg L^{-1}.

The tanks designated for the heterotrophic treatment were prepared with 40% mature biofloc inoculum and seawater at a salinity of 20 ppt, up to a useful volume of 5 m^3. The biofloc inoculum was sourced from a shrimp cultivation system with a useful volume of 237 m^3, a density of 184 shrimp m^{-2}, and an average weight of 7.1 ± 1.2 g, cultivated for 68 days. The initial water quality parameters in the shrimp production tank before the experiment were as

follows: a temperature of 25.6 °C, dissolved oxygen of 5.4 mg L^{-1}, pH of 7.47, alkalinity of 215.0 mg $CaCO_3$ L^{-1}, total ammoniacal nitrogen of 0.20 mg L^{-1}, nitrite of 0.20 mg L^{-1}, nitrate of 147.0 mg L^{-1}, phosphate of 4.0 mg L^{-1}, and total suspended solids of 700.00 mg L^{-1}.

2.5. Chemical and Physical Water Parameters

Water quality analyses were conducted on samples collected from the shrimp tanks, considering water homogenization due to the circulation within the systems. Temperature and dissolved oxygen were measured twice daily using a Pro-20 model (YSI Inc., Yellow Springs, OH, USA), and pH was measured daily with a bench pH meter (Seven2Go S7 Basic, Mettler Toledo, São Paulo, Brazil). Salinity was assessed twice a week using a Pro-20 model (YSI Inc., OH, USA), and, if necessary, fresh water was added to maintain salinity at 20. Alkalinity (mg $CaCO_3$ L^{-1}) was monitored twice a week following the APHA methodology [28], with calcium hydroxide added to both treatments when alkalinity fell below 150 mg $CaCO_3$ L^{-1}, as per Furtado et al. [20] recommendation.

Total ammoniacal nitrogen (mg L^{-1}) and nitrite (mg L^{-1}) were initially measured daily and then twice a week after nutrient stabilization, according to UNESCO methodology [29]. Nitrate (mg L^{-1}) and phosphate (mg L^{-1}) were measured twice a week, according to the method proposed by Aminot and Chaussepied [30]. Total suspended solids (mg L^{-1}–TSS) and settleable solids (ml L^{-1}–SS) were quantified twice a week, using the methodology described by Baumgarten et al. [31] and APHA [28], respectively. For the heterotrophic system, organic carbon (molasses) was added when the total ammoniacal nitrogen exceeded 1 mg L^{-1} to promote nitrogen uptake through heterotrophic bacteria growth, as proposed by Wasielesky et al. [32]. In the chemoautotrophic system, inorganic carbon (calcium hydroxide) was added when ammonia and nitrite concentrations exceeded 1 mg L^{-1} and 5 mg L^{-1}, respectively. In this treatment, alkalinity was maintained at 300 mg $CaCO_3$ L^{-1} for optimal nitrifying bacteria performance, as recommended by Furtado [20].

2.6. Macroalgae Growth and Biochemical Analysis

Macroalgae biomass was weighed every 15 days. Before the weighing process, the macroalgae were gently shaken inside the holding structure to eliminate any solids adhering to the surface. Subsequently, the circular holding structure was removed from the tank and set aside to air-dry for 10 min to remove excess water before weighing. The initial weight of macroalgae in each replicate was 502.7 ± 0.5 g. After each weighing, the extra macroalgae biomass was removed, ensuring that the initial weight of the macroalgae was maintained. The following formula was used to calculate the macroalgae specific growth rate (SGR) [33]:

$$\text{SGR (\% d}^{-1}\text{): } 100 \times [\ln(\text{final weight (g)}/\text{initial weight (g)})/(\text{final time} - \text{initial time})] \quad (1)$$

The nutrient absorption efficiency (NRR) of the macroalgae was calculated using the following formula [33]:

$$\text{NRR (\%): } 100 \times [(\text{nutrient concentration at initial time (mg L}^{-1}) - \text{nutrient concentration at final time (mg L}^{-1})) / \text{nutrient concentration at initial time (mg L}^{-1})] \quad (2)$$

At the conclusion of the experiment, random samples of macroalgae were collected from each replicate. Wet samples were weighed and then subjected to drying in an oven at 60 °C for 24 h after obtaining the dry weight. To determine the concentration of chlorophyll-a, chlorophyll-b, and carotenoids, 500 mg of the dry sample was macerated and then incubated in 5 mL of methanol in the dark for 60 min at 4 °C. After that, the solution was centrifuged (12,000 *g* × 10 min), and the supernatant was used to quantify the pigments. The wavelengths of 664 and 647 ηm were used to calculate chlorophyll a (Chla = 11.75 × A664 − 2.35 × A647), chlorophyll b (Chlb = 18.61 × A647 − 3.91 × A664), and carotenoids (Car = (1000 × A470 − 2.27 × Chla − 81.4 Chlb)/227), according to the methodology of Lichtenthaler & Wellburn [34].

Protein quantification was conducted using the Bradford method. An extract was obtained from the dried macroalgae, following the protocol of Barbarino & Lourenço [35], with the addition of 1 mL of sodium hydroxide and centrifugation. The extract and TCA (25%) were added in a ratio of 2.5:1 (*v*/*v*) to precipitate the protein and kept in an ice-cold bath for 30 min. The solution was then centrifuged and washed with dilutions of TCA (10 and 5%), removing the supernatant, until the protein pellet was formed. To the pellet suspension, 0.5 mL of sodium hydroxide (0.1 N) was added, and 20 µL of the solution was combined with 1 mL of the total protein kit for the final analysis procedure.

2.7. Feed Management and Performance of the Animals

Shrimp were fed twice daily with 1.6 mm feed (Guabi aqua QS 1–2 mm, Guabi Nutrition and Animal Health S.A., Campinas, São Paulo, Brazil), and weekly biometrics were conducted to adjust feed quantities following the method proposed by Jory et al. [36]. The tilapia were fed twice a day with commercial feed containing 40% protein (Guabi Tech, Guabi Nutrition and Animal Health S.A., Campinas, São Paulo, Brazil) at a rate of 1% of the biomass to encourage biofloc consumption. To evaluate shrimp performance, measurements were taken at the beginning, middle, and end of the experiment. Fish biometrics were conducted at the beginning

and end of the experiment. The animals' performance was assessed using the following formulas:

—Final average weight (g): final biomass of live animals (g)/total number of animals;

—Weekly weight gain (g week^{-1}): weight gain (g)/number of weeks;

—Final biomass (g): ∑ final weight of all live animals (g);

—Feed conversion rate (FCR) = ∑ feed offered (g)/(biomass gain (g));

—Survival (%) = (final number of animals/initial number of animals) × 100;

—Yield (kg m^{-3}): (final biomass (kg)/tank volume (m^3);

—Weight gain rate (%) = 100 × [(final mean weight − initial mean weight)/initial mean weight].

2.8. Statistical Analysis

The data mean (±standard deviation) values are presented in Tables 1 – 3. Data normality and homoscedasticity were assessed using the Shapiro–Wilk and Levene tests, respectively. Upon meeting these assumptions, a Student's *t*-test was employed to compare treatment differences. In cases where the assumptions of the Student's *t*-test were not met, the non-parametric Kruskal–Wallis test was utilized. Additionally, a one-way ANOVA followed by a Tukey post-hoc test was conducted to evaluate nitrate and phosphate concentrations over time in each treatment. A significance level of 5% ($p \leq 0.05$) was applied to all analyses. The tests were carried out using the PAST 4.03 2020 software [37].

3. Results

3.1. Physical and Chemical Parameters

During the 45-day trial period, there were significant differences ($p < 0.05$) observed in pH, alkalinity, and calcium hydroxide consumption between the treatments. The chemoautotrophic system exhibited the highest values, along with higher consumption of calcium hydroxide (Table 1).

Regarding nutrient levels, the chemoautotrophic system demostrated higher concentrations of ammonia and nitrite, reaching maximums of 3.1 and 20.0 mg L^{-1}, respectively. Conversely, the heterotrophic system exhibited higher concentrations of total suspended solids and settleable solids (Table 1). Significant nitrate and phosphate removal ($p < 0.05$) was observed in both treatments, although the heterotrophic treatment displayed a higher phosphate removal rate compared to the chemoautotrophic system.

Table 1. Water quality parameters (mean ± standard deviation) (maximum–minimum) of chemoautotrophic and heterotrophic biofloc systems during the 45 days of integrated cultivation of *Ulva lactuca* with *Oreochromis niloticus* and *Penaeus vannamei*.

	Treatments	
Parameters	**Chemoautotrophic**	**Heterotrophic**
Temperature (°C)	22.82 ± 0.30 (27.4–15.5)	22.67 ± 0.38 (27.3–14.9)
DO (mg L^{-1})	7.06 ± 0.04 (9.9–5.3)	6.96 ± 0.06 (9.7–5.2)
pH	8.15 ± 0.02 [a] (8.9–7.7)	7.89 ± 0.04 [b] (8.1–7.5)
Salinity (g L^{-1})	20.20 ± 0.26 (22.1–19.1)	21.83 ± 0.76 (23.3–20.3)
Alkalinity (mg $CaCO_3$ L^{-1})	280.00 ± 8.19 [a] (365.0–155.0)	189.58 ± 7.02 [b] (230.0–150.0)
TAN (mg L^{-1})	0.95 ± 0.07 [b] (3.1–0.0)	0.18 ± 0.08 [a] (1.9–0.0)
N—Nitrite (mg L^{-1})	7.96 ± 1.16 [b] (20.0–0.0)	0.86 ± 0.60 [a] (5.2–0.0)
N—Nitrate (mg L^{-1})	26.41 ± 2.72 (68.0–10.0)	26.04 ± 4.03 (75.0–15.0)
P—Phosphate (mg L^{-1})	1.04 ± 0.25 (2.0–0.3)	1.19 ± 0.21 (2.2–0.4)
SS (ml L^{-1})	0.39 ± 0.21 [a] (3.0–0.0)	8.44 ± 2.91 [b] (15.0–3.0)
TSS (mg L^{-1})	189.22 ± 26.02 [a] (270.0–70.0)	335.38 ± 47.92 [b] (452.5–175.0)
Calcium hydroxide (g L^{-1}) [#]	0.29 ± 0.02 [b] (0.32–0.28)	0.08 ± 0.03 [a] (0.10–0.04)
Water exchange (m^{-3}) [&]	2.0 ± 2.0 [a]	0.0 ± 0.0 [a]
	Removal rate	
Nitrate (%)	56.47 ± 4.93	57.00 ± 7.00
Phosphate (%)	47.75 ± 4.75 [b]	56.14 ± 1.14 [a]

DO (dissolved oxygen); TAN (total ammonium nitrogen); SS (settleable solids); TSS (total suspended solids). [#] Use of calcium hydroxide during cultivation. [&] Volume of water used for renovations. Different letters in the same line represent significant differences ($p \leq 0.05$) between treatments after Student's *t*-test.

Over the weeks of cultivation, there was a reduction in the concentration of nitrate and phosphate (Figures 2 and 3). The highest nitrate concentrations were observed at the beginning of cultivation, with a decrease from day 14 onward in both treatments (Figure 2). Similarly, phosphate concentrations were higher during the initial week, after which they stabilized in the heterotrophic treatment. In contrast, the chemoautotrophic treatment exhibited a decrease in phosphate concentration until the first week, followed by stabilization and a subsequent increase in the final week (Figure 3).

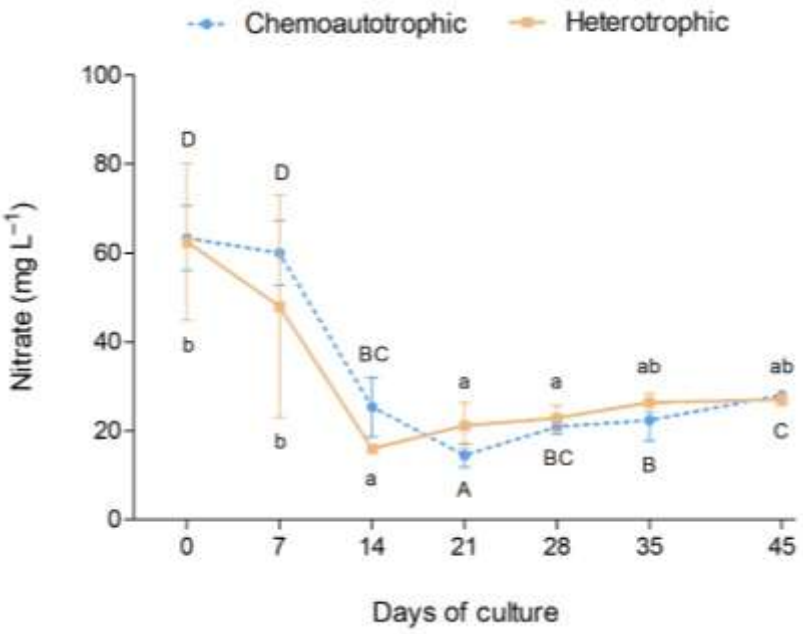


Figure 2. Average weekly nitrate concentrations (mg L^{-1}) during the experimental period in the chemoautotrophic (chemical fertilization prior to stocking) and heterotrophic (use of an inoculum from an ongoing biofloc shrimp cultivation). treatments in an integrated cultivation of *Ulva lactuca* with *Oreochromis niloticus* and *Penaeus vannamei*. Capital letters show differences between the chemoautotrophic treatments over time. Lowercase letters show statistical differences over time in the mature treatment.

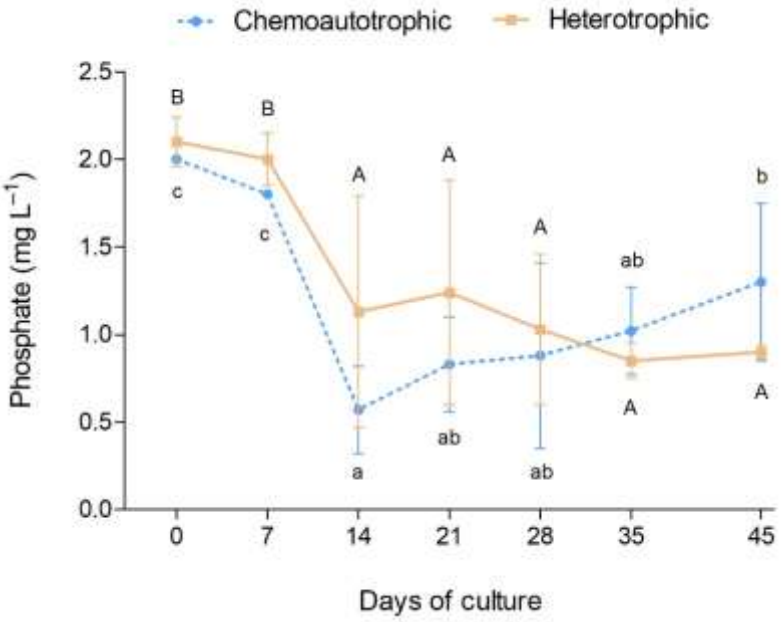


Figure 3. Weekly average phosphate concentrations (mg L^{-1}) during the experimental period in the chemoautotrophic (chemical fertilization prior to stocking) and heterotrophic (use of an inoculum from an ongoing biofloc shrimp cultivation) treatments in an integrated cultivation of *Ulva lactuca* with *Oreochromis niloticus* and *Penaeus vannamei*. Capital letters show differences between the chemoautotrophic treatments over time. Lowercase letters show statistical differences over time in the mature treatment.

Total suspended solids exhibited significant differences ($p < 0.05$) between treatments during most of the experimental weeks. High concentrations of solids reaching 452 mg L^{-1} were observed in the heterotrophic treatment, in contrast to maximum concentrations of 270 mg L^{-1} in the chemoautotrophic treatment (Figure 4).

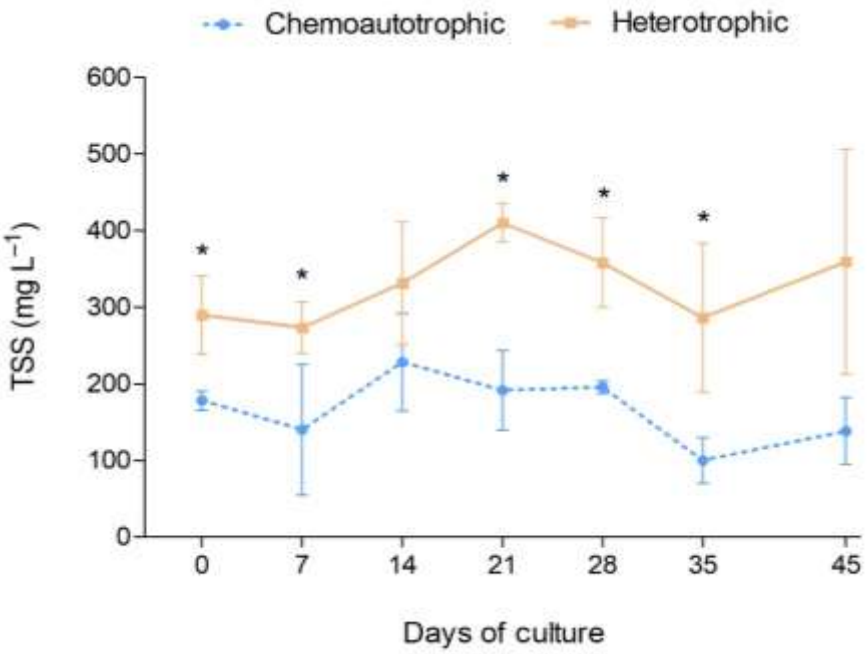


Figure 4. Average weekly concentrations of total suspended solids (mg L^{-1}) during the experimental period in the chemoautotrophic (chemical fertilization prior to stocking) and heterotrophic (use of an inoculum from an ongoing biofloc shrimp cultivation) treatments in an integrated cultivation of *Ulva lactuca* with *Oreochromis niloticus* and *Penaeus vannamei*. An asterisk (*) means a statistical difference on the same day between treatments.

3.2. Macroalgae Growth and Biochemical Analysis

There was an increase in macroalgae biomass in both treatments, with higher concentrations of protein in the macroalgae tissue in the heterotrophic treatment ($p < 0.05$). However, no significant differences ($p \geq 0.05$) were found in biomass gain, chlorophyll-a, chlorophyll-b, or carotenoids between the treatments (Table 2).

Table 2. Performance and biochemistry of the macroalgae (mean ± standard deviation) in the chemoautotrophic and heterotrophic treatments at the end of 45 days of an integrated culture of *Ulva lactuca* with *Oreochromis niloticus* and *Penaeus vannamei*.

	Treatments	
	Chemoautotrophic	**Heterotrophic**
Initial mean weight (g–FW)	502.76 ± 0.65	502.64 ± 0.44
Biomass gain (g–FW)	964.63 ± 290.57	708.11 ± 141.70

Protein (%)	15.13 ± 0.56 [b]	18.49 ± 0.56 [a]
Chlorophyll-*a* (mg g^{-1})	2.20 ± 0.02	2.18 ± 0.06
Chlorophyll-*b* (mg g^{-1})	3.28 ± 0.02	3.26 ± 0.08
Carotenoids (mg g^{-1})	0.06 ± 0.00	0.04 ± 0.02

Different letters in the same line represent significant differences ($p \leq 0.05$) between treatments after Student's *t*-test.

The specific growth rate indicates that the growth of macroalgae in the chemoautotrophic treatment remained consistent throughout the entire experiment, with no significant difference ($p \geq 0.05$) observed between weighings. In contrast, for the heterotrophic treatment, the highest growth rate was recorded on day 21, followed by a subsequent decrease in growth rate. Notably, a significant difference in growth rate was only detected in the last weighing among the treatments (Figure 5).

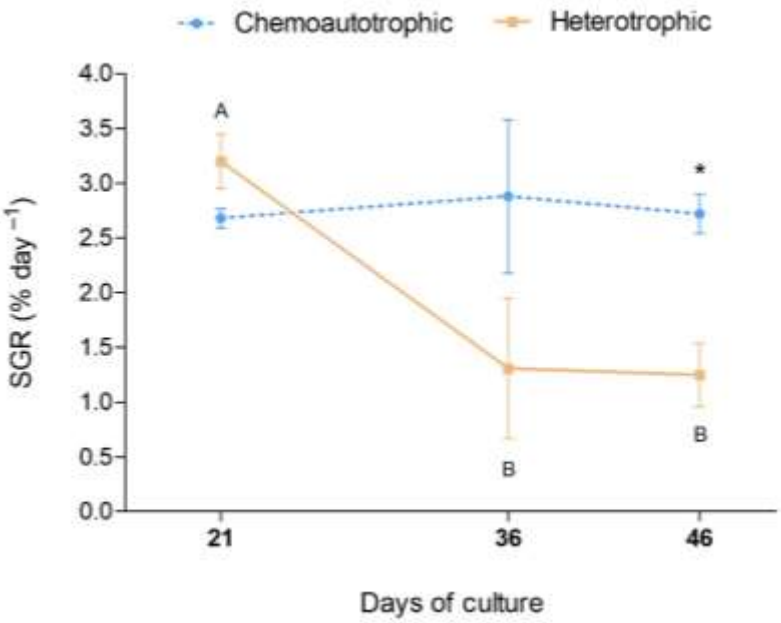


Figure 5. Macroalgae specific growth rate (% day^{-1}) in the chemoautotrophic (chemical fertilization prior to stocking) and heterotrophic (use of an inoculum from an ongoing biofloc shrimp cultivation) treatments in an integrated cultivation of *Ulva lactuca* with *Oreochromis niloticus* and *Penaeus vannamei*. An asterisk (*) means a statistical difference on the same day between treatments. Lowercase letters mean differences in the same treatment between sampling days.

3.3. Performance of the Animals

Shrimp performance remained unaffected by the different biofloc strategies, with no discernible differences observed between treatments. However, the performance of the fish exhibited a significant difference ($p < 0.05$) between treatments, with a higher final weight,

weight gain, and weight gain rate recorded in the heterotrophic treatment compared to the chemoautotrophic treatment (Table 3).

Table 3. Animal performance (mean ± standard deviation) in the chemoautotrophic (chemical fertilization prior to stocking) and heterotrophic (use of an inoculum from an ongoing biofloc shrimp cultivation) treatments at the end of 45 days of an integrated cultivation of *Ulva lactuca* with *Oreochromis niloticus* and *Penaeus vannamei*.

	Treatments	
	Chemoautotrophic	**Heterotrophic**
Shrimp		
Final mean weight (g)	9.60 ± 0.60	10.75 ± 0.49
WWG (g week^{-1}) [#]	0.41 ± 0.10	0.60 ± 0.08
Final biomass (kg)	10.02 ± 0.43	10.79 ± 0.60
FCR [##]	2.16 ± 0.38	2.36 ± 0.24
Survival (%)	84.71 ± 4.50	84.08 ± 5.22
Fish		
Final mean weight (g)	447.55 ± 1.05 [b]	456.25 ± 3.25 [a]
WWG (g week^{-1}) [#]	5.93 ± 0.18 [b]	7.38 ± 0.55 [a]
Final biomass (kg)	2.89 ± 0.54	2.98 ± 0.25
FCR [##]	1.03 ± 0.10	1.18 ± 0.02
Survival (%)	90.48 ± 16.50	95.24 ± 8.25
WGR (%) [###]	8.63 ± 0.25 [b]	10.74 ± 0.79 [a]

[#] WGW (weekly weight gain); [##] FCR (food conversion rate); [###] WGR (weight gain rate). Lowercase letters mean differences between treatments.

4. Discussion

It is widely acknowledged that the utilization of biofloc technology, compared to conventional cultivation methods, leads to reduced water consumption and enhanced control over water quality parameters [16]. The various fertilization strategies employed in this experiment resulted in differences in water quality maintenance, organism performance, water usage, and inputs. The chemoautotrophic system utilizes more inputs, such as sodium hydroxide, compared to the heterotrophic system. This variance is necessary to maintain optimal alkalinity values. In the chemoautotrophic system, the optimal functioning of nitrifying bacteria occurs at values maintained at around 300 mg $CaCO_3$ L^{-1}, consequently resulting in higher pH values [20]. As a result, there is a more frequent application of sodium hydroxide to correct these values and stimulate the growth of nitrifying bacteria with higher alkalinity.

Furthermore, differences in nutrient concentrations were observed between the adopted systems, which play a crucial role in macroalgae development. According to Messyasz et al. [38], most marine *Ulva* species thrive in environments with high concentrations of ammonia or nitrate. Ammonia, originating from waste feed and animal excretion, is the primary nitrogenous compound formed in the system and can be lethal to cultivated organisms at low levels [39]. In both systems in this study, an initial increase in ammonia concentration was observed due to animal stocking. Wasielesky et al. [32] suggest that the use of organic carbon fertilization could promote the growth of heterotrophic bacteria in the system, which consume produced ammonia and generate bacterial biomass. For the chemoautotrophic system, only inorganic fertilization with calcium hydroxide was employed to encourage the growth of nitrifying bacteria with higher alkalinity [20]. However, the slow establishment of nitrifying bacteria resulted in maximum values of 3.1 mg L^{-1} of ammonia in the system, which were higher than those found in the heterotrophic system. Nevertheless, according to Lin & Chen [39], the values obtained in our study were not toxic to the organisms.

