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**LAÍS COURA SORANÇO**

# Ecological Functioning of Aquaculture Systems: Effects of Species Interactions, Feed Inputs, and Nutrient Processing

Juiz de Fora

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**LAÍS COURA SORANÇO**

# Ecological Functioning of Aquaculture Systems: Effects of Species Interactions, Feed Inputs, and Nutrient Processing

Tese apresentada ao Programa de Pós-graduação em Biodiversidade e Conservação da Natureza da Universidade Federal de Juiz de Fora, como requisito parcial para obtenção do título de Doutora. Área de concentração: Comportamento, Ecologia e Sistemática.

Orientador: Dr. Nathan Oliveira Barros

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**Laís Coura Soranço**

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Aprovada em 19 de maio de 2026.

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Juiz de Fora, 09/06/2026.

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Finally, I would like to thank everyone who, in some way, contributed to the completion of this work.

**Summary**

## Resumo

A aquicultura expandiu-se rapidamente nas últimas décadas, tornando-se uma importante contribuição para a segurança alimentar global e para a produção de proteína animal. No entanto, sua sustentabilidade depende de uma melhor compreensão dos processos biológicos, comportamentais e biogeoquímicos que regulam a eficiência produtiva e os impactos ambientais dos sistemas de cultivo. Este estudo integra abordagens complementares para investigar como as características das espécies, os insumos alimentares e as vias de ciclagem de nutrientes interagem na estruturação de sistemas aquícolas de água doce. Primeiramente, avaliamos o desenvolvimento, o comportamento dos peixes e a qualidade da água sob diferentes estratégias de produção, demonstrando que as interações entre as espécies influenciam o desempenho de crescimento, a distribuição espacial e importantes parâmetros ambientais. Esses resultados destacam a importância da composição de espécies e da dinâmica comportamental na determinação tanto da produtividade quanto do funcionamento do sistema. Em seguida, investigamos o papel da ração como um fator determinante da dinâmica do carbono, com foco na decomposição da ração não consumida e em sua contribuição para as emissões de gases de efeito estufa. Nossos resultados mostram que as características da ração regulam fortemente as taxas de decomposição e a produção associada de $CO_2$ e $CH_4$, ressaltando a importância das propriedades da ração e de seu manejo no controle das emissões de carbono em sistemas aquícolas. Por fim, avaliamos a dinâmica dos nutrientes após a ingestão da ração, quantificando sua partição entre as vias dissolvida e particulada. Demonstramos que a maior parte dos nutrientes é rapidamente transferida para a fração dissolvida por meio da excreção e de processos microbianos, enquanto apenas uma fração limitada é exportada como resíduos particulados. Essa partição desempenha um papel central na determinação da qualidade da água e da disponibilidade de nutrientes no sistema. Em conjunto, esses resultados demonstram que os sistemas aquícolas são estruturados pela interação entre características específicas das espécies, os insumos alimentares e as vias de processamento de nutrientes. Ao integrar processos biológicos, comportamentais e biogeoquímicos, este estudo proporciona uma compreensão mais abrangente do funcionamento da aquicultura e contribui para o desenvolvimento de estratégias de produção mais eficientes e ambientalmente sustentáveis.

Palavras-chave: Aquicultura; Dinâmica do carbono; Nutrientes dissolvidos; Ração para peixes; Comportamento de peixes; Emissão de gases do efeito estuda; Growth performance; Ciclo de nutrientes; Matéria orgânica particulada; Policultura; Qualidade da água.

## Abstract

Aquaculture has rapidly expanded in recent decades, becoming a key contributor to global food security and animal protein production. However, its sustainability depends on a better understanding of the biological, behavioral, and biogeochemical processes that regulate production efficiency and environmental impacts in farming systems. This study integrates complementary approaches to investigate how species traits, feed inputs, and nutrient pathways interact to shape freshwater aquaculture systems. First, we evaluated fish development, behavior, and water quality under different production strategies, demonstrating that species interactions influence growth performance, spatial distribution, and key environmental parameters. These results highlight the importance of species composition and behavioral dynamics in determining both productivity and system functioning. Second, we investigated the role of feed as a driver of carbon dynamics, focusing on the decomposition of uneaten feed and its contribution to greenhouse gas emissions. Our results show that feed characteristics strongly regulate decomposition rates and associated $CO_2$ and $CH_4$ production, emphasizing the importance of feed properties and management in controlling carbon emissions in aquaculture systems. Finally, we assessed nutrient dynamics following feed ingestion by quantifying the partitioning between dissolved and particulate pathways. We demonstrate that most nutrients are rapidly transferred to the dissolved pool through excretion and microbial processes, while only a limited fraction is exported as particulate waste. This partitioning plays a central role in shaping water quality and nutrient availability within the system. Together, these findings demonstrate that aquaculture systems are structured by the interaction between species-specific traits, feed inputs, and nutrient processing pathways. By linking biological, behavioral, and biogeochemical processes, this study provides a more integrated understanding of aquaculture functioning and supports the development of more efficient and environmentally sustainable production strategies.



Keywords: Aquaculture; Carbon dynamics; Dissolved nutrients; Fishfeed; Fish behavior; Greenhouse gas emissions; Growth performance; Nutrient cycling; Particulate organic matter; Polyculture; Water quality.

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## List of Abbreviations

| | |
|---|---|
| AIC | Akaike Information Criterion |
| ANCOVA | Analysis of covariance |
| ANOVA | Analysis of variance |
| C | Carbon |
| CEUA | Comitê de Ética no Uso de Animais |
| $CO_2$ | Carbon dioxide |
| $CH_4$ | Methane |
| CM | Center of mass |
| C:N | Carbon to nitrogen ratio |
| DO | Dissolved oxygen |
| FCR | Feed conversion ratio |
| GLMM | Generalized linear mixed model |
| H | Shannon entropy |
| HSD | Honestly Significant Difference (Tukey's test) |
| ICP-OES | Inductively coupled plasma optical emission spectrometry |
| $I_8$ | Morisita index |
| LMM | Linear mixed-effects model |
| MR | Mortality rate |
| N | Nitrogen |
| NaOH | Sodium hydroxide |
| $NH_3$-N | Ammonia nitrogen |
| $NO_3^-$-N | Nitrate nitrogen |
| $NO_2^-$-N | Nitrite nitrogen |
| NPOC | Non-purgeable organic carbon |
| NTU | Nephelometric turbidity units |
| OM | Organic matter |
| P | Phosphorus |
| PCA | Principal Component Analysis |
| PERMANOVA | Permutational multivariate analysis of variance |
| PI | Preference index |
| $PO_4^{3-}$-P | Phosphate phosphorus |
| PR | Preference ratio |
| $SiO_2$ | Silica |
| SS | Suspended solids |
| TC | Total carbon |
| TN | Total nitrogen |
| TOC-L | Total organic carbon analyzer (modelo Shimadzu) |
| TON – | Total organic nitrogen |
| TP | Total phosphorus |

| | |
|---|---|
| TS | Total solids |
| UFJF | Universidade Federal de Juiz de Fora |
| UNEMAT | Universidade do Estado de Mato Grosso |
| UV-Vis | Ultraviolet-visible spectroscopy |
| YSI | Yellow Springs Instruments (marca do multiparâmetro) |

## Animal Use Ethics Committee

EPAMIG

**COMISSÃO DE ÉTICA NO USO DE ANIMAIS - EPAMIG**

**(CEUA / EPAMIG)**

**DECLARAÇÃO**

Declaramos que o projeto intitulado "**Dinâmica do desenvolvimento de diferentes espécies de peixes em pisciculturas no estado de Minas Gerais" (Protocolo 16/2022)**, em que se utilizará animais da espécie de peixes, sob responsabilidade do(a) pesquisador(a) **Dr. Giovanni Resende de Oliveira**, está de acordo com os princípios éticos e de experimentação animal da Empresa de Pesquisa Agropecuária de Minas Gerais e que foi aprovado pela referida instituição.

Belo Horizonte, 01 de dezembro de 2022

Comissão de Ética no Uso de Animais da EPAMIG
(CEUA / EPAMIG)

Cristiane Viana Guimarães Ladeira
Coordenadora da CEUA / EPAMIG

**Empresa de Pesquisa Agropecuária de Minas Gerais**
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**Vinculada à Secretaria de Estado da Agricultura, Pecuária e Abastecimento**

**PARECER 004/2021**

**DADOS DO PROCESSO 003/2021 - CEUA/UNEMAT**

**Projeto de Pesquisa**: O balanço biogeoquímico em aquiculturas tropicais: implicações sobre transformações ecossistêmicas e mudanças climáticas nos recursos hídricos.

**Professor:** Ernandes Sobreira Oliveira Junior

**Áreas temáticas**: Ciências Ambientais

**Instituição Proponente**: Universidade do Estado de Mato Grosso - UNEMAT

**Patrocinador Principal:** UNEMAT.

**DADOS DO PARECER**

**Número do Parecer: 004/2021** **Data da Relatoria: 13/07/2021**

**APRESENTAÇÃO:**

O projeto de pesquisa em análise apresenta em seu resumo: "A aquicultura depende fundamentalmente dos ecossistemas nos quais está inserida, sendo impossível produzir sem provocar alterações ambientais, que podem ocorrer durante a fase de implantação e também durante a sua operação de um sistema de cultivo (VALENTI, 2002).

Seu impacto está relacionado diretamente coma liberação de efluentes ricos em nutrientes (principalmente N e P), causando eutroficação em corpos d'água naturais (VALENTI, 2002), e com a introdução ou suspensão de substâncias toxicas, como os metais pesados, podem causar diversos danos ao meio devido a sua capacidade de bioacumulação, que ser definida como o processo pelo essas substâncias tóxicas se acumulam nos organismos vivos, onde a sua captação é considerada passiva e envolve gradientes de difusão criados pela adsorção ou ligação do metal ao tecido e à superfície celular (BERVOETS et al., 2013).

Sendo assim, como foco, o projeto atuará na investigação dos recursos hídricos relativos às águas represadas para uso comercial (pisciculturas), nascentes, e impactos antrópicos nos corpos d'água naturais e promoverá uma ampla abordagem da quantificação, qualificação e medidas de uso, gestão e manejo dos corpos d'água estaduais."

**OBJETIVO GERAL:**

Caracterizar a piscicultura do Brasil: tipos de tanques, espécies cultivadas e a qualidade da água.

Artigo 1. Avaliar a variação no balanço anual de C, N e P em tanques de piscicultura brasileiros

1.1 Analisar o balanço dos nutrientes (entrada, saída e estoque) de acordo com o tipo de sistema de cultivo;

1.2 Analisar o balanço dos nutrientes de acordo com as espécies cultivadas (peixes bentófagos, nectônicos);

1.3 Analisar o balanço dos nutrientes de acordo com o tipo de manejo dos tanques (seco periodicamente versus permanentemente inundado)

Artigo 2. Caracterizar a biodiversidade (peixes, macroinvertebrados e perifíton) nos tributários, tanques e efluentes

Artigo 3. Caracterizar o efeito do efluente da piscicultura na qualidade da água e sedimento de cursos d'água à jusante dos tanques

Artigo 4. Caracterizar a poluição total brasileira de C, N e P aos sistemas à jusante

Artigo 5. Caracterizar a dinâmica dos metais pesados As, Cd, Pb, Cr, Cl, Mn e THg em sistemas piscícolas tropicais no influxo, efluxo e taques

5.1 Identificar a presença de metais pesados na cadeia trófica (plantas de forrageio, água, solo e peixes) dos sistemas piscícolas

5.2 Qualificar os sistemas piscícolas de acordo com as concentrações de metais pesados encontrados

**COMENTÁRIOS E CONSIDERAÇÕES SOBRE O PROJETO:**

**QUANTO AOS CUIDADOS E MANEJO DOS ANIMAIS E RISCOS AOS PESQUISADORES:**

A realização deste projeto em cooperação internacional, entre a UNEMAT e a Radboud, analisam, em geral, a dinâmica de nutrientes e metais pesados em ambientes piscícolas semiabertos.

Quanto a metodologia encontra-se detalhada quanto aos sítios de coleta, amostragem e informam o quantitativo da amostra e demais informações para as técnicas de coleta e técnicas laboratoriais necessários.

O manejo da espécie em estudo para a coleta e eutanásia encontram-se em consonância com as técnicas utilizadas usualmente nos estudos em ambientes piscícolas e não apresenta riscos aos pesquisadores.

**CONSIDERAÇÕES SOBRE OS TERMOS DE APRESENTAÇÃO OBRIGATÓRIA:**

Os documentos apresentados encontram-se de acordo com as exigências institucionais.

**RECOMENDAÇÕES:**

Não há recomendações.

**CONCLUSÕES OU PENDÊNCIAS E LISTA DE INADEQUAÇÕES:**

A Comissão de Ética no Uso de Animais da Universidade do Estado de Mato Grosso CEUA/UNEMAT após análise do protocolo em comento, de acordo com a Lei nº 11.794 de 08/10/2008 é de parecer **FAVORÁVEL** ao desenvolvimento do projeto: **O balanço biogeoquímico em aquiculturas tropicais: implicações sobre transformações ecossistêmicas e mudanças climáticas nos recursos hídricos.**

**SITUAÇÃO DO PARECER:**
**FAVORÁVEL**

Cáceres - MT, 15/07/2021.

**Kleber Pelicia**
**Coordenador da Comissão de Ética no Uso de Animais - Ceua**
**UNEMAT/ PRPPG/Portaria n. 1595/2020**

## General Introduction

Aquaculture has shown a remarkable expansion in recent decades, becoming one of the main contributors to global animal protein production and playing a central role in food security. This growth is driven by increasing human population demands and the need for efficient and economically viable protein sources (Xu *et al*., 2022; Shi, 2023; FAO, 2024; Hussain *et al*., 2024). In this context, freshwater aquaculture stands out, particularly due to the cultivation of species such as Nile tilapia (*Oreochromis niloticus*) and grass carp (*Ctenopharyngodon idella*), which are widely distributed, present high growth rates, and are well adapted to diverse farming conditions (Froehlich *et al*., 2018; Geletu & Zhao, 2023; FAO, 2024). As a result, these species account for more than 11 million tonnes of global production and are commonly reared in pond-based systems, where feed inputs constitute the primary source of energy and nutrients (FAO, 2024; PEIXE BR, 2024). In 2022, aquaculture reached an animal protein production of 94.4 million tons, of which inland aquaculture contributed 59.1 million tons. Most of this production (89%) was destined for direct human consumption, highlighting the increasing importance of aquaculture for global food security (FAO, 2024).

Beyond its contribution to food production, aquaculture has become an important economic sector. By 2032, fish production is expected to grow further, reaching 205 million tons. In Latin America and the Caribbean, aquaculture production showed a 3.3% increase in 2022 compared to the previous year (FAO, 2024). In ten years, aquaculture increased by 53% in Brazil (PEIXE BR, 2024). The growth in the aquaculture sector is not only driven by the increasing global demand for food but also supported by its competitiveness as a viable animal protein source, offering a more cost-effective solution when compared to beef, pork, and chicken (Amorim & Tosta, 2020; Hussain *et al*., 2024). Furthermore, with its global valuation standing at around USD 313 billion, the economic significance of aquaculture is evident (FAO, 2024). The rapid expansion of the sector, however, has also increased the need for regulatory frameworks capable of ensuring environmentally sustainable production.

In Brazil, the expansion of aquaculture has been accompanied by the development of a regulatory framework aimed at promoting sustainable production while minimizing environmental impacts. The National Policy for the Sustainable Development of Aquaculture and Fisheries, established by National Policy for the Sustainable Development of Aquaculture and Fisheries (Law No. 11,959/2009), provides the legal basis for the sector and seeks to reconcile production growth with the sustainable use of aquatic resources, environmental conservation, and socioeconomic development (BRASIL, 2009a). Furthermore, the environmental licensing of aquaculture enterprises is regulated by CONAMA Resolution No. 413/2009, which establishes specific criteria for aquaculture activities based on their size and potential environmental impact (BRASIL, 2009b). In addition, the use of federal water bodies for aquaculture activities is governed by Decree No. 10,576/2020, which regulates the allocation of physical spaces in Union-owned waters for aquaculture purposes (BRASIL, 2020).

Considering the continuous expansion of aquaculture, there is an increasing need to better understand the factors that influence both production efficiency and system sustainability (Garlock *et al*., 2024). Among these factors, the development and growth of cultured species play a central role, as they directly determine productivity and resource use efficiency (Naylor *et al*., 2021; Ranjan *et al*., 2026). Fish growth is closely linked to feeding rates, nutrient assimilation, and metabolic activity, which vary throughout the production cycle (Shi *et al*., 2024; Ai *et al*., 2025). These processes are not only biologically driven but are also strongly influenced by environmental conditions and management practices, making it essential to understand how these elements interact to optimize production systems (Abd El-Hack *et al*., 2022; Aljehani *et al*., 2025; Turlybek *et al*., 2025).

In addition to physiological processes, fish behaviour represents a key component in aquaculture systems (Wurts & Durborow, 1992; Mei *et al*., 2022). Behavioural patterns such as feeding activity, spatial distribution, social interactions, and responses to environmental conditions are directly associated with animal welfare and can significantly affect growth performance and productivity (Patro *et al*., 2023; Maia *et al*., 2024). Stressful conditions, often observed in intensive systems, may compromise fish health, reduce feeding efficiency, and impair development (Sánchez-Velázquez *et al*., 2024). On the other hand, favourable environmental and management conditions can promote better welfare, reflected in improved growth and system performance (Sahu *et al*., 2008; Pietsch, 2025). Therefore, understanding behavioural dynamics and their relationship with environmental variables is essential for the development of more efficient and sustainable aquaculture strategies (Martins *et al*., 2011; Bonini-Campos *et al*., 2019).

Beyond individual performance, the structure of aquaculture systems and the combination of cultured species can strongly influence resource use efficiency and environmental conditions (Kim *et al*., 2022; Turlybek *et al*., 2025. Integrated production strategies, such as polyculture, have been increasingly proposed as sustainable alternatives because they promote complementary resource use among species occupying different ecological niches (Kim *et al*., 2022; Flores-Iwasaki *et al*., 2025; Pant & Kumar, 2025). Through differences in feeding habits, habitat use, and behavioural interactions, species may reduce competition, improve feed utilization, and influence key water quality parameters (Hossain *et al*., 2023; Pant & Kumar, 2025). Consequently, species composition becomes an important driver of productivity, animal welfare, and ecosystem functioning within aquaculture systems. Understanding how these interactions affect both fish development and environmental conditions is therefore essential for designing more efficient and sustainable production systems.

Despite its benefits, aquaculture expansion is associated with significant environmental challenges. Aquaculture systems have been recognized as potential sources of greenhouse gas emissions, particularly carbon dioxide ($CO_2$) and methane ($CH_4$), largely driven by the decomposition of organic matter in water and sediments (Huang *et al*., 2020; Kosten *et al*., 2020; Gephart *et al*., 2021; Yadava *et al*., 2024). A key yet insufficiently explored component of aquaculture-related emissions is the fraction of feed that is not consumed. Uneaten feed represents a direct and concentrated source of organic matter that is rapidly processed in sediments and the water column, where microbial pathways can lead to the production of greenhouse gases, particularly methane under anaerobic conditions (Stichnothe *et al*., 2008; Huang *et al*., 2020; Kosten *et al*., 2020). While feed is widely recognized as the main indirect contributor to emissions through its production chain, its role as a direct driver of in situ greenhouse gas (GHG) generation remains poorly quantified (Yang *et al*., 2021; Castilla-Gavilán *et al*., 2024; Yadava *et al*., 2024).

The transformation of this material within aquaculture systems is governed by complex interactions among microbial communities and environmental conditions, making its contribution to total emissions difficult to isolate under farming conditions. As a result, emissions associated with feed degradation are likely underestimated, representing a critical gap in current assessments of the carbon footprint of aquaculture. Therefore, quantifying the fate of uneaten feed and its contribution to greenhouse gas production is essential for improving current assessments of the environmental footprint of fish farming. As countries are legally bound under the Paris Agreement to reduce GHG emissions significantly (Rogelj *et al*., 2016), improving current estimates of aquaculture-related emissions is essential, particularly for economies reliant on fish farming.

In contrast, the fraction of feed that is ingested follows a biologically mediated pathway, in which organic matter is processed through fish metabolism and subsequently redistributed within the system. After consumption, nutrients can be incorporated into biomass or released via excretion and egestion, contributing to dissolved and particulate pools (Halvorson & Atkinson, 2019; Yang *et al*., 2021). These processes represent major internal pathways of nutrient input, particularly for carbon, nitrogen and phosphorus, and play a central role in shaping nutrient availability and distribution in the water column and sediments (Boyd & McNevin, 2023; Syropoulou *et al*., 2024).

Under intensive production conditions, this pathway becomes particularly relevant, as the release of nutrients contributes to the elevated organic matter and nutrient loads that can compromise water quality. (Munguti *et al*., 2021; Nathanailides *et al*., 2023; Dalbem Barbosa *et al*. 2024). The accumulation of nitrogen and phosphorus compounds in the water column may stimulate excessive primary production, reduce dissolved oxygen levels, and negatively affect both cultured organisms and surrounding ecosystems (Coldebella *et al*., 2018; Guan, *et al*., 2026). Collectively, these processes contribute to shifts in biogeochemical dynamics, reinforcing the role of metabolically derived waste as a key driver of environmental change in aquaculture systems.

Furthermore, species-specific traits strongly modulate these pathways. Differences in feeding strategy and digestive physiology influence both the efficiency of nutrient assimilation and the relative contribution of dissolved and particulate outputs (NRC, 2011). Herbivorous species, such as grass carp, typically exhibit lower digestibility, resulting in reduced nutrient assimilation and increased loss of ingested material (Cui *et al*., 1992; Kurniasih *et al*., 2024). Consequently, a greater fraction of ingested nutrients may be released as particulate waste through egestion. In contrast, omnivorous species such as Nile tilapia generally show greater digestive efficiency, releasing a larger proportion of nutrients in dissolved forms via excretion (Neto & Ostrensky, 2013; Kurniasih *et al*., 2024). Consequently, species composition can directly influence nutrient cycling pathways and environmental conditions within aquaculture systems.

Given the economic importance of aquaculture and its rapid expansion, it becomes essential to better understand the interconnected processes that govern these systems. This includes not only the development and behaviour of cultured species, but also nutrient dynamics, feed utilization, and their associated environmental impacts. Advancing this integrated understanding is crucial for supporting the development of more sustainable and efficient aquaculture practices.

The overall objective of this thesis was to investigate how species interactions, feed inputs, and nutrient processing pathways influence the functioning, productivity, and environmental sustainability of freshwater aquaculture systems. To achieve this objective, the following specific objectives were addressed: To evaluate water quality in earthen ponds throughout a complete production cycle under monoculture and polyculture systems involving Nile tilapia (*Oreochromis niloticus*) and grass carp (*Ctenopharyngodon idella*); To assess fish growth, survival, and productive performance under different cultivation strategies and examine their relationships with environmental conditions and management practices; To investigate behavioural responses and spatial distribution patterns of fish reared in monoculture and polyculture systems, evaluating their implications for animal welfare and production performance; To quantify the degradation dynamics of fish feed under aquaculture conditions and assess its contribution to greenhouse gas production, particularly $CO_2$ and $CH_4$ emissions; To evaluate the fate of nutrients following feed ingestion by quantifying dissolved and particulate nutrient pathways and comparing nutrient processing among fish species with different feeding strategies.

Together, these objectives provide an integrated assessment of the biological, behavioural, and biogeochemical processes that regulate the functioning and sustainability of freshwater aquaculture systems.

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## CHAPTER I: Polyculture as a Sustainable Strategy in Aquaculture: Insights from Tilapia and Carp Cultivation

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### Abstract

Aquaculture is one of the fastest-growing food production sectors worldwide, but its expansion raises concerns regarding water quality and the intensive use of natural resources. Polyculture, the simultaneous farming of different species, has emerged as a potentially sustainable alternative by promoting complementary resource use among species, improving production efficiency, and potentially reducing environmental impacts. This study evaluated water quality, fish performance, and behavioural responses of Nile tilapia (*Oreochromis niloticus*) and grass carp (*Ctenopharyngodon idella*) reared in monoculture and polyculture systems throughout a complete production cycle. The main findings show that water turbidity was significantly greater in tilapia ponds and less evident in carp monoculture. Furthermore, carp monoculture maintained a stable effluent temperature during harvesting, while systems containing tilapia (both monoculture and polyculture) experienced significant water warming, with a mean temperature difference of 2.5 to 2.8 °C. Regarding performance, carp raised in polyculture exhibited significantly higher growth than those in monoculture; in contrast, tilapia growth did not vary between systems. Remarkably, polyculture also drastically reduced aggression among tilapias and promoted spatial niche partitioning, leading to greater use of the upper water column by grass carp. These findings indicate that polyculture can provide both environmental and production benefits, representing a promising strategy for improving aquaculture sustainability without compromising productivity.

Keywords: Growth performance, Temperature, Fish farming, Weight, Water quality

**Data Availability Statement**

All data, metadata, and tools used in this work are available with the corresponding author.

**Author Contribution Statement**

All authors contributed to the conception and development of the work, in analyzing the results and writing the manuscript.

## 1. Introduction

Aquaculture has become the fastest-growing sector of animal food production globally, playing a critical role in food security, and economic development (FAO, 2024). However, its rapid expansion has intensified concerns regarding environmental sustainability and stimulated the search for more efficient production strategies. Due to the increase in the aquaculture sector, there is a growing concern about the potential environmental trade-offs of aquaculture, such as water pollution, increase in water temperature of the effluents, greenhouse gas emissions, and intensive use of natural resources (Amorim & Tosta, 2020; Kosten *et al*., 2020). For instance, some studies have shown increased total phosphorus and chlorophyll concentrations in aquaculture effluents (Rosini *et al*., 2019; Mercante *et al*., 2020). Rosini *et al*. (2019) also observed a decrease in dissolved oxygen concentrations and higher concentrations of ammonium and levels of electrical conductivity in cage-cultivated areas. Therefore, considering the economic importance of fish farming and its current expansion, it is important to better understand the impacts of aquaculture on the water quality and support new management techniques.

In addition to environmental aspects, another key factor associated with the productivity of aquaculture systems is the welfare of the fish kept in ponds, which is directly related to the animals' stress levels (Wurts & Durborow, 1992). In general, stress conditions are common in farming environments and can compromise the health of the organisms, resulting in reduced zootechnical performance and lower productivity (Sahu *et al*., 2008; Pietsch, 2025). On the other hand, when environmental and management conditions favour fish welfare, better individual development can be observed, positively reflecting on system productivity. Such conditions can be assessed by observing the behavioural repertoire of the organisms, both at the individual level and in groupings such as shoals (Martins *et al*., 2011). Therefore, understanding which environmental factors and management practices contribute to promoting welfare in artificial farming systems is essential for developing more efficient farming strategies with the potential to optimize production (Martins *et al*., 2011; Bonini-Campos *et al*., 2019).

Besides environmental quality and animal welfare, the long-term sustainability of aquaculture also depends on productive and economic performance. As aquaculture continues to expand worldwide and gains increasing economic importance, improving production efficiency has become essential for maintaining the profitability and competitiveness of fish farming (FAO, 2024). Productive indicators such as growth rate, survival, biomass yield, and feed conversion ratio (FCR) are widely used to evaluate system performance and serve as indirect measures of economic viability, particularly because feed typically represents the largest operational cost in aquaculture (Stone *et al*., 2024; Barreto *et al*., 2026). Consequently, production strategies that improve these indicators while reducing resource consumption and environmental impacts are considered fundamental for the sustainable development of the sector.

Given these environmental and production challenges, integrated farming systems have received increasing attention as sustainable alternatives to conventional monoculture. These systems seek to improve resource-use efficiency by combining species with complementary ecological functions, thereby enhancing productivity while reducing environmental impacts. A key ecological mechanism underlying the success of these systems is niche partitioning. An ecological niche encompasses the range of resources, environmental conditions, and spatial habitats used by a species, whereas niche partitioning occurs when coexisting species exploit these resources differently, thereby reducing interspecific competition and increasing resource-use efficiency (Nahon *et al*., 2020; Ríha *et al*., 2025). Among these approaches, polyculture—the simultaneous cultivation of two or more species in the same production system—has emerged as a promising strategy for sustainable aquaculture. By taking advantage of niche partitioning through differences in feeding habits, habitat use, and behavioural patterns, polyculture systems can promote complementary exploitation of available resources, improving feed utilization, enhance water quality, increase total yield, and improve economic returns (Wang & Lu, 2015; Shrestha *et al*., 2018; Pant & Kumar, 2025).

In this context, it is important to consider the role of the most representative species in global aquaculture. In 2022, cyprinids accounted for the largest share of global production, representing 22% of the total (FAO, 2024). This family includes the grass carp (*Ctenopharyngodon idella*), an herbivorous fish with a total production exceeding 6 million tons, making it one of the most cultivated fish globally over the past decade (Pedrazzani *et al*., 2022; FAO, 2024). The polyculture of grass carp with other carp species is among the most common aquaculture practices in the world (Hernández *et al*., 2014; Shrestha *et al*., 2018). However, the low consumer acceptance of some carp species has encouraged the diversification of these systems through the introduction of new species, such as Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758), a species of great economic interest, whose production reached 5 million tons in 2022 (Hernández *et al*., 2014; Geletu & Zhao, 2023; FAO, 2024). Although integrated and polyculture systems are widely used in several Asian countries, their adoption and scientific evaluation remain comparatively limited in Brazil, particularly for combinations involving Nile tilapia and grass carp (Wang & Lu, 2015; Valenti *et al*., 2021; Hossain *et al*., 2023). Nile tilapia and grass carp represent a potentially complementary species combination due to their distinct feeding habits and ecological niches. While Nile tilapia is an omnivorous species that exploits feed resources throughout the water column, grass carp is primarily herbivorous and capable of consuming aquatic vegetation and other plant-derived resources. This ecological complementarity may reduce resource competition and improve overall system efficiency (Wang & Lu, 2015; Hossain *et al*., 2023)

Although polyculture systems have been widely adopted worldwide and have demonstrated important environmental and productive benefits, information regarding specific species combinations remains uneven. In particular, the combined cultivation of Nile tilapia and grass carp has received comparatively little scientific attention, despite the economic importance of both species and their potentially complementary ecological roles. Consequently, important questions remain regarding the effects of this production strategy on water quality, fish performance, and behavioural interactions, limiting the development of science-based management practices for these systems. Our study addresses these gaps by thoroughly evaluating water quality in excavated ponds throughout a complete production cycle involving these two species. We also assess the growth and mortality rates of the fish, analyzing how these metrics correlate with water quality parameters and management practices. We also aim to explore the behavioural dimensions of fish reared in polyculture systems, correlating these data with water quality parameters and management practices to assess their effects on animal welfare and, consequently, on productive performance. By doing so, we aim to comprehensively understand the interactions between these species in a polyculture system and their implications for environmental sustainable aquaculture management.

