

UNIVERSIDADE FEDERAL DE MINAS GERAIS – UFMG

ESCOLA DE VETERINÁRIA

PÓS-GRADUAÇÃO EM ZOOTECNIA

Naiara Pinho Alves

**Parâmetros abióticos e tecnologias de produção: pH em sistema de bioflocos com
Colossoma macropomum e ritmo circadiano na aquaponia com *Tinca Tinca***

Belo Horizonte - MG
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Colossoma macropomum e ritmo circadiano na aquaponia com *Tinca Tinca***

Tese apresentada ao Programa de Pós-Graduação
em Zootecnia da Escola de Veterinária da
Universidade Federal de Minas Gerais como
requisito para obtenção do grau de Doutora em
Zootecnia.

Área de concentração: Produção
Animal/Aquacultura

Orientadora: Dra. Cintia Labussière Nakayama
Co-orientador: Dr. Ronald Kennedy Luz

Belo Horizonte - MG
2025

A474p Alves, Naiara Pinho, 1994-
Parâmetros abióticos e tecnologias de produção: pH em sistema de bioflocos com *Colossoma macropomum* e ritmo circadiano da aquaponia com Tinca Tinca/ Naiara Pinho Alves.- 2025.
123 f: il.

Orientadora: Cíntia Labussiere Nakayama
Co-orientador: Ronald Kennedy Luz
Tese (Doutorado) apresentada à Faculdade de Medicina Veterinária da UFMG, como requisito parcial para obtenção do título de Doutora em Zootecnia.
Área de Concentração: Produção Animal e Aquacultura.
Bibliografia f. 25 a 38.

1. Produção animal - Teses – 2. Aquicultura – Teses – 3. Sustentabilidade – Teses - 4. Tambaqui - Criação - Teses – I. Nakayama, Cíntia Labussiere - II. Luz, Ronald Kennedy - III. Universidade Federal de Minas Gerais, Escola de Veterinária - IV. Título.

CDD – 639.3

Bibliotecária responsável Cristiane Patrícia Gomes CRB 2569
Biblioteca da Escola de Veterinária, UFMG.



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ATA DE DEFESA DE TESE DA ALUNA NAIARA PINHO ALVES

Às 14:00 horas do dia 23 de maio de 2025, reuniu-se, a Comissão Examinadora de Tese, aprovada por Ad referendum no dia 15/05/2025, para julgar, em exame final, a defesa da tese intitulada: **Parâmetros abióticos e tecnologias de produção: pH em sistema de bioflocos com *Colossoma macropomum* e ritmo circadiano na aquaponia com Tinca Tinca**, como requisito final para a obtenção do Grau de **Doutor (a) em Zootecnia, área de concentração Produção Animal**.

Abrendo a sessão, o Presidente da Comissão, Profa. Cintia Labussiere Nakayama, após dar a conhecer aos presentes o teor das Normas Regulamentares da Defesa de Tese, passou a palavra ao (a) candidato (a), para apresentação de seu trabalho. Seguiu-se a arguição pelos examinadores, com a respectiva defesa do candidato (a). Logo após, a Comissão se reuniu, sem a presença do candidato e do público, para julgamento da tese, tendo sido atribuídas as seguintes indicações:

	Aprovada	Reprovada
Prof.(a)/Dr.(a) <u>Cintia Labussiere Nakayama</u>	☒	☐
Prof.(a)/Dr.(a) <u>Ligia Uribe Gonçalves</u>	☒	☐
Prof.(a)/Dr.(a) <u>Gabriel Bernardes Martins</u>	☒	☐
Prof.(a)/Dr.(a) <u>Galileu Crovatto Veras</u>	☒	☐
Prof.(a)/Dr.(a) <u>Gisele Cristina Favero</u>	☒	☐

Pelas indicações, o (a) candidato (a) foi considerado (a): ☒ Aprovado (a)

☐ Reprovado (a)

Para concluir o Doutorado, o(a) candidato(a) deverá entregar 03 volumes encadernados da versão final da tese acatando, se houver, as modificações sugeridas pela banca, e a comprovação de submissão de pelo menos um artigo científico em periódico recomendado pelo Colegiado dos Cursos. Para tanto terá o prazo máximo de 60 dias a contar da data defesa.

O resultado final, foi comunicado publicamente ao (a) candidato (a) pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora e encaminhada juntamente com um exemplar da tese apresentada para defesa.

Belo Horizonte, 23 de maio de 2025.

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DEDICATÓRIA

Dedico a sincronicidade da vida.

AGRADECIMENTOS

À UFMG e a UMU por toda infraestrutura necessária à execução das atividades.

Ao CNPq e a CAPES, pelo financiamento para o projeto, a bolsa de doutorado, e a bolsa de doutorado sanduíche.

Ao corpo docente, discente e técnico do Setor de Aquacultura do programa de Pós-Graduação em Zootecnia da EV/UFMG por todo aprendizado e convívio, vocês contribuíram para a minha formação acadêmica, profissional e pessoal.

Em especial, quero agradecer a minha orientadora Dr^a. Cíntia Labussière Nakayama, pela oportunidade, confiança, e disponibilidade para a realização dos trabalhos acadêmicos. Ao meu coorientador Dr. Ronald Kennedy Luz, pela disponibilidade, fornecimento dos animais, e empréstimo dos equipamentos.

Aos docentes: Dr^a. Paula Adriane Perez Ribeiro e Dr. Galileu Crovatto Veras, obrigada pelo empréstimo dos materiais, e escuta para sanar dúvidas.

As técnicas: Dr^a. Suellen Cristina Moreira de Sales e Dr^a. Érika Ramos de Alvarenga, obrigada pelo apoio técnico durante a execução deste trabalho.

À banca de qualificação, Dr^a. Gisele Cristina Favero, Dr^a. Janessa Sampaio de Abreu, e Dr. Gabriel Bernardes Martins, pela disponibilidade e pelas contribuições que enriqueceram esta tese.

Aos colegas da rede LAQUA-SE (SOS Naiara), obrigada por toda a companhia, conversas e risadas. Vocês foram fundamentais, e representam perfeitamente a frase “Juntos somos mais fortes”.

Ao corpo docente e técnico de Fisiologia Animal da UMU, que me acolheu durante o período de seis meses. Em especial, ao meu supervisor do doutorado sanduíche Dr. Francisco Javier Sanchez Vazquez. Obrigada pela oportunidade e confiança.

Aos docentes: Dr. José Fernando López-Olmeda, e Dr^a Luísa Maria Vera Andujar, pela escuta, auxílio e esclarecimentos de dúvidas.

Ao técnico José Oli, pela disponibilidade e ajuda durante todo o processo experimental. Ao Dr. Alfonso Navarro Rodenas, pelas análises das alfaces.

As colegas de “Master”, obrigada pela companhia, conversas e ajuda com o espanhol, rs. “*¡Qué alegría fue haberte conocidas!*”

À banca de defesa, Dr^a. Ligia Uribe Gonçalves, Dr^a Gisele Cristina Favero, Dr. Galileu Crovatto Veras, e Dr. Gabriel Bernardes Martins, pela disponibilidade e pelas contribuições para o aprimoramento final da tese.

Aos meus ancestrais e familiares, minha profunda gratidão.

Aos amigos que tenho e aos que fiz durante essa jornada, obrigada! Vocês tornaram essa caminhada muito mais leve.

À todas as pessoas que estiveram presentes, em algum momento, ao longo desse período, obrigada! A academia faz parte da vida, mas não é somente ela.

Ao Divino, obrigada! Sou grata por todos os ensinamentos, e experiências. Mesmo diante dos obstáculos, seguirei lutando pelo que acredito, sendo a protagonista da minha história.

Aho! Axé! Assim é!

EPÍGRAFE

“O sentido da vida é estar vivo. É tão claro, tão óbvio e tão simples. Mesmo assim, todo mundo não para de correr em pânico, como se fosse necessário conseguir alguma coisa além de si próprio”.

Alan Watts

RESUMO

Diversos sistemas vêm sendo adotados para o cultivo de peixes, e a escolha do modelo mais adequado depende das características regionais, dos objetivos da produção, aspectos ambientais, sociais do local de implantação, entre outros. Entre os sistemas intensivos, destaca-se a tecnologia de bioflocos (BFT – Biofloc Technology), reconhecida por seu uso mais eficiente e sustentável da água, além da possibilidade de intensificação da produção e maior lucratividade. Já a aquaponia tem ganhado destaque por integrar a produção de alimentos de forma mais ecológica, que une a aquicultura e a hidroponia em um ecossistema simbiótico, minimizando os impactos ambientais. Ambos os sistemas compartilham um aspecto fundamental: a ciclagem de nutrientes, que envolve a conversão da amônia em nitrato por meio da ação das bactérias *Nitrosomonas* e *Nitrobacter*, tornando o processo mais seguro e ambientalmente mais sustentável. Desta forma, o estudo foi separado em três partes: o primeiro experimento buscou entender melhor como as diferentes faixas de pH influenciam nas condições fisiológicas e desempenho zootécnico de *Colossoma macropomum*, correlacionando com a qualidade de água em sistema de bioflocos. Foi realizado durante 60 dias, sendo dividido em duas fases de crescimento (Fase 1- alevinos e Fase 2- juvenis). As faixas de tratamentos utilizadas foram: pH 5,5-6,0; pH 6,5-7,0; pH 7,5-8,0, denominados de BFT_{5,5}, BFT_{6,5} e BFT_{7,5}, respectivamente. Ao longo de todo período experimental, os resultados indicaram a influência dos pHs na qualidade de água, desempenho zootécnico e parâmetros sanguíneos de *C. macropomum*. Diante disso, o segundo experimento buscou entender as respostas fisiológicas de *C. macropomum* frente à exposição ao ar quando mantidos em diferentes pHs em BFT. O *C. macropomum* é uma espécie que é caracterizada pelo bom desenvolvimento em parâmetros de água variados que explica sua capacidade de adaptar nesses ambientes por meio da morfologia, fisiologia e comportamento. Logo após a finalização do primeiro experimento, o segundo experimento foi iniciado, onde os juvenis foram mantidos em jejum de 24h antes do teste de exposição ao ar (AE). Imediatamente ao início do experimento, um grupo de peixes (n= 12/tratamento) foi submetido a coleta de sangue para a determinação de níveis basais dos parâmetros sanguíneos, e posteriormente foram realizadas mais quatro coletas de sangue, imediatamente após a exposição ao ar (IAE), e nos tempos 6, 12 e 24 h após exposição ao ar (6hEA, 12hAE e 24hAE, respectivamente). Após 30 min de exposição ao ar, e 24h de recuperação, a sobrevivência dos *C. macropomum* foi de 100%, em todos os tratamentos. Os parâmetros de qualidade de água não apresentaram diferenças ($p>0.05$), exceto amônia total (TAN) que foram maiores em BFT_{5,5} ($p=0.0253$) e PO_4^{3-} que foi menor em BFT_{7,5} ($p<0.0276$). As análises sanguíneas dos juvenis de *C. macropomum* mantidos em diferentes pHs em BFT apresentaram

efeitos do pH de manutenção, do tempo de coleta e de suas interações, confirmando que as repostas fisiológicas podem ser diferentes, dependendo do pH em que *C. macropomum* são mantidos. Por fim, a literatura relata que resíduos de peixes e restos de ração são utilizados como fertilizante para as plantas e, após a filtragem e purificação da água pelas plantas, ela é devolvida para uso pelos peixes. Diante disso, o terceiro estudo teve como objetivo avaliar os efeitos do fotoperíodo na sincronização e dessincronização em sistemas aquapônicos acoplados. O conjunto experimental foi composto por oito sistemas aquapônicos autônomos dispostos em dois grupos experimentais: quatro sistemas com peixes e plantas sob o mesmo ciclo claro-escuro (LD) (grupo sincronizado) e quatro sistemas com peixes e plantas sob LD reverso (grupo dessincronizado). Durante o período experimental, o oxigênio dissolvido na água foi mantido em $7,0 \pm 0,4$ mg/L, pH $6,7 \pm 0,4$, temperatura em $23,0 \pm 0,1$ °C, e apenas K (14.10 ppm) e Mg (9.28 ppm) apresentaram diferenças significativas ($p = 0,0286$), com valores mais elevados no sistema sincronizado nas semanas 5 e 2, respectivamente. Em relação à produção de alface, a biomassa fresca, a taxa de crescimento da massa fresca e a massa seca das mudas de alface não apresentaram diferenças significativas ($p > 0,05$). Os resultados demonstram que a qualidade da água e a produção de alface não foram influenciadas pela sincronização e dessincronização dos sistemas aquapônicos utilizados, exceto para eficiência do uso da água (WUE), que apresentou comportamentos diferentes. Em conclusão, o uso de sistemas fechados, como o biofloco e a aquaponia, é uma abordagem agrícola que permite a reciclagem de nutrientes e resíduos por meio da produção contínua, segura e sustentável.

Palavras-chave: Produção aquícola sustentável, Ciclagem de nutrientes, Fisiologia de peixes, Qualidade da água.

ABSTRACT

Several systems have been adopted for fish farming, and the choice of the most suitable model depends on regional characteristics, production goals, and environmental and social aspects of the implementation site, among other factors. Among intensive systems, Biofloc Technology (BFT) stands out for its more efficient and sustainable use of water, as well as its potential for production intensification and increased profitability. Aquaponics has also gained prominence by integrating food production in a more ecological way, combining aquaculture and hydroponics in a symbiotic ecosystem that minimizes environmental impacts. Both systems share a fundamental feature: nutrient cycling, which involves the conversion of ammonia into nitrate through the action of *Nitrosomonas* and *Nitrobacter* bacteria, making the process safer and more environmentally sustainable. Thus, the study was divided into three parts. The first experiment aimed to better understand how different pH ranges influence the physiological conditions and zootechnical performance of *Colossoma macropomum*, correlating these responses with water quality in a biofloc system. The experiment lasted 60 days and was divided into two growth phases (Phase 1 - fry and Phase 2 – juvenile). The pH treatments used were 5.5-6.0, 6.5-7.0, and 7.5-8.0, referred to as BFT_{5.5}, BFT_{6.5}, and BFT_{7.5}, respectively. Throughout the experimental period, the results indicated that pH influenced water quality, zootechnical performance, and blood parameters of *C. macropomum*. Based on these findings, the second experiment aimed to evaluate the physiological responses of *C. macropomum* to air exposure when maintained at different pH levels in BFT systems. *C. macropomum* is characterized by good development under a wide range of water parameters, which explains its ability to adapt to different environments through morphological, physiological, and behavioral mechanisms. After the conclusion of the first experiment, the second experiment was initiated, and juveniles were fasted for 24 h prior to the air exposure (AE) test. At the beginning of the experiment, a group of fish (n = 12 per treatment) was subjected to blood sampling to determine baseline blood parameters. Subsequently, four additional blood samplings were performed: immediately after air exposure (IAE) and at 6, 12, and 24 h after exposure (6hAE, 12hAE, and 24hAE, respectively). After 30 min of air exposure and 24 h of recovery, the survival rate of *C. macropomum* was 100% in all treatments. Water quality parameters showed no differences ($p > 0.05$), except for total ammonia nitrogen (TAN), which was higher in BFT_{5.5} ($p = 0.0253$), and phosphate (PO_4^{3-}), which was lower in BFT_{7.5} ($p < 0.0276$). Blood analyses of juveniles maintained at different pH levels in BFT systems showed effects of maintenance pH, sampling time, and their interactions, confirming that physiological responses may vary depending on the pH conditions. Finally, the literature reports that fish waste and uneaten feed can be used as fertilizer for plants and, after filtration and purification by plants, the water is returned for fish use.

Therefore, the third study aimed to evaluate the effects of photoperiod on synchronization and desynchronization in coupled aquaponic systems. The experimental setup consisted of eight autonomous aquaponic systems arranged into two experimental groups: four systems with fish and plants under the same light–dark cycle (LD) (synchronized group) and four systems with fish and plants under a reversed LD cycle (desynchronized group). During the experimental period, dissolved oxygen was maintained at 7.0 ± 0.4 mg/L, pH at 6.7 ± 0.4 , temperature at 23.0 ± 0.1 °C, and only K (14.10 ppm) and Mg (9.28 ppm) showed significant differences ($p = 0.0286$), with higher values in the synchronized system during weeks 5 and 2, respectively. Regarding lettuce production, fresh biomass, fresh mass growth rate, and dry mass of lettuce seedlings did not show significant differences ($p > 0.05$). The results demonstrated that water quality and lettuce production were not influenced by system synchronization or desynchronization, except for water use efficiency (WUE), which showed different responses. In conclusion, the use of closed systems, such as biofloc technology and aquaponics, represents an agricultural approach that enables nutrient and waste recycling through continuous, safe, and sustainable production.

Keywords: Sustainable aquaculture production, Nutrient cycling, Fish physiology, Water quality.

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LISTA DE ABREVIATÖES

AOB - bactérias oxidadoras de amônio

OD - oxigênio dissolvido

CA - conversão alimentar

CAA - conversão alimentar aparente

CaCO₃ - carbonato de cálcio

CaO - óxido de cálcio

CHCM - concentração de hemoglobina corpuscular média

CL_{50 96h} – concentração letal média

COD - carbono orgânico dissolvido

CV - compressor radial

EE – extrato etéreo

Eri - eritrócitos

FCR - *feed conversion ratio*

Hb - hemoglobina

HCM - hemoglobina corpuscular média

Ht - hematócrito

IHS - índice hepatossomático

IVF - índice de volume do floco

NOB - bactérias oxidadoras de nitrito

PB – proteína bruto

pH - potencial hidrogeniônico

RBC – eritrócitos

rpm - rotações por minuto

SS - sólidos sedimentáveis

SST - sólidos suspensos totais

TAN - nitrogênio amoniacal total

TCE - taxa de crescimento específico

VCM - volume corpuscular médio

LISTA DE SIGLAS

ANOVA - *Variance analysis*

AOC - Associação de Químicos Analíticos Oficiais

BFT - *Biofloc Technology*

CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

CNPQ - Conselho Nacional de Desenvolvimento Científico e Tecnológico

CEUA - Comissão de ética no uso de animal

EV/UFMG – Escola Veterinária da Universidade Federal de Minas Gerais

FAO - Organização das Nações Unidas para Agricultura e Alimentação

IBGE - Instituto Brasileiro de Geografia e Estatística

LAQUA - Laboratório de Aquacultura

MAPA - Ministério da Agricultura, Pecuária e Abastecimento

Peixe Br - Associação Brasileira de Piscicultura

RAS- *Recirculating Aquaculture System*

UFMG - Universidade Federal de Minas Gerais

UMU – *Universidad de Murcia*

LISTA DE SÍMBOLOS

~ - aproximadamente
dL - decilitro
% - porcentagem
C - carbono
°C - graus Celsius
Ca²⁺ - cálcio
Cl⁻ - cloro
cm - centímetro
C:N - relação carbono e nitrogênio
CO₂ - dióxido de carbono
FL - fentolitro
g - grama
g/L - grama por litro
h - hora
H⁺ - hidrogênio
H₂O - água
= - igual
Kg - quilo
Kg /m³ - quilo por metro cúbico
km - quilômetro
L – litro
L/h - litro por hora
ln - logaritmo natural
± - mais ou menos
> - maior
< - menor
≤ - menor igual
m³ - metros cúbicos
mg - miligrama
mg CaCO₃/L - miligrama de carbonato de cálcio por litro
mg/L - miligrama por litro
min - minuto
mm - milímetro

mmol - milimol

ml - mililitro

/ - por

n - número

Na⁺ - sódio

Na₂HCO₃ - bicarbonato de sódio

NH₃ - amônia não ionizada ou amônia gasosa

NH₄⁺ - cátion monovalente amônia ionizada ou íon amônio

N-NH₃ - nitrogênio na forma de amônia não ionizada

N-NO₂⁻ - nitrogênio na forma de nitrito

NO₂⁻ - ânion monovalente do elemento químico nitrito

O₂ - oxigênio

p - *P* - *value*

pg - picograma

W - watt

μL – microlitro

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1. Introdução Geral

Entre os sistemas intensivos para produção aquícola, a tecnologia de produção em biofloc (BFT - “Biofloc Technology”), destaca-se por ser uma técnica de produção baseada na adição externa de carbono orgânico ao sistema, considerando a relação carbono: nitrogênio (C:N). Já a aquaponia tem ganhado destaque por integrar a produção de alimentos de forma mais ecológica, ao unir a aquicultura e a hidroponia em um ecossistema simbiótico, minimizando os impactos ambientais (Tyson, Treadwel e Simonne, 2011). Assim, em ambos os sistemas ocorre a estimulação da produção de bactérias heterotróficas responsáveis pela ciclagem dos nitrogenados acumulados, permitindo o uso e reuso da água (Avnimelech, 1999).

O nitrogênio presente na água, sob a forma de amônia total (TAN), é resultado da excreção dos organismos cultivados e das sobras de ração no sistema. Devido ao seu grau de toxicidade para os peixes, é considerado o produto de maior preocupação na produção aquícola (Kapoor e Viraraghavan, 1997). A toxicidade da TAN é controlada pela ação das bactérias (heterotróficas e quimioautotróficas) que tornam o ambiente seguro para a produção de peixes (Avnimelech, 2009). As bactérias quimioautotróficas, também conhecidas por nitrificantes, realizam o processo de nitrificação, onde a amônia é oxidada a nitrito (nitritação) e o nitrito é oxidado a nitrato (nitratação) (Tchobanoglous, Burton e Stensel, 2003).

Esse processo micribiológico, embora essencial para a estabilidade do sistema, promove alterações físico-químicas no ambiente aquático, como a liberação de H^+ , consumo de carbono inorgânico, acidificação da água, e redução da alcalinidade (Avnimelech, 2015). Nesse Nesse contexto, o pH da água assume papel central, uma vez que influencia diretamente a atividade das bactérias nitrificantes e, simultaneamente, afeta o desempenho e a saúde dos organismos cultivados (Gieseke et al., 2006a; Villaverde, García-Encina e Fdz-Polanco, 1997; Wagner et al., 2021).

As variações de pH podem estar associadas a diversos fatores, como o tipo de solo, o modelo de sistema produtivo (aberto ou fechado; extensivo ou intensivo), a densidade de estocagem, a produção de fitoplâncton e os níveis de alcalinidade da água (Gieseke et al., 2006b; Wagner et al., 2021). Para minimizar oscilações acentuadas e manter o pH em faixas mais próximas à neutralidade, os sistemas podem ser tamponados por meio da adição de carbonatos e bicarbonatos, garantindo maior estabilidade ao ambiente aquático (Van Loosdrecht e Jetten, 1998).

Alterações repentinas ou extremas no ambiente aquático representam importantes agentes estressores para os organismos aquáticos, uma vez que reduzem sua capacidade de manter a homeostase (Oba, Mariano e dos Santos, 2009). As estratégias fisiológicas adaptativas frente a essas variações podem ser avaliadas por meio de análises hematológicas e bioquímicas, que fornecem

indicadores sensíveis do estado de saúde e do nível de estresse dos peixes (Val, Silva e Almeida-Val, 1998).

Entre os fatores de estresse comumente enfrentados na produção aquícola, a exposição ao ar representa um desafio significativo para espécies dependentes da respiração branquial para a obtenção de oxigênio. Para lidar com essa condição, os peixes podem apresentar adaptações morfológicas, fisiológicas e comportamentais (Aride *et al.*, 2016; Val *et al.*, 2022). Em diversas espécies amazônicas, destaca-se a expansão labial como uma das adaptações mais evidentes em condições de hipóxia, processo associado à ativação de canais catiônicos e iônicos, que controlam o tônus muscular liso e o volume celular e fluxo de líquidos nos tecidos labiais (Araújo-Lima e Gomes, 2005; Fagundes, 2012).

Além disso, o pH do meio também é responsável por interferir em múltiplos processos fisiológicos (Almeida-Val e Hochachka, 1995), levando ao comprometimento da osmorregulação através do vazamento de eletrólitos do epitélio branquial em exposição a ambientes ácidos (Aride, Roubach e Val, 2007). Apesar disso, nas bacias amazônicas, o perfil de águas ácidas é comum, (Wood *et al.*, 1998), o que favorece a adaptação e o desenvolvimento de espécies nativas a essas condições ambientais.

No contexto da aquaponia, outro fator relevante diz respeito à dinâmica temporal da assimilação de nutrientes pelas plantas. Evidências indicam que as taxas de assimilação de nitrogênio vegetal são reguladas pelo ritmo circadiano (Farré e Weise, 2012). A sincronização dos ritmos circadianos de peixes e plantas pode otimizar os diferentes processos fisiológicos e ambos os organismos, aumentando a eficiência do sistema aquapônico como um todo (López-Olmeda, 2017). No entanto, estudos apontam para a ocorrência de dessincronização nos fluxos de nitrogênio entre peixes e plantas nesses sistemas (Obirikorang *et al.*, 2015). Essa dessincronização das taxas de excreção e absorção de nitrogênio pode comprometer a nitrificação (Obirikorang *et al.*, 2015), o desenvolvimento dos organismos cultivados, e a eficiência produtiva global.

Diante desse cenário, o presente estudo parte do pressuposto que uso de sistemas fechados, como o bioflocos e a aquaponia, constitui uma abordagem produtiva capaz de promover a reciclagem de nutrientes e resíduos por meio de uma produção contínua, segura e sustentável. Além disso, considera-se que a qualidade da água nesses sistemas exerce influência direta sobre a manutenção das comunidades bacterianas nitrificantes, o desempenho zootécnico e as condições fisiológicas de peixes e plantas.

2. Revisão bibliográfica

2.1 Sistemas de produção

Diversos sistemas de produção (extensivo, semi-intensivo e intensivo) têm sido utilizados na produção de espécies de peixes do Amazonas (Oliveira *et al.*, 2012) e da região norte do Brasil. As principais modalidades de produção são: viveiros escavados, tanques-redes, barragens e canais de igarapé (Dairiki e Silva, Da, 2011; Pantoja-Lima *et al.*, 2015), com predominância de viveiros escavados e em barragens (Melo, Izel e Rodrigues, 2001; Nakauth, 2015; Oliveira *et al.*, 2012; Pantoja-Lima *et al.*, 2015). Porém, a escolha do tipo de sistema utilizado, irá depender das características da região, objetivo da produção, e está relacionada com questões ambientais e sociais da localidade onde será inserido (Crepaldi *et al.*, 2006).