The produced ammonia is oxidized into nitrite by ammonium-oxidizing bacteria and subsequently into nitrate by nitrite-oxidizing bacteria. However, the observed increase in nitrite levels in the chemoautotrophic treatment suggests that the nitrite-oxidizing bacteria were not fully established in the system to facilitate this transformation. Despite the use of artificial substrate in this experiment, it is likely that the bacterial population was insufficient to oxidize the nitrite produced following the stocking of shrimp. The use of artificial substrate in the system is necessary for bacterial adherence and to increase their numbers [40]. According to Lin & Chen [41], the safe level for nitrite at a salinity of 25 is 15.2 mg L^{-1}, and concentrations exceeding this limit can be lethal to shrimp. Consequently, in our experiment, we carried out partial water exchange, and a reduction in shrimp and fish feeding was necessary to control nitrite levels in the system, resulting in higher water usage than in the heterotrophic system and the dilution of nutrients.

In biofloc systems, elevated concentrations of nitrate and phosphate are common in long-term production due to low water exchange rates and high animal densities, providing an advantageous environment for macroalgae development. Carneiro [42] noted that when macroalgae inhabit eutrophicated environments, they tend to absorb significant nutrient concentrations initially for storage, serving as a precautionary measure in case of sudden nutrient depletion. Additionally, Hanisak et al. [43] suggested that a constant high nitrogen availability in the environment does not necessarily result in increased removal, as macroalgae nitrogen absorption capacity saturates quickly at high concentrations. This phenomenon may

have occurred in both treatments in our study, resulting in a substantial reduction in nitrate and phosphate concentrations at the onset of cultivation. Following the second week, nutrient stabilization occurred. It is documented that 57% of nitrogen is lost from the water daily, with an increase over time [44], suggesting that the stabilization of these nutrients in the experiment may be attributed to the continuous absorption carried out by the macroalgae. Studies such as Massocato et al. [45] have demonstrated that 85% of the nitrate from a fish cultivation was absorbed within the first five days of algae cultivation.

Phosphorus is also another compound accumulated in the system and produced daily through waste feed [44]. It is an important element in photosynthesis and the transfer of energy from macroalgae [46], which shows the advantage of integrating macroalgae into closed systems. Phosphorus absorption is connected with nitrogen absorption, with an ideal ratio of 30:1 (nitrogen:phosphorus), so that phosphorus or nitrogen are not limiting [47]. The higher removal rate found in the heterotrophic treatment may be linked to the pH values. According to Rathod et al. [48], higher phosphate absorption occurs at pH levels below neutrality. The maintenance of high alkalinity and pH in the chemoautotrophic treatment may have negatively impacted phosphate absorption.

The utilization of macroalgae as a biofilter has advanced due to their excellent performance in nutrient absorption, ease of management, and high biomass production [49]. Alencar et al. [50] demonstrated that the macroalgae *Ulva lactuca* absorbed 94% of the ammonia concentration in an integrated cultivation with shrimp. Conversely, the impact of the organic load generated in macroalgae cultivation remains poorly understood. Due to the intensive production of bacterial biomass, the heterotrophic system in this study exhibited higher concentrations of total suspended solids and settleable solids. In contrast, the chemoautotrophic system, with its use of inorganic fertilizers and water exchange, exhibited a lower organic load, with a maximum of 270 mg L^{-1}. Similar outcomes were reported by Ferreira et al. [17] in their study of the two biofloc systems. Despite the absence of a significant difference in macroalgae biomass gain between the treatments, a higher growth rate was observed toward the end of cultivation in the chemoautotrophic treatment, potentially attributable to the lower solids content in the system compared to the heterotrophic system. The accumulation of microbial biomass and waste in the heterotrophic system intensified toward the end of cultivation, likely directly affecting macroalgae growth. Carvalho et al. [51] demonstrated that the presence of macroalgae in the heterotrophic system led to solid deposition due to the formation of a physical barrier, reducing light exposure for the macroalgae and consequently impacting their performance.

Despite the lower concentration of solids in the chemoautotrophic system, they still accumulated on the surface of the macroalgae, representing one of the challenges of biofloc systems. Studies like Resende et al. [6] reported significantly higher growth rates, with a maximum growth rate of 15.33 ± 2.87% day^{-1} when macroalgae were cultivated freely in tanks with fish farm effluent, characterized by minimal solids concentrations. The results found in our experiment are in agreement with studies by Martins et al. [52], who observed a growth rate of 3.0 ± 0.6% day^{-1} with the macroalga *Ulva ohnoi* in a biofloc system. Studies with red algae in biofloc have also been carried out, showing a maximum growth rate of 1.19 ± 0.04% day^{-1} [53], similar to those observed in our heterotrophic treatment results in the last weeks of cultivation. However, unlike studies such as Carvalho et al. [25] and Legarda et al. [14], which did not observe macroalgae growth in biofloc systems, our use of partial harvests might have reduced macroalgae density in the cultivation structure and minimized shading, resulting in improved biomass production. Biancacci et al. [54] showed that the use of partial harvests in the cultivation of the macroalga *Macrocystis pyrifera* promoted greater biomass gain, a lower incidence of epiphytes, and a change in the macroalgae biochemical composition.

In addition to serving as a bioremediator, macroalgae possess economic value, as the biomass they produce can be utilized in the pharmaceutical and food industries [55], thereby fostering sustainability and profitability in production. Macroalgae serve as vital sources of nutrients and vitamins and possess antioxidant and immunostimulant properties [56]. The higher protein values observed in macroalgae from the heterotrophic system may be attributed to reduced luminosity in the system due to the gradual accumulation of solids over the cultivation period. Ganesan et al. [57] showed a correlation between high pigment concentrations in low-light and salinity environments in their study on the macroalga *Ulva fasciata*, indicating potential adaptations to environmental conditions. The observed high values of chlorophyll-a and chlorophyll-b in our study compared to those reported by Silva et al. [58] may be linked to the necessity of increasing pigment concentrations in macroalgae to maximize photosynthesis, likely due to reduced light penetration caused by suspended particles in a biofloc system. Similar trends were noted by Fillit et al. [59], who reported increased pigment concentrations during periods of low light availability.

In the integrated system, all species must have productivity in cultivation and economic potential [60]. Despite the elevated nitrite concentrations in the chemoautotrophic system, shrimp and fish performance was not affected. However, growth outcomes and survival in both treatments were lower than those reported by Ferreira et al. [17] in their study on shrimp cultivation in chemoautotrophic, heterotrophic, and mature systems. This can be attributed to

temperature differences between the studies. The minimum temperature recorded in our experiment was 14.9 °C, directly impacting the survival of the organisms. Furthermore, the overall average temperature in our study (22.0 °C) was lower compared to studies conducted with shrimp and fish [61], which also influenced the growth of the animals due to their decreased metabolism. Fish performance in terms of weight gain was superior in the heterotrophic system compared to the chemoautotrophic system, possibly due to the higher availability of suspended organic matter. The reduced feed supply aimed to induce floc consumption in the system, as demonstrated by Holanda et al. [7], with floc serving as a supplementary food source for organisms [62]. Hence, the higher concentration of total suspended solids in the heterotrophic system might have positively influenced fish weight gain. Similar results were reported by Poli et al. [8], who observed lower fish growth in an integrated system with chemoautotrophic floc.

The use of integrated multi-trophic systems aims to balance system productivity with sustainability, ensuring that all organisms adapt to the cultivation conditions. According to Khanjani et al. [63], the utilization of integrated systems has been consistently increasing, highlighting potential species for inclusion in the system, with crustaceans being among the most commonly produced target species. Zimmermann et al. [64] discuss the future of tilapia production, emphasizing multitrophic cultivation and biofloc technology as promising systems for maximizing production, considering greater sustainability, biosecurity, and increased density. However, the integration of macroalgae into biofloc systems has not yet been fully stabilized. The inclusion of macroalgae in biofloc systems has presented challenges due to their low productivity [14,15,25], but their role as bioremediators in nutrient absorption has shown promise, as demonstrated by the data presented in this study. Furthermore, the production of macroalgae biomass with an increase in nitrogen content in tissues, as reported by Legarda et al. [14] and Carvalho et al. [25], adds value to the product and enhances its applicability. The incorporation of macroalgae produced in integrated systems into fish and shrimp feed has yielded significant results, as evidenced by Marinho et al. [65] and Valente et al. [66]. Improved methods for managing the incorporation of macroalgae into biofloc systems are needed to enhance production and sustainability in intensive production systems.

5. Conclusions

The use of macroalgae in an integrated system with organic fertilization proved to be viable for increasing biomass production and nitrate and phosphate absorption, improving the system's sustainability. The use of a system with a low concentration of solids, as in the

chemoautotrophic system, promoted better growth rates for the macroalgae. However, the use of an inoculum from a heterotrophic system intensified the removal of phosphate and nitrate and increased the protein content of the macroalgae. A better maintenance of water quality was found in the heterotrophic system with the use of organic fertilization, without the need for water renewal. Finally, the heterotrophic system contributed to the better performance of the tilapia, with an increase in weight gain and a higher average final weight.

Author Contributions: Conceptualization, A.C., J.Z., A.P.C., F.N.V., M.O., G.T., and L.H.P.; data curation, A.C.; formal analysis, A.C. and H.B.; funding acquisition, L.H.P.; investigation, H.B., J.Z., A.P.C., F.N.V., M.O., and L.H.P.; methodology, A.C., H.B., A.P.C., F.N.V., and L.H.P.; project administration, G.T. and L.H.P.; supervision, F.N.V., G.T., and L.H.P.; validation, A.C.; visualization, J.Z., A.P.C., M.O., G.T., and L.H.P.; writing—original draft, A.C.; writing—review and editing, H.B., J.Z., A.P.C., F.N.V., M.O., G.T., and L.H.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the ASTRAL Project—H2020 grant agreement 863034.

Institutional Review Board Statement: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors. The experiment was approved by the Ethics and Animal Welfare Committee of FURG (Case number 23116.005895/2016-42).

Data Availability Statement: Data are contained within the article.

Acknowledgments: Special thanks to the Brazilian Council of Research (CNPq), the Coordination for the Improvement of Higher Level or Education Personnel (CAPES), and the Rio Grande do Sul State Government. Luís H. Poersch and Felipe N. Vieira received a productivity research fellowship from CNPq.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Gordalina, M.; Pinheiro, H.M.; Mateus, M.; da Fonseca, M.M.R.; Cesário, M.T. Macroalgae as Protein Sources—A Review on Protein Bioactivity, Extraction, Purification and Characterization. *Appl. Sci.* **2021**, *11*, 7969. https://doi.org/10.3390/app11177969.
2. Wan, A.H.L.; Davies, S.J.; Soler-Vila, A.; Fitzgerald, R.; Johnson, M.P. Macroalgae as a Sustainable Aquafeed Ingredient. *Rev. Aquac.* **2019**, *11*, 458–492.
3. Chopin, T. Marine Aquaculture in Canada: Well-Established Monocultures of Finfish and Shellfish and an Emerging Integrated Multi-Trophic Aquaculture (IMTA) Approach Including Seaweeds, Other Invertebrates, and Microbial Communities. *Fisheries* **2015**, *40*, 28–31. https://doi.org/10.1080/03632415.2014.986571.
4. Queirós, A.S.; Circuncisão, A.R.; Pereira, E.; Válega, M.; Abreu, M.H.; Silva, A.M.S.; Cardoso, S.M. Valuable Nutrients from *Ulva rigida*: Modulation by Seasonal and Cultivation Factors. *Appl. Sci.* **2021**, *11*, 6137. https://doi.org/10.3390/app11136137.
5. Troell, M.; Joyce, A.; Chopin, T.; Neori, A.; Buschmann, A.H.; Fang, J.G. Ecological Engineering in Aquaculture—Potential for Integrated Multi-Trophic Aquaculture (IMTA) in Marine Offshore Systems. *Aquaculture* **2009**, *297*, 1–9. https://doi.org/10.1016/j.aquaculture.2009.09.010.
6. Resende, L.; Flores, J.; Moreira, C.; Pacheco, D.; Baeta, A.; Garcia, A.C.; Rocha, A.C.S. Effective and Low-Maintenance IMTA System as Effluent Treatment Unit for Promoting Sustainability in Coastal Aquaculture. *Appl. Sci.* **2022**, *12*, 398. https://doi.org/10.3390/app12010398.
7. Holanda, M.; Wasielesky, W.; de Lara, G.R.; Poersch, L.H. Production of Marine Shrimp Integrated with Tilapia at High Densities and in a Biofloc System: Choosing the Best Spatial Configuration. *Fishes* **2022**, *7*, 283. https://doi.org/10.3390/fishes7050283.
8. Poli, M.A.; Legarda, E.C.; de Lorenzo, M.A.; Pinheiro, I.; Martins, M.A.; Seiffert, W.Q.; do Nascimento Vieira, F. Integrated Multitrophic Aquaculture Applied to Shrimp Rearing in a Biofloc System. *Aquaculture* **2019**, *511*, 734274. https://doi.org/10.1016/j.aquaculture.2019.734274.
9. de Souza, R.L.; de Lima, E.C.R.; de Melo, F.P.; Ferreira, M.G.P.; de Souza Correia, E. The Culture of Nile Tilapia at Different Salinities Using a Biofloc System. *Rev. Cienc. Agron.* **2019**, *50*, 267–275. https://doi.org/10.5935/1806-6690.20190031.
10. Li, E.; Chen, L.; Zeng, C.; Chen, X.; Yu, N.; Lai, Q.; Qin, J.G. Growth, Body Composition, Respiration and Ambient Ammonia Nitrogen Tolerance of the Juvenile White Shrimp, *Litopenaeus vannamei*, at Different Salinities. *Aquaculture* **2007**, *265*, 385–390. https://doi.org/10.1016/j.aquaculture.2007.02.018.
11. Natori, M.M.; Sussel, F.R.; Santos, E.D.; Previero, T.D.C.; Viegas, E.M.M.; Gameiro, A.H. Desenvolvimento Da Carcinicultura Marinha No Brasil e No Mundo: Avanços Tecnológicos e Desafios. *Informações econômicas* **2011**, *41*, 61–73.
12. FAO. *State of the World Fisheries and Aquaculture—2022 (SOFIA)*; FAO: Rome, Italy, 2022; ISBN 9789251072257.
13. Brito, L.O.; Arantes, R.; Magnotti, C.; Derner, R.; Pchara, F.; Olivera, A.; Vinatea, L. Water Quality and Growth of Pacific White Shrimp *Litopenaeus vannamei* (Boone) in Co-Culture with Green Seaweed *Ulva lactuca* (Linaeus) in Intensive System. *Aquac. Int.* **2014**, *22*, 497–508. https://doi.org/10.1007/s10499-013-9659-0.
14. Legarda, E.C.; da Silva, D.; Miranda, C.S.; Pereira, P.K.M.; Martins, M.A.; Machado, C.; de Lorenzo, M.A.; Hayashi, L.; do Nascimento Vieira, F. Sea Lettuce Integrated with Pacific White Shrimp and Mullet Cultivation in Biofloc Impact System Performance and the Sea Lettuce Nutritional Composition. *Aquaculture* **2021**, *534*, 736265. https://doi.org/10.1016/j.aquaculture.2020.736265.

15. de Morais, A.P.M.; Santos, I.L.; Carneiro, R.F.S.; Routledge, E.A.B.; Hayashi, L.; de Lorenzo, M.A.; do Nascimento Vieira, F. Integrated Multitrophic Aquaculture System Applied to Shrimp, Tilapia, and Seaweed (*Ulva ohnoi*) Using Biofloc Technology. *Aquaculture* **2023**, *572*, 739492. https://doi.org/10.1016/j.aquaculture.2023.739492.
16. Crab, R.; Avnimelech, Y.; Defoirdt, T.; Bossier, P.; Verstraete, W. Nitrogen Removal Techniques in Aquaculture for a Sustainable Production. *Aquaculture* **2007**, *270*, 1–14. https://doi.org/10.1016/j.aquaculture.2007.05.006.
17. Ferreira, G.S.; Santos, D.; Schmachtl, F.; Machado, C.; Fernandes, V.; Bögner, M.; Schleder, D.D.; Seiffert, W.Q.; Vieira, F.N. Heterotrophic, Chemoautotrophic and Mature Approaches in Biofloc System for Pacific White Shrimp. *Aquaculture* **2021**, *533*, 736099. https://doi.org/10.1016/j.aquaculture.2020.736099.
18. Ebeling, J.M.; Timmons, M.B.; Bisogni, J.J. Engineering Analysis of the Stoichiometry of Photoautotrophic, Autotrophic, and Heterotrophic Removal of Ammonia-Nitrogen in Aquaculture Systems. *Aquaculture* **2006**, *257*, 346–358. https://doi.org/10.1016/j.aquaculture.2006.03.019.
19. Gaona, C.A.P.; de Almeida, M.S.; Viau, V.; Poersch, L.H.; Wasielesky, W. Effect of Different Total Suspended Solids Levels on a *Litopenaeus vannamei* (Boone, 1931) BFT Culture System during Biofloc Formation. *Aquac. Res.* **2017**, *48*, 1070–1079. https://doi.org/10.1111/are.12949.
20. Furtado, P.S.; Poersch, L.H.; Wasielesky, W. Effect of Calcium Hydroxide, Carbonate and Sodium Bicarbonate on Water Quality and Zootechnical Performance of Shrimp *Litopenaeus vannamei* Reared in Biofloc Technology (BFT) Systems. *Aquaculture* **2011**, *321*, 130–135. https://doi.org/10.1016/j.aquaculture.2011.08.034.
21. Furtado, P.S.; Campos, B.R.; Serra, F.P.; Klosterhoff, M.; Romano, L.A.; Wasielesky, W. Effects of Nitrate Toxicity in the Pacific White Shrimp, *Litopenaeus vannamei*, Reared with Biofloc Technology (BFT). *Aquac. Int.* **2015**, *23*, 315–327. https://doi.org/10.1007/s10499-014-9817-z.
22. Macedo, C.F.; Sipaúba-Tavares, L. Eutrophication and Water Quality in Pisciculture: Consequences and Recommendations. *Bol. Do Inst. De Pesca* **2010**, *36*, 149–163.
23. Brandão, H.; Xavier, Í.V.; Santana, G.K.K.; Santana, H.J.K.; Krummenauer, D.; Wasielesky, W. Heterotrophic versus Mixed BFT System: Impacts on Water Use, Suspended Solids Production and Growth Performance of *Litopenaeus vannamei*. *Aquac. Eng.* **2021**, *95*, 102194. https://doi.org/10.1016/j.aquaeng.2021.102194.
24. Krummenauer, D.; Samocha, T.; Poersch, L.; Lara, G.; Wasielesky, W. The Reuse of Water on the Culture of Pacific White Shrimp, *Litopenaeus vannamei*, in BFT System. *J. World Aquac. Soc.* **2014**, *45*, 3–14. https://doi.org/10.1111/jwas.12093.
25. Carvalho, A.; Costa, L.C. de O.; Holanda, M.; Poersch, L.H.; Turan, G. Influence of Total Suspended Solids on the Growth of the Sea Lettuce *Ulva lactuca* Integrated with the Pacific White Shrimp *Litopenaeus vannamei* in a Biofloc System. *Fishes* **2023**, *8*, 163. https://doi.org/10.3390/fishes8030163.
26. Copertino, M.D.S.; Tormena, T.; Seeliger, U. Biofiltering Efficiency, Uptake and Assimilation Rates of *Ulva clathrata* (Roth) J. Agardh (Clorophyceae) Cultivated in Shrimp Aquaculture Waste Water. *J. Appl. Phycol.* **2009**, *21*, 31–45. https://doi.org/10.1007/s10811-008-9357-x.
27. Duke, C.S.; Litaker, W.; Ramus, J. Effects of the Temperature, Nitrogen Supply and Tissue Nitrogen on Ammonium Uptake Rates of the Chlorophyte Seaweeds *Ulva Curvata* and *Codium Decorticatum*. *J. Phycol.* **1989**, *25*, 113–120.
28. American Public Health Association (APHA). *Standard Methods for the Examination of Water and Waste Water*, 16th ed.; American Public Health Association: Washington, DC, USA, 1989.
29. UNESCO. *Chemical Methods for Use in Marine Environmental Monitoring*; Intergovernmental Oceanographic Commission: Paris, France, 1983.

30. Aminot, A.; Chaussepied, M. *Manuel Des Analyses Chimiques En Milieu Marin*; Centre National pour l'exploitation des océans: *Paris, France*, 1983;.
31. Baumgarten, M.D.G.Z.; De Barros Rocha, J.M.; Niencheski, L.F.H. *Manual de Análises Em Oceanografia Química*; FURG: Rio Grande, Brazil, 1996.
32. Wasielesky, W.; Krummenauer, D.; Lara, G.; Fóes, G. Cultivo de Camarões Em Sistema de Bioflocos: Realidades e Perspectivas. **2013**, *15*, 30–36.
33. Loureiro, R.R.; Reis, R.P.; Critchley, A.T. In Vitro Cultivation of Three *Kappaphycus alvarezii* (Rhodophyta, Areschougiaceae) Variants (Green, Red and Brown) Exposed to a Commercial Extract of the Brown Alga *Ascophyllum nodosum* (Fucaceae, Ochrophyta). *J. Appl. Phycol.* **2010**, *22*, 101–104. https://doi.org/10.1007/s10811-009-9412-2.
34. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of Total Carotenoids and Chlorophylls a and b of Leaf Extracts in Different Solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592. https://doi.org/10.1042/bst0110591.
35. Barbarino, E.; Lourenço, S.O. An Evaluation of Methods for Extraction and Quantification of Protein from Marine Macro- and Microalgae. *J. Appl. Phycol.* **2005**, *17*, 447–460. https://doi.org/10.1007/s10811-005-1641-4.
36. Jory, D.E.; Cabrera, T.R.; Dugger, D.M.; Fegan, D.; Lee, P.G.; Lawrence, L.; Jackson, C.J.; Mcintosh, R.P.; Castañeda, J.I.; Al, B.; et. A Global Review of Shrimp Feed Management: Status and Perspectives. *Aquaculture* **2011**, *318*, 104–152.
37. Øyvind Hammer; David, A.T. Harper; Paul, D. Ryan National Knowledge Resource Consortium - a National Gateway of S&T on-Line Resources for CSIR and DST Laboratories. *Curr. Sci.* **2013**, *105*, 1352–1357.
38. Messyasz, B.; Rybak, A. Abiotic Factors Affecting the Development of *Ulva* Sp. (Ulvophyceae; Chlorophyta) in Freshwater Ecosystems. *Aquat. Ecol.* **2011**, *45*, 75–87. https://doi.org/10.1007/s10452-010-9333-9.
39. Lin, Y.C.; Chen, J.C. Acute Toxicity of Ammonia on *Litopenaeus vannamei* Boone Juveniles at Different Salinity Levels. *J. Exp. Mar. Bio. Ecol.* **2001**, *259*, 109–119. https://doi.org/10.1016/S0022-0981(01)00227-1.
40. De Lara, G.R.; Poersch, L.H.; Wasielesky, W., Jr. The Quantity of Artificial Substrates Influences the Nitrogen Cycle in the Biofloc Culture System of *Litopenaeus vannamei*. *Aquac. Eng.* **2021**, *94*, 102171. https://doi.org/10.1016/j.aquaeng.2021.102171.
41. Lin, Y.C.; Chen, J.C. Acute Toxicity of Nitrite on *Litopenaeus vannamei* (Boone) Juveniles at Different Salinity Levels. *Aquaculture* **2003**, *224*, 193–201. https://doi.org/10.1016/S0044-8486(03)00220-5.
42. Carneiro, M.A.D.A. Estudo Do Crescimento, Eficiência de Biofiltração e Cinética de Absorção de Nutrientes (N-NH, N-NO e P-$PO4^{3}$) Da Macroalga *Gracilaria cervicornis* (Turner). *J. Agardh (Master's thesis, Universidade Federal do Rio Grande do Norte),* **2007**, *75*. https://repositorio.ufrn.br/jspui/handle/123456789/12509.
43. Hanisak, M.D. The Use of *Gracilaria tikvahiae* (Gracilariales, Rhodophyta) as a Model System to Understand the Nitrogen Nutrition of Cultured Seaweeds. *Hydrobiologia* **1990**, *204–205*, 79–87. https://doi.org/10.1007/BF00040218.
44. Da Silva, K.R.; Wasielesky, W.; Abreu, P.C. Nitrogen and Phosphorus Dynamics in the Biofloc Production of the Pacific White Shrimp, *Litopenaeus vannamei*. *J. World Aquac. Soc.* **2013**, *44*, 30–41. https://doi.org/10.1111/jwas.12009.
45. Massocato, T.F.; Robles-Carnero, V.; Moreira, B.R.; Castro-Varela, P.; Pinheiro-Silva, L.; Oliveira, W. da S.; Vega, J.; Avilés, A.; Bonomi-Barufi, J.; Rörig, L.R.; et al. Growth, Biofiltration and Photosynthetic Performance of *Ulva* Spp. Cultivated in Fishpond Effluents: An Outdoor Study. *Front. Mar. Sci.* **2022**, *9*, 981468. https://doi.org/10.3389/fmars.2022.981468.