## 2. Material and Methods

### *2.1. Study site and general experimental design*

To understand the effects of integrated fish farming systems on water quality, fish performance, and behavioural responses, an experiment was conducted at the Agricultural Research Company of Minas Gerais (EPAMIG) (21°28’33.7” S, 42°43’16” W). The experimental farm is located in the municipality of Leopoldina, southeastern Brazil, where rainfall periods are concentrated from October to March. We used the Meteum platform to obtain data on air temperature (°C), precipitation (mm $h^{-1}$), and wind speed (m $s^{-1}$) throughout the cultivation period from (March 2022 till March 2023).

### *2.2. Trial 1: Pond-scale evaluation of monoculture and polyculture systems*

#### *2.2.1. Experimental setup*

In March 2022, *O. niloticus* and *C. idella* fingerlings obtained from a commercial hatchery supplying fish to the region were randomly stocked into nine earthen ponds (**Fig. 1A** and **B**). Each pond had a surface area of 540 m², an average depth of 1.5 m, and a volume of approximately 810 m³. Prior to stocking, a subsample of fish was weighed and measured to characterize the initial population, with both species presenting an average initial weight of approximately 0.5 g. Three treatments with triplicates were tested: monoculture of *O. niloticus* (named Tilapia Mono), monoculture of *C. idella* (named Carp Mono) and polyculture ponds with both species (named Poly) (**Fig. 1C**). The polyculture followed the 1:9 ratio criteria for cultivating two species in the same pond (Ostrensky & Boeger, 1998; Yang & Huang, 2011; Wang & Lu, 2015), meaning 630 *O. niloticus* and 70 *C. idella* were raised together totalling 700 individuals (≈ 0,43 g/$m^3$).

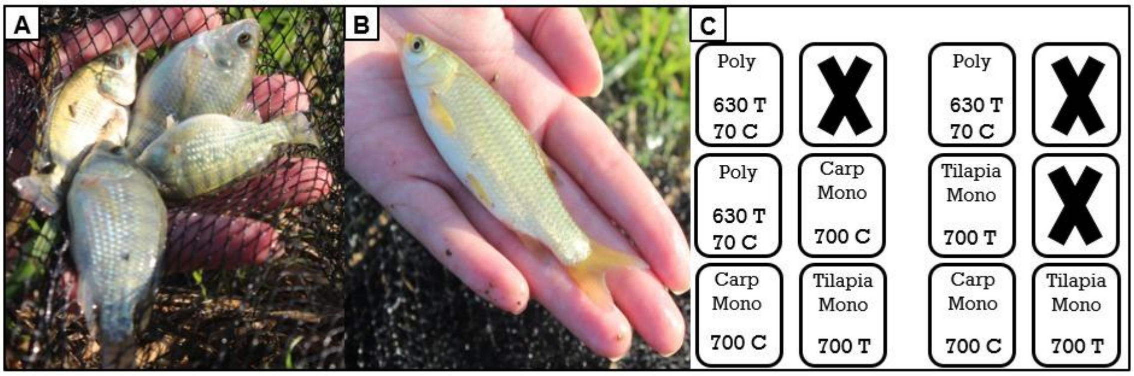


**Fig 1** Overview of experimental sampling. (**A**) *Oreochromis niloticus*, (**B**) *Ctenopharyngodon idella*, and (**C**) Arrangement and identification of the experimental tanks according to treatment.

The fish were fed twice daily with an extruded commercial diet, adjusted from 45% crude protein in the beginning to 28% crude protein in the adult stage. The nine excavated ponds were filled with water from a nearby stream (Samambaia stream). During the cultivation period, water replenishments were done only during the driest period of the year, when necessary, and except during the drainage for the fish harvest, the ponds had no water outflow. The production system adopted in this study can be classified as a semi-intensive aquaculture system. Semi-intensive systems are widely adopted in Brazilian freshwater aquaculture, particularly by small-scale producers, due to their relatively low capital requirements, reliance on natural productivity supplemented by feed inputs, and manageable operational complexity (Valenti *et al*., 2021).

*2.2.2. Fish growth and production performance*

Every month, throughout the 12-month cultivation period, 40 individuals were sampled from each pond using a casting net (8- and 25-mm mesh size) and a dragging net (12 mm mesh size) and immersed in water containing Eugenol (50 mg $L^{-1}$) until they reached anaesthesia stage II, characterized as deep sedation, and then weighted and measured for their total length (cm), standard length (cm), head length (cm), and height (cm) (Ostrensky & Boeger, 1998; Vidal *et al*., 2008; Medeiros-Silva *et al*., 2014). After 13 months of cultivation (March 2023), all the ponds were emptied, and all the fish were harvested, where the total number of individuals was counted, and a total of 90 individuals per pond, regardless of specie, were measured and weighed.

The feed conversion ratio (FCR) was calculated using total fish weight gain and total fish feed, according to the equation (Ostrensky & Boeger, 1998; Gontijo *et al*., 2008):

$$FCR = \frac{(Fish\, feed\ (kg))}{(Weight\ gain\ (kg))}$$

The growth curves were calculated based on Von Bertalanffy's nonlinear regression model (Burbank *et al*., 2023), applying the following equation:

$$L_a = L_\infty\,(1 - e^{-K(a - t_0)})$$

where $L_a$ is the length-at-age in cm, $L_\infty$ is the asymptotic length in cm, K is the growth coefficient, a is the age of the farmed fish in months, and $t_0$ is the theoretical starting age, with the length of the fish supposedly being zero. The model was adjusted to describe total length growth.

The mortality rate (MR) percentage was calculated following the difference between the initial and final number of fish per pound.

#### *2.2.3. Water quality monitoring*

Every month, the water temperature (°C), pH, turbidity (NTU), conductivity (µS $cm^{-1}$), and dissolved oxygen at the surface and the bottom of the ponds were registered using a multiparameter probe (YSI Pro DSS4 port digital); and water transparency (m) with a Secchi disk. Integrated water column samples were collected using a custom-made sampler, consisting of a PVC tube equipped with a bottom valve to ensure closure underwater. After collecting, the samples were stored in plastic bottles and refrigerated for later analysis. Total solids (TS) and total suspended solids (SS) were determined by the gravimetric method at 103–105 °C according to Standard Methods 2540-B and 2540-D, respectively (APHA, 2017). The determination of total nitrogen (TN) employed the sodium hydroxide (NaOH) digestion technique, while nitrate ($NO_3^-$-N) was quantified through the addition of sulfuric acid (Crumpton *et al.*, 1992). Nitrite ($NO_2^-$-N) concentrations were determined following the colorimetric method (4500-NO2–-B), whereas ammonia ($NH_3$-N) was quantified using flow injection analysis (4500-NH3-H; APHA, 2017). Silica concentrations were determined by the colorimetric molybdate method (4500-$SiO_2$ C) following acid digestion. For total phosphorus (TP), the persulfate digestion method was utilized, and phosphate ($PO_4^{3-}$-P) concentrations were measured with the ascorbic acid method (4500-PE). Total pigments were determined spectrophotometrically after extraction with 90% acetone, according to Standard Methods 10200 H (APHA, 2017). A PerkinElmer® LAMBDA 365 UV-Vis spectrophotometer was used to read nutrient concentrations, which were calculated through the corresponding calibration curve equation.

Water temperature was recorded every five minutes throughout the entire 13-month cultivation period using loggers (HOBO, Onset Computer Corp., Pocasset, MA) placed about 75 cm above the bottom of each pond. Additionally, 24 hours before the fish harvest, the water temperature was recorded in the inlet, and during the harvest in the outlet. During fish harvest, water management was adjusted according to the husbandry requirements of each species, following common practices adopted in commercial fish farms (Lines & Spence, 2012; Pavlidis *et al.*, 2023). Accordingly, Carp Mono ponds received a continuous inflow of fresh water throughout the harvest, whereas no additional water was supplied to Tilapia Mono ponds (Mohammed *et al.*, 2024; Pedrazzani *et al.*, 2024; Emam *et al.*, 2025). In Poly ponds, fresh water was supplied during most of the harvest and discontinued near its completion to accommodate the presence of both species.

### *2.3. Trial 2: Behavioral monitoring*

#### *2.3.1. Experimental setup*

To compare behavioural responses among fish reared under different cultivation systems, ten fish were collected from each pond (n = 30 fish per treatment). In the Poly systems, priority was given to collecting eight tilapia and two carp per pond (**Fig. 2**). The fish were then transferred to nine experimental tanks (1 m$^3$) containing clear water, with three replicate tanks per treatment and 10 fish per tank, totalling 90 individuals. All systems were equipped with aerators and underwent regular siphoning procedures to maintain appropriate environmental conditions. Water quality parameters — temperature, pH, ammonia, turbidity, conductivity, and dissolved oxygen — were monitored daily using a multiparameter probe (YSI Pro DSS4 port digital).

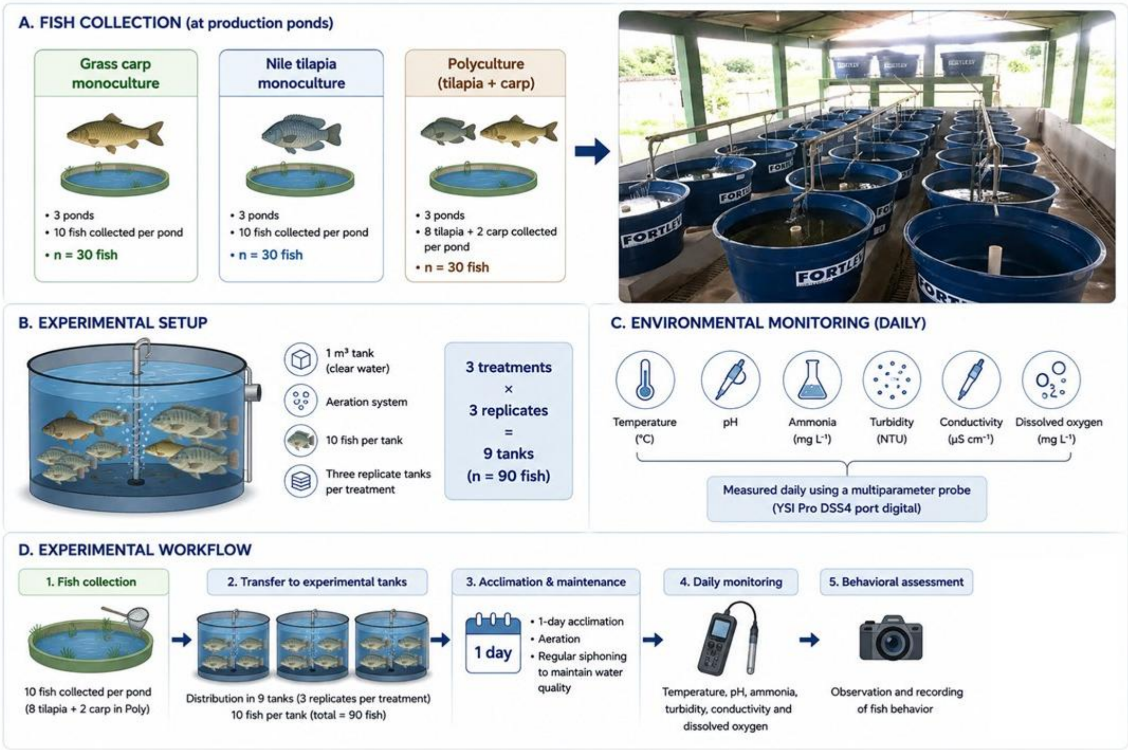


**Fig 2** Experimental design of the behavioral trial. Fish from grass carp monoculture, Nile tilapia monoculture, and polyculture were collected from three production ponds per treatment and transferred to nine 1 m³ experimental tanks (three replicate tanks per treatment; 10 fish per tank). Experimental tanks were maintained under standardized conditions with continuous aeration, and water quality (temperature, pH, ammonia, turbidity, conductivity, and dissolved oxygen) was monitored daily using a YSI Pro DSS multiparameter probe. Behavioral responses were then compared among the three cultivation systems.

Immediately after transfer fishes to a new place, each tank was filmed for 10 minutes to assess the initial behavioural response to handling. After a 24-hour acclimation period, the fish were subjected to a battery of behavioural tests every other day for one week. All observations were conducted in the morning and recorded using two GoPro HERO3 cameras (GoPro Inc., San Mateo, CA, USA), positioned to obtain lateral and top-view footage of each tank. To assess the condition factor of the animals, all fish were weighed (in grams) and measured (in centimeters) after the experiments.

*2.3.2. Behavioural assays*

The behavioral trial was designed to compare stress responses, activity levels, feeding behaviour, social interactions, spatial distribution, and shoaling organization among fish originating from different cultivation systems (Tilapia Mono, Carp Mono, and Poly).

To assess overall activity levels, 10-minute observations were conducted. After the initial 5 minutes of observation, the fish were fed, and both the amount of feed provided, and the total feeding time were recorded. Next, a 3-minute shoal responsiveness test was performed, during which a novel object was introduced into the center of the pond, and the number of fish per quadrant was recorded every minute (Dong *et al*., 2020). This was followed by a 3-minute social preference test, in which the shoal was placed in the center of the pond, flanked by two perforated screens — one containing a carp and the other a tilapia — to evaluate species preference (Dong *et al*., 2020). Throughout all tests, the behaviours expressed and their frequency of occurrence within the shoal were recorded, along with any clinical sign's indicative of disease (Martins *et al*., 2011).

### *2.3.3. Spatial distribution and shoal metrics*

To evaluate the spatial distribution and locomotion of fish in the experimental tanks, each tank was subdivided into 16 quadrants (Q1-Q16), with assigned x and y coordinates. The number of fish in each quadrant was recorded at each minute of observation. Fish positions were determined based on the anterior region of the body (Bonini-Campos *et al*., 2019). To investigate potential niche partitioning between Nile tilapia and grass carp, behavioural metrics describing spatial distribution, quadrant preference, feeding behaviour, and interspecific interactions were evaluated throughout the behavioural assays.

The spatial distribution of fish during the behavioural trials was used to calculate the relative proportion of individuals in each quadrant. The preference index (PI) was calculated as the difference between the observed frequency of fish in each quadrant, weighted by time, and the expected frequency under a uniform distribution. From the quadrants with positive PI, the relative preference ratio (PR) was calculated, which expresses the proportion of use among the preferred locations (Maia & Volpato, 2016). Furthermore, three indices were estimated to characterize the shoal: Shannon entropy (H); uniformity of distribution), variance of proportions (spatial dispersion), and Morisita's index (aggregation). The analyses were subsequently grouped by treatment.

The Shannon entropy, used to assess the uniformity of fish distribution across quadrants, was calculated as (Zhao *et al*., 2016; Kadota *et al*., 2024):

$$H = -\sum_{i=1}^{k} p_i \log_2 p_i$$

where *pi* is the proportion of fish in quadrant $i$, and $k$ is the total number of quadrants. The variance of proportions $\sigma^2$, applied as an indicator of spatial dispersion, was calculated as (Puy *et al*., 2024a):

$$\sigma^2 = \frac{1}{k}\sum_{i=1}^{k}(p_i - \bar{p})^2$$

where $\bar{p}$ is the mean proportion of individuals across quadrants. The Morisita's index ($I_S$), used to evaluate the degree of aggregation, was calculated as (Morisita, 1971):

$$I_\delta = \frac{\sum_{i=1}^{k} x_i(x_i - 1)}{N\,(N-1)}$$

where xi is the number of individuals in quadrant *i*, *n* is the number of quadrants, and *N* is the total number of individuals.

The center of mass (CM) of the school was calculated as the weighted average of the quadrant positions by the number of fish present, using the following equations (Puy *et al.*, 2024b):

$$x_{CM} = \frac{\sum_{i=1}^{N} x_i \times N_i}{\sum_{i=1}^{N} N_i}$$

$$y_{CM} = \frac{\sum_{i=1}^{N} y_i \times N_i}{\sum_{i=1}^{N} N_i}$$

Where $x_i$ and $y_i$ are the coordinates of quadrant, and $N_i$ is the number of fish in that quadrant. The successive displacement of the CM was calculated as the Euclidean distance between consecutive time points, allowing the assessment of temporal changes in collective movement.

### 2.4. Data analysis

#### 2.4.1. Trial 1: Pond-scale evaluation of monoculture and polyculture systems

To verify differences in water quality parameters between depths in the ponds (*i.e.*, surface and bottom), we employed linear mixed-effects models (LMM) using the *lmerTest*() function from the lme4 package (Bates *et al.*, 2015; Kuznetsova *et al.*, 2017). In these models, sampling depth was included as a fixed effect, while pond identity and sampling date were treated as random effects to account for repeated measurements over time and the hierarchical structure of the data. For each water quality parameter (water temperature, dissolved oxygen, conductivity, pH, and turbidity), a model was constructed with depth (surface or bottom) as a fixed effect, and days into the experiment and pond as random intercepts to account for temporal variability. For each model, residual analysis was performed to ensure the appropriateness of the model fit. Residuals were plotted using histograms and Q-Q plots to assess normality. In cases where normality was not satisfied, a generalized linear mixed model (GLMM) was considered with a Gamma family and log link function to handle potential deviations from normality. To fit Gamma family, a fixed value was added to exclude zeros when necessary. The models were compared using the Akaike Information Criterion (AIC). The model with the lower AIC was selected as the better fit, despite the lack of perfect residual normality in some cases.

When the differences between the surface and bottom were observed, the water parameter was considered separately. A similar approach was followed to verify then the differences among the treatments (*i.e.*, Tilapia Mono, Carp Mono, and Poly). We examined the effects of fish presence and temporal changes (measured as days) on water quality parameters, including temperature, dissolved oxygen, and turbidity at both surface and bottom depths, together with electrical conductivity, pH, water transparency and nutrients (*i.e.*, nitrate, nitrite, ammonium, inorganic and organic nitrogen, total nitrogen, phosphate, total phosphorus, total solids, suspended solids, and total pigments). We employed LMM using the different treatments and days into the experiment as fixed effects, while the number of the pond was kept as a random effect to account for potential variability among ponds. The residuals were also evaluated, and when deviation from normality was observed, GLMM using the function (*glmer*()) was used instead. The models were compared, and the best fit was used. When differences were observed, the model was subjected to analysis of variance (ANOVA) using the car package's *Anova*() function (Type III sum of squares) (Fox & Weisberg, 2019). Post hoc pairwise comparisons were conducted using the emmeans package with *emmeans*() to assess differences among treatments (Lenth, 2024). The same statistical approach was used to verify differences among the treatments in terms of biometry parameters (*i.e.*, total length, standard length, head length, height, and weight), and water temperature during the harvesting.

Because mortality was evaluated only at the end of the cultivation cycle (366 days), comparisons among the four experimental groups were performed using a one-way ANOVA, after confirming the assumptions of residual normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test). Pairwise comparisons were subsequently performed using Tukey-adjusted estimated marginal means (emmeans package). Additionally, Spearman's correlation was utilized to explore the relationship between precipitation and turbidity and, to explore the correlation between fingerlings biomass and FCR. All statistical analyzes and graphics were performed using R software version 4.5.1. (RStudio Team, 2025), adopting a significance level of $\alpha = 0.05$.

*2.4.2. Trial 2: Behavioural monitoring*

Behavioural responses were analyzed separately according to the nature of each variable and experimental design. All statistical analyzes and graphics were performed using R software version 4.5.1. (RStudio Team, 2025), adopting a significance level of $\alpha = 0.05$. To verify whether the fish used in the behavioural assays were morphometrically representative of the experimental populations, biometric variables measured before the behavioural trials were compared with those obtained at harvest using the Kruskal–Wallis test.

Environmental variation during the behavioural trials was assessed using Z-score standardized variables. Principal component analysis (PCA) was employed to reduce dimensionality and reveal patterns of variation among treatments over time. A nested PERMANOVA with Euclidean distance evaluated the effects of time, treatments, and variation among replicates.

For each behavioural assay, Shannon entropy, variance and Morisita index were analysed independently using Linear Mixed Models (LMMs), with treatment, sampling day and their interaction as fixed effects and tank identity as a random effect. Models were fitted using the *lmer*() function from the *lme4* package (Bates *et al*., 2015). The significance of fixed effects was assessed by analysis of variance (ANOVA), and significant models were followed by pairwise comparisons using estimated marginal means from the *emmeans* package (Lenth, 2024), with Tukey-adjusted P-values.

The same analytical framework was applied to the remaining behavioural variables, with modifications according to the response variable and experimental design. Fish displacement was modelled as the successive distance travelled by the centre of mass between consecutive observations, considering the combination of species and culture system (Carp Mono, Carp Poly, Tilapia Mono and Tilapia Poly) as a fixed effect, and observation time (minutes nested within sampling day) together with tank identity as random effects. The distance between the centre of mass of each species and the novel object was analysed using two LMMs: the first compared treatments, whereas the second included species, culture system (monoculture or polyculture), and their interaction as fixed effects while maintaining the same random-effects structure. Spatial selectivity was evaluated separately for each species using the standard deviation of the Preference Index (PI) among quadrants as the response variable, with culture system included as a fixed effect and sampling day as a random effect.

Furthermore, the variation in the PR profile across culture systems was assessed using a Permutational Multivariate Analysis of Variance (PERMANOVA). Based on the Bray-Curtis dissimilarity index (adonis2 function, *vegan* package), the test modelled the preference matrix of each species as a function of the culture factor. To determine the nature of any significant differences detected, the mean number of preferred quadrants was compared between culture systems via a Welch's T-test. This subsequent analysis evaluated whether interspecific interactions resulted in more selective or exploratory behaviour.

To evaluate the differences in aggressive behaviour among treatments, we used a two-way ANOVA with farming system (Tilapia Mono, Carp Mono, and Poly) and behavioural trial type as independent variables. Additionally, we conducted a Student's t-test on each subgroup to investigate interaction differences between conspecifics and heterospecifics. For the analysis of freezing behaviour, time data were converted to minutes, and a total freezing metric was calculated by multiplying the time by the number of fish of each species in the tank. Freezing behaviour was subsequently analysed using the same LMM framework described for the behavioural indices.

The non-parametric Kruskal-Wallis test was used to assess overall differences in organism-environment interactions among the three treatments, followed by Dunn's post-hoc test with Bonferroni correction to identify pairwise differences between groups. The effect of polyculture on the feeding time of each species was assessed by comparing monoculture and polyculture data using the Mann-Whitney U test, selected due to non-normal data distribution (Shapiro-Wilk). Direct competition between carp and tilapia in the polyculture system was analyzed with the Wilcoxon Signed-Rank test for paired data, after verifying the normality of the differences.

## 3. Results

### *3.1. Trial 1: Pond-scale evaluation of monoculture and polyculture systems*

#### *3.1.1. Environmental conditions and water quality*

During the cultivation period, the average air temperature ranged from 19.1°C to 25.6°C, with lower values from June to August 2022 and higher from January to March 2023 (**Fig. S1**). The monthly accumulated average precipitation varied from 0.007 mm $h^{-1}$ to 0.4 mm $h^{-1}$, peaking in December 2022 (**Fig. S1**). The initial analysis showed significant differences between surface and bottom for temperature, dissolved oxygen, and turbidity ($p < 0.05$; LMM), with higher temperature and dissolved oxygen at the surface and higher turbidity at the bottom. No significant differences were observed for conductivity and pH between depths ($p > 0.05$; LMM). The comparison between treatments throughout the cultivation period revealed no significant effects ($p > 0.05$; LMM) for bottom temperature, dissolved oxygen at surface and bottom, bottom turbidity, transparency, $NH_3$-N, TS, and SS.

On the other hand, most of the analyzed variables showed significant changes over time within each treatment ($p < 0.05$; GLMM), while differences between treatments were observed only in specific cases. Surface temperature showed statistically significant temporal variations ($p < 0.05$; LMM), characterized by an initial decline in values followed by a consistent increase during the final stages of cultivation (**Fig. S2**). The pH analysis revealed continuous fluctuations over time across all treatments (**Fig. S2**), with highly significant statistical differences ($p < 0.05$; LMM) observed between several days, indicating consistent variation of the variable throughout the experiment. Conductivity showed statistically significant variations over time in all treatments ($p < 0.05$; LMM), with an initial increase up to day 47, followed by a significant decline in the subsequent days (Fig. S2). $SiO_2$ concentrations decreased significantly over time in the Carp Mono and Poly treatments ($p < 0.05$; LMM). Turbidity was significantly higher in the later stages of cultivation ($p < 0.05$; LMM). This increase was more pronounced in Tilapia Mono, followed by Poly, and was least evident in Carp Mono.

$NO_3^-$-N, TON, and TN concentrations decreased significantly after day 1 across all treatments ($p < 0.05$; LMM), showing a general declining trend over time followed by later fluctuations. $NO_2^-$-N showed a significant decreased over time in the Carp Mono and Tilapia Mono ($p < 0.05$; LMM). $PO_4^{3-}$-P concentrations varied significantly over time ($p < 0.05$; GLMM), with distinct patterns among treatments. Carp Mono showed significantly higher values compared to those with tilapia and mixed systems, especially during intermediate cultivation phases. Significant differences were observed between day 147 and other sampling days within the Carp Mono, indicating a peak in concentrations at this time. In the Tilapia Mono and Poly, increases in $PO_4^{3-}$-P concentrations over time were also significant, but less pronounced. Total phosphorus (TP) concentrations varied significantly over time in the carp treatment ($p < 0.05$; LMM), with the highest values recorded on day 147. On that same day, carp ponds showed significantly higher TP concentrations compared to the tilapia and Poly treatments. The mixed-species ponds exhibited the lowest TP levels, closely followed by the tilapia ponds. Total pigment concentrations in carp ponds decreased significantly over time ($p < 0.05$; LMM).

Water temperature monitoring showed significant variation over time ($p < 0.05$; LMM), revealing a well-defined temporal pattern throughout the experimental period (**Fig. S3**). Although the range of temperature variation was smaller in the tilapia monoculture (20 to 33.3 °C) compared to the other treatments — Carp Mono (18.7 to 33 °C) and Poly (19.1 to 35.5 °C) — no significant effect of the farming system on water temperature was detected ($p > 0.05$; LMM), indicating that the different groups exhibited similar trajectories of temperature variation throughout the experiment.

During harvest, water inflow management was adjusted according to the husbandry requirements of each species, following standard practices commonly adopted in commercial fish farms. Significant differences in water temperature were observed between the affluent and effluent in both the Tilapia Mono (average difference of 2.8 °C) and Poly systems (2.5 °C), with the effluent showing significantly higher temperatures in both cases ($p < 0.05$; GLMM; **Fig. 3**). In contrast, in the carp monoculture, the average difference was only 0.04 °C, with no statistically significant change ($p > 0.05$; GLMM). Additionally, the water temperature in the effluent of the Tilapia Mono and Poly treatments was significantly higher than that observed in the effluent of the Carp Mono ($p < 0.05$; GLMM), indicating greater water heating in systems with tilapia.

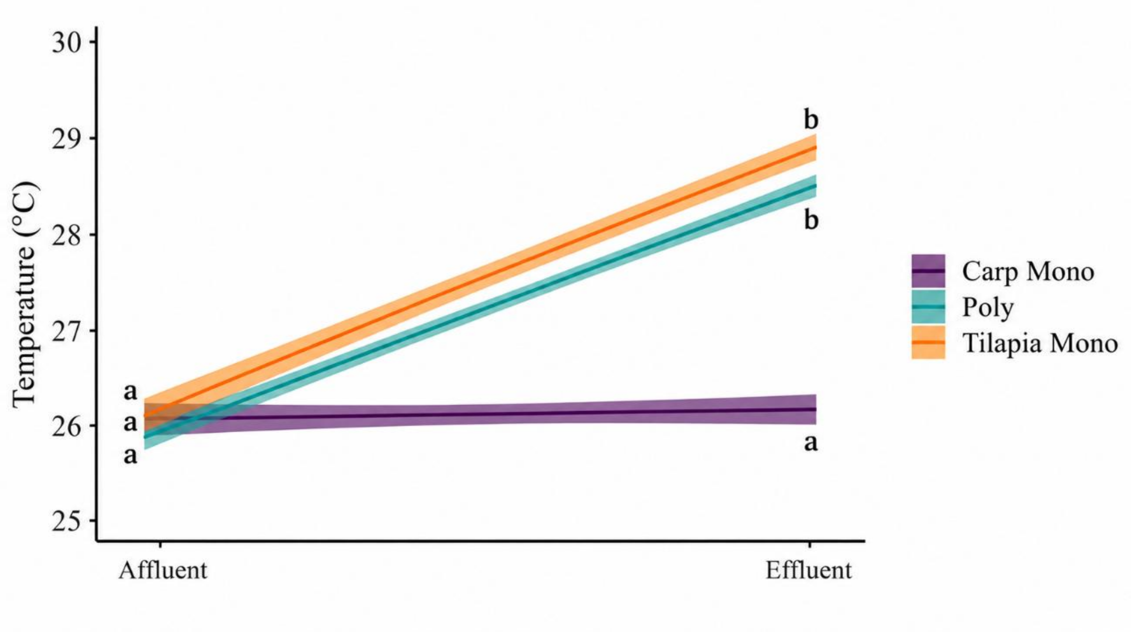


**Fig 3** Average water temperature during the harvesting processes in the affluent and the effluent of the evaluated fishponds. Different lowercase letters indicate significant differences among affluent and effluent temperatures within and between farming systems ($p < 0.05$; GLMM).

Fish species composition (monoculture or polyculture) did not alter the relationship between precipitation and turbidity, as positive correlations were observed in all treatments ($p < 0.05$; Spearman correlation). Although the association appeared weaker in Tilapia Mono, the overall trend remained consistent across systems (**Fig. S4**). Moreover, the correlation was more pronounced at the surface than at the bottom.