2.1.1 Tecnologia de biofloco (BFT)

Dentre o sistema intensivo, a tecnologia em biofloco (BFT - “Biofloc Technology”) destaca-se por suas características de uso mais sustentável em relação ao uso e reuso da água, intensificação e possibilidade de maior lucratividade (Avnimelech, 1999). Esse aproveitamento da água é possível em razão da microbiota presente nesse sistema, especialmente das bactérias (Martínez-Córdova *et al.*, 2015). Essa tecnologia, além de reduzir a descarga de efluentes (nitrogenados), promove biossegurança e impede escapes dos peixes para o ambiente (Avnimelech, 2007).

O acúmulo de nitrogenados em BFT (fig. 1) é decorrente da excreção dos peixes/camarões e das sobras de rações, que é representada pela amônia total (TAN), composto nitrogenado mais tóxico aos organismos aquáticos. E, para contornar essa situação, faz-se a adição de fonte externa de carbono orgânico considerando a relação de carbono: nitrogênio (C:N). A sua proporção pode variar de 20:1 a 6:1, tendo como principal objetivo a produção em biomassa das bactérias heterotróficas (Khanjani e Sharifinia, 2020).

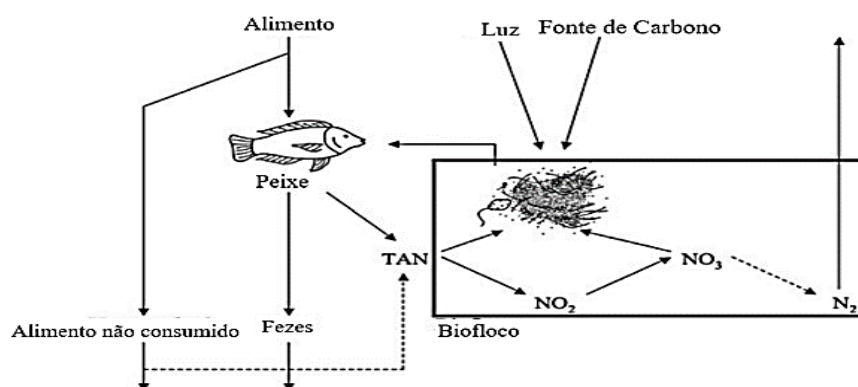


Figura 1. Ciclo do nitrogênio em sistema BFT. Fonte: Adaptada de Crab et al. (2007)

As bactérias heterotróficas são responsáveis pela assimilação do nitrogênio amoniacal, tornando o ambiente mais seguro às espécies produzidas (Ebeling, Timmons e Bisogni, 2006; Emerenciano *et al.*, 2017). Ademais, o fitoplâncton também utiliza os compostos nitrogenados (TAN e nitrato) e o fosfato para construir estruturas celulares, proteínas e açúcares, formando uma biomassa fitoplanctônica (Ray, Dillon e Lotz, 2010), que por sua vez, torna-se base de alimentação aos zooplanktons que serão utilizados como alimentos de peixes e camarões (Gaye-Siessegger, Focken e Becker, 2006).

A comunidade microbiana, que compõe o biofloco, apresenta-se bem diversificada para cada unidade de produção em BFT e é influenciada pelos parâmetros de água (Avnimelech, 2015; Ebeling, Timmons e Bisogni, 2006; Gallardo-Colli *et al.*, 2019; Hargreaves, 2013; Vinatea *et al.*, 2010). Essa diversidade microbiana é responsável por variações nos teores de proteína total, extrato etéreo, matéria seca etc. (Ray e Lotz, 2014). Estudos demonstram que teores de proteína bruta e extrato etéreo no biofloco podem variar de 12 e 50% e 0,5 a 46%, respectivamente (Emerenciano *et al.*, 2022; Hargreaves, 2013). Fatores como a fonte de carbono orgânico, relação de C:N, intensidades de luz, salinidade, alcalinidade, dureza da água, entre outros, são capazes de modificar a comunidade microbiana e consequentemente influenciar as variações bromatológicas (Azim e Little, 2008; Colwell, 2006).

Diante do exposto, fica evidente que a compreensão dos grupos, abundância e diversidade dos microrganismos, bem como seus papéis na dinâmica ecológica são importantes para uma operação do sistema no BFT (Fiałkowska Ą e Pajdak-Stó, 2008).

2.2.1 Aquaponia

Com o objetivo de integrar a produção de alimentos e reduzir os impactos ambientais gerados pelos sistemas de produção, a aquaponia, tem despertado o interesse por ser um sistema que combina a aquicultura (cultivo de organismos aquáticos) e a hidroponia (cultivo de plantas) em um ecossistema simbiótico (Danaher *et al.*, 2013; Shete *et al.*, 2016; Thomas *et al.*, 2019). Os resíduos metabólicos dos peixes são aproveitados na forma de nutrientes para o desenvolvimento das plantas, tornando-o eficiente e sustentável (Tyson, Treadwel e Simonne, 2011). Este processo é baseado no tratamento de efluente pelas bactérias nitrificantes presentes no filtro biológico, que convertem a amônia em nitrito, e posteriormente em nitrato (Nuwansi *et al.*, 2021) (fig.2).

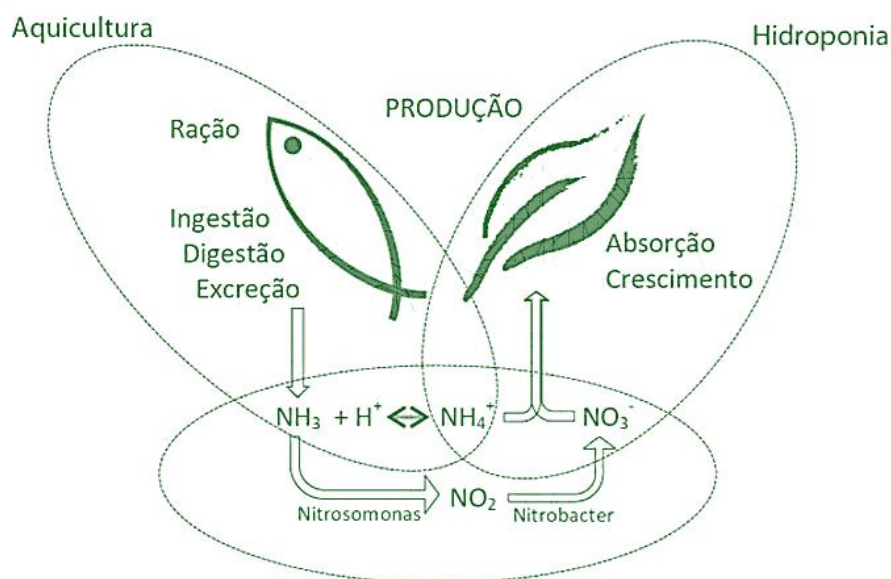


Figura 2. Processos biológicos do sistema aquapônico. Fonte: Adaptada de Carneiro (2015)

Neste sistema, a ciclagem de nutrientes permite que a amônia, o principal resíduo metabólico de peixes, e a substância mais tóxica dentro do sistema, seja oxidada pelas bactérias *Nitrosomonas* em nitrito e *Nitrobacter* em nitrato (Salam, Asadujjaman e Rahman, 2013). O nitrato é o último produto gerado no processo de nitrificação, e usado como nutriente pelas plantas (Hundley2 e Navarro3, 2013; Love *et al.*, 2014).

Há evidências de que as taxas de assimilação de nitrogênio nas plantas são reguladas através do ritmo circadiano (Farré e Weise, 2012). A sincronização dos ritmos circadianos de peixes e plantas pode otimizar os diferentes processos fisiológicos (López-Olmeda, 2017) tornando o sistema aquapônico mais eficiente. Todavia, estudos sugerem que há uma dessincronização nos fluxos de nitrogênio entre peixes e plantas em sistemas aquapônicos (Obirikorang *et al.*, 2015). Essa dessincronização das taxas de excreção e absorção de nitrogênio pode afetar o processo de nitrificação (Obirikorang *et al.*, 2015), e consequentemente, o desenvolvimento de peixes e plantas, assim como a eficiência do sistema como um todo.

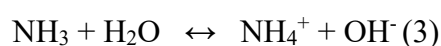
Contudo, as vantagens produtivas dos sistemas aquapônicos são muitas, desde a eficiência hídrica, uso de pequenas áreas, produção contínua, saudável e sustentável (Falanghe *et al.*, 2015; Thomas *et al.*, 2019)e, mesmo apresentando as concentrações mais baixas de alguns nutrientes (Bittsanszky *et al.*, 2016; Delaide *et al.*, 2016; Graber e Junge, 2009) como cálcio (Ca), potássio (K), magnésio (Mg) e ferro (Fe) (Tidwell, 2012), a produção ainda pode ser melhor do que no solo (Rakocy *et al.*, 2004).

2.2 Nitrogênio no meio

O nitrogênio é um dos elementos mais importantes presentes nas águas de produção. Ele apresenta-se de formas múltiplas, conforme estado de oxidação (Hulth *et al.*, 2004): nitrogênio amoniacal, nitrogênio orgânico, nitrito e nitrato (He, Xue e Wang, 2009).

2.2.1 Amônia

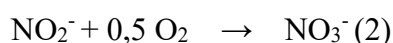
A amônia é o principal produto da excreção dos peixes e é gerado após a degradação das proteínas, proveniente, principalmente das rações comerciais. A amônia (eq.3) é um gás extremamente solúvel na água, e se apresenta de acordo com a seguinte reação de equilíbrio:



As membranas celulares dos peixes são permeáveis à amônia não ionizada (NH_3) devido a afinidade com compostos lipofílicos, o que facilita atravessar a membrana, tornando-se mais tóxica aos peixes. Por outro lado, a amônia ionizada (NH_4^+) é de natureza lipofóbica. No sistema de produção, a soma das formas ionizada (NH_4^+) e não-ionizada (NH_3) compõem a chamada amônia total (TAN).

2.2.2 Nitrificação

A nitrificação é o termo usado para descrever o processo da remoção biológica de nitrogênio, em que a amônia é oxidada a nitrito (nitritação) e o nitrito é oxidado a nitrato (nitratação) (Metcalf, Eddy e Tchobanoglous, 2004). A oxidação biológica da TAN ocorre por meio das bactérias oxidadoras de amônio (AOB) e bactérias oxidadoras de nitrito (NOB). Essas bactérias nitrificantes obtêm a energia para o crescimento por meio do carbono (CO_2) inorgânico e são classificadas como bactérias autotróficas (Kapoor e Viraraghavan, 1997). As AOB oxidam o amônio a nitrito (Eq. 1), são dos gêneros: *Nitrosomonas*, *Nitrosococcus* e *Nitrospira*, sendo as mais estudadas as *Nitrosomonas*. Já as NOB, oxidam o nitrito a nitrato (Eq. 2) e são dos gêneros: *Nitrospira*, *Nitrobacter*, *Nitrospina* e *Nitrococcus*; sendo as mais estudadas as *Nitrobacter* (Antileo, Roeckel e Wiesmann, 2003; Beltran, 2008; Ciudad *et al.*, 2007).



Nas equações (1) e (2) é possível verificar a demanda por O₂ para a nitrificação (Ruiz, Jeison e Chamy, 2003). Por isso, em condições de oxigenação insuficiente, as *Nitrosomonas* e *Nitrobacter* diminuem a atividade bioquímica devido a sua característica aeróbica (Cervantes-Carrillo, Pérez e Gómez, 2000).

2.2.3 Potencial hidrogeniônico (pH) e o sistema

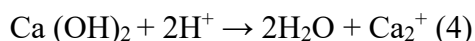
O pH é utilizado numa escala de 1 a 14 e indica se o ambiente está ácido, neutro ou básico, resultado de uma equação logarítmica de H⁺ (Sørensen, 1909). A concentração dos íons H⁺ indicam reação ácida, enquanto os íons OH⁻ indicam reação básica ou alcalina na água e a presença de mais ou menos H⁺ ou OH⁻ determina a condição da água em relação ao pH.

Nos sistemas aquáticos, o pH pode ser modificado pelos processos químicos e biológicos que ocorrem na água (Arana, 2004). Por exemplo, no processo de nitrificação ocorre a redução do pH devido a produção de H⁺, e maior consumo de carbono inorgânico proveniente da alcalinidade. Azim e Little, (2008) relatam outro exemplo de alteração do pH, o rápido crescimento das bactérias heterotróficas, e elevado consumo de O₂ e produção de CO₂, favorece a acidificação do meio. Isso acontece, porque o aumento de CO₂ em virtude da respiração dos organismos presentes, diminui o pH na água (Swann e Ozil, 1994).

Por outro lado, o pH afeta a nitrificação, devido a ativação-desativação das bactérias nitrificantes (Bock e Wagner, 2006; Gieseke *et al.*, 2006b; Villacorta Correa, 1997). Para oxidação biológica de nitrogênio, considera-se uma faixa ideal de pH (7,0 a 9,0) (Ford, Churchwell e Kachtick, 1980; Henze *et al.*, 1997; Sharma e Ahlert, 1977). Em pH alcalino (> 7,0), o equilíbrio químico é deslocado no sentido da formação de amônia (NH₃) até a formação de nitrito, favorecendo as atividades das AOB sobre as NOB. Entretanto, em um pH < 6,0 tanto as AOB quanto as NOB diminuem sua atividade (Ciudad *et al.*, 2007), e no pH < 5,0 essas bactérias não são capazes de realizar a nitrificação (Polanco, Villaverde e Garcia, 1996).

2.2.4 Alcalinidade

A alcalinidade refere-se às bases tituláveis presentes na água, na presença dos íons bicarbonatos (HCO₃⁻), carbonatos (CO₃²⁻) e hidroxila (OH⁻) que produzem uma reação de neutralização (eq. 4), que atua na elevação do pH (Martins *et al.*, 2017; Vinatea, 1997). Seu tamponamento (eq. 5) está associado a alcalinidade total expressa em mg/L de CaCO₃ (Boyd, Tucker e Somridhivej, 2016).





Na aquicultura, suas concentrações variam de acordo com o tipo de sistema, solo e os organismos produzidos (Gieseke et al., 2006b; Wagner et al., 2021). Boyd e Tucker (1998) recomendam concentrações de 75 mg de CaCO_3 /L como demanda mínima de alcalinidade em sistema de recirculação de água. Chen et al. (2006) relaram uma concentração ótima para atuação das bactérias nitrificantes de 200 mg/L de CaCO_3 , enquanto, Ebeling, Timmons e Bisogni, (2006) recomendam concentrações entre 100 e 150 mg/L de CaCO_3 . Essas recomendações indicam a necessidade da adição de compostos alcalinizantes para manter o pH estável ao longo do dia (Boyd, Tucker e Somridhivej, 2016; Boyd *et al.*, 2011). O bicarbonato de sódio (NaHCO_3), por exemplo, é um composto seguro e eficaz no aumento da alcalinidade, e possui rápida diluição na água, e ao se dissociar, produz HCO_3^- e Na^+ (Loyless e Malone, 1997).

2.2.5 Dureza

A relação dureza:alcalinidade está diretamente associada ao equilíbrio ácido-base da água, determinante para a vida aquática (Arana, 2004). A dureza total é representada pela soma dos cátions divalentes, principalmente Ca^{2+} e Mg^{2+} , expressa em mg de CaCO_3 /L (Boyd, Tucker e Somridhivej, 2016). Ela é classificada como: leve (50-150), dura (150-300) e muito dura (> 300 mg de CaCO_3 /L) (Boyd, 2015). Além disso, a partir da dureza total, é possível demonstrar a dureza de Ca^{2+} , que representa 0,4 do valor de dureza total (Boyd, Tucker e Somridhivej, 2016). Em água doce, o intervalo de dureza é bastante heterogêneo, podendo possuir valores entre 5-75 mg/L em regiões úmidas, até 1000 mg/L em áreas com solo calcário (Boyd, 2015).

A principal função de Ca^{2+} para os teleósteos está relacionada a calcificação óssea e promoção do aumento da rigidez das “*tight junctions*” nas brânquias, o que evita a perda iônica (Wood, 2001). Essa estratégia serve para evitar a perda iônica na água em pH acidificado (Baldisserotto, 2011).

2.3 *Colossoma macropomum*

2.4.1 Produção de *C. macropomum*

Em cativeiro, o sucesso da produção de *C. macropomum* (fig. 2) é atribuído à sua facilidade de adaptação aos sistemas produtivos, como o êxito reprodutivo por meio do domínio das técnicas de reprodução em cativeiro (Kubtiza, 2004), boa aceitação a rações comerciais (Zaniboni Filho e Weingartner, 2007), e desempenho zootécnico (López-Elías *et al.*, 2015). Essas características, contribuíram para que o tambaqui se tornasse a principal espécie nativa produzida no Brasil, tendo

como consequência, a diminuição da sua exploração no ambiente natural (Aride, P. H.R. *et al.*, 2016; Lobo *et al.*, 2015).

O principal produtor da espécie é o estado de Rondônia com 41.554 ton, seguido de Maranhão com 10.735 ton e Roraima, com 10.450 ton (Embrapa, 2020). Apesar do grande potencial produtivo do tambaqui, ainda existem gargalos que estagnam o seu crescimento no setor (Oliveira *et al.*, 2012). Alguns dos problemas relacionados a produção da espécie, está no mercado consumidor muito regionalizado, com destaque nas regiões Norte e Centro-Oeste, além das questões ambientais legais, segurança sanitária, falta de recursos financeiros para reinvestimento e expansão da produção, somados a pandemia por COVID-19 que acarretou o aumento do preço de insumos (IBGE, 2013; PeixeBR, 2020). Ademais, na sua maioria, os piscicultores não possuem conhecimento ou direcionamento técnico para explorar melhor a atividade. Sem planejamento e gerenciamento adequado, acabam não tendo o resultado esperado (Padua, Aguiar e Boijink, 2008). Principalmente pela escassez de dados em condição de produção natural, pois muitos dos trabalhos científicos são realizados em laboratórios, onde o ambiente é totalmente controlado.

Contudo, o tambaqui apresenta uma capacidade alta de popularização em escala nacional pelo sabor agradável da sua carne e o preço competitivo (Gentil *et al.*, 2020). A espécie é destaque nas exportações da aquicultura brasileira (Pedroza Filho e Rocha, 2019), evidenciando o interesse da inserção do tambaqui na alimentação (Woynárovich e Anrooy, 2019).

No período de 2013 a 2018, o tambaqui *C. macropomum* representou 20% da produção total, com 102 mil toneladas, registrando um crescimento de 15,6%, cerca de 2,6% de aumento anual (IBGE, 2020). Após esse período, a espécie apresentou um recuo, até 2021 conforme a figura 3.

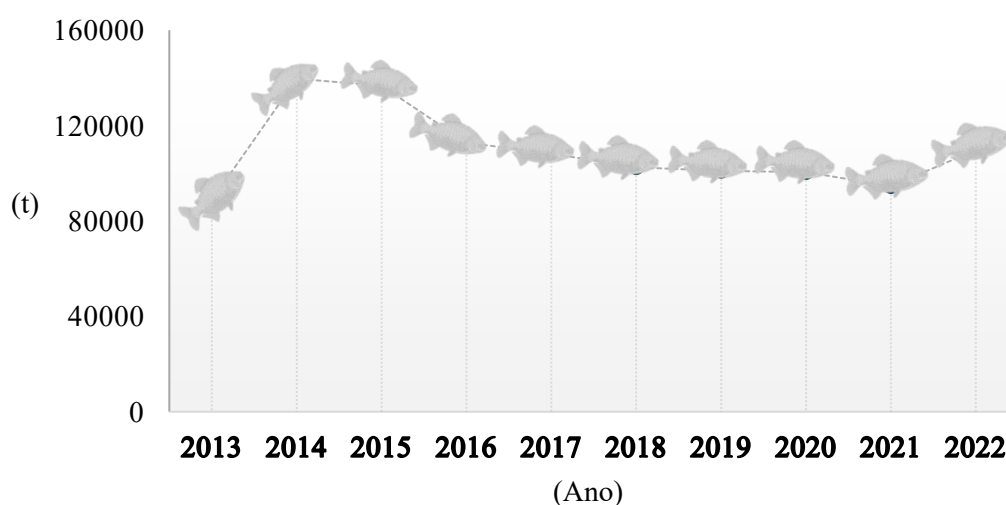


Figura 3. Produção (ton.) de *Colossoma macropomum* de 2013 a 2022. Fonte: Adaptada do IBGE (2022).

O processo de consolidação da produção do tambaqui é baseada na divisão da sua produção em três fases: (1) a larvicultura, em que os peixes são criados desde a eclosão até o peso médio individual de 0,5 a 1g (período de 30 a 45 dias), (2) juvenis, em que o peso médio individual alcança 40 e 50 g (~ 60 dias), e (3) engorda, em que o tempo pode variar dependendo do peso desejado para abate, de 800 g a 2 kg (Dairiki e Silva, Da, 2011; Lima, 2013). Contudo, o mercado nacional sofre pela falta ou dificuldade de aquisição das formas jovens e de qualidade (Brabo *et al.*, 2015; Guerreiro, Streit e Rotta, 2015).

2.4.2 Características e hábitat

De acordo com os dados, somente a Bacia Amazônica compreende 2.411 espécies de peixes, sendo 21 % correspondente a gêneros, e 45 % são espécies endêmicas da bacia (Reis *et al.*, 2017). Dentre esses peixes, o *C. macropomum*, conhecido popularmente como tambaqui, é uma espécie nativa dos rios Solimões/Amazonas, Orinoco e seus afluentes (Araújo-Lima e Gomes, 2005; Baldisserotto, 2014). Pertencente a família Serrasalminidae, ordem Characiformes e classe Actinopterygii (Araújo-Lima e Gomes, 2005), maior espécie em tamanho, da ordem dos Caracídeos, podendo atingir > 1m de comprimento total (CT) e 45 kg (Araujo-Lima e Goulding, 1997) (fig.4).



Figura 4. Juvenil de *Colossoma macropomum*. Arquivo: Pessoal

Neste meio de águas ricas em nutrientes, as temperaturas variam de 25 a 34 °C e pH 4 a 8 (Araújo-Lima e Gomes, 2005; Izel *et al.*, 2014) e embora a ocorrência de teleósteos em águas naturalmente acidificadas seja conhecida há muito tempo (Brown e Jewell, 1926; Jewell e Brown, 1924), poucos estudos fisiológicos foram (Dederen *et al.*, 1986; Freda e McDonald, 1988; Gonzalez e Dunson, 1987, 1989a; b; Mc Donald e Milligan, 1992). Todavia, muitas espécies amazônicas,

como o *C. macropomum*, migram de águas claras circuneutras (pH 6,2) para se alimentar em águas ácidas do rio Negro (pH 5,0 -5,5) e seus afluentes (pH $\leq$ 4) (Goulding, 1980; Goulding e Carvalho, 1982; Roubach e Saint-Paul, 1994; Val - Almeida e Hochachka, 1995). No rio Negro, o pH é em torno de 5,5, com níveis de Na^+ , Cl^- , Ca^{2+} geralmente inferiores a 50 mg/L, e os riachos florestais exibem rotineiramente até 2 unidades de pH mais baixos (Furch, 1984; Henderson e Walker, 1996; Val - Almeida e Hochachka, 1995).

Os valores de dureza e alcalinidade para melhor crescimento de *C. macropomum* não são determinados, porém, no estado do Amazonas, sistemas produtivos de tambaqui apresentam águas com baixíssima dureza (1 a 10 mg/L) quando não há adição de alcalinizantes nos viveiros. Quando o sistema é alcalinizado, nestas regiões, a dureza pode chegar a 40 mg/L (Baldiasserotto, 2014).

De comportamento diurno, o tambaqui, tem como preferências regiões com o clima quente e rios de águas claras com boa luminosidade (Mendonça *et al.*, 2012). Na fase larval a alimentação consiste em zooplâncton, especialmente cladóceros e copépodes (Baldiasserotto, 2014). Na fase de juvenil (comprimento total < 60 cm) passa a ser incorporado na dieta pequenas sementes e frutas, sendo as sementes mais abundantes durante a enchente, quando as plantas frutificam (Araujo-Lima e Goulding, 1997; Baldiasserotto, 2014). Os adultos se alimentam principalmente durante a enchente e cheia, e migram durante a vazante e seca (Costa, 1998), vivendo em áreas alagas restritas, até atingirem idade de 5 e 6 anos (Goulding, 1979, 1980; Goulding e Carvalho, 1982; Villacorta Correa, 1997).

Conforme descrito anteriormente, o regime das chuvas e seca promove uma alternância do ambiente, obrigando o tambaqui a explorar ampla variedade de alimentos (Rodrigues, 2014). Para se adaptar à variedade de alimentos, a espécie possui dentes molariformes, que propicia alimentar-se de frutos e sementes e numerosas cerdas brânquias, prolongadas para retenção de zooplâncton e fitoplâncton, atendendo dessa forma o hábito onívoro da espécie (Dairiki e Silva, Da, 2011; Goulding e Carvalho, 1982). Um fato curioso da ingestão de frutos e sementes é que os adultos selvagens se tornam um importante dispersor de sementes na região (Araujo-Lima e Goulding, 1997).

Na natureza, durante o período reprodutivo, o tambaqui migra em busca de local para a reprodução (Santos M.Q. *et al.*, 2013; Woynarovich, 1993; Zaniboni Filho e Barbosa, 1996). A migração ocorre entre os meses de setembro a fevereiro (Villacorta-correa e Saint-paul, 1999), e a desova acontece naturalmente com a liberação dos gametas na água, onde eles são fertilizados (Fauvel, Suquet e Cosson, 2010). Contudo, em ambientes artificiais, o tambaqui necessita da aplicação exógena de hormônio para atingir a maturação final dos ovócitos e desovar (Ihering, Von, 1937; Silva *et al.*, 1981).