46. Tsagkamilis, P.; Danielidis, D.; Dring, M.J.; Katsaros, C. Removal of Phosphate by the Green Seaweed *Ulva lactuca* in a Small-Scale Sewage Treatment Plant (Ios Island, Aegean Sea, Greece). *J. Appl. Phycol.* **2010**, *22*, 331–339. https://doi.org/10.1007/s10811-009-9463-4.
47. Zirino, A.; Elwany, H.; Facca, C.; Maicu', F.; Neira, C.; Mendoza, G. Nitrogen to Phosphorus Ratio in the Venice (Italy) Lagoon (2001–2010) and Its Relation to Macroalgae. *Mar. Chem.* **2016**, *180*, 33–41. https://doi.org/10.1016/j.marchem.2016.01.002.
48. Rathod, M.; Mody, K.; Basha, S. Efficient Removal of Phosphate from Aqueous Solutions by Red Seaweed, *Kappaphycus alverezii*. *J. Clean. Prod.* **2014**, *84*, 484–493. https://doi.org/10.1016/j.jclepro.2014.03.064.
49. Chopin, T.; Buschmann, A.H.; Halling, C.; Troell, M.; Kautsky, N.; Neori, A.; Kraemer, G.P.; Zertuche-González, J.A.; Yarish, C.; Neefus, C. Integrating Seaweeds into Marine Aquaculture Systems: A Key toward Sustainability. *J. Phycol.* **2001**, *37*, 975–986. https://doi.org/10.1046/j.1529-8817.2001.01137.x.
50. Alencar, J.R. de; Junior, P.A.H.; Celino, J.J. Cultivo de Camarão Branco *Litopenaeus vannamei* (Boone, 1931) Com a Macroalga *Ulva lactuca* Linneaus (Chlorophyta) No Tratamento de Efluentes Em Sistema Fechado de Recirculação. *Rev. Biol. e Ciências da Terra* **2010**, *10*, 117–137.
51. Carvalho, A.; Costa, L.C. de O.; Holanda, M.; Gonçalves, M.; Santos, J.; Costa, C.S.B.; Turan, G.; Poersch, L.H. Growth of the Macroalgae *Ulva lactuca* Cultivated at Different Depths in a Biofloc Integrated System with Shrimp and Fish. *Phycology* **2023**, *3*, 280–293. https://doi.org/10.3390/phycology3020018.
52. Martins, M.A.; da SILVA, V.F.; Tarapuez, P.R.; Hayashi, L.; Vieira, F.D.N. Cultivation of the Seaweed *Ulva* Spp. With Effluent from a Shrimp Biofloc Rearing System: Different Species and Stocking Density. *Bol. do Inst. Pesca* **2020**, v. *46, n. 03*. https://doi.org/10.20950/1678-2305.2020.46.3.602.
53. Pires, C.M.; Bazzo, G.C.; Barreto, P.L.M.; do Espírito Santo, C.M.; Ventura, T.F.B.; Pedra, A.G.L.M.; Rover, T.; McGovern, M.; Hayashi, L. Cultivation of the Red Seaweed *Kappaphycus alvarezii* Using Biofloc Effluent. *J. Appl. Phycol.* **2021**, *33*, 1047–1058. https://doi.org/10.1007/s10811-020-02335-6.
54. Biancacci, C.; Visch, W.; Callahan, D.L.; Farrington, G.; Francis, D.S.; Lamb, P.; McVilly, A.; Nardelli, A.; Sanderson, J.C.; Schwoerbel, J.; et al. Optimisation of At-Sea Culture and Harvest Conditions for Cultivated *Macrocystis pyrifera*: Yield, Biofouling and Biochemical Composition of Cultured Biomass. *Front. Mar. Sci.* **2022**, *9*, 951538. https://doi.org/10.3389/fmars.2022.951538.
55. Mabeau, S.; Fleurence, J. Seaweed in Food Products: Biochemical and Nutritional Aspects. *Trends Food Sci. Technol.* **1993**, *4*, 103–107. https://doi.org/10.1016/0924-2244(93)90091-N.
56. Peñalver, R.; Lorenzo, J.M.; Ros, G.; Amarowicz, R.; Pateiro, M.; Nieto, G. Seaweeds as a Functional Ingredient for a Healthy Diet. *Mar. Drugs* **2020**, *18*, 301. https://doi.org/10.3390/md18060301.
57. Ganesan, M.; Mairh, O.P.; Eswaran, K.; Subba Rao, P.V. Effect of Salinity, Light Intensity and Nitrogen Source on Growth and Composition of *Ulva fasciata* Delile (Chlorophyta, Ulvales). *Indian J. Mar. Sci.* **1999**, *28*, 70–73.
58. Silva, A.F.R.; Abreu, H.; Silva, A.M.S.; Cardoso, S.M. Effect of Oven-Drying on the Recovery of Valuable Compounds from *Ulva rigida*, *Gracilaria* Sp. and *Fucus vesiculosus*. *Mar. Drugs* **2019**, *17*, 90. https://doi.org/10.3390/md17020090.
59. Fillit, M. Seasonal Changes in the Photosynthetic Capacities and Pigment Content of *Ulva rigida* in a Mediterranean Coastal Lagoon. *Bot. Mar.* **1995**, *38*, 271–280. https://doi.org/10.1515/botm.1995.38.1-6.271.

60. Buck, B.H.; Troell, M.F.; Krause, G.; Angel, D.L.; Grote, B.; Chopin, T. State of the Art and Challenges for Offshore Integrated Multi-Trophic Aquaculture (IMTA). *Front. Mar. Sci.* **2018**, *5*, 165. https://doi.org/10.3389/fmars.2018.00165.
61. Wyban, J.; Walsh, W.A.; Godin, D.M. Temperature Effects on Growth, Feeding Rate and Feed Conversion of the Pacific White Shrimp (*Penaeus vannamei*). *Aquaculture* **1995**, *138*, 267–279. https://doi.org/10.1016/0044-8486(95)00032-1.
62. Ahmad, I.; Babitha Rani, A.M.; Verma, A.K.; Maqsood, M. Biofloc Technology: An Emerging Avenue in Aquatic Animal Healthcare and Nutrition. *Aquac. Int.* **2017**, *25*, 1215–1226. https://doi.org/10.1007/s10499-016-0108-8.
63. Khanjani, M.H.; Zahedi, S.; Mohammadi, A. Integrated Multitrophic Aquaculture (IMTA) as an Environmentally Friendly System for Sustainable Aquaculture: Functionality, Species, and Application of Biofloc Technology (BFT). *Environ. Sci. Pollut. Res.* **2022**, *29*, 67513–67531. https://doi.org/10.1007/s11356-022-22371-8.
64. Zimmermann, S.; Kiessling, A.; Zhang, J. The Future of Intensive Tilapia Production and the Circular Bioeconomy without Effluents: Biofloc Technology, Recirculation Aquaculture Systems, Bio-RAS, Partitioned Aquaculture Systems and Integrated Multitrophic Aquaculture. *Rev. Aquac.* **2023**, *15*, 22–31. https://doi.org/10.1111/raq.12744.
65. Marinho, G.; Nunes, C.; Sousa-Pinto, I.; Pereira, R.; Rema, P.; Valente, L.M.P. The IMTA-Cultivated Chlorophyta *Ulva* Spp. as a Sustainable Ingredient in Nile Tilapia (*Oreochromis niloticus*) Diets. *J. Appl. Phycol.* **2013**, *25*, 1359–1367. https://doi.org/10.1007/s10811-012-9965-3.
66. Valente, L.M.P.; Araújo, M.; Batista, S.; Peixoto, M.J.; Sousa-Pinto, I.; Brotas, V.; Cunha, L.M.; Rema, P. Carotenoid Deposition, Flesh Quality and Immunological Response of Nile Tilapia Fed Increasing Levels of IMTA-Cultivated *Ulva* spp. *J. Appl. Phycol.* **2016**, *28*, 691–701. https://doi.org/10.1007/s10811-015-0590-9.

CAPÍTULO 6: UTILIZAÇÃO DA MACROALGA *Ulva lactuca* PARA TRATAMENTO DE EFLUENTE DE CULTIVO DE CAMARÃO *P. vannamei* EM SISTEMA DE BIOFLOCOS COM DIFERENTES FERTILIZAÇÕES.

Andrezza Carvalho[1], Navinésia Rungo[1], Alessandro Cardozo[1], Gamze Turan[2], Luis H. Poersch[1].

[1] Marine Aquaculture Station, Institute of Oceanography, Federal University of Rio Grande—FURG, Rua do Hotel, no.2, Cassino, Rio Grande 96210-030, RS, Brazil;

[2] Aquaculture Department, Fisheries Faculty, Ege University, Izmir 35100, Turkey;

Autor correspondete: andrezzachagas@hotmail.com

Resumo

O objetivo deste trabalho foi avaliar o crescimento e a absorção de nutrientes pela macroalga *Ulva lactuca* em efluentes de um cultivo integrado com camarão (*Penaeus vannamei*) e tilápia (*Oreochromis niloticus*) em sistema BFT, dominados por bactérias heterotróficas e quimioautotróficas. O experimento teve a duração de 15 dias e foi realizado em seis tanques circulares de 3,5 m³ com aeração constante, que estavam desacoplados do sistema IMTA. Em função do tipo de fertilizante utilizado, cada tratamento foi dominado por bactérias Quimioautotróficas ou Heterotróficas. As macroalgas foram inseridas em cada tratamento na densidade de 1 g L^{-1} em um total de 6 tanques divididos entre os dois tipos de fertilização. As condições iniciais dos tratamentos apresentaram diferenças significativas nos níveis de nitrato e sólidos suspensos totais (SST). Durante o cultivo, o sistema quimioautotrófico destacou-se por apresentar maior alcalinidade (290,00 ± 36,33 mg $CaCO_3$ L^{-1}) e pH (8,40 ± 0,04). O sistema heterotrófico registrou maiores concentrações de sólidos suspensos totais (303,33 ± 68,25 mg L^{-1}) e uma taxa de remoção de nitrato de 28% durante o período experimental, sendo o dobro do encontrado no sistema Quimioautotrófico. A relação nitrogênio e fosfato encontrada no tratamento Heterotrófico promoveu um melhor desempenho em absorção para a macroalga. Esses resultados indicam que a *U. lactuca* pode ser utilizada em sistemas heterotróficos para maximizar o aproveitamento de nutrientes, contribuindo tanto para o tratamento de efluentes quanto para a produção de biomassa de alta qualidade, com implicações positivas para a sustentabilidade da aquicultura integrada.

Palavras-chave: *Ulva lactuca*, bioflocos, quimioautotrófico, heterotrófico, qualidade da água, sustentabilidade.

1. Introdução

O investimento na produção de macroalgas do gênero *Ulva* tem evoluído durante os anos devido aos avanços na reprodução em laboratório, no cultivo em larga escala e na aplicação da

biomassa produzida. Sua reprodução difere de outros grupos de macroalgas, ocorrendo através da formação e liberação de zoósporos ou gametas, podendo ser induzida através de diversos fatores abióticos como intensidade luminosa, temperatura e fragmentação do talo (Balar and Mantri, 2020). E quando em seu estágio adulto possuem morfologia dinâmica de acordo com as variáveis ambientais (Schiavon et al., 2012), mostrando sua adaptabilidade as condições ambientais em que são cultivadas.

A produção de macroalgas em áreas abertas pode estar sujeita a disponibilidade de nutrientes, herbivoria e dinâmica de correntes (FAO, 2024), podendo-se utilizar modelos multi-escalas que levam em consideração a luz, temperatura e nutrientes em diferentes épocas do ano para obtenção de maior crescimento e sequestro de nutrientes, uma ferramenta importante para a maximização da produção sustentável de macroalgas (Zollmann et al., 2021). A produção em terra também tem se mostrado promissora, o trabalho realizado por Mata et al. (2016) mostraram que a produção de *Ulva ohnoi* em seis tanques de fibra de vidro com 10 m^{-3} de volume útil produziram 138 toneladas de peso seco ha^{-1} ano^{-1}, com variações altas de produtividade de biomassa durante o ano. Essa produção está diretamente interligada com a disponibilidade de nutrientes limitantes no ambiente aquático, como nitrato e fostato (Hadley et al., 2015; Lundberg et al., 1989). De acordo com Shakouri and Balouch, (2020) testando diferentes proporções de nitrato e fosfato para maximizar a produção de biomassa de macroalgas, constataram que melhores resultados foram encontrados nas concentrações de 30 mg L^{-1} de nitrato e 15 mg L^{-1} de fosfato.

O uso de fertilizantes químicos para entrada de nutrientes pode representar um alto custo na produção em terra (Mansilla et al., 2014). Para isso, como forma de reaproveitamento de resíduos inorgânicos produzidos através de atividade aquícolas, tem-se realizado a inserção de macroalgas no sistema, através de cultivos integrados ou tratamentos de efluente. O uso de macroalgas como agente biorremediador em cultivos de peixes e camarões tem aumentado cada vez mais como forma de mitigar impactos na produção e liberação de nutrientes. Martins et al. (2020) utilizaram a macroalga *Ulva ohnoi* no tratamento do efluente de camarão obtendo alta produtividade com uma taxa de crescimento específica de 3 % dia^{-1}. Portanto a utilização de macroalgas como consumidor inorgânico se torna viável através da necessidade de absorção do nitrogênio e fosforo como fonte de energia, realização de processos fisiológicos e bioquímicos essenciais para crescimento e reprodução (Duke et al., 1989).

Portanto o tratamento do efluente gerado na aquicultura busca levar maior sustentabilidade com uma maior diversificação de produtos gerados no cultivo. Entre os sistemas intensivos de produção de camarão e tilápia, se destaca o sistema de bioflocos (BFT) que permite o uso de

altas densidades de estocagem sem comprometer a qualidade de água ou uso de renovações (Krummenauer et al., 2011; Wasielesky et al., 2013). No sistema de bioflocos, o controle dos nitrogenados ocorre por meio da indução ao crescimento de microrganismos na água que irão ciclar compostos nitrogenados do sistema e convertendo em biomassa bacteriana (Wasielesky et al., 2013). Diferentes fertilizações iniciais no sistema de bioflocos irão proporcionar o crescimento de diferentes bactérias, podendo ser bactérias heterotróficas, bactérias quimioautotróficas ou um sistema misto com ambas as bactérias presentes no sistema (Brandão et al., 2021). A utilização de carbono orgânico irá favorecer o crescimento de bactérias heterotróficas, que utilizarão a amônia para crescimento de biomassa bacteriana, acarretando em uma maior produção de sólidos suspensos totais (bioflocos) no sistema (Gaona et al., 2017). Já a utilização de carbono inorgânico resultará na predominância de bactérias nitrificantes, que irão trabalhar na oxidação da amônia ao nitrito e posteriormente ao nitrato, sendo tal composto acumulado em altas quantidades no cultivo (Brandão et al., 2021).

Em ambos os sistemas de fertilização utilizados irá ocorrer o acúmulo de nutrientes e matéria orgânica, que quando despejada no corpo de água sem tratamento prévio poderá acarretar em problemas de saúde humana e eutrofização do ambiente (Macedo and Sipaúba-Tavares, 2010). Com isso a inserção da macroalga no efluente do cultivo poderá ser uma opção de tratamento de água para despejo ou reutilização para um novo ciclo. A sustentabilidade aquícola é definida na produção de organismos aquáticos para atender a demanda necessária, sem prejudicar o ecossistema ao redor ou esgotar recursos naturais necessário para manutenção da atividade (Boyd et al., 2020). No entanto pouco se sabe sobre a manutenção da qualidade de água do sistema de bioflocos quando não estão inseridas espécies ativas no sistema para movimentação do floco. Além disso, altas concentrações de nutrientes poderá ser um fator estressante para a macroalga, sendo um gatilho para reprodução e causando perda da biomassa (Copertino et al., 2009), assim como altas concentrações de sólidos poderá interferir na absorção de luz e realização da fotossíntese, causando baixa taxa de crescimento da macroalga (Carvalho et al., 2023b). Portanto, este trabalho teve como objetivo avaliar o crescimento e absorção de nutrientes pela macroalga *Ulva lactuca* em um efluente de sistema integrado com camarão e tilápia em bioflocos gerados a partir de fertilização orgânica e inorgânica.

2. Materiais e métodos

1.1 Localização e origem da macroalga

O experimento foi realizado em uma estufa agrícola, com aeração constante promovida por um soprador que injetava ar para mangueiras microperfuradas (aerotubes). A estufa estava

situada na Estação Marinha de Aquacultura (EMA), Instituto de Oceanografia, na Universidade Federal do Rio Grande, localizada na praia do Cassino, Rio Grande, Rio Grande do Sul.

As macroalgas foram provenientes de um cultivo de manutenção na Estação Marinha de Aquacultura, produzidas em um tanque circular de 1 $m^{3,}$ aeração constante e 5% de inóculo de sistema de cultivo de camarão em bioflocos, mantendo na concentração de 35.1 ± 2.74 e 2.24 ± 1.2 mg L^{-1} de nitrato e fosfato respectivamente.

1.2 Caracterização do sistema de bioflocos

Para realização do experimento foram utilizados dois efluentes com 55 dias de produção com salinidade 20 de um cultivo integrado com camarão *Penaeus vannemei*, peixe *Oreochromis niloticus* e a macroalga *Ulva lactuca* em diferentes sistemas de bioflocos.

O primeiro efluente foi proveniente de um cultivo integrado com sistema quimioautotrófico. Nesse sistema houve uma fertilização química prévia a estocagem dos animais, com duração de 35 dias, utilizando cloreto de amônio e nitrito de sódio na concentração de 1 mg L^{-1} na água e o uso de substrato artificial para fixação das bactérias. Após os 35 dias de formação e estabilização das bactérias do sistema quimioautotrófico, foi feito o inóculo de 30% nos três tanques e estocagem dos animais. Durante o período experimental de 55 dias foram realizadas apenas fertilizações inorgânicas com bicarbonato de sódio para correção da alcalinidade para melhor efetividade das bactérias (Furtado et al., 2011), a temperatura média foi mantida em 22.82 ± 0.30 ºC, e os nutrientes tiveram como média 0.95 ± 0.07 mg L^{-1} para amônia, 7.96 ± 1.16 mg L^{-1} de nitrito, 26.41 ± 2.72 mg L^{-1} de nitrato, 1.04 ± 0.25 mg L^{-1} de fosfato, e os sólidos suspensos totais com média de 189.22 ± 26.02 mg L^{-1}. Devido ao aumento da concentração de nitrito no início do cultivo nesse tratamento foram necessárias renovações de água como forma de manejo e controle do nitrito.

O segundo efluente foi obtido de um cultivo integrado em sistema heterotrófico, com o uso de fertilizações orgânicas. Como início desse sistema foi utilizado 40% de um inóculo de biofloco maduro proveniente de um cultivo de engorda de camarão *P. vannamei* com 68 dias de produção e uma densidade de 184 camarões m^{-2} e 60% de água em salinidade 20 ppt. O uso de fertilizações com carbono orgânico, melaço, foram realizadas quando a amônia passasse de 1 mg L^{-1} (Ebeling et al., 2006) e o uso de bicarbonato de sódio foi realizado para manutenção da alcalinidade. Nesse sistema, durante os 55 dias de cultivo em sistema integrado a temperatura média foi mantida em 22.67 ± 0.38 ºC, e os nutrientes tiveram como média 0.18 ± 0.08 mg L^{-1} para amônia, 0.86 ± 0.60 mg L^{-1} de nitrito, 26.04 ± 4.03 mg L^{-1} de nitrato, 1.19 ± 0.21 mg L^{-1}

de fosfato, e os sólidos suspensos totais com média de 335.38 ± 47.92 mg L^{-1}. Não foram realizadas renovações de água nesse sistema.

1.3 Delineamento experimental

Ao final do cultivo integrado de ambos os sistemas, foi realizada a despesca dos animais e a água foi mantida nos tanques para realização do experimento, que teve duração de 15 dias. Foram realizados dois tratamentos com três réplicas cada, sendo eles: Quimioautotrófico – uso de fertilizações inorgânicas para manutenção da qualidade de água do sistema; e Heterotrófico – uso de fertilizações orgânicas com melaço para manutenção da qualidade de água do sistema.

O experimento foi realizado em tanques circulares com 3.5 m^3 de volume útil e aeração constante por mangueiras microperfuradas (aerotubes). Uma densidade de 1 g L^{-1} de macroalga foi adotada, tendo peso médio inicial de 3.52 ± 0.10 g. A macroalga foi disposta no tanque dentro de uma única estrutura flutuante em cada tanque de cultivo, com uma profundidade de coluna de água de 0.10 m para manutenção das macroalgas próximo a superfície, e as dimensão de 1 m x 1 m x 0.30 m (comprimento x largura x altura). Os parâmetros iniciais de nutrientes e sólidos de cada tratamento estão descritos na tabela abaixo (Tabela 1).

Tabela 1. Parâmetros iniciais (média ± desvio padrão) de nutrientes e sólidos nos tratamentos Quimioautotrófico e Heterotrófico.

	Tratamentos	
Parâmetros	**Quimioautotrófico**	**Heterotrófico**
Nitrogênio amoniacal total (mg L^{-1})	0.07 ± 0.03	0.07 ± 0.03
Nitrito (mg L^{-1})	0.11 ± 0.03	0.15 ± 0.03
Nitrato (mg L^{-1})	25.00 ± 0.00 a	32.33 ± 2.08 b
Fosfato (mg L^{-1})	2.43 ± 0.21	2.17 ± 0.35
Sólidos suspensos totais (mg L^{-1})	165.00 ± 77.78 a	346.67 ± 115.58 b

1.4 Qualidade de água

A temperatura (ºC), oxigênio dissolvido (mg L^{-1}) e pH foram mensurados diariamente com o auxílio de uma sonda multiparâmetro Pro-20 model (YSI Inc., Yellow Springs, OH, USA) e um pHmetro de bancada (Seven2Go S7 Basic, Mettler Toledo, São Paulo, SP, Brazil). A

salinidade (ppt) e alcalinidade (mg $CaCO_3$ L^{-1}) foram mensuradas uma vez na semana utilizando uma sonda Pro-20 model (YSI Inc., Yellow Springs, OH, USA) e a metodologia proposta por APHA, (2005) respectivamente. O nitrogênio amoniacal total (mg L^{-1}) e nitrito (mg L^{-1}) foram mensurados diariamente de acordo com a metodologia proposta por Unesco, (1983). O nitrato (mg L^{-1}) e fosfato (mg L^{-1}) foram mensurados quatro vezes na semana de acordo com a metodologia proposta por Aminot, A., Chaussepied, (1983). Os sólidos suspensos totais (mg L^{-1}) foram realizados no início e ao final do experimento de acordo com a metodologia proposta por Baumgarten et al., (1996).

1.5 Desempenho da macroalga

Foi realizada uma pesagem inicial e final da macroalga no experimento. O procedimento de pesagem consistiu na retirada da estrutura flutuante da água, e deixada ao ar livre por 10 minutos para remover o excesso de água e por fim pesadas em balança digital (BL3200H, MARTE®, Santa Rita do Sapucaí, Minas Gerais, Brazil). A fórmula abaixo foi utilizada para mensurar a taxa de crescimento relativa (TCR) e taxa de remoção de nutrientes (TRN) pela macroalga.

TCR (%d^{-1}): 100 × [ln (peso final (g)/peso inicial (g))/(tempo final – tempo inicial)]

TRN (%): 100 × [(concentração de nutrientes no tempo inicial (mg L^{-1}) – concentração de nutrientes no tempo final (mg L^{-1}))/ concentração de nutrientes no tempo inicial (mg L^{-1})]

1.6 Análise estatística

Os dados são representados por média ± desvio padrão. A normalidade e homocedasticidade dos dados foram avaliadas utilizando o teste de Shapiro-Wilk e Levene respectivamente. Quando os pressupostos foram atendidos foi realizado um test t de Student para avaliar diferenças entre os tratamentos. Nos casos em que as premissas do teste t de Student não foram atendidas, foi utilizado o teste não paramétrico de Kruskal-Wallis. Foi aplicado um nível de significância de 5% ($p \leq 0,05$) a todas as análises.

3. Resultados

3.1 Qualidade de água

Os resultados mostraram uma maior concentração da alcalinidade e pH no tratamento Quimioautotrófico comparado ao Heterotrófico. Em relação aos nutrientes, o nitrogênio amoniacal total e nitrato apresentaram menores concentrações no tratamento Quimioautotrófico, assim como a concentração de sólidos suspensos totais que apresentou diferença significativa ($p \leq 0.05$) com o tratamento Heterotrófico (Tabela 2).

Tabela 2. Parâmetros médios de qualidade de água (média ± desvio padrão) durantes os 15 dias de cultivo, nos tratamentos Quimioautotrófico e Heterotrófico.

	Tratamentos	
Parâmetros	**Quimioautotrófico**	**Heterotrófico**
Temperatura (ºC)	17.76 ± 1.19	17.21 ± 1.34
OD (mg L^{-1})	6.88 ± 0.32	6.99 ± 0.35
Ph	8.40 ± 0.04 [a]	8.29 ± 0.04 [b]
Salinidade	20.08 ± 0.50	21.74 ±1.33
Alcalinidade (mg $CaCO_3$ L^{-1})	290.00 ± 36.33 [a]	218.33 ± 34.74 [b]
Nitrogênio amoniacal total (mg L^{-1})	0.12 ± 0.02 [a]	0.19 ± 0.05 [b]
Nitrito (mg L^{-1})	0.11 ± 0.02	0.10 ± 0.04
Nitrato (mg L^{-1})	22.36 ± 1.93 [a]	27.33 ± 3.13 [b]
Fosfato (mg L^{-1})	3.54 ± 0.72	3.27 ± 0.70
Sólidos suspensos totais (mg L^{-1})	157.50 ± 81.32 [a]	303.33 ± 68.25 [b]

Letras diferentes na mesma linha indicam diferença significativa entre os tratamentos após teste t de Student.