*3.1.2. Fish performance*

Carp total length, head length, and body height are affected by the type of farming system (Mono vs. Poly). At harvest, the total length of the fish ranged from 17.7 to 48 cm (**Fig. 4**), with a significant increase over time in all treatments ($p < 0.05$; LMM). Tilapia Mono consistently exhibited greater growth than Carp Mono at all sampling times during the 13-month monitoring period. Tilapia Mono reached the highest average length (32.8 ± 1.7 cm), while Carp Mono had the lowest (23 ± 1.3 cm). However, carp reared in Poly achieved a considerably higher average (30.5 ± 4.4 cm), with significantly greater growth compared to Carp Mono. In contrast, tilapia did not show statistically significant differences between Mono and Poly systems, maintaining similar average lengths (31.5 ± 0.8 cm). The behaviour of standard length, head length, and height values followed the same significance patterns observed for total length (**Fig. 4**).

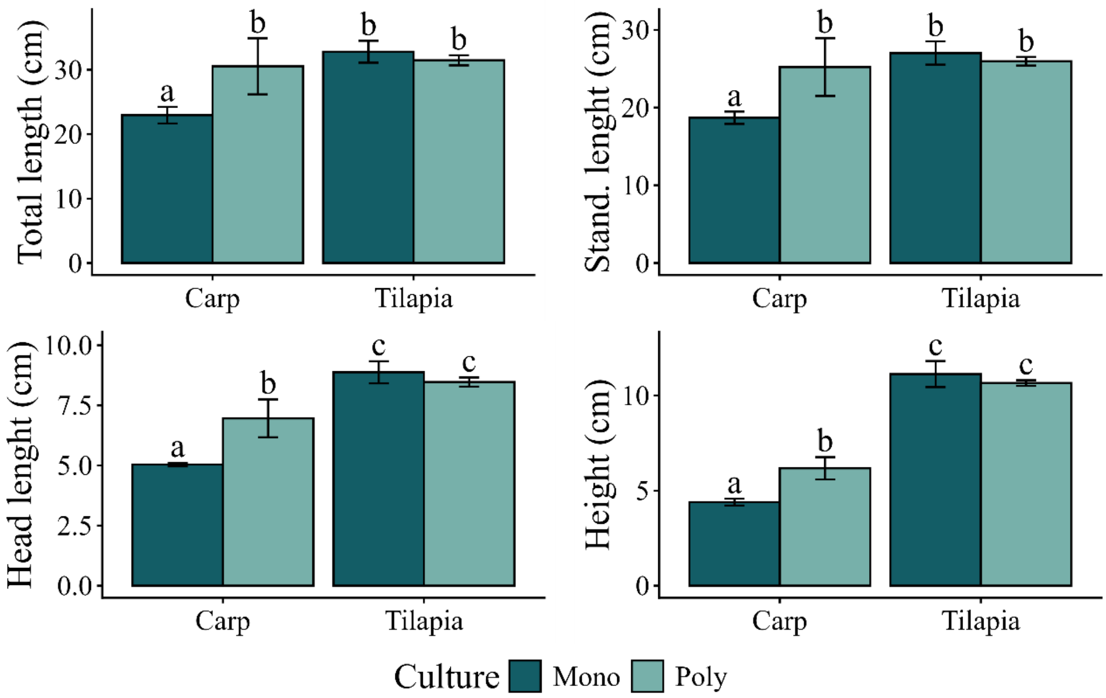


**Fig 4** Results of total length (cm), average length (cm), head length (cm), and height (cm), obtained throughout a cultivation cycle in three treatments: carp monoculture, tilapia monoculture, and polyculture of these species. The bars are represented by mean and standard error. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

Carp and tilapia mean body weight was significantly affected by the type of farming system (Mono vs. Poly). The tilapia, in monoculture (0.6 ± 0.1 kg) and polyculture (0.5 ± 0.03 kg) systems, exhibited significantly body weights than carp in either system at several time points during the experiment. Additionally, carp raised in Poly were heavier (0.2 ± 0.1 kg) than those maintained in Carp Mono (0.1 ± 0.01 kg) ($p < 0.05$; LMM; **Fig. 5**). No significant differences in the body weight of Carp Mono were observed over time ($p > 0.05$). In contrast, Carp Poly showed progressive increase in mean body weight over time, with statistically significant differences between time points ($p < 0.05$; LMM). For tilapia, both monoculture and polyculture systems showed significant differences over time as well ($p < 0.05$; LMM), indicating continuous growth. However, no statistically significant differences were found between the two systems for tilapia ($p > 0.05$; LMM; **Fig. 5**), suggesting that the type of culture system had little effect on weight for this species. The FCR allowed to see high feed conversion values for Carp Mono (5.8 ± 0.4) compared to Tilapia Mono (2.2 ± 0.1) and Poly (2.3 ± 0.2).

The growth curves showed that the Poly, which presented an average final biomass of 241.7 ± 46.7 kg, exhibited values similar to the Tilapia Mono, which weighed 275.7 ± 5 kg (**Fig. 5**). In contrast, the Carp Mono presented an average final biomass of 61.7 ± 5.3 kg, a significantly lower value than the other two species ($p < 0.05$; LMM; **Fig. 5**).

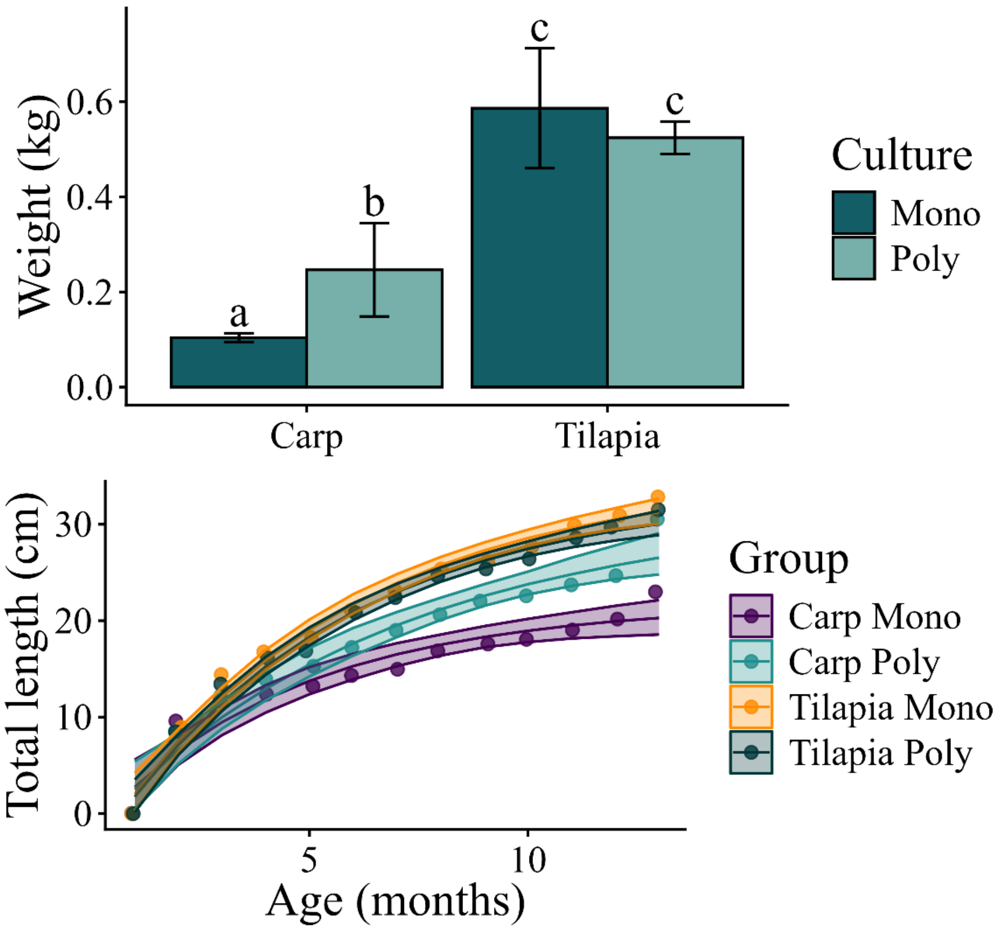


**Fig 5** Comparison between the average growth of fish in polyculture treatment and monoculture ponds. Growth curve, based on total length (cm) as a function of age (month), in each treatment (grass carp and tilapia monoculture and polyculture). Error bars correspond to the standard deviation

Total mortality did not differ significantly among farming systems or species at the end of the cultivation cycle ($p > 0.05$; ANOVA). Although Carp Poly presented the highest average mortality (37.1 ± 13.4%), followed by Tilapia Mono (30.8 ± 10.9%), Tilapia Poly (22.0 ± 13.4%) and Carp Mono (17.0 ± 10.9%), these differences were not statistically significant.

After 200 days of cultivation, the presence of tilapia fingerlings and minnows was detected in some of the examined ponds. However, the actual volume of fingerlings in each pond could only be accounted for during the harvesting. In the case of Carp Mono ponds, only a small biomass of *Astyanax* sp. fingerlings was identified. On the other hand, in ponds dedicated to Tilapia Mono and Poly, where a reduced number of *Astyanax* sp. were also observed, the volume of tilapia fingerlings and juveniles proved slightly more expressive. For monoculture treatment, the average biomass of tilapia juveniles was recorded as 75 ± 19.5 kg, while in polyculture, this average biomass was 48.5 ± 16.2 kg.

The results indicate a significant association ($p < 0.05$; Spearman correlation) between fingerlings biomass and feed conversion ratio in Tilapia Mono and Poly systems. In both cases, the positive correlation suggests that as fry biomass increases, the feed conversion ratio also increases. In Carp Mono, the correlation was weak and not significant ($p > 0.05$), indicating that, for this treatment, the relationship between fingerlings biomass and feed conversion ratio is not consistent.

### *3.2. Trial 2: Behavioural monitoring*

The mean weight of the test Carps was 0.103 ± 0.038 kg in monoculture and 0.209 ± 0.088 kg in polyculture, while Tilapia weighed 0.626 ± 0.158 kg in monoculture and 0.634 ± 0.137 kg in polyculture (**Fig. 6**). The fish used in the behavioural tests were compared with the biometric data of the animals at the time of harvest, and no significant differences were observed between the groups ($p > 0.05$; Kruskal-Wallis), indicating a similar morphometric profile.

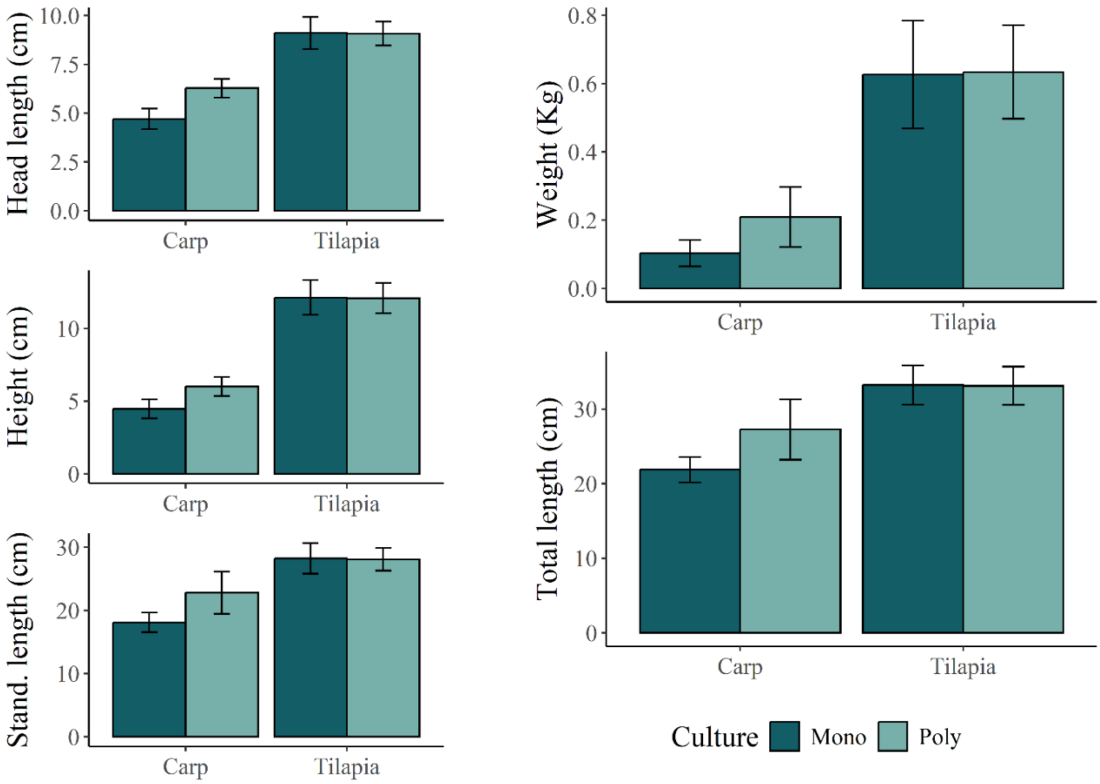


**Fig 6** Mean values of weight (Kg), total length (cm), standard length (cm), head length (cm), and height (cm) of the individuals evaluated in the behavioral assay, considering three treatments: carp monoculture, tilapia monoculture, and polyculture of these species. The bars are represented by mean and standard error.

The analysis of water quality indicated that the replicates were homogeneous within each treatment ($p > 0.05$; PERMANOVA), while significant differences were observed between sampling days and among treatments with different species ($p < 0.05$; PERMANOVA). Complementarily, the PCA biplot of the environmental variables (PC1 = 39.8%; PC2 = 21.1%) showed a clear separation both between days and among treatments, reinforcing the temporal and environmental distinctions between groups. Separation along the PC1 axis was positively associated with dissolved oxygen and temperature, and negatively with conductivity and turbidity. In turn, the PC2 axis mainly represented variations in pH and $NH_3$ (**Fig. 7**).

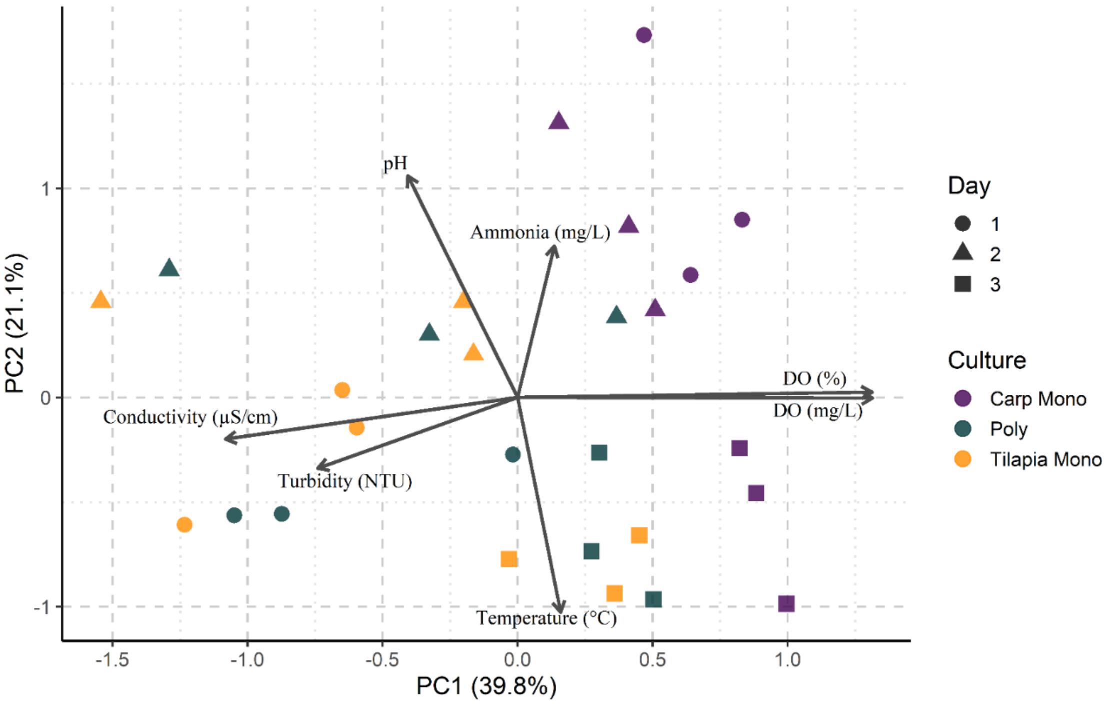


**Fig 7** Principal Component Analysis (PCA) of environmental variables in each tank used in the behavioral assay, considering different fish cultures and monitoring days. Each point represents a treatment on a specific day, and different shapes indicate the collection days. Arrows show the direction and magnitude of each environmental variable's contribution to the principal components. DO represents dissolved oxygen.

Analysis of shoaling behaviour revealed that Carp Mono exhibited significantly lower entropy, higher variance, and higher Morisita index ($p < 0.05$; LMM), indicating more aggregated shoals and irregular spatial distribution (**Fig 8A**). In contrast, Poly and Tilapia Mono did not differ from each other ($p > 0.05$; LMM), showing higher entropy, lower variance, and lower aggregation, reflecting more dispersed and uniform shoals. The effect of time was consistent across all treatments, with entropy increasing and variance and aggregation decreasing. A consistent pattern was observed over the days in the activity, feeding, and object response assays (**Fig 8B** and **Fig S5**). This pattern was characterized by an increase in entropy and a decrease in variance and behavioural aggregation, regardless of the treatment. In contrast, for social behaviour, the time scale of minutes, rather than days, was the main predictor of changes (**Fig 8C**). In this case, the Poly and Tilapia Mono led to lower overall aggregation, and the effect of minutes was particularly evident in the Poly, where the passage of time resulted in higher entropy and lower variance ($p < 0.05$; LMM).

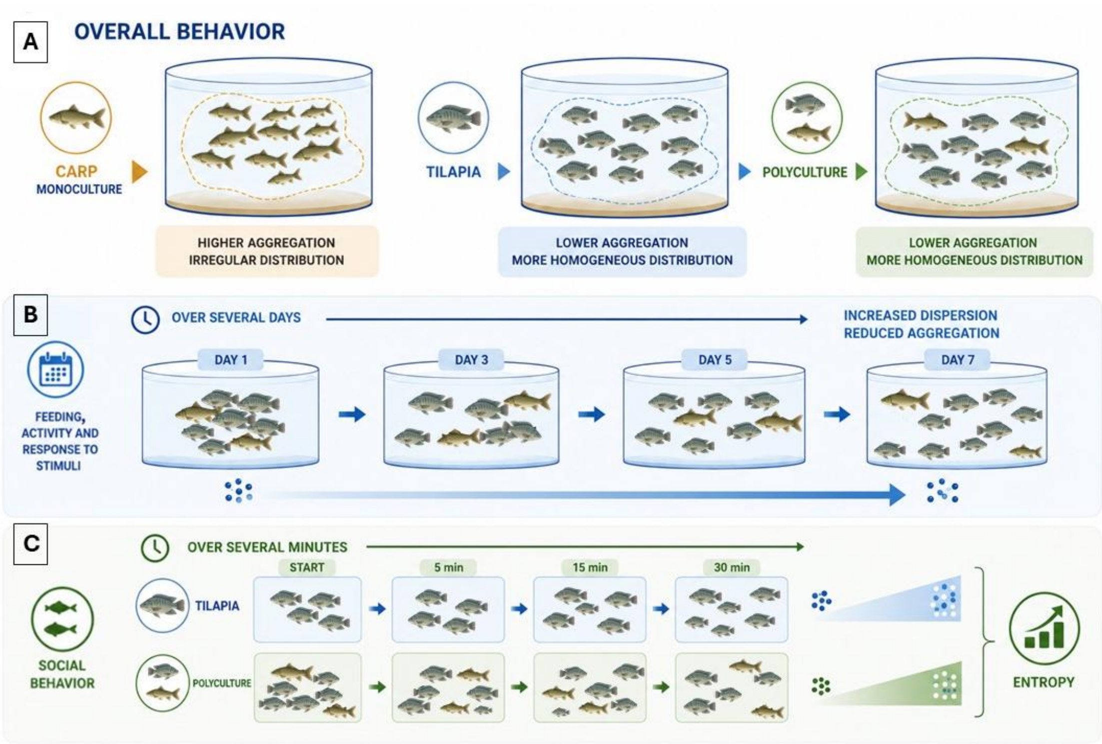


**Fig 8** Conceptual framework of the spatial behavior analyses performed in this study. (A) Comparison of the general aggregation patterns observed in carp monoculture, tilapia monoculture, and polyculture. (B) Temporal changes in fish aggregation over consecutive days, with increasing dispersion and reduced aggregation following feeding, activity and response to stimuli. (C) Short-term dynamics illustrating changes in fish spatial organization during the social behavior test. Spatial entropy was used as a quantitative metric of fish distribution, with higher entropy indicating a more homogeneous and less aggregated distribution of individuals.

The locomotion trial prior to feeding revealed differences in displacement according to species and treatment (**Fig 9A**). On average, Carp Poly showed the highest displacement (0.65 ± 0.39 quadrant units), followed by Carp Mono (0.39 ± 0.24), Tilapia Poly (0.32 ± 0.13), and Tilapia Mono, which exhibited the lowest displacement (0.18 ± 0.10). Carp Poly moved significantly more than Carp Mono ($p < 0.05$; LMM), whereas the differences between Tilapia Mono and Tilapia Poly were not significant ($p > 0.05$; LMM). The analysis of spatial selectivity indicated unilateral interaction. The presence of Tilapia significantly altered the behaviour of Carp, which shifted from a highly selective pattern of space use in monoculture to a more generalist one in polyculture ($p < 0.05$; LMM; **Fig 10A**). The reverse, however, was not observed, as tilapia maintained their pattern of space use regardless of the carp's presence ($p > 0.05$; LMM).

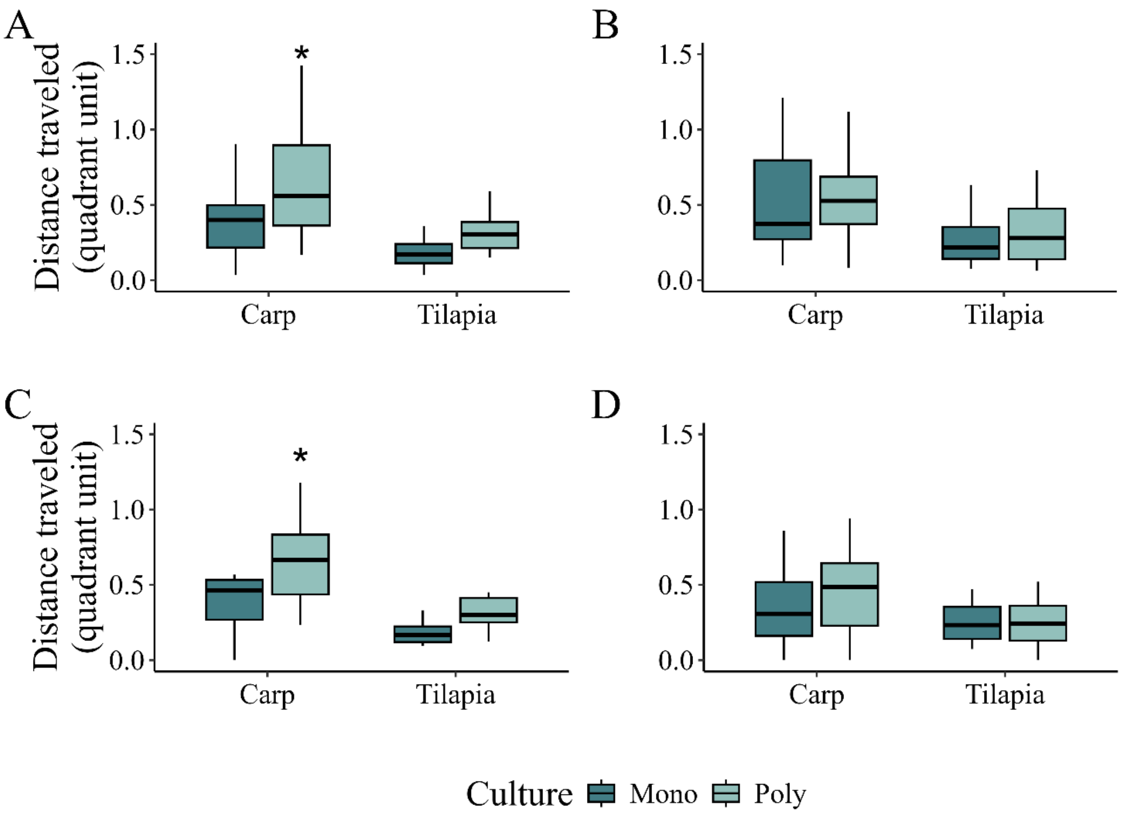


**Fig 9** Displacement of the center of mass for carp and tilapia in monoculture and polyculture systems in response to different stimuli. Each panel represents a specific experimental context: (A) Baseline activity, (B) Feeding stimulus, (C) Novel object stimulus, and (D) Social stimulus. Asterisks denote a statistically significant difference ($p < 0.05$) between groups, as determined by a Tukey's post-hoc test following a

linear mixed-effects model.

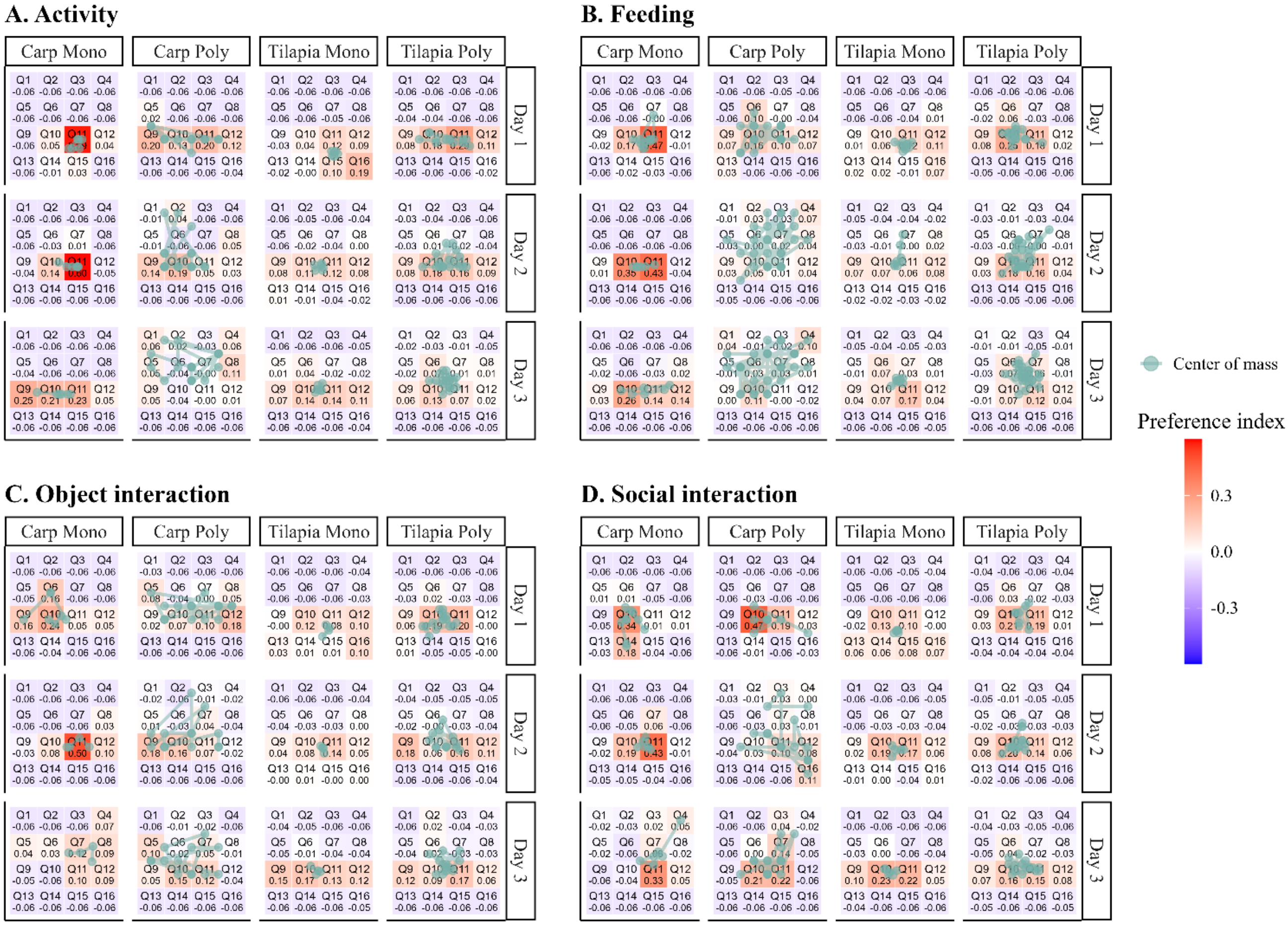


**Fig 10** Analysis of spatial use and center of mass trajectory for grass carp and Nile tilapia in monoculture and polyculture systems into four distinct behavioral contexts: (A) general locomotor activity, (B) feeding behavior, (C) novel object interaction, (D) social preference. Spatial preference is visualized by a heatmap of the Preference Index (PI). Red colors indicate preference (PI > 0), blue indicates avoidance (PI < 0), and light shades represent neutral use (PI ≈ 0). The trajectory of the group's center of mass (CM), calculated each minute, is overlaid as points and connecting lines. Within each context, sub-panels are arranged by observation day (rows) and experimental treatment (columns).

During feeding, although there was no longer a difference in the displacement of carps between monoculture (0.59 ± 0.44) and polyculture (0.56 ± 0.28; **Fig 9B**), the way each species utilized the habitat was profoundly altered ($p < 0.05$; LMM; **Fig 10B**). Carp, which were selective when alone, became generalists in the presence of tilapia. Tilapia exhibited the opposite behaviour: from being generalists in monoculture, they became significantly more selective in polyculture, preferring specific areas. In monoculture, carp displayed a strong, consistent preference for the lower zone (PI < 0.15), while tilapia, despite a similar initial preference (PI < 0.10), exhibited a gradual upward trend in the water column (PI > 0.41), though they continued to prefer the lower zone across all measurements. In polyculture, carp used the upper zone more extensively than tilapia each day, ultimately showing a preference for the upper layer (PI > 0.53) from the second day of the study. The feeding performance of Carp Mono was significantly lower than that of the Tilapia Mono and Poly ($p < 0.05$, Dunn). Regarding the exploratory behaviour, while the tilapia's approach time to the feed was not affected by the presence of the other species ($p > 0.05$, Mann-Whitney), the carp was significantly reduced ($p < 0.05$, Mann-Whitney). However, in direct competition within the Poly, there was no statistical difference in the latency to consume between the two species ($p > 0.05$, Wilcoxon).

