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FLUXO DE GASES DO EFEITO ESTUFA (CO_2, CH_4, N_2O) EM MONOCULTIVOS DE CARCINICULTURA

MOSSORÓ – RN
2023

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Defesa apresentada ao Mestrado do Programa de Pós-Graduação em Ecologia e Conservação da Universidade Federal Rural do Semi-Árido como requisito para obtenção título de Mestre.

Linha de Pesquisa: Ecologia e conservação de ecossistemas aquáticos

Orientador: Prof. Dr. Gustavo Henrique Gonzaga da Silva - UFERSA

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Defendida em: 29 / 05 / 2023.

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À minha mãe, Reginalda Augusta da Rocha...

Que acreditou em mim quando ninguém mais acreditava

Que ofereceu cuidados quando estive necessitado

Que pediu a Deus a realização dos meus sonhos,

quando os seus próprios não foram realizados

A você, que merece todo o meu amor...

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APRESENTAÇÃO:

Este trabalho de dissertação aborda uma temática ambiental no contexto da aquicultura. Dedicou-se a compreender o fluxo difusivo e ebulitivo de três dos principais gases causadores do efeito estufa (dióxido de carbono, metano e óxido nitroso) gerados a apartir de atividades aquícolas em monocultivos de camarão marinho (*Litipenaeus vannamei*) em diferentes densidades de estocagem (baixa, média e alta).

O trabalho está organizado em dois capítulos. O capítulo I é a parte introdutória, aborda a temática geral da pesquisa e a problemática em torno do tema. Possui introdução geral e referencial teórico, abordando os caminhos de emissão de GEE, os objetivos e hipóteses e material e métodos, contendo os processos metodológicos e imagens das atividades realizadas em campo. Este capítulo foi elaborado no intuito de auxiliar futuros discentes de pós-graduação que queiram desenvolver outras pesquisas com escopo semelhante.

O capítulo II é a pesquisa propriamente dita. Possui uma introdução mais específica, material e métodos, os resultados e a discussão da dinâmica do fluxo de GEE e, as considerações finais. Este capítulo está escrito em formato de artigo científico no idioma inglês, visando facilitar a submissão ao periódico Aquatic Living Resource (https://www.alr-journal.org/) e para aprimorar as minhas habilidades de escrita científica.

RESUMO GERAL

A principal causa do aquecimento global é o acúmulo de gases que absorvem a radiação infravermelha na atmosfera terrestre, fenômeno conhecido como efeito estufa. O dióxido de carbono (CO_2), o metano (CH_4) e o óxido nitroso (NO_2) são os principais GEEs emitidos a partir das atividades antrópicas. As atividades realizadas em ecossistemas aquáticos, como a aquicultura, têm contribuído para o acúmulo desses gases. Desta forma, conhecer a dinâmica de emissão de GEE é de extrema importância para a avaliação da sustentabilidade ambiental da carcinicultura. Assim, é relevante quantificar o fluxo de GEE proveniente da carcinicultura. Diante do exposto, este trabalho objetiva avaliar a dinâmica do fluxo de GEE em sistemas de monocultivos de camarão marinho (*Litopenaeus vannamei*). A pesquisa foi realizada em duas fazendas de aquicultura, ambas localizadas no semiárido brasileiro. Com a finalidade de mensurar a produção de GEE, foram monitorados três cultivos de camarão. A dinâmica da emissão difusiva e ebulitiva dos gases CO_2, CH_4, e N_2O foi monitorada nos períodos diurno (06h) e noturno (18h). Os resultados indicaram que a densidade de estocagem é o principal regulador das emissões ebulitivas de CO_2. Foi observada uma tendência decrescente nas emissões difusivas de CO_2. A aeração desempenhou um papel importante no controle das emissões difusivas e ebulitivas de CH_4 e N_2O, reduzindo suas emissões. As emissões difusivas de CH_4 superaram as emissões ebulitivas, e a presença de oxigênio suprimiu a via anóxica para N_2O, fazendo os viveiros atuarem como sumidouros para esse gás. Este estudo destaca a influência da densidade de estocagem, aeração, parâmetros físico-químicos e abundância de fitoplâncton no fluxo de GEE. E, ressalta a importância de considerar e otimizar esses fatores no manejo de sistemas de cultivo de camarão, visando alcançar práticas sustentáveis e ecologicamente menos impactante e mitigar as emissões de GEE.

Palavras-chave: Gases do efeito estufa; camarão marinho; *Litopenaeus vannamei*; dióxido de carbono, metano, óxido nitroso.

ABSTRACT

The main cause of global warming comes from the accumulation of gases that absorb infrared radiation in the Earth atmosphere, a phenomenon known as the greenhouse effect. Methane (CH_4), nitrous oxide (NO_2) and, carbon dioxide (CO_2) are the main greenhouse gases (GHG) emitted from anthropogenic activities. Activities carried out in aquatic ecosystems, such as aquaculture, have also contributed to the accumulation of gases. Thus, knowing the dynamics of GHG emissions is extremely important for assessing the environmental sustainability of shrimp farming. In this context, it is relevant to quantify the flux of greenhouse gases from productive activities such as shrimp farming. The aims of this work were to evaluate the GHG flux dynamics in marine shrimp (*Litopenaeus vannamei*) monoculture systems and verify whether different stocking densities of marine shrimp influence the GHG emissions flux. The study was carried out in two aquaculture farms, both located in the Brazilian semiarid region. In order to measure the production of greenhouse gases, three cultures of marine shrimp were monitored. The dynamics of the diffusive and ebullitive emission of CO_2, CH_4, and N_2O were monitored in the three culture units during the daytime (6:00 am) and nighttime (6:00 pm) according to the sampling of each pond. The results showed the stocking density as the main regulator for ebullitive CO_2 emissions. A decreasing trend in diffusive CO_2 emissions was observed. Aeration played a crucial role in controlling the diffusive and ebullitive emissions of CH_4 and N_2O, effectively reducing their release. The diffusive CH_4 emissions exceeded ebullitive emissions and the oxygen content suppressed the anoxic pathway for N_2O and the ponds acted as a sink for this gas. This study showed the influence of stocking density, aeration, physicochemical parameters and phytoplankton abundance in the flux of GHG and highlights the importance of considering and optimizing them in the design and management of shrimp farm systems to achieve sustainable and environmentally friendly practices while mitigating GHG emissions.

Keywords: Greenhouse gases; marine shrimp; *Litopenaeus vannamei*; carbon dioxide, methane, nitrous oxide.

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1 INTRODUÇÃO GERAL

A principal causa do aquecimento global é o acúmulo de gases que absorvem a radiação infravermelha na atmosfera terrestre, fenômeno esse conhecido como efeito estufa (Cloy; Smith, 2017), sendo que nas últimas décadas, as atividades humanas têm contribuído significativamente para o aumento das emissões de CO_2 (Friedlingstein *et al*., 2022), metano e óxido nitroso (Jones *et al*., 2023). As emissões antrópicas de gases do efeito estufa (GEE) corresponderam a 59 $GtCO_2$-eq (± 6.6) no ano de 2019, sendo a contribuição de cada gás equivalente a: 45 $GtCO_2$-eq (± 5.5) para o dióxido de carbono (CO_2), 11 $GtCO_2$-eq (± 3.2) para o metano (CH_4) e, 2.7 $GtCO_2$-eq (± 1.6) para o óxido nitroso (N_2O) (IPCC, 2022).

A aquicultura é uma importante atividade produtora de proteína para a alimentação humana (Cai; Zhou, 2019), mas que também gera impactos ambientais (Ottinger; Clauss; Kuenzer, 2016; Soares; Henry-Silva, 2019), como por exemplo, possíveis emissões de dióxido de carbono, metano e óxido nitroso para a atmosfera. (Yang *et al*., 2015). A emissão aquícola corresponde a 263 $MtCO_2$eq, o que representa 0,49% do total das emissões antrópicas de GEE (Macleod *et al*., 2020). As emissões de CH_4 e N_2O a partir das atividades aquícolas são estimadas em 1,8% e 0,3% das emissões de globais, respectivamente, considerando apenas as emissões dos 21 principais produtores mundiais (Yuan *et al*., 2019).

O impacto gerado por este setor é variável e depende do tipo de espécies cultivadas e da intensidade de produção (Efole Ewoukem *et al*., 2012). Estima-se que aproximadamente 16,6 milhões de toneladas de carbono estão submersos em viveiros de aquicultura a nível mundial, demonstrando que o acúmulo de carbono no sedimento dos viveiros pode ser um importante mecanismo de sequestro desse elemento químico, podendo gerar créditos de carbono pela aquicultura (Boyd *et al*., 2010). Entretanto, vale enfatizar que as estimativas de emissão de GEE entre os anos de 2010 a 2019 apresentaram a maior média já registradas em uma década (~56 $GtCO_2$-eq yr^{-1}) (IPCC, 2022).

A produção de óxido nitroso (N_2O) em sistemas aquícolas ocorre por meio de processos de nitrificação e desnitrificação (Yuan *et al*., 2021). Os mecanismos que regulam a produção de N_2O na aquicultura estão relacionados com os parâmetros operacionais, como fertilização e condições ambientais, visto que tais parâmetros afetam diretamente a nitrificação e desnitrificação (Hu *et al*., 2013). Em águas oxigenadas da camada do epilímnio, a principal via de produção de N_2O é a nitrificação. Enquanto que, na interseção óxico-anóxica, entre o epilímnio e o hipolímnio, ocorrem tanto processos aeróbicos quanto anaeróbicos (Webb *et al*., 2019).

Desta forma, conhecer a dinâmica de emissão de gases gerados é de extrema relevância para a avaliação da sustentabilidade ambiental da aquicultura (Valenti *et al*., 2011; Schott *et al*., 2016). Os dados na literatura sobre medidas de fluxos de gases do efeito estufa ainda são escassos, principalmente de óxido nitroso em regiões de clima tropical. Assim, é importante quantificar o fluxo de gases de efeito estufa provenientes de atividades produtivas como a carcinicultura. Diante do exposto, esse trabalho objetiva avaliar a dinâmica de fluxo de gases do efeito estufa em sistemas de monocultivo de camarão marinho (*Litopenaeus vannamei*).