No experimento foi constatado um decréscimo das concentrações de nitrato ao longo do experimento, em ambos os tratamentos. O tratamento Heterotrófico apresentou maior concentração inicial de nitrato, apresentando diferenças significativas ($p \leq 0.05$) com o tratamento Quimioautotrófico durante os dias de cultivo. No entanto, ao final do experimento não houve diferença significativa ($p > 0.05$) entre os tratamentos, mostrando concentração semelhante de nitrato (Figura 1). A concentração de fosfato não diferiu entre os tratamentos ($p > 0.05$), no entanto apresentou um acréscimo ao longo do experimento (Figura 2).

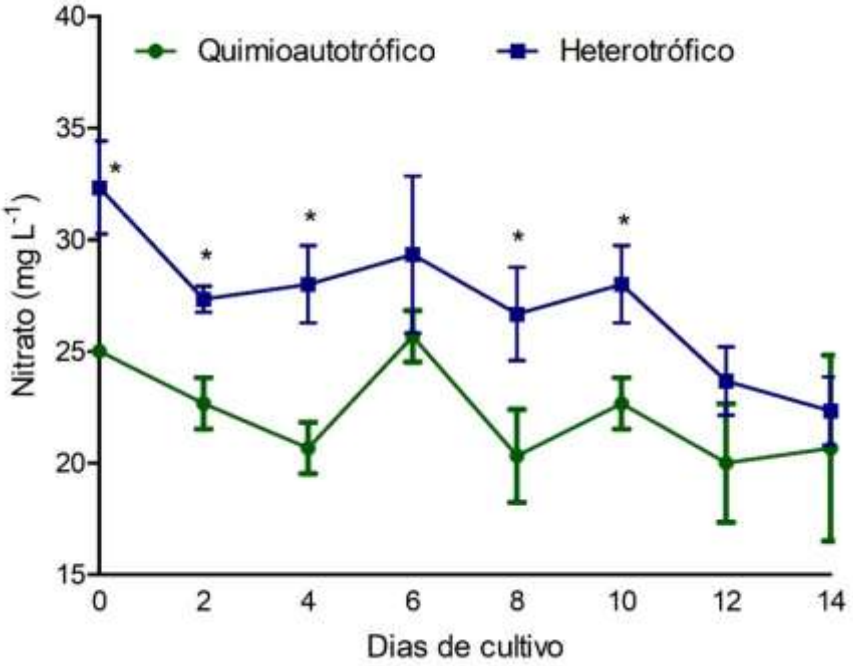


Figura 1. Concentração média de nitrato (mg L^{-1}) durante os dias de cultivo nos tratamentos Quimioautotrófico e Hetrotrófico. Asterisco (*) significam diferença significativa entre os tratamentos no mesmo dia.

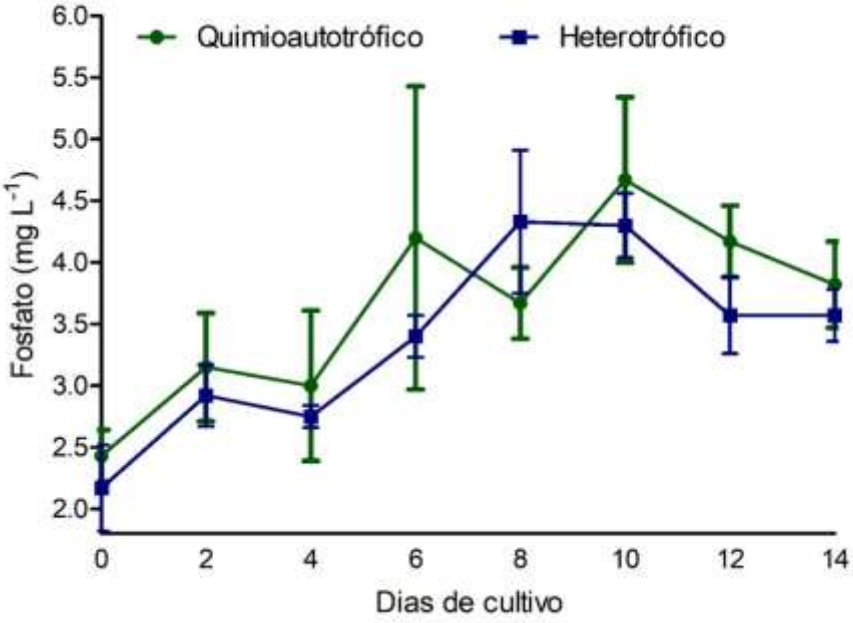


Figura 2. Concentração média de fosfato (mg L^{-1}) durante os dias de cultivo nos tratamentos Quimioautotrófico e Hetrotrófico.

3.2 Desempenho da macroalga

Não houve diferença significativa ($p > 0.05$) entre o peso médio final da macroalga entre os tratamentos, mostrando valores positivos de ganho de biomassa em ambos os tratamentos. No entanto, foi constatado uma taxa de remoção de nitrato maior no tratamento Heterotrófico comparado ao Quimioautotrófico (Tabela 3).

Tabela 3. Desempenho da macroalga (média ± desvio padrão) durante os 15 dias de cultivo, nos tratamentos Quimioautotrófico e Heterotrófico.

	Tratamentos	
Parâmetros	**Quimioautotrófico**	**Heterotrófico**
Peso médio final (kg)	5.98 ± 0.13	6.47 ± 1.03
TCR (% dia $^{-1}$)	3.53 ± 0.15	3.99 ± 1.12
Ganho de biomassa (kg)	2.46 ± 0.13	2.95 ± 1.03
TRN – nitrato (%)	14.67 ± 7.25 b	28.02 ± 10.71 a

Letras diferentes na mesma linha indicam diferença significativa entre os tratamentos após teste t de Student.

4 Discussão

Os diferentes tipos de fertilização no sistema de bioflocos poderão afetar a concentração dos nutrientes e sólidos do sistema, além de interferir no desempenho dos organismos. A manutenção do sistema quimioautotrófico ocorre com a inserção de carbono inorgânico, como bicarbonato de sódio e carbonato de cálcio, com o objetivo de elevar a alcalinidade do sistema para melhor estabelecimento e atividade das bactérias nitrificantes (Ebeling et al., 2006). De acordo com Furtado et al., (2011) a alcalinidade deve ser mantida acima de 150 mg CaCO3 L^{-1}, no entanto para sistemas quimioautotróficos a alcalinidade pode ser mantida em 300 mg CaCO3 L^{-1}. Com isso, os maiores valores da alcalinidade e pH encontrados no tratamento quimioautotrófico refletem a necessidade de manter o sistema com maiores concentrações para garantir o funcionamento eficiente.

No entanto, durante o período experimental não foi necessária a realização de correções com carbono inorgânico para elevar a alcalinidade. Sabe-se que as bactérias nitrificantes consomem 7.14 da alcalinidade, resultando em 1.69 g de carbono inorgânico, para cada grama de amônia convertida em nitrato no sistema quimioautotrófico, em comparação ao sistema heterotrófico que opera com o consumo de 3.57g da alcalinidade, resultando em 0.86 g de carbono inorgânico para conversão de um grama de amônia em biomassa

bacteriana (Ebeling et al., 2006). Essa manutenção da alcalinidade em ambos os tratamentos pode ter sido promovida devido ao consumo de dióxido de carbono pela macroalga. De acordo com Chopin, (2015) as macroalgas auxiliam no combate a acidificação dos oceanos, sendo importantes na ecologia marinha. Com isso, o cultivo de macroalgas poderá diminuir a quantidade de insumos utilizados na produção. Legarda et al., (2021) e Carvalho et al., (2024) utilizaram 0.38 e 0.07 g L^{-1} de carbono inorgânico em 35 e 45 dias de cultivo integrado com camarão e macroalgas, apresentado valores de utilização de carbono inorgânico inferiores comparado a sistemas de monocultivo.

Além da manutenção da alcalinidade, a inserção da macroalga para tratamento de efluente tem como finalidade a biorremediação com absorção dos nutrientes acumulados durante o período de cultivo, de acordo com Rio, (1996) a utilização de 2.5 g L^{-1} de peso fresco da macroalga *Ulva rigida* foi capaz de absorver em média 1.7 g de nitrogênio inorgânico dissolvido m^2 d^{-1} no tratamento de efluente de um cultivo de *Sparus aurata.* Em comparação a sistemas convencionais com renovação de água, o sistema de bioflocos opera como sistema fechado, resultando no acúmulo de nitrato e fosfato (Da Silva et al., 2013; Luo et al., 2020). Neste estudo, a concentração média de nitrato no tratamento Quimioautotrófico se mostrou inferior ao tratamento Heterotrófico, tal resultado foi decorrente das menores concentrações de nitrato no tratamento Quimioautotrófico no início do experimento. No entanto, quando avaliado todo o período experimental, o tratamento Heterotrófico apresentou o dobro da taxa de remoção de nitrato comparado ao sistema Quimioautotrófico, sendo mensurado que ao final do cultivo ambos os tratamentos apresentavam valores semelhantes de nitrato.

Tal resultado pode estar associado com melhores condições físico e químicas no tratamento Heterotrófico que resultou na maximização da absorção de nitrato. Para a macroalga, o balanceamento da relação nitrogênio e fosforo é essencial para melhores taxas de remoção, de acordo com Wheeler and Björnsäter, (1992) a relação N:P no tecido da macroalga é superior a 10 sendo diferente do encontrado para fitoplâncton, provavelmente devido a uma maior demanda de nitrogênio pela macroalga. Portanto uma maior disponibilidade de nitrogênio presente no tratamento Heterotrófico possibilitou que tal nutriente não fosse limitante, em comparação ao tratamento Quimioautotrófico. Além da disponibilidade de nitrogênio, o balanço N:P na relação 30:1 (N:P) poderá promover a maximização do crescimento e taxa de remoção de nutrientes pela macroalga (Harrison and Hurd, 2001), com isso o tratamento Heterotrófico também apresentou uma relação N:P mais

próxima do ideal, em comparação ao tratamento Quimioautotrófico que inicialmente teve uma relação abaixo de 30:1 o que pode ter resultado na limitação de nitrogênio.

Os resultados de taxa de remoção foram inferiores ao encontrado por Carvalho et al., (2024b) com o mesmo sistema de fertilizações no bioflocos em sistema integrado com camarão e peixe, obtendo uma taxa de remoção de nitrato de 57% e 47% para fosfato. A produção constante de nutrientes no sistema integrado se torna uma fonte de alimento disponível para a macroalga, sendo mínima a limitação de nitrogênio ou fosforo, diferente do tratamento de efluente que com a redução de algum dos nutrientes causará limitação e desbalanceamento da relação ideal. A produção de nitrogênio é proveniente da excreção dos animais, e lixiviação da ração (Da Silva et al., 2013), fatores que não estão presentes quando se trabalha apenas com o efluente da produção aquícola. Utilizando a macroalga *Ulva ohnoi* para tratamento de efluente de camarão Martins et al., (2020) tiveram como um resultado a absorção de 58% do nitrato em três semanas de experimento utilizando o dobro de biomassa comparado a este experimento.

O fosfato representa um nutriente importante para realização da fotossíntese e formação de tecidos, e quando limitante no meio poderá interferir no crescimento da macroalga (Zirino et al., 2016). Neste experimento, os valores de fosfato foram crescentes no decorrer dos dias de cultivo em ambos os tratamentos, podendo estar associado a uma maior produção de fosfato nos tanques em paralelo a um menor consumo de fosfato pela macroalga. Apesar de ser um nutriente importante para o crescimento da macroalga, o requerimento de fosfato em relação ao nitrogênio é menor, de acordo com Shakouri and Balouch, (2020) para melhor crescimento da macroalga *Ulva rigida* é necessário 30 mg L^{-1} de nitrato e 15 mg L^{-1} de fosfato, portanto se utiliza mais nitrogênio disponível no meio do que o fosfato, e assim a macroalga não foi capaz de realizar uma taxa de remoção do nutriente.

De acordo com Da Silva et al., (2013), apesar do fósforo não apresentar concentrações tóxicas, as altas concentrações acumuladas podem vir a desencadear blooms de fitoplancton e eutrofização, sendo esse fósforo produzido através da lixiviação da ração e morte de organismos. Neste experimento, como não foi realizada oferta de ração, o fósforo produzido pode ter sido proveniente da decomposição do biofloco. O floco microbiano é formado por um aglomerado de bactérias, fitoplâncton e zooplâncton (Reis et al., 2019), e a morte desses organismos liberam o nitrogênio e fosfato acumulados no tecido.

Diferentes de sistemas de recirculação e renovação de água, o sistema de bioflocos opera com uma elevada carga orgânica formada por agregados microbianos (Gaona et al., 2017).

No sistema quimioautotrófico a concentração de sólidos foi inferior ao sistema heterotrófico, tal resultado também foi encontrado por Ferreira et al., (2021). Isso se deve ao uso do carbono orgânico no sistema heterotrófico, que tem como função a manutenção da relação Carbono:nitrogênio para favorecimento de bactérias que irão consumir a amônia e transformar em biomassa bacteriana e assim aumentar a concentração de sólidos suspensos totais no sistema (Hostins et al., 2019). No entanto, o sistema de tratamento de efluente apesar da aeração intensa, não haviam animais como camarão e peixe para realizar a movimentação da água e suspensão dos sólidos, portanto em alguns pontos do tanque, pode ter ocorrido a formação de zonas de decantação e decomposição do floco, o que ocasionou o aumento do fosfato na água. A inserção de um organismo séssil como a macroalga também auxilia na decantação dos sólidos, formando uma barreira na movimentação da água e sua deposição sobre a lamina da macroalga (Carvalho et al., 2023a).

Esse aprisionamento de sólidos na superfície da macroalga também pode afetar seu crescimento devido à baixa incidência de luz e realização da fotossíntese. No entanto, diferente dos trabalhos realizados por Carvalho et al., (2023ª), Morais et al., (2023), Legarda et al., (2021) com cultivo de macroalga em sistema integrado com bioflocos que apresentaram perda de biomassa ou taxa de crescimento relativa de 0.14% dia^{-1}, neste estudo as taxas foram superiores apresentando como média geral de 3.76 ± 0.76 % dia^{-1}. Essa alta taxa de crescimento pode estar relacionada com a estabilização na produção de sólidos quando se opera com tratamento de efluente, além da possível decantação de sólidos no fundo do tanque, tornando a água mais clara. Em sistema integrado a produção de sólidos é contínua devido a presença dos organismos (Gaona et al., 2017), podendo ser um fator estressante para as macroalgas e que não é evidenciado quando se trabalha com tratamento de efluente. Além da concentração de sólidos, a temperatura também pode ser um fator importante para maiores taxas de crescimento da macroalga, de acordo com Hiraoka and Oka, (2008) a maior taxa de crescimento para a *Ulva fasciata* utilizando água salgada de profundidade foi de 22 ºC, no entanto foi ainda constatado um crescimento em águas com 13ºC e um decréscimo de biomassa com temperaturas superiores a 22ºC. Portanto os valores médios de temperatura em nosso trabalho podem ter contribuído para uma melhor taxa de crescimento.

Os resultados foram similares aos encontrados por Resende et al., (2022) com taxa de crescimento específica de 3.82 % dia^{-1} na primeira semana de cultivo da macroalga *Ulva* no efluente da piscicultura, destacando que o experimento foi realizado em condições

controladas de laboratório e com uso de malha para filtração prévia do efluente, diferente das condições empregadas neste experimento. Os resultados obtidos neste experimento foram superiores ao encontrado por Ramos and Gallardo, (2021) usando *Ulva lactuca* para tratamento de efluente de *Seriola lalandi* obtendo apenas 0.78 % dia^{-1} provavelmente pela baixa concentração de nutrientes encontrada no efluente. Portanto, devido à alta disponibilidade de nitrogênio e fosforo no sistema de bioflocos decorrente da ação das bactérias e nula renovação de água, o efluente do sistema de bioflocos se torna uma alternativa viável para o crescimento e biorremediação da macroalga.

5 Conclusão

O uso do efluente com fertilização orgânica para cultivo da macroalga *Ulva lactuca* se mostrou viável com taxa de remoção de 28% do nitrato e uma taxa de crescimento relativa de 3.99%. A utilização de macroalgas como biorremediador além da remoção de nitrogenados também atuou no controle do pH e alcalinidade do sistema, reduzindo o uso de insumos. Entretanto, melhores formas de manejo devem ser adotadas para manutenção dos sólidos suspensos totais para que não ocorra a decomposição do bioflocos no sistema.

Referências

Aminot, A., Chaussepied, M., 1983. Manuel des analyses chimiques en milieu marin. Centre National pour l'exploitation des océans, França.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, (21st edn). ed. American Public Health Association, Washington, DC, USA.

Balar, N.B., Mantri, V.A., 2020. Insights into life cycle patterns, spore formation, induction of reproduction, biochemical and molecular aspects of sporulation in green algal genus Ulva: implications for commercial cultivation. J. Appl. Phycol. 32, 473–484. https://doi.org/10.1007/s10811-019-01959-7

Baumgarten, M. D. G. Z., De Barros Rocha, J. M., & Niencheski, L.F.H., 1996. Manual de análises em oceanografia química.

Boyd, C.E., D'Abramo, L.R., Glencross, B.D., Huyben, D.C., Juarez, L.M., Lockwood, G.S., McNevin, A.A., Tacon, A.G.J., Teletchea, F., Tomasso, J.R., Tucker, C.S., Valenti, W.C., 2020. Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. J. World Aquac. Soc. 51, 578–633. https://doi.org/10.1111/jwas.12714

Brandão, H., Xavier, Í.V., Santana, G.K.K., Santana, H.J.K., Krummenauer, D., Wasielesky, W., 2021. Heterotrophic versus mixed BFT system: Impacts on water use, suspended solids production and growth performance of *Litopenaeus vannamei*. Aquac. Eng. 95. https://doi.org/10.1016/j.aquaeng.2021.102194

Carvalho, A., Braga, Í., Chaar, F., Cardozo, A.P., Monserrat, J.M., Ramírez, J.R.B., Wasielesky, W., Poersch, L.H., 2024a. Production of the Macroalgae *Ulva lactuca* Integrated with the Shrimp *Penaeus vannamei* in a Biofloc System: Effect of Total Suspended Solids and Nutrient Concentrations. Phycology 4, 37–52. https://doi.org/10.3390/phycology4010002

Carvalho, A., Brandão, H., Zemor, J.C., Cardozo, A.P., Vieira, F.N., Okamoto, M.H., Turan, G., Poersch, L.H., 2024b. Effect of Organic or Inorganic Fertilization on Microbial Flocs Production in Integrated Cultivation of *Ulva lactuca* with *Oreochromis niloticus* and *Penaeus vannamei*. Fishes 9. https://doi.org/10.3390/fishes9060191

Carvalho, A., Costa, L.C. de O., Holanda, M., Gonçalves, M., Santos, J., Costa, C.S.B., Turan, G., Poersch, L.H., 2023a. Growth of the Macroalgae *Ulva lactuca* Cultivated at Different Depths in a Biofloc Integrated System with Shrimp and Fish. Phycology 3, 280–293. https://doi.org/10.3390/phycology3020018

Carvalho, A., Costa, L.C. de O., Holanda, M., Poersch, L.H., Turan, G., 2023b. Influence of Total Suspended Solids on the Growth of the Sea Lettuce *Ulva lactuca* Integrated with the Pacific White Shrimp *Litopenaeus vannamei* in a Biofloc System. Fishes 8, 163. https://doi.org/10.3390/fishes8030163

Chopin, T., 2015. Marine Aquaculture in Canada: Well-Established Monocultures of Finfish and Shellfish and an Emerging Integrated Multi-Trophic Aquaculture (IMTA) Approach Including Seaweeds, Other Invertebrates, and Microbial Communities. Fisheries 40, 28–31. https://doi.org/10.1080/03632415.2014.986571

Copertino, M.D.S., Tormena, T., Seeliger, U., 2009. Biofiltering efficiency, uptake and assimilation rates of *Ulva clathrata* (Roth) J. Agardh (Clorophyceae) cultivated in shrimp aquaculture waste

water. J. Appl. Phycol. 21, 31–45. https://doi.org/10.1007/s10811-008-9357-x

Da Silva, K.R., Wasielesky, W., Abreu, P.C., 2013. Nitrogen and Phosphorus Dynamics in the Biofloc Production of the Pacific White Shrimp, *Litopenaeus vannamei*. J. World Aquac. Soc. 44, 30–41. https://doi.org/10.1111/jwas.12009

de Morais, A.P.M., Santos, I.L., Carneiro, R.F.S., Routledge, E.A.B., Hayashi, L., de Lorenzo, M.A., do Nascimento Vieira, F., 2023. Integrated multitrophic aquaculture system applied to shrimp, tilapia, and seaweed (*Ulva ohnoi*) using biofloc technology. Aquaculture 572. https://doi.org/10.1016/j.aquaculture.2023.739492

Duke, C.S., Litaker W., Ramus J, 1989. Effects of the temperature, nitrogen supply and tissue nitrogen on ammonium uptake rates of the Chlorophyte seaweeds *Ulva curvata* and *Codium decorticatum*. J. Phycol.

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

FAO, 2024, 2024. World fisheries and aquaculture in review, State of the World Fisheries and Aquaculture.

Ferreira, G.S., Santos, D., Schmachtl, F., Machado, C., Fernandes, V., Bögner, M., Schleder, D.D., Seiffert, W.Q., Vieira, F.N., 2021. Heterotrophic, chemoautotrophic and mature approaches in biofloc system for Pacific white shrimp. Aquaculture 533. https://doi.org/10.1016/j.aquaculture.2020.736099

Furtado, P.S., Poersch, L.H., Wasielesky, W., 2011. Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical performance of shrimp *Litopenaeus vannamei* reared in bio-flocs technology (BFT) systems. Aquaculture 321, 130–135. https://doi.org/10.1016/j.aquaculture.2011.08.034

Gaona, C.A.P., de Almeida, M.S., Viau, V., Poersch, L.H., Wasielesky, W., 2017. Effect of different total suspended solids levels on a *Litopenaeus vannamei* (Boone, 1931) BFT culture system during biofloc formation. Aquac. Res. 48, 1070–1079. https://doi.org/10.1111/are.12949

Hadley, S., Wild-Allen, K., Johnson, C., Macleod, C., 2015. Modeling macroalgae growth and nutrient dynamics for integrated multi-trophic aquaculture. J. Appl. Phycol. 27, 901–916. https://doi.org/10.1007/s10811-014-0370-y

Harrison, P.J., Hurd, C.L., 2001. Nutrient physiology of seaweeds: Application of concepts to aquaculture. Cah. Biol. Mar. 42, 71–82.

Hiraoka, M., Oka, N., 2008. Tank cultivation of *Ulva prolifera* in deep seawater using a new "germling cluster" method. J. Appl. Phycol. 20, 97–102. https://doi.org/10.1007/s10811-007-9186-3

Hostins, B., Wasielesky, W., Decamp, O., Bossier, P., De Schryver, P., 2019. Managing input C/N ratio to reduce the risk of Acute Hepatopancreatic Necrosis Disease (AHPND) outbreaks in biofloc systems – A laboratory study. Aquaculture 508, 60–65. https://doi.org/10.1016/j.aquaculture.2019.04.055

Krummenauer, D., Peixoto, S., Cavalli, R.O., Poersch, L.H., Wasielesky, W., 2011. Superintensive culture of white shrimp, *Litopenaeus vannamei*, in a biofloc technology system in Southern Brazil at different stocking densities. J. World Aquac. Soc. 42, 726–733. https://doi.org/10.1111/j.1749-

7345.2011.00507.x

Legarda, E.C., da Silva, D., Miranda, C.S., Pereira, P.K.M., Martins, M.A., Machado, C., de Lorenzo, M.A., Hayashi, L., do Nascimento Vieira, F., 2021. Sea lettuce integrated with Pacific white shrimp and mullet cultivation in biofloc impact system performance and the sea lettuce nutritional composition. Aquaculture 534, 736265. https://doi.org/10.1016/j.aquaculture.2020.736265

Lundberg, P., Weich, R.G., Jensén, P., Vogel, H.J., 1989. Phosphorus-31 and Nitrogen- 14 NMR Studies of the Uptake of Phosphorus and Nitrogen Compounds in the Marine Macroalgae Ulva lactuca . Plant Physiol. 89, 1380–1387. https://doi.org/10.1104/pp.89.4.1380

Luo, G., Xu, J., Meng, H., 2020. Nitrate accumulation in biofloc aquaculture systems. Aquaculture 520, 734675. https://doi.org/10.1016/j.aquaculture.2019.734675

Macedo, C.F., Sipaúba-Tavares, L., 2010. Eutrofização E Qualidade Da Água Na Piscicultura : Consequências E Recomendações Eutrophication and Water Quality in Pisciculture : Consequences and Recommendations. Bol. Inst. Pesca SãoPaulo, 149–163.