The pattern of spatial reorganization observed in the feeding assay was even more pronounced in the object test, where carp in polyculture not only had the highest displacement of all groups (0.86 ± 0.49; $p < 0.05$; LMM; **Fig 9C**), but their spatial selectivity was also drastically reduced. In contrast, Tilapia again became more selective in the presence of carp ($p < 0.05$; LMM; **Fig 10C**). Analysis of the fish's distance to the novel object revealed a strong and consistent behavioural difference between the species across both culture systems. In both monoculture and polyculture ($p < 0.05$; LMM), carp remained significantly farther from the object than tilapia. Moreover, the transition from a monoculture to a polyculture environment did not significantly change tilapia's behaviour ($p > 0.05$; LMM). No significant differences were found among the treatment groups for either fish displacement during the social preference trial (**Fig 9D**) or for spatial selectivity ($p > 0.05$; LMM; **Fig 10D**). Still, the analysis of the Preference Index (PI) revealed distinct behavioural patterns for each species and treatment during the social trial. In monoculture, carp showed a preference for the zone containing the test carp (PI = 62.20 ± 75.50), while tilapia preferred the zone with the test tilapia (PI = 7 ± 11.0). In polyculture, preference patterns changed significantly. Carp reversed their preference to the zone containing the test tilapia (PI = 9.33 ± 9.25), while tilapia in polyculture did not exhibit an expressive preference (PI = 0.17 ± 2.89), adopting a more balanced space-use behaviour.

Analysis of the PR profile revealed that the culture system induced significant and species-specific behavioural modifications, with particularly strong effects during locomotion and feeding trials. The spatial use profile of Carp differed significantly between monoculture and polyculture ($p < 0.05$; PERMANOVA), as did that of Tilapia ($p < 0.05$; PERMANOVA). Subsequent analyses unveiled asymmetrical responses to this interspecific interaction. In polyculture, Carp significantly expanded its spatial niche by utilizing a greater number of preferred quadrants (mean = 4.50) in comparison to monoculture (mean = 2.58; $p < 0.05$; T-test). This expansion was characterized by a distinct shift in spatial patterning: whereas in monoculture their preference was concentrated in the central zone (Q9, Q10, and Q11), in the polyculture system, their preferred quadrants moved to new, peripheral locations (Q4, Q8, and Q16). Tilapia showed an inverse pattern, occupying more quadrants in monoculture than in polyculture ($p < 0.05$; T-test), rather, its adaptation also was characterized by a spatial relocation of its preferred areas to new quadrants.

During the trials, Carp Mono formed shoals and were not aggressive toward one another. In contrast, Tilapias Mono did not move in shoals and displayed high levels of aggression. In Poly, both species stopped exhibiting shoaling behaviour. Aggression among tilapias decreased significantly ($p < 0.05$, T-test), while aggression between the species (carps and tilapias) was rare and sporadic. The analysis of freezing behaviour showed no significant effects for any treatment or species ($p > 0.05$; LMM). Tilapias exhibited similar freezing times between treatments throughout the assays, with a slight increase during the feeding moment (Tilapia Mono: 2.00 min ± 1.42; Poly: 1.80 min ± 1.48). In the monoculture, a secondary peak was observed during the social assay (2.26 min ± 1.42), while the lowest value occurred in the object assay (0.11 min ± 1.42). In the polyculture, freezing remained low and relatively constant, with values approaching zero in the social assay. For carps, mean freezing times were higher during the feeding and activity moments in the monoculture treatment (5.56 min ± 3.60), representing the main peaks observed. There was a progressive reduction in the object (3.33 min ± 3.60) and social (0.00 ± 3.60) moments, while in the polyculture treatment, values remained close to zero and stable across assays.

Regarding social interaction, Tilapia Mono showed a keen interest in the novel species. They interacted with the test carp 10 times, double the interactions observed with the test tilapia. In sharp contrast, tilapia from the polyculture system interacted minimally with both stimulus fish. Meanwhile, carp displayed low levels of social interaction across both treatments. In terms of environmental stimuli, while interaction with a test object did not differ among treatments ($p > 0.05$, Kruskal-Wallis), the presence of the camera elicited a significantly greater response from tilapia than from carp ($p < 0.05$, Dunn).

## 4. Discussion

In this study, we found that polyculture systems demonstrated significant advantages over monoculture cultivation, particularly for carp. Carp reared in polyculture exhibited greater mean body weight and larger biometric measurements than those reared in monoculture. Moreover, polyculture did not result in a general deterioration of water quality and, for some parameters, showed values comparable to or more favourable than those observed under monoculture conditions. Together, these findings suggest that integrating grass carp with Nile tilapia can improve carp production while maintaining suitable water quality throughout the production cycle.

### *4.1. Water quality*

The water quality results are crucial indicators of the health of the cultivation system and its potential environmental impact (Sinha & Banerjee, 2025). Given the discharge of these untreated effluents into adjacent water bodies, it's essential to consider how these parameters affect natural aquatic ecosystems (Yusoff *et al*., 2024). Tilapia Mono, which exhibited lower water transparency, may produce effluents with higher organic load and lower quality due to increased turbidity (Zhang *et al*., 2022). These conditions can degrade the water quality of receiving ecosystems, promoting eutrophication and reducing local biodiversity (Huser *et al*., 2022; Liu *et al*., 2024). In contrast, Poly demonstrated greater environmental management efficiency, with better dissolved oxygen concentration and lower conductivity, indicating a reduced concentration of nutrients and minerals. This suggests a less severe environmental impact when these effluents are released into natural resources. Therefore, mixed cultivation emerges as a more sustainable alternative for producers aiming to minimize the environmental impact of their operations while maintaining high productivity levels (Holanda *et al*., 2023).

Thermal stratification and the consequent formation of low dissolved oxygen zones at the bottom of ponds are critical challenges in aquaculture management (Avnimelech *et al*., 1992; Netto & Arana, 2005; Sriyasak *et al*., 2013). However, such conditions were not observed in our study, a result attributable to the shallow depth of the systems (average of 1.5 m), which facilitated constant mixing of the water column. This vertical homogeneity eliminates a significant stress factor for the fish and ensures that the observed performance results were not influenced by variations in water quality between the different pond layers.

Ponds with longer periods of water inflow during harvesting showed reduced temperatures in the effluents compared to others. Concerns about water temperature during harvest have already been addressed in the literature, as this parameter affects animal stress levels and, consequently, the quality of the final product (Papaharisis *et al*., 2019; Prabu *et al*., 2019). It is speculated that tilapia has a higher resistance in comparison to other fishes, such as carp, which potentially prompts fish farmers to implement diverse harvesting strategies (Sátiro *et al*., 2018). This distinction in their physiological responses likely necessitates different handling techniques during the harvesting process. Understanding these species-specific responses is pivotal in tailoring efficient and species-appropriate management practices, ultimately influencing the success and sustainability of aquaculture operations.

To our knowledge, few studies have specifically quantified the increase in water temperature of aquaculture effluents during fish harvest, making direct comparisons difficult. Nevertheless, harvest has been recognized as one of the periods of greatest alteration in effluent quality due to sediment resuspension, fish activity, and management practices (Coldebella *et al*., 2018). In the present study, the increase of approximately 2.5–2.8 °C observed in systems containing tilapia suggests that harvesting procedures may also temporarily modify the thermal characteristics of discharged water, reinforcing the importance of adopting management practices that minimize thermal alterations.

Furthermore, the water after harvesting is normally sent to water bodies adjacent to the effluent. Therefore, changes in the effluent quality may have subsequent impacts on the downstream systems (Ahmad *et al*., 2021). Especially when considering that temperature is a critical variable, directly and indirectly influencing a wide range of biological processes, with effects that can vary depending on the affected system, the organisms present, nutrient availability, biological organization, and other factors (Jabiol *et al*., 2020). Previous studies indicate that even subtle increases in water temperature can affect organisms at different trophic levels in various ways. Lessard & Hayes (2003) reported that temperature increases due to the presence of dams altered macroinvertebrate communities and led to population declines in some fish species. Gossiaus *et al*. (2020) documented metabolic changes, higher growth rates in invertebrates, and increased competition for resources. Additionally, other studies highlight adverse effects on fish physiology and reproduction, impairing embryonic and larval development (Ashal-Ud-Doulah *et al*., 2021; Yu *et al*., 2022). Moreover, there is prior evidence suggesting that rising temperatures, along with the consequent increase in fish activity, tend to elevate parasitic infection rates in affected systems, which can lead to the spread of diseases and increased mortality among organisms (Gopko *et al*., 2020).

In this sense, using different management practices, like water inflow during all harvesting, can improve the final product of the fish farm and allow for the mitigation of possible environmental impacts in locations close to the farming system. However, this practice must be aligned with the sustainable use of water to prevent excessive consumption of this resource. Thus, establishing appropriate renewal rates for maintaining the pond during harvest is crucial. Additionally, diluted effluent in larger water volumes can be repurposed for use in other cultivation sectors. From a regulatory perspective, although Brazilian legislation does not establish specific temperature limits for aquaculture effluents, aquaculture enterprises must comply with environmental licensing requirements established by CONAMA Resolution No. 413/2009, while the discharge of effluents must meet the general conditions defined by CONAMA Resolution No. 430/2011 and preserve the water quality standards established by CONAMA Resolution No. 357/2005 (BRASIL, 2005, 2009, 2011). Therefore, management practices that reduce thermal alterations in harvesting effluents, such as continuous water renewal, may contribute to minimizing impacts on receiving water bodies and to supporting compliance with environmental regulations.

### *4.2. Fish performance*

The growth curve observed in our study follows a similar pattern to previous studies, although the fish, especially the carp, had a lower average weight than expected (Gontijo *et al*., 2008; Aguiar *et al*., 2021). This could be related to the long cold periods during the experiment. As shown by Santos *et al*. (2013), the greatest weight gains occurred during the warmest periods of the year, with temperatures above 28°C. While temperatures between 22 and 27°C were present in the first 200 experimental days, showed little evidence of weight gain. This scenario is because temperature has a strong influence on fish behaviour. Elevated temperatures increase the overall activity of the fish, raising their energy demands, which leads to higher growth rates and improved feed conversion ratios (Bartolini *et al*., 2015). In the same sense the high feed conversion ratio observed throughout the year, in addition to being linked to the presence of fingerlings at culture, may be linked to low fish activity in the coldest periods of the experiment. This scenario highlights that feed management cannot be static and must be dynamically adjusted in response to thermal conditions to align nutrient supply with the metabolic activity of the fish, a critical factor for successful cultivation (Hamed *et al*., 2024). Moreover, the positive correlation between fingerlings biomass and the FCR suggest that the increase in fingerlings biomass in monoculture Tilapia and in polyculture ponds may be associated with a decrease in feed efficiency.

The absence of a true stomach in grass carp may explain the reduced growth observed in the monoculture of this species, as the daily amount of feed was divided to be offered only a few times during the daytime (Mokhtar *et al*., 2021). Furthermore, omnivorous fish feeds used in Brazil use tilapia as a test model, which is an advantageous factor over other species that do not yet have a well-defined nutritional technological package, such as grass carp (Ng & Romana, 2013; Alemayehu *et al*., 2018; Makwinja & Geremew, 2020). The higher growth of tilapia may be related to the genetic improvement of the species for better feed conversion, as opposed to grass carp, which has not been included in genetic improvement programs, especially for this purpose (Verdal *et al*., 2018; Rode *et al*., 2020).

Carp showed higher weight and growth when cultivated in polyculture. The discrepancy in the growth curves of carp in monoculture and polyculture systems is not entirely new in the literature, as a similar situation has been reported for other teleost species, including different carp species (Papoutsoglou *et al*., 2001; Candido *et al*., 2006; Shahin *et al*., 2011; Ritter *et al*., 2013; Wang & Lu, 2015; Barbosa, 2017; Barros *et al*., 2020). Nevertheless, the ideal proportions for establishing fortuitous polyculture and the reasons for this scenario are still a subject of better understanding. Studies have been showing that the presence of tilapia provides a favourable environment for the development of carp, while a smaller number of carp in relation to tilapia allows lower competition (Papoutsoglou *et al*., 2001; Wang & Lu, 2015). Moreover, behavioural modifications have also been proposed to justify this improvement in production, as they may be linked to a lower stress rate (Papoutsoglou *et al*., 2001; Wang & Lu, 2015). Some studies also point to an improvement in the physiological condition of carp and tilapia raised in polyculture, with changes in parameters such as lipid content, hematocrit, cholesterol, triglycerides, pH, albumin, plasma Cl level, $pCO_2$ and $pO_2$ (Papoutsoglou *et al*., 2001).

Additionally, mortality did not differ among farming systems, indicating that the improved growth of grass carp under polyculture was achieved without compromising fish survival. Survival is one of the main indicators of production efficiency in aquaculture because it directly influences final biomass, production costs, and economic return (Kankainen *et al*., 2012). The similar mortality observed among treatments suggests that fish losses were more likely associated with common husbandry conditions experienced throughout the production cycle than with the farming system itself. Therefore, the higher growth observed for carp in polyculture cannot be attributed to differences in survival, but rather to improved individual performance.

### *4.3. Behavioural experiment*

The main conclusion of this behavioural analysis is that the polyculture of Nile tilapia and grass carp induces profound and beneficial changes that extend beyond simple coexistence. Instead of a sum of individual behaviours, a synergistic remodelling was observed, transforming the interaction between the species into a driver for system optimization. The most notable effects were the drastic reduction in intraspecific aggression among tilapias and the promotion of spatial niche partitioning, where the carp began to actively explore the upper water column. These behavioural adjustments not only mitigate direct competition but also create a more stable and productive culture environment, suggesting that the benefits of polyculture are fundamentally rooted in the behavioural plasticity of the species involved.

The analysis of quadrant preference revealed a clear competitive hierarchy and an asymmetrical spatial dynamic between tilapia and carp in the polyculture system. Tilapia emerges as the spatially dominant species, exhibiting a remarkably stable and resilient behaviour by maintaining a consistent preference for the central zone of quadrants Q10 and Q11, regardless of the carp's presence. In contrast, the carp demonstrates significant behavioural plasticity; although it also prefers the same zone in monoculture, its distribution in polyculture becomes much more variable and unpredictable. Under the apparent competitive pressure from the tilapia, the carp is frequently displaced to peripheral and new quadrants (such as Q4, Q8, and Q16), indicating a strategy of avoidance or the forced exploration of alternative spatial niches. Therefore, the interaction between the species is not one of simple coexistence but rather a complex interplay of spatial overlap in moments of resource sharing and of clear segregation, where the more reactive behaviour of the carp suggests it is actively outcompeted by the tilapia in the dispute for preferential space. This phenomenon is a classic example of niche partitioning, where the subordinate species (carp) avoids interference competition with the dominant species (tilapia) by shifting its spatial niche to an underutilized area (Říha *et al*., 2025). This adaptation is crucial for explaining the superior zootechnical performance of carp in polyculture, as the reduction in competitive stress and access to new resources likely facilitated its growth.

Beyond its ecological benefits, polyculture resulted in a substantial improvement in animal welfare for both cultured species. The presence of carp diluted agonistic interactions among tilapias, significantly decreasing the frequency of aggressive behaviours that are energetically costly and detrimental in high-density monocultures (Gonçalves-de-Freitas *et al*., 2019). Complementarily, carp, which exhibited peaks of "freezing" behaviour (an indicator of fear and stress) in monoculture, had this behaviour practically eliminated in the presence of tilapia. This indicates that the polyculture environment promoted a state of greater security, corroborating the findings of Yao *et al*. (2024), who demonstrated that duoculture can reduce social interaction costs, such as aggression, and improve growth rates in fish species. Therefore, the improvement in animal welfare is not merely a secondary outcome but a central factor contributing to the system's sustainability and productive efficiency.

Our study also allows for the discernment between plastic behaviours, shaped by the social environment, and more consistent, intrinsic personality traits of each species (Castanheira *et al*., 2017). Space utilization, activity level, and aggression demonstrated high plasticity, changing drastically in response to the presence of another species. In contrast, traits such as boldness and shyness, measured by the distance to a novel object, appeared to be consistent: carp remained consistently shyer than tilapia in both culture systems. This distinction is fundamental for aquaculture management, as it suggests that while a species' predisposition to be shy cannot be altered, it is possible to create an environment (in this case, polyculture) where this trait does not become a productive disadvantage, allowing the animal to adapt through other behavioural pathways.

In summary, the observed behavioural modifications are not mere ethological curiosities, they represent the mechanistic basis that explains the productive benefits and enhanced sustainability of the polyculture system. The behavioural patterns observed in the present study suggest the occurrence of niche partitioning between Nile tilapia and grass carp, promoting a more complete utilization of the pond's trophic and spatial resources, while the reduction in stress and aggression improved the overall welfare and energy efficiency of the fish. Together, these factors create a more resilient and productive system. Therefore, the practice of tilapia and grass carp polyculture transcends simple biomass production, evolving into a form of "ecological management" where the farmer actively manages behavioural interactions to foster a culture ecosystem that is, in part, self-regulated and optimized by the very nature of the fish.

## 5. Conclusion

Our findings provide a deeper understanding of the interactions between growth, behaviour, and environmental impact in semi-intensive pond systems. The results indicate that polyculture, particularly involving carp and tilapia, offers significant benefits that are driven by profound behavioural adaptations. Crucially, the polyculture environment drastically reduced intraspecific aggression in tilapia, a known constraint in monoculture, while promoting a classic case of niche partitioning where carp shifted their spatial use to the upper water column. This behavioural synergy directly explains the enhanced weight gain observed in carp and contributes to a more stable and efficient production system. These findings, combined with environmental observations such as the reduced effluent heating in carp systems with constant water inflow, enable the development of more holistic management strategies. By integrating species with complementary behaviours and carefully managing water flow, it is possible to support decision-making aimed at more sustainable food production, where managing ecological interactions is as vital as controlling physicochemical parameters. Ultimately, this holistic approach allows the polyculture system to achieve a final biomass comparable to that of the intensive tilapia monoculture, proving that high productivity and ecological sustainability are not mutually exclusive goals.

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## Ethics Approval

All stages involving the maintenance and manipulation of fish were submitted to the Ethics Committees on Animal Use of the State University of Mato Grosso (UNEMAT) and the Agricultural Research Company of Minas Gerais (EPAMIG). They were approved by these institutions under Opinion 004/2021 (The biogeochemical balance in tropical aquaculture: implications for ecosystem transformations and climate change in water resources) - UNEMAT, and under Protocol 16/2022 (Dynamics of the development of different fish species in fish farms in the state of Minas Gerais) - EPAMIG.

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**Supplementary Material**

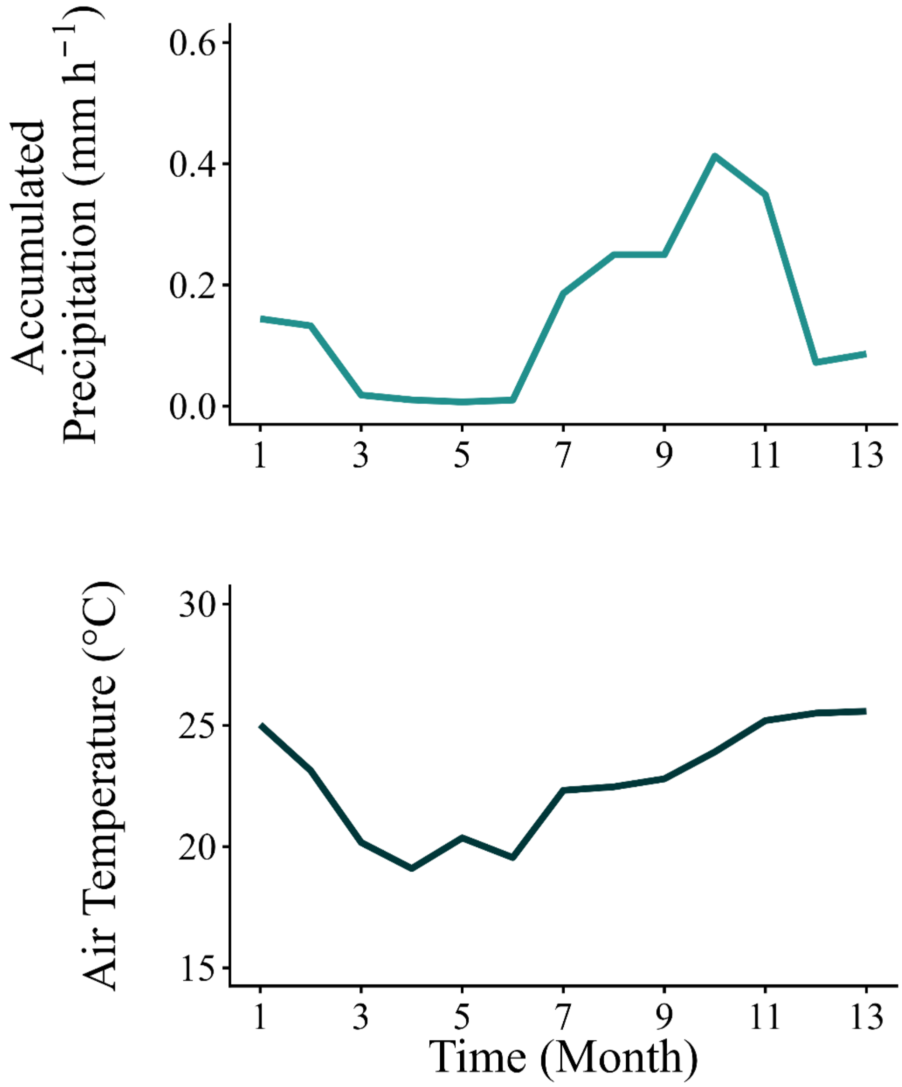


**Fig S1** Variation of air temperature (°C), and accumulated precipitation (mm h$^{-1}$) throughout the study period.

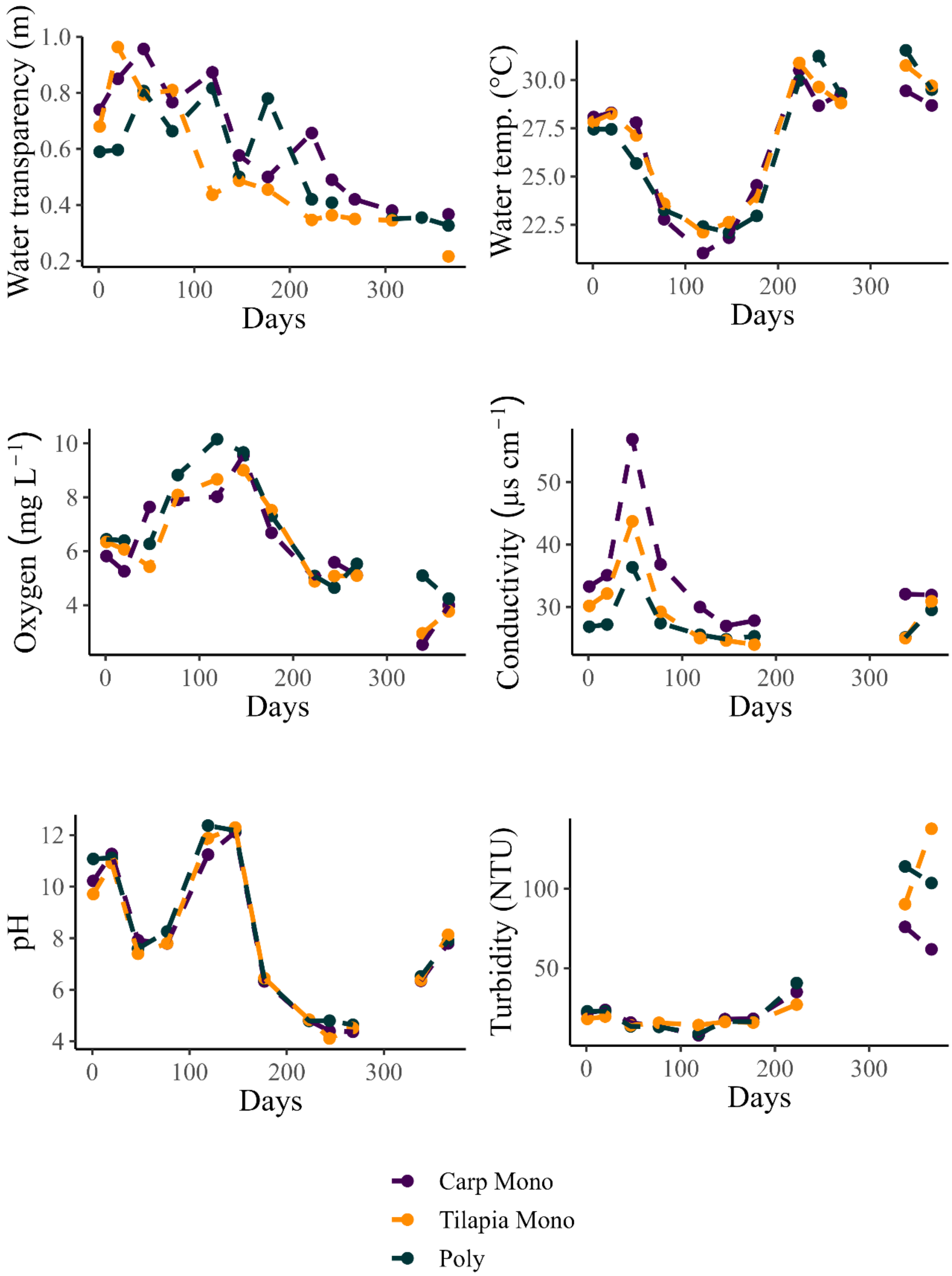


**Fig S2** Results of water transparency (m), water temperature (ºC), dissolved oxygen (mg $L^{-1}$), conductivity (µs $cm^{-1}$), pH, and turbidity (NTU) measured during a cultivation cycle in excavated ponds with monoculture (Carp Mono and Tilapia Mono) and polyculture systems of carp and tilapia (Poly).

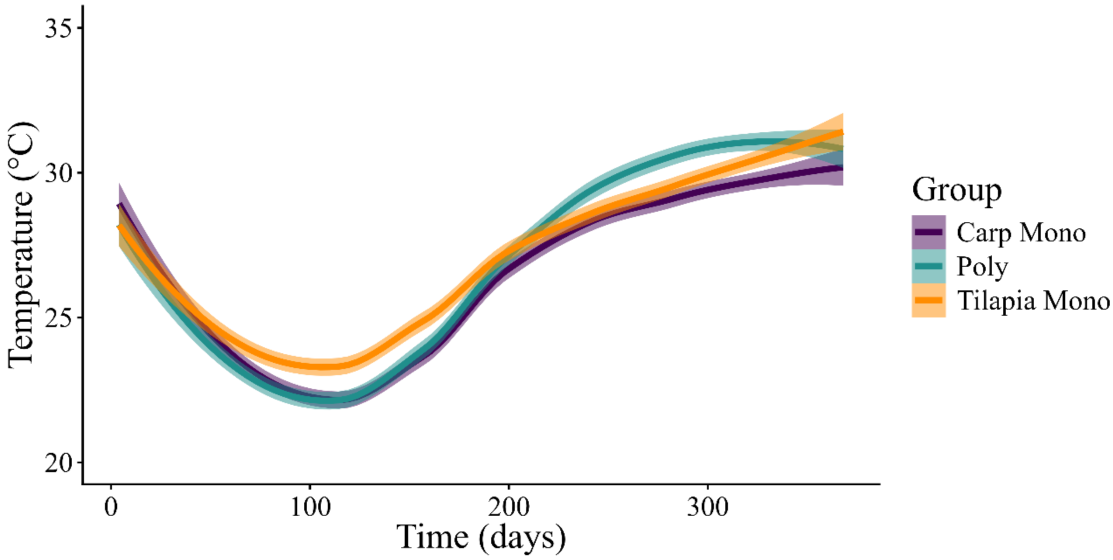


**Fig S3** Water temperature monitoring using datalogger sensors (HOBO, Onset Computer Corp., Pocasset, MA) throughout the entire cultivation period in different treatments represented by the different colors (Carp Mono, Tilapia Mono and Poly).