2 REFERENCIAL TEÓRICO

2.1 Principais gases do efeito estufa e fontes antropogênicas de emissão

O gás metano (CH_4), o óxido nitroso (N_2O) e o dióxido de carbono (CO_2) são os principais gases de efeito estufa (GEE) emitidos a partir das atividades humanas (Yang *et al*., 2015). Estudos climáticos têm demonstrado diversos impactos atrelados ao acúmulo desses gases e às mudanças climáticas, tais como: o aumento do nível e da acidificação dos oceanos, o derretimento das calotas polares e as alterações nos padrões de precipitação (IPCC, 2022). Esses eventos são consequência da absorção da radiação solar infravermelha pelos gases acumulados na atmosfera terrestre, causando o seu aquecimento e gerando distúrbios nos ecossistemas (Cloy; Smith, 2017).

As concentrações atmosféricas de gases têm aumentado rapidamente desde o século XVIII devido ao advento da industrialização e do crescimento da população humana. No ano de 2011, estas concentrações já atingiam valores superiores aos relatados para o período pré-industrial, cerca de 40% a mais para o dióxido de carbono; 150% para o metano; e 20% para o óxido nitroso (IPCC, 2013). Em 2016, valores ainda mais altos foram aferidos: 145% para o CO_2 (403.3±0.1ppm); 257% para o CH_4 (1853±2 ppb); e 122% para o N_2O (328.9±0.1 ppb) (WMO, 2017). Em 2019, o total das emissões antrópicas foram: 59 $GtCO_2$-eq (± 6.6), sendo a contribuição indvidual de cada gás equivalente a: 45 $GtCO_2$-eq (± 5.5) para o dióxido de carbono (CO_2), 11 $GtCO_2$-eq (± 3.2) para o metano (CH_4) e, 2.7 $GtCO_2$-eq (± 1.6) para o óxido nitroso (N_2O) (IPCC, 2022).

Dentre as fontes antropogênicas de emissão de GEE, as mais significativas são as atividades agropecuárias (IPCC, 2016; Osório; Azevedo, 2014). A contribuição desse setor para o aumento das concentrações desses três importantes gases do efeito estufa é de aproximadamente 25% referente ao CO_2; 65% para o CH_4; e 90% para o NO_2 (IPCC, 2016). No entanto, outras atividades também estão relacionadas com as emissões de GEE, como a produção de energia elétrica e a aquicultura. Estudos em hidrelétricas demonstraram que estes ambientes não apresentam emissões nula de gases, como se pensava anteriormente (Chen *et al*., 2011). A aquicultura, por sua vez, uma das principais produtoras de proteína animal para a alimentação humana (Cai; Zhou, 2019) apesar de reduzir a pressão de pesca sobre os ecossistemas naturais, também tem causado diversos impactos ambientais negativos a partir da emissão dos principais gases do efeito estufa (Yang *et al*., 2015).

A porcentagem de emissão e os respectivos potenciais de aquecimento global são distintos para os três gases. O dióxido de carbono (CO_2), dentre os GEE, é o gás individual mais importante, pois apresenta um forçamento radiativo total (impacto que um gás tem no balanço de energia da Terra) de 64%, ademais, corresponde a 82% das emissões antropogênicas de gases. No entanto, gases traços, como o CH_4 e o N_2O, possuem contribuição substancial sob a perspectiva de seus altos potenciais de aquecimento global, cerca de 27 e 273 vezes maior do que o CO_2, respectivamente, o que significa que são mais eficientes como gases indutores do efeito estufa (IPCC, 2022).

2.2 Tipos de emissões e fatores que influenciam no fluxo de GEE

Há dois modos distintos pelos quais os gases podem ser emitidos para a atmosfera a partir dos ecossistemas aquáticos: emissões difusivas e emissões ebulitivas. Na emissão difusiva, ocorre a difusão molecular dos gases através da coluna d'água, sendo, em seguida, liberados no ar. Enquanto que, nos processos ebulitivos, as bolhas são formadas naturalmente no fundo do corpo hídrico, sendo periodicamente liberadas.

Em ecossistemas aquáticos, a pressão antrópica das atividades aquícolas ocasiona a produção de GEE (Ottinger; Clauss; Kuenzer, 2016; Soares; Henry-Silva, 2019). Nessas atividades, o fluxo de gases e o impacto ambiental gerado dependem do tipo de manejo empregado, das espécies cultivadas e dos tipos de sistemas de produção. Alguns sistemas de cultivo são considerados pouco impactantes, podendo oferecer, inclusive, benefícios ambientais (Valenti *et al*., 2011), como o acúmulo de carbono no sedimento (Boyd *et al*., 2010). Dentro dessa perspectiva, terão influência as espécies cultivadas e as densidades de estocagem utilizadas (Efole Ewoukem *et al*., 2012). Fatores como a qualidade da água, a quantidade de oxigênio disponível, as características físico-químicas do ambiente e a qualidade do alimento ofertado aos organismos cultivados também influenciam a dinâmica do fluxo de gases (Hu *et al*., 2012).

O dióxido de carbono é um gás presente na atmosfera terrestre, apresentando distintas formas de emissão. Dentre elas, se destacam a queima de combustíveis fósseis e diversos outros materiais biológicos. O ciclo do carbono se completa com o sequestro de CO_2 da atmosfera pelas plantas, por meio do processo fotossintético. É importante ressaltar que, a influência das atividades antrópicas tem alterado a capacidade dos sumidouros naturais em remover o CO_2 e armazená-lo no solo e nas florestas (EPA, 2022).

O gás metano (CH_4) é formado, tanto em condições ambientais de anoxia, a partir da decomposição da matéria orgânica no sedimento dos ambientes aquáticos (Lofton *et al*., 2015), quanto na presença de oxigênio (O_2), por meio de microorganismos planctônicos (Tang *et al*., 2016). O transporte do gás metano (CH_4) produzido no sedimento pode ocorrer por meio dos processos de ebulição e difusão através da coluna d’água ou mediado por plantas (Yang *et al*., 2015). Sendo as emissões ebulitivas responsáveis por cerca de 90% das emissões totais de CH_4 em sistemas aquícolas (Yang *et al*., 2020).

É importante enfatizar que metade da emissão global de metano provém dos ecossistemas aquáticos, sendo as atividades antrópicas responsáveis por grande parte dessas emissões, aproximadamente 41 a 53%. O impacto do gás metano sobre o aumento das temperaturas globais foi, inicialmente, subestimado pelos cientístas devido a cálculos imprecisos dessas emissões. Dentro dessa perspectiva, o metano apresenta uma forte atuação no controle da temperatura da Terra, visto que é mais potente comparado ao dióxido de carbono (Rosentreter *et al*., 2021). Já o óxido nitroso (N_2O), nos sistemas aquícolas, pode ser produzido durante os processos de nitrificação e desnitrificação microbianas. Os mecanismos exatos de produção de N_2O na aquicultura estão relacionados com parâmetros operacionais específicos, como fertilizações, condições ambientais, e oxigênio dissolvido (OD), pois nos sistemas de aquicultura, esses parâmetros operacionais afetam diretamente a nitrificação e desnitrificação (Hu *et al*., 2013).

3 HIPÓTESE

Monocultivos de camarão marinho (*Litopenaeus vannamei*), em baixa densidade de estocagem, emitem menor quantidade de GEE, quando comparados a monocultivos com maiores densidades de estocagem, devido ao menor aporte de matéria orgânica proveniente do alimento artificial.

4 OBJETIVO

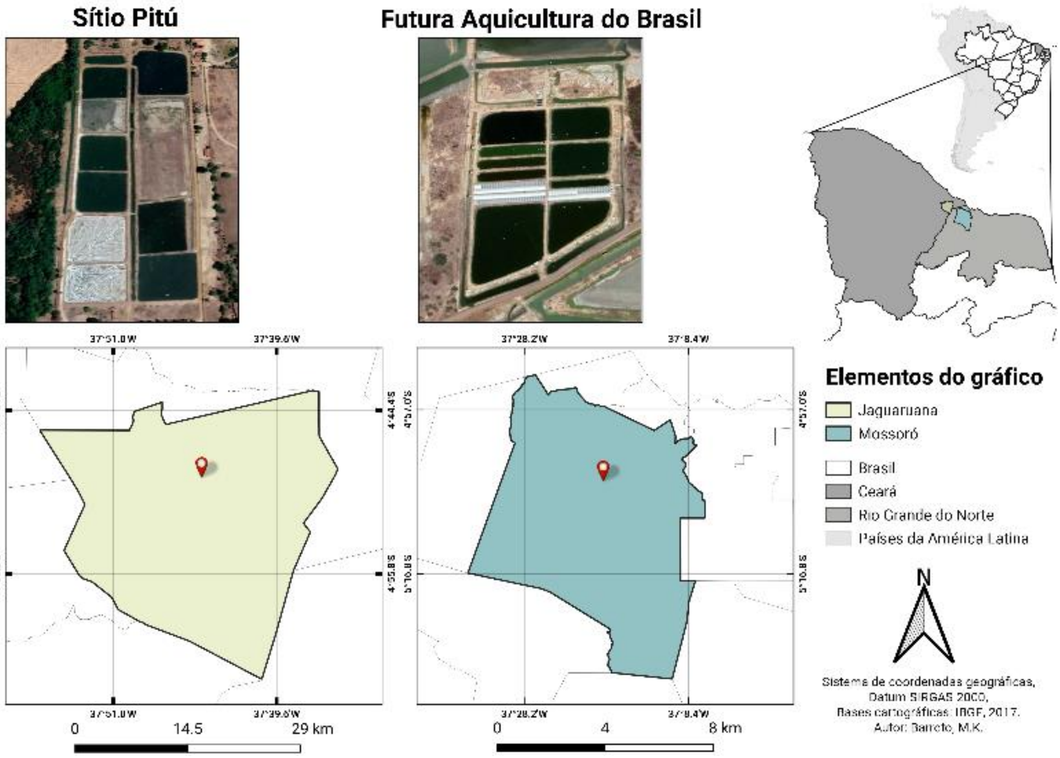
Avaliar a dinâmica do fluxo de gases do efeito estufa em sistemas de monocultivo de camarão marinho (*Litopenaeus vannamei)* no semiárido brasileiro.