Mansilla, A., Rodriguez, J.P., Souza, J.M.C., Rosenfeld, S., Ojeda, J., Yokoya, N.S., 2014. Growth responses to temperature, salinity and nutrient variations, and biomass variation and phenology of *Ahnfeltia plicata* (Rhodophyta, Ahnfeltiales): a commercially interesting agarophyte from the Magellanic Region, Chile. J. Appl. Phycol. 26. https://doi.org/10.1007/s10811-013-0150-0

Martins, M.A., da SILVA, V.F., Tarapuez, P.R., Hayashi, L., Vieira, F.D.N., 2020. Cultivation of the seaweed *Ulva* spp. With effluent from a shrimp biofloc rearing system: Different species and stocking density. Bol. do Inst. Pesca. https://doi.org/10.20950/1678-2305.2020.46.3.602

Mata, L., Magnusson, M., Paul, N.A., de Nys, R., 2016. The intensive land-based production of the green seaweeds Derbesia tenuissima and *Ulva ohnoi*: biomass and bioproducts. J. Appl. Phycol. 28, 365–375. https://doi.org/10.1007/s10811-015-0561-1

Miguel Jimenez del Rio, Z.R.& G.G.-R., 1996. *Ulva rigida* (Ulvales, Chlorophyta) tank culture as biofilters for dissolved inorganic nitrogen from fishpond effluents.

Ramos, R., Gallardo, S., 2021. Capacidad de biofiltración de nutrientes y crecimiento de macroalgas utilizando efluentes generados en el cultivo del pez dorado *Seriola lalandi* (Perciformes: Carangidae). Rev. Biol. Mar. Oceanogr. https://doi.org/10.22370/rbmo.2021.56.1.2795

Reis, W.G., Wasielesky, W., Abreu, P.C., Brandão, H., Krummenauer, D., 2019. Rearing of the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931)in BFT system with different photoperiods: Effects on the microbial community, water quality and zootechnical performance. Aquaculture 508, 19–29. https://doi.org/10.1016/j.aquaculture.2019.04.067

Resende, L., Flores, J., Moreira, C., Pacheco, D., Baeta, A., Garcia, A.C., Rocha, A.C.S., 2022. Effective and Low-Maintenance IMTA System as Effluent Treatment Unit for Promoting Sustainability in Coastal Aquaculture. Appl. Sci. 12. https://doi.org/10.3390/app12010398

Schiavon, M., Moro, I., Pilon-Smits, E.A.H., Matozzo, V., Malagoli, M., Dalla Vecchia, F., 2012. Accumulation of selenium in *Ulva* sp. and effects on morphology, ultrastructure and antioxidant enzymes and metabolites. Aquat. Toxicol. 122–123, 222–231. https://doi.org/10.1016/j.aquatox.2012.06.014

Shakouri, A., Balouch, G.M., 2020. The effects of nitrate and phosphate on growth of algae, *Ulva rigida*. Iran. J. Fish. Sci. 19, 59–66. https://doi.org/10.22092/ijfs.2018.117843

Unesco, 1983. Chemical methods for use in marine environmental monitoring. Intergovernmental Oceanographic Commission.

Wasielesky, W., Krummenauer, D., Lara, G., Fóes, G., 2013. Cultivo de camarões em sistema de bioflocos : realidades e perspectivas 30–36.

Wheeler, P.A., Björnsäter, B.R., 1992. Seasonal Fluctuations in Tissue Nitrogen, Phosphorus, and N:P for Five Macroalgal Species Common To the Pacific Northwest Coast. J. Phycol. https://doi.org/10.1111/j.0022-3646.1992.00001.x

Zirino, A., Elwany, H., Facca, C., Maicu', F., Neira, C., Mendoza, G., 2016. Nitrogen to phosphorus ratio in the Venice (Italy) Lagoon (2001-2010) and its relation to macroalgae. Mar. Chem. 180, 33–41. https://doi.org/10.1016/j.marchem.2016.01.002

Zollmann, M., Rubinsky, B., Liberzon, A., Golberg, A., 2021. Multi-scale modeling of intensive macroalgae cultivation and marine nitrogen sequestration. Commun. Biol. 4. https://doi.org/10.1038/s42003-021-02371-z

CAPÍTULO 7: AVALIAÇÃO DO USO DA MACROALGA *Ulva lactuca* PRODUZIDA EM SISTEMA INTEGRADO COM BIOFLOCOS NA DIETA DA TILÁPIA *Oreochromis niloticus*

Andrezza Carvalho[1 *], Larissa Müller[4], Victor Torres[2], Marcelo Borges Tesser[3], Juliane Ventura-Lima[4], Gamze Turan[5], Marcelo Pias[6], Luís Poersch[1]

[1] Estação Marinha de Aquacultura, Instituto de Oceanografia, Universidade Federal do Rio Grande—FURG, Rua do Hotel, no. 2, Cassino, Rio Grande 96210-030, RS, Brazil.
[2] Environmental Science Center, Qatar University, P.O. Box: 2713, Doha, Qatar.
[3] Laboratório de Nutrição de Organismos Aquáticos, Instituto de Oceanografia, Universidade Federal de Grande-FURG-Rua do Hotel, 02, Cassino, 96201-900, Brazil.
[4] Instituto de Ciências Biológicas (ICB), Universidade Federal de Rio Grande, FURG, Rio Grande, RS, Brazil.
[5] Ege University Fisheries Faculty, Aquaculture Department 35100, Bornova, Izmir, Turkey.
[6] Centro de Ciencias Computacionais, Universidade Federal do Rio Grande – FURG, Av. Itália, km 8, Carreiros, Rio Grande, RS, Brazil.

*Corresponding Author: andrezzachagas@hotmail.com

Resumo

A biomassa de macroalgas produzidas em sistemas integrados com bioflocos pode se tornar um produto de alta qualidade nutricional para substituição de ingredientes na dieta para a tilápia, aplicando conceitos da economia circular. Com isso, este trabalho teve como objetivo avaliar diferentes níveis de inclusão da macroalga *Ulva lactuca* no desempenho zootécnico, hematológico e capacidade antioxidante na tilápia-do-Nilo *Oreochromis niloticus*. Foram realizadas quatro dietas isoproteicas (40%) e isolipídicas (8%), com inclusões de 5, 10 e 15% de farinha de macroalga na dieta, e um tratamento controle sem a inclusão da macroalga. O experimento teve duração de 42 dias em um sistema de recirculação de água. Ao final do experimento, foi realizado a pesagem dos animais, coleta de sangue e composição proximal. Para avaliar o potencial benéfico da inclusão da biomassa de algas, um teste de estresse com salinidade foi realizado nos peixes e após foram realizadas coletas para análises bioquímicas. Não houve diferenças significativas no desempenho zootécnico e composição bromatológica da carcaça entre os tratamentos. Os resultados após teste de estresse mostraram que a inclusão de 10% de macroalga na ração resultou em maior contagem de granulócitos, enquanto a capacidade antioxidante obteve melhores resultados nas inclusões de 5 e 10% de macroalga, seguido por uma modulação do sistema antioxidante evidenciado pelo aumento da atividade da GST e níveis de GSH. No entanto, apenas no tratamento 5% de inclusão não ocorreu oxidação proteica e lipídica comparado aos tratamentos com maior inclusão, provavelmente associado

aos pigmentos antioxidantes presentes na macroalga. Portanto, menores inclusões de macroalga (5%) na dieta da tilápia são indicadas para melhora na capacidade antioxidante do organismo frente ao estresse, além de apresentar maior sustentabilidade e economia circular no sistema.

Palavras-chave: Biocompostos; Macroalga; Dieta; Estresse; Antioxidante.

1. Introdução

A produção de macroalgas em sistema integrado tem apresentado resultados significativos, tais como o aumento de produtividade e remoção de nutrientes do cultivo (Ben-Ari et al., 2014; Queirós et al., 2021; Resende et al., 2022). Essa associação de organismos de diferentes níveis tróficos é definida como Aquicultura Multitrófica Integrada (IMTA), que consiste na escolha de consumidores orgânicos e/ou inorgânicos com a finalidade de reaproveitar resíduos produzidos pela espécie de maior nível trófico (Chopin, 2015). Com isso, a disponibilidade de nutrientes durante o cultivo permite que as macroalgas sejam cultivadas em sistemas integrados, podendo apresentar taxa de crescimento maior quando comparado com cultivos em mar aberto, além de remover cerca de 90% da amônia disponível no sistema (Alencar et al., 2010). Resende et al. (2022) reportaram o incremento de nitrogênio no tecido e capacidade de absorção de amônia, nitrato e fosfato pela macroalga *Ulva* spp. quando cultivada em sistema integrado em escala piloto com Dourada (*Sparus aurata)* e Robalo (*Dicentrarchus labrax*).

Com isso, a inserção de macroalgas em sistemas intensivos com baixa troca de água pode auxiliar no reaproveitamento de resíduos inorgânicos para formação de biomassa. A tecnologia de bioflocos (BFT) é classificado como um sistema intensivo por permitir o aumento da densidade de organismos e a manutenção da qualidade de água através do crescimento da comunidade microbiana, como bactérias, microalgas e protozoários (Khanjani et al., 2023). As bactérias presentes podem oxidar a amônia a nitrito e posteriormente a nitrato ou transformar a amônia em biomassa bacteriana (Ebeling et al., 2006). Nesse sistema, o de nitrato e fosfato representam mais de 80% dos compostos inorgânicos produzidos e acumulados no sistema em 42 dias de cultivo de acordo com Silva et al. (2013) sendo propício a inserção de macroalgas no sistema para reaproveitamento dos nutrientes. Carvalho et al. (2024) mostraram que uma remoção de 55% de nitrato e 31% de fosfato foi realizada pela macroalga em sistema integrado com camarão em bioflocos.

A alta disponibilidade de nutrientes e elevada carga orgânica presentes no sistema de bioflocos podem alterar a composição nutricional da macroalga. Devido a baixa penetração de

luz causada pelo acumulo de sólidos em suspensão (Reis et al., 2019), as macroalgas cultivadas em sistema de bioflocos tendem a aumentar a clorofila a e b para maximização da fotossíntese (Carvalho et al., 2024; Levavasseur, 1989). Além do incremento da clorofila, as macroalgas *Ulva lactuca* produzidas no sistema de bioflocos apresentam maior concentração proteica comparativamente as produzidas em solução laboratorial (Carvalho et al., 2023). Este fato é devido a alta disponibilidade de nutrientes. Assim, ambientes diferentes podem ter efeitos no valor nutricional das macroalgas como salinidades abaixo de 25 ppt, que influenciam a concentração de aminoácidos e ácidos graxos nas macroalgas (Xu et al., 2018).

Com isso, a produção de biomassa de macroalgas em sistema integrado com bioflocos com elevado teor nutricional pode fornecer um produto de qualidade que poderá retornar ao sistema de produção como aditivo na ração de organismos aquáticos (Montgomery and Gerking, 1980). Diferente da economia linear que consiste na produção e venda do produto, a proposta da economia circular possui um conceito mais amplo e visa a obtenção de produtos ou subprodutos e sua inserção novamente no sistema de cultivo (Kirchherr et al., 2017). Portanto, com essa prática os recursos produzidos no sistema são reutilizados várias vezes em um circuito fechado (Cornejo-Ponce et al., 2020). O termo economia circular reflete na adoção de práticas relacionadas a melhor utilização dos nutrientes produzidos, o gerenciamento de resíduos e a utilização de novos ingredientes para substituição na ração (Cooney et al., 2023). Tal conceito tem sido promovido pela Comissão Europeia como economia verde aplicada em setores marítimos e costeiros, como estímulo a novas práticas de gestão, devido aos recursos limitados e grandes impactos ambientais, com foco em fornecer uma aquicultura sustentável (Campanati et al., 2021).

De acordo Mwendwa et al. (2023) a produção de macroalgas para fins comerciais possui elevado retorno financeiro, com o aumento de infraestruturas em comunidades locais, principalmente quando associado com sistemas de produções integrados. Essa produção quando aplicada em dietas podem diminuir custos com a ração comercial associada com melhora no desempenho como observado por Saleh et al. (2014). Marinho et al., (2013) testando inclusões de 10, 15 e 20 % de farinha de macroalga *Clorhophyta* produzida no sistema IMTA na dieta para a tilápia, constatou que as dietas com até 10% de inclusão de macroalgas são possíveis sem prejudicar o desempenho zootécnico da espécie. A utilização da tilápia *O. niloticus* em estudos com substituição de ingredientes se deve ao hábito alimentar onívoro da espécie, além da facilidade de obtenção de juvenis, ao rápido crescimento e à possibilidade de cultivo em diversos locais, sendo uma espécie amplamente produzida no Brasil e com grande interesse econômico (Santos et al., 2020). Podendo ser cultivada em salinidade de 0 a 16 sem afetar seu

desempenho zootécnico de acordo com Souza et al. (2019), sendo promissora para sua produção em cultivos integrados.

Uma vantagem da utilização de macroalgas em dietas para aquicultura é seu teor de compostos fenólicos, sendo um composto que está interligado com o aumento da palatabilidade da ração (Yildiz et al., 2012). Além disso os compostos fenólicos possuem propriedades antioxidantes (He et al., 2016), que em cultivos de altas densidades podem ajudar a remover o excesso de espécies reativas de oxigênio (ROS) bem como restabelecer o sistema antioxidante dos organismos. As macroalgas verdes também possuem características imunoestimulantes através da presença de um polissacarídeo sulfatado chamado Ulvana, podendo estar interligado na redução da incidência de doenças crônicas, propriedades antivirais e antioxidantes (Kidgell et al., 2019). O ambiente de cultivo da macroalga também pode interferir na produção de biocompostos, como resultado apresentado por Xu et al., (2018) mostrando que a quantidade de nutrientes afeta positivamente habilidade da capacidade antioxidante e concentração de polifenois totais na macroalga.

O grupo de macroalgas verdes por apresentar ausência ou menor concentração de polissacarídeos como alginatos, carragenana e ágar (Ju et al., 2023), representa uma opção promissora na aplicação em formulação de rações. Entretanto, apesar do hábito onívoro da tilápia proporcionar a utilização de uma dieta diversificada, o uso de alguns alimentos de baixa digestibilidade podem influenciar no desempenho do animal, custo da ração e qualidade de água do cultivo (Oliva-Teles, 2012). Por serem formadas por células vegetais com parede celular, as macroalgas podem apresentar em sua composição alto teor de fibras (Ju et al., 2023), que quando inseridas na ração de organismos aquáticos podem atuar como fator antinutricional (Mhlongo and Mnisi, 2023). Com isso, o objetivo deste trabalho foi avaliar diferentes níveis de inclusão da macroalga *Ulva lactuca* proveniente de um cultivo em sistema integrado com bioflocos no desempenho zootécnico e respostas antioxidantes da tilápia *Oreochromis niloticus*.

2. Materiais e métodos

2.1 Local e procedência dos animais

O experimento foi realizado na Estação Marinha de Aquacultura – EMA, tendo duração de 42 dias, com o número de processo do CEUA 000476.0013263/2024. Os peixes foram provenientes de uma piscicultura comercial, alimentados com uma ração comercial GuabiTech Inicial Larvas com 55% de proteína bruta, pó (Guabi Nutrition and Animal Health S.A., Campinas, São Paulo, SP, Brazil) por um período de aclimatação de três semanas,

posteriormente a salinidade foi ajustada gradualmente até a salinidade desejada de 15 ppt para início do experimento.

As macroalgas foram provenientes de cultivo integrado com o camarão *Litopenaeus vannamei* e a tilápia *Oreochromis niloticus* em sistema de bioflocos, com tempo de cultivo de 70 dias, e valores médios de nitrato e fosfato de 63.06 ± 26.00 mg L^{-1} de nitrato e 5.71 ± 3.97 mg L^{-1} de fosfato (Carvalho et al., 2024). A biomassa de macroalgas foi coletada e levada ao laboratório para lavagem em água do doce. Posteriormente foi colocada em estufa para secar em 60 ºC por 24 horas, triturada até chegar em pó e retirada amostras para análise centesimal (Tabela 1).

Tabela 1. Composição proximal da macroalga *Ulva lactuca* cultivada em bioflocos.

	Proteína	Lipídio	Fibra	Cinzas
Farinha de Ulva	19.31 ± 0.30	0.46 ± 0.04	10.34 ± 0.72	29.05 ± 0.63

2.2 Delineamento experimental

Para a realização do experimento, foram fabricadas quatro rações isoproteícas (40% de proteína bruta) e isolipídicas (8% de extrato etéreo) no Laboratório de Nutrição de Organismos Aquáticos – LANOA na FURG, sendo elas: Controle - ração controle produzida em laboratório sem adição de macroalgas; T5 - 5% de inclusão de macroalgas na ração; T10 - 10% de inclusão de macroalgas; T15 - 15% de inclusão de macroalgas, todas as dietas experimentais testadas em quadruplicata (Tabela 2).

O experimento foi realizado em tanques com recirculação de água que continha um macrocosmo de 800 L de volume útil, com a presença de substrato artificial (0.60 m^2), e por meio de uma bomba submersa (SPA 4000 L/h, BOYU©, Guangdong, China) a água recirculava para 16 unidades com 50 L de volume útil a uma velocidade de 2.60 L min^{-1}. Para retirada da matéria orgânica no fundo das caixas, os tanques eram sifonados três vezes na semana.

Foram utilizados dez peixes por tanque com peso inicial de 0.94 ± 0.01. Os peixes foram alimentados a uma taxa fixa de 6% da biomassa total por dia, sendo dividido em 3 alimentações durante o dia (8:00am, 12:00pm e 17:00pm) (Huang et al., 2015). Foi realizada uma biometria inicial e final, o ajuste de ração foi realizado com o uso de um fator de conversão alimentar de 1:1 até a sexta semana e de 1:5 na última semana. Ao final do experimento os peixes foram anestesiados com benzocaína (De Miranda Cabral Gontijo et al., 2003) e pesados para a determinação das variáveis de desempenho zootécnico. As amostras para composição proximal

e hematológica foram retiradas ao final do experimento, após anestesia e eutanásia dos animais com benzocaína, na concentração de 400 ppm (De Miranda Cabral Gontijo et al., 2003).

Tabela 2. Composição dos ingredientes e análise de proximidade das dietas experimentais (% de matéria seca) contendo farinha de *U. lactuca* em diferentes níveis.

Ingredientes	Dietas experimentais			
	Controle	T5	T10	T15
Farinha de peixe	40.00	40.00	40.00	40.00
Farelo de soja	33.00	32.00	31.00	30.00
Farelo de trigo	12.00	8.00	4.00	0.00
Gelatina	2.00	2.00	2.00	2.00
Óleo de soja	2.00	2.00	2.00	2.00
Óleo de peixe	2.00	2.00	2.00	2.00
Celulose	4.00	4.00	4.00	4.00
Vitamina/mineral mix	5.00	5.00	5.00	5.00
Farinha de Ulva	0.00	5.00	10.00	15.00
Composição proximal (%)				
Proteína	42.44	41.82	41.37	39.98
Lipídeo	8.23	8.07	8.05	8.64
Cinzas	16.28	17.45	19.01	20.02
Energia bruta	17.63	17.34	17.32	17.42

Energia bruta: Expresso em MJ/kg peso úmido da raçaõ

Após a coleta de amostras e pesagem dos animais, foram selecionados três peixes de cada tanque dos tratamentos Controle, T5, T10 e T15 para realização de um teste de estresse salino. Com isso os peixes foram transferidos e mantidos por 24 horas em novas unidades experimentais feitas de tanques de polietileno com 30 L de volume útil, e ocorreu a redução da salinidade de 15 para 0 ppt sem aclimatação. Como controle do estresse salino, foram coletados três peixes do tratamento Controle e mantidos na salinidade do experimento (15 ppt). Ao final, para o estresse salino foram utilizadas15 unidades experimentais e 5 tratamentos, sendo eles, Sal15 (controle sem alga e na salinidade 15 ppt de cultivo), Sal0C0 (controle sem alga na salinidade 0 ppt), Sal0T5 (dieta com 5% de macroalga na salinidade 0 ppt), Sal0T10 (dieta com 10% de na salinidade 0 ppt) e Sal0T15 (dieta com 15% de macroalga na salinidade 0 ppt). Após as 24hs de exposição, os 3 peixes de cada tanque (9 de cada tratamento) foram eutanasiados por congelamento e dissecados. Foram coletados as brânquias, fígado e músculo de cada peixe e conservados a -80 ºC para futuras análises bioquímicas.

2.3 Qualidade de água e desempenho zootécnico

Para o monitoramento da qualidade da água, a temperatura (°C), oxigênio dissolvido (OD, mg L^{-1}) e pH foram medidos diariamente com o auxílio de uma sonda multiparâmetros (YSI, modelo Pro-20, EUA) e um pHmetro de bancada (Mettler Toledo, FEP20, Brasil). A salinidade

(ppt) foi medida duas vezes na semana usando uma sonda multiparâmetros (YSI, modelo Pro-20, EUA). O nitrogênio amoniacal total (ou TAN, mg L^{-1}) e nitrito (mg L^{-1}) foram analisados de acordo com a metodologia da Unesco (1983) e Bendschneider, K., Robinson, R.J., (1952) diariamente até a estabilização do sistema e após foram realizados duas vezes na semana. Nitrato (mg L^{-1}) e fosfato (mg L^{-1}) foram analisados pela metodologia descrita por Aminot, A., Chaussepied, (1983) e monitorados semanalmente. A alcalinidade total (mg $CaCO_3$ L^{-1}) foi monitorada de acordo com a metodologia apresentada por APHA, (2005) e foi medida duas vezes por semana. Para manter o $CaCO_3$ acima de 150 mg L^{-1} foi utilizado bicarbonato de sódio (FURTADO, 2011).

Para avaliação da performance dos animais, foram analisadas as seguintes fórmulas:

1. Peso médio final (g): biomassa final de animais vivos (g) / número total de animais;
2. Ganho de peso semanal (g $semana^{-1}$): ganho de peso (g) / número de semanas
3. Taxa de crescimento específico (% d^{-1}): 100 × [ln (peso final (g) – peso inicial (g)) / (tempo de cultivo)].
4. Fator de conversão alimentar (FCA) = ∑ração oferecida (g) / (biomassa final (g) - biomassa inicial (g));
5. Sobrevivência (%) = (número final de animais / número inicial de animais) × 100;

2.4 Composição proximal e hematológica dos peixes

Amostras de músculo de peixes foram coletadas de cada unidade experimental no início e ao final do experimento. As amostras foram pesadas para determinar o peso úmido e colocadas em estufa a 60°C por 24 horas, e depois deste tempo foram pesadas novamente para obter o peso seco. Em seguida, as amostras foram trituradas até ficarem pó.

O teor de nitrogênio dos peixes foi determinado pelo método de titulação de Kjeldahl segundo (AOAC, 1997) no Laboratório de Nutrição de Organismos Aquáticos-LANOA (EMA, FURG, Rio Grande, Brasil). O teor de lipídeos foi determinado utilizando uma extração a frio pelo método Bligh- Dyer, o teor de cinzas foi obtido por método gravimétrico em forno mufla a 600 ºC, ambos descritos por (AOAC, 1997). A fibra bruta pelo método de lavagens de meio ácido e básico.

Para análises hematológicas, foram selecionados três peixes por tanque, totalizando nove por tratamento, anestesiados em água limpa com 50 mg L^{-1} de cloridrato de benzocaína e realizado uma punção caudal para a retirada de sangue, esfregaço e corados com May-Grünwald-Giemsa (Rosenfeld, G., 1947), para realização da contagem de linfócitos e granulócitos.

2.5 Análise bioquímica

Ao final do teste de estresse, nove peixes de cada tratamento foram eutanasiados por congelamento, e três tecidos foram coletados, sendo eles brânquias, fígado e músculo de cada peixe e conservados em ultrafreezer a -80 ºC. A homogeneização dos tecidos (1:5; w/v) ocorreu em tampão Tris-HCl (100 mM, pH 7,75) com EDTA (2 mM) e Mg2+ (5 mM). O homogeneizado passou por centrifugação 20,000g (14,010rpm) por 10 minutos a 4.0 ºC, e foi utilizado para as seguintes análises. A determinação das proteínas totais foi realizada pelo método de Biureto.

A capacidade antioxidante total contra radicais peroxil (ACAP) foi determinada conforme a metodologia de Amado et al., (2009) com a detecção de espécies reativas de oxigênio. A leitura foi realizada em microplaca contendo 10 µl de extrato de tecido extrato do tecido, 7,5 µl da solução ABAP (2,2-azobis-2-methylpropionamidine dihydrochloride) em concentração final de 20 µM, e por último 10 µl H2DCF-DA (2′, 7′ dichlorofluorescein diacetate) na concentração de 40 µM. A leitura da placa ocorreu em fluorímetro (Victor 2, Perkin Elmer), no comprimento de onda de 485/520 nm mantido na temperatura de 37°C. Sendo o resultado interpretado como menor área maior a capacidade antioxidante.

Os níveis de peroxidação lipídica foram determinados através da quantificação de substâncias reativas ao ácido tiobarbitúrico (TBARS) proposto por Oakes and Van Der Kraak, (2003). Para isso foi utilizado 10 µL do homogeneizado e adicionado 20 µl da solução de hidroxitolueno butilado (BHT, 67 µM), 150 µl de ácido acético a 20%, 150 µl da solução de TBA a 0.8%, 50 µl de água milli Q e 20 µl de dodecil sulfato de sódio 8.1%. Posteriormente, as amostras passaram por banho maria a 95 ºC por 30 minutos e 10 minutos de descanso em temperatura ambiente. Após o tempo foi adicionado 100 µl de água milli Q e 500 µl de n-butanol, e as amostras foram levadas ao vortex e posteriormente centrifugadas a 3000 rpm por 10 minutos a 15ºC para separar as fases. 150 µl do sobrenadante foi retirado e colocado em uma microplaca e medido a fluorescência em 553 nm para emissão e 515 nm para exitação em um fluorímetro (Victor 2, Perkin Elmer). Os resultados são expressos em MDA por mg de tecido.