2.4.3 Mecanismos adaptativos à exposição ao ar e variação de pH

Em ambientes de produção, o tambaqui apresenta bom desenvolvimento em águas com alcalinidade e dureza superior a 30 mg/L, temperaturas entre 27 e 30 °C (Izel *et al.*, 2014) e a faixa de pH entre 4,0 e 6,5 (Aride, Roubach e Val, 2007). Mas, como vários peixes de água doce, o tambaqui enfrenta uma série de desafios ambientais que exigem adaptações morfológicas, fisiológicas e comportamentais para a sobrevivência (Aride, P. H.R. *et al.*, 2016; Val e Oliveira, 2021).

A expansão labial é a adaptação morfológica mais marcante em várias espécies de peixes amazônicos em relação a variação do oxigênio no ambiente (Araújo-Lima e Gomes, 2005) (fig. 5). A função da expansão dos lábios inferiores é facilitar a captura do oxigênio na coluna d'água por meio da respiração na superfície aquática (ASR) (Val - Almeida e Hochachka, 1995). Fagundes, (2012), analisando a expressão gênica propôs que canais catiônicos são ativados para facilitar essa expansão. Entretanto, Saint-Paul, (1984) descreve que a expansão do lábio inferior do tambaqui não está diretamente relacionada com a troca gasosa, mas sim, para canalizar a água da interface com o ar, onde é mais rica em oxigênio.

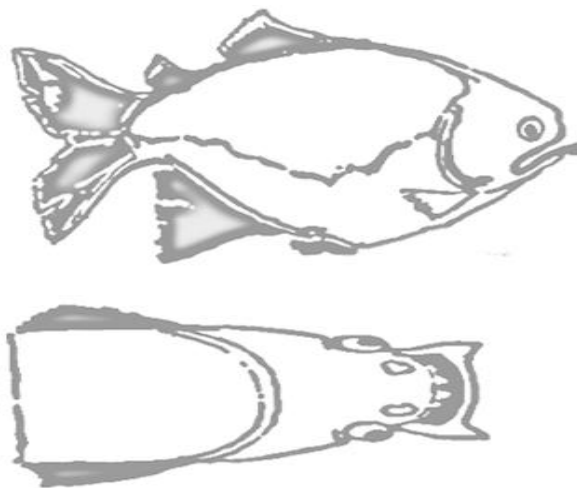


Figura 5. Desenho esquemático (vista lateral e superior) da expansão dos lábios inferiores de *Colossoma macropomum*. Fonte: Adaptada de Val e Oliveira. (2021).

A resiliência do tambaqui também é descrita em relação ao pH (Aride, Roubach e Val, 2007; Wood *et al.*, 2018). A variação de pH, especialmente em valores extremos de acidez ou alcalinidade, pode representar um desafio adicional para essa espécie. Em um estudo sobre o efeito da diminuição do pH de 6 para 3,5, Heisler (2011) observou que das três espécies estudadas (*Hoplosternum littorale*, *Brycon amazonicus* e *C. macropomum*), o *C. macropomum* foi o que apresentou menos

distúrbios de regulação iônica, e o único que se recuperou completamente ao retonar ao pH 6 (Aride, Roubach e Val, 2007) ao submeter o tambaqui a diferentes pHs (4, 6 e 8) descrevem ajuste significativos na hematologia do animal, principalmente quando exposto ao pH 8. Nesse sentido, *C. macropomum* demonstra uma considerável capacidade de tolerar variações no pH da água, especialmente em faixas que vão de 6,0 a 8,0, que são as condições de pH típicas dos habitats naturais dessa espécie (Tavares-Dias e Moraes, 2007).

Variações no pH podem interferir em múltiplos processos fisiológicos (Val - Almeida e Hochachka, 1995), como o comprometimento da osmorregulação devido o vazamento de eletrólitos através do epitélio branquial em exposição a ambientes ácidos (Henrique Rocha Aride e Luis Val, 2007). As principais respostas a situação de estresse nesses ambientes de pH entre 4,0 e 5,5 estão relacionadas a um distúrbio da ionorregulação por altos níveis externos de H^+ (Oba, Mariano e Santos, dos, 2009). A inibição da captação ativa de Na^+ e Cl^- nas brânquias, combinado com a estimulação de fluxos iônicos passivos, causa reduções nos níveis plasmáticos de Na^+ e Cl^- . A redução do pH na água está associada a acidose sanguínea que diminui os níveis de oxigênio no sangue. E com o aumento de catecolamina circulante, pode ocorrer altas taxas de ventilação branquial nos peixes (Heisler, 2011). Todavia, nas bacias amazônicas o perfil de águas ácidas é comum (Wood *et al.*, 1998) tendo como um agente estressor direto na taxa de crescimento a exposição em ambiente alcalino, como é observado em estudos com *C. macropomum* (Alves, Luz e Nakayama, 2025; Henrique Rocha Aride e Luis Val, 2007).

Estudos apontam que em pH ácido (pH até 4) as alterações internas de *C. macropomum* foram insignificantes, indicam uma maior tolerância a H^+ . O limite sugerido para o dano letal aos mecanismos de transporte branquial e/ou permeabilidade de *C. macropomum* está entre o pH 3,0 e 3,5 (Mc Donald e Wood, 1981; Wood, 1989; Wood *et al.*, 1998).

Em pH baixo, os fluxos corporais e a água externa, mudam drasticamente. Wood *et al.*, (1998) observaram que o potencial trans epitelial de *C. macropomum* aumentou de um valor altamente negativo (-23 mV) no pH 6,5, para um valor altamente positivo (+35 mV) em pH 3. A presença de altos gradientes de pressão parcial entre o sangue e água indica uma baixa capacidade de difusão branquial (Saint-Paul, 1984). A baixa capacidade de difusão branquial minimiza as perdas iônicas e a carga ionorregulatória em ambientes ácidos, pois, em condições de pH 6,5, os teleósteos de água doce têm os níveis de íons plasmáticos mais elevados. Os estudos confirmam que em espécies acidófilas os distúrbios iônicos são menores em ambientes de pH mais baixo (Mc Donald e Milligan, 1992; Wood *et al.*, 1998).

Por outro lado, estudos relatam a relação da elevação da glicose em razão da mobilização de catecolaminas isoladamente ou em combinação com o cortisol em pH 4 (Reid, Moon e Perry, 1992;

Ye, Randall e He, 1991). A ação dos glicocorticoides no organismo dos peixes, cujos níveis de cortisol no sangue são elevados, afetam às modificações fisiometabólicas, observadas por meio do aumento do número de eritrócitos e da queda no Volume Corpuscular Médio (Vosylienė, 1999DC).

Wood et al. (2018), ao expor tambaquis de diferentes tamanhos em pH 8, observaram que os tambaquis juvenis, pesando entre 4 e 15g, tiveram uma inibição inicial da taxa de excreção de amônia que é seguida por uma restauração subsequente aos níveis de controle. Todavia, autores demonstram o baixo crescimento alcançado em comparação a valores normais de crescimento para o tambaqui em ambientes alcalinos (Henrique Rocha Aride e Luis Val, 2007).

2.4 Uso da hematologia e bioquímica sanguínea como ferramenta para avaliação da homeostase

O ambiente aquático pode passar por alterações rápidas ou extremas gerando situação de estresse, devido a redução da habilidade de manutenção da homeostase dos organismos aquáticos (Oba, Mariano e Santos, dos, 2009). O termo “estressor” é empregado para descrever qualquer elemento capaz de alterar a homeostase dos peixes (Smith, 1982), e pode ser de ordem química e física do ambiente (Costa *et al.*, 2004; Urbinati e Carneiro, 2004).

Em peixes, dois eixos neuroendócrinos são acionados em condições de estresse: o eixo hipotálamo-sistema nervoso simpático - células cromafins (HSC), que resulta na liberação de catecolaminas (CAs) (adrenalina e noradrenalina), e o eixo hipotálamo-hipófise-interrenal (HHI) que libera corticosteroides (cortisol e cortisona) (McLeay, 1973). A liberação de CAs e de cortisol tem como consequências alterações metabólicas e bioquímicas como a hiperglicemia, hiperlactacemia, depleção das reservas glicogênicas, lipólise e inibição da síntese proteica (Oba, Mariano e Santos, dos, 2009).

As CAs exercem influência no sistema cardiovascular, promovendo em modificações no fluxo sanguíneo, aumento da perfusão branquial, alteração na capacidade de transporte de oxigênio pelo sangue. Essas reações fazem com que os peixes tentem manter o suprimento de oxigênio para os tecidos e restaurar o equilíbrio osmótico e iônico. Por outro lado, os corticosteroides estimulam os mecanismos de transporte iônico presentes nas brânquias e nos rins, auxiliando na manutenção da homeostase hidroeletrolítica (Oba, Mariano e dos Santos, 2009).

Essas respostas fisiológicas são observadas através de análises hematológicas e bioquímicas em peixes, permitindo acompanhar o quadro homeostático desses animais a situações de estresse (Kori-Siakpere, Ake e Idoge, 2005). No eritrograma, por exemplo, a saúde dos animais pode ser avaliada por meio das células vermelhas do sangue, identificando quadros anêmicos (Val *et al.*,

1998). As características dessas células como sua coloração vermelha, é conferida em razão do grupo prostético formado por ferro e grupamentos porfirínicos, e geralmente configurada por 96% de globinas e 4% de heme (Valero *et al.*, 2016). A quantidade e morfologia dos eritrócitos, auxiliam a compreender o estado sanitário dos peixes (Drumond *et al.*, 2010; Signor *et al.*, 2017; Tavares-Dias *et al.*, 2009).

No que se refere à bioquímica sanguínea, alterações relacionadas à má nutrição, distúrbios ionoregulatórios e processos infecciosos (Chagas *et al.*, 2005; Liebl *et al.*, 2022) em peixes, podem ser detectadas por meio da avaliação das concentrações das proteínas totais. Isso porque as proteínas totais são compostos bioquímicos importantes na constituição de tecido, incluindo o tecido sanguíneo (Oliveira *et al.*, 2017), e seu aumento pode estar associado à hemólise (Hrubec e Smith, 1999), enquanto, a sua diminuição pode ocorrer em consequência da redução da síntese proteica por disfunção hepática, do aumento da permeabilidade vascular ou da degradação proteica pelas enzimas proteolíticas que são liberadas durante processos infecciosos (Stoskopf, 1993).

Lopes, Biondo e Santos, (2007) relatam que as perdas sanguíneas ou anemias hemorrágicas em peixes, podem ser agudas ou crônicas, e que, a alteração do hematócrito mediante o estresse, ocasiona a hemoconcentração ou hemodiluição. Na hemoconcentração, há o aumento do hematócrito devido a liberação dos eritrócitos pelo baço, e na hemodiluição, há a redução nos valores do hematócrito (Tavares-Dias e Mataqueiro, 2004).

A dinâmica do fluxo sanguíneo está relacionada ao volume corpuscular médio (VCM), enquanto a hemoglobina corpuscular média (HCM) reflete a eficiência função respiratória (Houston, Robinson e Gerrard, 1990). Ambos constituem importantes indicadores do estado fisiológico dos peixes.

Vale ressaltar que, embora a hematologia seja uma ferramenta amplamente utilizada para avaliação da homeostase, é necessária uma padronização dos processos e parâmetros analisados (Castro *et al.*, 2021; Kori-Siakpere, Ake e Idoge, 2005), devido à ampla variação interespecífica observada (Tavares-Dias e Mataqueiro, 2004; Tavares-Dias e Moraes, 2007). Um exemplo dessa variabilidade é a concentração de glicose sanguínea. Estudos demonstram que a concentração de glicose depende do hábito de vida da espécie, pela capacidade locomotora (Carneiro e Amaral, 1979), pelo tipo de sistema de cultivo, parâmetros de qualidade de água, e pela dieta a que estão submetidos (Tavares-Dias *et al.*, 2009).

Villacorta-Correa e Saint-Paul (1999) descrevem que o *C. macropomum* em condições naturais, apresentam hábito alimentar onívoro e comportamento migratório. Esses autores relacionam o processo de migração da espécie a uma estratégia alimentar voltada ao acúmulo de reservas energéticas sob a forma de glicose e compostos lipídicos. Assim, o aumento ou acúmulo

de lipídicos como colesterol e triglicerídeos, juntamente com as proteínas totais e glicose, pode ser interpretado como indicativo de reservas energéticas.

3. OBJETIVOS

3.1 Objetivo Geral

Verificar a sinergia entre os parâmetros do ambiente aquático e as tecnologias de produção, utilizando diferentes faixas de pH em sistema de bioflocos e os efeitos da sincronização e dessincronização do fotoperíodo em sistema aquapônicos.

3.2 Objetivos Específicos

- ✓ Avaliar a hematologia e bioquímica sanguínea de *Colossoma macropomum*;
- ✓ Avaliar o índice hepatossomático de *C. macropomum*;
- ✓ Avaliar os parâmetros do desempenho zootécnico de *C. macropomum*;
- ✓ Avaliar as repostas fisiológicas de *C. macropomum* a um agente estressor (exposição ao ar) quando mantidos em diferentes pHs em BFT;
- ✓ Avaliar os parâmetros de qualidade da água em sistema BFT;
- ✓ Avaliação bromatológica do biofloco em BFT;
- ✓ Avaliar a qualidade de água na sincronização e dessincronização do fotoperíodo em sistemas aquapônicos;
- ✓ Avaliar a produção vegetal na sincronização e dessincronização do fotoperíodo em sistemas aquapônicos.

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CAPÍTULO 1

Artigo publicado na revista AQUACULTURE

Aquaculture 599 (2025) 742087



Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquacultureProduction of *Colossoma macropomum* in BFT using different pH ranges

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ARTICLE INFO

Keywords:

Biofloc

Water quality

Tambaqui

ABSTRACT

Colossoma macropomum is an important species produced in Latin America. Its widespread domestic and international popularity can be attributed to its high productivity, adaptability to various production systems, and desirable meat flavor. Additionally, this species can tolerate wide variations in water quality, including pH ranges. Thus, this study aimed to evaluate the water quality, zootechnical performance, and physiological conditions of *C. macropomum* and biofloc bromatology, using different pH ranges in biofloc technology (BFT). The experiment was conducted over 60 days and divided into two growth phases (Phase 1, 30 days, and Phase 2, 30 days). The treatment pH ranges used were pH 5.5–6.0, 6.5–7.0, and 7.5–8.0, denominated BFT_{5.5}, BFT_{6.5}, and BFT_{7.5}, respectively. Throughout the experimental period, the results indicated the influence of pH on water quality, particularly in the nitrification process, with higher values of TAN and NO_3^- in BFT_{5.5} ($p < 0.05$). However, the concentrations were considered safe for the species. In Phase 1, growth (final weight and total length) was higher in BFT_{5.5} ($p < 0.05$). In Phase 2, the best zootechnical performance results (weight, total length, weight gain, biomass, specific growth rate, feed conversion, and productivity) of *C. macropomum* were observed in BFT_{5.5} ($p < 0.05$). Glucose (122 ± 78.61 mg/dL) and cholesterol (114 ± 32.92 mg/dL) concentrations were higher in BFT_{5.5} ($p < 0.05$), in Phase 2. Juveniles maintained in BFT_{7.5} showed hemoconcentration in both phases of the experiment. Crude protein and ether extract of biofloc did not show differences ($p > 0.05$) between treatments. Despite BFT_{5.5} presenting the best performance results to *C. macropomum*, further studies are needed on pH ranges over the entire production cycle to monitor system dynamics in BFT, particularly the nitrification process and the zootechnical performance of *C. macropomum*. This research is essential before establishing an optimal production range for the species in BFT.

1. Introduction

Biofloc technology (BFT) is based on the principle of more sustainable water use, which is made possible through nutrient cycling performed by microorganisms in the floc (Khanjani et al., 2024a). However, BFT requires strict water quality control, with pH being one of its critical parameters (Khanjani et al., 2024b). The challenge of maintaining the ideal water pH is linked to achieving higher efficiency in bacterial activation (Bock and Wagner, 2006; Gieseke et al., 2006; Villaverde et al., 1997). On the other hand, the rapid growth rate of bacteria and other microorganisms in this system results in high oxygen consumption and CO_2 production, leading to medium acidification (Azim and Little, 2008; Hargreaves, 2013; Martins et al., 2019). Consequently, pH influences the chemical equilibrium of the water, altering ammonia toxicity, the nitrification process, and fish homeostasis (Arana, 2004; Golombieski et al., 2013; Hargreaves, 2013).

Although the occurrence of teleost in naturally acidified waters has been known for a long time (Brown and Jewell, 1926; Jewell and Brown, 1924), few physiological studies have been conducted in low pH waters (< 5.5) (Dederen et al., 1986; Freda and McDonald, 1988; Gonzalez and Dunson, 1989a, 1989b; Gonzalez and Dunson, 1987; McDonald and Milligan, 1992). However, these studies confirm that in acidophilic species, ionic disturbances are less severe in lower pH environments. In fish, water pH between 4.0 and 5.5 results in ionoregulation disturbances due to high external concentration of H^+ (Oba et al., 2009). Among these disturbances is the inhibition of active Na^+ and Cl^- uptake in the gills, combined with the stimulation of passive ionic fluxes, causing reductions in plasma Na^+ and Cl^- concentrations (Wood et al., 1998). This effect results in decreased blood volume and increased hematocrit and plasma proteins. The combination of high blood viscosity and low blood volume leads to circulatory insufficiency, which can be fatal for the fish (Milligan and Wood, 1982). In *Salmo gairdneri*

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Received 12 September 2024; Received in revised form 11 December 2024; Accepted 25 December 2024

Available online 27 December 2024

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Production of *Colossoma macropomum* in BFT using different pH ranges

Abstract

Colossoma macropomum is an important species produced in Latin America. Its widespread domestic and international popularity can be attributed to its high productivity, adaptability to various production systems, and desirable meat flavor. Additionally, this species can tolerate wide variations in water quality, including pH ranges. Thus, this study aimed to evaluate the water quality, zootechnical performance, and physiological conditions of *C. macropomum* and biofloc bromatology, using different pH ranges in biofloc technology (BFT). The experiment was conducted over 60 days and divided into two growth phases (Phase 1, 30 days, and Phase 2, 30 days). The treatment pH ranges used were pH 5.5–6.0, 6.5–7.0, and 7.5–8.0, denominated BFT_{5.5}, BFT_{6.5}, and BFT_{7.5}, respectively. Throughout the experimental period, the results indicated the influence of pH on water quality, particularly in the nitrification process, with higher values of TAN and NNO_2^- in BFT_{5.5} ($p < 0.05$). However, the concentrations were considered safe for the species. In Phase 1, growth (final weight and total length) was higher in BFT_{5.5} ($p < 0.05$). In Phase 2, the best zootechnical performance results (weight, total length, weight gain, biomass, specific growth rate, feed conversion, and productivity) of *C. macropomum* were observed in BFT_{5.5} ($p < 0.05$). Glucose (122 ± 78.61 mg/dL) and cholesterol (114 ± 32.92 mg/dL) concentrations were higher in BFT_{5.5} ($p < 0.05$), in Phase 2. Juveniles maintained in BFT_{7.5} showed hemoconcentration in both phases of the experiment. Crude protein and ether extract of biofloc did not show differences ($p > 0.05$) between treatments. Despite BFT_{5.5} presenting the best performance results to *C. macropomum*, further studies are needed on pH ranges over the entire production cycle to monitor system dynamics in BFT, particularly the nitrification process and the zootechnical performance of *C. macropomum*. This research is essential before establishing an optimal production range for the species in BFT.

Keywords: Biofloc; Water quality; Tambaqui

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achieving higher efficiency in bacterial activation (Bock and Wagner, 2006; Gieseke et al., 2006; Villaverde et al., 1997). On the other hand, the rapid growth rate of bacteria and other microorganisms in this system results in high oxygen consumption and CO₂ production, leading to medium acidification (Azim and Little, 2008; Hargreaves, 2013; Martins et al., 2019). Consequently, pH influences the chemical equilibrium of the water, altering ammonia toxicity, the nitrification process, and fish homeostasis (Arana, 2004; Golombieski et al., 2013; Hargreaves, 2013).

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Many Amazonian species, such as *C. macropomum*, migrate from circumneutral waters (pH 6.2) to the acidic waters of the Rio Negro (pH 5.0–5.5) and its tributaries (pH ≤ 4) for feeding and reproduction (Furch, 1984; Goulding, 1980; Goulding and Carvalho, 1982; Henderson and Walker, 1996; Roubach and Saint-Paul, 1994; Val-Almeida and Hochachka, 1995), where the pH tends to be up to 2 units lower (Furch, 1984; Val-Almeida and Hochachka, 1995). In captivity, *C. macropomum* shows better growth in acidic waters, with a pH between 4 and 6 (Aride et al., 2007). Wood et al. (1998) reported that exposure of *C. macropomum* to a pH of 3 maintained unchanged hematocrit and hemoglobin concentrations. However, little is known about their

ionoregulatory, hematological, and blood-biochemical functions in acidic environments (Saint-Paul, 1984).

Given the above, the importance of pH for the functioning of the BFT system, the composition of biofloc, zootechnical performance, and hematological responses in fish is evident. Therefore, the objective of the present study was to evaluate the effects of different pH ranges in BFT on water quality, biofloc bromatological analysis, zootechnical performance, and physiological conditions for *C. macropomum*.

2. Material na methods

The study was conducted at the Aquaculture Laboratory (LAQUA) of the Federal University of Minas Gerais (UFMG) in Brazil, following the approval of the protocol by the Animal Use Ethics Committee (CEUA – 95/2022). The experiment was divided into two growth phases (Phase 1 and Phase 2), totaling 60 days.

2.1 Biofloc maturation process and water pH adjustment

The biofloc system were compound by three independent tanks (each with a working volume of 800 L), wich were filled with water from an artesian well. Each tank contained 11 juvenile of *C. macropomum* weighing approximately 139.61 g (density of 13.75 fish/m³–1.91 kg/m³) sourced from the Larviculture sector (LAQUA - UFMG). The juveniles were fed three times daily (9 AM, 12 PM, and 3 PM) with a commercial diet (32 % protein, 6 % ether extract, 4–6 mm – Aquos Tropical 32) fixed at 76.5 g per tank. For aeration and water movement, the tanks were equipped with porous hoses connected to a radial compressor (2HP - IBRAM Brazil) and thermostatic heaters (500 W - Ocean Tech) to maintain the temperature at ~28 °C. An organic carbon source (corn starch) was added at C:N ratio (20:1) when the total ammonia nitrogen (TAN) reached 0.5 mg/L (adapted from Avnimelech, 2015) to produce biofloc. After 30 days, the BFT systems were considered mature when the nitrite reached a safe level for the fish (< 0.5 mg/L) (Da Costa et al., 2004). The photoperiod was maintained at 12 h of light and 12 h of darkness.

The pH adjustment was done concurrently with the biofloc maturation process within the following treatment ranges: pH 5.5–6.0; 6.5–7.0; 7.5–8.0, denominated BFT_{5.5}, BFT_{6.5}, and BFT_{7.5}, respectively. In the BFT_{5.5} and BFT_{6.5} treatments, the pH ranges reached their respective ranges during the biofloc maturation without needing external acidification (Ebeling et al., 2006, Martins et al., 2017). However, in BFT_{7.5}, sodium bicarbonate (Na₂HCO₃) had to be added. Once the proposed pH ranges were achieved, the water from each tank was transferred to the respective systems for the experimental design.

2.2 Experimental design

The experimental design consisted of three pH treatments, each with four replicates. Three biofloc systems with water recirculation were utilized, following the method described by Wasielesky et al. (2006). Each system comprised four tanks (each with 80 L) and a sump tank (250 L) for water homogenization (Fig. 1). In the sump tank (Fig. 1-A), two thermostat heaters (500 W - Ocean Tech) and a submersible pump (3700 L/h, Aquarium) were installed to distribute water (Fig. 1-D) to the four replicate tanks (Fig. 1-B). For aeration (Fig. 1-C), all tanks were equipped with porous hoses connected to a radial compressor (2 CV - IBRAM). A photoperiod of 12 h of light and 12 h of darkness was maintained throughout the experimental period.

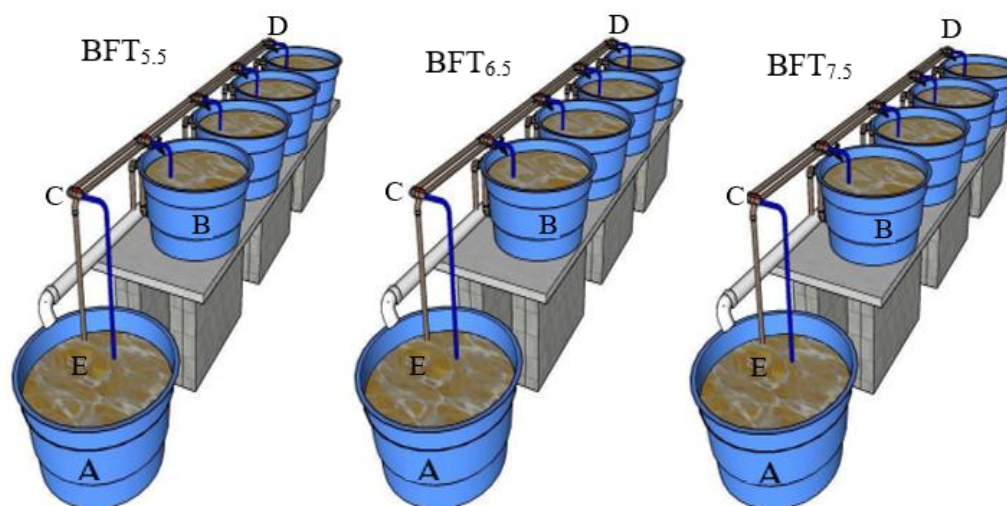


Figure 1. Three experimental biofloc units with water recirculation to produce *Colossoma macropomum* under different water pH ranges (BFT_{5.5}, pH 5.5–6.0; BFT_{6.5}, pH 6.5–7.0; and BFT_{7.5}, pH 7.5–8.0). A – sump, B – replicate tanks, C – aeration system, D – water inlet, E – water outlet.