5 MATERIAL E MÉTODOS

5.1 Área de estudo

A pesquisa foi realizada em duas empresas, fazenda Futura Aquicultura do Brasil, localizada no município de Mossoró, no estado do Rio Grande do Norte (5.1919° S, 37.3424° W), e na Fazenda Sítio Pitú, localizada no município de Jaguaruana, no estado do Ceará, Brasil, semiárido brasileiro (4.8378° S, 37.7829° W) (fig. 01; fig. 02, a e b).

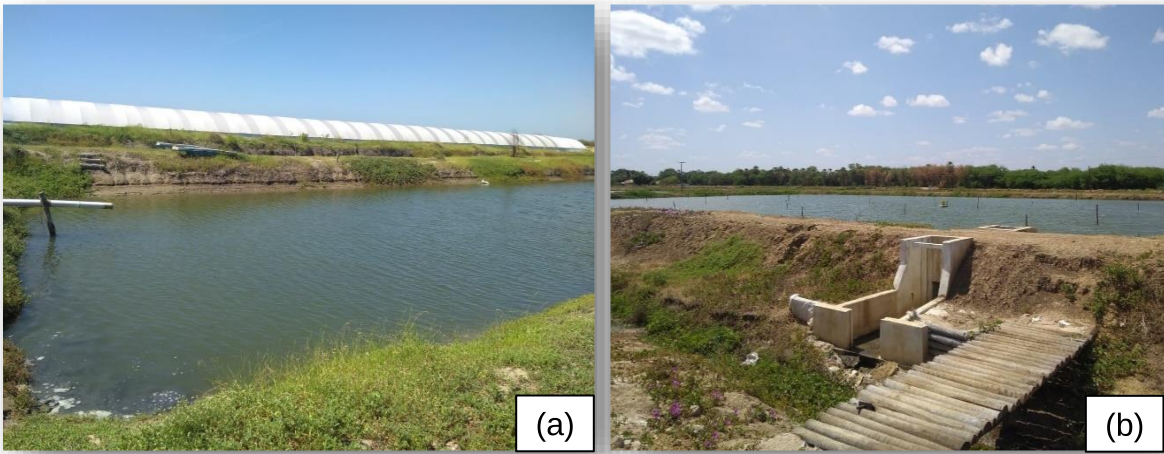
Figure 01 – Localização geográfica da área de estudo.



Fonte: elaborado pelo autor.

A fazenda Futura Aquicultura do Brasil possui 80 viveiros de engorda com área variado de 0,16 a 6 ha, com área tota del de 800 hectares. A fazenda Sítio Pitú possui 6 viveiros de 0,5 ha a 0,7 ha, e uma área total de 2,7 hectares. Ambas empresas cultivam camarões marinhos (*Litopenaeus vannamei*). A região é caracterizada por clima semiárido muito quente (BSwh), com estação chuvosa ocorrendo no verão com atraso para o outono; temperatura média de 27,4 ºC; precipitação pluviométrica anual média de 685,3 mm e umidade relativa média do ar de 68,9%.

Figura 02 – Imagens das fazendas: (a) Cultivos na fazenda Futura Aquicultura do Brasil e, (b) Cultivo na fazenda Sítio Sítio Pitú.



Fonte: elaborado pelo autor.

5.2 Delineamento experimental

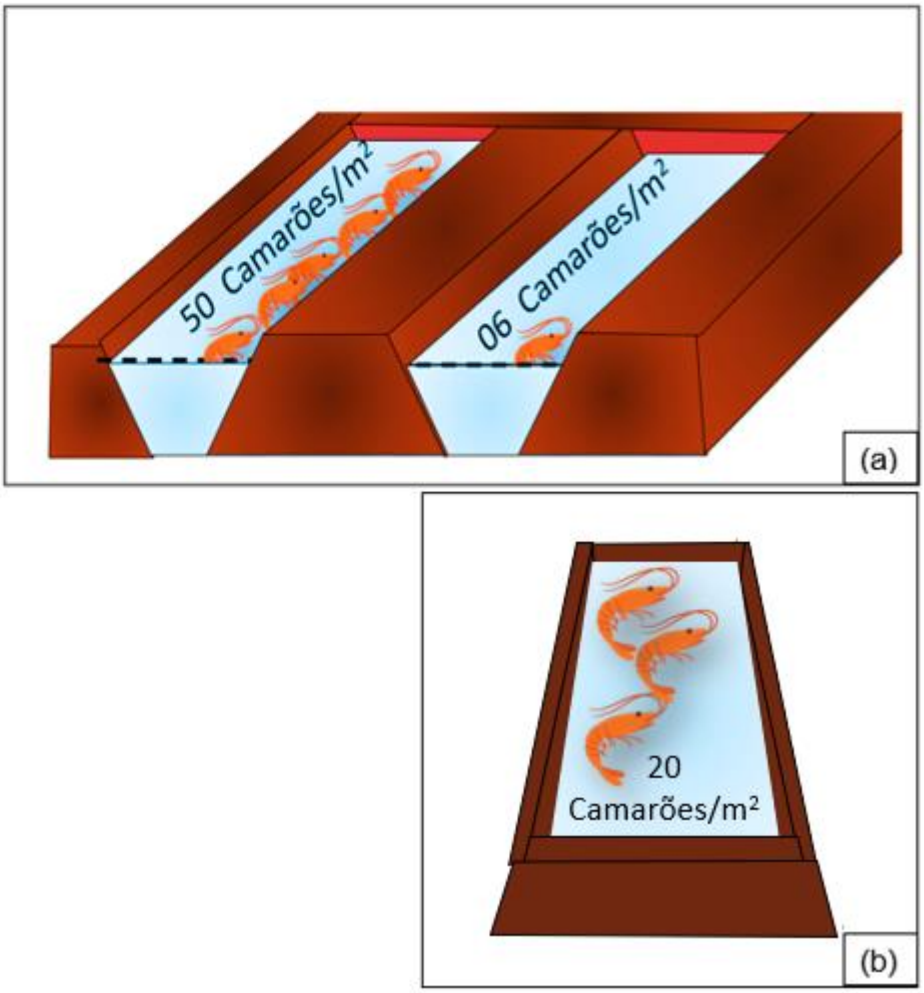
Com a finalidade de quantificar a produção de gases do efeito estufa foram analisados três sistemas de cultivo (tabela 01) de camarão marinho (*L. vannamei*). Dois cultivos foram conduzidos na fazenda Futura Aquicultura do Brasil (fig. 04, a), com densidades de 6 camarões/m^2 (C6) e 50 camarões/m^2 (C50) e um cultivo conduzido na Fazenda Sítio Sítio Pitú (fig. 04, b), com densidade de 20 camarões/m^2 (C20).

Tabela 01 - Descrição dos viveiros

	C6	**C20**	**C50**
Área (m^2)	2000	5000	6000
Profundidade (m)	1,5	1,0	1,5
Hectares (ha)	0,2	0,5	0,6
Tempo de cultivo (dias)	120	120	64
Densidade de estocagem (camarões/m^2**)**	6	20	50

Fonte: elaborado pelo autor.

Figura 03 – Esquematização dos cultivos. (a) Cultivos na fazenda Futura Aquicultura do Brasil e, (b) Cultivo na fazenda Sítio Sítio Pitú.



Fonte: elaborado pelo autor.

5.3 Detalhamento e manejo

Antes da estocagem inicial das pós-larvas de camarão foi adicionado probiótico aos viveiros, com o intuito de obter melhor qualidade de água e saúde dos organismos cultivados. Não foram realizadas fertilizações iniciais em nenhum dos cultivos. O método de arraçoamento foi o voleio, utilizando ração peletizada com 35% de teor de proteína bruta, ofertadas triturada nas fases iniciais dos cultivos. Foram ofertadas duas refeições diárias.

5.4 Procedimento de coleta

Foram realizadas quatro campanhas de coleta em C6 e C20 e duas campanhas em C50, sendo colatadas amostras de água superficial e de gases. A primeira ocorreu antes do povoamento dos organismos (controle); a segunda, após 40 dias; a terceira, após 70 dias e; a quarta, ao final do experimento, com aproximadamente 90 dias. Em cada coleta, foram realizadas amostragens em três pontos em cada sistema de cultivo.

As análises de nutrientes das amostras superficiais ocorreram de acordo com os seguintes métodos: nitrogênio total (Koroleff, 1976); fósforo total e ortofosfato (Golterman *et al*., 1978); amônia, nitrito e nitrato (Mackereth *et al*., 1978); carbono orgânico e inorgânico (analisador de carbono VARIO-TOC); carbono total (CT) e clorofila *a* (APHA, 2005). A transparência da água foi obtida com um disco de Secchi, a salinidade com um refratômetro e, os valores de temperatura, pH e oxigênio dissolvido, com um multisensor de variáveis limnológicas (HORIBA U-50).

5.5 Quantificação dos Gases

A dinâmica das emissões difusivas (fig. 05, a) e ebulitivas (fig. 05, b) dos gases dióxido de carbono (CO_2), metano (CH_4) e óxido nitroso (N_2O) foram monitoradas nas três unidades de cultivo nos períodos diurno (iniciando às 06h00) e noturno (iniciando às 18h00) nos três pontos de amostragem, de acordo com as campanhas de coleta para cada cultivo. Foi utilizada uma câmara de difusão para estimar o fluxo difusivo superficial (Santos *et al*., 2005). Após a instalação da câmara, foram retiradas amostras de gases com o auxílio de uma seringa de 30 mL, nos tempos de 0 min, 1 min, 2 min e 4 min e, imediatamente, armazenadas em câmaras gasométricas.