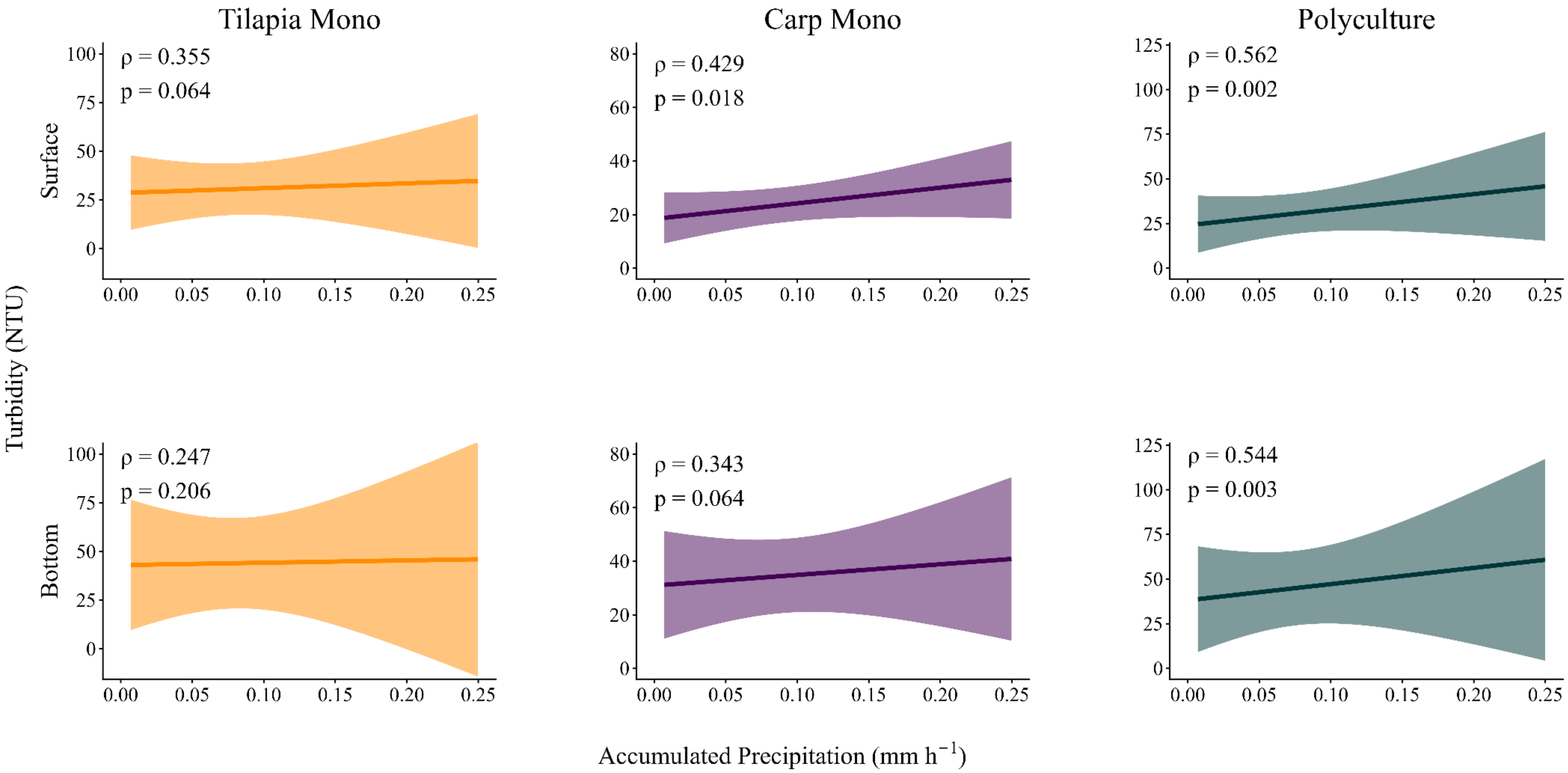


**Fig S4** Linear regression between precipitation and turbidity in the different treatments (Carp Mono, Tilapia Mono and Poly) and in the surface and bottom water layer. Spearman's correlation between the variables allowed us to infer whether the comparison had a significant or non-significant effect, in addition to the correlation strength according to the treatment and the measurement site.

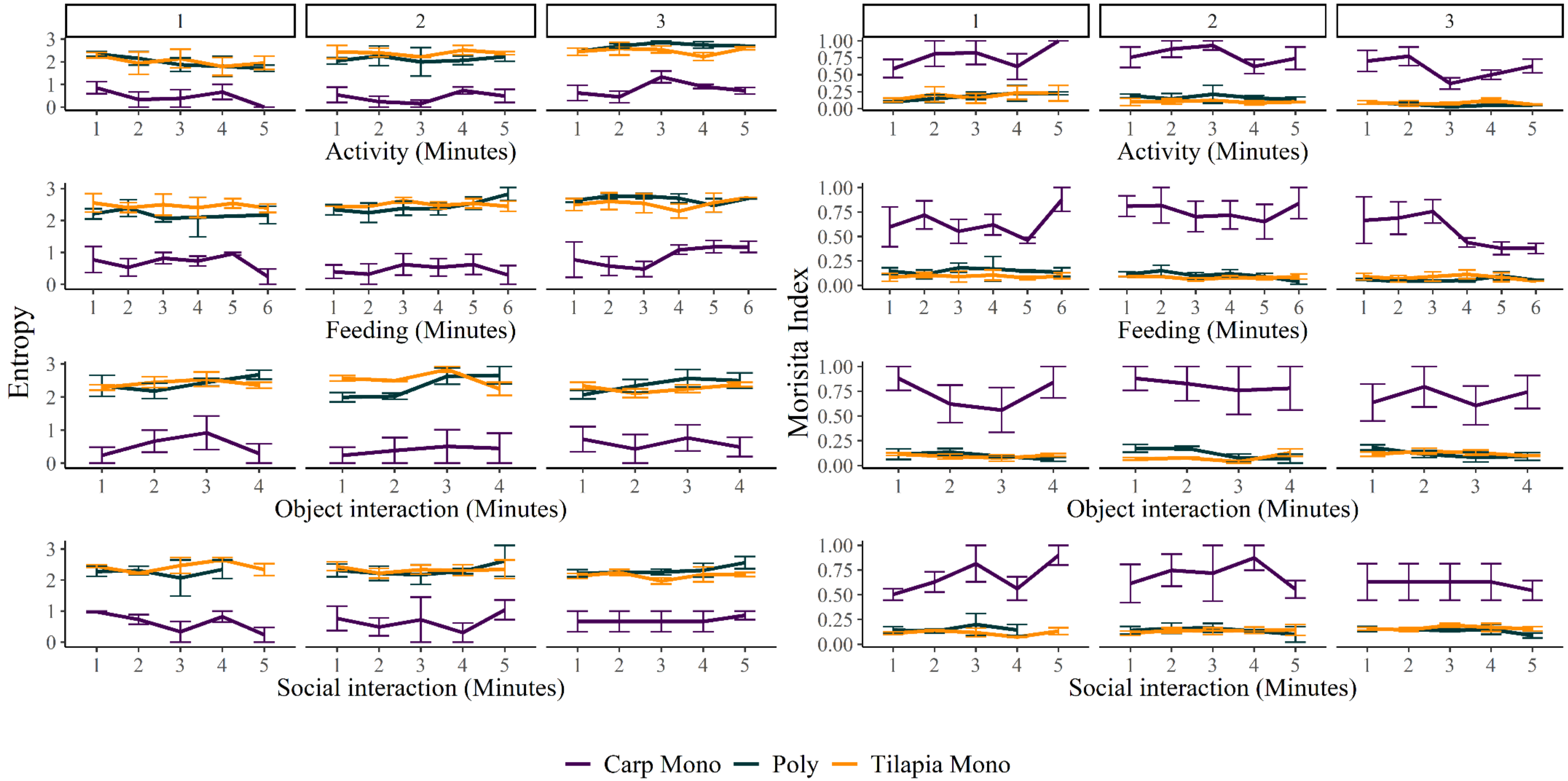


**Fig S5** Mean (± SD) values of spatial distribution indices in behavioral assays. Shannon entropy indicates distribution uniformity, and the Morisita index measures individual aggregation. Results are presented by behavioral assay, treatments, and evaluation days.

## CHAPTER II: A combined in situ–ex situ assessment of fish feed fate and associated carbon emissions[1]

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### Abstract

Aquaculture plays a key role in addressing the global protein demand. However, its long-term sustainability depends on minimizing its environmental impacts, particularly eutrophication and greenhouse gas (GHG) emissions. The fate of fish feed that is not ingested by the fish remains an overlooked factor, yet it is crucial for accurately assessing the carbon footprint of fish production. To assess the fate of undigested fish feed under different conditions, we used complementary *in situ* and *ex situ* approaches to separate the effect of feed composition from the influence of pond conditions. First, we performed an *in situ* experiment to investigate the breakdown of four common fish feed types, and secondly, quantified their associated carbon dioxide ($CO_2$) and methane ($CH_4$) production in an *ex situ* experiment. We explored potential drivers, including feed physicochemical properties. Results showed that the feeds varied significantly in their properties (CN ratio range: 5.4–10.7; decay constant k range:

0.023–0.05 $h^{-1}$). This variation was critical: feeds with lower physical stability and lower CN ratios disintegrated fastest. In *ex situ* incubations, $CO_2$ production significantly increased with the addition of all feed types compared to controls that contained only fishpond sediment (±15 nmol $g^{-1}$ $h^{-1}$). Conversely, $CH_4$ production was minimal across all treatments even under anoxic conditions. Our findings indicate that while feed decomposition directly stimulated $CO_2$ production, it did not constitute a determining factorin $CH_4$ production in this system. Critically, we show that feed physicochemical properties, particularly physical stability and CN ratio, are key regulators of the rate of these microbial processes. To effectively reduce aquaculture's carbon footprint, future development must embed precision feeding (i.e. prevention of overfeeding), along with improved feed composition and stability, into comprehensive GHG mitigation strategies.

Keywords: Carbon dioxide; Eutrophication; Fish Farm; Methane; Pisciculture

[1]Article published in the journal *Journal of Cleaner Production*, v.544, 2026 (Appendix 1). https://doi.org/10.1016/j.jclepro.2026.147711

# 1. Introduction

The rapid growth of the global human population has intensified the demand for high-quality protein sources, driving the expansion of aquaculture (Shi, 2023). Aquaculture is widely regarded as an effective solution to bridge the seafood supply-demand gap globally (Subasinghe *et al*., 2009). In 2022, global aquaculture production reached 94.4 million tonnes – a 7.6% increase compared to 2020 –, and the projection is to reach 205 million tonnes by 2032 (FAO, 2024). Therefore, aquaculture is a key section of the global economy, with an estimated global market value of USD 313 billion (FAO, 2024). Aquaculture is also recognized as a more economically viable protein source than terrestrial livestock (Amorim *and* Tosta, 2020).

Globally, freshwater aquaculture is dominated by a small number of species, notably carps and tilapias, which consistently rank among the most abundant farmed fish groups. According to FAO (2024), grass carp (*Ctenopharyngodon idella*) alone produced approximately 6.2 million tonnes in 2022, making it the world's most farmed fish species, followed closely by Nile tilapia (*Oreochromis niloticus*), with about 5.3 million tonnes. Together, these groups support a substantial share of global freshwater aquaculture and are predominantly cultivated in pond-based systems where formulated feed is the main source of organic matter.

However, aquaculture is not without environmental challenges. Aquaculture is estimated to contribute approximately 0.49% of global anthropogenic greenhouse gas (GHG) emissions (MacLeod *et al*., 2020), with the majority attributed to feed production, transportation, waste processing and energy use in farming systems (Gephart *et al*., 2021; Yadava *et al*., 2024). Fishponds can be GHG emission hotspots due to high organic matter (OM) inputs from leftover fish feed, fish excreta, aquatic plant growth, and runoff from surrounding areas (Huang *et al*., 2020; Kosten *et al*., 2020). In anaerobic sediments, OM can be converted into methane ($CH_4$) (Stichnothe *et al*., 2008) – a GHG with a significantly higher Global Warming Potential (GWP) compared to carbon dioxide ($CO_2$), raising concerns (IPCC, 2023). The first study on GHG emissions from aquaculture was published about 15 years ago (Strangmann *et al*., 2008), and research in this field has since expanded significantly (Yuan *et al*., 2019). Recent studies have highlighted feed as the main indirect source of emissions in the sector, particularly due to emissions associated with its production chain (Castilla-Gavilán *et al*., 2024; Yadava *et al*., 2024). However, significant knowledge gaps remain, especially concerning direct emissions resulting from feed degradation within culture tanks. Although there is evidence that fish feed contributes to nutrient enrichment and GHG emissions (Yang *et al*., 2021), the behavior and transformation of feed in pond environments, as well as the GHG emissions associated with its decomposition, remain poorly understood and widely overlooked in the literature. Yet, it is crucial for accurately assessing the carbon footprint of fish production. As countries are legally bound under the Paris Agreement to reduce GHG emissions significantly (Rogelj *et al*., 2016), improving current estimates of aquaculture-related emissions is essential, particularly for economies reliant on fish farming.

Fish feed decomposition and associated GHG emissions depend strongly on environmental conditions. Isolating the processes that occur during feed breakdown *in situ* is extremely challenging due to the variability of fishpond biogeochemistry. Therefore, we designed a combined approach using controlled *ex situ* incubations and an *in situ* disintegration experiment. The *ex situ* experiment allowed us to isolate the direct effect of feed composition on $CO_2$ and $CH_4$ production while keeping temperature, oxygen, sediment mass and initial chemical conditions constant. In contrast, the *in situ* experiment captured realistic disintegration dynamics under farm conditions. Here, we use disintegration to refer to the combined processes of physical breakdown of the feed into smaller particles or dissolved constituents and the microbial decomposition of the organic matter from which the feed is composed. Together, these approaches provide a mechanistic understanding of how feed physicochemical traits modulate microbial processing and gas production. In this context, physical stability is expressed as the resistance of feed pellets to structural breakdown in water and is quantitatively represented by their disintegration rate, with higher decay constants indicating lower physical stability.

Therefore, this study aims to: (1) investigate the *in situ* disintegration rate of four different fish feed types; (2) determine associated $CO_2$ and $CH_4$ production through an *ex situ* incubation experiment; and (3) assess physical and chemical characteristics of the fish feeds and its relation to the disintegration and GHG production rates. We expected: (1) rapid fish feed disintegration due to its labile nature; (2) $CO_2$ and $CH_4$ production associated with fish feed addition; and (3) the highest disintegration rates in low C:N ratio fish feeds.

## 2. Methods

*2.1. Fish feed*

Four types of fish feed were used in the disintegration experiment (hereafter referred to as the *in situ* experiment), while three types were tested in the GHG production experiment (hereafter referred to as the *ex situ* experiment). The four feed formulations tested were selected because they are commonly used in intensive production systems in Brazil, and because they represent the spectrum of pellet sizes and nutritional compositions (e.g., different protein and energy content) applied in the main cultivation phases (**Table 1**).

**Table 1:** Nutritional composition and physical characteristics of different types of extruded fish feed basis used in the decomposition and gas emission tests were obtained from the information provided on the respective product labels.

| | Feed 1 | Feed 2 | Feed 3 | Feed 4 |
|---|---|---|---|---|
| **Crude protein (%)** | 35-40 | 32 | 22 | 36 |
| **Granulometry (mm)** | 2-3 | 4-6 | 8-10 | 3-4 |
| **Crude fiber (g kg$^{-1}$)** | 60 | 100 | 140 | 60 |
| **Calcium (g kg$^{-1}$)** | 20 | 15-30 | 10-30 | 20 |
| **Phosphorus (g kg$^{-1}$)** | 6 | 2.5 | 7 | 6 |
| **Vitamin C (mg kg$^{-1}$)** | 400 | 200 | 100 | 400 |
| **Ethereal extract (g kg$^{-1}$)** | 65 | 50 | 30 | 65 |

| | | | | |
|---|---|---|---|---|
| **Mineral matter (g kg$^{-1}$)** | 140 | 140 | 140 | 140 |
| **Moisture (g kg$^{-1}$)** | 100 | 130 | 130 | 100 |
| **Vitamin E (Ul kg$^{-1}$)** | 125 | - | - | 125 |

*2.2. In situ experiment*

To investigate fish feed disintegration rates, an *in situ* exclosure was set up in an excavated fishpond with tilapias (1.5 m deep) in Leopoldina, Minas Gerais, Brazil (-21.475021 S, -42.717961 W). The region's climate is classified as Aw, characterized by hot, rainy summers and mild, dry winters (Köppen *and* Geiger, 1928). The exclosure consisted of a polyvinyl chloride (PVC) frame covered with transparent plastic on the sides, leaving the top and bottom open to the environment while preventing fish entry (**Fig. S1a**). Disintegration was assessed using the bag method (Velthuis, 2018). Mesh bags (≤ 1 mm mesh size) contained 4 g of one of the four fish feed types (Feed 1, 2, 3 and 4) or no fish feed – as a control. All mesh bag contained an Eppendorf tube with a label. The mesh allowed water exchange while retaining the feed. Triplicates were suspended using floating foam attached to the PVC frame at two water column depths (**Fig. S1b**): at the surface, exposed to oxygen (aerobic), and at the bottom, anchored with fishing sinkers and in contact with sediment (anaerobic). Every eight hours, a set of replicates was removed from the fishpond, and the weight was recorded before and after drying at 35°C. Water temperature (°C), pH, and dissolved oxygen (mg L$^{-1}$) were measured at both depths using a multiparameter probe (YSI Pro DSS4 Port Digital). The experiment lasted 72 hours (*i.e.*, nine measuring time points). Air temperature and precipitation were measured using a weather station (Hobo U30 Station). Each bag and its contents were weighed before the experiment to correct for initial weight variations.

*2.3. Ex situ experiment*

To investigate GHG production associated with the disintegration of fish feed, a series of incubations was conducted for each fish feed type using water and sediment from the same fishpond as the *in situ* experiment. Water and organic matter-rich sediment (top 4 cm) were collected a day before incubation and kept refrigerated until use (as in Grasset *et al.,* 2018). Each replicate was then prepared in a 120 mL glass bottle, containing 5 g of sediment, 30 mL of water, and 3 g of feed (either type 1, 2 or 3, selected to represent the C/N gradient identified in our *in situ* screening, as Diets 2 and 4 were chemically very similar; **Fig. S2**). Incubations were conducted under oxic and anoxic conditions. Oxic bottles were flushed with oxygen ($O_2$), and anoxic bottles were flushed with nitrogen ($N_2$) for two minutes before sealing (as in Grasset *et al*., 2019). Bottles were incubated at 27°C in a dark room for all experiment. Additionally, an oxic incubation containing only water and feed was included. Control bottles were also prepared with water only (oxic condition) or both water and sediment (oxic and anoxic conditions). In total, the experiment included nine treatments and three controls, each with five replicates (n = 60). Gas samples (2 mL) were collected daily using a syringe with a three-way valve and injected into a portable gas analyzer (Los Gatos Research Inc., Mountain View, CA). To maintain stable pressure, bottles were refilled with either $N_2$ (anoxic bottles) or atmospheric air (oxic bottles) after each sampling. The incubations were monitored for a total of 21 days, to quantify mainly the gas production directly associated with feed decomposition and to exclude confounding gas production from subsequent, secondary biological activities (Struik *et al.*, 2024).

We opted for *ex situ* incubations to because in the pond environments, the influence of uneaten feed on total pond emissions would be difficult to disentangle because of the large an variable overall emissions. In addition, the ex situ incubations allowed us to maintaining fixed temperatures, standardized sediment masses, controlled oxygen regimes, and stable water chemistry, allowing us to attribute observed GHG production directly to feed decomposition rather than to environmental variability. In contrast, the *in situ* experiment was necessary to quantify real-world feed disintegration rates under commercial pond conditions. Together, this two-tiered approach provides both mechanistic clarity and ecological relevance.

*2.4. Physical-chemical characterization*

A series of laboratory analyses were performed to assess the physico-chemical characteristics of fish feed, sediment, and water. The moisture content of fish feeds and sediment was determined by weight loss after drying at 100°C for 2 hours. OM content was assessed via loss on ignition at 500°C for 4 hours (Goldin, 1987). Elemental composition was analyzed after extraction using inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Fischer Scientific, Bremen, Germany). Total carbon (C) and total nitrogen (N) content were quantified using a CNS elemental analyzer (Vario MICRO cube; Elementar Analysensysteme, Langenselbold, Germany).

Total nitrogen (TN) was quantified in the water samples using the sodium hydroxide (NaOH) digestion method (APHA, 2017; Crumpton *et al.*, 1992). Nitrate ($NO_{3-}$) was measured after sulfuric acid addition (Crumpton *et al.*, 1992). Nitrite ($NO_2^-$) was quantified via the Colorimetric Method (4500-$NO_2^-$; APHA, 2017). Ammonia ($NH_3$) was measured using Flow Injection Analysis (4500-$NH_{3;}$ APHA, 2017). Total phosphorus (TP) was quantified using the persulfate digestion method (APHA, 2017). Orthophosphate ($PO_4^{3-}$) was quantified following the ascorbic acid method (4500-PE; APHA, 2017). Nutrient concentrations were quantified using a PerkinElmer® LAMBDA 365 UV-Vis spectrophotometer and calculated using a calibration curve equation. Dissolved inorganic nitrogen (DIN) was determined as the sum of $NO_2^-$, $NO_3^-$, and $NH_3$ concentrations, while total organic nitrogen (TON) was calculated as the difference between total TN and DIN.

*2.5. Data analysis*

Fish feed disintegration was modeled using an exponential decay equation (Adair *et al.*, 2010; Forney *and* Rothman, 2012):

$$R_t = R_0 \times exp^{-kt}$$

Where: $R_t$ is the remaining feed percentage at time *t*; $R_0$ is the initial feed percentage; *t* is the incubation time in hours; *k* is the decay rate.

To assess the fit, the equation was first ln-transformed to linearize the relationship. Model fit for this linearized equation was then assessed using $R^2$ values. The normality of disintegration data was checked using the Shapiro-Wilk test, histograms and Q-Q plots. Statistical comparisons between the decay rates of the different fish feeds were made using paired t-tests (parametric data) or Kruskal-Wallis tests, followed by the Dunn test (non-parametric data).

Linear mixed-effects models (LMMs) from the *lme4* package (Bates *et al*., 2015) were used to assess differences in water quality between surface and bottom layers in the *in situ* experiment. For the *ex situ* experiment, differences in $CO_2$ and $CH_4$ production rates were analyzed using generalized linear mixed models (GLMMs) from the *glmer* package, considering the Gamma family with a logarithmic link function to account for potential deviations from normality. The last was necessary since residuals did not meet the assumptions for LMMs. When required, a fixed value was added to the data to avoid null values. In cases where significant differences were detected, the models were subjected to analysis of variance (ANOVA) using the *car* package, with Type III sum of squares (Fox and Weisberg, 2019). For post hoc multiple comparisons, the *emmeans* package was used to assess differences between treatments (Lenth, 2024).

Gas peaks were converted to nanomolar units using calibration curves and the ideal gas law (as in Grasset *et al.,* 2021). Peaks were corrected for dilution effects. The production rate was calculated by dividing the slope by the dry weight of the sample (i.e. either fishfeed, sediment or fishfeed + sediment depending on the treatment).

All statistical analyzes and graphics were performed using R software version 4.3.3. (R Core Team, 2024).

## 3. Results

### *3.1. In situ experiment: disintegration of fish feeds*

The fish feeds were almost completely disintegrated within 72 hours (**Fig. 1**). Decay constants (k) ranged from 0.02 to 0.04 $h^{-1}$ at the surface of the water column and between 0.03 and 0.05 $h^{-1}$ at the bottom, with no significant difference in the percentage of fish feed remaining between the depths for most time points (T-test: $p < 0.05$; **Fig. 1**). Feed 1 exhibited the highest decay rates (0.05 $h^{-1}$), whereas Feed 3 had the lowest decay rate (0.023 $h^{-1}$) (Dunn test: $p < 0.05$; **Fig. 1**).

Dissolved oxygen concentrations were higher at the surface of the water column (average ± standard deviation = 5.6 ± 0.3 mg $L^{-1}$) than at the bottom (5.2 ± 0.3 mg $L^{-1}$; LMM: $p < 0.05$; **Ta ble S2**). The pH values were also higher at the surface than at the bottom (surface = 4.5 ± 0.4; bottom = 4.3 ± 0.3; LMM: $p < 0.05$; **Table S1)**. No significant difference was found for temperature between the water depths, with mean values of 29.3 ± 0.9°C at the surface and 29.1 ± 1.1°C at the bottom (LMM: $p > 0.05$; **Table S1**). During the experimental period, climatological data indicated an average air temperature of 24.1°C and the occurrence of precipitation events. However, the rain intensity was predominantly low, characterized as 'light rain' (generally below 2.5 mm $h^{-1}$). The experiment also recorded several intervals of null precipitation (0 mm $h^{-1}$), which were interspersed with a light rain.

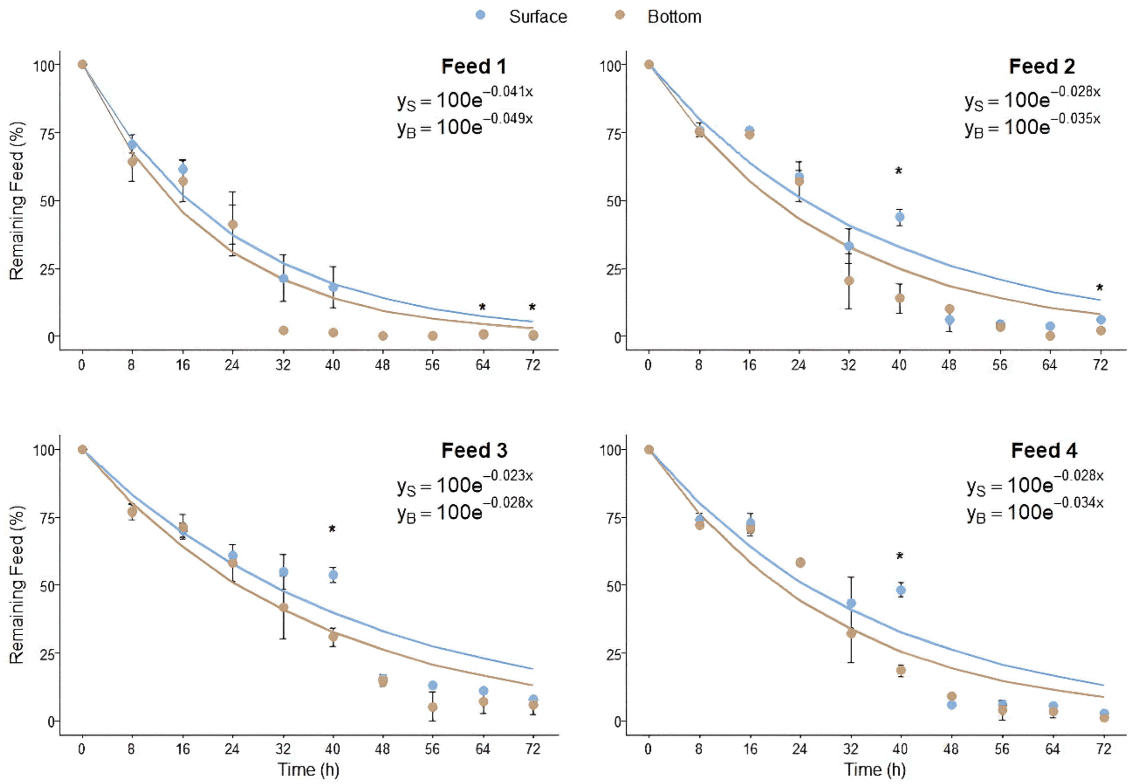


**Fig. 1.** The percentage of fish feed remaining at the bottom and surface of the water column over 72 hours. Data is shown as average and standard deviation. $Y_S$ indicates the decay equation for samples at the surface, while $Y_B$ represents samples at the bottom of the fishpond. The asterisk (*) indicates statistical significance between water column positions (T-test: $p < 0.05$). The error bars indicate the standard deviation.

*3.2. Ex situ experiment: GHG production rates associated with fish feed addition*

$CH_4$ production was generally minimal across all treatments, except in the controls with sediment, which showed higher $CH_4$ production than the feed addition treatments (GLMM: $p < 0.05$; **Fig. 2a**). Under oxic conditions and without sediment, $CH_4$ production associated with Feed 1 was significantly higher than that of the respective control (GLMM: $p < 0.05$). Contrary to $CH_4$ production results, $CO_2$ production significantly increased after adding all fish feed types compared to their controls, regardless of the conditions (GLMM: $p < 0.05$; **Fig. 2b**).

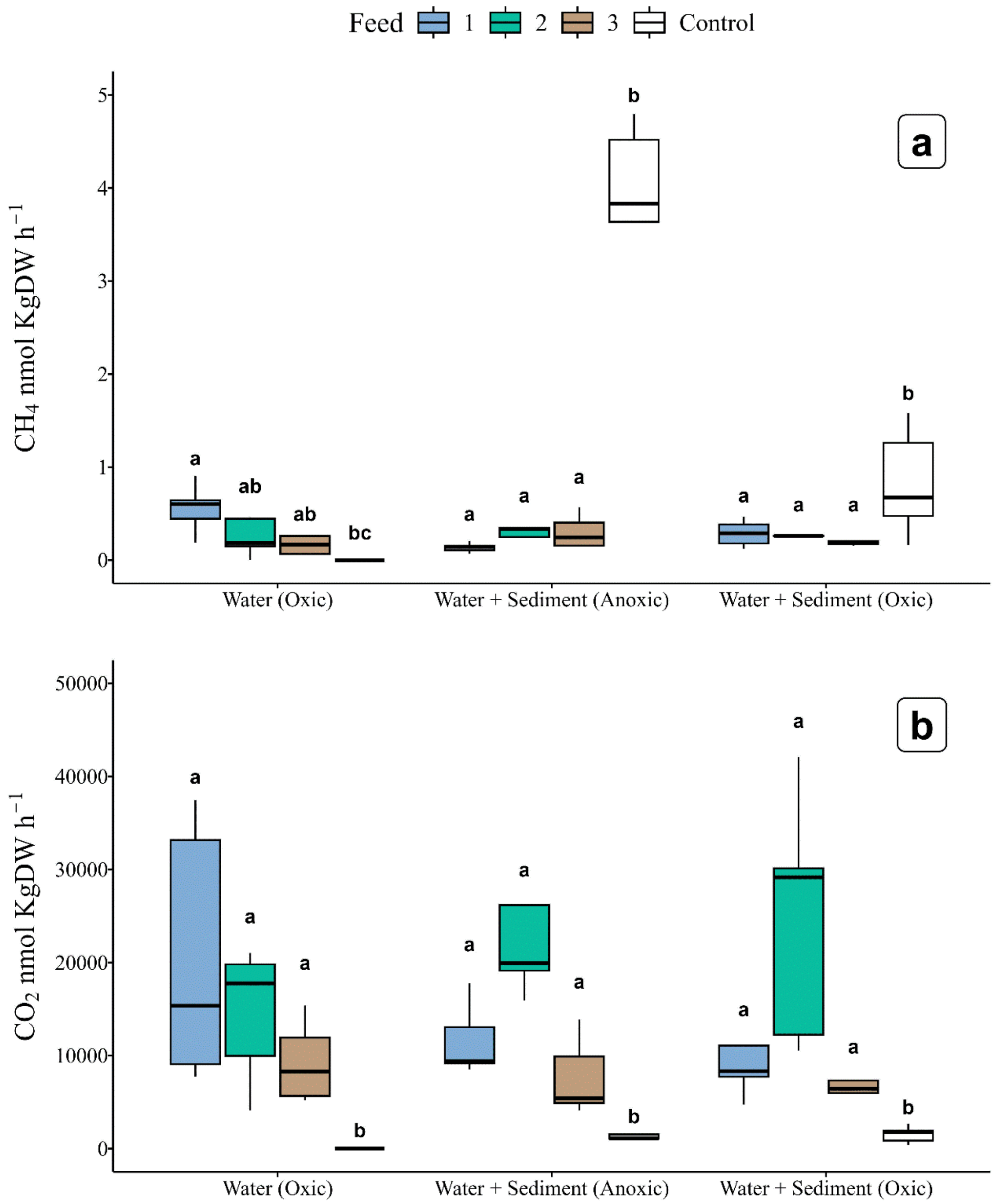


**Fig. 2.** (a) Methane ($CH_4$) and (b) carbon dioxide ($CO_2$) production (nmol $Kg^{-1}$ dry weight $h^{-1}$) under three different conditions: (1) water and feed addition in oxic conditions, (2) water, sediment and feed addition in anoxic conditions, and (3) water, sediment and feed addition in oxic conditions. Different letters indicate significant differences among samples (GLMM: $p < 0.05$).