2.3 Juvenile growth

Phase 1

The 288 juveniles of *C. macropomum* (3.93 ± 0.02 g; 6.69 ± 0.93 cm) were distributed at a density of $n = 24$ juveniles/tank (1.18 kg/m³) and fed three times a day (9 h, 12 h, and 15 h) with a commercial diet containing 45 % crude protein (45 % crude protein, 8 % ether extract, 2–3 mm, Aquos 45 – Total) at 5 % of the biomass, without leftovers collection, for 30 days.

Phase 2

In this phase, the density was adjusted to $n = 12$ juveniles/tank (2.15 kg/m^3) from Phase 1. The juveniles were fed three times a day (9 h, 12 h, and 15 h) during 30 days with a commercial diet containing 32 % protein (32 % crude protein, 6 % ether extract, 4 to 6 mm, Aquos Tropical 32), at 5 % of the biomass, with leftover feed collected for drying in an oven (65°C) 30 min after its supplying.

2.4 Water quality

Temperature, dissolved oxygen (DO - using an AK87 probe Brazil), and pH (pH meter – AK90 Brazil) were measurement daily. Analyses of settleable solids (SS) (using an Imhoff cone –15 min - Avnimelech, 2015), total ammonia nitrogen (TAN) ($\text{NH}_3 + \text{NH}_4^+$) (APHA, 2017), and nitrite (NO_2^-) (Aminot and Chaussepied, 1983) were performed three times per week. Ionized ammonia (NH_3) was calculated using Boyd and Tucker's formula (1998), where T = temperature:

$$\text{Amônia ionizada} = \frac{\text{Amônia total}}{1 + \log \{0,09018 + [2729,92/(273,15 + T)] - \text{pH}\}}$$

Alkalinity and hardness (APHA, 2017) were measured once a week. Nitrate (NO_3^-) (Monteiro et al., 2003), total suspended solids (TSS) (Eaton et al., 1995), and floc volume index (FVI, = SS/TSS, mL/g) (Yousuf, 2013) were conducted at the beginning and end of the experimental period. For solids control, when SS exceeded 20 mL/L , a clarification process was performed (Gaona et al., 2016). Ions (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , and PO_4^{3-} , mg/L) were analyzed in ionic chromatographer (Metrohm ECO IC1), the samples of water were collected at the end of each experimental phase.

When necessary, the pH was adjusted using sodium bicarbonate (Na_2HCO_3).

2.5 Zootechnical performance

The juveniles of *C. macropomum* were weighed using a precision balance (0.01 g; AD5002 Marte Brazil) and counted every 15 days throughout the experimental period (Phase 1 and Phase 2).

The evaluation of zootechnical performance was conducted using the following formulas:

a- Weight Gain (g) = Final weight – Initial weight;

b- Apparent Feed Conversion Ratio (AFCR) = Feed offered / Weight gain (Phase 1) and Feed Conversion Ratio (FCR) = Feed consumed / Weight gain (Phase 2);

c- Specific Growth Rate (SGR) (%/day) = $[(\ln \text{Final weight} - \ln \text{Initial weight}) / \text{time}] \times 100$;

d- Biomass (g) = Total number of fish $\times$ final weight;

e- Productivity (kg/m^3) = Biomass / Tank volume in m^3 ;

f- Survival Rate (%) = (Number of fish at the end of the experiment / Initial number of fish) $\times 100$.

2.6 Blood analyses

Blood samples from 12 juveniles were collected via caudal puncture at the end of each experimental phase using insulin syringes (1 mL – Solidor) containing sodium heparin (5000 I.U./mL – Hepamax-S). Blood aliquots were used for hematocrit determination via the Micro-Hematocrit method with capillary tubes (Goldenfarb et al., 1971). For hemoglobin analysis, 10 μL of blood was added and homogenized in 2 mL of colorimetric reagent (Bioclin® Brazil). Plasma protein was determined via plasma readings on a manual refractometer (RHC 200-ATC, Huake Instrument Co China). Biochemical analyses (glucose, triglycerides, and cholesterol) were performed by centrifuging blood at 4000 rpm for 10 min to remove plasma, and their concentrations were determined using commercial kits (Bioclin® Brazil). All sample readings were performed on a spectrophotometer (Bioclin® 100 - Quibasa LTDA Brazil).

A 10 μL aliquot of whole blood was diluted in 2.0 mL of formalin-citrate solution to quantify erythrocytes. A biological microscope (BX51, Olympus) helped quantify them in a Neubauer chamber ($\times 10^6 \mu\text{L}$). Hematometric indices, related to mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were calculated from the equations of Brown and Wu (1976), where Ht corresponds to hematocrit, Hb to hemoglobin, and RBC (Red Blood Cells) to erythrocytes:

$$\text{MCV} = (\text{Ht} \times 10) / \text{RBC}$$

$$\text{MCH} = (\text{Hb} \times 10) / \text{RBC}$$

$$\text{MCHC} = (\text{Hb} \times 10) / \text{Ht}$$

2.7 Hepatosomatic index

For the evaluation of the hepatosomatic index, after blood collection, the fish were euthanized with 285 mg of eugenol/ L (Mattioli et al., 2017), and the liver samples were collected ($n = 12$ juveniles per treatment). The hepatosomatic index was calculated using the formula: Hepatosomatic Index (HSI) (%) = (liver weight / body weight) $\times 100$.

2.8 Bromatological analyses of biofloc

The bromatological analyses of the bioflocs was conducted only at the end of Phase 2 (after 60 days of cultivation). Biofloc samples were collected via decantation, dried in a digital oven

(65 °C – Nova Ética), and stored at –20 °C for later analysis. The determination of dry matter, ash, ether extract, and crude protein was performed according to the Association of Official Analytical Chemists (AOAC, 2009). Dry matter was determined by drying samples at 105 °C until constant weight; ash content by incineration in a muffle furnace at 600 °C for 4 h; ether extract by extraction with petroleum ether and ethyl ether in a Soxtec System HT apparatus (after acid hydrolysis); and crude protein by measuring nitrogen ($N \times 6.25$) using the Kjeldahl method.

2.9 Statistical analyses

The data were analyzed for normality and homoscedasticity. When the assumptions for Analyses of Variance (ANOVA) were met post hoc Duncan test was applied. Non-parametric data were analyzed by Kruskal-Wallis test. All test used 5 % significance level. Percentage data were transformed using the arcsine of the square root. Only non-transformed data are presented.

3. Results

Phase 1

3.1.1.1. Water quality

Water quality parameters showed significant differences (TAN, NH_3 , NO_2^- , pH, alkalinity, TSS, and SS; $p < 0.05$), except for NO_3^- , hardness, and FVI. TAN and NO_2^- concentrations were higher in BFT_{5.5} ($p < 0.0001$) (tabela 1). NH_3 ($p < 0.0001$), pH ($p < 0.0001$), and alkalinity ($p = 0.0013$) were lower in BFT_{5.5}. For TSS ($p = 0.002$) and SS ($p = 0.0162$), the lowest values were in BFT_{7.5}. Due to sample issues, hardness was measured only once, indicating 200, 230, and 150 mg of CaCO_3/L in treatments BFT_{5.5}, BFT_{6.5}, and BFT_{7.5}, respectively. At the start of the experiment, there were no SS; therefore, FVI records refer to the end of Phase 1 and showed no differences among treatments ($p > 0.05$). The ions, only PO_4^{3-} was different ($p = 0.0276$) and lower in BFT_{7.5}.

Table 1. Mean and standard deviation ($\pm$) of water quality parameters (TAN, NH_3 , NO_2^- , NO_3^- (mg/L), pH, alkalinity (mg CaCO_3/L), TSS (mg/L), SS (mL/L), ions (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , and PO_4^{3-} , mg/L) in the production of *Colossoma macropomum* in a BFT system with different pH ranges during Phase 1.

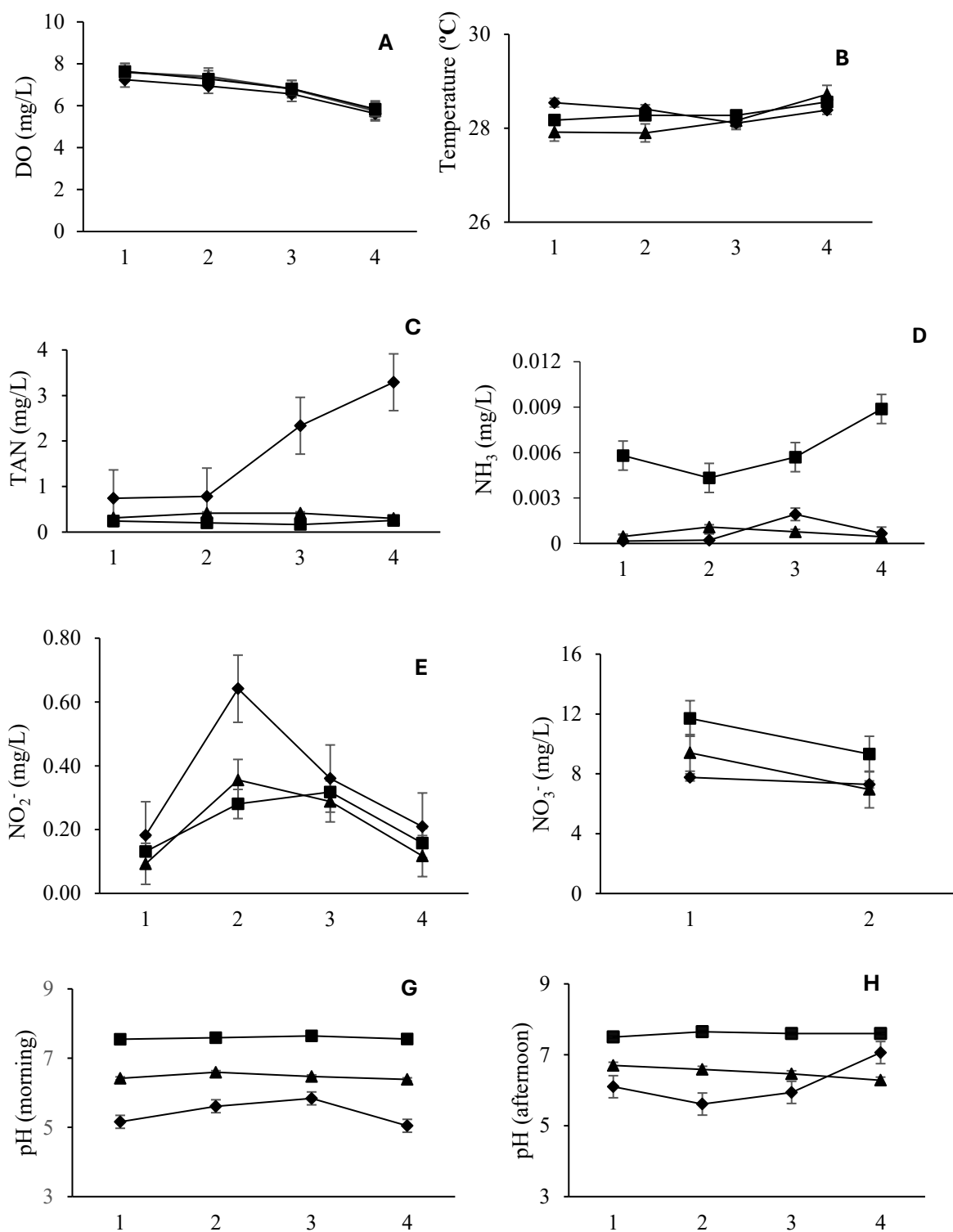
pH			
BFT _{5.5}	BFT _{6.5}	BFT _{7.5}	P value

Temperature (°C)	28.36±0.48 ^a	28.19±0.45 ^b	28.33±0.41 ^a	0.0023
TAN (mg/L)	2.54±2.63 ^a	0.34±0.11 ^b	0.25±0.10 ^c	<0.0001
NH ₃ (mg/L)	0.00061±0.00083 ^b	0.00064±0.0004 ^b	0.01±0.0081 ^a	<0.0001
NO ₂ ⁻ (mg/L)	0.45±0.68 ^a	0.22±0.26 ^b	0.24±0.26 ^{ab}	0.0225
NO ₃ ⁻ (mg/L)	7.29±1.58	6.95±0.35	9.32±0.01	-
pH	5.40±0.42 ^c	6.47±0.17 ^b	7.58±0.14 ^a	<0.0001
Alkalinity (mg CaCO ₃ /L)	24.38±22.59 ^b	25.0±7.76 ^b	65.0±21.04 ^a	0.0013
TSS (mg/L)	158.00±13.86 ^{ab}	198.33±21.177 ^a	96.67±26.94 ^b	0.0002
SS (mL/L)	17.44±15.15 ^a	7.83±8.39 ^{ab}	3.38±1.56 ^b	0.0162
FVI (mL/g)	42.85±49.55	11.13±13.08	14.39±19.35	-
*Na ⁺ (mg/L)	58.47±14.91	79.98±0.29	88.36±10.87	-
*K ⁺ (mg/L)	65.30±37.23	87.29±34.37	71.78±17.92	-
*Ca ⁺² (mg/L)	77.93±11.30	70.54±2.16	57.62±1.22	-
*Mg ⁺² (mg/L)	8.99±2.79	10.4±0.14	10.45±2.78	-
*Cl ⁻ (mg/L)	52.26±17.99	35.09±0.18	44.62±1.68	-
*PO ₄ ⁻³ (mg/L)	49.86±5.41 ^a	42.42±0.17 ^a	28.25±4.36 ^b	0.0276

Letters on the same line represent significant differences according to ANOVA Duncan* test or non-parametric Kruskal-Wallis test, ($p < 0.05$). BFT_{5.5}, pH 5.5-6.0; BFT_{6.5}, pH 6.5-7.0; and BFT_{7.5}, pH 7.5-8.0.

DO curves across all treatments were similar, ranging from 6.55 ± 1.09 to 6.83 ± 1.21 mg/L (Fig. 2A) ($p > 0.05$). The temperature (28.29 ± 0.45 °C) remained stable across all treatments throughout the experimental period (Fig. 2B) ($p < 0.05$). From week 1, the concentrations of TAN (Fig. 2C) in BFT_{5.5} were higher ($p < 0.05$), reaching the highest concentration (3.2 mg/L) at the end of the experimental period (week 4). Conversely, NH₃ concentrations in BFT_{7.5} were higher ($p < 0.05$) throughout the period. BFT_{5.5} exhibited higher NO₂⁻ concentrations (0.64 mg/L) in week 2 (Fig. 2E) ($p < 0.05$). NO₃⁻ concentration showed a reduction until the end of this phase, with no difference between treatments ($p > 0.05$) (Fig. 2F). The pH values (morning and afternoon) remained within the proposed treatment ranges (Fig. 2G and H) ($p < 0.05$). In BFT_{7.5}, alkalinity was higher compared to the other treatments throughout Phase 1 (Fig. 2I). From week 2, there was an increase in SS in all treatments, with the highest SS values observed in week 4 for BFT_{5.5} and BFT_{6.5} ($p < 0.05$) (Fig. 2J). TSS showed higher values ($p < 0.05$) in BFT_{6.5} (198.33 mg/L) (Fig. 2K).

FVI did not show difference ($p > 0.05$), but a gradual increase was observed in all treatments (Fig. 2L).



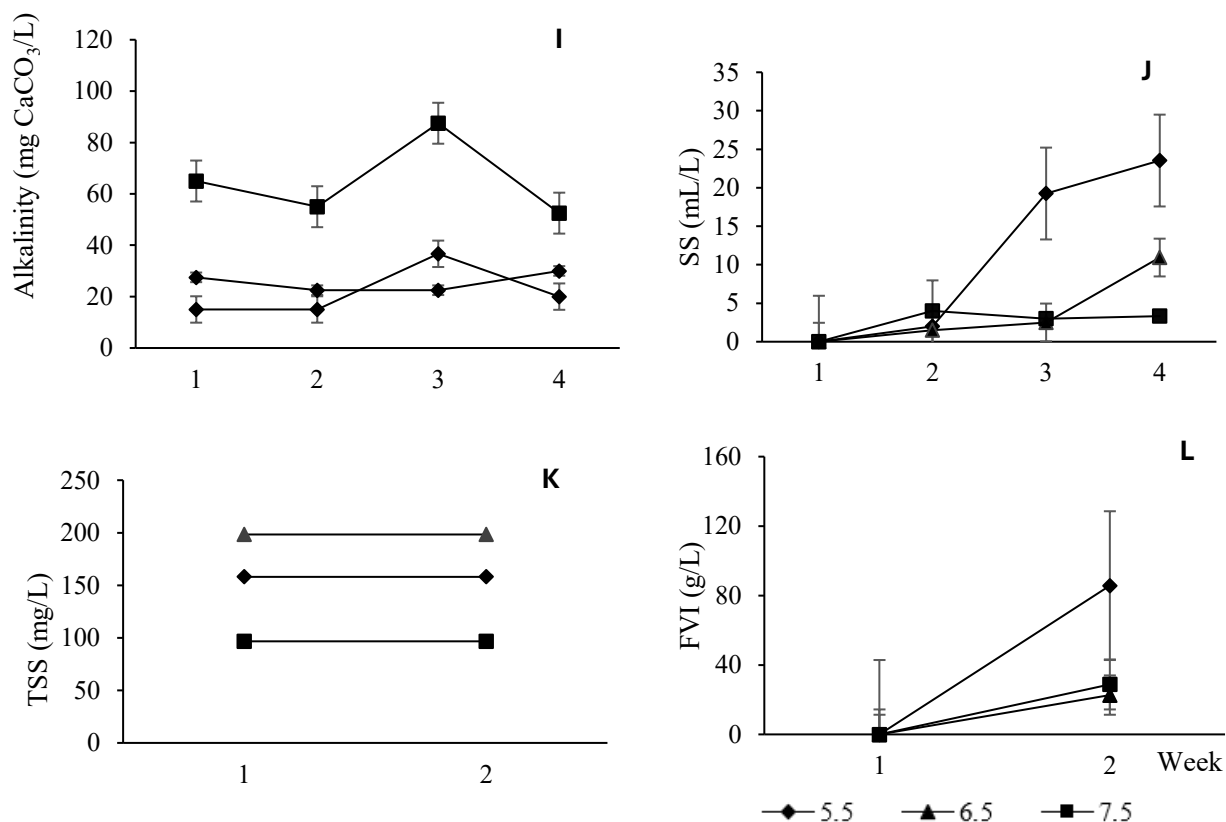


Figure 2. Temporal curves of water quality parameters. A- DO (mg/L), B- Temperature (°C), C- TAN (mg/L), D- NH₃ (mg/L), E- NO₂⁻ (mg/L), F- NO₃⁻, G- pH (morning), H- pH (afternoon), I- Alkalinity (mg CaCO₃/L), J- SS (mL/L), K- TSS (mg/L) and L- FVI (g/L), on week. Production of *Colossoma macropomum* juveniles in BFT systems using different ranges of pH (BFT_{5.5}, pH 5.5-6.0; BFT_{6.5}, pH 6.5-7.0 and BFT_{7.5}, pH 7.5-8.0) Phase 1. Mean ± SD.

3.1.1.2. Zootechnical performance

The zootechnical performance data of *C. macropomum* showed differences for final weight ($p = 0.0031$) and final total length ($p = 0.0034$), with the highest values in BFT_{5.5} (Table 2). Weight gain, biomass, SGR, feed ration, AFCR, production, and survival rate did not show statistical differences ($p > 0.05$).

Table 2. Zootechnical performance parameters (final weight, final length, weight gain, biomass, SGR, feed ration, AFCR, productivity, and survival rate) of *Colossoma macropomum* in the BFT system at different pH ranges, after 30 days of cultivation (end of Phase 1).

pH			
BFT _{5.5}	BFT _{6.5}	BFT _{7.5}	P value

Final weight (g)	15.78±5.46 ^a	13.79±5.28 ^b	13.53±4.88 ^b	0.0031
Final length (cm)	10.34±1.44 ^a	10.08±2.18 ^b	9.73±1.18 ^b	0.0034
Weight gain (g)	11.38±1.26	8.88±1.82	9.84±0.68	-
Biomass (g)	360.75±38.80	300.45±40.73	314.78±15.13	-
SGR (%/day)	4.60±0.57	4.17±0.71	4.67±0.15	-
Feed ration (g)	226.17±35.42	188.90±10.86	179.34±10.11	-
AFCR	0.89±0.25	0.91±0.16	0.77±0.11	-
Productivity (kg/m ³)	4.51±0.49	3.76±0.51	3.93±0.19	-
Survival rate (%)	96.88±3.99	98.88±3.99	98.96±2.08	-

Different letters in the same row represent significant differences by the Kruskal-Wallis non-parametric test ($p < 0.05$). BFT_{5.5}, pH 5.5-6.0; BFT_{6.5}, pH 6.5-7.0; and BFT_{7.5}, pH 7.5-8.0.

3.1.1.3 Blood analyses

In the blood analyses of juvenile *C. macropomum*, statistical differences were observed ($p < 0.05$), as shown in Table 3. The highest concentrations of plasma protein (4.13 ± 0.16 g/dL), glucose (81.08 ± 17.11 mg/dL), mean corpuscular hemoglobin (MCH) (87.34 ± 17.09 pg), and mean corpuscular hemoglobin concentration (MCHC) (37.61 ± 10.32 g/dL) were found in the BFT_{5.5} treatment group. Hematocrit was higher in the BFT_{7.5} group ($29.40 \pm 7.81\%$). Hemoglobin, triglycerides, cholesterol, erythrocytes, and mean corpuscular volume (MCV) did not show significant differences among the treatments ($p > 0.05$).

Table 3 . Blood analyses (plasma protein, hematocrit, hemoglobin, glucose, triglycerides, cholesterol, erythrocytes, MCV, MCH, MCHC) of *Colossoma macropomum* produced in BFT system with different pH ranges after 30 days of cultivation (end of Phase 1).

	pH			P-value
	BFT _{5.5}	BFT _{6.5}	BFT _{7.5}	
Plasma Protein (g/dL)	4.13±0.16 ^a	3.98±0.21 ^b	3.9±0.22 ^b	0.0358
Hematocrit (%)	20.63±5.34 ^b	23.11±9.48 ^{ab}	29.40±7.81 ^a	0.0358
Hemoglobin (g/L)	7.62±2.34	6.01±1.41	6.86±1.35	-
Glucose (mg/dL)	81.08±17.11 ^a	48.34±12.80 ^b	50.16±12.13 ^b	0.0001
Triglycerides (mg/dL)	125.17±42.16	116.87±44.80	117.67±36.62	-
Cholesterol (mg/dL)	69.50±10.81	66.85±11.76	64.36±11.38	-
Erythrocytes (RBC uL/10 ⁶)	0.90±0.27	0.98±0.30	1.08±0.36	-
MCV (fL)	238.89±44.29	233.49±130.04	264.03±125.0	-
MCH (pg)	87.34±17.09 ^a	73.09±46.93 ^b	67.20±17.53 ^b	0.0236
MCHC (g/dL)	37.61±10.32 ^a	28.05±12.18 ^b	26.16±8.56 ^b	0.0366

Different letters in the same row represent significant differences by the Kruskal-Wallis non-parametric test ($p < 0.05$). BFT_{5.5}, pH 5.5-6.0; BFT_{6.5}, pH 6.5-7.0; and BFT_{7.5}, pH 7.5-8.0.

Phase 2

3.1.2.1. Water quality

The highest values of TAN and hardness were found in BFT_{5.5} (Table 4). NH₃ and pH were lower in BFT_{5.5} ($p < 0.0001$). Alkalinity was higher in BFT_{7.5} ($p < 0.05$). No differences were observed for NO₃⁻, TSS, SS, and FVI ($p > 0.05$). Ca²⁺, Mg²⁺, Cl⁻, and PO₄³⁻ were higher in BFT_{5.5} ($p < 0.05$).