Foram utilizados funis invertidos submersos, com diâmetro de 0,0707 m^2, para estimar as emissões por ebulição (Santos *et al*., 2005). Os funis foram instalados abaixo da superfície da água e, um frasco previamente preenchido com água, foi fixado ao seu topo, o qual coletou os gases. Após um período de 24 horas, os frascos foram retirados e transportados ao laboratório, onde os gases foram retirados e armazenados em câmaras gasométricas para a determinação das concentrações de metano (CH_4), dióxido de carbono (CO_2) e óxido nitroso (N_2O) pelo método cromatográfico (modelo cromatográfico Hplc Shimadzu).

Figura 05 – Coleta de gases: (a) gases difusivos e, (b) gases ebulitivos.

Fonte: elaborado pelo autor.

5.6 Variáveis zootécnicas

Com a finalidade de realizar biometrias (fig. 06) e quantificar as variáveis zootécnicas, foram capturados quinzenalmente aproximadamente 10% dos camarões com o auxílio de uma tarrafa e, os organismos, imediatamente devolvidos aos viveiros após as pesagens. O cálculo para o ganho de biomassa consiste na diferença entre a biomassa média individual atual e a biomassa média individual da pesagem anterior. A sobrevivência dos organismos, expressa em percentual, foi calculada com base na quantidade de animais despescados, dividido pelo número de indivíduos estocados e multiplicado por cem. A produção dos cultivos foi obtida por meio da soma de biomassa, em quilos, de todos os animais despescados e extrapolada para a área de um hectare.

O Fator de Conversão Alimentar Aparente (FCAA), foi calculado com base na ração total ofertada, dividido pela soma das biomassas finais dos organismos cultivados. A Taxa de Conversão Proteica (TCP) (quantidade de proteína bruta necessária para produzir um quilograma de pescado vivo), foi obtida multiplicando o FCAA pelo percentual de proteína bruta presente na ração (35%) e divido por 100.

Figura 06 – biometria dos camarões.

Fonte: elaborado pelo autor.

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CAPÍTULO II

GREENHOUSE GAS EMISSION FLUX (CO_2, CH_4, N_2O) FROM SHRIMP MONOCULTURE

ABSTRACT

Greenhouse gases (GHG) and their atmospheric rising concentrations have been one of the most important international subjects due to their huge impact on climate change (IPCC, 2019). Aquaculture has crucial importance to the human food supply and nutrition since it is one of the major sectors providing animal protein, besides reducing the pressure on the fisheries stocks from natural resources worldwide (NAYLOR *et al*., 2000). On the other hand, it represents a threat to the environment by generating waste. Thus, this work aims to evaluate the GHG flux dynamics in marine shrimp (*Litopenaeus vannamei*) monoculture systems. The study was carried out in two aquaculture farms, both located in the Brazilian semiarid region. In order to measure the production of greenhouse gases, three cultures of marine shrimp were monitored. The dynamics of the diffusive and ebullitive emission of CO_2, CH_4, and N_2O were monitored in the three units during the daytime (6:00 am) and nighttime (6:00 pm). The results showed the stocking density as the main regulator for ebullitive CO_2 emissions. A decreasing trend in diffusive CO_2 emissions was observed. Aeration played a crucial role in controlling the diffusive and ebullitive emissions of CH_4 and N_2O, effectively reducing their release. The diffusive CH_4 emissions exceeded ebullitive emissions and the oxygen content suppressed the anoxic pathway for N_2O and the ponds acted as a sink for this gas. This work aims to evaluate the dynamics of greenhouse gas flow in marine shrimp (*Litopenaeus vannamei*) monoculture systems in the Brazilian semi-arid region and to verify whether lower stocking densities emit less greenhouse gases when compared to higher densities.

Keywords: Greenhouse gases; marine shrimp; *Litopenaeus vannamei*; carbon dioxide, methane, nitrous oxide.

1 INTRODUCTION

Greenhouse gases (GHG) and their rising atmospheric concentrations have been one of the most important international subjects due to their huge impact on climate change. The anthropogenic emissions accounted for 59 $GtCO_2$-eq (± 6.6) in 2019, at a lower annual growth rate (1.3%, 2010‑2019) compared to the last decadal growth rate (2.1%, 2000‑2009). This data still represents a threat to the environment since its annual average (56 $GtCO_2$-eq yr^{-1}, 2010‑2019) was the greatest decadal mean recorded (IPCC, 2022).

The aquatic ecosystems show a great potential to contribute to the increase of global warming by GHG emissions, which is enhanced for human activities such as aquaculture (Ottinger, 2016; Soares; Henry-SILVA, 2019). The contribution of aquaculture activities to GHG emissions was equivalent to 263 $MtCO_2$-eq in 2017, which represents 0.49% of total anthropogenic GHG emissions (Macleod *et al*., 2020).

Aquaculture has crucial importance to the human food supply and nutrition, since it is one of the major sectors providing animal protein (Cai; Zhou, 2019; FAO, 2022). Its advantages is a short production cycle, a small space demand, a sophisticated production technological level (Samuel-Fitwi *et al*., 2012), and reduction of the pressure on the fisheries stocks from natural resources worldwide. Among 178 million tonnes of global aquaculture, 88 million tonnes were from aquaculture (49%) (FAO, 2022). On the other hand, it represents a threat to the environment by generating waste. The culture of *Litopenaeus vannamei*, for example, when carried out at high stocking density, generates a huge amount of nutrients, particulate matter, high turbidity, and high particle sedimentation rates (Bessa Júnior *et al.,* 2021; Henry-Silva *et al*., 2023).

Moreover, it is characterized by low nutrient utilization, where 70% of the nitrogen is wasted by discarding or excreted by the shrimps in dissolved forms or particles (Troell *et al*., 1999). The uneaten food and animal feces release nutrients into the water body causing eutrophication (Thomsen *et al*., 2020) and emission of GHG, which carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are the main gases emitted (Yang, P. *et al*., 2015). It is important to emphasize that the intensity of those impacts is variable and depends on different factors such as the type of culturing species and the production intensity rate (Ewoukem *et al*., 2012). Additionally, considering the amount of anthropogenic global emissions, among those three greenhouse gases, the most important are carbon dioxide and methane, and the individual contribution of each gas is equivalent to: 45 GtCO2-eq (± 5.5) for CO_2, 11 GtCO2-eq (± 3.2) for CH_4 and, 2.7 GtCO2-eq (± 1.6) for N_2O (IPCC, 2022). Methane and nitrous oxide, despite being trace gases, need to have attention on them due to their high global warming potential, approximately, 27 and 273 times higher than carbon dioxide in a period of 100 years, respectively (Forster *et al*., 2021). It shows clearly their substantial contribution to global warming, which means they are much more efficient as greenhouse gases compared to carbon dioxide.

The Brazilian shrimp farming accounts for around 10% of the global production, which sets Brazil as the third Latin American biggest producer (FAO, 2017) and 99% of total Brazilian shrimp production occurs in the northeastern region (IBGE, 2019). Although, there is still a lack of data about shrimp farming greenhouse gases emissions, particularly, nitrous oxide. Within this perspective, this work aimed to evaluate the dynamic of greenhouse gas flux from monocultures of shrimp farming systems and verify whether different stocking densities of marine shrimp (*L. vannamei*) influence the (GHG) emissions flux. Our hypothesis is: marine shrimp monocultures at low stocking density emit less GHG when compared to higher-densities monocultures due to the lower input of organic matter from the artificial food.

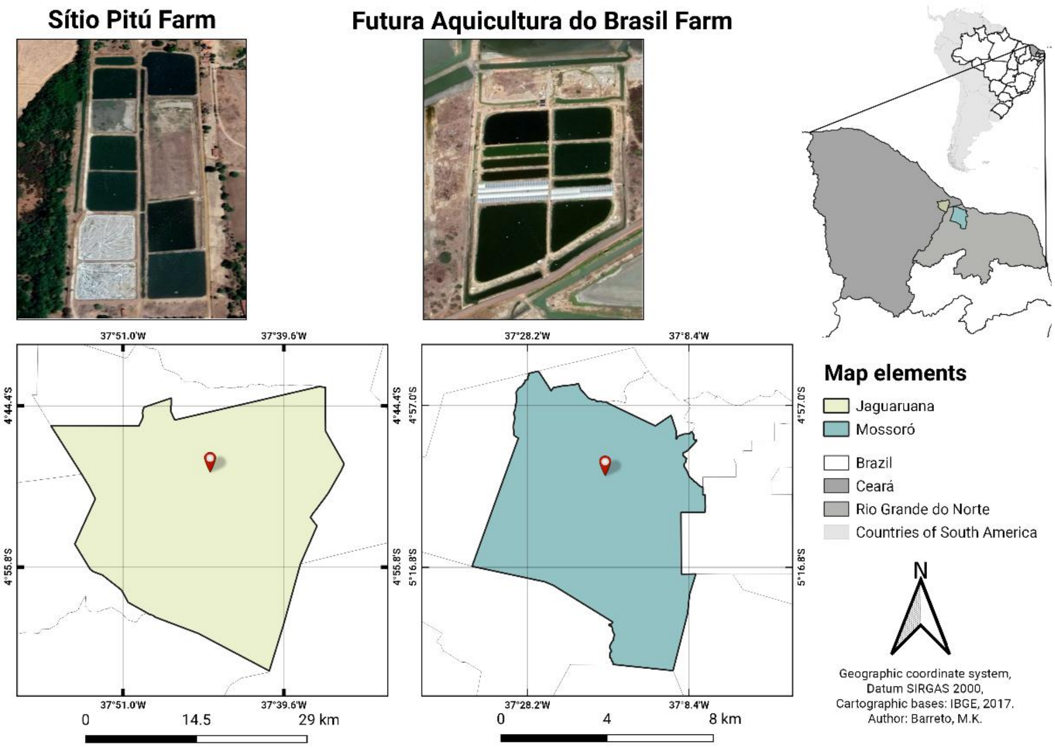
2 MATERIALS AND METHODS

2.1 Study Area

The study was carried out in two aquaculture farms: on the “Futura Aquicultura do Brasil” farm, located in the city of Mossoró, state of Rio Grande do Norte, and on the “Sítio Pitú” Farm, located in the city of Jaguaruana, state of Ceará, Brazil, both located in the Brazilian semi-arid region. The Futura Aquicultura do Brasil farm has 80 shrimp ponds with, approximately, 0.16 to 6.0 ha, totaling an area of 800 hectares. The Sítio Pitú farm has 6.0 shrimp ponds ranging from 0.5 ha to 0.7 ha, covering a total area of 2.7 hectares. Both shrimp farms cultivate Marine

Shrimp (*L. vannamei*). The climate is considered hot/semiarid (BSw'h', according to Köpper Classification), which has a rainy season occurring from January to June; an average temperature of 27.4 ºC; an average annual rainfall of 685.3 mm and average relative humidity of the air of 68.9%.