A atividade da enzima glutationa-S-transferase (GST) foi determinada seguindo a metodologia de (Habig et al, 1974). Seguindo o procedimento de 15 µl de amostra homogeneizada, 10 µl de GSH 25 Mm e 80 µl de CDNB, e um meio de reação de fosfato de potássio aquecido a 25 ºC em banho maria. As amostras foram lidas em espectrofotômetro (BiotelELx 800) na absorbância de 340 nm.

A concentração de glutationa reduzida (GSH) foi mensurada de acordo com a metodologia proposta por Sedlak and Lindsay, (1968). Após a incubação de 240 µl de extrato de amostra

com de 28 µl de ácido tricloroacético na concentração final de 5% (TCA), foi realizada a centrifugação por 10 minutos a 20000 g a 4ºC, e após o a centrifugação foi retirado 100 µl do sobrenadante para a montagem da placa, e adicionado 200 µl de Tris-Base 0,4M com pH 8,9, e 10 µl of DTNB (5,5′-dithiobis(2-nitrobenzoic acid)). A placa foi mantida em temperatura ambiente por 15 minutos e posteriormente foi feita a leitura em espectrofotômetro (BiotelELx 800) na absorbância de 405 nm, sendo o resultado expresso em µmoles de GSH por mg de proteína. Para a medição do grupo sufidrila (P-SH) foi utilizado o pellet formado na análise anterior, ressuspenso com 240 µl de tampão de homogeneização. Então, 20 µl do extrato obtido foi adicionado em uma microplaca juntamente com 160 µl de Tris-Base 0,2M; com pH 8,2 e 10 µl de DTNB. A placa foi lida em espectrofotômetro (BiotelELx 800) na absorbância de 405 nm, após tempo de incubação de 15 minutos.

2.6 Análise estatística

A normalidade e a homocedasticidade dos dados foram verificadas utilizando os testes de Shapiro-Wilk e Levene, respectivamente. Quando os pressupostos da ANOVA foram atendidos, foi realizada uma ANOVA de uma via, seguida do teste de post-hoc de Tukey para verificar a diferença entre os tratamentos. Quando os pressupostos da ANOVA não foram satisfeitos, foi utilizado o teste não paramétrico de Kruskall Wallis. O nível de significância de 5% ($p<0,05$) foi aplicado em todas as análises.

3. Resultados

3.1 Desempenho dos peixes e qualidade de água

Os parâmetros de qualidade de água foram iguais entre os tratamentos. A temperatura da água foi mantida em 27.57 ± 1.42 ºC, o oxigênio dissolvido em 6.26 ± 2.18 mg L^{-1}, o pH 8.18 ± 0.24, a salinidade foi mantida em 15.61 ± 0.62 ppt e a alcalinidade em 163.49 ± 16.43 mg $CaCO_3$ L^{-1}. Os nutrientes foram mantidos na média de 0.12 ± 0.07, 0.10 ± 0.15, 18.69 ± 7.29 e 0.85 ± 0.54 mg L^{-1}, para nitrogênio amoniacal total, nitrito, nitrato e fosfato, respectivamente. A performance dos animais está representada na tabela 3, onde não mostrou diferenças significativas ($p \geq 0.05$) entre os tratamentos.

Tabela 3. Performance da tilápia nos tratamentos Controle (sem inclusão da macroalga), T5 (5% de inclusão da macroalga na dieta), T10 (10% de inclusão da macroalga na dieta) e T15 (15% de inclusão da macroalga na dieta) nos 42 dias de cultivo.

	Tratamentos			
Parâmetros	Controle	T5	T10	T15
Peso médio final (g)	10.78 ± 0.51	11.44 ± 0.84	10.35 ± 0.92	10.33 ± 0.15

Ganho de peso (g)	9.85 ± 051	10.50 ± 0.83	9.41 ± 0.92	9.39 ± 0.15
SGR (% dia^{-1})	5.82 ± 0.11	5.95 ± 0.15	5.71 ± 0.20	5.70 ± 0.40
FCA	1.00 ± 0.07	0.96 ± 0.09	0.99 ± 0.06	1.02 ± 0.02
Sobrevivência (%)	87.50 ± 15.00	85.00 ± 5.77	95.00 ± 5.77	90.0 ± 0.00

3.2 Composição proximal e hematológica dos peixes

A composição proximal dos peixes não apresentou diferenças significativas ($p \geq 0.05$) entre os tratamentos. Para os parâmetros hematológicos, houve diferença na concentração de granulócitos entre os tratamentos, apresentando maiores concentrações nos tratamentos com inclusão da macroalga na ração comparado ao controle (Tabela 4 e 5).

Tabela 4. Composição proximal e hematológica do *O. niloticus* ao final dos 42 dias de experimentos nos tratamentos Controle (sem inclusão da macroalga), T5 (5% de inclusão da macroalga na dieta), T10 (10% de inclusão da macroalga na dieta) e T15 (15% de inclusão da macroalga na dieta).

	Tratamentos			
Parâmetros	Controle	T5	T10	T15
Umidade (%)	77.31 ± 1.23	77.95 ± 0.30	77.68 ± 0.18	77.58 ± 0.27
Proteína bruta (%)	14.23 ± 0.63	13.73 ± 0.74	13.67 ± 0.79	13.81 ± 0.96
Lipídio (%)	3.72 ± 0.53	3.33 ± 0.30	3.74 ± 0.35	3.55 ± 0.28
Cinzas (%)	4.04 ± 0.17	4.08 ± 0.21	4.01 ± 0.11	4.09 ± 0.18

Tabela 5. Composição hematológica de *O. niloticus* ao final dos 42 dias de experimentos nos tratamentos Controle (sem inclusão da macroalga), T5 (5% de inclusão da macroalga na dieta), T10 (10% de inclusão da macroalga na dieta) e T15 (15% de inclusão da macroalga na dieta).

	Tratamentos			
Parâmetros	Controle	T5	T10	T15
Linfócitos (× 10^{-4} cel ml^{-1})	273.50 ± 97.65	240.00 ± 78.50	209.60 ± 78.54	278.50 ± 186.76
Granulócitos (× 10^{-4} cel ml^{-1})	10.50 ± 9.20 ^{b}	26.17 ± 20.73 ^{ab}	36.60 ± 36.84 ^{a}	23.17 ± 13.98 ^{ab}

Letras minúsculas na mesma linha representam diferença significativa ($p<0.05$) entre os tratamentos após uma ANOVA de uma via.

3.3 Análises bioquímicas

Os resultados de capacidade antioxidante total (ACAP) apresentados na Figura 1a e 1b mostraram que nas brânquias o tratamento com 10% de inclusão de macroalga após sofrer o

estresse apresentou maior capacidade antioxidante comparado ao tratamento sem estresse e sem macroalga, ao contrário do apresentado no músculo que apresentou maior capacidade antioxidante no tratamento com 5% de inclusão de macroalga após estresse comparado ao tratamento controle sem alga após estresse. Ambos os resultados mostraram a influência da inclusão de macroalga no aumento da capacidade antioxidante comparado ao controle sem inclusão.

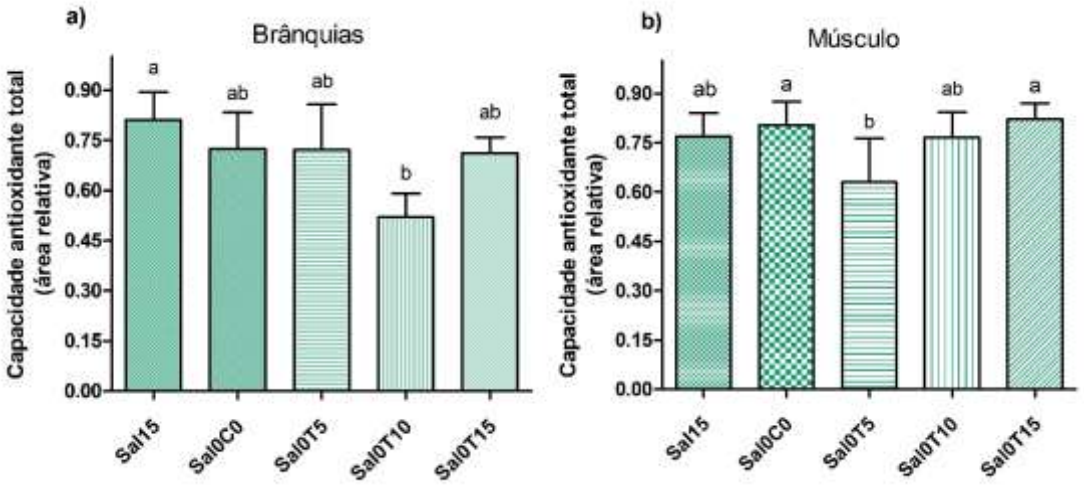


Figura 1. Capacidade antioxidante total – ACAP (média ± desvio padrão) nas brânquias (a) e musculo (b) ao final do estresse salino, nos tratamentos Sal15 (controle sem alga e na salinidade 15 de cultivo), Sal0C0 (controle sem alga após estresse salino), Sal0T5 (dieta com 5% de macroalga após estresse salino), Sal0T10 (dieta com 10% de macroalga após estresse salino) e Sal0T15 (dieta com 15% de macroalga após estresse salino). Letras diferentes representam diferença significativa entre os tratamentos.

Nas brânquias, foi observado que que todos os níveis de inclusão aumentaram dos níveis de GSH de maneira gradual, enquanto o estresse salino não diferenciou do controle (salinidade 15). No musculo, foi constatado que apenas o tratamento com 10% de inclusão de macroalga na dieta apresentou uma maior concentração da enzima em comparação ao tratamento controle sem o estresse (Figura 2a e 2b).

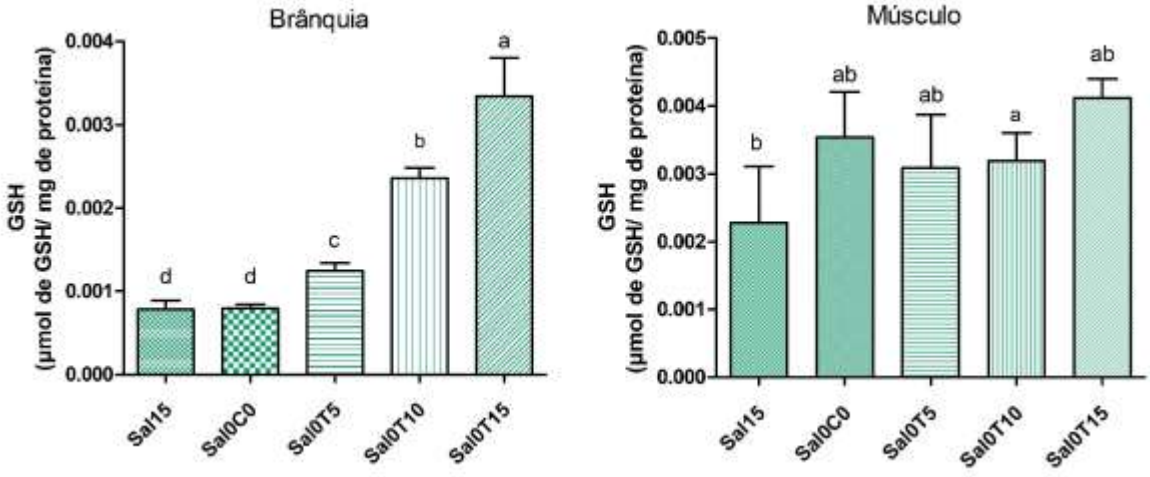


Figura 2. Concentração de glutationa reduzida - GSH (média ± desvio padrão) nas brânquias (a) e musculo (b) ao final do estresse salino, nos tratamentos Sal15 (controle sem alga e na salinidade 15 de cultivo), Sal0C0 (controle sem alga após estresse salino), Sal0T5 (dieta com 5% de macroalga após estresse salino), Sal0T10 (dieta com 10% de macroalga após estresse salino) e Sal0T15 (dieta com 15% de macroalga após estresse salino). Letras diferentes representam diferença significativa entre os tratamentos.

No músculo, o estresse salino mostrou reduzir a capacidade de detoxificação (evidenciada pela diminuição na atividade da GST) e os animais que receberam a ração com as inclusões mostraram restabelecer a atividade da enzima comparada com os animais submetidos ao estresse salino. No fígado, o estresse salino não induziu modificações na atividade da GST, entretanto, no grupo que recebeu 10% de inclusão mostrou aumentar a atividade da enzima comparado com o grupo controle sem choque salino (Figura 3a e 3b).

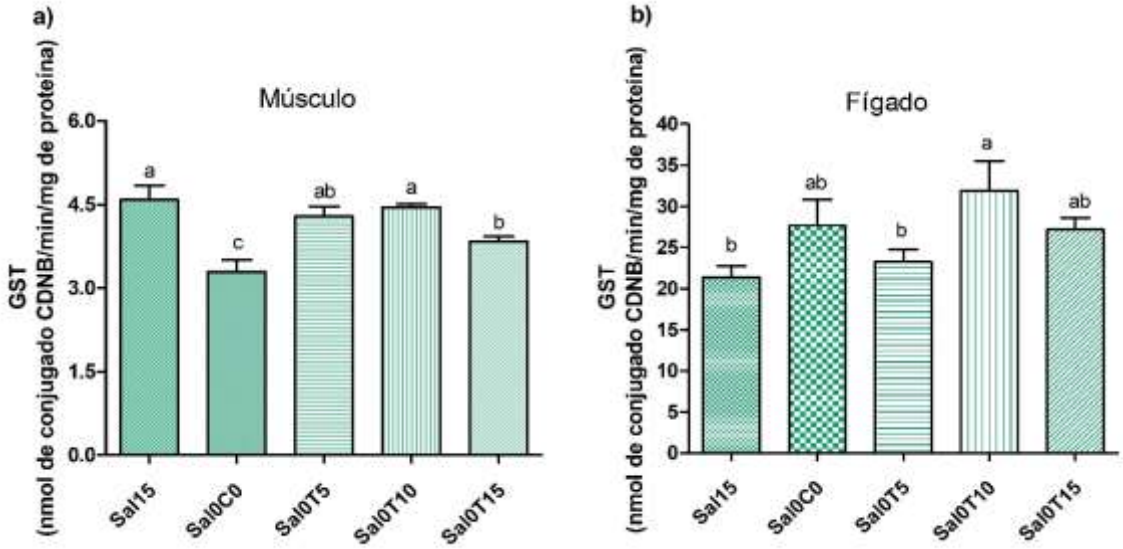


Figura 3. Atividade da glutationa S-transferase – GST (média ± desvio padrão) nas brânquias (a) e musculo (b) ao final do estresse salino, nos tratamentos Sal15 (controle sem alga e na salinidade 15 de cultivo), Sal0C0 (controle sem alga após estresse salino), Sal0T5 (dieta com 5% de macroalga após estresse salino), Sal0T10 (dieta

com 10% de macroalga após estresse salino) e Sal0T15 (dieta com 15% de macroalga após estresse salino). Letras diferentes representam diferença significativa entre os tratamentos.

No músculo, o tratamento com 15% de inclusão da macroalga na dieta após estresse mostrou aumentar os níveis de SH indicando um aumento do estado redutor. Nas brânquias, esse resultado foi observado no grupo que recebeu 5 % de inclusão (Figura 4a e 4b).

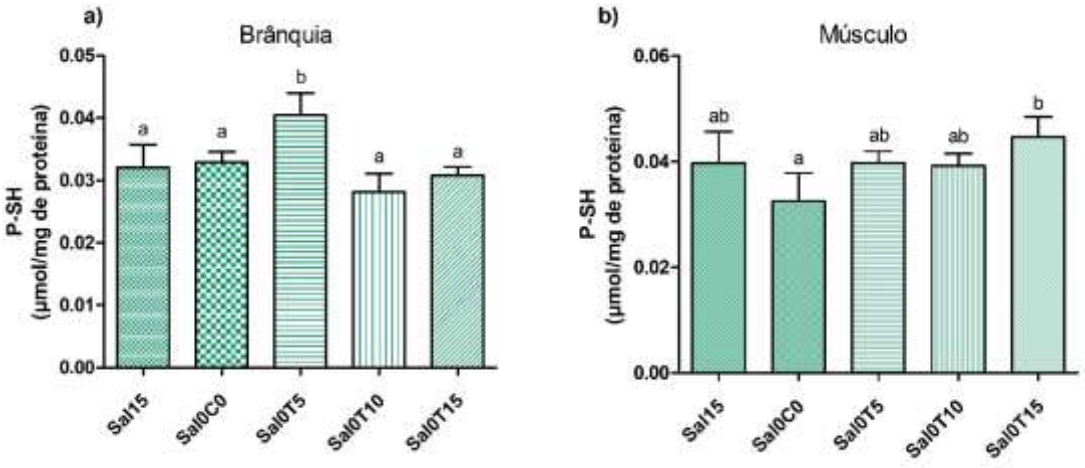


Figura 4. Concentração dos grupos sulfidrilas associadas com proteína – P-SH (média ± desvio padrão) nas brânquias (a) e musculo (b) ao final do estresse salino, nos tratamentos Sal15 (controle sem alga e na salinidade 15 de cultivo), Sal0C0 (controle sem alga após estresse salino), Sal0T5 (dieta com 5% de macroalga após estresse salino), Sal0T10 (dieta com 10% de macroalga após estresse salino) e Sal0T15 (dieta com 15% de macroalga após estresse salino). Letras diferentes representam diferença significativa entre os tratamentos.

No músculo, a inclusão de 10 e 15 % mostrou aumentar o dano lipídico comparado com os outros tratamentos (Figura 5).

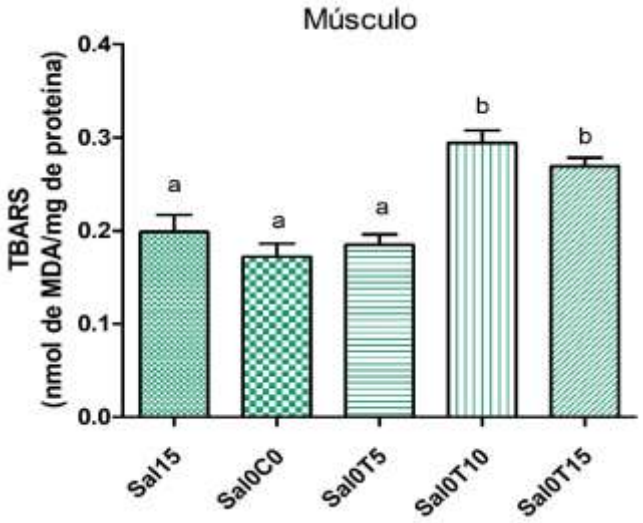


Figura 5. Concentração de substâncias reativas ao ácido tiobarbitúrico - TBARS (média ± desvio padrão) nas brânquias (a) e musculo (b) ao final do estresse salino, nos tratamentos Sal15 (controle sem alga e na salinidade 15 de cultivo), Sal0C0 (controle sem alga após estresse salino), Sal0T5 (dieta com 5% de macroalga após estresse

salino), Sal0T10 (dieta com 10% de macroalga após estresse salino) e Sal0T15 (dieta com 15% de macroalga após estresse salino). Letras diferentes representam diferença significativa entre os tratamentos.

4. Discussão

A composição nutricional da macroalga pode variar de acordo com as variáveis do ambiente em que são cultivadas, com isso, neste estudo o teor de proteína da macroalga cultivada em sistema integrado com bioflocos foi de 19%, sendo superior aos estudos apresentados por Limiñana et al., (2023) que obtiveram 14% de proteína de macroalgas coletadas no ambiente, e por Angell et al., (2017) trabalhando com *Ulva ohnoi* coletada do ambiente com o valor de 13.6% de proteína na macroalga. Esse incremento proteico encontrado no presente estudo pode estar associado as altas concentrações de nitrogênio disponíveis para absorção no sistema de bioflocos, assim como uma alta carga de produção de sólidos ocasionou um incremento na clorofila-a como forma de maximizar a realização da fotossíntese encontrado por Carvalho et al., (2024). Legarda et al. (2021) trabalhando em sistema integrado com camarão, tainha e macroalga mostraram um incremento de nitrogênio na macroalga, sendo de 1% de nitrogênio no início do experimento para 2.9% ao final do experimento associado com o aumento da clorofila-a e carotenoides, mostrando um incremento no valor nutricional da macroalga quando cultivada em associação com outros organismos, dito isto tal resultado está associado a uma maior afinidade das macroalgas de utilizar amônia na biossíntese das proteínas (Copertino et al., 2009).

Esse aumento proteico e de biocompostos no tecido da macroalga torna sua aplicabilidade na inclusão de rações para organismos aquáticos uma atividade promissora e sustentável. Marinho et al., (2013) testaram 10, 15 e 20% de inclusão de macroalga na dieta e substituição da farinha de peixe para a tilápia, tendo como resultado que até 10% de inclusão o desempenho do animal não foi afetado, sendo a digestibilidade, a retenção aparente de proteína e energia fatores essenciais para o sucesso ou fracasso da substituição (Qiu et al., 2018). Resultado semelhante foi encontrado por Ergün et al., (2009) para tilápia, mostrando que pequenas porcentagens de inclusão da macroalga *Ulva* (5 e 10%) não interferiram negativamente no desempenho do animal comparado a dieta controle. Já para uma espécie carnívora como alevinos de robalo *Dicentrarchus labrax,* foi encontrado que a inclusão de até 5% da farinha de macroalga promoveu melhorias em crescimento e taxa de sobrevivência, relacionado com o teor de proteína, minerais e perfil de aminoácidos essenciais presentes na macroalga (Wassef et al., 2013).

No presente estudo, a inclusão da macroalga na dieta ocasionou a substituição dos ingredientes de fontes vegetais terrestres, como farelo de soja e farelo de trigo, e apesar da

farinha de macroalga possuir menor energia e digestibilidade proteica como apresentado por Qiu et al., (2018), não interferiu no desempenho zootécnico da tilápia em nenhum nível de inclusão nesse experimento. Suryaningrum and Samsudin, (2020) avaliando os índices de digestibilidade da *U. lactuca* para a tilápia, obtiveram valores de 82% para proteína, 92% para lipídio, 63% para cinzas e 74% energia, mostrando bons índices e viabilidade de inclusões na dieta. Os resultados do presente trabalho foram semelhantes aos encontrados por Silva et al., (2015) testando a inclusão de 10% de *Gracilaria* proveniente de um cultivo em sistema integrado na dieta da tilápia, mostrando que não houveram diferenças no desempenho zootécnico ou composição do corpo da tilápia entre o tratamento com *Ulva* e o controle. Com isso, a aplicação da biomassa de macroalgas produzida no sistema integrado com bioflocos na ração da tilápia reflete a aplicação da economia circular, que consiste na adoção de práticas sustentáveis relacionadas a uma melhor utilização dos nutrientes produzidos e o gerenciamento de resíduos (Cooney et al., 2023).

Diversos estudos apontam situações diferentes com o efeito da inclusão da macroalga na composição proximal da carcaça do animal. Um estudo realizado por Saleh et al., (2014) testaram a substituição da farinha de trigo por farinha de macroalga, mostrando que maiores inclusões (75 e 100 g kg^{-1}) obtiveram maior crescimento e mudanças na carcaça da tilápia com maiores valores de proteína e menores de lipídio com o aumento da inclusão de macroalga na ração. Azaza et al., (2008) também encontrou diminuição dos valores de lipídio com aumento da inclusão de macroalgas, entretanto os valores de proteína não foram alterados. De acordo com Ortiz et al., (2006) o perfil de vitaminas e minerais na macroalga podem interferir diretamente na composição corporal dos organismos, como exemplo a presença de vitamina C na macroalga pode promover um aumento do metabolismo lipídico no animal e mudar sua composição corporal (Nakagawa, 1997). As tilápias alimentadas com inclusão de macroalga não apresentaram diferenças na composição proximal dos animais entre os tratamentos, portanto a inclusão de até 15% de farinha de *Ulva* na ração não interferiu na composição do corpo da tilápia, estando os resultados semelhantes aos encontrados por Ergün et al., (2009) testando as mesmas concentrações de inclusão de macroalga na dieta.