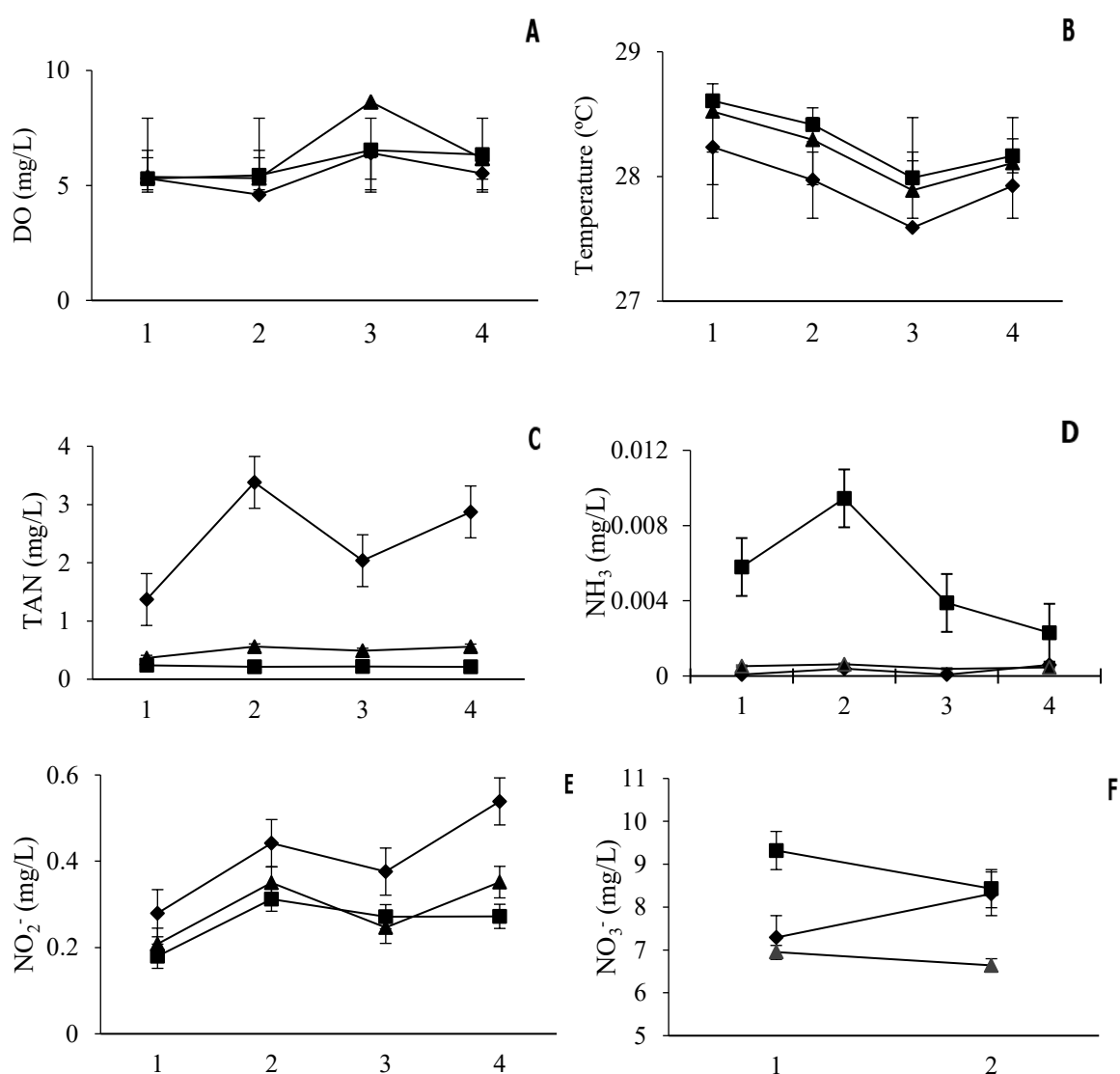
Table 4. Water quality parameters (temperature °C, TAN - mg/L, NH₃ mg/L, NO₂⁻ mg/L, NO₃⁻ mg/L, pH, alkalinity mg CaCO₃/L, hardness mg CaCO₃/L, TSS mg/L, SS mL/L, FVI mL/g, and ions Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, and PO₄³⁻, (mg/L)) in *Colossoma macropomum* production in a BFT system with different pH ranges during Phase 2.

	pH			P value
	BFT _{5.5}	BFT _{6.5}	BFT _{7.5}	
Temperature (°C)	27.93±0.62 ^b	28.20±0.60 ^a	28.29±0.45 ^a	<0.0001
TAN (mg/L)	2.55±2.04 ^a	0.48±0.18 ^b	0.22±0.07 ^c	<0.0001
NH ₃ (mg/L)	0.00023±0.00032 ^c	0.00052±0.00032 ^b	0.01±0.0046 ^a	<0.0001
NO ₂ ⁻ (mg/L)	1.85±1.7 ^a	0.43±0.21 ^b	0.20±0.10 ^c	0.0045
NO ₃ ⁻ (mg/L)	8.31±0.01	6.64±0.01	8.43±1.11	-
pH	5.07±0.47 ^c	6.13±0.33 ^b	7.50±0.23 ^a	<0.0001
Alkalinity (mg CaCO ₃ /L)	8.75±6.94 ^b	20.63±6.23 ^b	73.13±13.55 ^a	<0.0001
Hardness	314.29±83.24 ^a	222.50±37.27 ^b	187.50±38.08 ^b	0.0001
TSS (mg/L)	357.33±232.31	273.34±87.90	235.0±162.33	-
SS (mL/L)	17.83±16.57	19.12±11.73	17.66±9.60	-
FVI (mL/g)	70.02±8.35	85.10±49.48	68.84±30.60	-
*Na ⁺ (mg/L)	158.36±29.20	117.10±27.64	145.18±8.92	-
*K ⁺ (mg/L)	141.37±35.28	88.09±19.25	106.81±5.41	-
*Ca ²⁺ (mg/L)	128.13±14.34 ^a	81.43±6.10 ^b	59.50±5.75 ^b	0.0124
*Mg ²⁺ (mg/L)	18.19±2.31 ^a	10.95±2.23 ^b	11.35±0.24 ^b	0.0497
*Cl ⁻ (mg/L)	124.07±24.75 ^a	58.85±13.79 ^b	70.71±5.79 ^b	0.0567
*PO ₄ ³⁻ (mg/L)	115.13±8.55 ^a	73.45±4.91 ^b	48.95±6.13 ^c	0.0051

Letters on the same line represent significant differences according to ANOVA Duncan* test or non-parametric Kruskal-Wallis test, ($p < 0.05$). BFT_{5.5}, pH 5.5-6.0; BFT_{6.5}, pH 6.5-7.0; and BFT_{7.5}, pH 7.5-8.0.

Figure 3 presents the water quality data. DO concentrations followed a similar pattern for all treatments, despite an increase in week 3 (Fig. 3A) ($p > 0.05$). Temperature (28.14 ± 0.56 °C) remained stable across all treatments (Fig. 3B) ($p > 0.05$). TAN (Fig. 3C) reached a maximum

concentration of 3.3 mg/L in week 2 in BFT_{5.5} ($p < 0.05$). NH_3 increased by 0.009 mg/L in BFT_{7.5} (Fig. 3D). The highest concentration of NO_2^- (0.54 mg/L) was observed in week 4 in BFT_{5.5} (Fig. 3E) ($p < 0.05$). NO_3^- (Fig. 3F) showed no difference during this experimental phase ($p > 0.05$). The pH (morning and afternoon) remained within the proposed range in all treatments ($p < 0.05$) (Fig. 3G and 3H, respectively). Alkalinity remained higher in BFT_{7.5} throughout the weeks (Fig. 3I) ($p < 0.05$). Fig. 3J shows an increase in total hardness across all treatments, with the highest values at the end of the experimental period for BFT_{5.5} ($p < 0.05$). SS curves show variations during the experimental weeks, with higher SS at the end of week 4 in BFT_{5.5} (Fig. 3K) ($p < 0.05$). TSS increased in all treatments (Fig. 3L) but without differences among the treatments ($p > 0.05$). FVI did not show statistical differences among the treatments ($p > 0.05$) (Fig. 3M).



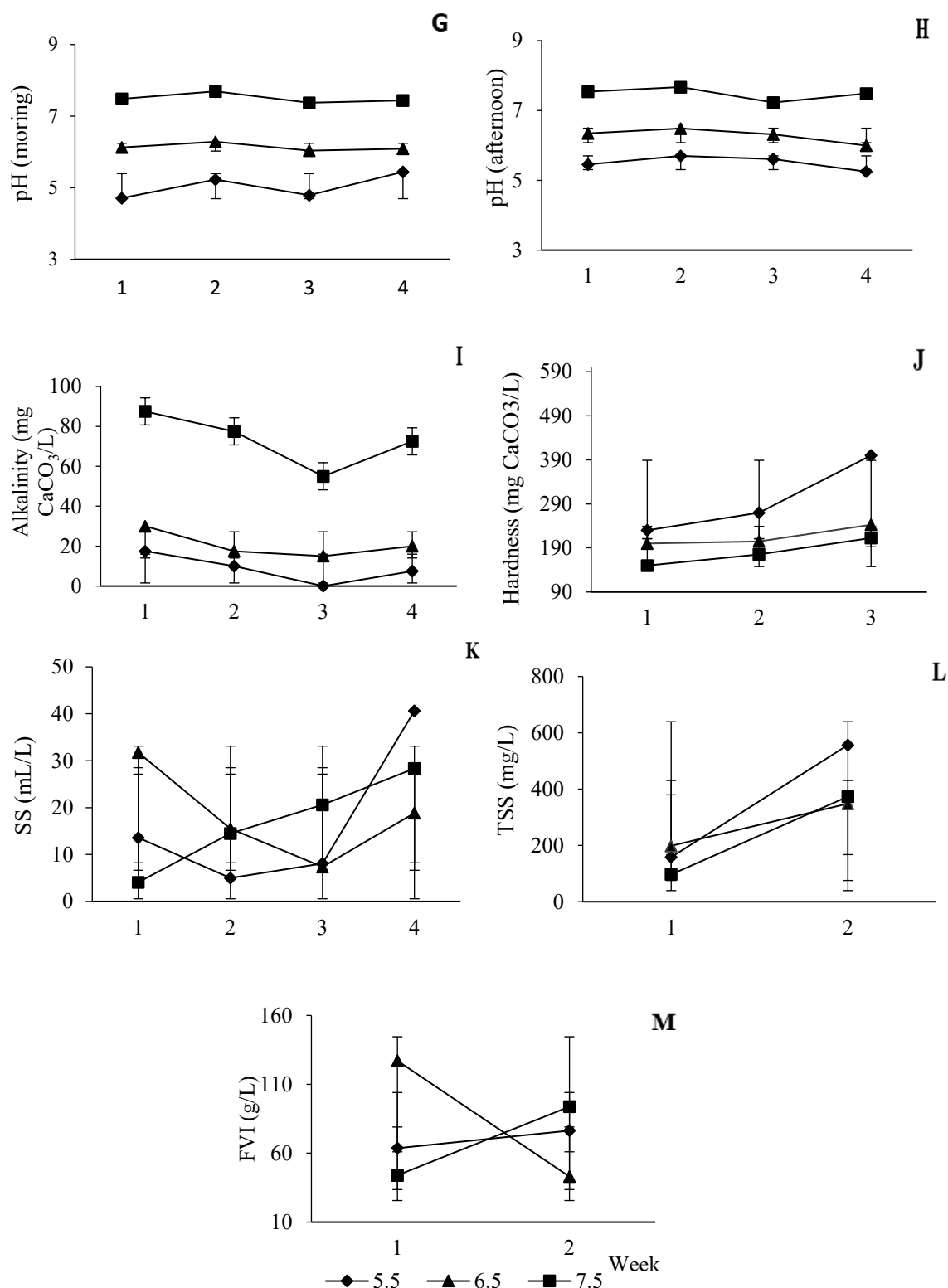


Figure 3. Temporal curves of water quality parameters. A- DO (mg/L), B- Temperature (°C), C- TAN (mg/L), D- NH₃ (mg/L), E- NO₂⁻ (mg/L), F- NO₃⁻, G- pH (morning), H- pH (afternoon), I- Alkalinity (mg CaCO₃/L), J- Hardness (mg CaCO₃/L), K- SS (mL/L), L- TSS (mg/L) and M- FVI (g/L), on week, during production of *Colossoma macropomum* juveniles in BFT systems using

different ranges of pH (BFT_{5.5}, pH 5.5-6.0; BFT_{6.5}, pH 6.5-7.0 and BFT_{7.5}, pH 7.5-8.0). Phase 2. Mean $\pm$ SD.

3.1.2.2. Zootechnical performance

The zootechnical performance data for *C. macropomum* (Table 5) showed the highest values for final weight, final total length, weight gain, biomass, SGR (3.97 ± 0.43 %/day), feed intake, and productivity in BFT_{5.5} ($p < 0.05$). The FCR was lower in BFT_{5.5} ($p < 0.05$). The survival rate did not differ among treatments ($p > 0.05$).

Table 5. Zootechnical performance parameters (final weight, final length, weight gain, biomass, SGR, feed ration, FCR, productivity, and survival rate) of *Colossoma macropomum* in the BFT system at different pH ranges, after 30 days of cultivation (end of Phase 2).

	pH			P value
	BFT _{5.5}	BFT _{6.5}	BFT _{7.5}	
Final weight (g)	49.90 $\pm$ 18.52 ^a	22.81 $\pm$ 7.77 ^b	23.08 $\pm$ 8.54 ^b	< 0.0001
Final length (cm)	14.61 $\pm$ 1.69 ^a	11.53 $\pm$ 1.28 ^b	11.72 $\pm$ 1.32 ^b	< 0.0001
Weight gain (g)	34.79 $\pm$ 4.84 ^a	10.26 $\pm$ 0.77 ^b	10.29 $\pm$ 1.76 ^b	0.0107
Biomass (g)	998.78 $\pm$ 86.81 ^a	444.89 $\pm$ 31.78 ^b	444.55 $\pm$ 49.98 ^b	0.0132
SGR (%/day)	3.97 $\pm$ 0.43 ^a	2.0 $\pm$ 0.20 ^b	1.96 $\pm$ 0.27 ^b	0.0132
Feed ration (g)	1039.93 $\pm$ 64.33 ^a	474.93 $\pm$ 36.74 ^b	498.58 $\pm$ 50.32 ^b	0.0187
FCR	1.51 $\pm$ 0.19 ^b	2.52 $\pm$ 0.57 ^a	2.69 $\pm$ 0.49 ^a	0.0066
Productivity (kg/m ³)	12.48 $\pm$ 1.09 ^a	5.56 $\pm$ 0.40 ^b	5.56 $\pm$ 0.62 ^b	0.0132
Survival rate (%)	100	97.50 $\pm$ 5.0	96.25 $\pm$ 7.50	-

Different letters in the same row represent significant differences by the Kruskal-Wallis non-parametric test, ($p < 0.05$). BFT_{5.5}, pH 5.5-6.0; BFT_{6.5}, pH 6.5-7.0; and BFT_{7.5}, pH 7.5-8.0.

3.1.2.3 Blood analyses

Differences were observed for plasma protein, glucose, and cholesterol, with higher values in BFT_{5.5} ($p < 0.05$), while hematocrit (35.58 ± 3.8 %) was higher in BFT_{7.5} ($p < 0.05$) (Table 6). Hemoglobin, triglycerides, erythrocytes, MCV, MCH, and MCHC did not show differences between the treatments ($p > 0.05$).

Table 6. Blood analyses (plasma protein, hematocrit, hemoglobin, glucose, triglycerides, cholesterol, erythrocytes, MCV, MCH, MCHC) of *Colossoma macropomum* produced in BFT system with different pH ranges after 30 days of cultivation (end of Phase 2).

	pH			P-value
	BFT _{5,5}	BFT _{6,5}	BFT _{7,5}	
Plasma Protein (g/dL)	4.75±0.22 ^a	4.15±0.30 ^b	4.23±0.34 ^b	0.0004
Hematocrit (%)	26.36±3.20 ^b	24.0±3.16 ^b	35.58±3.82 ^a	< 0.0001
Hemoglobin (g/L)	16.50±8.38	17.31±9.27	15.07±10.0	-
Glucose (mg/dL)	122.71±132.62 ^a	65.55±29.60 ^b	59.12±22.26 ^b	0.0044
Tryglycerides (mg/dL)	189.08±40.23	157.10±95.39	178.51±75.99	-
Cholesterol (mg/dL)	114.24±32.92 ^a	57.33±22.19 ^b	62.29±33.72 ^b	< 0.0001
Erythrocytes (RBC uL/10 ⁶)	1.79±0.66	2.15±1.18	2.86±1.22	-
MCV (fL)	167.51±54.99	158.74±105.34	131.79±51.31	-
MCH (pg)	122.18±78.61	134.49±161.39	52.60±38.23	-
MCHC (g/dL)	62.44±36.81	79.04±46.94	48.87±35.76	-

Different letters in the same row represent significant differences by the Kruskal-Wallis non-parametric test ($p < 0.05$). BFT_{5,5}, pH 5.5-6.0; BFT_{6,5}, pH 6.5-7.0; and BFT_{7,5}, pH 7.5-8.0.

Table 7. Biofloc bromatological analysis (ash, crude protein, ether extract, and dry matter) produced in a BFT system with different pH ranges in the production of *Colossoma macropomum*.

	pH			P-value
	BFT _{5,5}	BFT _{6,5}	BFT _{7,5}	
Ash (%)	5.71±0.18 ^b	6.26±0.07 ^{ab}	7.18±0.06 ^a	0.0002
Crude protein (%)	21.46±0.04	21.40±0.17	22.16±0.26	-
Ether extract (%)	0.21±0.29	0.19±0.26	0.02±0.59	-
Dry matter (%)	94.29±0.18 ^a	93.74±0.07 ^{ab}	92.82±0.06 ^b	0.0002

Letras diferentes na mesma linha representam diferenças significativas por teste não-paramétrico Kruskal-Wallis ($p < 0,05$). BFT_{5,5}, pH 5.5-6.0; BFT_{6,5}, pH 6.5-7.0; and BFT_{7,5}, pH 7.5-8.0.

3.1.2.4. Hepatosomatic Index

The HSI of juvenile *C. macropomum* were 1.83 ± 0.35 , 1.54 ± 0.41 , and 1.55 ± 0.79 to BFT_{5,5}, BFT_{6,5}, BFT_{7,5}, respectively, and did not show any differences between the treatments ($p > 0.05$).

3.1.2.5. Bromatological analyses

The bromatological analyses of the bioflocs showed higher ash values in BFT_{7,5} compared to BFT_{5,5} and higher dry matter in BFT_{5,5} compared to BFT_{7,5} ($p < 0.05$) (Table 5). Total protein and ether extract did not show differences ($p > 0.05$).

4. Discussion

The different pH ranges in the BFT system affected the water quality, zootechnical performance, and blood parameters of *C. macropomum*.

4.1. Phase 1

Although the temperature showed difference between treatments the values were considered without impact biological. Temperature ($\sim 28^\circ\text{C}$) as well dissolved oxygen ($\text{DO} > 3 \text{ mg/L}$) remained within optimal conditions for the growth of *C. macropomum* (Izel-Silva et al., 2020; Saint-Paul, 1984).

The highest concentrations of TAN occurred in BFT_{5.5}, it was associated with reduced bacterial activity in the nitrification process, in acidic conditions (Grommen et al., 2002; Khanjani et al., 2024b). However, TAN ($< 3 \text{ mg/L}$) (Oba et al., 2009; Santos et al., 2021) and NO_2^- ($< 0.5 \text{ mg/L}$) (Costa et al., 2004) were kept at safe concentrations for the species. Bastos et al. (2017) and Hurtado and Bomfim (2022) determined the CL_{50} of NH_3 in *C. macropomum* to be 0.064 and 7.76 mg/L, respectively. The differing CL_{50} values for NH_3 in these studies can be explained by variations in temperature, pH, alkalinity, and water hardness to which the animals were exposed (Becker & Baldisserotto, 2020). Nonetheless, using these values as a reference, the highest recorded NH_3 ($\sim 0.01 \text{ mg/L}$) was considered safe for *C. macropomum* in BFT_{7.5}. The different concentrations of NH_3 in the present study are explained by the direct relationship between NH_3 and pH, where at higher pH there is an increase in the concentration of NH_3 (Boyd & Tucker, 1998).

Information regarding NO_3^- toxicity for fish is still limited, including for *C. macropomum*, but this nitrogenous compound must be monitored in fish farms because fish absorb NO_3^- through their gills, leading to histopathological changes, endocrinal disorder, especially in closed systems where this compound accumulates (Yu et al., 2021). Banerjee et al. (2023) considered *Poecilia reticulata* one of the most sensitive freshwater fish to NO_3^- with a $\text{CL}_{50-96\text{h}}$ of 191 mg/L (Rubin & Elmaraghy, 1977). Therefore, the NO_3^- values in this study ($< 10 \text{ mg/L}$) were considered low and safe for *C. macropomum*.

The lowest alkalinity values in BFT_{5.5} and BFT_{6.5} are attributed to consumption of alkalinity by nitrifying bacteria in the system, that use carbonate and bicarbonate as inorganic nutrients (Pandey et al., 2014, Hargreaves, 2013, Martins et al., 2017). On the other hand, the higher alkalinity is related to the addition of sodium bicarbonate (Martins et al, 2019) to raise the pH in BFT_{7.5}. Holanda Cavalcante et al. (2014) suggest that the alkalinity of fish production systems should be between 55 and 150 mg of CaCO_3/L . However, Santos et al. (2024) reported satisfactory growth of

C. macropomum with alkalinity between 11 and 16 mg of CaCO_3/L . These values are close to the lowest alkalinity in the present study. But alkalinity must be analyzed and in conjunction with pH because the low values of these parameters, for a long period of time, can collapse the BFT system (Avnimelech, 2015).

The floc formation depends mainly on the external addition of organic carbon into the BFT system (Avnimelech, 2015; Samocha et al., 2007) and the highest value of SS in BFT_{5.5} compare to BFT_{7.5} was attributed to the addition of organic carbon to control TAN. Another relationship to be consider in the production of solids/flocs are the ions present in the water. Cations such as Na^+ , Ca^{+2} and Mg^{+2} are considering important to interact with negative charge of the polymers that allow adhesion between microorganisms, helping in the formation of flocs (Nguyen et al., 2009; Sanin & Vesilind, 2000; Sobeck & Higgins, 2002). But, for this Phase of the study, it was not possible to establish this relationship between cations and the production of solids in the different pH ranges.

Although the limits for SS and TSS for *C. macropomum* have not yet been determined in the literature, it was possible to control and maintain the solids (SS and TSS), in all treatment, within the range suggested for fish in BFT (SS: 5-50 mL/L and TSS < 500 mL/L) (Avnimelech, 2015; Pellegrin et al., 2021; Soaudy et al., 2023) and without clarification. And also, the lowest production of SS and TSS in the alkaline medium (BFT_{7.5}) can be considered an advantage in solids control (Martins et al., 2019).

Even though the presence of solids/biofloc in the system is important, and their control is necessary (De Schryver et al., 2008; Khanjani et al., 2024b) to not compromised the excessive reduction of dissolved oxygen nor oscillation of the nitrification process (Martins et al., 2019). And another way to maintain water quality is through the relationship SS and SST, with FVI (Yousuf, 2013) which stabilishes quality of the floc through the density (Yuvarajan, 2021).

In effluent treatment using activated sludge, as in aquaculture (BFT), it is recommended that the FVI remain between 40 to 60 mL/g due to the excellent sedimentation capacity of the floc at these densities (De Schryver et al., 2008). The authors consider these values similar to aquaculture's because aeration can resuspend these dense decanted flocs, keeping them in the water column without significant problems. Unlike what happens when $\text{FVI} > 200 \text{ mL/g}$ (very low density), which results in bulk sludge. Therefore, the FVI values of all treatment are close to those indicated, moving away from a bulking sludge situation (De Schryver et al., 2008).

Other parameter to discuss solids is phosphorus, that in excessive amounts can cause environmental eutrophication (Nguyen et al., 2022). The phosphate (PO_4^{-3}) in BFT is part cycling by microorganism, including heterotrophic bacteria, that results in solids/bioflocs in the system (Martins et al., 2019; Silva et al., 2009; Longnecker et al., 2010) and part of PO_4^{-3} tends to

accumulate over time (Khanjani et al., 2024b). In the present study, the pH at BFT_{5.5} and BFT_{6.5} resulted in the highest concentration of PO₄⁻³, but only BFT_{5.5} generated more SS in relation to BFT_{7.5}. In this case, it was not possible to establish a direct relationship between higher concentrations of PO₄⁻³ and the generation of more solids, but it is clear that lower pH presented more anions PO₄⁻³. Corroborating with others authors that shows higher dissociation of anions PO₄⁻³ in an acid pH environment (Silva et al., 2009).

For *C. macropomum*, as well as other species from the waters of the Rio Negro in the Amazon, excess H⁺ (low pH) and very soft water (low Ca²⁺) compete with Na⁺ and Cl⁻ from the water, increasing ion losses to the environment (Baldiasserotto & Gomes, 2014; McDonald & Wood, 1981; Val et al., 1998; Wood et al., 1998). At the same time, excess H⁺ in soft water results in an increase in Cl⁻ cells, gill ventilation, and lamellar thickening, hindering gas exchange (Becker & Baldiasserotto, 2020). But to many species the exposure to an acidic environment, with hard water (elevated Ca²⁺) (similar to BFT_{5.5}, for example) provides considerable protection against the effects of ion regulation by high external H⁺ concentrations, due to the competition between Ca²⁺ and H⁺, thus reducing branchial fluxes and plasmatic of Na⁺ and Cl⁻ (McDonald & Wood, 1981; McDonald et al., 1983; Wood, 1989). It is possible that this homeostatic regulation occurred at lower pH in the present study.

The migratory behavior of Amazonian fish, such as *C. macropomum*, is governed by rainfall regimes, making them move from regions of circumneutral waters (pH 6) to the acidic waters of the Rio Negro (pH 5.0 - 5.5), for example, and its tributaries (pH ≤ 4) and vice versa (Goulding, 1980; Goulding and Carvalho, 1982; Roubach and Saint-Paul, 1994; Val-Almeida and Hochachka, 1995). This migratory capability demonstrates the species ability to occupy different pH environments.

Previous studies reveal that *C. macropomum* exhibits better zootechnical performance in acidic pH, ranging from 4 to 6 (Aride et al., 2007; Val et al., 1998). This is possible due to its ability to maintain homeostasis in an acidic environment (Wood et al., 1998). At the same time, the present study showed that the acidic environment (BFT_{5.5}), associated with extremely hard water, promoted better growth (final weight and final length) compared to the other treatments, and with similar feed ration between treatments. Weight gain, biomass, SGR, AFCR, and productivity were not impacted by the different pH ranges and are in agreement with the work with *C. macropomum* (Dias et al., 2018, Assis et al., 2020, dos Santos et al., 2021, Ananias et al., 2023).

The growth of *C. macropomum* in BFT_{5.5} was accompanied by changes in plasma protein, hematocrit, glucose and MCH and MCHC, demonstrating the adaptation of *C. macropomum* to the environment, mainly in BFT_{5.5} in relation to BFT_{7.5}.

Plasma protein is essential in the constitution of blood tissue (Oliveira et al., 2017), and its increase is associated with hemolysis or responses to infectious processes (Hrubec & Smith, 1999, Khanjani et al., 2023a). Despite the differences observed in this study, the plasma protein values are similar to those reported in other studies on *C. macropomum*, where the animals were considered healthy (Bussons et al., 2021; Liebl et al., 2022; Nakayama et al., 2022; Tavares-Dias & Moraes, 2010).

The higher values of MCH and MCHC and the maintenance of hematocrit within the normal range for the species (Assis et al., 2020) in BFT_{5.5} indicate that *C. macropomum* adjusted to the environment, as the control of branchial flow and hematocrit concentration was maintained even during acidic exposure (Wood, 1989).

Studies describe that when fish are kept in an acidic pH environment, acidification accompanied by reduced blood oxygen concentrations are among the consequences, a situation that results in the release of catecholamines and the development of hyperglycemia (Aride et al., 2007; McDonald & Wood, 1981). Associating this information with the present study, the high glucose would be linked to adaptation of *C. macropomum* at acidic pH in BFT_{5.5}, as observed in *Salmo gairdneri* at pH 4 (Reid et al., 1992; Ye et al., 1991).