Figure 01 – Geographic location of the study area.



Source: Elaborated by the authors.

2.2 Experimental design

In order to measure the flux of greenhouse gases, three cultures of marine shrimp (*Litopenaeus vannamei*) were monitored. Two systems were conducted on the "Futura Aquicultura do Brasil" farm, one with 6 shrimp/m^2 (C6) and another with 50 shrimp/m^2 (C50) and; and one culture on "Sítio Pitú" farm, with 20 shrimp/m^2 (C20). The culturing pond C6, had an area of 0,2 ha and 1.5 m of depth. The culturing pond C20, had an area of 0,5 ha and 1 m of depth, and; the culturing pond C50, had an area of 0,6 ha and 1,5 m of depth. The ponds had cultivation times of 120, 120, and 64 days, respectively.

Four sampling campaigns were carried out in experiments C6 and C20, and only two sampling campaigns were in C50 due to the economic crisis faced by the farm occasioned by the COVID-19 pandemic. The first sampling (control) occurred before the initial storage; the second, after 40 days; the third, after 70 days and the fourth at the end of the experiment, with approximately 90 days. In each system, the samples were collected at three sampling points.

2.3 Shrimp ponds management

Before the initial storage of the shrimp post-larvae, probiotic was added to the ponds in order to obtain better water quality and shrimp health. Initial fertilization was not performed in any of the cultures. The supply of ration followed the volley method, using a pelleted ration with 35% of protein content, which was triturated in the initial phases of the culturing period. Two daily meals were offered.

2.4 Laboratory analysis

The flux of diffusive and ebullitive emissions of methane (CH_4), nitrous oxide (N_2O), and carbon dioxide (CO_2) was monitored in all culture units during the daytime (6:00 am) and nighttime (6:00 pm). A diffusion chamber was used to estimate surface diffusive flux (Santos *et al*., 2005) and it was put face down on the water, so the gases coming from the water column could concentrate into the chamber. After installation, gas samples were taken with the aid of a 30 mL syringe, at times of 0, 1, 2, and 4 min and immediately stored in gasometric chambers.

Submerged inverted funnels with a diameter of 0.0707 m^2 were used to estimate ebullitive emissions (Santos *et al*., 2005). The funnels were fixed just below the water surface and a water-filled bottle was attached to its top, which collected the gas bobbles. The funnels stayed in the water for 24 hours and the bottles were removed and transported to the laboratory, where the gases were removed and stored in gasometric chambers to determine the concentrations of methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) by chromatographic method (chromatographic model HPLC SHIMADZU). Other variables were also measured in order to verify if there is an influence of them on gas fluxes. That includes physical-chemical parameters: temperature, dissolved oxygen (DO), pH, electrical conductivity, and, turbidity with the aid of a multisensor probe of limnological parameters; salinity, with the aid of a refractometer, and; transparency, using a

Secchi disk. And nutrient parameters: total phosphorus (TP), total nitrogen (TN), orthophosphate, nitrate, nitrite, ammonia, chlorophyll *a*, and organic and inorganic carbon in the water column.

2.5 Zootechnical parameters

Biometrics measurements were performed biweekly in each system with the aid of a cast net. The organisms were returned to the ponds after the biometry. Other zootechnical parameters measured at the end of the experiment were: final mean weight (g), apparent feed conversion factor (AFC), and survival rate (%).

3 RESULTS

In order to characterize the systems is presented zootechnical parameters (table 01) and limnological characteristics of the water column (table 02).

Table 01. Zootechnical parameters.

PARAMETERS	C6	C20	C50
Final average weight (g)	12.0	12.5	6.0
Apparent feed conversion ratio	0.8	1.7	1.5
Protein conversion ratio	0.3	0.6	0.5
Mean biomass produced (kg)	80.0	752.0	598.0
Biomass (kg/ha)	996.7	1,504.0	400.0
Survival (%)	55,5	40.0	33,3
Cycles per year	3	3	3
Density (shrimp/m^2)	6.0	20.0	50.0

Subtitle: C6, culture with 6 shrimps/m^2; C20, culture with 20 shrimps/m^2 and C50, culture with 50 shrimps/m^2.

3.1 Ebullitive and Diffusive flux of gases

Variations were identified for ebullitive and diffusive fluxes, and distinct emission patterns for each gas analyzed were also found over the study period.

Table 02. Limnological characteristics of the water column.

	C6		C20		C50	
PARAMETERS	Fist	Final	Fist	Final	Fist	Final
Salinity (ppt)	8.0 ±1.4	12.8 ±1.6	0.5 ±0.0	30.0 ±0.0	10.9 ±0.1	17.1 ±1.7
Transparency (cm)	100.0 ±0.0	35.0 ±0.0	30.0 ±0.0	25.0 ±0.0	30.0 ±0.0	30.0 ±0.0
Temperature (ºC)	28.9 ±0.3	27.1 ±0.0	27.4 ±0.3	27.5 ±0.1	26.3 ±0.1	27.2 ±0.1
pH	8.4 ±0.1	7.5 ±0.3	7.6 ±0.2	8.2 ±0.1	8.5 ±0.2	8.1 ±0.1
Conductivity (us/cm)	14.4 ±1.7	22.6 ±1.8	1.0 ±0.0	0.5 ±0.0	18.3 ±0.1	29.1 ±2.1
Turbidity (NTU)	23.1 ±10.1	84.1 ±7.1	112.0 ±9.2	118.0 ±1.4	139.7 ±3.1	7.7 ±2.1
DO (mg/l)	10.4 ±0.3	8.2 ±1.5	9.4 ±1.5	8.1 ±1.0	10.0 ±2.2	7.7 ±2.1
% DO	140.6 ±5.2	109.7 ±21.1	120.9 ±19.3	104.4 ±12.5	133.3 ±29.8	104.5 ±29.1
TDS (g/L)	9.2 ±0.8	14.6 ±1.1	0.6 ±0.0	0.3 ±0.0	11.4 ±0.1	18.6 ±1.1
Chlorophyll *a* (ug/L)	5.8 ±0.3	47.5 ±8.0	101.5 ±27.6	68.0 ±2.2	206.0 ±25.7	371.6 ±40.3
Total nitrogen (ug/L)	1205.3 ±124.4	1092.6 ±138.9	1577.0 ±183.7	901.1 ±44.3	3666.4 ±248.3	2117.6 ±345.0
Ammonia (ug/L)	158.7 ±64.8	607.9 ±91.2	8.5 ±2.7	0.0 ±0.0	632.1 ±26.2	638.1 ±23.3
Nitrate (ug/L)	375.5 ±65.8	712.5 ±23.6	507.4 ±49.2	290.0 ±64.2	573.2 ±26.3	611.3 ±8.5
Nitrite (ug/L)	2.3 ±0.4	0.4 ±0.2	2.6 ±0.8	1.8 ±0.2	1.9 ±0.9	0.5 ±0.4
Total phosphorus (ug/L)	11.0 ±0.9	28.0 ±2.5	185.4 ±4.9	122.4 ±5.2	91.6 ±2.7	51.1 ±1.7
Orthophosphate (ug/L)	2.8 ±0.0	3.6 ±0.4	5.0 ±0.1	9.5 ±1.1	4.4 ±0.3	22.2 ±5.0

Title: Mean values and standard deviation of limnological parameters over the study period. Subtitle: DO, Dissolved oxygen; TDS, total dissolved solids; C6, culture with 6 shrimps/m^2; C20, culture with 20 shrimps/m^2 and C50, culture with 50 shrimps/m^2.

3.1.1 Carbon dioxide

Differently than expected, the emissions of ebullitive CO_2 were higher at the beginning of the study compared to the end in the ponds C6 (6 shrimps/m^2) and C20 (20 shrimps/m^2), but slightly bigger in C50 (50 shrimps/m^2). The pond C50 showed the greater mean value (50.99 mg. m-^{2}. day^{-1}) at the end of the cultivation time, followed by C20 (3.12 mg. m-^{2}. day-^{1}) and C6 (0.05 mg. m-^{2}. day^{-1}) (fig.02). Carbon dioxide also presented higher values than the other gases.

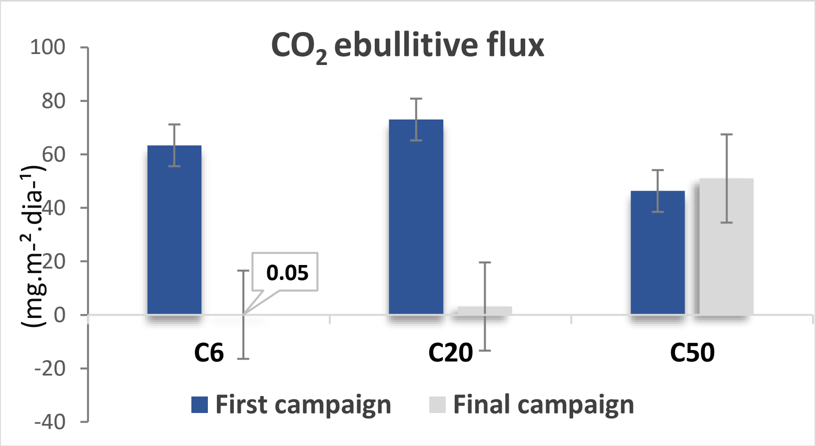


Fig. 02. Mean values and standard error of CO_2 ebullitive flux over the study period (mg. m^{-2}. day^{-1}) for each culture. Subtitle: CO_2, Carbon dioxide; C6, C20 and C50 culture with 6, 20 and 50 shrimps/m^2, respectively.