*3.3. Physical-chemical characterization*

The C content from the fish feeds ranged from 373.4 to 411.2 mg $g^{-1}$ and N from 37.8 mg $g^{-1}$ to 75.2 mg $g^{-1}$, with C:N ratio varying from 5.4 to 10.7. The OM from the fish feeds varied from 76.5 to 81.2% (**Table 2**). The sediment had 0.35 mg of C $g^{-1}$, 0.05 mg of N $g^{-1}$, a C:N ratio of 7.9, and an OM of 14.8%. Additional information on the nutritional composition and elemental content (**Table S2**) is available in the supporting information.

The $NO_2^-$, $NO_3$- and $NH_3$ concentrations in the water from the fish pond were 7.37 µg $L^{-1}$, 568.64 µg $L^{-1}$ and 555.72 µg $L^{-1}$, respectively. DIN was 1131.74 µg $L^{-1}$, TON was 2629.74 µg $L^{-1}$, and TN was 3761.47 µg $L^{-1}$. $PO_4^{3-}$ concentration was 44.53 µg $L^{-1}$, and TP was 181.47 µg $L^{-1}$.

**Table 2:** Results of carbon (C) and nitrogen (N) concentrations in mg $g^{-1}$, C:N ratio, and percentage of organic matter (OM) in the sediment and fish feed used in the *in situ* and *ex situ* experiments.

| | Feed 1 | Feed 2 | Feed 3 | Feed 4 | Sediment |
|---|---|---|---|---|---|
| **N mg $g^{-1}$** | 75.2 | 47.2 | 37.8 | 47.7 | 0.05 |
| **C mg $g^{-1}$** | 405.1 | 411.2 | 405.9 | 373.4 | 0.35 |
| **C:N ratio** | 5.4 | 8.7 | 10.7 | 7.8 | 7.9 |
| **OM %** | 81.2 | 76.5 | 79.9 | 80.7 | 14.8 |

## 4. Discussion

Although microbial pathways governing the transformation of organic matter in aquatic systems including aquaculture ponds remain incompletely resolved, the patterns emerging from our experiments provide useful insight into the dominant processes. Rapid $CO_2$ production is consistent with heterotrophic metabolism of labile substrates released from the fish feed, paralleling responses observed for other organic inputs that stimulate both aerobic and anaerobic respiration (Ferreira & Chauvet, 2011; Grasset *et al*., 2018). (Ferreira *&* Chauvet, 2011; Grasset *et al*., 2018). In contrast, the persistently low $CH_4$ production indicates limited activation of methanogenic pathways, which require strongly anoxic microzones and are constrained by substrate quality and electron-acceptor availability (Donis *et al*., 2017; Nayak *et al*., 2020). The absence of substantial $CH_4$ accumulation may further reflect concurrent methane oxidation by methanotrophs under oxic or anoxic conditions (Guerrero-Cruz *et al*., 2021). While microbial communities were not characterized, the observed gas production patterns provide indirect but robust evidence of the dominant microbial processes shaping feed degradation in our system.

### *4.1. Fish feed disintegration rates*

Our results confirm the fast disintegration rates of fish feeds (within 72 hours). This supports our first hypothesis and aligns with previous studies, indicating that high protein concentrations accelerate disintegration (Torres-Beristain *et al*., 2006; Ferreira *and* Chauvet, 2011). Furthermore, the lack of a statistically significant difference in disintegration between the sediment and water column might be related to the experimental pond's shallowness (1.5 m), likely limiting water column stratification and reducing temperature-driven variations (Ferreira and Chauvet, 2011).

Feed composition plays a role in determining disintegration rates (Kristensen *et al*., 1995; Torres-Beristain *et al*., 2006). As we expected, feeds with a lower C:N ratio, typically protein-rich, exhibited higher disintegration rates (*k*) compared to those with a higher C:N ratio, aligning with previous research (Torres-Beristain *et al*., 2006). However, this trend is also consistent with intrinsic properties of the feed, such as reduced particle size, which increases surface area exposure (Hargrave, 1972; Parnas, 1975; Torres-Beristain *et al*., 2006). Here, the feeds with lower C:N are also the smaller ones, and the enhanced surface area may also explain the increased disintegration rate.

The organic matter content (14.8%) and carbon concentration (0.35 mg $g^{-1}$) observed in the pond sediment reflect fundamental differences between aquatic sediments and terrestrial soils. In aquaculture ponds, organic matter is continuously supplied through uneaten feed, feces, and autochthonous production, resulting in high inputs of labile and recently deposited material (Go *et al*., 2023). In contrast to soils, where organic carbon can be stabilized over long periods through aggregation and mineral–organic associations, aquatic sediments are subject to rapid microbial mineralization, physical disturbance, and resuspension, which limit long-term preservation (Boix-Fayos *et al*., 2015). Consistently, Huang *et al*. (2024) reported low carbon concentrations in sediments from aquaculture systems, emphasizing that high organic matter inputs do not necessarily result in carbon accumulation in sediments. Moreover, Boix-Fayos *et al*. (2015) demonstrated that sediments generally store substantially less carbon than soils due to shorter residence times and weaker mineral–organic associations.

*4.2. GHG production associated with fish feed addition*

Contrary to our initial hypothesis, we found no clear evidence that fish feed addition increased $CH_4$ production rates, except for a slight increase after adding Feed 1 under anoxic conditions. Possibly anoxic micro patches occurred in the incubation enabling $CH_4$ production. As expected, $CH_4$ production remained low under oxic conditions since methanogenesis is primarily an anoxic process (Donis *et al*., 2017; Nayak *et al*., 2020; Struik *et al*., 2024). However, $CH_4$ production remained unexpectedly low in anoxic bottles after feed addition, with $CH_4$ production rates being even lower than those observed in the feed-free control, suggesting that the feed might contain components that inhibit methanogens. Firstly, the extruded feeds used in the experiment also contained $O_2$ (Hernández *et al*., 2014), which is gradually released as the pellets dissolve, potentially influencing microbial activity. It is also plausible that the low $CH_4$ production during incubation may be related to the presence of compounds with antimicrobial activity in the feed formulations. Considering that the microbial community is highly sensitive to antibiotics (Ni *et al*., 2023; Bollinger *et al*., 2024), the presence of antimicrobial components in the feeds could inhibit methanogens, interfering with methane production. The effectiveness of these compounds may vary depending on their concentration and spectrum of activity (Raza *et al.*, 2022). It is also important to consider that the sediment used may contain residual antibiotics from previous cultivation cycles, serving as a reservoir for these substances (Feng *et al*., 2022). The presence of antibiotic agents in aquaculture raises ecological and sanitary concerns, including the selection of resistant bacteria and potential disruptions to aquatic microbiota (Lima *et al.*, 2006; Zhou *et al*., 2021; Raza *et al.*, 2022). Another inhibitory factor could be antioxidants present in the tested feeds, such as ascorbic acid and tocopherol. These compounds, listed in varying proportions on product labels, are commonly used in fish feed to enhance preservation and immune function but can also act as antimicrobial agents, inhibiting bacterial growth (Elnagar *et al*., 2024; Hu *et al*., 2025; Sherif *et al*., 2024).

In contrast to $CH_4$ production, feed addition increased $CO_2$ production across all conditions. $CH_4$ can be oxidized to $CO_2$ by methanotrophic microorganisms, either aerobically using $O_2$ as an electron acceptor or anaerobically through alternative electron acceptors, depending on environmental availability (Guerrero-Cruz *et al*., 2021; Struik *et al*., 2024). Certain elements present in high concentrations in the water and feeds, such as sulfur (S) and phosphorus (P), may have influenced anaerobic $CH_4$ oxidation, also supporting the low $CH_4$ values and the elevated $CO_2$ levels observed. Sulfur, primarily in the form of sulfate ($SO_4^{2-}$), is a key electron acceptor in this process, enabling anaerobic methanotrophic archaea to convert $CH_4$ into $CO_2$ in symbiosis with sulfate-reducing bacteria (Fan *et al*., 2020; Struik *et al*., 2024). Phosphorus (P) is also linked to the methane cycle and may shape the methane-oxidizing bacterial community composition (Du *et al*., 2024; Nijman *et al*., 2022). The incubated water also contained high levels of nitrogen compounds, which could also serve as alternative electron acceptors for microbial communities (Du *et al.*, 2024; Chen *et al.*, 2022; Nijman *et al*., 2022).

Despite the observed increases in $CO_2$ concentrations following feed addition in our study, these emissions originate from carbon that was previously sequestered from the atmosphere by plants used as feed ingredients (Gephart *et al*., 2021; Yadava *et al*., 2024). As such, from a global carbon balance perspective, this release does not represent a net addition of $CO_2$ to the environment, but rather a re-emission within the short carbon cycle. In this cycle, carbon fixed via photosynthesis is subsequently returned to the atmosphere through organic matter decomposition, without significantly altering global carbon stocks. Nevertheless, the carbon footprint of feed production extends beyond this biogenic emission. The conversion of natural ecosystems into agricultural land for feed cultivation can release large quantities of long-stored carbon from soils and vegetation and diminish the capacity of these systems to function as carbon sinks (Gephart *et al*., 2021; Castilla-Gavilán *et al*., 2024). Therefore, while the $CO_2$ emissions observed in our experiment are part of a short cycle atmospheric loop, the indirect effects of feed production — particularly those related to land-use change — remain a major contributor to the overall carbon footprint of aquaculture (MacLeod *et al*., 2020).

While we observed no or only minimal $CH_4$ production attributable to fish feed, contrasting results were reported by Waldemer and Koschorreck (2023), who documented elevated methane emissions in areas containing uneaten feed. However, it is important to note that the studies were conducted under markedly different conditions. This study was carried out in a controlled laboratory environment, simulating a production system in a tropical climate and using sediments and residues derived from tilapia farming. In contrast, the previous study was conducted under field conditions in a temperate climate, involving aquaculture species with ecological habits distinct from those of tilapia. These methodological and ecological differences can significantly influence microbial dynamics, directly affecting methane production processes. Furthermore, the feeds used in the temperate-climate study had a higher protein content (Waldemer *et al*., 2024), than the the formulations used in the present study. This characteristic may be related to the observed emissions, especially considering that, among the feeds tested here, only Feed 1 — which had the highest protein content — resulted in methane emissions significantly higher than the control. Finally, the different rates observed by feed type may be linked to the nutritional composition (Klanjscek *et al*., 2011; Torres-Beristain *et al*., 2006). Previous research suggested that protein-rich feeds may result in lower $CO_2$ emissions (Klanjscek *et al*., 2011), which aligns with our findings.

A central consideration of this study is the extrapolation of ex situ results to field conditions. We used an *ex situ* approach to isolate the effect of feed composition on greenhouse gas emissions, which cannot be achieved *in situ* due to confounding environmental variables. Although the absolute emission rates reported are specific to our tropical regime and the feeds we used, the underlying biogeochemical mechanism is universal (Conant *et al*., 2011; Tang *et al*., 2017). Our results revealed clear contrasts in the decay dynamics of different feeds: nitrogen-rich feeds that consisted of smaller pellets decayed faster and driving $CO_2$ production than coarser and nitrogen-poorer feeds. Because $CO_2$ and $CH_4$ production rates are temperature dependent, and the decay rates of the same feed would likely be lower in cooler climatic zones (Moras *et al*., 2024). However, temperature primarily influences the magnitude of these processes rather than their relative patterns, as the differences observed among feeds arise from intrinsic physicochemical properties (Tang *et al*., 2017). Therefore, optimizing the physical stability and composition of the feed is a GHG mitigation strategy with global applicability.

## 5. Conclusion

Both $CO_2$ and $CH_4$ production associated with fish feed decomposition are highly dependent on environmental conditions. Our experimental design, which combined controlled *ex situ* incubations with *in situ* disintegration tests, allowed us to distinguish the intrinsic effect of feed properties from the context-dependent responses driven by pond biogeochemistry.

Our findings confirm the rapid disintegration of fish feeds, leading to nutrient release into the water column and GHG emissions. Notably, fish feed appears to contribute more to $CO_2$ production than $CH_4$, which is a positive result given the higher global warming potential of $CH_4$. However, in our settings, $CH_4$ production seems to be limited by components in the feed and deserve further research. From the carbon balance perspective, since feed production incorporates $CO_2$, its decomposition does not introduce additional $CO_2$ into the atmosphere. However, practitioners can minimize environmental impact by optimizing feed formulations and reducing excess feed to prevent leftovers.

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## CRediT authorship contribution statement

**Laís Coura Soranço:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Project administration, Supervision. **Jesper Wien:** Writing – review & editing, Validation, Formal analysis, Visualization. **Ana Paula Dalbem Barbosa:** Writing – review & editing, Resources, Data curation. **Anderson Machado de Melo Júnior:** Writing – review & editing, Investigation, Data curation. **Giovana Machado Cardoso:** Methodology, Investigation. **Vitor Luís da Cunha Duque:** Methodology, Investigation, Visualization. **Caique Rodrigues Soares:** Methodology, Investigation. **Gabrielle Quadra:** Writing – review & editing, Data curation. **Tarcila Silva:** Writing – review & editing. **Giovanni Resende de Oliveira:** Validation, Formal analysis. **Sarian Kosten:** Supervision. **Nathan Oliveira Barros:** Supervision, Conceptualization, Funding acquisition, Writing – review & editing.

## Declaration of competing interest

The authors declare that there are no financial or personal relationships that could have inappropriately influenced the work presented in this article.

## Data availability

All data, metadata, and tools used in this work are available with the corresponding author.

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## Ethics approval

This study did not require prior approval from the Ethics Committees on Animal Use, as the experiments conducted did not involve the handling of living organisms.

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## Supplementary Material


**Fig. S1:** Feed degradation experiment. (a) Surface view of the in-situ experiment; (b) schematic representation of a single replicate, illustrating the spatial organization of components, including feed placement, used to monitor breakdown.

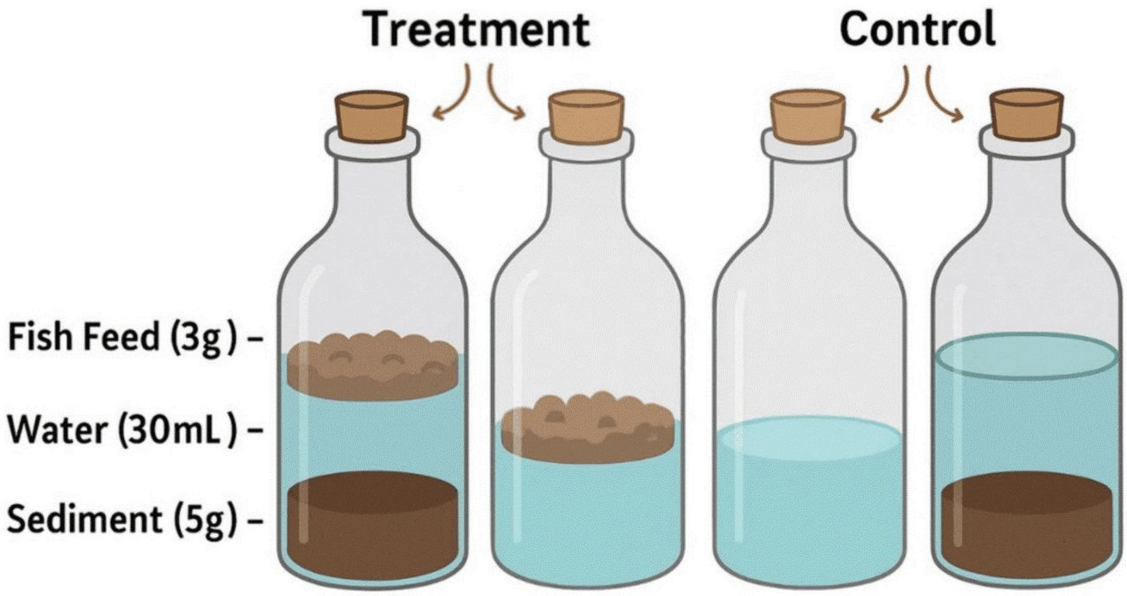


**Fig. S2:** Diagram illustrating the experimental treatments and controls used in the ex-situ trial. The greenhouse gas emission analysis experiment included treatments consisting of sediment, water, and fish feed, incubated under oxic and anoxic conditions.

**Table S1:** Water quality parameters measured over 72 hours in an experimental pond. Data for both the surface and bottom of the pond are presented. Measurements were taken every eight hours. Temperature (T); Dissolved oxygen (DO).

| Time | Surface | | | Bottom | | |
|---|---|---|---|---|---|---|
| | T (°C) | DO (mg/L) | pH | T (°C) | DO (mg/L) | pH |
| **0** | 30.5 | 6.1 | 4.3 | 30.9 | 5.0 | 4.0 |
| **8** | 29.6 | 5.6 | 4.5 | 30.3 | 4.9 | 4.2 |
| **16** | 28.5 | 5.8 | 4.4 | 28.6 | 5.3 | 4.3 |
| **24** | 29.1 | 5.4 | 4.1 | 29.0 | 5.1 | 3.9 |
| **32** | 28.2 | 5.7 | 4.4 | 28.4 | 5.8 | 4.3 |
| **40** | 28.8 | 5.6 | 4.2 | 28.9 | 4.9 | 4.1 |
| **48** | 28.6 | 5.7 | 4.4 | 28.2 | 5.7 | 4.2 |
| **56** | 29.7 | 5.1 | 4.6 | 27.9 | 4.9 | 4.3 |
| **64** | 28.5 | 5.8 | 4.9 | 27.9 | 5.5 | 4.6 |
| **72** | 31.2 | 5.3 | 5.5 | 30.9 | 5.1 | 5.1 |

**Table S2:** Elemental composition of the initial sediment and feed used in the ex-situ experiment, determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Fischer Scientific, Bremen, Germany).

| Elemental content (ppm) | Sediment | Feed 1 | Feed 2 | Feed 3 |
|---|---|---|---|---|
| **Aluminum (Al)** | 56.0 | 0.4 | 0.4 | 0.8 |
| **Calcium (Ca)** | 1.1 | 36.7 | 53.7 | 17.4 |
| **Iron (Fe)** | 88.6 | 1.1 | 0.9 | 0.9 |
| **Potassium (K)** | 0.7 | 7.6 | 15.1 | 21.2 |
| **Magnesium (Mg)** | 0.7 | 2.4 | 4.9 | 6.3 |
| **Manganese (Mn)** | 0.4 | 0.1 | 0.2 | 0.1 |
| **Sodium (Na)** | 0.1 | 8.2 | 12.0 | 8.3 |
| **Phosphorus (P)** | 1.4 | 24.9 | 21.4 | 15.4 |
| **Sulfur (S)** | 0.4 | 13.2 | 5.4 | 4.5 |
| **Silicon (Si)** | 0.9 | 1.3 | 0.5 | 0.6 |
| **Zinc (Zn)** | 0.1 | 0.3 | 0.2 | 0.2 |

# CHAPTER III: Partitioning of dissolved and particulate nutrient pathways reveals species-specific controls in freshwater aquaculture

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## Abstract

Understanding how nutrients are partitioned between dissolved and particulate pathways is essential for predicting the environmental impacts of aquaculture systems. This study investigated the short-term dynamics of carbon (C), nitrogen (N), and phosphorus (P) in freshwater experimental tanks containing Nile tilapia (*Oreochromis niloticus*), grass carp (*Ctenopharyngodon idella*), and a control. Nutrient inputs from feed, particulate outputs via feces, and dissolved concentrations in the water column were quantified over a 24 h period. Particulate export accounted for a minor fraction of total nutrient fluxes, indicating that most ingested material was retained and internally processed. Dissolved pathways dominated nutrient dynamics, with rapid accumulation of inorganic nitrogen (especially ammonia) and phosphorus in fish treatments, particularly in Nile tilapia. In contrast, dissolved organic carbon remained relatively stable, suggesting tight coupling between carbon release and respiration. Nutrient export patterns differed among elements. Nitrogen exhibited consistently high export across treatments, reflecting its high mobility in dissolved forms. Phosphorus showed intermediate behavior, with partial retention in fish treatments. Carbon export was significantly lower in the presence of fish, indicating enhanced assimilation and respiratory losses. Species-specific effects were evident, with Nile tilapia promoting greater nutrient accumulation and stronger changes in water quality than grass carp, although carbon export was primarily driven by biomass. These findings show that short-term nutrient dynamics in aquaculture systems are governed mainly by internal transformation processes rather than particulate export, highlighting the importance of dissolved pathways and species-specific metabolism in shaping environmental impacts.

Keywords: Aquaculture, Nutrient cycling, Dissolved nutrients, Particulate organic matter

## 1. Introduction

Fish production has become increasingly central to global food supply systems, particularly in light of population growth and the rising demand for nutritious protein sources. In this context, aquaculture has emerged as a key contributor to meeting global seafood demand and is widely regarded as an effective means of addressing the imbalance between supply and consumption of aquatic products (Shi, 2023; Subasinghe *et al*., 2009). In 2022, global aquaculture output reached 94.4 million tonnes, representing a 7.6% increase relative to 2020, and projections indicate continued expansion to approximately 205 million tonnes by 2032 (FAO, 2024). This growth underscores the economic significance of the sector, which is currently valued at approximately USD 313 billion, and highlights its greater cost efficiency relative to land-based livestock protein production (FAO, 2024).

In freshwater systems, aquaculture production is concentrated in a limited number of species that account for a substantial proportion of global output. Carps and tilapias dominate global aquaculture, with grass carp (*Ctenopharyngodon idella*) as the most produced species and Nile tilapia (*Oreochromis niloticus*) also representing a major share of global production (FAO, 2024). These species are predominantly cultivated in pond-based systems, where formulated feeds and fertilizers are the primary source of nutrients, shaping the dynamics of production and nutrient cycling (Dalbem Barbosa *et al*., 2024).

Despite its strategic importance for global food security and economic development, aquaculture is also associated with environmental challenges that must be addressed to ensure long-term sustainability. Among the main challenges is the excessive input of nutrients from feed and fertilizers, excretion and egestion by cultured fish, with nitrogen and phosphorus being the primary elements released (Munguti *et al*., 2021; Nathanailides *et al*., 2023; Dalbem Barbosa *et al*., 2024). The accumulation of these compounds in the water column and sediment can increase concentrations of nitrogenous and phosphorus forms, promote excessive microalgal growth and impair the performance of cultured species, mainly due to reductions in dissolved oxygen levels (Coldebella *et al*., 2018; Guan *et al*., 2026).

Feces represent the main fraction of organic waste generated in aquaculture systems, and their production is directly influenced by both feeding rates and feed quality. As fish biomass increases throughout the production cycle, the release of organic wastes intensifies, enhancing microbial decomposition processes and nitrogenous excretion, which in turn elevate the availability of key nutrients such as carbon, phosphorus, and nitrogen, thereby altering the biogeochemical dynamics of aquaculture environments (Sarà, 2007; Rosini *et al*., 2019).

Furthermore, differences in feeding strategy and digestive physiology among fish species can influence both the quantity and form of waste produced (NRC, 2011). Herbivorous species such as grass carp typically exhibit lower digestibility, resulting in lower nutrient assimilation and greater loss of ingested material (Cui *et al*., 1992; Kurniasih *et al*., 2024). Consequently, a greater fraction of ingested nutrients may be released as particulate waste through egestion. Whereas omnivorous species such as Nile tilapia, which generally exhibit higher digestive efficiency and nutrient assimilation, may release a greater proportion of nutrients in dissolved forms through excretion (Neto & Ostrensky, 2013; Kurniasih *et al.*, 2024).

Therefore, this study aims to (1) Quantify the deposition of nitrogen, phosphorus, and carbon associated with fish feces, assessing the contribution of particulate organic matter in closed-pond aquaculture systems; (2) Determine the concentration and dynamics of dissolved forms of nitrogen, phosphorus, and carbon in the water column resulting from fish metabolic excretion and the decomposition of organic residues; (3) Provide insights into the environmental impacts of aquaculture intensification, contributing to the development of more sustainable feeding and environmental management strategies.

Based on these objectives, we hypothesize that: Fish feces are a major pathway for the deposition of particulate nitrogen, phosphorus, and carbon in aquaculture systems, with grass carp (*C. idella*) contributing a relatively larger particulate fraction due to its lower digestive efficiency; Dissolved forms of nitrogen, phosphorus, and carbon in the water column are primarily driven by fish metabolic excretion and dominate short-term nutrient dynamics, with Nile tilapia (*O. niloticus*) promoting greater dissolved nutrient accumulation than grass carp; The combined accumulation of particulate and dissolved nutrients enhances internal nutrient loading, increasing the potential for environmental impacts such as eutrophication in aquaculture systems.

## 2. Methods

*2.1. Experiment setup*

To evaluate the contributions of feces and the water column as sources, three experimental treatments were established: adult grass carp (*C. idella*), adult Nile tilapia (*O. niloticus*), and a control (no fish). Each treatment was conducted in triplicate, and the fish treatments contained 10 individuals (n = 60) (**Figure 1**). The fish used in this experiment originated from a previous study that monitored a full aquaculture production cycle to evaluate greenhouse gas emissions. After the completion of that experiment, individuals were maintained under quarantine conditions prior to the start of the present study. Before the experiment, all fish underwent an acclimation period for one day, during which body length and weight were measured. Fish were maintained for 24 h in 1 $m^3$ tanks filled with local stream water and equipped with an air-compression aeration system, with no water renewal during the exposure period. The tanks were covered with mesh to allow gas exchange while preventing fish escape. They were also equipped with a false bottom consisting of a mesh grid placed near the tank bottom to avoid contact between the fish and the underlying material. Fish were fed to apparent satiation twice (at 0h and 4h), eating the total amount offered, without leftovers.

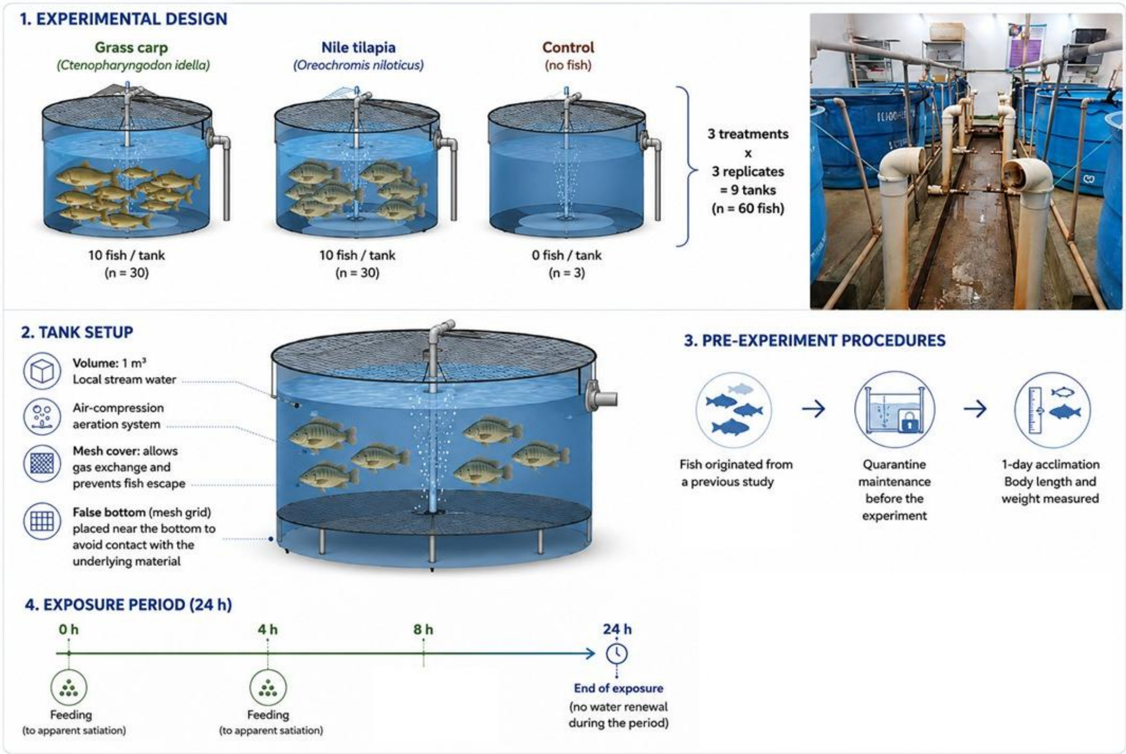


**Figure 1:** Experimental design showing the three treatments (grass carp, Nile tilapia, and control), tank configuration, and exposure timeline. Each treatment was conducted in triplicate using 1 m³ tanks. Fish treatments contained 10 adult fish per tank. Tanks were operated under static-water conditions with aeration, mesh covers, and a false-bottom mesh grid. Fish were acclimated for one day before the 24h experiment and fed to apparent satiation at 0, and 4h.

*2.2. Water quality characterization*

Water quality variables were monitored at four time points throughout the experiment: at the beginning of the assay (0 h), after 4, 8, and 24 h. This sampling design was adopted because nitrogen excretion in fish is not constant, but rather occurs in postprandial pulses, typically increasing within the first hours after feeding and peaking between approximately 4 and 10 h, depending on species and environmental conditions (Ott *et al*., 2025). At each sampling time, water temperature (°C), pH, turbidity (NTU), conductivity (µS $cm^{-1}$), and dissolved oxygen were measured with a multiparameter probe (YSI Pro DSS4 port digital), allowing a temporal assessment of environmental conditions within the system.