The increase in hematocrit in BFT_{7.5} indicates a reduction in plasma volume and can result from erythrocyte edema and a decrease in plasma ions (Milligan & Wood, 1982). The result of the present study is contrary to that described for *C. macropomum* maintained at pH 8, where a reduction in hematocrit was observed (Aride et al., 2007). Despite these differing results, the increase and decrease in hematocrit appears to be related to the different environments and system to which *C. macropomum* were subjected.

4.2 Phase 2

The same way on Phase 1, in the Phase 2, temperature, DO, TAN, NH₃, and NO₂⁻ were kept within optimal conditions and safe to growth of the *C. macropomum* (Izel et al., 2020; Saint-Paul, 1984; Oba et al., 2009; Santos et al., 2021; Costa et al., 2004; Bastos et al., 2017; Hurtado & Bomfim, 2022).

But it is worth highlighting that at this Phase, it becomes clearer how pH affected the concentrations of nitrogenous compounds (TAN, NH₃ e NO₂⁻) in BFT_{5.5}, with lower efficiency of the nitrification process (Grommen et al., 2022; Khanjani et al., 2024b). At the same time in BFT_{5.5}, there is a significant reduction in alkalinity due to the consumption of carbonates by nitrifying bacteria (Ebeling et al., 2006; Pandey et al., 2014).

No difference was found for TSS, SS or FVI, but what draws attention is that these parameters were higher than in Phase 1, demonstrating the accumulation of solids in the systems, despite still being considered safe values (Avnimelech, 2015; Pellegrin et al., 2021; Soaudy et al., 2023; De Schryver et al., 2008).

The higher values of Ca^{2+} , Mg^{2+} , Cl^- , and PO_4^{3-} ions in BFT_{5.5} are explained by Le Chatelier's equilibrium principle, in with there is greater solubility of these ion in acidic pH (Hillert, 1995). And the increase of Ca^{2+} and Mg^{2+} resulted in an increase in hardness (Boyd et al., 2016), a fact observed in BFT_{5.5}.

Discussing the increase in cations from the perspective of bioflocculation and the increase in solids (Nguyen et al., 2009; Sobeck & Higgins, 2002, Lou et al., 2013), in this Phase, the higher concentration of these ions in BFT_{5.5} did not result in more solids produced (TSS and SS). Only TSS increased in all treatments, in relation to Phase 1, demonstrating the accumulation of suspended solids in the system over time (Avnimelech, 2015). Although more studies should be explored the ions in BFT, it is known that increasing ions in water has the ability to improve the survival of fish exposed to low pH (Leivestad & Muniz, 1976).

At this Phase, *C. macropomum* also showed higher growth (weight and length), as in Phase 1. However, more parameters (weight gain, biomass, SGR, FCR and productivity) clearly showed better performance in BFT_{5.5}. Obviously, the highest weight gain reflects the highest biomass and productivity, but weight gain was almost 3.5 times higher in BFT_{5.5}, demonstrating the influence of pH on performance of *C. macropomum* in BFT. As *C. macropomum* started Phase 2 with a larger size class than in Phase 1, the question arises, "Do larger *C. macropomum* adapt better in BFT_{5.5}?"

Despite these differences in zootechnical performance, the survival rate (> 96%) was considered high in all treatments.

Blood parameters, just like in Phase 1, plasma protein, hematocrit and glucose continue to be significantly modulated in BFT_{5.5}. Additionally, cholesterol, a precursor of the stress hormone responsible for activating the gluconeogenesis pathway, is present in the catecholamine metabolic route, which responds by releasing more glucose into the blood (Godavarthy et al., 2012), its values increased in this phase, demonstrating the regulation mechanism of *C. macropomum* in BFT_{5.5}.

Despite the differences in blood parameters and zootechnical performance indices among the treatments, the HSI of *C. macropomum* was not altered, and the values are close to those verified for *C. macropomum* in BFT (Nakayama et al., 2022).

The diversified profile of biofloc composition is related to the abundance and variety of the microbial community (Azim & Little, 2008; Colwell, 2006) and is intrinsically linked to water quality parameters, alkalinity, organic carbon sources, among others (Martins et al., 2017; Oliveira

et al., 2024, Khanjani et al., 2023b). In the present study, the biofloc levels of crude protein (CP) (> 20 %) and ether extract (EE) (< 0.5 %) were not influenced by the different pH ranges. The values are in line with others studies indicating varied CP contents (12 to 50 %) (Emerenciano et al., 2022) and EE (0.5 to 46 %) (Hargreaves, 2013).

Despite ashes being higher in BFT_{7.5} compared to BFT_{5.5}, the values are close to those (5-10 %) found in the literature (Azim & Little, 2008; Ekasari et al., 2010; López-Elías et al., 2015). The ash content in the samples refers to inorganic material. In this case, adding an alkalizing agent (sodium bicarbonate) may be primarily responsible for the higher ash content in BFT_{7.5}.

5. Conclusions

In this study, the nitrification process was more efficient at pH ranges of 6.5 and 7.5. However, TAN and NO₂⁻ concentrations were kept within safe limits for *C. macropomum* at pH 5.5. Zootechnical performance was better in BFT_{5.5}, in Phase 1 and Phase 2, which is associated with higher glucose and cholesterol concentrations in *C. macropomum*.

Despite the results indicating that *C. macropomum* can be produced across a wide pH range with optimal performance at pH 5.5 range, further long-term studies are needed. These studies should monitor the BFT system's dynamics and the entire production cycle at various pH ranges.

Acknowledgments

This research received support from Conselho National de Desenvolvimento Científico e Tecnológico (CNPq-Brazil - Project No. 402952/2021-9), Coordination for the Improvement of Higher Education Personnel, and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG-Brazil). R.K. LUZ received a research fellowship from Conselho National de Desenvolvimento Científico e Tecnológico (CNPq Process 402840/2023-2).

Author contributions

Naiara Pinho Alves: Conceptualization, Methodology, Validation, Formal analysis, Resources, Investigation, Writing Original Draft, Writing Reviewing and Editing, Visualization, Funding Acquisition. **Ronald Kennedy Luz:** Writing Reviewing and Editing, Visualization, Project Administration, Funding Acquisition. **Cintia Labussiere Nakayama:** Supervision, Methodology, Formal analysis, Resources Investigation, Writing Reviewing and Editing, Visualization, Funding Acquisition.

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CAPÍTULO 2

A formatação do manuscrito foi baseada na revista AQUACULTURE

Physiological responses of *Colossoma macropomum* reared in acidic pH, neutral and alkaline pH in the BFT system under air exposure stress challenge

Abstract

Water pH and management practices, such as exposure to air, can act as stressors and modulate the physiological responses of fish farmed in intensive systems. This study evaluated how different pH ranges in biofloc systems (BFT) modulate the hematological, biochemical and hepatic responses of *Colossoma macropomum* juveniles subjected to exposure to air. Fish ($n = 180$) were kept for 60 days in BFT under three pH ranges: 5.5–6.0 (BFT_{5.5}), 6.5–7.0 (BFT_{6.5}) and 7.5–8.0 (BFT_{7.5}), and then exposed to air for 30 min. Blood samples were collected before exposure (baseline), immediately after and 6, 12 and 24 h after challenge. Survival was 100% in all treatments. The water quality parameters remained within adequate conditions, with higher concentrations of TAN in BFT_{5.5} and lower concentrations of PO_4^{3-} in BFT_{7.5}. The physiological analyses indicated effects of maintenance pH, collection time and their interactions. Hematocrit showed higher baseline values in BFT_{7.5} and transient increase after exposure to air in BFT_{5.5} and BFT_{6.5}. Glucose increased immediately after exposure to air in all treatments, returning to baseline levels within 6 h, with no effect of pH. Lactate increased after exposure to air, with higher values in BFT_{5.5}. Triglycerides and plasma proteins showed treatment effect, with higher values in BFT_{5.5}, while cholesterol remained within the physiological range of the species, with greater temporal variation only in BFT_{5.5}. AST activity and hepatosomatic index showed a late increase at 24 h after exposure to air, especially at BFT_{5.5}. Despite the variations observed, all parameters returned to levels considered physiological, indicating efficient recovery and absence of severe metabolic impairment. The results demonstrate that *C. macropomum* has high resistance to exposure to air in BFT systems, and the magnitude of physiological responses is modulated by the pH of cultivation.

Keywords: Aquaculture systems, blood biochemistry, hematology, metabolic responses, stress physiology.

1. Introduction

Intensive aquaculture exposes fish to highly dynamic environments, in which physical, chemical, and operational variations can act as stressors, challenging the maintenance of physiological homeostasis (Oba et al., 2009). The term "stressor" is used to describe any element capable of altering the homeostasis of fish (Smith, 1982) and can be triggered by chemical and physical variations in the environment (Costa et al., 2004; Urbinati & Carneiro, 2004). Among these stressors, routine management practices such as capture, biometrics, classification, and transport often involve exposure to air, which is considered a relevant acute stressor for species that rely majorly on gill respiration (Araújo-Lima and Gomes, 2005; Braum, 1983; do Carmo Neves et al., 2020). The intensity of the physiological response to this type of challenge can vary according to the environmental conditions to which the fish are previously subjected. and can be triggered by chemical and physical variations in the environment.

Exposure to stressors in fish results in the activation of the hypothalamic-pituitary-sympathetic nervous system axis - chromaffin cells (HSC) and the hypothalamic-pituitary-interrenal (HPI) axis, which is triggered by catecholamines (adrenaline and noradrenaline) and, subsequently, by corticosteroids (cortisol and cortisone) (McLeay, 1973). These responses trigger a series of physiological and metabolic adjustments, including hyperglycemia, increased plasma lactate, lipid mobilization, hematological changes, and modulation of liver activity; these changes ensure adequate energy and oxygenation during and after stress (Oba et al., 2009).

In this context, hematological and biochemical analyses, as well as hepatosomatic indices (HSI), are widely used tools to assess the physiological status, adaptive capacity, and post-stress recovery patterns in farmed fish (dos Santos Silva et al., 2024; Kori-Siakpere et al., 2005; Sousa et al., 2021). Changes in these parameters do not necessarily indicate pathological impairment, but may reflect transient physiological adjustments in response to environmental and management challenges

Among the environmental factors that modulate the stress response, water pH plays a central role in regulating acid-base balance, osmoregulation, and energy metabolism in fish (Fromm, 1980; Roberts & Powell, 2003; Val-Almeida & Hochachka, 1995). Under acidic pH conditions, the increased external concentration of hydrogen ions (H^+) can intensify ionic fluxes through the gills, altering electrolyte excretion and acid-base dynamics (Aride et al., 2007; Oba et al., 2009). Despite this, studies demonstrate that tambaqui (*Colossoma macropomum*) exhibits tolerance to acidic environments, with reduced ionoregulatory disturbances and recovery of ionic fluxes after stressful situations (Wilson et al., 1999; Wood et al., 1998).

C. macropomum is one of the main native species in Brazilian aquaculture, standing out for its hardiness, wide physical plasticity, and ability to adapt to different environmental conditions, including pH variations and distributed obesity (Aride et al., 2007; Izel et al., 2014; Saint-Paul, 1984). These characteristics make it a particularly suitable species for intensive farming systems, where fish are frequently exposed to environmental and management challenges.

Among these systems, biofloc technology (BFT) has been widely adopted to promote greater efficiency in water use, control of nitrogen compounds, and additional supply of nutrients and bioactive compounds (Avnimelech, 2015; Schryver et al., 2008). However, BFT is characterized by high microbial activity, which can result in fluctuations in water pH, frequently leading to slightly acidic conditions. These pH variations, although common in the system, can interact with management stressors, modulating the physiological responses of the fish and their recovery processes.

Recent studies indicate that the bioactive compounds present in bioflocs can favor the innate immune response and physiological stability of fish (Haridas et al., 2021; Mansour and Esteban, 2017; Nageswari et al., 2022). However, there is still little information on how different pH ranges in biofloc systems influence the acute stress response and the physiological recovery pattern of *C. macropomum*, especially when subjected to air exposure.

Given this scenario, the present study aimed to evaluate how different maintenance pH values in BFT systems modulate the hematological, biochemical, and hepatic responses of *Colossoma macropomum* exposed to air for 30 minutes, as well as the pattern of physiological recovery after the stressor. The results contribute to the understanding of the interaction between environmental conditions and management challenges, providing support for improving cultivation practices and the well-being of the species in intensive systems.

2. Material and Methods

The experiment was developed at the Aquaculture Laboratory of the Federal University of Minas Gerais – Brazil, after approval of the protocol by the Ethics Committee on the Use of Animals in Experimentation (CEUA – 95/2022).

2.1 Experimental conditions

C. macropomum (1.94 ± 12.94 g; 12.62 ± 1.44 cm) were distributed and maintained in BFT with different pH ranges (5.5–6.0; 6.5–7.0 and 7.5–8.0, designated BFT_{5.5}; BFT_{6.5} and BFT_{7.5}, respectively) in a micro-macrocosm model (Wasielesky et al., 2006) for 60 days. Each system consisted of four replicate tanks (80 L, 15 fish/tank) and a sump tank (250 L) for water

homogenization, containing a submersible pump (3700 L/h, Aquarium China) and two thermostatic heaters (500 W - Ocean Tech). All tanks were equipped with a porous hose connected to a radial compressor (2CV – IBRAM Brazil) for oxygenation and water movement. The photoperiod, throughout the experimental period, was maintained at 12 h of light and 12 h of darkness, and the fish were fed three times a day (9 h, 12 h and 15 h) with a commercial diet containing (45% crude protein, 8 % ether extract, 2-3 mm, Aquos 45 – Total) at 5% of the biomass.

2.2 Challenge test: Exposure to air

After the conditioning period (60 days), under the respective pH conditions, all fish were fasted for 24 hours before the experimental stressor and during the subsequent 72-hour sampling period. Before the start of the stress challenge, blood samples were collected from a group of juveniles ($n = 12/\text{treatment}$) to determine baseline levels of blood parameters.

Next, the stress challenge was performed using synchronized air exposure (AE). All fish were captured using a medium square net (medium trawl net) (20×14.5 cm) and kept out of the water for 30 minutes. After the challenge, the fish were returned to their respective culture tanks (do Carmo Neves et al., 2022).

Blood samples were collected immediately after air exposure (AE) and 6, 12, and 24 hours (6 h AE, 12 h AE, and 24 h AE, respectively) after the challenge. After blood collection, juveniles were euthanized by immersion in a eugenol solution (285 mg eugenol/L, (Mattioli et al., 2017) (Figure 1). Survival was assessed using the following formula: $\text{Survival rate (\%)} = (\text{number of fish at the end of the experiment} / \text{initial number of fish}) \times 100$.

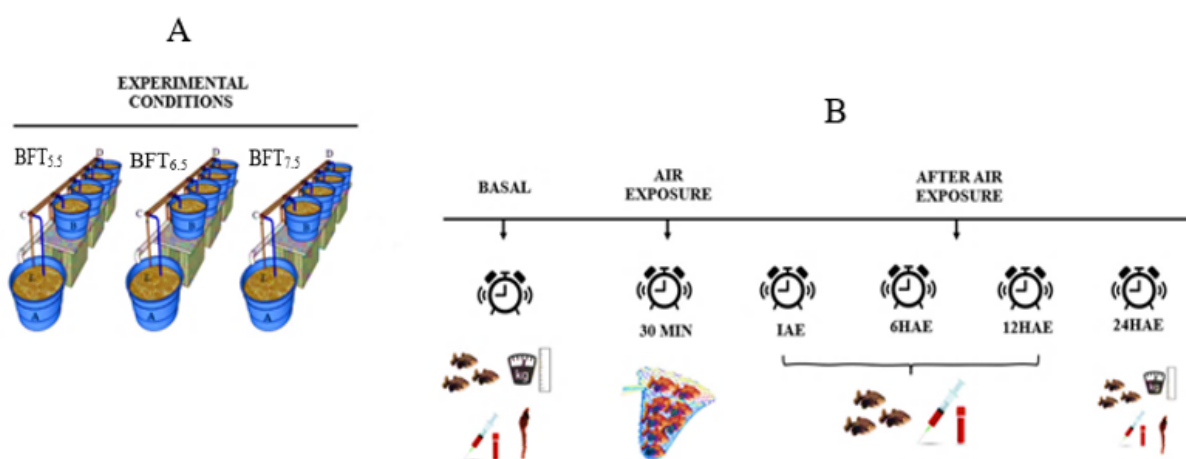


Figure 1. Schematic representation of the experimental design used to evaluate the physiological responses of *Colossoma macropomum* produced in a biofloc technology (BFT)

system under different water pH ranges and subjected to an air exposure challenge. (A) Experimental conditions of the BFT system in different pH ranges (BFT_{5.5}, BFT_{6.5}, e BFT_{7.5}). (B) Blood collection schedule before (basal), immediately after 30 minutes (IAE) and during the recovery period (6, 12 and 24 hours after air exposure).

2.3 Water quality

Water temperature, dissolved oxygen (DO - by means of AK87 Akso Brasil probe), pH (pH meter - AK90 Akso Brasil), alkalinity (Rice et al., 2012), total ammonia (TAN) (Rice et al., 2012), nitrite (NO₂⁻) (Aminot and Chaussepied, 1983), settleable solids (SS) by means of Imhoff cone (15 min - (Avnimelech, 2015), hardness (NBR 5761, 1984) and total suspended solids (TSS) (Eaton et al., 1995) were measured at the beginning and end of the air exposure test. Ions (Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, and PO₄³⁻ mg/L) were analyzed in ion chromatograph (Metrohm ECO IC1), the water samples were collected at the end of each experimental phase.

2.4 Blood Tests

Blood collections were performed by caudal puncture, with insulin syringes (1 mL – Solidor) containing 10% sodium heparin (5.000 IU/mL – Hepamax-S) of the total volume of blood collected. Blood aliquots were used to determine hematocrit by the microhematocrit method, using capillary microtubes (Goldenfarb et al., 1971). For hemoglobin analysis, 10 µL of blood were added and homogenized in 2.0 mL of colorimetric reagent (Drabkin's reagent). Plasma protein was determined by collecting plasma and reading it on a manual refractometer (RHC 200-ATC, Huake Instrument Co). Biochemical analyses (glucose, triglycerides, cholesterol, lactate, alanine aminotransferase (AST) and aspartate aminotransferase (ALT) were performed by centrifuging the blood at 4.000 rpm for 10 minutes to remove the plasma, and their dosages were performed using commercial kits (Bioclin® Brasil). All sample readings were performed using a spectrophotometer (Bioclin® 100 - Quibasa LTDA). Lactate, ALT and AST analyses were performed before and 24 hours after exposure to air.

2.5 Hepatosomatic index

Liver ($n = 12$ fish/treatment) was collected at the beginning and end of the experimental period, after euthanasia. The following formula was used to calculate the hepatosomatic index: Hepatosomatic index (HI) = (liver weight / body weight) × 100.

2.6 Statistical Analysis

The experimental design was completely randomized, with subsampling, in which treatments were applied to experimental units and evaluations were performed at different times using different animals. Data were previously assessed for normality and homoscedasticity; once these assumptions were met, they were subjected to analysis of variance (ANOVA). When significant differences were detected, means were compared using Tukey's test for hematological, biochemical, and water quality variables, and Duncan's test for ions. Variables that did not meet the ANOVA assumptions were analyzed using the non-parametric Kruskal-Wallis test, adopting a significance level of 5 %. Data expressed as percentages were transformed using the arcsine of the square root; only the untransformed values are presented.

3. Results

After 30 min of air exposure, and 24 h of recovery, the survival of *C. macropomum* was 100%, in all treatments.

3.1 Water quality

The water quality parameters DO, Temperature, SS, NO_2^- , TSS, Na^+ , K^+ , Ca^{+2} , Mg^{+2} e Cl^- did not show differences ($p > 0.05$). The pH ($p = 0.0018$) varied as proposed for the treatments. The SS was larger in BFT_{5.5} ($p < 0.001$), TAN concentrations were higher in BFT_{5.5} ($p = 0.0253$) (Table 1) and PO_4^{3-} , lower in BFT_{7.5} ($p < 0.0276$) (Table 2).

Table 1. OD, Temperature, TAN, NO_2^- , (mg/L), pH, TSS (mL/L), SS (mL/L) (mean $\pm$ SD) of *Colossoma macropomum* produced in a BFT system under different water pH ranges before a 30-minute air exposure challenge.

Water parameters	pH			P value
	BFT _{5.5}	BFT _{6.5}	BFT _{7.5}	
DO (mg/L)	5.38 $\pm$ 0.37	6.62 $\pm$ 0.37	6.59 $\pm$ 0.37	0.0540
Temperature (°C)	27.53 $\pm$ 0,05	27.63 $\pm$ 0.05	27.43 $\pm$ 0.05	0.0928
pH	5.55 $\pm$ 0.10 ^c	6.40 $\pm$ 0.10 ^b	7.48 $\pm$ 0.10 ^a	0.0018
SS (mL/L)	28.75 $\pm$ 8.07	15.00 $\pm$ 8.07	30.00 $\pm$ 8.07	<0.001
TAN (mg/L)	3.32 $\pm$ 0.41 ^a	0.71 $\pm$ 0.41 ^b	0.28 $\pm$ 0.41 ^b	0.0253
NO_2^- (mg/L)	0.51 $\pm$ 0.55	0.43 $\pm$ 0.05	0.30 $\pm$ 0.08	0.7013

TSS (mg/L)	498.67 ± 61.02	350.00 ± 105.69	373.34 ± 74.73	0.6283
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Letters on the same line represent significant differences according to ANOVA test Tukey ($p < 0.05$). BFT_{5.5}, pH 5.5-6.0; BFT_{6.5}, pH 6.5-7.0; and BFT_{7.5}, pH 7.5-8.0. (DO – dissolved oxygen. pH = hydrogen potential, SS = settleable solids, TAN = total ammonia, NO₂⁻ = nitrite, TSS = total suspended solids).

Table 2. Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, and PO₄³⁻ (mg/L) (mean ± SD) of *Colossoma macropomum* produced in a BFT system under different water pH ranges before a 30-minute air exposure challenge.

Water ions	pH			P value
	BFT _{5.5}	BFT _{6.5}	BFT _{7.5}	
K ⁺ (mg/L)	65.30 ± 37.23	87.29 ± 34.37	71.78 ± 17.92	-
Ca ²⁺ (mg/L)	77.93 ± 11.30	70.54 ± 2.16	57.62 ± 1.22	-
Mg ²⁺ (mg/L)	8.99 ± 2.79	10.4 ± 0.14	10.45 ± 2.78	-
Cl ⁻ (mg/L)	52.26 ± 17.99	35.09 ± 0.18	44.62 ± 1.68	-
PO ₄ ³⁻ (mg/L)	49.86 ± 5.41 ^a	42.42 ± 0.17 ^a	28.25 ± 4.36 ^b	0.0276

Letters on the same line represent significant differences according to ANOVA test Duncan ($p < 0.05$). BFT_{5.5}, pH 5.5-6.0; BFT_{6.5}, pH 6.5-7.0; and BFT_{7.5}, pH 7.5-8.0. (Na⁺ = sodium, K⁺ = potassium, Ca²⁺ = calcium, Mg²⁺ = magnesium, Cl⁻ = chlorine and PO₄³⁻ = phosphate).

3.2 Blood Tests

Blood analyses of juvenile *C. macropomum* maintained at different pH ranges in a BFT system showed a significant effect of both blood collection time and pH range on hematocrit, cholesterol, and lactate values (Table 3). Furthermore, a significant interaction was observed between time and pH ($p < 0.0001$), indicating that the responses of these variables over time differed between the pH ranges evaluated.

Table 3. Hematocrito (%), colesterol (mg/dL), and lactato (nmol/dL) (mean ± SE) of *Colossoma macropomum* produced in a BFT system under different water pH ranges before a 30-minute air exposure challenge.

BFTs	Tempo	Hematocrito (%)	Colesterol (mg/dL)	Lactato (nmol/dL)
			114.24 ± 5.44 ^{bA}	8.15 ± 1.82 ^{bA}

BFT _{5.5}	Basal	26.38 ± 1.06 ^{cB}		
	IAE	31.46 ± 1.11 ^{aA}	96.65 ± 5.44 ^{dA}	-
	6hAE	29.58 ± 1.01 ^{bA}	102.32 ± 5.44 ^{cA}	-
	12hAE	19.13 ± 1.06 ^{dC}	122.76 ± 5.44 ^{aA}	-
	24hAE	26.38 ± 1.06 ^{cB}	84.92 ± 6.29 ^{eB}	37.05 ± 1.9 ^{aA}
BFT _{6.5}	Basal	23.95 ± 1.06 ^{cC}	57.33 ± 5.44 ^{cE}	7.78 ± 1.82 ^{bA}
	IAE	31.17 ± 1.01 ^{aA}	68.36 ± 5.44 ^{bD}	-
	6hAE	24.06 ± 1.11 ^{bC}	65.04 ± 5.44 ^{bD}	-
	12hAE	21.59 ± 1.06 ^{dB}	70.74 ± 5.44 ^{aC}	-
	24hAE	23.95 ± 1.06 ^{cC}	56.13 ± 5.69 ^{cE}	24.06 ± 1.91 ^{aB}
BFT _{7.5}	Basal	32.58 ± 1.01 ^{aA}	62.29 ± 5.44 ^{aD}	7.09 ± 1.82 ^{bA}
	IAE	29.60 ± 1.17 ^{bA}	57.75 ± 5.44 ^{bE}	-
	6hAE	27.92 ± 1.06 ^{dB}	58.66 ± 5.44 ^{aD}	-
	12hAE	27.96 ± 1.11 ^{cA}	51.88 ± 5.44 ^{bE}	-
	24hAE	32.58 ± 1.01 ^{aA}	57.82 ± 5.68 ^{bE}	23.68 ± 1.90 ^{aB}
<i>P-value</i> (BFTs x T)		<0.0001	0.0056	0.0013
CV (%)		12.83	25.10	36.03

Lowercase letters compare collection times within each pH range, while uppercase letters compare pH ranges within each time, according to Tukey's test ($p < 0.05$). BFT_{5.5}, pH 5.5-6.0; BFT_{6.5}, pH 6.5-7.0; and BFT_{7.5}, pH 7.5-8.0. (pH = Hydrogen potential. IAE = immediately after expusere, AE = after expuser and CV = coefficient of variation).