Carbon dioxide diffusive mean flux exhibited different patterns among the systems (fig.03, a and b). C6 presented only positive values, whereas the other two systems showed absorption of CO_2 at different intensities. In C20, the emissions occurred only in the first campaign during the day and presented greater absorption values at the beginning and the end of the study. In contrast, C50 absorbed CO_2 in the first campaign all day long, and in the final campaign, only at night, but had a final positive balance in diffusive flux (37.59 mg. m^{-2}. day^{-1}) at the end due to day emission, which compensated the absorption.

Comparing the initial of the cultivation period to the end, we can see a tendency to decrease in C20 and an increasing tendency in C6 and C50 (fig.03, c). It is needed to highlight that C50 could have shown higher averages if it had had a longer cultivation time.

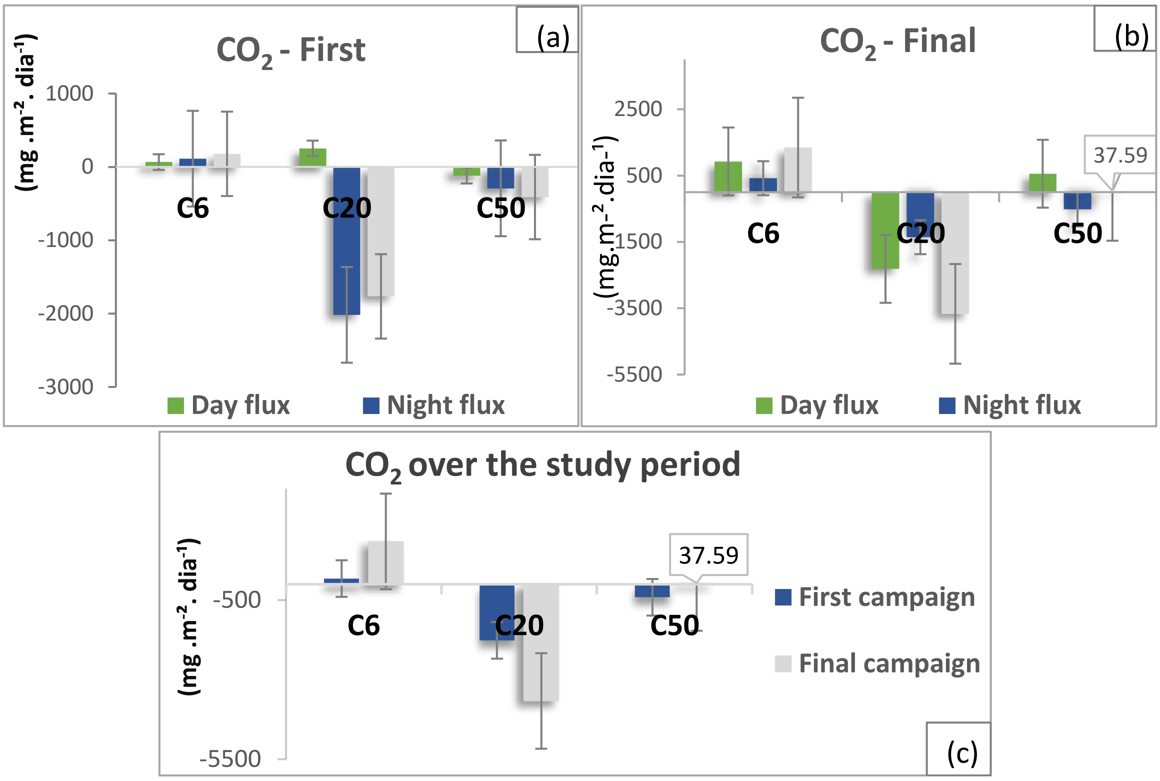


Fig. 03. Mean values and standard error of CO_2 diffusive fluxes (mg. m^{-2}. day^{-1}) for each culture (a) in the first campaign and, (b) in the final campaign; and, (c) mean values and standard error of CO_2 diffusive fluxes over the study period for each culture. Subtitle: CO_2, Carbon dioxide, C6, C20 and C50, culture with 6, 20 and 50 shrimps/m^2, respectively.

3.1.2 Methane

Methane ebullitive mean values were close to zero in all systems over the study period (fig.04), except in C20 (8.63 mg. m^{-2}. day^{-1}) which exhibited higher values at the end of the cultivation period.

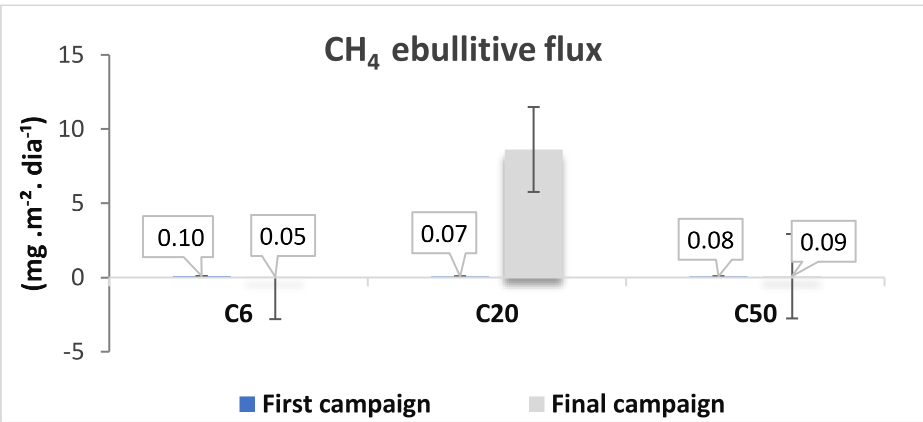


Fig. 04. Mean values and standard error of CH_4 ebullitive fluxes (mg. m^{-2}. day^{-1}) over the study period for each culture. Subtitle: CH_4, Methane; C6, C20 and C50 culture with 6, 20 and 50 shrimps/m^2, respectively.

The diffusive flux of methane was positive during the entire period of cultivation, and the three ponds acted as a source of CH_4. The shorter emissions were found in the pond C50. The higher emissions occurred at night than during the day for the three ponds (fig. 05, a and b) during the study period, except for C50 in the first campaign. The biggest differences between night and day emissions were found in C6 (first campaign) and in C20 (final campaign), while C50 did not show a big variation. A decreasing pattern was also observed in the ponds C6 and C50, whereas the opposite occurred in C20 (fig. 05, c).

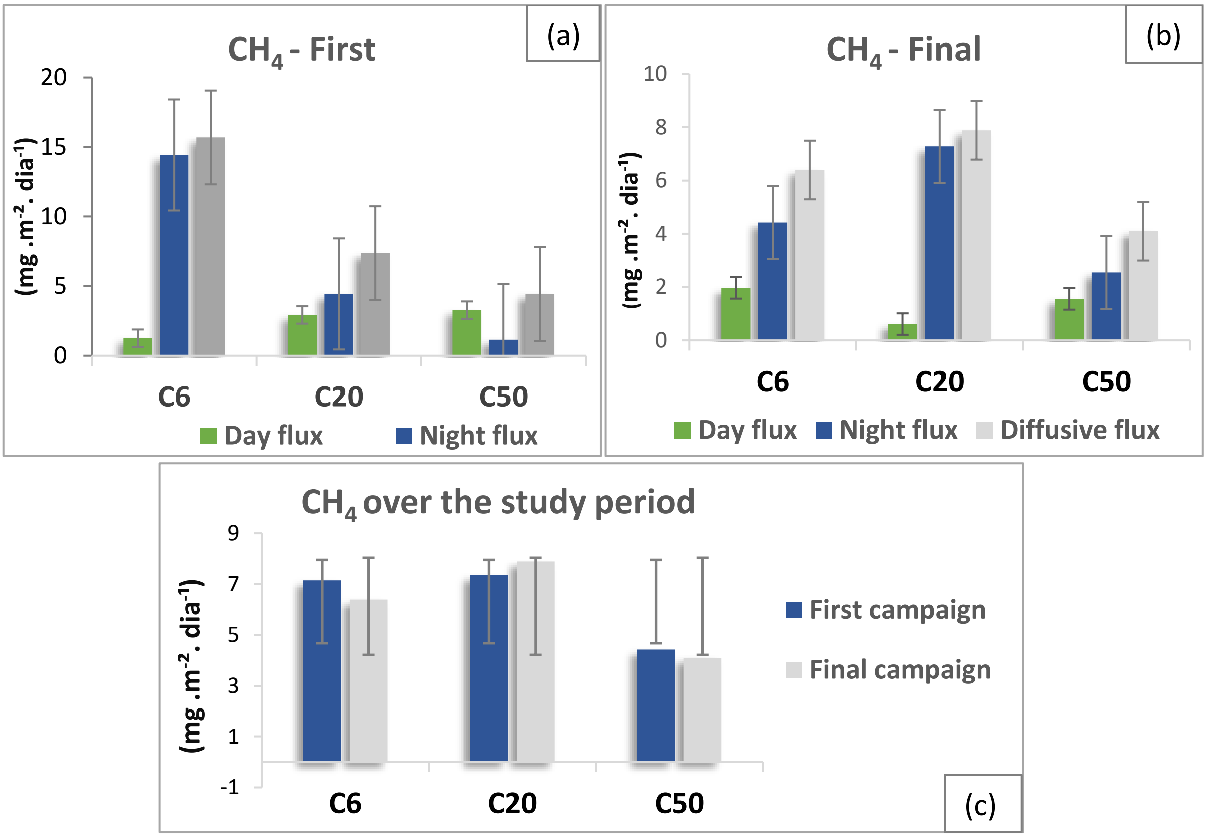


Fig. 05. Mean values and standard error of CH_4 diffusive fluxes (mg. m^{-2}. day^{-1}) for each culture (a) in the first campaign and, (b) in the final campaign; and, (c) mean values and standard error of CH_4 diffusive fluxes over the study period for each culture. Subtitle: CH_4, Methane; C6, C20 and C50 culture with 6, 20 and 50 shrimps/m^2, respectively.