Water samples were collected from each sampling time. Total nitrogen (TN) was quantified using the sodium hydroxide (NaOH) digestion method (APHA, 2017; Crumpton *et al*., 1992). Nitrate ($NO_3^-$-N) was measured after sulfuric acid addition (Crumpton *et al*., 1992). Nitrite ($NO_2^-$-N) was quantified via the Colorimetric Method (4500-$NO_2^-$; APHA, 2017). Ammonia ($NH_3$-N) was measured by flow injection analysis (4500-$NH_3$; APHA, 2017). Total phosphorus (TP) was quantified using the persulfate digestion method (APHA, 2017), and orthophosphate ($PO_4^{3-}$-P) was quantified following the ascorbic acid method (4500-P; APHA, 2017). Nutrient concentrations were determined using a PerkinElmer® LAMBDA 365 UV-Vis spectrophotometer and calculated from calibration curve equation. Total organic nitrogen (TON) was calculated as the difference between TN and the sum of $NO_2^-$-N, $NO_3^-$-N, and $NH_3$-N. Total solids (TS) and total suspended solids (SS) were determined gravimetrically at 103–105 °C (2540-B; 2540-D; APHA, 2017). The concentration of non-purgeable organic carbon (NPOC) was measured following Sugimura & Suzuki (1988) using a TOC-L analyzer (Shimadzu).

### *2.3. Fish feed and feces characterization*

At the end of the experimental period, the composition of fish feces and feed was determined. The moisture content of solid material was estimated from mass loss after drying at 100°C for 2 h. Organic matter (OM) content was assessed by loss on ignition at 500°C for 4 h (Goldin, 1987). Elemental composition was analyzed after extraction using inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Fisher Scientific, Bremen, Germany). Total carbon (TC), Total phosphorus (TP), and total nitrogen (TN) content were quantified using a CNS elemental analyzer (Vario MICRO cube; Elementar Analysensysteme, Langenselbold, Germany).

### *2.4. Data analysis*

For the biometric data, consistency among experimental replicates within each treatment was first assessed by testing variation among tanks within each treatment. Residual normality and homogeneity of variances were assessed using the Shapiro–Wilk and Levene tests, respectively, using *stats* and *car* packages (Fox & Weisberg, 2019). When these assumptions were met, differences among replicates were evaluated using one-way analysis of variance (ANOVA). When the assumptions were violated, the nonparametric Kruskal–Wallis test was applied. These analyses verified whether the experimental tanks behaved similarly, thereby supporting their use as true replicates in subsequent analyses.

Differences in water quality parameters among treatments were evaluated using linear mixed-effects models (LMMs) fitted with the *lme4* and *lmerTest* packages (Bates *et al*., 2015; Kuznetsova *et al*., 2017). Water temperature, dissolved oxygen, electrical conductivity, pH, turbidity, dissolved nutrient concentrations ($NO_3^-$-N, $NO_2^-$-N, $NH_3$-N, TON, TN, $PO_4^{3-}$-P, TP, NPOC), total and suspended solids (TS and SS), treatment (Nile tilapia, carp, and control), time (hours) were included as fixed effects, and tank was included as a random intercept. To disentangle species and body size effects, a three-step modeling approach was applied. A baseline model including treatment and time was first fitted, followed by an alternative model including mean weight per experimental unit (mean-centered) as an additional covariate. The models were compared using likelihood ratio tests under maximum likelihood estimation, and mean weight was retained only when it significantly improved model fit. Model assumptions were verified by inspection of residuals. When significant effects were detected, the model was evaluated using Type III analysis of variance with the *Anova*() function from the *car* package (Fox *&* Weisberg, 2019). Post hoc pairwise comparisons were conducted using the emmeans package with *emmeans*() to assess differences among treatments (Lenth, 2024).

The solid fraction was analyzed separately to evaluate treatment effects on particulate organic matter and solid-phase carbon, nitrogen, and phosphorus associated with feed inputs and fecal outputs. Nutrient concentration of initial and final water was evaluated using Student's T-tests after verifying the normality of the initial–final differences. In addition, the influence of treatment and mean fish weight on the exported solid fraction was assessed using analysis of covariance (ANCOVA), with treatment as a fixed factor and mean weight as a continuous covariate. Models included the interaction term between treatment and weight, and effects were evaluated using Type III sums of squares. In the absence of significant interactions, main effects were interpreted under a common-slope framework.

Finally, the overall nutrient balance of the system was assessed considering the combined solid (feed or fecal material) and dissolved fractions in the water column. Differences in nutrient inputs (feed and initial water) among treatments were evaluated at the tank level. After visual inspection of the data and assessment of residual normality and homogeneity of variances using the Shapiro–Wilk and Levene tests, respectively, differences among treatments were analyzed using the Kruskal–Wallis test, followed by Dunn's post hoc test with Bonferroni correction when appropriate. Potential differences in treatment outputs were performed using one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) test.

Within each treatment, nutrient inputs and outputs (fecal material and final water) were compared to evaluate short-term nutrient retention or release using paired t-tests or Wilcoxon signed-rank tests depending on whether the normality assumption (Shapiro–Wilk test) was met. Differences among treatments in excreted nutrient fractions were evaluated using ANOVA, with pairwise comparisons performed using Tukey's HSD test when significant effects were detected. The influence of fish weight on nutrient excretion was assessed using ANCOVA, with the excreted fraction as the response variable, mean weight per tank as a continuous covariate, and treatment as a fixed factor. Models included the interaction between weight and treatment and were evaluated using Type III sums of squares. In the absence of significant interactions, treatment and weight effects were interpreted under a common-slope framework.

All statistical analyzes and graphics were produced in R version 4.5.1 (R Core Team, 2025).

### *2.5. Ethics Committee on the Use of Animals*

All procedures involving the maintenance and handling of fish were approved by the Animal Ethics Committees (Comitê de Ética em Uso Animal - CEUA) of the State University of Mato Grosso (UNEMAT) under Opinion No. 004/2021 (Biogeochemical balance in tropical aquaculture: implications for ecosystem transformations and climate change in water resources) and the Agricultural Research Company of Minas Gerais (EPAMIG) under Protocol No. 16/2022 (Development dynamics of different fish species in aquaculture systems in the state of Minas Gerais).

## 3. Results

### *3.1. Fish biometry*

Fish biometric data were consistent within treatments, with no significant differences among experimental replicates ($p > 0.05$; ANOVA/Kruskal–Wallis), supporting their use as true replicates in subsequent analyses. Nile tilapia had a higher mean body mass (592.7 ± 104.2 g) than grass carp (290.2 ± 231.2 g), reflecting interspecific differences in size. Nile tilapia also exhibited greater mean body height (10.6 ± 0.8 cm) and head length (8.7 ± 0.5 cm) than grass carp (5.3 ± 1.2 cm and 6.8 ± 1.4 cm, respectively). Total and partial lengths followed similar patterns, with slightly higher average values in Nile tilapia (30.9 ± 5.6 cm and 27.0 ± 1.5 cm) than in grass carp (29.4 ± 6.9 cm and 24.8 ± 5.7 cm).

*3.2. Temporal dynamics of physico-chemical water parameters*

Physical and chemical conditions remained relatively stable throughout the experimental period, with some parameters showed significant temporal and treatment-related variation. Water temperature did not differ significantly among treatments ($p > 0.05$; LMM), although all groups exhibited significant temporal variation over the 24 h period ($p < 0.05$; LMM) (**Figure 2A**). The lowest dissolved oxygen value was recorded in Nile tilapia treatment at 8 h (1.5 mg $L^{-1}$) (**Figure 2B**). Significant temporal variation was detected only in Nile tilapia treatment, which showed reduced oxygen concentration at 4 h (4.4 mg $L^{-1}$) and 8 h (3.3 mg $L^{-1}$) compared with the control at 4h (8.7 mg $L^{-1}$) and 8h (8.5 mg $L^{-1}$) ($p < 0.05$; LMM).

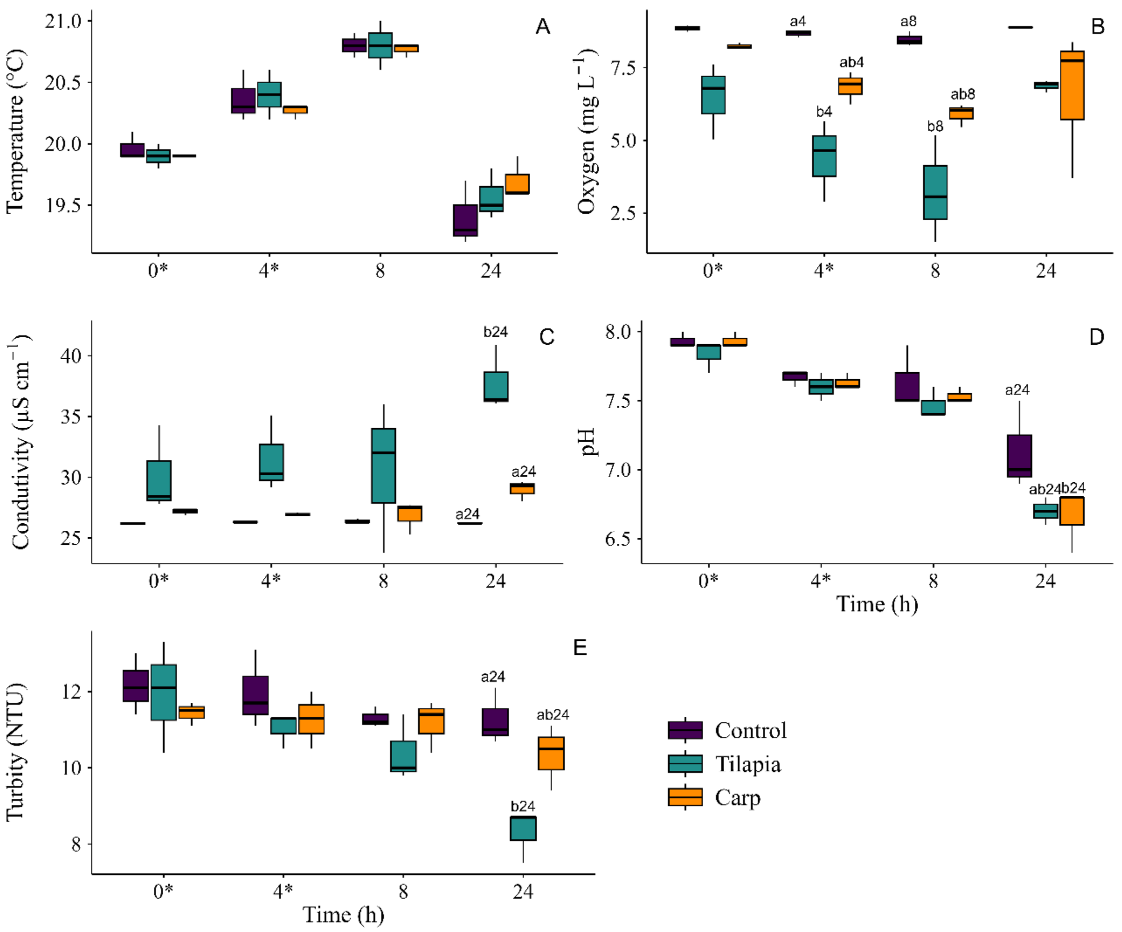


**Figure 2:** Temporal variation in physical and chemical water quality parameters during the experimental period in tilapia, grass carp, and control treatments. Panels show changes in water temperature (°C) (**A**), dissolved oxygen concentration (mg $L^{-1}$) (**B**), electrical conductivity (µS $cm^{-1}$) (**C**), pH (**D**), and turbidity (NTU) (**E**) measured at 0, 4, 8, and 24 h. Error bars indicate standard deviation. Different lowercase

letters indicate statistically significant differences among treatments at the same sampling time ($p < 0.05$). Statistical analyses were performed using linear mixed-effects models. *Fish were fed immediately before measurements at 0 and 4 h.

Conductivity increased significantly over time in Nile tilapia treatment ($p < 0.05$; LMM) and differed among treatments at 24 h, with higher mean values in Nile tilapia (37.8 $\mu S\ cm^{-1}$) compared with carp (28.9 $\mu S\ cm^{-1}$) and control (26.2 $\mu S\ cm^{-1}$) ($p < 0.05$; LMM; **Figure 2C**). Water pH decreased significantly over time in all treatments ($p < 0.05$; LMM), declining by approximately 2 pH units from 0 to 24 h. At 24 h, grass carp treatment had significantly lower mean pH values (6.7) than the control (7.1) ($p < 0.05$; LMM; **Figure 2D**). Turbidity decreased over time in Nile tilapia treatment from 12 NTU at 0 h to 8.3 NTU at 24 h ($p < 0.05$; LMM), while at 24 h, control exhibited significantly higher turbidity (mean = 11.3 NTU) than Nile tilapia treatment ($p < 0.05$; LMM; **Figure 2E**).

*3.3. Temporal dynamics of dissolved nutrients*

Nitrogen concentrations in the water column showed distinct temporal patterns among the evaluated forms. Mean $NO_2^-$-N concentrations remained consistently low across treatments (**Figure 3A**), particularly relative to other nitrogen forms, with no significant variation over time or among treatments ($p > 0.05$; LMM), although models including mean fish weight provided a better fit ($\chi^2 = 6.06$, df = 1, $p = 0.01$), indicating a measurable negative influence of biomass. In contrast, $NO_3^-$-N concentrations increased significantly over time in fish treatments ($p < 0.05$; LMM; **Figure 3B**), and this pattern was better explained by models that included mean fish weight ($\chi^2 = 5$, df = 1, $p = 0.03$), indicating a measurable positive influence of biomass. $NH_3$-N accumulated over time in all treatments ($p < 0.05$; LMM), with the strongest increase observed in the Nile tilapia treatment (**Figure 3C**). At 24h, ammonia concentrations differed significantly among all treatments, following the pattern Nile tilapia > grass carp > control ($p < 0.05$; LMM), with mean values of 509.3, 157.2, and 18.6 $\mu g\ L^{-1}$.

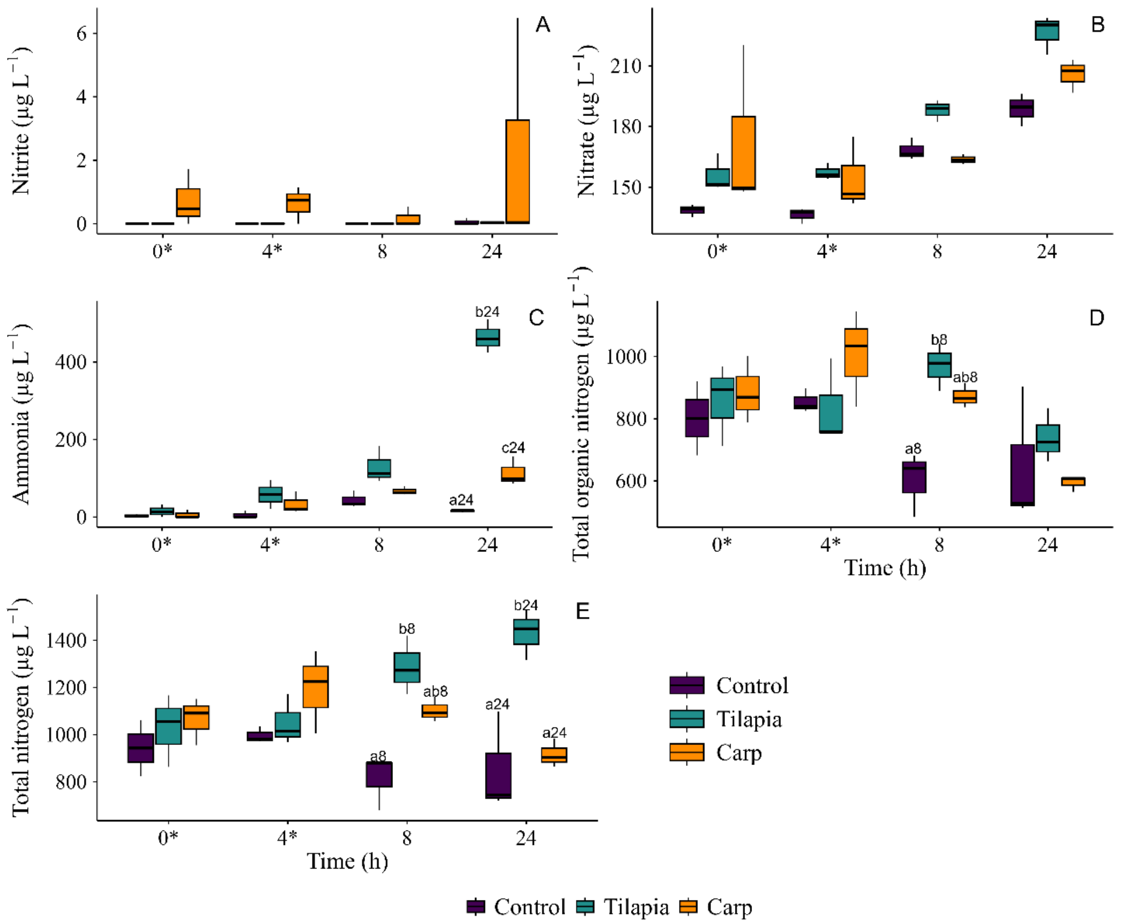


**Figure 3:** Temporal variation in dissolved nitrogen forms in the water column during the experimental period in Nile tilapia, grass carp, and control treatments. Panels show changes in nitrite ($NO_2^-$-N) (**A**), nitrate ($NO_3^-$-N) (**B**), ammonia ($NH_3$-N) (**C**), total organic nitrogen (TON) (**D**), and total nitrogen (TN) (**E**) measured at 0, 4, 8, and 24 h. Error bars indicate standard deviation. Different lowercase letters indicate statistically significant differences among treatments at the same sampling time ($p < 0.05$). Statistical analyses were performed using linear mixed-effects models. *Fish were fed immediately before measurements at 0 and 4 h.

TON and TN showed limited temporal variation, although some significant differences were detected in specific treatments and sampling times ($p < 0.05$; LMM; **Figure 3D** and **3E**). Carp treatment initially exhibited the highest TN values, followed by a slight decline over time, whereas tilapia treatment showed an increase after 4 h. For TN, concentrations in tilapia treatment were significantly higher than in the control at 8 h and higher than both the control and grass carp treatments at 24 h. In addition, TN in tilapia treatment significantly increased from 0 to 24 h. TON showed a broadly similar to TN. In general, nitrogen concentrations in tilapia treatment increased initially but declined after 8 h, whereas in carp treatment TON significantly decreased from 4 to 24 h. A significant difference among treatments was detected at 8 h, when TON concentrations were higher in tilapia than in control. The control consistently showed the lowest concentrations of $NO_3^-$-N, $NO_2^-$-N, $NH_3$-N, TON, and TN throughout the experiment.

Model comparisons indicated that including mean fish weight significantly improved model fit for $PO_4^{3-}$-P ($\chi^2 = 4.78$, df = 1, $p = 0.03$) and TP ($\chi^2 = 4.21$, df = 1, $p = 0.04$), highlighting the positive influence of body size on phosphorus dynamics. $PO_4^{3-}$-P and TP increased significantly over time in tilapia treatment ($p < 0.05$; LMM), while no significant temporal changes were detected in carp and control treatments ($p > 0.05$; LMM; **Figure 4A, B**). In tilapia treatment, $PO_4^{3-}$-P concentrations increased from approximately 15.7 µg $L^{-1}$ at 0 h to values exceeding 55.5 µg $L^{-1}$ at 24 h, whereas TP rose from approximately 29.9 µg $L^{-1}$ to 109 µg $L^{-1}$ at the end of the experimental period.

At 24 h, significant differences among treatments were observed for both $PO_4^{3-}$-P and TP ($p < 0.05$; LMM), with Nile tilapia ($PO_4^{3-}$-P: 46.3 µg $L^{-1}$, TP: 86 µg $L^{-1}$) showing higher concentrations than the grass carp ($PO_4^{3-}$-P: 21.2 µg $L^{-1}$, TP: 36.5 µg $L^{-1}$) and control treatments ($PO_4^{3-}$-P: 16.8 µg $L^{-1}$, TP: 25 µg $L^{-1}$). However, after accounting for mean fish weight, the difference between tilapia and carp was no longer significant ($p > 0.05$; LMM). NPOC, TS, and SS did not vary significantly among treatments or over time during the experimental period ($p > 0.05$; LMM; **Figure 4C**). Mean NPOC values averaged across the experimental period were 2.3, 1.2, and 2.4 mg $L^{-1}$ in the control, carp, and tilapia treatment, respectively. In the same order, mean TS values were 28.8, 32.8, and 25.8 mg $L^{-1}$, and mean SS values were 9.3, 13.2, and 10.7 mg $L^{-1}$.

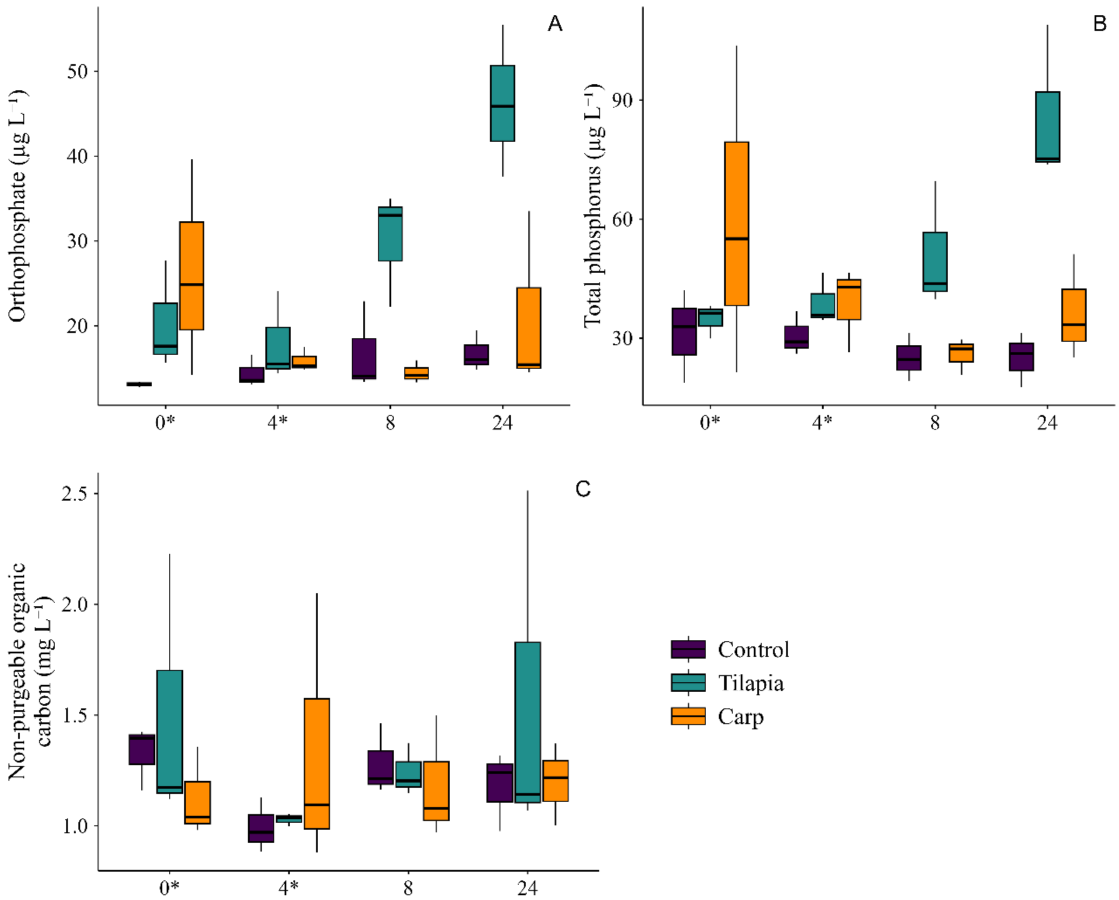


**Figure 4:** Temporal variation in dissolved phosphorus and organic carbon in the water column during the experimental period in tilapia, grass carp, and control treatments. Panels show changes in orthophosphate ($PO_4^{3-}$-P) (**A**), total phosphorus (TP) (**B**), and non-purgeable organic carbon (NPOC) (**C**) measured at 0,

4, 8, and 24 h. Error bars indicate standard deviation. Statistical analyses were performed using linear mixed-effects models. *Fish were fed immediately before measurements at 0 and 4 h.

*3.4. Composition and export of particulate nutrients*

The nutrients associated with feed inputs differed significantly between Nile tilapia and grass carp treatments for all evaluated parameters ($p < 0.05$; Student T-test). TC inputs ranged from 1.5 to 9.9 g, TN from 0.2 to 1.2 g, TP from 0.09 to 0.55 mg, and organic matter from 3.1 to 19.9 g across treatments. Nile tilapia treatment exhibited higher input values (TC: 7.1 g, TN: 836.3 mg, TP: 0.4 mg, OM: 14.3 g) than the carp (TC: 1.8 g, TN: 209.1 mg, TP: 0.1 mg, OM: 3.6 g) ($p < 0.05$; Student T-test) (**Figure 5**). The composition of the solid fraction associated with fecal outputs also differed between species for most parameters. TC, TN, and OM outputs varied significantly between Nile tilapia (TC: 1.3 g, TN: 73.2 mg, OM: 3.6g) and grass carp (TC: 0.3g, TN: 21.5 mg, OM: 0.8 g) ($p < 0.05$; Student T-test), whereas TP output did not differ significantly between treatments ($p > 0.05$; Student T-test) (**Figure 5**). Comparisons between feed inputs and fecal outputs indicated that, for all nutrients and treatments, input loads were significantly higher than output loads ($p < 0.05$; Paired T-tests). The exported fraction of organic matter, carbon, nitrogen, and phosphorus was not significantly affected by fish weight or treatment ($p > 0.05$; ANCOVA).

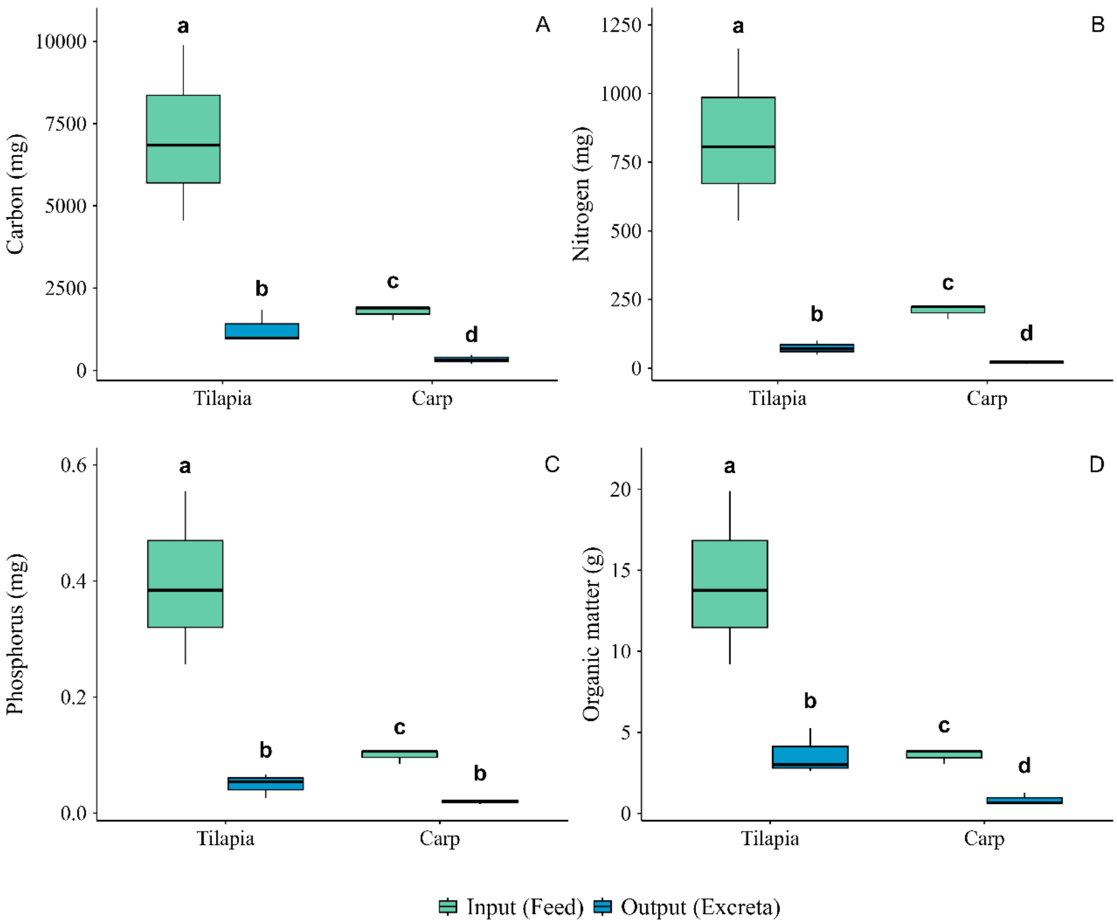


**Figure 5:** Input and output loads of solid-phase organic matter and nutrients in Nile tilapia and grass carp treatments. Panels show carbon (C) (**A**), nitrogen (N) (**B**), phosphorus (P) (**C**), and organic matter (OM)

(**D**) associated with feed inputs and fecal outputs. Error bars indicate standard deviation. Different lowercase letters indicate statistically significant differences among treatments at the same sampling time ($p < 0.05$). Statistical analyses were performed using Student and paired T-tests.

### *3.5. Nutrient partitioning and short-term system balance*

Absolute nutrient inputs and outputs differed among treatments (**Figure 6**). For nutrient inputs, significant differences among treatments were detected for TC, TN, and TP ($p < 0.05$; Kruskal–Wallis). Post hoc tests revealed that, for all three elements, tilapia treatment had significantly higher input loads than the control ($p < 0.05$; Dunn), while no significant differences were detected between carp and control treatments ($p > 0.05$; Dunn). For nutrient outputs, TP loads associated with dissolved and solid fractions did not differ significantly among treatments ($p = 0.07$; ANOVA), although a consistent trend of higher values in the Nile tilapia treatment was observed. In contrast, carbon and nitrogen outputs differed significantly among treatments ($p < 0.05$; ANOVA). Post hoc tests indicated significant differences between tilapia and carp and between tilapia and control ($p < 0.05$; Tukey), while no significant differences were detected between grass carp and control ($p > 0.05$; Tukey).