On the other hand, a significant effect of collection time was observed only on hemoglobin ($p < 0.0001$), glucose ($p < 0.0001$), triglycerides ($p < 0.0001$), and AST ($p = 0.0012$) values (Figure 2). There was no significant effect of pH range on hemoglobin ($p = 0.2698$), glucose ($p = 0.0560$), and AST ($p = 0.3785$), nor was there an interaction between time and pH for these variables ($p > 0.05$).

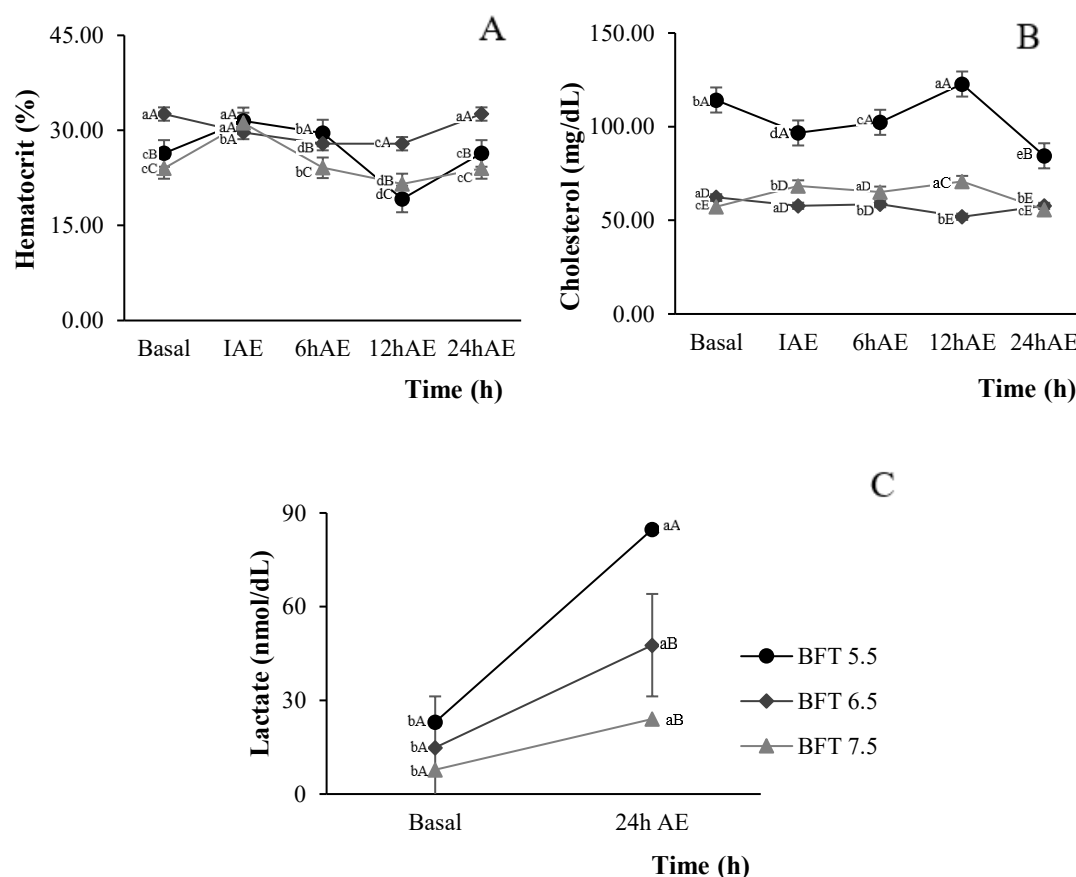


Figure 2. Hematocrit (A), cholesterol (B), and lactate (C) responses of *Colossoma macropomum* reared in a BFT system under different water pH ranges before and after a 30 minute air exposure challenge. Lowercase letters compare collection times within each pH range, while uppercase letters compare pH ranges within each time, according to Tukey's test ($p < 0.05$). BFT_{5.5}, pH 5.5-6.0; BFT_{6.5}, pH 6.5-7.0; and BFT_{7.5}, pH 7.5-8.0.

The highest hemoglobin values were recorded at the beginning of the experiment, with a significant reduction after the challenge, remaining relatively stable at subsequent time points. Glucose levels showed significant variation throughout the experimental period, increasing after the air exposure challenge, followed by a progressive reduction at the final time points. AST activity showed higher values after 24 hours of air exposure compared to the beginning of the experiment (Table 4).

Table 4. Hemoglobin (g/dL), glucose (mg/dL), triglycerides (mg/dL), and AST (U/L) (mean $\pm$ SE) of *Colossoma macropomum* reared in a BFT system under different water pH ranges before and after a 30-minute air exposure challenge.

Time	Hemoglobin (g/dL)	Glucose (mg/dL)	Triglycerides (mg/dL)	AST (U/L)
Basal	16.3 ± 1.85 ^a	82.46 ± 8.80 ^b	174.90 ± 9.39 ^{ab}	79.06 ± 7.60 ^b
IAE	10.9 ± 1.85 ^b	154.69 ± 8.80 ^a	191.82 ± 9.39 ^a	-
6hAE	7.2 ± 1.85 ^b	98.42 ± 8.80 ^b	169.93 ± 9.39 ^{ab}	-
12hAE	6.9 ± 1.85 ^b	64.31 ± 8.80 ^b	139.47 ± 9.39 ^{bc}	-
24hAE	7.5 ± 1.85 ^b	68.70 ± 9.19 ^b	126.74 ± 9.96 ^c	127.95 ± 11.4 ^a

Letters on the same line represent significant differences according to ANOVA test Tukey ($p < 0.05$). BFT_{5.5}, pH 5.5-6.0; BFT_{6.5}, pH 6.5-7.0; and BFT_{7.5}, pH 7.5-8.0. (AST = aspartate aminotransferase, IAE = Immediately after air exposure, AE = after air exposure, and CV = coefficient of variation.).

Although triglyceride levels were significantly influenced by both collection time and experimental pH range ($p = 0.0034$), no significant interaction was observed between these factors ($p = 0.5420$), indicating that their effects occurred independently. The highest triglyceride values were observed at the initial time points, with a gradual reduction throughout the experimental period (Table 4).

On the other hand, there was no significant effect of collection time on plasma protein ($p = 0.4439$), nor any interaction between time and pH range ($p = 0.2509$). However, a significant effect of pH range was observed ($p < 0.0001$), with the highest plasma protein values recorded in animals maintained at BFT_{5.5}, followed by BFT_{6.5} and BFT_{7.5} (Table 5).

Table 5. Plasma protein (g/dL) and triglycerides (mg/dL) (mean ± SE) of *Colossoma macropomum* reared in a BFT system under different water pH ranges before and after a 30-minute air exposure challenge.

BFTs	Proteína plasmática (g/dL)	Triglicéridos (mg/dL)
BFT _{5.5}	4.80 ± 0.09 ^a	179.61 ± 7.40 ^a
BFT _{6.5}	4.20 ± 0.09 ^b	158.28 ± 7.34 ^{ab}
BFT _{7.5}	4.15 ± 0.09 ^c	143.83 ± 7.34 ^b
P-value	<0.0001	0.0032
CV (%)	7.10	34.96

Letters on the same line represent significant differences according to ANOVA test Tukey ($p < 0.05$). BFT_{5.5}, pH 5.5-6.0; BFT_{6.5}, pH 6.5-7.0; and BFT_{7.5}, pH 7.5-8.0. (AST = aspartate aminotransferase, IAE = Immediately after air exposure, AE = after air exposure, and CV = coefficient of variation.).

3.3 Hepatosomatic index

The hepatosomatic index (HSI) of *C. macropomum* showed a significant difference only as a function of pH, with higher values in BFT_{5.5} after 24 h AE ($p = 0.0076$). (Fig. 3).

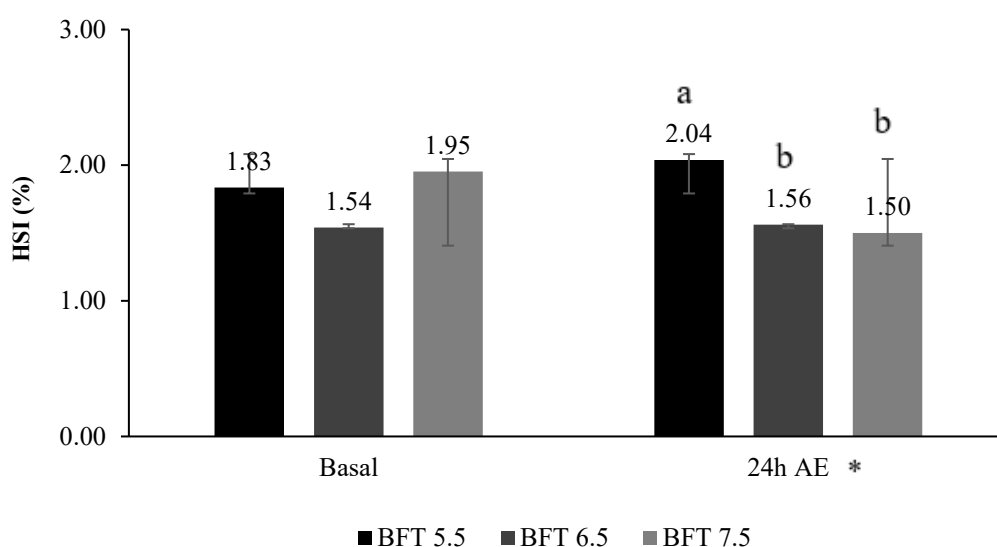


Figure 3. Hepatosomatic index (HSI, %) of *Colossoma macropomum* produced in a BFT system under different water pH ranges, at the beginning of the study and 24 hours after air exposure challenge (24 h AE). Values are expressed as mean $\pm$ SE. Different lowercase letters indicate significant differences between BFT treatments within the same sampling period (non-parametric Kruskal-Wallis test, $p = 0.0076$).

4. Discussion

Temperature, dissolved oxygen (DO), total soluble solids (TSS), and nitrogen dioxide (NO_2^-) were maintained at levels considered adequate and safe for the growth of *Colossoma macropomum* (Bastos et al., 2017; Costa et al., 2004; Hurtado and Bomfim, 2022; Izel-Silva et al., 2020; Oba et al., 2009; Saint-Paul, 1984; Santos M.Q. et al., 2013). Similarly, the values of soluble solids (SS) and total solids (TSS) remained consistent with well-managed biofloc systems (Avnimelech, 2015; Schryver et al., 2008), indicating adequate environmental conditions throughout the experimental period.

The higher concentration of TAN in BFT_{5.5} can be attributed to the lower efficiency of nitrification processes under acidic pH conditions, since the activity of nitrifying bacteria is directly influenced by the pH of the water, which directly affects the oxidation of TAN and NO_2^- (Grommen et al., 2002; Khanjani et al., 2023). Similarly, the increased concentrations of PO_4^{3-} in BFT_{5.5} are associated with the greater solubility of this ion at acidic pH, which favors the desorption of phosphate from the particulate phase to the dissolved phase (Hillert, 1995).

For fish that rely on gill respiration for gas exchange, exposure to air represents acute stress (Araújo-Lima and Gomes, 2005; Braum, 1983). Despite this, *C. macropomum* has recognized adaptive and physiological mechanisms that allow it to tolerate critical dissolved oxygen conditions

(Florindo et al., 2006; Robertson et al., 2015; Saint-Paul, 1984) and/or short periods of air exposure (do Carmo Neves et al., 2022). In the present study, the 100% survival rate after 24 hours confirms the species' resistance to air exposure for 30 minutes.

Variations in water pH interfere with the regulation of acid-base balance in aquatic organisms (Aride et al., 2007; Fromm, 1980; Roberts and Powell, 2003). This effect may explain the differences observed in the evaluated blood parameters. When exposed to pH variations, fish activate compensatory mechanisms, such as the excretion of hydrogen ions (H^+) or bicarbonates (HCO_3^-) through the gills, in order to restore acid-base balance and minimize the adverse effects associated with metabolic acidosis or alkalosis (Oba et al., 2009).

The study observed the effects of pH and/or collection time on hematocrit, cholesterol, and lactate, as well as the temporal effects on hemoglobin, glucose, triglycerides, and AST, in addition to the effects of treatment on plasma proteins and triglycerides. The variations observed in these parameters reflect important physiological adjustments related to oxygen transport capacity and the stress response imposed by air exposure.

Production of *C. macropomum* in BFT_{7.5} resulted in significantly higher baseline hematocrit values compared to the other treatments, which remained stable after the air exposure period. This pattern may be associated with greater efficiency in oxygen transport and maintenance of hematological homeostasis. Studies report that variations in water pH can influence hematocrit levels in *C. macropomum*, reflecting physiological adjustments associated with the oxygen-carrying capacity of the blood (Alves et al., 2025; Aride et al., 2007; Ranzani-Paiva and Tavares-Dias, 2004; Tavares-Dias and Mataqueiro, 2004).

In contrast, in the BFT_{5.5} and BFT_{6.5} treatments, baseline hematocrit values were within the normal range for the species (Alves et al., 2025). However, after air exposure, an immediate increase in this parameter was observed, characterizing a transient stress response. This increase may be related to the release of erythrocytes from the spleen or to hemoconcentration, mechanisms that temporarily increase oxygen transport capacity during episodes of acute hypoxia (Hillert, 1995). The gradual reduction in hematocrit throughout the recovery period in the BFT_{5.5} and BFT_{6.5} treatments indicates the reestablishment of physiological balance after the stressful stimulus.

In turn, hemoglobin showed a significant effect only over time, with a reduction after exposure to air and maintenance of values below baseline during recovery, regardless of culture pH. This behavior suggests that, although the number or proportion of erythrocytes may have transiently increased (as indicated by hematocrit), the hemoglobin concentration per unit volume of blood may have been modulated by adjustments in plasma volume or by the redistribution of blood components during the stress response (Oba et al., 2009). Despite this reduction, hemoglobin levels remained

within the physiological range described for *C. macropomum* (do Carmo Neves et al., 2022), indicating that there was no functional impairment of the hematological system.

Similar results were observed in *Oreochromis niloticus* exposed to air for 30 and 60 minutes, in which the temporary changes in hemoglobin did not exceed the physiological limits of the species (da Silva Liebl et al., 2022). These findings reinforce the idea that air exposure promotes transient hematological adjustments without causing permanent damage to the respiratory capacity of the fish.

Hematocrit levels showed responses dependent on both the pH of the culture medium and the time after challenge, while hemoglobin responded exclusively to the time factor, evidencing distinct but complementary regulatory mechanisms. These responses reflect the adaptive strategies of *C. macropomum* that ensure the maintenance of oxygen transport and physiological homeostasis, even under different pH conditions in BFT systems.

Plasma lactate concentrations increased significantly after air exposure, characterizing a typical metabolic response to acute stress in *C. macropomum*. Although this increase was observed in all treatments, its magnitude was greater in the BFT_{5.5} treatment, highlighting the modulating effect of pH on the physiological response to the stressor. Under basal conditions, no differences were observed between treatments, indicating that maintenance pH, in isolation, did not influence lactate levels in non-stressful situations.

Elevated lactate levels reflect the intensification of anaerobic metabolism resulting from temporary hypoxia and are considered a classic response to secondary stress in fish, frequently associated with increased plasma glucose (Wendelaar Bonga, 1997). Higher BFT_{5.5} values suggest that more acidic conditions may potentiate the reliance on anaerobic pathways during post-stress recovery, without indicating serious physiological impairment.

Plasma cholesterol in stressed fish has been associated with increased metabolic utilization, mainly as a precursor in the synthesis of corticosteroids, such as cortisol, which in turn is related to increased plasma glucose and lactate (da Silva Liebl et al., 2022; do Carmo Neves et al., 2022). In the present study, fish maintained in the BFT_{5.5} treatment showed higher cholesterol concentrations compared to the BFT_{6.5} and BFT_{7.5} treatments at all evaluated time points. Despite these differences, the values remained within the physiological range described for *C. macropomum* (do Carmo Neves et al., 2020), which does not indicate metabolic impairment. The interaction between treatment and time was more evident in BFT_{5.5}, with a significant reduction in cholesterol at 24 h AE, while in the other treatments (BFT_{6.5} and BFT_{7.5}) the values remained relatively constant, suggesting more pronounced metabolic adjustments under acidic pH conditions. This suggests that more acidic

conditions may intensify metabolic adjustments related to cholesterol dynamics in response to stress, without characterizing a pathological response

Glucose showed rapid mobilization after air exposure, with concentrations practically doubling after exposure, demonstrating the efficiency of the initial metabolic response. Six hours after air exposure, levels returned to baseline values and remained stable within normal conditions for the species (Assis et al., 2020; do Carmo Neves et al., 2020), with no effect of pH or interaction between treatment and time. Similar results were reported for *Piaractus mesopotamicus* (Abreu et al., 2009) and *C. macropomum* (do Carmo Neves et al., 2022), in which baseline glucose levels were restored within 24 hours after air exposure.

Unlike glucose, triglycerides showed an effect of the treatment, with higher values in BFT_{5.5}. Lipid mobilization is associated with the physiological adaptation of fish, ensuring the energy necessary for the functioning of the cardiovascular system and other metabolic functions during environmental challenges (Villacorta-Correa and Saint-Paul, 1999). The reduction observed only at 24 h AE suggests a delayed metabolic response, indicating that lipids act as a complementary energy source during the post-stress recovery phase.

Plasma proteins, which are important in osmotic regulation, metabolite transport, and the immune response of fish (Hrubec and Smith, 1999; Oliveira et al., 2017), showed variations between treatments, with higher values in BFT_{5.5}. However, the values recorded in all treatments remained within the range considered physiologically normal for *C. macropomum* (Alves et al., 2025; do Carmo Neves et al., 2022). The variations observed likely reflect physiological adjustments to the environment, and not a response associated with metabolic stress or immune impairment of *C. macropomum*, and although water pH can influence plasma protein dynamics, this effect did not show significant biological relevance under the experimental conditions evaluated.

The increase in the hepatosomatic index (HSI) in fish kept at pH_{5.5} after 24 hours of air exposure, associated with a delayed increase in aspartate aminotransferase (AST) activity during the same period, indicates an intensification of hepatic metabolic activity during the post-stress recovery process (Cardoso Roriz et al., 2015). The absence of an immediate increase in AST after the challenge suggests that air exposure did not cause severe tissue damage, but triggered transient physiological adjustments consistent with an adaptive process (Cardoso Roriz et al., 2015). Thus, the concomitant increase in HSI and AST reinforces the role of the liver as a central organ in restoring metabolic homeostasis, especially in fish kept at pH_{5.5}.

In general, the results indicate that *C. macropomum* maintained at pH_{5.5} showed more pronounced physiological responses when exposed to air, reflecting greater metabolic and hematological sensitivity to the acute stressor under these conditions. However, these responses do

not indicate physiological impairment, but rather transient adaptive adjustments to the imposed challenge.

Studies with *C. macropomum* demonstrate the species' high tolerance to acidic pH environments, in which ionoregulatory disturbances tend to be reduced and the recovery of ionic fluxes occurs efficiently after stress (Wilson et al., 1999; Wood et al., 1998). This adaptive capacity favors the species' performance and growth in naturally acidic environments (Alves et al., 2025; Aride et al., 2007). Thus, although pH_{5.5} potentiated the magnitude of physiological responses to stress, the data reinforces the adaptive robustness of *Colossoma macropomum* in the face of challenging environmental conditions.

5. Conclusion

The results demonstrate the capacity and resilience of *Colossoma macropomum* maintained at different pH levels in BFT medium, even after exposure to air for 30 minutes. The absence of mortality and the restoration of hematological and biochemical parameters to baseline levels within 24 hours highlight the species' ability to recover from an acute stressor, reinforcing its recognized adaptability to varied environmental conditions.

However, it was observed that fish kept at pH_{5.5} showed more pronounced changes in hematological, biochemical, and hepatosomatic indices compared to the other treatments, suggesting that the maintenance pH influences the magnitude of physiological responses to stress. These findings confirm that, although fish also exhibit high physiological robustness, metabolic and hematological adjustments to environmental challenges can vary according to pH, without necessarily compromising physiological function.

Acknowledgments

This research received support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Brazil).

Author contributions

Naiara Pinho Alves: Conceptualization, Methodology, Validation, Formal analysis, Resources, Investigation, Writing Original Draft, Writing Reviewing and Editing, Visualization, Funding Acquisition. **Ronald Kennedy Luz:** Writing Reviewing and Editing, Visualization, Project Administration, Funding Acquisition. **Cintia Labussiere Nakayama:** Supervision, Methodology,

Formal analysis, Resources Investigation, Writing Reviewing and Editing, Visualization, Funding Acquisition.

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CAPÍTULO 3

A formatação do manuscrito foi baseada na revista AQUACULTURE

Optimizing aquaponic systems by synchronizing fish and plant circadian rhythms

Abstract

Aquaculture plays an essential role in ensuring food security and promoting the health of the world's population through a source of high biological value protein. The use of closed systems such as aquaponics is an agricultural approach that allows the recycling of nutrients and waste through the combined production of fish and plants. Fish waste and feed residues are used as fertilizer for the plants, and after the plants filter and purify the water, it is returned to be used by the fish. The present study aimed to evaluate the effects of photoperiod on synchronization and desynchronization in coupled aquaponics systems. The experimental set consisted of eight autonomous aquaponic systems arranged in two experimental groups: four systems with fish and plants under the same light-dark cycle (LD) (synchronized group) and four systems with fish and plants under reverse LD (desynchronized group). During the experimental period, dissolved oxygen in the water was maintained at 7.0 ± 0.4 mg/L, pH 6.7 ± 0.4 , temperature at 23.0 ± 0.1 °C, and only except for K with higher values (14.10 ppm) and Mg (9.28 ppm) with higher values in the synchronized system in weeks 5 and 2, respectively ($p = 0.0286$). Regarding lettuce production, fresh biomass, growth rate of fresh mass and dry mass of lettuce seedlings did not show significant differences ($p > 0.05$). The results demonstrate that water quality and lettuce production were not influenced by the synchronization and desynchronization of the aquaponic systems used, except for water use efficiency (WUE), which showed different behaviors.

Keywords: Aquaculture, photoperiod, nutrients, water quality.

1. Introduction

Due to the high number of people worldwide living in precarious food conditions, the importance of maintaining a balance between food production and nutrition for future generations is highlighted (FAO, 2022). However, food production depends on the availability of resources, such as land, fresh water, fossil energy and nutrients (Conijn et al., 2018). In view of this, aquaculture plays a fundamental role in eliminating hunger and promoting the health of the world population (Sgnaulin et al., 2018), through a source of high biological value protein (Gonçalves, 2011). Furthermore, biochemical flow limits food supply, as do climate change (Steffen et al., 2015). Therefore, nutrient recycling, waste prevention and dietary changes are essential to transform current food production (Conijn et al., 2018; Kahiluoto et al., 2014).

In aquaculture, the use of closed aquaculture systems (Timmons et al., 2018) is encouraged, since water is recycled and fish farming density is high. An example of this type of system is aquaponics. Aquaponics is an agricultural approach that, through the recycling of nutrients and waste, fits into a sustainable aquaculture system, especially in arid regions or with non-arable soils (Bittsanszky et al., 2016; Goddek and Körner, 2019; Kotzen and Appelbaum, 2010).

Aquaponic systems offer several advantages when it comes to producing food in an innovative and sustainable way, such as the synergistic effects of increasing CO₂ concentration for greenhouse crops and the reduction of total thermal energy consumption when cultivating fish and crops in the same space (Körner et al., 2017). Furthermore, it is a proposed solution for the use of marginal land in urban areas for food production (Bernstein, 2011). The combination of a recirculating aquaculture system (fish or shrimp) with hydroponic production (plants) that avoids the discharge of aquaculture effluents enriched in dissolved nitrogen and phosphorus (Buzby and Lin, 2014; Van Rijn, 2013), and the fertilization of soilless crops with what can be considered an organic solution (Goddek and Körner, 2019; Yogev et al., 2016). Furthermore, aquaponics produces plant growth comparable to conventional hydroponics, and even with lower concentrations of most nutrients (Bittsanszky et al., 2016; Delaide et al., 2016; Graber and Junge, 2009), production can still be better than in soil (Rakocy et al., 2004).

Briefly, in aquaponic systems, nutrients absorbed by plants are derived from leftover feed and fish excretion residues. Ammonia is the main metabolic end product of dietary protein intake, being metabolized in the mitochondria of the fish liver (Weihrauch et al., 2009). Peak ammonia excretion and peak nitrogen uptake in plants occur at different light phases during the day, suggesting a desynchronization between the metabolic rhythms of fish and plants (Obirikorang et al., 2015).

This temporal disharmony can harm the productivity of aquaponic systems. In view of this, the present study investigated the effects of photoperiod on synchronization and desynchronization in coupled aquaponic systems. The evaluation of the different treatments focused on water quality and plant production.

2. Material and Methods

The experiment was carried out at the Department of Physiology of the University of Murcia – Spain, during a period of five weeks. The experimental set up was composed of eight autonomous aquaponic systems arranged in two experimental groups: four systems with fish and plants under the same light-dark (LD) cycle (synchronized group), and four systems with fish and plants under reversed LD (desynchronized group) (Fig.1).

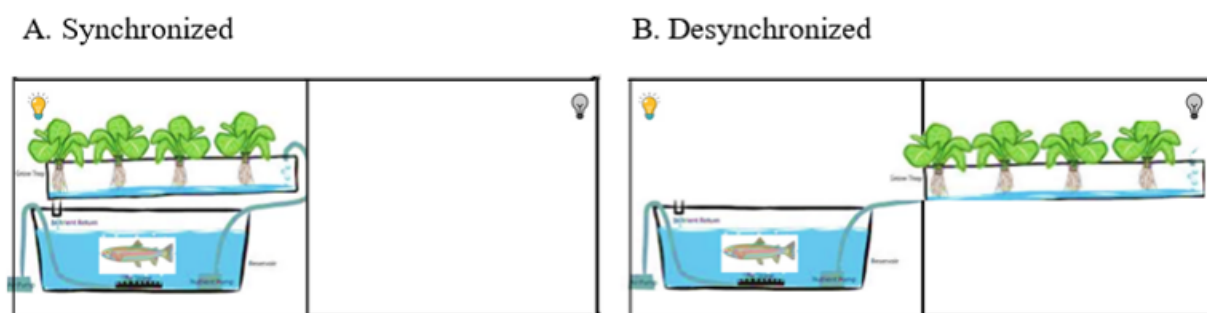


Figure 1. Schematic diagram of the experimental set up representing fish tanks and plant trays under the same LD (A, synchronized) or reversed (B, desynchronized).