3.1.3 Nitrous oxide

Nitrous oxide concentrations were also very low and close to zero over the sampling periods (fig.06).

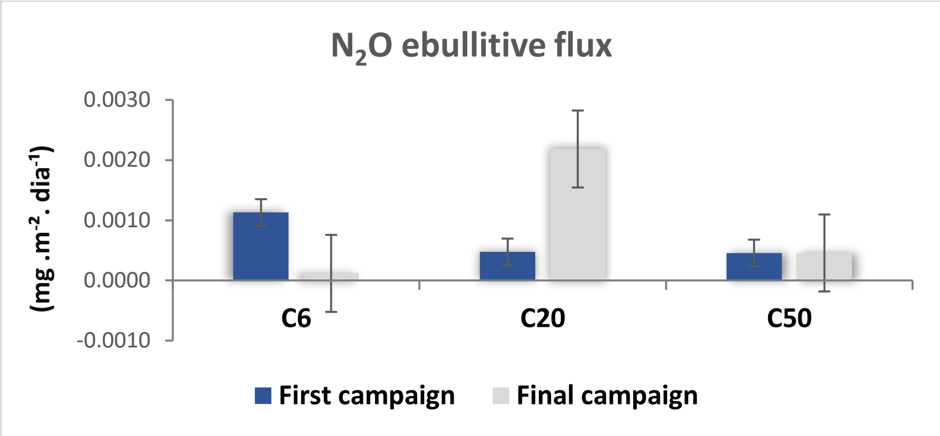


Fig. 06. Mean values and standard error of N2O ebullitive fluxes (mg. m^{-2}. day^{-1}) over the study period for each culture. Subtitle: N_2O; Nitrous oxide; C6, C20 and C50 culture with 6, 20 and 50 shrimps/m^2, respectively.

There was variation on nitrous oxide diffusive flux among systems and sampling periods (fig. 07, a and b).

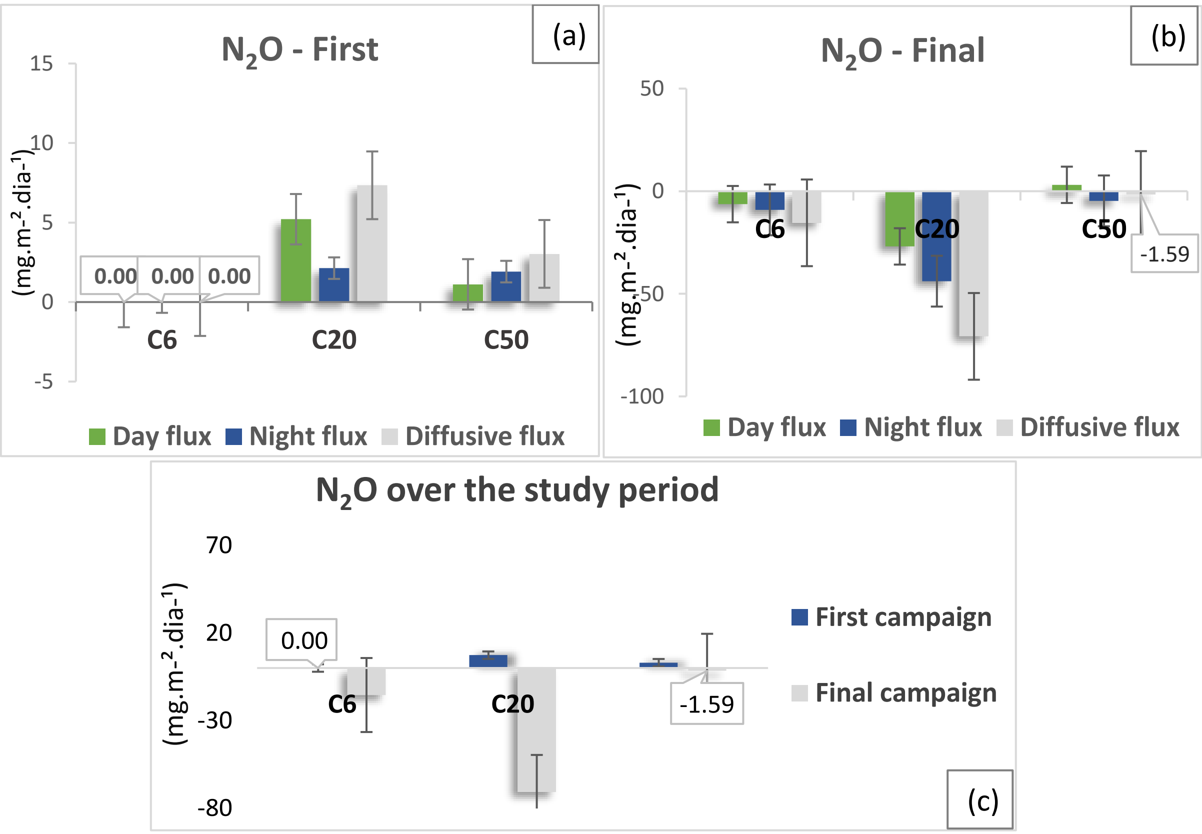


Fig. 07. (Mean values and standard error of N_2O diffusive fluxes (mg.m^{-2}. day^{-1}) for each culture (a) in the first campaign and, (b) in the final campaign; and, (c) mean values and standard error of N_2O diffusive fluxes over the study period for each culture. Subtitle: N_2O, Nitrous oxide; C6, C20 and C50 culture with 6, 20 and 50 shrimps/m^2, respectively.

C6 did not show flux of N_2O in the first campaign and absorbed this gas at the end of the sampling period, mainly at night. C20, differently, emitted N_2O at the beginning, mostly in the day, and the opposite occurred at the end, wherein there was gas absorption, largely at night. C50 also emitted gas at the beginning and acted as a sink for nitrous oxide at the end of the study, since the emissions during the day were not enough to compensate for the absorption at night, resulting in a negative diffusive flux. The flux of N_2O throughout the sampling period shows that all ponds absorbed gas and exhibited a decreasing pattern towards the end of the production cycle (fig. 07, c). It is important to emphasize again, that C50 could have been a stronger sink for N_2O if it had had a longer period of cultivation time.

3.2 Total gas fluxes

The sum of ebullitive and diffusive gases revealed the final balance of gases in each campaign (fig.08).

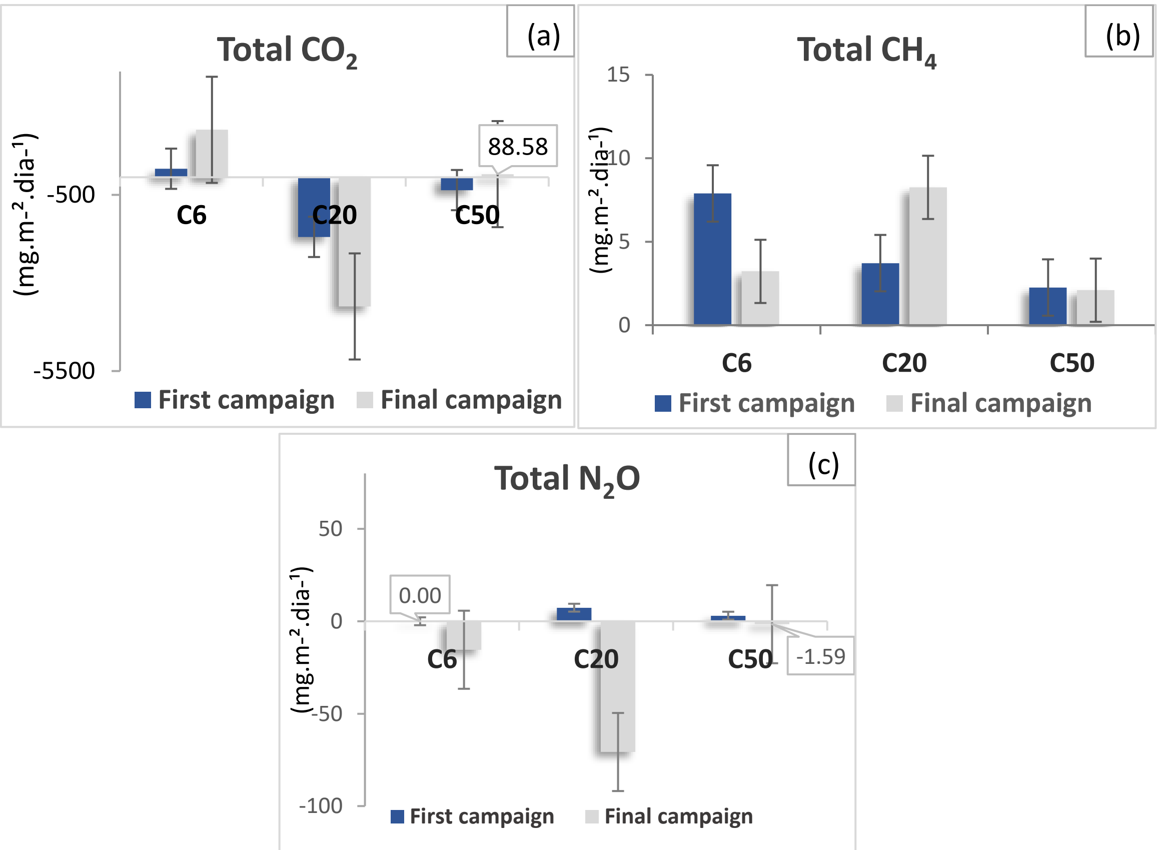


Fig. 08. Mean values and standard error of total flux of gases (mg. m^{-2}. day $^{-1}$) of (a) CO_2; (b) CH_4 and, (c) N2O. Subtitle: CO_2, Carbon dioxide; CH_4; Methane; N2O, Nitrous oxide; C6, C20 and C50 culture with 6, 20 and 50 shrimps/m^2.