Além dos parâmetros zootécnicos e bromatológicos, os parâmetros de sangue como contagem de células podem caracterizar a saúde do peixe e efeitos fisiológicos associados ao sistema de cultivo e alimentação (Satheeshkumar et al., 2012). A contagem de linfócitos e granulócitos neste estudo se mantiveram de acordo com os trabalhos de Jerônimo et al., (2011) e Charlie-Silva et al., (2019), mostrando que o ambiente de cultivo não apresentava fatores altos de estresse ou baixa sanidade. Os linfócitos são indicadores de inflamação em situação de

estresse (Jerônimo et al., 2011), os resultados apresentados indicam que tais processos não foram identificados nos organismos amostrados. Já o número de granulócitos pode estar associado com parâmetros de qualidade de água, Jerônimo et al., (2011) associa o aumento dos granulócitos como mecanismo de defesa quando obtiveram temperaturas inferiores do que o recomendado para o cultivo de tilápia. A qualidade de água do presente trabalho se manteve em condições ideais e sem diferença entre os tratamentos, portanto o aumento das concentrações de granulócitos no tratamento 10% comparado ao controle pode estar associada com a inclusão da macroalga na dieta. Amar et al., (2004) reportaram que incluir ingredientes ricos em carotenoides podem estimular o sistema imune de peixes, isso poderia explicar o incremento na produção de granulócitos no presente experimento, estimulando a produção dos mesmos pela presença de carotenoides na macroalga. Contrário aos presentes resultados, Mendonça et al., (2019) perceberam que a inclusão de macroalga *Gracilaria dominguensis* afetou o crescimento da tainha ao inseri-la na dieta em uma inclusão de até 15%, porém sem efeito na contagem de granulócitos e linfócitos. Além dos carotenoides, de acordo com Abdelrhman et al., (2022) alguns fatores como a presença de polissacarídeos e carboidratos sulfatados em macroalgas marinhas podem estimular a imunidade do animal.

No cultivo de organismos aquáticos, situações de estresse relacionados a qualidade de água, manejo e biossegurança podem ser comuns. O termo estresse pode ser caracterizado como uma alteração da homeostase causada por um agente estressor (Barton and Iwama, 1991) e afetam diretamente o desempenho zootécnico e fisiológico dos animais. Situações estressoras no cultivo como mudanças de pH, variações de temperatura e salinidade podem induzir uma situação pró-oxidativa nos diferentes tecidos dos animais e para tentar minimizar essa situação os organismos recrutam respostas antioxidantes como interceptação de ROS ou modulação dos sistemas antioxidante (enzimático ou não enzimático) que por sua vez, demandam energia de outros processos fisiológicos como crescimento e reprodução (Halliwell and Gutteridge, 1985). Tecidos como brânquia, fígado e músculo podem dar respostas fisiológicas sobre a ação do estresse no animal, tais como funções osmorregulatórias, processos de biotransformação e detoxificação de xenobióticos (Bernet et al., 1999). Uma alternativa para economizar energia do animal para lidar com situações pró-oxidantes é ofertar dieta com boa capacidade antioxidante como a utilização de macroalgas que proporcionam substancias bioativas como carotenoides, que podem ter concentrações superiores se produzidas em sistemas BFT em comparação a algas coletadas do ambiente (Poljsak et al., 2013).

Para a tilápia, apesar de ser um organismo que suporta grandes variações de salinidade entre 0 e 16 (de Souza et al., 2019), a realização de uma mudança abrupta pode provocar uma situação

pró-oxidante com a formação de espécies reativas de oxigênio e alterações do sistema antioxidante (Vieira et al., 2018). Os sistemas antioxidantes de defesa são ativados quando situações de estresse, seja por mudanças na qualidade de agua ou pelo incremento na densidade de cultivo, assim a dieta pode ser uma ferramenta para incrementar resistência (Oliva-Teles, 2012). Nesse estudo, os dados da brânquia mostraram que o aumento dos níveis de inclusão de macroalga na dieta proporcionou o aumento dos níveis glutationa reduzida (GSH) e aumento da capacidade antioxidante (ACAP), e aumentos nos grupos P-SH. Sabe-se que a GSH representa um dos primeiros mecanismos de defesa contra a ação dos radicais livres (Ventura-Lima et al., 2009). Assim, Jiang et al., (2016) indicaram que a dieta com inclusão de curcumina pode ser um modulador da biossíntese da GSH mediante a regulação da expressão gênica. Em situações de estresse a brânquia representa o tecido mais afetado por estar em contato direto com possíveis contaminantes dissolvidos na água, sendo importante o recrutamento de moléculas antioxidantes para manutenção da homeostase (Bernet et al., 1999), com isso o uso de macroalgas na inclusão em dietas representa uma alternativa para utilização da biomassa produzida e uma entrada exógena de enzimas antioxidantes. De acordo com Tziveleka et al., (2021) por serem aptas a se desenvolver em ambientes estressantes, as macroalgas possuem mecanismos de defesas como aumento de enzimas antioxidantes como polifenois e carotenoides que representam características importantes na aplicação da biomassa produzida.

O músculo que é parte comestível do peixe, é interessante que se observe a ausência de danos e melhora na capacidade antioxidante frente a condições de estresse. Neste estudo, se observou que nas menores inclusões como 5 e 10 % mesmo quando os animais foram submetidos ao estresse salino, houve uma melhora na capacidade de detoxificação e ausência de dano lipídico. De acordo com Srikanth et al., (2013) diversos antioxidantes como a GST, GSH e SOD são reportadas no organismo para manutenção do equilíbrio entre a produção e eliminação de ROS. Este efeito antioxidante das algas pode ser devido ao fato que as macroalgas do gênero *Ulva* possuem em sua composição diversos metabólitos importantes, como compostos fenólicos, carotenoides, clorofilas e minerais (Tziveleka et al., 2021). De acordo com Pérez-gálvez et al., (2020) carotenoides e clorofilas podem ser caracterizados como antioxidantes que buscam prevenir/interceptar a ROS, com isso a inclusão de macroalgas na dieta pode conferir maior preparação ao animal na prevenção de danos oxidativos. No entanto, as inclusões de 10 e 15% da macroalga na dieta proporcionaram peroxidação lipídica em comparação aos tratamentos controles. De acordo com Poljsak et al., (2013) a quantidade inapropriada de antioxidantes pode converter compostos bioativos em pro-oxidantes, ocasionado pela neutralização dos radicais fisiologicamente benéficos, o que pode ter ocorrido

nos maiores níveis de inclusão de macroalga. Algumas características antinutricionais, como alto teor de cinzas e fibras também podem afetar fisiologicamente o animal, tendo em vista que comparado ao farelo de soja o coeficiente de digestibilidade do nitrogênio da macroalga são inferiores (Bikker et al., 2020).

A atividade da GST também aumentou no fígado na concentração de 10% de inclusão de macroalgas comparado ao controle sem estresse. A GST tem como papel a biotransformação e eliminação de compostos tóxicos na célula (Goto et al., 2009), e nesse processo a GSH é utilizada como co-substrato na detoxificação para manutenção da homeostase, portanto seus níveis podem ser alterados pela atividade da GST (Liu et al., 2020). No entanto, em nosso estudo diferente do acumulo de GSH e atividade da GST que ocorreram nas brânquias, os níveis de GSH no fígado não aumentaram, o que provavelmente indica que estavam sendo utilizados para a detoxificação de algum composto acumulado no organismo em inclusões de 10 e 15%, podendo estar relacionado ao estresse de salinidade ou a possíveis características antinutricionais da macroalga (Suryaningrum and Samsudin, 2020). Tais questões podem ser melhoradas com o uso de diferentes processos de extração e processos de fermentação da macroalga como alternativas para aumentar a disponibilidade de nutrientes e podendo minimizar possíveis efeitos fisiológicos no animal (Hardjani et al., 2017). Portanto, a obtenção de um subproduto da aquicultura integrada de melhor valor nutricional e rico em antioxidantes se torna vantajoso ambientalmente e economicamente, podendo ser usado no sistema como biorremediador e voltar ao sistema na substituição de ingredientes de origem terrestre vegetal, aplicando os conceitos da economia circular (Colombo et al., 2023). Tal conceito representa economia verde na busca por uma aquicultura sustentável e reciclagem de resíduos (Campanati et al., 2021).

5. Conclusão

O uso da farinha de macroalgas produzidas em sistema integrado com bioflocos na inclusão de 5% na ração da tilápia possibilitou desempenho zootécnico semelhante ao tratamento controle sem a macroalga, com a substituição parcial do farelo de soja e trigo por um ingrediente alternativo. A composição proximal e análise hematológica não foi influenciada pela inclusão da macroalga na dieta. Os resultados de estresse oxidativo após a mudança brusca de salinidade mostram uma maior capacidade antioxidante no tratamento 5% de inclusão, associado com um aumento da glutationa reduzida (GSH) e grupos sufidrilas (P-SH), bem como melhora na capacidade de detoxificação além da ausência de peroxidação lipídica comparado aos tratamentos controle sem e com estresse de salinidade.

Referências

Abdelrhman, A.M., Ashour, M., Al-Zahaby, M.A., Sharawy, Z.Z., Nazmi, H., Zaki, M.A.A., Ahmed, N.H., Ahmed, S.R., El-Haroun, E., Van Doan, H., Goda, A.M.A., 2022. Effect of polysaccharides derived from brown macroalgae *Sargassum dentifolium* on growth performance, serum biochemical, digestive histology and enzyme activity of hybrid red tilapia. Aquac. Reports 25, 101212. https://doi.org/10.1016/j.aqrep.2022.101212

Alencar, J.R. de, Junior, P.A.H., Celino, J.J., 2010. Cultivo de Camarão Branco *Litopenaeus Vannamei* (Boone , 1931) com a Macro- alga *Ulva Lacuata Linneaus* (Chlorophyta) no Tratamento de Efluentes em Sistema Fechado de Recirculação. Rev. Biol. e Ciências da Terra 10, 117–137.

Amado, L.L., Garcia, M.L., Ramos, P.B., Freitas, R.F., Zafalon, B., Ferreira, J.L.R., Yunes, J.S., Monserrat, J.M., 2009. A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: Application to evaluate microcystins toxicity. Sci. Total Environ. 407, 2115–2123. https://doi.org/10.1016/j.scitotenv.2008.11.038

Amar, E.C., Kiron, V., Satoh, S., Watanabe, T., 2004. Enhancement of innate immunity in rainbow trout (*Oncorhynchus mykiss* Walbaum) associated with dietary intake of carotenoids from natural products. Fish Shellfish Immunol. 16, 527–537. https://doi.org/10.1016/j.fsi.2003.09.004

Aminot, A., Chaussepied, M., 1983. Manuel des analyses chimiques en milieu marin. Centre National pour l'exploitation des océans, França.

Angell, A.R., Paul, N.A., Nys, R. De, 2017. A comparison of protocols for isolating and concentrating protein from the green seaweed *Ulva ohnoi* 1011–1026. https://doi.org/10.1007/s10811-016-0972-7

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, (21st edn). ed. American Public Health Association, Washington, DC, USA.

Arcos Limiñana, V., Benoist, T., Anton Sempere, S., Maestre Pérez, S.E., Prats Moya, M.S., 2023. Chemical composition of sustainable Mediterranean macroalgae obtained from land-based and sea-based aquaculture systems. Food Biosci. 54. https://doi.org/10.1016/j.fbio.2023.102902

Azaza, B.M.S., Mensi, F., Ksouri, J., Dhraief, M.N., Brini, B., Abdelmouleh, A., Kraı, M.M., 2008. Growth of Nile tilapia (*Oreochromis niloticus* L .) fed with diets containing graded levels of green algae ulva meal (*Ulva rigida*) reared in geothermal waters of southern Tunisia 24, 202–207. https://doi.org/10.1111/j.1439-0426.2007.01017.x

Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu. Rev. Fish Dis. 1, 3–26. https://doi.org/10.1016/0959-8030(91)90019-G

Ben-Ari, T., Neori, A., Ben-Ezra, D., Shauli, L., Odintsov, V., Shpigel, M., 2014. Management

of *Ulva lactuca* as a biofilter of mariculture effluents in IMTA system. Aquaculture 434, 493–498. https://doi.org/10.1016/j.aquaculture.2014.08.034

Bendschneider, K., Robinson, R.J., 1952. New spectrophotometric method for the determination of nitrite in water. Fresenius Environ. Bull.

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., Wahli, T., 1999. Histopathology in fish: Proposal for a protocol to assess aquatic pollution. J. Fish Dis. 22, 25–34. https://doi.org/10.1046/j.1365-2761.1999.00134.x

Bikker, P., Stokvis, L., van Krimpen, M.M., van Wikselaar, P.G., Cone, J.W., 2020. Evaluation of seaweeds from marine waters in Northwestern Europe for application in animal nutrition. Anim. Feed Sci. Technol. 263. https://doi.org/10.1016/j.anifeedsci.2020.114460

Campanati, C., Willer, D., Schubert, J., Aldridge, D.C., 2021. Sustainable Intensification of Aquaculture through Nutrient Recycling and Circular Economies: More Fish, Less Waste, Blue Growth. Rev. Fish. Sci. Aquac. https://doi.org/10.1080/23308249.2021.1897520

Carvalho, A., Braga, Í., Chaar, F., Cardozo, A.P., Monserrat, J.M., Ramírez, J.R.B., Wasielesky, W., Poersch, L.H., 2024. Production of the Macroalgae Ulva lactuca Integrated with the Shrimp *Penaeus vannamei* in a Biofloc System: Effect of Total Suspended Solids and Nutrient Concentrations. Phycology 4, 37–52. https://doi.org/10.3390/phycology4010002

Carvalho, A., Costa, L.C. de O., Holanda, M., Poersch, L.H., Turan, G., 2023. Influence of Total Suspended Solids on the Growth of the Sea Lettuce *Ulva lactuca* Integrated with the Pacific White Shrimp *Litopenaeus vannamei* in a Biofloc System. Fishes 8, 163. https://doi.org/10.3390/fishes8030163

Caxico Vieira, C.A.S., Vieira, J.S., Bastos, M.S., Zancanela, V., Barbosa, L.T., Gasparino, E., Del Vesco, A.P., 2018. Expression of genes related to antioxidant activity in Nile tilapia kept under salinity stress and fed diets containing different levels of vitamin C. J. Toxicol. Environ. Heal. - Part A Curr. Issues 81, 20–30. https://doi.org/10.1080/15287394.2017.1401968

Charlie-Silva, I., Klein, A., Gomes, J.M.M., Prado, E.J.R., Moraes, A.C., Eto, S.F., Fernandes, D.C., Fagliari, J.J., Junior, J.D.C., Lima, C., Lopes-Ferreira, M., Conceição, K., Manrique, W.G., Belo, M.A.A., 2019. Acute-phase proteins during inflammatory reaction by bacterial infection: Fish-model. Sci. Rep. 9, 1–13. https://doi.org/10.1038/s41598-019-41312-z

Chopin, T., 2015. Marine Aquaculture in Canada: Well-Established Monocultures of Finfish and Shellfish and an Emerging Integrated Multi-Trophic Aquaculture (IMTA) Approach Including Seaweeds, Other Invertebrates, and Microbial Communities. Fisheries 40, 28–31. https://doi.org/10.1080/03632415.2014.986571

Habig, W., Pabst, M. J., & Jakoby, W. B. (1974). The first enzymatic step in mercapturic acid

formation. Glutathione-S-transferase. J. Biol. Chem, 249, 7130-7139.. https://doi.org/10.1515/9781773851365-038

Colombo, S.M., Roy, K., Mraz, J., Wan, A.H.L., Davies, S.J., Tibbetts, S.M., Øverland, M., Francis, D.S., Rocker, M.M., Gasco, L., Spencer, E., Metian, M., Trushenski, J.T., Turchini, G.M., 2023. Towards achieving circularity and sustainability in feeds for farmed blue foods. Rev. Aquac. 15, 1115–1141. https://doi.org/10.1111/raq.12766

Cooney, R., de Sousa, D.B., Fernández-Ríos, A., Mellett, S., Rowan, N., Morse, A.P., Hayes, M., Laso, J., Regueiro, L., Wan, A.H., Clifford, E., 2023. A circular economy framework for seafood waste valorisation to meet challenges and opportunities for intensive production and sustainability. J. Clean. Prod. 392. https://doi.org/10.1016/j.jclepro.2023.136283

Copertino, M.D.S., Tormena, T., Seeliger, U., 2009. Biofiltering efficiency, uptake and assimilation rates of *Ulva clathrata* (Roth) J. Agardh (Clorophyceae) cultivated in shrimp aquaculture waste water. J. Appl. Phycol. 21, 31–45. https://doi.org/10.1007/s10811-008-9357-x

Cornejo-Ponce, L., Vilca-Salinas, P., Lienqueo-Aburto, H., Arenas, M.J., Pepe-Victoriano, R., Carpio, E., Rodríguez, J., 2020. Integrated aquaculture recirculation system (Iars) supported by solar energy as a circular economy alternative for resilient communities in arid/semi-arid zones in southern south america: A case study in the camarones town. Water (Switzerland). https://doi.org/10.3390/w12123469

Da Silva, K.R., Wasielesky, W., Abreu, P.C., 2013. Nitrogen and Phosphorus Dynamics in the Biofloc Production of the Pacific White Shrimp, *Litopenaeus vannamei*. J. World Aquac. Soc. 44, 30–41. https://doi.org/10.1111/jwas.12009

de Mendonça, A.J.C., Rosas, V.T., Monserrat, J.M., Romano, L.A., Tesser, M.B., 2019. The inclusion of algae *Gracilaria domingensis* in the diet of mullet juveniles (*Mugil liza*) improves the immune response. J. Appl. Aquac. 31, 210–223. https://doi.org/10.1080/10454438.2018.1539693

De Miranda Cabral Gontijo, Á.M., Barreto, R.E., Speit, G., Valenzuela Reyes, V.A., Volpato, G.L., Favero Salvadori, D.M., 2003. Anesthesia of fish with benzocaine does not interfere with comet assay results. Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 534, 165–172. https://doi.org/10.1016/S1383-5718(02)00276-0

de Souza, R.L., de Lima, E.C.R., de Melo, F.P., Ferreira, M.G.P., de Souza Correia, E., 2019. The culture of Nile tilapia at different salinities using a biofloc system. Rev. Cienc. Agron. 50, 267–275. https://doi.org/10.5935/1806-6690.20190031

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

Ergün, S., Soyutürk, M., Güroy, B., Güroy, D., Merrifield, D., 2009. Influence of Ulva meal on growth, feed utilization, and body composition of juvenile Nile tilapia (*Oreochromis niloticus*) at two levels of dietary lipid. Aquac. Int. 17, 355–361. https://doi.org/10.1007/s10499-008-9207-5

FURTADO, P.S., 2011. O Efeito do Hidróxido de Cálcio, do Carbonato e Bicarbonato de Sódio na Qualidade de Água e no Desempenho Zootécnico do Camarão *Litopenaeus vannamei* Cultivado com Tecnologia de Bioflocos (BFT). Furg 3, 1–48.

Goto, S., Kawakatsu, M., Izumi, S. ichi, Urata, Y., Kageyama, K., Ihara, Y., Koji, T., Kondo, T., 2009. Glutathione S-transferase π localizes in mitochondria and protects against oxidative stress. Free Radic. Biol. Med. 46, 1392–1403. https://doi.org/10.1016/j.freeradbiomed.2009.02.025

Halliwell, B., Gutteridge, J.M.C., 1985. Fee Radicals in Biology and Medicine Carcinogenesis. J. Free Radic. Biol. Med. 1, 331–334.

Hardjani, D.K., Suantika, G., Aditiawati, P., 2017. Nutritional profile of red seaweed K*appaphycus alvarezii* after fermentation using saccharomyces cerevisiae as a feed supplement for white shrimp *Litopenaeus vannamei* nutritional profile of fermented red Seaweed. J. Pure Appl. Microbiol. 11, 1637–1645. https://doi.org/10.22207/JPAM.11.4.01

He, J., Xu, Y., Chen, H., Sun, P., 2016. Extraction, structural characterization, and potential antioxidant activity of the polysaccharides from four seaweeds. Int. J. Mol. Sci. 17. https://doi.org/10.3390/ijms17121988

Huang, Q., Huang, K., Ma, Y., Qin, X., Wen, Y., Sun, L., Tang, L., 2015. Feeding frequency and rate effects on growth and physiology of juvenile genetically improved farmed nile tilapia. N. Am. J. Aquac. 77, 503–512. https://doi.org/10.1080/15222055.2015.1066472

Jerônimo, G., Laffitte, L., Speck, G., Martins, M., 2011. Seasonal influence on the hematological parameters in cultured Nile tilapia from southern Brazil. Brazilian J. Biol. 71, 719–725. https://doi.org/10.1590/s1519-69842011000400017

Jiang, J., Wu, X.Y., Zhou, X.Q., Feng, L., Liu, Y., Jiang, W.D., Wu, P., Zhao, Y., 2016. Effects of dietary curcumin supplementation on growth performance, intestinal digestive enzyme activities and antioxidant capacity of crucian carp *Carassius auratus*. Aquaculture 463, 174–180. https://doi.org/10.1016/j.aquaculture.2016.05.040

Ju, J., Yang, J., Zhang, W., Wei, Y., Yuan, H., Tan, Y., 2023. Seaweed polysaccharide fibers: Solution properties, processing and applications. J. Mater. Sci. Technol. 140, 1–18. https://doi.org/10.1016/j.jmst.2022.07.053

Khanjani, M.H., Mozanzadeh, M.T., Sharifinia, M., Emerenciano, M.G.C., 2023. Biofloc: A sustainable dietary supplement, nutritional value and functional properties. Aquaculture 562, 738757. https://doi.org/10.1016/J.AQUACULTURE.2022.738757

Kidgell, J.T., Magnusson, M., de Nys, R., Glasson, C.R.K., 2019. Ulvan: A systematic review of extraction, composition and function. Algal Res. 39, 101422. https://doi.org/10.1016/j.algal.2019.101422

Kirchherr, J., Reike, D., Hekkert, M., 2017. Conceptualizing the circular economy: An analysis of 114 definitions. Resour. Conserv. Recycl. 127, 221–232. https://doi.org/10.1016/j.resconrec.2017.09.005

Legarda, E.C., da Silva, D., Miranda, C.S., Pereira, P.K.M., Martins, M.A., Machado, C., de Lorenzo, M.A., Hayashi, L., do Nascimento Vieira, F., 2021. Sea lettuce integrated with Pacific white shrimp and mullet cultivation in biofloc impact system performance and the sea lettuce nutritional composition. Aquaculture 534. https://doi.org/10.1016/j.aquaculture.2020.736265

Levavasseur, G., 1989. Analyse comparée des complexes pigment-protéines de chlorophycophytes marines benthiques. Phycologia 28, 1–14. https://doi.org/10.2216/i0031-8884-28-1-1.1

Liu, J.D., Liu, W. Bin, Zhang, C.Y., Xu, C.Y., Zheng, X.C., Zhang, D.D., Chi, C., 2020. Dietary glutathione supplementation enhances antioxidant activity and protects against lipopolysaccharide-induced acute hepatopancreatic injury and cell apoptosis in Chinese mitten crab, *Eriocheir sinensis*. Fish Shellfish Immunol. 97, 440–454. https://doi.org/10.1016/j.fsi.2019.12.049

Marinho, G., Nunes, C., Sousa-Pinto, I., Pereira, R., Rema, P., Valente, L.M.P., 2013. The IMTA-cultivated Chlorophyta Ulva spp. as a sustainable ingredient in Nile tilapia (*Oreochromis niloticus*) diets. J. Appl. Phycol. 25, 1359–1367. https://doi.org/10.1007/s10811-012-9965-3

Mhlongo, G., Mnisi, C.M., 2023. Effect of seaweed (*Ecklonia maxima*) on apparent nutrient digestibility, growth performance, and physiological and meat quality parameters in Boschveld cockerels. Poult. Sci. 102, 102361. https://doi.org/10.1016/j.psj.2022.102361

Montgomery, W.L., Gerking, S.D., 1980. Marine macroalgae as foods for fishes: an evaluation of potential food quality. Environ. Biol. Fishes 5, 143–153. https://doi.org/10.1007/BF02391621

Mwendwa, R., Wawire, M., Kahenya, P., 2023. Potential for Use of Seaweed as a Fish Feed Ingredient: A Review. J. Agric. Sci. 15, 96. https://doi.org/10.5539/jas.v15n2p96

Nakagawa, H., 1997. Effect of dietary algae on improvement H Nakagawa of lipid metabolism in fish mg FA / g g 345–348.

Oakes, K.D., Van Der Kraak, G.J., 2003. Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. Aquat. Toxicol. 63, 447–463. https://doi.org/10.1016/S0166-445X(02)00204-7

Oliva-Teles, A., 2012. Nutrition and health of aquaculture fish. J. Fish Dis. 35, 83–108.

https://doi.org/10.1111/j.1365-2761.2011.01333.x

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C., Navarrete, E., Osorio, A., Rios, A., 2006. Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. Food Chem. 99, 98–104. https://doi.org/10.1016/j.foodchem.2005.07.027

Pérez-gálvez, A., Viera, I., Roca, M., 2020. Carotenoids and chlorophylls as antioxidants. Antioxidants 9, 1–39. https://doi.org/10.3390/antiox9060505

Poljsak, B., Šuput, D., Milisav, I., 2013. Achieving the balance between ROS and antioxidants: When to use the synthetic antioxidants. Oxid. Med. Cell. Longev. 2013. https://doi.org/10.1155/2013/956792

Qiu, X., Neori, A., Kim, J.K., Yarish, C., Shpigel, M., Guttman, L., Ben Ezra, D., Odintsov, V., Davis, D.A., 2018. Evaluation of green seaweed *Ulva* sp. as a replacement of fish meal in plant-based practical diets for Pacific white shrimp, *Litopenaeus vannamei*. J. Appl. Phycol. 30, 1305–1316. https://doi.org/10.1007/s10811-017-1278-0

Queirós, A.S., Circuncisão, A.R., Pereira, E., Válega, M., Abreu, M.H., Silva, A.M.S., Cardoso, S.M., 2021. Valuable nutrients from *Ulva rigida*: Modulation by seasonal and cultivation factors. Appl. Sci. 11. https://doi.org/10.3390/app11136137

Reis, W.G., Wasielesky, W., Abreu, P.C., Brandão, H., Krummenauer, D., 2019. Rearing of the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931)in BFT system with different photoperiods: Effects on the microbial community, water quality and zootechnical performance. Aquaculture 508, 19–29. https://doi.org/10.1016/j.aquaculture.2019.04.067

Resende, L., Flores, J., Moreira, C., Pacheco, D., Baeta, A., Garcia, A.C., Rocha, A.C.S., 2022. Effective and Low-Maintenance IMTA System as Effluent Treatment Unit for Promoting Sustainability in Coastal Aquaculture. Appl. Sci. 12. https://doi.org/10.3390/app12010398

Rosenfeld, G., 1947. Corante pancrômico para hematologia e citologia clínica, 20th ed. Inst. Butantan.