The aquaponic systems in which the fish tanks and hydroponic plants trays shared the same LD cycle were named as "synchronized" (SL), while those systems in which the fish and plants were maintained under opposite LD cycles were named as "desynchronized" groups.

2.1 Lighting conditions

To simulate the photoperiod, an artificial lighting system composed of four LED panels (300W, CERoHS) was used, which provided an optimized light spectrum for plant growth through the combination of red, blue and white wavelengths. The lettuces and fish were grown in systems that were in an LD 12h:12h, with lights on at 07:00 h (ZeitgeberTime = 0, ZT = 0 h).

2.2 Aquaponics System

Eighty-eight tenca juveniles (*Tinca tinca*) with 11.0 ± 2.20 g; 9.8 ± 1.0 cm were randomly distributed in each experimental tank after being weighed on a precision scale (0.01 g, SKX2201 OHAUS) and measured on an ichthyometer (Aquactic Biotechnology). The average initial biomass per tank was 120.9 ± 5.3 g.

Each aquaponic system was composed of a fish tank (90 L) with 11 fish, and a hydroponic tray of 0.24 m² and 15 cm water deep. Each hydroponic unit contained five lettuces (*Lactuca sativa* L.) held in a phenolic floating foam (20 × 20 × 15 mm). The water from the fish tank was pumped up to the hydroponic units after being filtered by an external filter (300 L/h, EHEIM classic 150). Filtration was carried out by two main stages: first by capturing solid particles using a combination of coarse and fine filter media, and second by hollow ceramic cylinders, which enable denitrification by bacterial communities, and mechanical filtration.

Before the beginning of the experiment, the biological filters were matured, with the addition of bacteria (Kiwoko-Start) according to the manufacturer's recommendation. To avoid iron and potassium deficiencies, each hydroponic system was supplemented with Fe-DTPA (COMPO, Chelated Iron DTPA 13 %) at a concentration of 40 µmol/L in water and potassium in the form of potassium sulfate (K₂SO₄, Culvers) at 10 µmol/L, following the protocol developed by Tsoumlakou et al., (2022).

Fish feeding was carried out once a day with automatic feeders, providing a single meal in the middle of the night (ZT18). An isoprotein diet (1.7mm) contained 46 % CP, 10 % fat and 0.9 % crude fiber (Alltech Coppens, Vital) was used.

2.3 Water quality

During the experimental period, the dissolved oxygen the water was maintained in 7.0 ± 0.4 mg/L, pH 6.7 ± 0.4 , temperature at 23.0 ± 0.1 °C and the ambient temperature at 20.0 ± 0.5 °C. Water samples were collected weekly, and frozen at -18 °C for further analysis. Concentrations of total organic carbon (TOC), total inorganic carbon (IC) and total carbon (TC) were performed by elemental analysis of liquids (Analytikjena N/C 3100). calcium (Ca), potassium (k), magnesium (Mg), and iron (Fe), by plasma optical emission spectrometer (ICP-OES/ Agilente 5110), and anions: fluoride, chloride, nitrite, nitrate, sulfate, and phosphate in ion chromatography (Dionex ICS-2100), according to U.S. Environmental Protection Agency Procedure 300.0 (EPA, 1993).

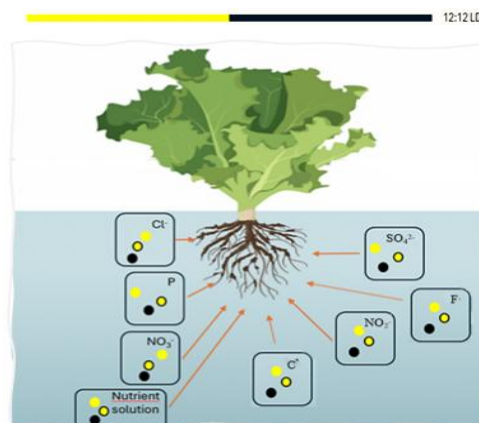


Figure 2. Photoperiod regime and nutrient absorption dynamics by the root system of cultivated lettuce.

2.4 *Lactuca sativa* L. production

Before being installed in the hydroponic beds of aquaponic systems, lettuce seedlings (*Lactuca sativa* L.) were weighed and their roots were carefully wiped to remove all dirt. The mean initial biomass of each hydroponic system was 19.35 ± 1.28 g. The analysis of water use efficiency (WUE), assimilation rate (A), stomatal conductance (gsw), difference in carbon dioxide concentration (ΔCO_2), difference in water vapor concentration ($\Delta\text{H}_2\text{O}$) by means of the portable photosynthesis system (LI-6800 XT, LI-COR, Lincoln, NE, USA), were performed fortnightly. At the end of the experiment the physiological parameters of the lettuce were analyzed by weighing the plants of each experimental unit to determine the growth rate, which was calculated according to the following equation:

$$\text{Weight Gain (\%)} = (\text{Final Weight} - \text{Initial Weight} / \text{Initial Weight}) \times 100$$

2.5 Statistics

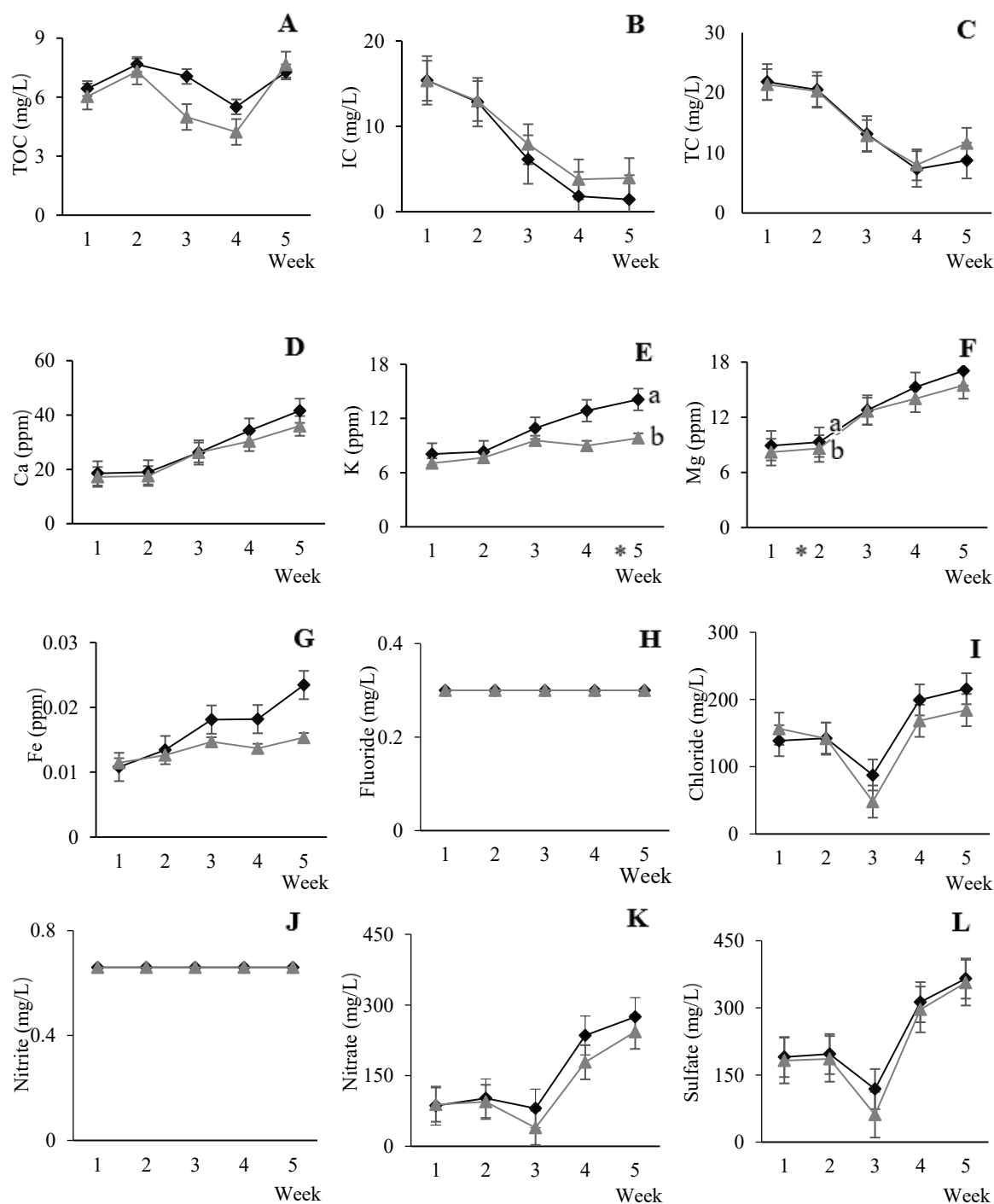
The data were analyzed for normality and homoscedasticity, and when the assumptions for ANOVA analysis of variance were not met, the data were analyzed by the nonparametric Kruskal Wallis test with 5 % significance. Percentage data were transformed into arcsine of the square root; only untransformed data are presented.

3. Results

3.1 Water quality

Water quality parameters do not show significant differences between synchronized and desynchronized systems, $p > 0.05$, with higher values (14.10 ppm) and Mg (9.28 ppm) with higher

values in the synchronized system in weeks 5 and 2, respectively ($p = 0.0286$) (fig. 3, H and F). However, an oscillation was observed during the experimental weeks among the other water parameters (TOC (mg/L) (fig. 6, A), IC (mg/L) (fig. 6, B), TC (mg/L) (fig. 6, C), Ca (ppm) (fig. 6, D), Fe (ppm) (fig. 6, G), chloride (mg/L) (fig. 6, I), nitrate (mg/L) (K), sulfate (mg/L) (fig. 6, L) and phosphate (mg/L) (fig. 6, M), except fluoride (mg/L) (fig. 6, H) and nitrate (mg/L) (fig. 6, J), which remained stable.



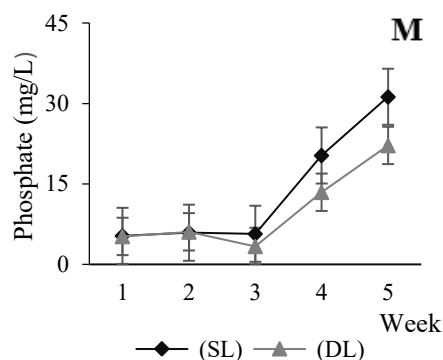


Figure 3. Weekly curves of water quality parameters: A, TOC (mg/L); B, IC (mg/L); C, TC (mg/L); D, Ca (ppm); E, Fe (ppm); F, K(ppm); G, Mg (ppm); H, fluorine (mg/L); I, chloride (mg/L); J, nitrite (mg/L); K, nitrate (mg/L); L, sulfate (mg/L) and M, phosphatase (mg/L) in synchronized and desynchronized systems, respectively, during the experimental period. Different letters in the same week represent significant differences by non-parametric Kruskal-Wallis test, $p < 0.05$. (SL - synchronized system, DL -desynchronized, Ca - calcium, Fe - iron, K- potassium, Mg - magnesium, TOC - total organic carbon, IC - total inorganic carbon, TC - total carbon).

3.2 *Lactuca sativa* L production

Based on the nonparametric Kruskal-Wallis test, it is possible to observe that the fresh biomass (fig. 4), the growth rate of fresh weight and the dry weight (fig. 5) of the lettuce seedlings did not present significant differences ($p > 0.05$).

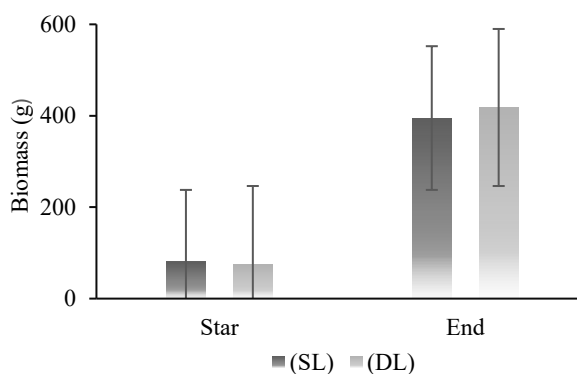


Figure 4. Average fresh biomass production of lettuce grown in aquaponics in synchronized and desynchronized systems. Analysis by the non-parametric Kruskal-Wallis test, $p > 0.05$. Analysis by the non-parametric Kruskal-Wallis test, $p > 0.05$. (SL - synchronized system, DL -desynchronized).

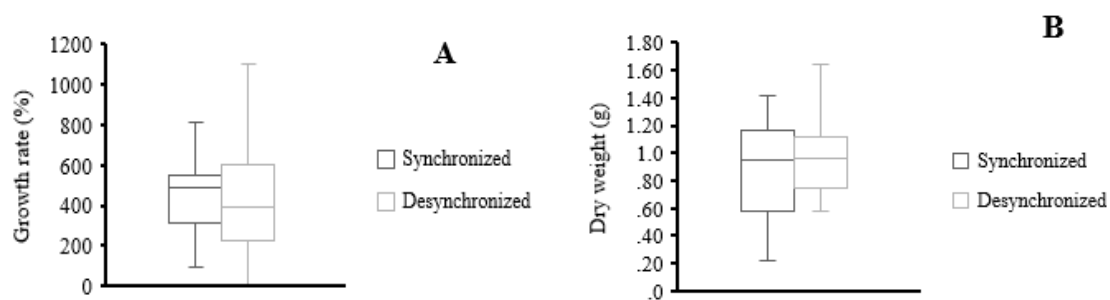


Figure 5. A, Fresh weight growth rate (g) and B, Dry weight (g) of lettuce in the synchronized and desynchronized systems. Analysis by the non-parametric Kruskal-Wallis test, $p > 0.05$. (SL - synchronized system, DL -desynchronized).

The measurements of the weekly gas exchange curves of lettuce leaves did not show significant differences until week 3, $p > 0.05$. In week 5, significant differences were observed for WEA, GS and ΔH_2O , $p < 0.05$ between the synchronized and desynchronized systems.

WUE varied throughout the experimental period, with higher concentrations ($125.98 \mu\text{molCO}_2 \cdot \text{mmolH}_2\text{O}^{-1}$) in week 5 in the desynchronized system ($p = 0.0498$) (fig. 6, A). In contrast, GS and ΔH_2O showed similar behavior, with higher concentrations ($0.08 \text{ mmolH}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; $0.60 \text{ mmolH}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, respectively) in the synchronized system ($p = 0.0014$) in the same experimental week (fig. 6, C and E).

A and ΔCO_2 did not present statistical differences ($p > 0.05$), but a gradual oscillation was observed in the experimental weeks in the synchronized and desynchronized systems (fig. 6, B and D). But with different behaviors. The concentration of A during the experimental weeks showed a decrease in concentrations from $2.88 \mu\text{molCO}_2 \cdot \text{mmolH}_2\text{O}^{-1}$ to $1.64 \mu\text{molCO}_2 \cdot \text{mmolH}_2\text{O}^{-1}$ and $2.71 \mu\text{molCO}_2 \cdot \text{mmolH}_2\text{O}^{-1}$ to $1.11 \mu\text{molCO}_2 \cdot \text{mmolH}_2\text{O}^{-1}$ in synchronized and desynchronized systems, respectively. While the concentrations of ΔCO_2 maintained a constant elevation with the highest concentrations in week 5 with -0.78 in the desynchronized system and -1.63 in the desynchronized system.

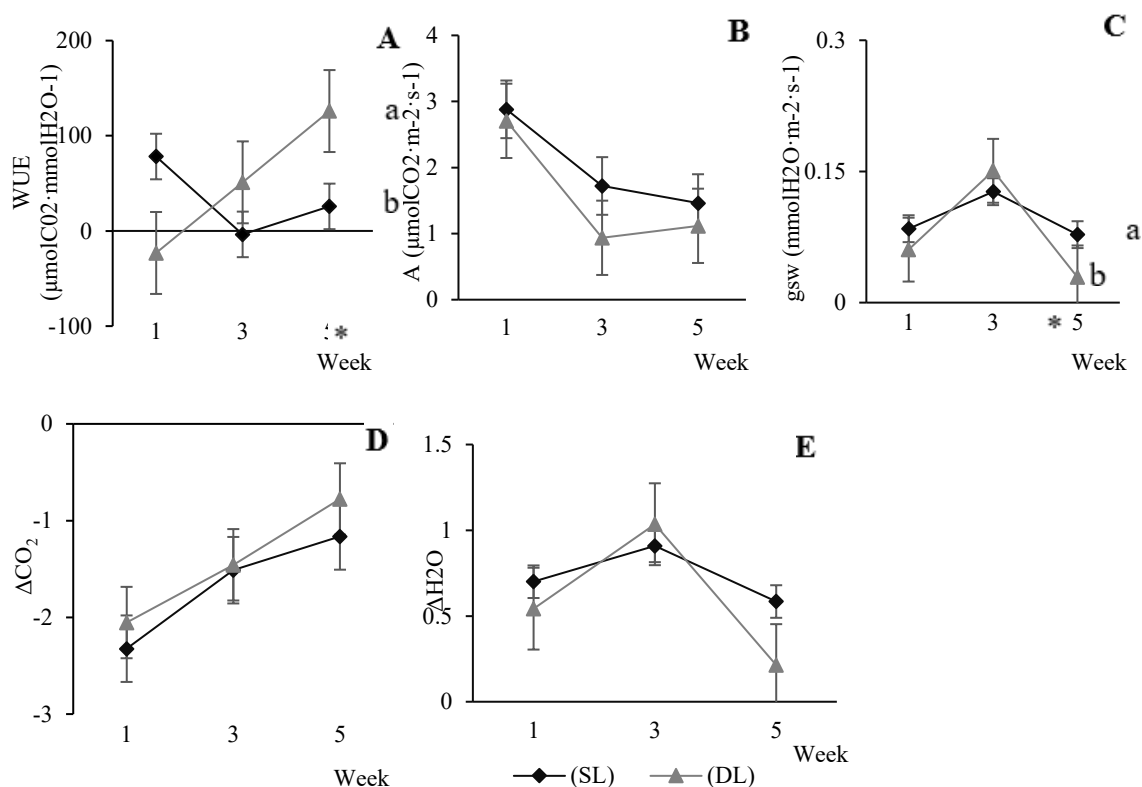


Figure 6. Measurements of gas exchange in lettuce leaves in aquaponics (A, WUE ($\mu\text{molCO}_2 \cdot \text{mmolH}_2\text{O}^{-1}$); B, A ($\mu\text{molCO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); C, gsw ($\text{mmolH}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); D, ΔCO_2 and E, $\Delta\text{H}_2\text{O}$ in the synchronized and desynchronized systems. Different letters in the same week represent significant differences by non-parametric Kruskal-Wallis test, $p < 0.05$.

3. Discussion

Water quality

Water quality parameters are essential for the success of an aquaponic system, as they directly influence fish health, plant growth, and the balance of the integrated ecosystem. In the present study, dissolved oxygen, temperature, and pH were maintained at conditions suitable for the optimal functioning of aquaponic systems (Nuwansi et al., 2021; Souza et al., 2021; Teixeira et al., 2006).

The ionic balance should also be monitored and adjusted according to the needs of each system. The higher Ca concentrations in the final period in both treatments in this study are probably related to its addition through the feed provided, since calcium has high reactivity (Adler et al., 1996). But the concentrations obtained are within those presented in a study with experimental/commercial aquaponic cultivation (Rakocy, 2007), essential for maintaining the cellular structure of plants (Hawkesford, 2012; Maathuis, 2009; Malavolta et al., 2006).

Potassium influences the photosynthesis process by regulating transpiration and the entry of CO_2 into the leaves, and this process is essential for maintaining osmotic and ionic balance in plant

cells (Hasanuzzaman et al., 2018; Hawkesford, 2012; Maathuis, 2009). Authors report that when there is a deficiency of this micronutrient, there is an accumulation of soluble nitrogen compounds and a lower accumulation of nitrate, which was not confirmed in the present study (Faquin et al., 1994; Rincón et al., 2005). On the contrary, K concentrations showed a slight increase in the last week with higher values in the synchronized system. Furthermore, lettuces showed no significant difference in growth (Mengel et al., 2001).

As a constituent compound of chlorophyll, Mg deficiency is observed through a decrease in the photosynthetic rate, which promotes symptoms such as discoloration of leaf tissues, characterized as leaf necrosis (Rincón et al., 2005). In the present study, these symptoms were not observed.

Iron supplementation is recommended in aquaponic systems, since iron from the feed is insufficient. In the present study, supplementation was performed only before the start of the experiment, since the plants did not show signs of deficiency, although the concentrations were below the recommended level (Rakocy, 2007).

Fluoride and nitrite remained stable throughout the experimental period. Nitrite values are in accordance with the recommended level for fish < 1.0 mg/L (Jiang et al., 2014), and its stable concentration throughout the experimental period confirms the efficiency of the nitrification process.

Chlorine showed a slight increase in the last experimental weeks, but its concentrations are below those inadequate for growing plants and fish. For nitrate, variations were probably due to absorption by plants. In a production system, its proportion varies according to the amount of protein in the feed and the fish population density (Endut et al., 2010). Concentrations > 300 mg/L are reported as harmless to *Oreochromis niloticus* (Graber and Junge, 2009), values close to those found in the present study. This result indicates that, although the observed concentrations remained below levels considered harmful to fish, nitrate accumulation occurred in the final weeks of the experiment, suggesting a possible imbalance between the fish stocking rate and plant biomass.

Sulfate influences the electrical conductivity of the aquaponic system; however, its additional addition is not necessary, as the concentrations present in the system are adequate to ensure plant development (Tidwell, 2012). Phosphorus is essential for energy transfer and cell growth (Boyd and Tucker, 1998). As in other studies, the present work demonstrates the accumulation of phosphorus over time (Avnimelech, 2009), with concentrations > 20 mg/L for growth and constant biomass in plants (Pinho et al., 2021; Santos et al., 2005). The accumulation of phosphorus is probably related to the low density of vegetation (lettuce), which led to an imbalance between plant biomass and the stocking rate of fish.

Lactuca sativa L production

Results suggest that synchronized and non-synchronized systems do not present differences in terms of productivity in the cultivation of lettuce seedlings in aquaponics.

In lettuce cultivation, growth results showed no difference between treatments. Different data were found with *Oreochromis niloticus*, using the same experimental design (François, 2025). This probably occurred because *Tinca tinca* is a nocturnal feeding species with a slow metabolism (Perrow et al., 1996), which may have influenced the acrophase of nitrogen absorption between plants and fish. However, the plants showed good development, which confirms that they were in suitable environmental conditions.

Analyses of gas exchange trends in lettuce leaves demonstrate that the inverse relationship between gsw and A up to week 3 may indicate an accumulation of nutrients in the aquaponic system in both treatments. This may have influenced the gsw values being $>0.65 \text{ mmolH}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, favoring stress in the plants (Guerra et al., 2017; Soares et al., 2015), and a deficiency in the maintenance of A.

In an aquaponic system, plants have continuous access to water, favoring $>\text{gsw}$ and $>\Delta\text{H}_2\text{O}$, a result found in the present study (da Silva et al., 2015).

In the present study, the increase in ΔCO_2 reflects lower photosynthesis in accordance with the decrease in A in both treatments. Based on the plant's CO_2 assimilation rates, reduced growth would be expected. However, this pattern was not found in this study. This could be explained by increased maintenance respiration, since photosynthesis alone does not drive biomass production (Lambers et al., 1983; Zhu et al., 2010). The difference in WUE between the systems may indicate that the desynchronized system had better water use efficiency.

4. Conclusion

The results show that water quality and lettuce production were not influenced by the synchronization and desynchronization of the aquaponic systems used, except for WUE, which showed different behaviors. Studies should be carried out, mainly in relation to the ammonia excretion rate of *Tinca tinca*, in order to verify whether this may have influenced the results of the present study.

Acknowledgments

This research received support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Brazil).

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Considerações Finais

De modo geral, os resultados obtidos nos Artigos 1 e 2 confirmaram as hipóteses propostas, evidenciando a eficiência da fonte de carbono avaliada e a adaptabilidade de *Collossoma macropomum* a diferentes faixas de pH. Quanto à aplicabilidade, a utilização de pH 5,5 em sistemas de produção comercial mostrou-se viável em condições controladas e em pequena escala, desde que acompanhada de manejo rigoroso da qualidade da água e da utilização de sistemas eficientes de tamponamento. Considerando que *C. macropomum* é uma espécie nativa de ambientes naturalmente ácidos, essa estratégia pode representar uma alternativa promissora para sistemas produtivos localizados em regiões amazônicas ou em áreas com características hidroquímicas semelhantes.

No entanto, os resultados observados indicam a necessidade de investigações adicionais que incluam análises histológicas, de íons, de pH sanguíneo e de estresse oxidativo, as quais podem contribuir para o melhor entendimento dos mecanismos fisiológicos envolvidos na resposta da espécie às diferentes condições ambientais avaliadas.

Em relação aos resultados do terceiro artigo, conduzido com *Tinca tinca* na Espanha, estes podem ser considerados como referência para o contexto brasileiro com cautela, uma vez que diferenças fisiológicas entre as espécies, especialmente no metabolismo e nos mecanismos de excreção de amônia, limitam extrapolações diretas. Ainda assim, os princípios associados à dessincronização fotoperiódica demonstram potencial de aplicação em outros sistemas e espécies aquícolas. Dessa forma, destaca-se a necessidade de novos estudos com espécies nativas, conduzidos sob condições ambientais brasileiras, a fim de aprofundar a compreensão dos processos envolvidos e ampliar a aplicabilidade dos resultados obtidos.