Carbon dioxide had a negative final balance only in C20 and C50 (first campaign). The highest emission occurred in C20 (-3666.19 mg. m^{-2}. day^{-1}) at the end of the cultivation period. Methane was the only gas with a positive final balance in all ponds. C50 showed the low mean values for CH_4 while the other two ponds presented higher values, being greater in C20 at the end of the cycle period (8.26 mg. m^{-2}. day^{-1}) and in C6 at the beginning (8.26, 7.89 mg. m^{-2}. day^{-1}, respectively). The mean values for nitrous oxide were positive only in C20 and C50 in the first campaign, and presented the biggest negative means in C20 in the final campaign (-70.75 mg.m-². day^{-1}).

4 DISCUSSIONS

4.1 GHG emission driving factors

4.1.1 Carbon dioxide driving factors

Differently than expected, the mean values for ebullitive carbon dioxide were very low in the present study. The flux of CO_2 is affected by biogeochemical conditions and the uptake of CO_2 by the phytoplankton to use it in photosynthesis processes can drop the CO_2 concentrations to negative values (Zhang *et al*., 2022). Physicochemical factors, such as high pH, can also induce the dissolution of atmospheric CO_2 (Aimé *et al*., 2018; Zhang *et al*., 2019). The high concentrations of chlorophyll *a* and pH in this work support this statement for C6 and C20.

In contrast, C50 had the highest chlorophyll *a* and pH concentration among all ponds, but had the highest emission of carbon dioxide via the ebullition pathway. Thus, the greater concentration in C50 was due to the higher stocking density, which results in the emission of CO_2 by the respiratory processes (Scofield *et al*., 2016) and to the greater nutrient input required to feed the shrimps. The cultures of shrimp farming can also exhibit significantly higher overall respiratory emissions of GHG compared to cultures of larger-sized animals, primarily due to the greater stocking density (Zhang *et al*., 2022).

Carbon dioxide diffusive flux demonstrated different sink-source transition patterns among the systems and sampling periods. The patterns observed indicate that different factors regulated the diffusive flux of CO_2 in each pond. The diurnal variation identified in the sampling periods can be related to the management practice and the phytoplankton content.

The stocking density did not appear to regulate the diffusive flux of CO_2 in this study, as the two ponds with greater stocking densities had lower values than the one with the lowest stocking density. From this perspective, the greater emission of C6 may be related to the smaller size of the pond, which can result in a higher CO_2 production per unit area by the respiratory process (Natchimuthu *et al*., 2017). The lower uptake of CO_2 by the microbiota can also explain the higher values in C6, since it had the lowest chlorophyll *a* concentration.

A study, carried out in a reservoir, also found the lowest concentration of chlorophyll *a* following the highest CO_2 emission, indicating a negative correlation between those two variables even in an environment with different characteristics (Zhang *et al*., 2021). Other studies also have reported a negative correlation between CO_2 and chlorophyll *a* (Xiao *et al*., 2020; Yu *et al*., 2022). In contrast, the absorption of CO_2 via the diffusive pathway in C20 and C50 can be attributed to a strong phytoplankton uptake by photosynthetic process (Yang *et al*., 2018; Zhang *et al*., 2022). The high levels of chlorophyll *a* found in C20 and C50 (68.0 and 371.6 ug/L in the final campaign, respectively) in the present study, provide supporting evidence for this statement.

4.1.2 Methane driving factors

Methane ebullitive emission was lower than diffusive emissions, (except in C6 in the final campaign) which is uncommon, considering that the ebullition process had been reported as a major pathway for CH_4 (Wu *et al*., 2019; Yang *et al*., 2020; Yang *et al*., 2023; Zhang *et al*., 2020). High variability for CH_4 diffusive flux was already demonstrated and strong emission was found near feeding sites, while a substantial decrease was observed far from the feeding sites, where the emissions achieved less than 1% of contribution (Waldemer; Koschorreck, 2023). Methane is strongly sensible to the managing practices applied by farmers such as aeration, water changing, and drainage processes (Kauffman *et al*., 2018; Liu *et al*., 2016; Maher *et al*., 2019). Aeration increases the concentration of dissolved oxygen (DO), which reduces the anaerobic methanogenesis process which requires a low-DO environmental concentration, and, consequently, reduces methane emission by 75% (Yuan *et* al., 2019; Zhang *et al*., 2022). The action of the aerators also breaks physically the methane bubbles released into the water column and reduces the anoxic condition of sediment, which decreases the contribution of the ebullitive process (Tong *et al*., 2021; Yang *et al*., 2023). Furthermore, high nitrate content can inhibit CH_4 emissions (Hu *et al*, 2017; Yang *et al*., 2018). The high concentration of nitrate and dissolved oxygen (DO) supports this statement in this study. The aeration was probably the main factor that influenced the ebullitive methane emissions.

The methane diffusive flux was higher than the ebullitive flux. Despite the aeration reducing the CH_4 ebullitive emission (Kauffman *et al*., 2018; Liu *et al*., 2016; Maher *et al*., 2019; Xu *et al*., 2022; Yuan *et al*., 2019) the diffusion of CH_4 is enhanced by accelerating the transference of gas from water to the air (Hu *et al*., 2013; Kosten *et al*., 2020; Yang, H. *et al*., 2015). Otherwise, in oxygenated waters, a phytoplankton-rich environment can produce CH_4 (Günthel *et al*., 2019; Tang *et al*., 2016).

The GHG flux cannot be attributed only to the stocking density, as other factors can be at play in influencing its emissions. The size of the earthen ponds shows some influence, where smaller systems (less than one hectare) present higher production of CH_4 than bigger ones (Zhang *et al*., 2022). This can be explained by the factor that a large size system has bigger water-air interface contact which enhances the diffusion of oxygen within the water column and reduces the anoxic methanogenesis process. Temperature and nutrient supply also influence the flux of methane.

4.1.3 Nitrous oxide driving factors

The low means of ebullitive N_2O were in accord with previous studies which showed lower values for nitrous oxide (N_2O) compared to the other GHGs (IPCC, 2022; Soares; Henry-Silva, 2019; Waldemer; Koschorreck, 2023). The flux of N_2O is regulated by fertilization (operational parameter), dissolved oxygen (physicochemical factor) and particular environmental conditions (Hu *et al*., 2013).

The emission and absorption of N_2O are associated with nitrification and denitrification (Williams; Crutzen, 2010) in aerobic and anaerobic reactions conducted by microorganisms (Yang, H. *et al*., 2015; Daims *et al*., 2016; Li *et al*., 2021) occurring in the water column (Maavara *et al*., 2019; Yuan *et al*., 2021) and in the sediment (Yang *et al*., 2022). The depletion of oxygen in anoxic environments, significantly increases N_2O emissions (Hu *et al*., 2012; Tan *et al*., 2020). In this study, the well-oxygenated waters, suggest that the denitrification processes were reduced by aeration, leading to low emission of ebullitive nitrous oxide.

The diffusive flux of N_2O frequently shows a positive balance, differently than what was found in the present study. All systems exhibited a similar pattern throughout the study period showing a decreasing tendency towards to end of the cycling period and acting as a sink for N_2O. The mechanisms regulating the N_2O pathways are complex and depend on many factors.

The emission and absorption of N_2O depends on the balance between the microbial processes involved in the formation and consumption of this gas in the sediment (Yang *et al*., 2022). A study reported that the shrimp farm tends to decrease the emission of N_2O by converting coastal wetlands in aquaculture ponds in an environment with low nitrogen availability. It appears to change the populations of sediment microorganisms (ammonium oxidizing archaea and denitrifying bacteria) (Yang *et al*., 2022). The uptake of N_2O was also observed in high eutrophic agricultural ponds (Webb *et al*., 2019), in boreal aquatic ecosystems (Soued *et al*., 2016) and, in temperate freshwater at a lower contribution proportion (Waldemer; Koschorreck, 2023). This demonstrates the variety of factors that can control the flux of N_2O in many environments. The negative values in this study suggest that the formation of N_2O was outpaced by the consumption process.

5 CONCLUSIONS

The findings of this study corroborated partially our initial hypothesis that monocultures of marine shrimp at lower stocking densities emit a lower quantity of greenhouse gases compared to monocultures at higher densities. The stocking density played a crucial role in regulating the ebullitive emissions of CO_2 in shrimp ponds, while the diffusive emissions were regulated by phytoplankton uptake. This highlights the importance of carefully managing and optimizing stocking densities to minimize CO_2 emissions in shrimp production. The decreasing trend observed in diffusive CO_2 emissions towards the end of the cultivation period suggests the potential for improved management strategies to further reduce CO2 emissions over time.

The aeration process proved to be a better regulator than stocking density for CH_4 and N_2O effectively reducing the release of these gases into the atmosphere. The study also revealed the complex dynamics of CH_4 emissions, with diffusive emissions outpacing ebullitive emissions. These findings contribute to our understanding of the complex dynamics of diffusive and ebullitive greenhouse gas emissions in shrimp farm systems and highlight the importance of considering factors such as stocking density, aeration and phytoplankton content in managing and mitigating greenhouse gas emissions in aquaculture practices. The well oxygenated waters suggest that the anoxic reactions, which is the main pathway for N_2O emission, were suppressed by the oxygen content. This highlights the potential of aeration as a mitigation strategy for reducing diffusive and ebullitive N_2O emissions and the potential of shrimp farm in acting as a sink for nitrous oxide.

This study has provided insights that many factors can influence and promote variability on the flux of gas throughout the culturing period and highlights the importance of considering and optimizing factors such as stocking density, aeration, physicochemical factors, phytoplankton abundance in the design and management of shrimp farm systems to achieve sustainable and environmentally friendly practices while mitigating GHG emissions.

SUGGESTIONS FOR FUTURE STUDIES:

The great challenge in carry out studies on farms is to control all variables at play influencing the flux of greenhouse gases. We suggest the monitoring of shrimp ponds in different stocking densities and different pond sizes in order to contrast the influence of those two variables. It is worth also to verify the microbiota on water column and in the sediment in order to describe their interaction with nutrient content and if the stocking density and the pond size changes the community of microorganism.

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