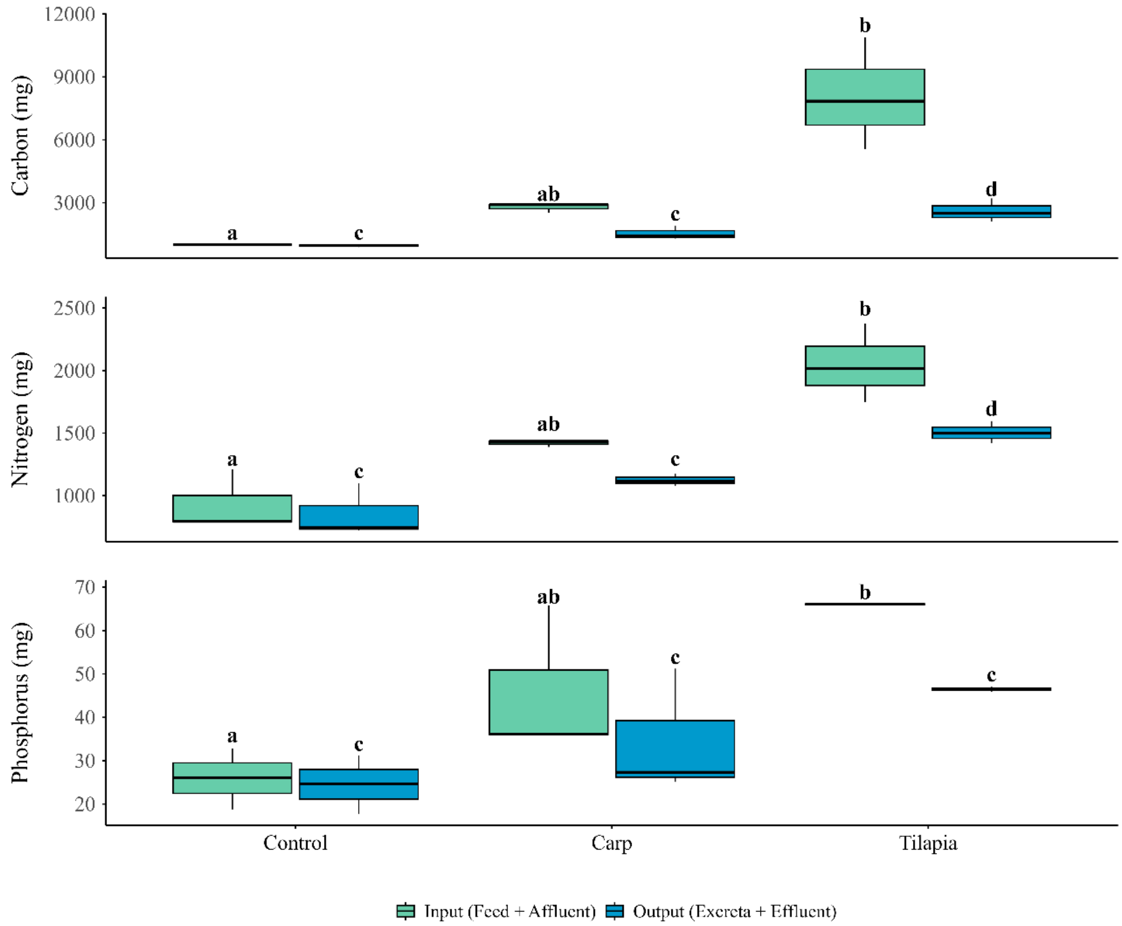


**Figure 6:** Input (feed + affluent) and output (excreta + effluent) loads of carbon (C), nitrogen (N), and phosphorus (P) in control, grass carp, and tilapia treatments. Panels show total carbon (TC), total nitrogen (TN), and total phosphorus (TP) loads (mg) associated with feed and water inputs (initial water) and fecal and water outputs (final water). Error bars indicate standard deviation. Different lowercase letters indicate

statistically significant differences among treatments ($p < 0.05$), as determined by ANOVA/Kruskal–Wallis followed by Tukey's or Dunn's post hoc tests.

Paired comparisons between nutrient inputs and outputs within each treatment revealed consistent differences in nutrient retention patterns. In the carp treatment, nutrient input was significantly higher than output for TC, TN, and TP ($p < 0.05$; Paired t-tests). In Nile tilapia treatment, inputs were significantly higher than outputs for TN and TP ($p < 0.05$; Paired t-tests), whereas carbon showed only a tendency toward higher input values ($p = 0.05$; Paired t-tests). In the control treatment, input loads were significantly higher than output loads for TP ($p < 0.05$; Paired t-tests) and TN ($p = 0.05$; Paired t-tests), whereas carbon inputs and outputs did not differ significantly ($p > 0.05$; Paired t-tests), indicating similar loads at the initial and final water.

Nutrient balance analyses integrating feed input with dissolved and solid outputs revealed distinct patterns of nutrient export among treatments (**Figure 7**). For TC, mean exported fractions were highest in the control treatment (93.9 ± 5.2%), followed by grass carp (55.7 ± 10.7%) and Nile tilapia (33.7 ± 9.7%). Analysis of variance indicated a highly significant treatment effect ($p < 0.05$; ANOVA). Post hoc comparisons revealed significant differences between both fish treatments and the control ($p < 0.05$; Tukey), while no significant difference was detected between Nile tilapia and grass carp ($p = 0.051$; Tukey). In addition, ANCOVA showed a trend toward an association between body mass and the exported fraction of TC ($p = 0.057$), while no significant interaction between biomass and treatment was detected.

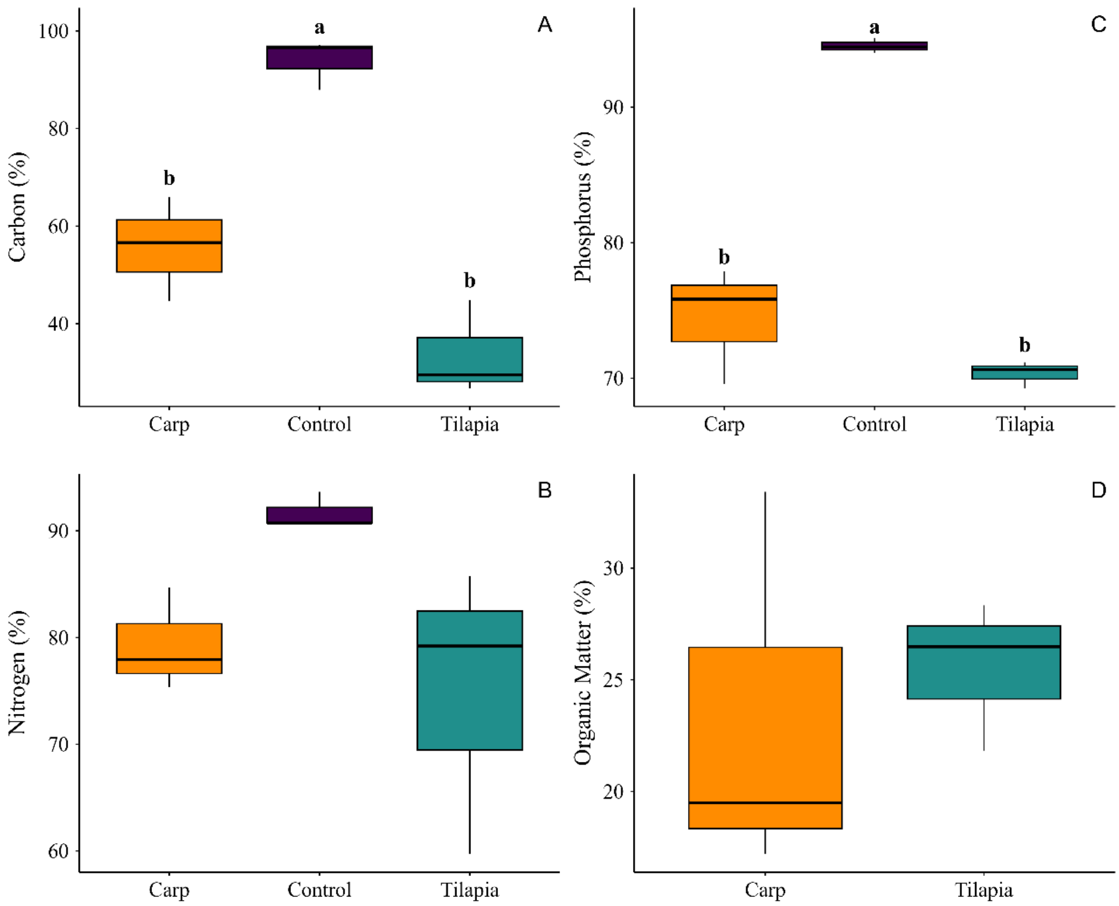

**Figure 7:** Exported fractions of carbon (C)(**A**), nitrogen (N)(**B**), phosphorus (P)(**C**), and organic matter (OM) (**D**) in control, grass carp, and tilapia treatments. Values represent the proportion of each nutrient exported relative to total inputs, considering dissolved and solid outputs. Error bars indicate standard deviation. Different lowercase letters indicate statistically significant differences among treatments ($p < 0.05$), as determined by ANOVA followed by Tukey's post hoc test.

TN exhibited high exported fractions in all treatments, with mean values of 94.5 ± 0.6% in the control, 74.4 ± 4.3% in grass carp, and 70.3 ± 1% in Nile tilapia. No significant treatment effect was detected for TN export ($p > 0.05$; ANOVA). Likewise, OM excretion did not differ significantly among treatments ($p > 0.05$; ANOVA), with mean excreted fractions of 25.5 ± 3.4% for tilapia and 23.4 ± 8.8% for carp. TP exports differed significantly among treatments ($p < 0.05$; ANOVA). Mean exported fractions were highest in control (94.5 ± 0.6%), followed by grass carp (74.4 ± 4.3%) and Nile tilapia (70.3 ± 1.0%). Post hoc test indicated significant differences between both fish treatments and the control ($p < 0.05$; Tukey), whereas no significant difference was detected between Nile tilapia and grass carp. No significant effects of mean weight on the exported fractions of TN, TP, and OM were observed ($p > 0.05$; ANCOVA).

## 4. Discussion

Understanding how nutrients are partitioned between dissolved and particulate pathways is central to predicting the environmental impacts of aquaculture systems. Our results suggest that, over the 24-hour observation period, nutrient dynamics were primarily driven by internal processing rather than direct export, with a substantial proportion of carbon, nitrogen, and phosphorus apparently being retained and rapidly transformed within the system. This internal cycling was primarily reflected in the accumulation of dissolved nutrients in the water column, whereas particulate outputs accounted for a comparatively small fraction of total nutrient fluxes. Notably, this dynamic was strongly modulated by fish species: Nile tilapia treatments exhibited higher total nutrient inputs, faster accumulation of nitrogen and phosphorus compounds, lower dissolved oxygen concentrations, and increased turbidity, whereas grass carp showed more moderate nutrient accumulation and environmental responses. These species-specific differences highlight the role of physiological and feeding traits in regulating nutrient cycling, indicating that the ecological footprint of aquaculture systems may be more closely linked to metabolic excretion and transformation processes than to the direct release of particulate wastes.

Biometric parameters were consistent among experimental replicates, indicating adequate experimental control and supporting their use as independent units in subsequent analyses. As expected, Nile tilapia exhibited higher body mass and more robust body dimensions than grass carp, reflecting species-specific growth patterns (Khalid & Naeem, 2017; Carvalho *et al*., 2022). Nile tilapia is widely recognized for its rapid growth and high weight gain in aquaculture systems (Rodríguez-Hernández *et al*., 2025). Because fish weight can influence metabolic activity and nutrient fluxes in aquaculture systems, it was incorporated as a covariate in the statistical analyses to distinguish biomass-related effects from species-specific responses (Barton *et al*., 2023). In most cases, species identity explained the observed patterns, while fish weight significantly affected only a few nutrients in the water column. These interspecific differences in biomass and body structure provide an important baseline for interpreting the contrasting effects of each species on nutrient cycling and water quality observed in this study.

Water temperature varied over time, likely because the low volume of experimental tanks reduced thermal buffering capacity and increases sensitivity to fluctuations in air temperature (O'Reilly *et al*., 2015; Woolway *et al*., 2026). However, no significant differences were detected among treatments, and values remained within a narrow and biologically suitable range. This indicates that temperature was effectively controlled across experimental units and is unlikely to have driven the observed differences in nutrient dynamics.

While particulate material derived from fish feces is often considered a major pathway of nutrient export in aquaculture systems (He *et al*., 2024; Lian *et al*., 2024), our results indicate that its role may be more limited in short time scales. The consistently higher nutrient loads associated with feed inputs than with fecal outputs indicate that only a fraction of the ingested material is rapidly exported as particulate matter, with a substantial proportion was retained within the system. These results suggest that particulate material acts less as an immediate export pathway and more as an intermediate compartment that can undergo further decomposition and contribute indirectly to dissolved nutrient pools. This pattern is consistent with recent studies showing that particulate organic matter is progressively remineralized by microbial processes, thereby linking particulate and dissolved pools in aquatic systems (Adyasari *et al*., 2023).

Although Nile tilapia exhibited higher absolute particulate input and output values than grass carp, this difference primarily reflects higher feed intake rather than intrinsic differences in particulate nutrient processing. This higher feed intake in Nile tilapia may be related to its widely reported voracious and opportunistic feeding behavior (Temesgen *et al*., 2022). After normalization as exported fractions, the effect of differential feeding was minimized, revealing that the proportion of nutrients released as particulate matter was relatively similar between species. This pattern suggests that, despite differences in appetite and total nutrient ingestion, the mechanisms governing particulate waste production operate in a comparable manner across species. The absence of a significant effect of fish biomass on particulate nutrient export reinforces that this pathway is not primarily driven by individual process. Rather, the consistency in exported fractions points to feed-related factors as the dominant control, suggesting that particulate waste production is more closely associated with diet composition than with metabolic processes (Syropoulou *et al*., 2024).

Dissolved nutrient dynamics revealed a consistent pattern of rapid transformation and accumulation in the water column, indicating that metabolic excretion coupled with subsequent biogeochemical processes was the dominant pathway regulating short-term nutrient availability. Nitrogen dynamics were marked by the accumulation of dissolved inorganic forms, particularly $NH_3$-N, which increased markedly over time. In Nile tilapia treatment, $NH_3$-N reached peak concentrations approximately 20 h after feeding, although peaks may occur as early as 6 h post-feeding (Obirikorang *et al*., 2020). The progressive increase in $NH_3$-N likely reflects the release of metabolic nitrogen through fish excretion following feeding events

(Gyamfi *et al*., 2024). This pattern suggests that direct excretion was the main source of dissolved inorganic nitrogen in the system, reinforcing its role as the primary pathway driving nitrogen availability.

$NO_2^-$-N concentrations remained consistently low, indicating rapid oxidation and suggesting that intermediate nitrification products did not accumulate in the water column. Accordingly, $NO_3^-$-N concentrations increased over time in treatments containing fish, supporting the occurrence of nitrification processes fueled by $NH_3$-N released by fish (Iber & Kasan, 2021; Yan *et al*., 2024). Models including fish biomass provided a better fit for both $NO_3^-$-N and $NO_2^-$-N dynamics, indicating that metabolic intensity contributed to these transformations. This pattern is consistent with findings from other aquaculture systems, where fish biomass has been identified as a key factor regulating nitrogen cycling (Yan et al., 2024). These patterns highlight the role of fish not only as sources of nitrogen inputs but also as drivers of rapid nitrogen transformation within tank systems.

Because nitrification is an oxygen-consuming process, the oxidation of $NH_3$-N to $NO_2^-$-N and $NO_3^-$-N may have contributed to the reductions in dissolved oxygen observed in fish tanks, particularly in tilapia tanks where $NH_3$-N accumulation was highest (Coldebella *et al*., 2018). In line with this, dissolved oxygen concentrations showed marked fluctuations in fish treatments, with reduced levels during the first hours after incubation. The lowest oxygen values were consistently observed after feeding. This pattern suggests that, in addition to oxygen consumption driven by nitrification, feeding-induced increases in metabolic activity combined with intensified microbial decomposition of organic residues further amplified oxygen demand. Together, these processes likely elevated oxygen consumption, reflecting both enhanced respiratory activity and stimulated heterotrophic processes associated with organic matter inputs (Baxa *et al*., 2021; Ibearugbulam *et al*., 2021; Godoy-Olmos *et al*., 2022; Lindholm-Lehto, 2023; Qiu *et al*., 2024; Stavrakidis-Zachou *et al*., 2025).

Lower $NH_3$-N concentrations observed in carp systems may also be partially explained by host-associated microbial processes. Recent studies have identified symbiotic association in the gills of carp species, where ammonia-oxidizing bacteria such as *Nitrosomonas* occur within gill tissue (Mes *et al*., 2024). These microorganisms do not merely transient, but appear to be adapted to this microenvironment, forming a stable component of the gill microbiome. This association may enhance the *in situ* oxidation of excreted $NH_3$-N, thereby reducing the accumulation in the surrounding water and contributing to the comparatively lower concentrations observed in carp systems.

Consistent with the patterns observed for $NH_3$-N, TN and TON reflect the integrated behavior of the nitrogen pool within the system, capturing the balance between continuous inputs and internal transformation processes. The temporal increases in TN and TON observed in fish treatments, especially in Nile tilapia, suggest that feed inputs and fecal material contributed additional organic nitrogen, further fueling these transformation processes (Iber & Kasan, 2021; Yan *et al*., 2024). The increase in TN, particularly in Nile tilapia treatment, indicates net accumulation in the water column, consistent with sustained inputs via excretion and limited short-term removal pathways, a pattern widely reported in aquaculture systems where fish metabolism is a continuous source of nitrogen (Blatt *et al*., 2025; Yan *et al*., 2024). Concurrently, variation in TON point to active exchanges between organic and inorganic fractions, suggesting that part of the nitrogen is temporarily retained in organic forms before further mineralized (Bai *et al*., 2023; Zhang *et al*., 2024). In this context, fish presence appears to intensify nitrogen cycling by simultaneously enhancing nitrogen inputs and maintaining these compounds in a reactive and transformable state.

In parallel with nitrogen dynamics, phosphorus concentrations also reflected the predominance of dissolved pathways, with marked increases over time, particularly in tilapia treatment. The accumulation of $PO_4^{3-}$-P and TP indicates that metabolic excretion was a major source of dissolved phosphorus in the water column, contributing to its rapid enrichment. In fish, a substantial fraction of excreted phosphorus is released in highly soluble forms, particularly via urinary pathways, making it immediately available in the dissolved pool (Sugiura, 2025). In addition, low phosphorus retention efficiency in aquaculture systems enhances P excretion rates, further increase phosphorus concentrations in the water column (Nathanailides *et al*., 2023). Together, these processes highlight the central role of metabolic nutrient recycling in driving phosphorus accumulation in aquatic systems.

Unlike nitrogen, however, phosphorus dynamics appeared to be more directly influenced by fish biomass, as evidenced by the improved model fit when body weight was considered. This suggests that phosphorus release may be more closely to the magnitude of metabolic inputs than to transformation processes. The larger body size of tilapia likely promoted greater phosphorus release into the water column through metabolic excretion and the processing of feed-derived organic matter. As fish biomass increases, both feeding activity and nutrient turnover intensify, leading to higher rates of phosphorus regeneration (Oliveira-Cunha *et al*., 2022). The temporal increase observed in tilapia tanks therefore likely reflect the combined effects of feed input and the higher metabolic demand associated with larger individuals.

Consistent with the accumulation of dissolved nitrogen and phosphorus, electrical conductivity increased over time, particularly in tilapia treatment. This increase became evident after the second feeding, reflecting the accumulation of dissolved ions derived from metabolic excretion and organic matter mineralization (Lindholm-Lehto, 2023).

In contrast to the accumulation patterns observed for nitrogen and phosphorus, NPOC remained relatively stable throughout the experimental period, indicating a distinct mode of regulation within the carbon cycle. This stability suggests that carbon released through excretion and organic matter decomposition was rapidly processed rather than accumulated in the water column. A significant fraction of this carbon is likely respired as $CO_2$ by microbial communities, while the remainder may be quickly assimilated and recycled within the system (Ende *et al*., 2024; Iber *et al*., 2025). This tight coupling between carbon release and consumption contrasts with the accumulation observed for nitrogen and phosphorus, highlighting fundamental differences in how these elements are processed over short time scales.

This tight coupling between carbon processing and respiratory pathways is also reflected in the progressive decline in pH observed across treatments, indicating system-wide acidification. The effect was more pronounced in fish treatments, which exhibited consistently lower pH values than the control, consistent with higher metabolic activity and organic matter processing. In addition to $CO_2$ production through respiration, this pattern also aligns with the accumulation and subsequent transformation of excreted $NH_3$-N, as nitrification contributes to proton release and further acidification of the water column (Lindholm-Lehto, 2023; Qiu *et al*., 2024). Together, these processes highlight the combined influence of carbon and nitrogen cycling on short-term changes in water chemistry.

Turbidity was unexpectedly higher in control treatment at 24h compared to fish treatments. Increased turbidity is often associated with fish interactions with bottoms, sediment disturbance and bioturbation (Collins, 2025). However, in our study, fish access to bottoms was restricted and resuspension effects were minimized. In contrast, suspended and total solids (SS and TS) did not differ significantly among treatments, indicating that differences in turbidity were not accompanied by changes in overall particulate concentrations. This apparent decoupling may be associated with differences in particle size distribution (Kim *et al*., 2025). The control treatment, which received only water inputs, may have contained a higher proportion of fine or colloidal particles that increased turbidity without substantially affecting measured suspended solids. In contrast, particulate inputs in fish treatments may have included larger particles, resulting in lower turbidity despite similar SS and TS values.

Total carbon export differed markedly among treatments, with the highest exported fractions observed in the control and lower values in fish treatments, particularly in tilapia. This pattern indicates that, in the absence of fish, carbon was more readily transferred out of the system, whereas fish presence promoted its retention and internal processing. Reduced export in fish treatments can be explained by the biological transformation of ingested carbon: a fraction is assimilated into biomass, while another portion is rapidly respired as $CO_2$ by fish and microbial communities (Yang *et al*., 2022; Liu *et al*., 2022; Dalbem Barbosa *et al*., 2024). These pathways reduce the amount of carbon remaining available for export. This interpretation is consistent with the stability of NPOC and the observed decline in pH, indicating that carbon was actively processed and partially converted to $CO_2$ within the system.

Although differences were initially observed between Nile tilapia and grass carp, after accounting for biomass, species identity was no longer a significant factor, indicating that carbon export was regulated by body size rather than intrinsic differences between species. The absence of significant interaction further indicates that the relationship between biomass and carbon export was consistent across treatments. As tilapia individuals were larger and exhibited lower exported fractions, this indicates that larger individuals tend to retain a greater proportion of carbon within the system. This pattern likely occurs because increasing body size elevates total metabolic demand, as metabolic and respiration rates scale positively with body mass in fish, resulting in greater internal processing of ingested carbon relative to direct export (Kraskura *et al*., 2023; Zaú *et al*., 2024; Pauly & Muller, 2025).

In contrast to carbon, nitrogen exhibited consistently high exported fractions across all treatments, with no significant differences among them. This pattern suggests that nitrogen remained highly mobile within the system, with a large proportion being transferred to the output regardless of fish presence. Such behavior is consistent with the predominance of dissolved inorganic nitrogen forms, particularly ammonia and nitrate, which are readily transported in the water column (Damashek & Francis, 2018; Yan *et al*., 2024). Although fish activity clearly enhanced nitrogen inputs and internal transformations, these processes did not substantially alter the proportion of nitrogen ultimately exported. This indicates that, over short time scales, nitrogen cycling is characterized by rapid turnover and limited retention, with dissolved pathways facilitating its continuous transfer through the system.

Phosphorus export showed an intermediate pattern between carbon and nitrogen, with significantly higher exported fractions in the control compared to fish treatments, while no differences were detected between species. This indicates that the presence promoted partial phosphorus retention within the system over the experimental period. Unlike nitrogen, which remained highly mobile and was continuously excreted, phosphorus is more tightly regulated biologically, being rapidly assimilated into biomass, particularly in growing organisms (Dalbem Barbosa *et al*., 2024; Sugiura, 2024; Xu *et al*., 2025). This tight biological regulation is further supported by physiological mechanisms controlling phosphorus uptake and retention, as several intestinal enzymes, membrane transporters, and regulatory pathways are involved in optimizing dietary phosphorus absorption in farmed fish (Nathanailides *et al*., 2023). The physiological control reduces its immediate release to the water column, contributing to lower export rates in fish treatments. However, in contrast to carbon, this retention was less pronounced, suggesting that phosphorus dynamics reflect a balance between biological assimilation and ongoing excretion. These results indicate that phosphorus occupies an intermediate position in terms of mobility, being neither as readily exported as nitrogen nor as strongly retained as carbon.

Organic matter export, measured exclusively in the particulate fraction, was low and did not differ among treatments or with fish biomass. This indicates that only a small proportion of ingested organic matter was released as fecal material, while most was retained through assimilation and metabolic processing. A substantial fraction was likely rapidly metabolized and converted into dissolved forms through microbial mineralization and excretion, resulting in the release of inorganic nutrients and dissolved carbon, alongside conversion to carbon dioxide via respiration (Arndt *et al*., 2013; Hou *et al*., 2022; Liu *et al*., 2023). Part of this organic matter may also have been temporarily incorporated into microbial biomass, further contributing to internal nutrient cycling (Nelson *et al*., 2023). Together, these processes contributed to the accumulation in the water column. These results reinforce that particulate organic matter represents a minor export pathway, with organic inputs being rapidly transformed, recycled, and retained within the system rather than exported in particulate form.

## 5. Conclusion

Despite the continuous nutrient input through feeding, nutrient balance analysis revealed that a substantial proportion of carbon, nitrogen, and phosphorus was retained within the system over the experimental period. This pattern is consistent with the observed accumulation of dissolved nutrients and the limited contribution of particulate export, indicating that internal processing pathways dominated short-term nutrient dynamics. Differences among treatments further highlight the role of fish activity in regulating nutrient fate. While control showed higher proportional export, the presence of fish, particularly Nile tilapia, was associated with increased nutrient retention and transformation within the water column. This suggests that fish-mediated processes, including metabolic excretion and enhanced biogeochemical cycling, promote the retention of nutrients in reactive dissolved forms rather than their direct export from the system. From an environmental perspective, the results indicate that the impacts of aquaculture systems may be more strongly linked to the accumulation and transformation of dissolved nutrients than to the direct release of particulate wastes, particularly over short time scales.

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CRediT authorship contribution statement

**Laís Coura Soranço:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Project administration, Supervision. **Ive Muzitano**: Project administration. **Ana Paula Dalbem Barbosa:** Writing – review & editing, Resources, Data curation. **Anderson Machado de Melo Júnior:** Writing – review & editing, Investigation, Data curation. **Giovana Machado Cardoso:** Methodology, Investigation. **Giovanni Resende de Oliveira:** Validation, Formal analysis. **Nathan Oliveira Barros:** Supervision, Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that there are no financial or personal relationships that could have inappropriately influenced the work presented in this article.

Data availability

All data, metadata, and tools used in this work are available from the corresponding author.

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## Ethics approval

All procedures involving the maintenance and handling of fish were reviewed and approved by the Animal Use Ethics Committees of the State University of Mato Grosso (UNEMAT) and the Agricultural Research Company of Minas Gerais (EPAMIG). Approval was granted under Opinion No. 004/2021 (“The biogeochemical balance in tropical aquaculture: implications for ecosystem transformations and

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## Final Considerations and Implications

This study demonstrates that aquaculture systems are governed by a set of interconnected processes in which feed inputs, species biology, and management strategies jointly determine both production outcomes and environmental impacts. By integrating different experimental approaches, this work advances the understanding of how these components interact to shape the functioning of freshwater aquaculture systems.

First, our findings reinforce the central role of feed as the primary driver of biogeochemical processes. The fate of feed-derived organic matter, particularly the fraction that is not consumed, plays a key role in regulating decomposition dynamics and associated greenhouse gas emissions. These processes highlight the importance of feed characteristics and stability in controlling carbon transformations within aquaculture systems, emphasizing that not only the quantity but also the quality of feed influences environmental outcomes.

Second, this study demonstrates that nutrient dynamics within aquaculture systems are primarily governed by the partitioning between dissolved and particulate pathways. After ingestion, only a limited fraction of nutrients is returned as particulate waste, while a substantial proportion is rapidly released in dissolved forms through metabolic excretion and subsequent microbial transformations. This predominance of dissolved pathways highlights their central role in driving short-term nutrient accumulation and water quality changes.

Third, species-specific traits emerge as a fundamental component influencing both nutrient pathways and system performance. Differences in feeding strategy, digestive efficiency, and metabolism between species such as Nile tilapia and grass carp lead to distinct patterns of nutrient assimilation and release. These differences extend beyond physiology, as fish behavior and social interactions also influence feeding efficiency, spatial distribution, and overall system dynamics, directly linking animal welfare to productivity and environmental impact.

Finally, this work highlights the importance of management strategies in modulating these processes. Polyculture systems demonstrate the potential to improve resource use efficiency and reduce negative environmental effects by promoting complementary interactions among species. However, the success of such approaches depends on a detailed understanding of species interactions, behavioral dynamics, and nutrient processing within the system.

Taken together, these findings demonstrate that aquaculture systems cannot be understood through isolated processes. Instead, they must be approached as integrated systems in which feed inputs, nutrient transformations, species biology, and management practices are tightly interconnected. Advancing this integrative perspective is essential for improving production efficiency while minimizing environmental impacts, ultimately supporting the development of more sustainable aquaculture practices.

## Appendix of Thesis

Appendix 1 - Article published in the Journal of Cleaner Production: Soranço, L. C., Quadra, G., Wien, J., Dalbem Barbosa, A. P., Melo Júnior, A. M., Cardoso, G. M., Duque, V. L. C., Soares, C. R., Silva, T., Oliveira, G. R., Kosten, S., Barros, N. O. A combined in situ-ex situ assessment of fish feed fate and associated carbon emissions. Journal of Cleaner Production, v.544, 2026. https://doi.org/10.1016/j.jclepro.2026.147711