Saleh, N.E., Shalaby, S.M., Sakr, E.M., Elmonem, A.I.A., Michael, F.R., 2014. Effect of Dietary Inclusion of *Ulva fasciata* on Red Hybrid Tilapia Growth and Carcass Composition. J. Appl. Aquac. 26, 197–207. https://doi.org/10.1080/10454438.2014.909279

Santos, I. L. M., Melatti, E., de Souza Correia, E., Ferreira, D. A., & da Silva, L.O.B. (2020)., 2020. Economic viability of tilapia cultivation (*Oreochromis niloticus*) in net tanks located in the municipality of Glória-BA.

Satheeshkumar, P., Ananthan, G., Kumar, D.S., Jagadeesan, L., 2012. Haematology and biochemical parameters of different feeding behaviour of teleost fishes from Vellar estuary, India. Comp. Clin. Path. 21, 1187–1191. https://doi.org/10.1007/s00580-011-1259-7

Sedlak, J., Lindsay, R.H., 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25, 192–205. https://doi.org/10.1016/0003-2697(68)90092-4

Silva, D.M., Valente, L.M.P., Pereira, R., Pires, M.A., Seixas, F., Rema, P., 2015. Evaluation of IMTA-produced seaweeds (*Gracilaria* , Porphyra , and Ulva) as dietary ingredients in Nile tilapia , *Oreochromis niloticus* L ., juveniles . Effects on growth performance and gut histology 1671–1680. https://doi.org/10.1007/s10811-014-0453-9

Srikanth, K., Pereira, E., Duarte, A.C., Ahmad, I., 2013. Glutathione and its dependent enzymes' modulatory responses to toxic metals and metalloids in fish-a review. Environ. Sci. Pollut. Res. 20, 2133–2149. https://doi.org/10.1007/s11356-012-1459-y

Suryaningrum, L.H., Samsudin, R., 2020. Nutrient digestibility of green seaweed Ulva meal and the influence on growth performance of Nile tilapia (*Oreochromis niloticus*). Emirates J. Food Agric. 32, 488–494. https://doi.org/10.9755/ejfa.2020.v32.i7.2131

Tziveleka, L.A., Tammam, M.A., Tzakou, O., Roussis, V., Ioannou, E., 2021. Metabolites with antioxidant activity from marine macroalgae. Antioxidants 10. https://doi.org/10.3390/antiox10091431

Unesco, 1983. Chemical methods for use in marine environmental monitoring. Intergovernmental Oceanographic Commission.

Ventura-Lima, J., de Castro, M.R., Acosta, D., Fattorini, D., Regoli, F., de Carvalho, L.M., Bohrer, D., Geracitano, L.A., Barros, D.M., Marins, L.F.F., da Silva, R.S., Bonan, C.D., Bogo, M.R., Monserrat, J.M., 2009. Effects of arsenic (As) exposure on the antioxidant status of gills of the zebrafish *Danio rerio* (Cyprinidae). Comp. Biochem. Physiol. - C Toxicol. Pharmacol. 149, 538–543. https://doi.org/10.1016/j.cbpc.2008.12.003

Wassef, E.A., El-Sayed, A.F.M., Sakr, E.M., 2013. *Pterocladia* (Rhodophyta) and *Ulva* (Chlorophyta) as feed supplements for European seabass, Dicentrarchus labrax L., fry. J. Appl. Phycol. 25, 1369–1376. https://doi.org/10.1007/s10811-013-9995-5

AOAC.Official Methods of Analysis; Association of Official Analytical Chemists: Arlington, VA, USA, 2005; p. 245..

Xu, P., Tan, H., Jin, W., Li, Y., Santhoshkumar, C., Li, P., Liu, W., 2018. Antioxidative and antimicrobial activities of intertidal seaweeds and possible effects of abiotic factors on these bioactivities. J. Oceanol. Limnol. 36, 2243–2256. https://doi.org/10.1007/s00343-019-7046-z

Yildiz, G., Celikler, S., Vatan, O., Dere, S., 2012. Determination of the anti-oxidative capacity and bioactive compounds in green seaweed *Ulva rigida* C. Agardh. Int. J. Food Prop. 15, 1182–1189. https://doi.org/10.1080/10942912.2010.517341

DISCUSSÃO GERAL

A sustentabilidade aquícola é definida na produção de organismos aquáticos para atender a demanda necessária, sem prejudicar o ecossistema ao redor ou esgotar recursos naturais necessários para manutenção da atividade (Boyd et al., 2020). De acordo com Campanati et al. (2021) uma maior eficiência no uso dos recursos e menor produção de resíduos são fatores chaves na intensificação da aquicultura de forma sustentável.

A utilização de sistemas que operam com mínima ou nula troca de água além de possuírem maior biossegurança se tornam sistemas mais sustentáveis, como a tecnologia de bioflocos e sistema simbiótico. O sistema simbiótico, caracterizado pelo pré-tratamento das fontes de carbono através da degradação causada por microrganismos, tem como função a manutenção da qualidade de água do sistema além de fornecer uma fonte complementar de alimento para os organismos cultivados (Khanjani et al., 2022). Devido essa manutenção da qualidade de água, os resultados mostraram um menor uso de água nos tratamentos com sistema simbiótico comparado ao cultivo em água clara. Sabe-se que no sistema convencional ocorre o acumulo da amônia devido à baixa concentração de microrganismos para oxidação do composto (Verdegem et al., 2006), diferente do sistema simbiótico onde ocorre o favorecimento da comunidade microbiana para controle dos compostos nitrogenados (Oladosu et al., 2016).

Além do sistema simbiótico, a utilização do sistema integrado neste experimento também mostrou que a inserção da macroalga promoveu uma menor concentração final de nitrato comparado aos tratamentos sem macroalga. A utilização de macroalgas como consumidor inorgânico tem se mostrado efetivo, assim como encontrado por Jones et al. (2001). Entretanto, para o fosfato, a produção desse composto no simbiótico é superior aos demais sistemas, provavelmente devido ao pré-6tratamento do farelo (Lima et al., 2021), sendo produzido um valor superior ao que a macroalga consegue absorver.

Além do acúmulo de nutrientes, a produção de biomassa bacteriana no sistema também é alta, o que influenciou negativamente no desempenho da ostra e macroalga. Para a macroalga, a deposição dos sólidos sobre a lamina fotossintetizante reduz a realização da fotossíntese (Summers et al., 2023), interferindo em seu crescimento, comparado ao tratamento em água clara. Para a ostra, apesar de ser um organismo filtrador, a predominância de partículas maiores que 1 e 3 µm, interferiu na alimentação e causou mortalidade total das ostras no sistema (Haven and Morales-alamo, 1970).

Em comparação ao sistema simbiótico, o sistema de bioflocos opera com a manipulação da relação carbono e nitrogênio da água para o crescimento de microrganismos (Khanjani et al., 2023). E por ser um sistema intensivo, ocorre o acúmulo de nutrientes no meio que podem

ser utilizados por um consumidor inorgânico. A inserção da macroalga no cultivo integrado com o camarão em um sistema de bioflocos com concentrações iniciais 56.67 ± 5.77, 1.55 ± 0.14 e 246.67 ± 2.89 mgL^{-1} de nitrato, fosfato e sólidos suspensos totais respectivamente, mostraram que a relação nitrogênio:fósforo do sistema é essencial para o desempenho da macroalga. De acordo com Zirino et al. (2016) a relação ideal para as macroalgas seria de 30:1 nitrogênio:fósforo, portanto a manutenção desses valores próximos ao ideal durante o cultivo promoveram melhor crescimento e absorção de nutrientes pela macroalga. Com isso, a integração da macroalga proporcionou uma taxa de remoção de nitrato de 55% e 31% de fosfato, mostrando ser um agente biorremediador e de grande aplicabilidade no sistema integrado.

No entanto, a produção constante de sólidos no sistema de bioflocos causada pela formação de biomassa bacteriana, acumulo de fezes e sobras de ração (Gaona et al., 2017) pode representar um problema para o crescimento das macroalgas. Por ser um organismo séssil, a macroalga funciona como uma barreira física na movimentação da água, aprisionando os sólidos sobre as laminas fotossintetizantes, como também constatado por Brito et al. (2016). Com isso, a profundidade da estrutura de cultivo interfere no crescimento da macroalga, e apesar de ter um menor espaço de estrutura para movimentação, a proximidade com a superfície promoveu um maior crescimento para a macroalga. De acordo com Reis et al. (2019) a penetração de luz diminui consideravelmente de acordo com a profundidade em sistema de biofloco, isso se deve a absorção e reflexão da luz causada pelas partículas em suspensão. Portanto a utilização de estruturas de cultivo com até 10 cm de profundidade aumentou a disponibilidade de luz para a realização da fotossíntese, gerando maiores taxas de crescimento especifica.

Essa alta produção de flocos microbianos, que são formados por agregados de bactérias, protozoários, microalgas, nematoides (Reis et al., 2019), também pode ser modificada com a inserção da macroalga no sistema. Nossos resultados mostraram uma menor concentração de diatomáceas, ciliados e cianofíceas, mostrando que uma competição por nutrientes gerou a exclusão ou diminuição de alguns microrganismos, sem afetar a qualidade de água ou desempenho dos animais. Assim como a presença de esporos foi encontrada nos tratamentos com maior densidade de macroalgas, devido a uma situação de estresse e reprodução, fazendo com que a macroalga liberasse esporos na água (Copertino et al., 2009).

Também no sistema de bioflocos as diferentes fertilizações adotadas além de influenciar na qualidade de água e custo, também irá afetar o desempenho dos organismos. O sistema quimioautotrófico é mantido através de fertilizações inorgânicas, resultando um maior ph,

alcalinidade e acumulo de nitrato (Pâmela et al., 2024), diferente do sistema heterotrófico com reuso de água e fertilização orgânica que possui uma maior geração de sólidos no sistema (Brandão et al., 2021). Apesar dessa formação de sólidos no sistema heterotrófico em termos de produção de biomassa não houve diferenças na produção final de macroalgas entre ambos os tratamentos, mostrando que a macroalga conseguiu se adaptar em ambos os sistemas. Em adição, devido a uma maior absorção de nutrientes pela macroalga no tratamento Heterotrófico, um maior teor de proteína foi encontrado no tecido da macroalga, portanto uma maior disponibilidade de nutrientes pode afetar a composição nutricional da macroalga (Msuya and Neori, 2008). Além do desempenho da macroalga, a qualidade de água também foi afetada pelo sistema de fertilização. No sistema Quimioautotrófico, devido ao não estabelecimento eficiente das bactérias no sistema foram necessárias renovações de água para controle do nitrito, interferindo na concentração de nutrientes para as macroalgas e sustentabilidade do sistema. Já o sistema heterotrófico com reúso de água promoveu melhor manutenção da qualidade de água, e melhor desemepenho em ganho de peso para a tilápia, provavelmente devido a maior disponibilidade de sólidos em suspensão (Poli et al., 2019). Portanto, de acordo com Krummenauer et al. (2014) além de maior sustentabilidade, o uso de água de um sistema maduro e fertilizações orgânicas promovem uma maior manutenção da qualidade de água em níveis ideais durante o cultivo e melhor desempenho das macroalgas.

Além do sistema integrado com espécies de diferentes níveis tróficos, as macroalgas também podem ser utilizadas para o tratamento de efluente aquícola (Rio, 1996). Durante 15 dias de cultivo a macroalga *U. lactuca* teve uma taxa de absorção de nitrato de 28% no tratamento heterotrófico, sendo o dobro do resultado encontrado no tratamento Quimioautotrófico, isso provavelmente se deve a uma maior disponibilidade de nitrogênio no tratamento Heterotrófico e um balanço mais próximo do ideal de nitrogênio e fósforo no sistema (Harrison and Hurd, 2001). Em tratamento de efluente a taxa de crescimento da macroalga foi superior aos demais experimento, tendo média geral de 3.76 ± 0.76 % dia^{-1}, isso se deve provavelmente a uma menor temperatura de cultivo, a ausência de manejo com outros organismos e não ocorrer a formação contínua de sólidos no sistema.

Por fim, a biomassa de macroalgas produzida possui biocompostos importantes, como pigmentos e compostos fenólicos que possuem propriedades antioxidantes e possível aumento na palatabilidade da ração (He et al., 2016; Yildiz et al., 2012), sendo um produto de interesse na inclusão em dietas. A utilização de até 15% de macroalga na dieta da tilápia não afetou o desempenho zootécnico e composição corporal do animal, semelhante ao encontrado por Ergün et al. (2009). No entanto, frente a um estresse de salinidade o tratamento 5% de inclusão da

macroalga promoveu ao animal uma melhor capacidade antioxidante com o aumento de enzimas, sem que ocorresse oxidação lipídica ou proteica. De acordo com Pérez-gálvez et al. (2020) carotenoides e clorofilas podem ser caracterizados como antioxidantes que buscam prevenir/interceptar a formação de espécies reativas de oxigênio, com isso a inclusão de macroalgas na dieta podem conferir maior preparação ao animal na prevenção de danos oxidativos.

Referências

Boyd, C.E., D'Abramo, L.R., Glencross, B.D., Huyben, D.C., Juarez, L.M., Lockwood, G.S., McNevin, A.A., Tacon, A.G.J., Teletchea, F., Tomasso, J.R., Tucker, C.S., Valenti, W.C., 2020. Achieving sustainable aquaculture: Historical and current perspectives and future needs and challenges. J. World Aquac. Soc. 51, 578–633. https://doi.org/10.1111/jwas.12714

Brandão, H., Xavier, Í.V., Santana, G.K.K., Santana, H.J.K., Krummenauer, D., Wasielesky, W., 2021. Heterotrophic versus mixed BFT system: Impacts on water use, suspended solids production and growth performance of Litopenaeus vannamei. Aquac. Eng. 95. https://doi.org/10.1016/j.aquaeng.2021.102194

Brito, L.O., Chagas, A.M., da Silva, E.P., Soares, R.B., Severi, W., Gálvez, A.O., 2016. Water quality, Vibrio density and growth of Pacific white shrimp Litopenaeus vannamei (Boone) in an integrated biofloc system with red seaweed Gracilaria birdiae (Greville). Aquac. Res. 47, 940–950. https://doi.org/10.1111/are.12552

Campanati, C., Willer, D., Schubert, J., Aldridge, D.C., 2021. Sustainable Intensification of Aquaculture through Nutrient Recycling and Circular Economies: More Fish, Less Waste, Blue Growth. Rev. Fish. Sci. Aquac. https://doi.org/10.1080/23308249.2021.1897520

Copertino, M.D.S., Tormena, T., Seeliger, U., 2009. Biofiltering efficiency, uptake and assimilation rates of Ulva clathrata (Roth) J. Agardh (Clorophyceae) cultivated in shrimp aquaculture waste water. J. Appl. Phycol. 21, 31–45. https://doi.org/10.1007/s10811-008-9357-x

Ergün, S., Soyutürk, M., Güroy, B., Güroy, D., Merrifield, D., 2009. Influence of Ulva meal on growth, feed utilization, and body composition of juvenile Nile tilapia (Oreochromis niloticus) at two levels of dietary lipid. Aquac. Int. 17, 355–361. https://doi.org/10.1007/s10499-008-9207-5

Gaona, C.A.P., de Almeida, M.S., Viau, V., Poersch, L.H., Wasielesky, W., 2017. Effect of different total suspended solids levels on a Litopenaeus vannamei (Boone, 1931) BFT culture system during biofloc formation. Aquac. Res. 48, 1070–1079. https://doi.org/10.1111/are.12949

Harrison, P.J., Hurd, C.L., 2001. Nutrient physiology of seaweeds: Application of concepts to aquaculture. Cah. Biol. Mar. 42, 71–82.

HAVEN, D.S., MORALES-ALAMO, R., 1970. Filtration of Particles From Suspension By the American Oyster Crassostrea Virginica . Biol. Bull. 139, 248–264. https://doi.org/10.2307/1540081

He, J., Xu, Y., Chen, H., Sun, P., 2016. Extraction, structural characterization, and potential antioxidant activity of the polysaccharides from four seaweeds. Int. J. Mol. Sci. 17. https://doi.org/10.3390/ijms17121988

Jones, A.B., Dennison, W.C., Preston, N.P., 2001. Integrated treatment of shrimp effluent by sedimentation, oyster filtration and macroalgal absorption: A laboratory scale study. Aquaculture 193, 155–178. https://doi.org/10.1016/S0044-8486(00)00486-5

Khanjani, M.H., Mozanzadeh, M.T., Fóes, G.K., 2022. Aquamimicry system: A sutiable strategy for shrimp aquaculture - A review. Ann. Anim. Sci. 22, 1201–1210. https://doi.org/10.2478/aoas-2022-0044

Khanjani, M.H., Mozanzadeh, M.T., Sharifinia, M., Emerenciano, M.G.C., 2023. Biofloc: A sustainable

dietary supplement, nutritional value and functional properties. Aquaculture 562, 738757. https://doi.org/10.1016/J.AQUACULTURE.2022.738757

Krummenauer, D., Samocha, T., Poersch, L., Lara, G., Wasielesky, W., 2014. The reuse of water on the culture of pacific white shrimp, litopenaeus vannamei, in BFT system. J. World Aquac. Soc. 45, 3–14. https://doi.org/10.1111/jwas.12093

Lima, P.C.M., Silva, A.E.M., Silva, D.A., Silva, S.M.B.C., Brito, L.O., Gálvez, A.O., 2021. Effect of stocking density of Crassostrea sp. in a multitrophic biofloc system with Litopenaeus vannamei in nursery. Aquaculture 530, 735913. https://doi.org/10.1016/j.aquaculture.2020.735913

Miguel Jimenez del Rio, Z.R.& G.G.-R., 1996. Ulva rigida (Ulvales, Chlorophyta) tank culture as biofilters for dissolved inorganic nitrogen from fishpond effluents.

Msuya, F.E., Neori, A., 2008. Effect of water aeration and nutrient load level on biomass yield, N uptake and protein content of the seaweed Ulva lactuca cultured in seawater tanks. J. Appl. Phycol. 20, 1021–1031. https://doi.org/10.1007/s10811-007-9300-6

Oladosu, Y., Rafii, M.Y., Abdullah, N., Magaji, U., Hussin, G., Ramli, A., Miah, G., 2016. Fermentation Quality and Additives: A Case of Rice Straw Silage. Biomed Res. Int. 2016. https://doi.org/10.1155/2016/7985167

Pâmela, R., Sena, O., Krummenauer, D., Jr, W.W., Augusto, O., Ferreira, L., Bezerra, A., Renato, J., Junior, S., Carvalho, A., Ravagnan, E., Bagi, A., Poersch, L.H.S., 2024. The Effects of Predominantly Chemoautotrophic Versus Heterotrophic Biofloc Systems on Nitrifying Bacteria , Planktonic Microorganisms , and Growth of Penaeus vannamei , and Oreochromis niloticus in an Integrated Multitrophic Culture.

Pérez-gálvez, A., Viera, I., Roca, M., 2020. Carotenoids and chlorophylls as antioxidants. Antioxidants 9, 1–39. https://doi.org/10.3390/antiox9060505

Poli, M.A., Legarda, E.C., de Lorenzo, M.A., Pinheiro, I., Martins, M.A., Seiffert, W.Q., do Nascimento Vieira, F., 2019. Integrated multitrophic aquaculture applied to shrimp rearing in a biofloc system. Aquaculture. https://doi.org/10.1016/j.aquaculture.2019.734274

Reis, W.G., Wasielesky, W., Abreu, P.C., Brandão, H., Krummenauer, D., 2019. Rearing of the Pacific white shrimp Litopenaeus vannamei (Boone, 1931)in BFT system with different photoperiods: Effects on the microbial community, water quality and zootechnical performance. Aquaculture 508, 19–29. https://doi.org/10.1016/j.aquaculture.2019.04.067

Summers, N., Fragoso, G.M., Johnsen, G., 2023. Photophysiologically active green, red, and brown macroalgae living in the Arctic Polar Night. Sci. Rep. 13, 1–16. https://doi.org/10.1038/s41598-023-44026-5

Verdegem, M.C.J., Bosma, R.H., Verreth, J.A.J., 2006. Reducing water use for animal production through aquaculture. Int. J. Water Resour. Dev. 22, 101–113. https://doi.org/10.1080/07900620500405544

Yildiz, G., Celikler, S., Vatan, O., Dere, S., 2012. Determination of the anti-oxidative capacity and bioactive compounds in green seaweed Ulva rigida C. Agardh. Int. J. Food Prop. 15, 1182–1189. https://doi.org/10.1080/10942912.2010.517341

Zirino, A., Elwany, H., Facca, C., Maicu', F., Neira, C., Mendoza, G., 2016. Nitrogen to phosphorus ratio in the Venice (Italy) Lagoon (2001-2010) and its relation to macroalgae. Mar. Chem. 180,

33–41. https://doi.org/10.1016/j.marchem.2016.01.002

CONCLUSÃO GERAL

A inserção da macroalga no cultivo multitrófico integrado com camarão e peixes em sistema de bioflocos se mostrou viável com a adoção das práticas de manejo realizadas, aumentando a produtividade do sistema, a sustentabilidade com absorção de nutrientes e rentabilidade com a aplicação da biomassa de macroalgas produzidas. Os experimentos realizados nesta tese mostraram que o sistema simbiótico afetou negativamente na sobrevivência da ostra *C. virginica* e o crescimento da macroalga *U. lactuca*, no entanto o camarão apresentou maior ganho de peso e menor fator de conversão alimentar quando comparado ao sistema integrado com ostra e macroalga. Em comparação, a adoção do sistema de bioflocos na concentração máxima de 250 mg L^{-1} de SST e 56 mg L^{-1} de nitrato promoveu maior remoção de nitrato e fosfato (55 e 31% respectivamente), associado com o aumento do teor de proteína e clorofila-a no tecido da macroalga quando cultivada em sistema integrado com camarão.

Melhores formas de cultivo da macroalga em escala piloto também aumentaram a produtividade do sistema, com isso, o uso de estruturas de cultivo com até 10 cm de profundidade possibilitou maior ganho de biomassa e taxa de crescimento relativa quando cultivada com camarão e peixe em bioflocos. Assim como a inserção da macroalga no sistema integrado não afetou negativamente a comunidade microbiana ou qualidade de água, proporcionando um decréscimo de cianofíceas, diatomáceas e ciliados. Além disso, a adoção de fertilizações organicas e reuso de água de um sistema de produção maduro, também possibilitou melhor estabilidade da qualidade de água sem o uso de renovações de água, maior ganho de peso para a tilápia, e maior taxa de remoção de nitrato e fosfato para a macroalga.

Além do cultivo integrado, a utilização da macroalga como biorremediador para o tratamento de efluente proveniente de um cultivo com bioflocos possibilitou uma remoção de 28% de nitrato e uma taxa de crescimento relativa de 4% dia^{-1} quando cultivada em sistema heterotrófico. Assim, essa biomassa produzida pode voltar ao sistema e possuir aplicação na formulação de ração de organismos aquáticos, aplicando o termo da economia circular. Com isso, a inclusão de 5% da macroalga produzida em sistema integrado com bioflocos na dieta da tilápia possibilitou uma maior resistência a eventos de estresse salino, com o aumento da capacidade antioxidante e menor oxidação proteica e lipídica.

ANEXO

Figura 1. Unidades experimentais de 0.60 m^{-3} para realização do experimento com sistema integrado com simbiótico (Capítulo 01).

Figura 2. Aquário com pós-larva de camarão, ostra e estrutura para macroalga para realização do experimento com sistema integrado com simbiótico (Capítulo 01).

Figura 3. A) Unidade experimental com 180 L de volume útil para camarão, e estrutura flutuante para inserção da macroalga no tanque. B) Pesagem das macroalgas (Capítulo 02).

Figura 4. Estufa agrícola com as unidades experimentais para camarão, peixe e macroalga (Capítulo 03).

Figura 5. Estrutura de cultivo para a macroalga no experimento com diferentes profundidades (Capítulo 03).

Figura 6. Sinais de esporulação da macroalga *Ulva lactuca*, “ghost tissue” (Capítulo 03).

Figura 7. Estufa agrícola para realização do experimento Quimioautotrófico e Heterotrófico (Capítulo 5 e 6).

Figura 8. Estrutura de cultivo da macroalga para o experimento tratamento de efluente (Capítulo 6).

Figura 9. Rações para a tilápia com as inclusões de 5, 10 e 15 % e ração controle (Capítulo 7).

Figura 10. Tilápia utilizada no experimento com ração na dieta (Capítulo 7).