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**Efeito de diferentes concentrações de sólidos suspensos totais em sistema de bioflocos
sobre o desempenho zootécnico, a condição intestinal e a atividade de enzimas
antioxidantes de juvenis de truta arco-íris *Oncorhynchus mykiss***

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Tese apresentada ao Programa de Pós-graduação em Ciência Animal, da Universidade do Estado de Santa Catarina (UDESC), como requisito parcial à obtenção do título de Doutora em Ciência Animal, área de concentração em Produção Animal. Orientador: Prof. Dr. Thiago El Hadi Perez Fabregat

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"O Escoteiro sorri e canta nas dificuldades."

*— Artigo 8º da Lei Escoteira (União dos
Escoteiros do Brasil)*

*Dedicado à Edemilson, Carmem e Henrique,
os amores da minha vida*

RESUMO

A intensificação da aquicultura tem aumentado a busca por sistemas de produção que permitam maior eficiência no uso da água e controle das condições de cultivo. Nesse contexto, a tecnologia de bioflocos (BFT) permite a reciclagem de nutrientes e o controle de compostos nitrogenados por meio da atividade microbiana. Apesar de sua ampla utilização com espécies mais tolerantes, a aplicação da tecnologia em espécies carnívoras e fases de desenvolvimento iniciais ainda apresenta desafios relacionados à concentração de sólidos suspensos, às exigências respiratórias e à qualidade da água. Assim, esta tese teve como objetivo investigar a aplicação da tecnologia de bioflocos na criação de espécies e fases consideradas sensíveis, com ênfase na truta arco-íris (*Oncorhynchus mykiss*). Inicialmente, foi realizada uma revisão sobre os principais fatores que determinam a adaptação do sistema de bioflocos a diferentes espécies e fases de desenvolvimento. A partir de uma revisão literária, foi proposto que o BFT pode ser manejado de acordo com as características fisiológicas e ecológicas da espécie, principalmente por meio do controle da relação carbono:nitrogênio e dos sólidos suspensos. Posteriormente, foi realizado um experimento com 225 alevinos de truta arco-íris, com peso inicial médio de $0,81 \pm 0,06$ g, distribuídos em 15 tanques circulares de 70 L, com 15 peixes por tanque, em delineamento inteiramente casualizado, com três tratamentos e cinco repetições. Foram avaliadas uma condição controle em sistema de recirculação de água e duas concentrações de sólidos suspensos totais em sistema de bioflocos, 250 e 350 mg L⁻¹, durante 56 dias. Ao final do período experimental, foram avaliados o desempenho zootécnico, a microbiota e a histomorfometria intestinal e a atividade de enzimas antioxidantes. A concentração de 350 mg L⁻¹ reduziu o peso final, o ganho de peso e a taxa de crescimento específico em relação ao controle, enquanto a concentração de 250 mg L⁻¹ não diferiu do controle para essas variáveis. O consumo de ração, conversão alimentar e a sobrevivência não diferiram entre os tratamentos. A concentração de bactérias ácido-láticas no intestino aumentou nos peixes mantidos em 350 mg L⁻¹, enquanto o número de células caliciformes foi menor nessa concentração. Não foram observadas diferenças na altura e largura das vilosidades intestinais ou na atividade das enzimas antioxidantes avaliadas. Os resultados indicam que a criação de alevinos de truta arco-íris em sistema de bioflocos é possível com concentrações de sólidos suspensos de até 250 mg L⁻¹, enquanto concentrações mais elevadas podem afetar o desempenho zootécnico e alguns parâmetros intestinais. Dessa forma, o manejo dos sólidos suspensos e das condições de qualidade da água deve ser ajustado às exigências da espécie e à fase de desenvolvimento.

Palavras-chave: Aquicultura intensiva; sólidos suspensos; microbiota intestinal; saúde intestinal; tecnologia de bioflocos.

ABSTRACT

The intensification of aquaculture has increased the demand for production systems that allow greater water-use efficiency and control of rearing conditions. In this context, biofloc technology (BFT) enables nutrient recycling and the control of nitrogenous compounds through microbial activity. Despite its widespread use with more tolerant species, the application of this technology to carnivorous species and early developmental stages still presents challenges related to suspended solids concentration, respiratory requirements, and water quality. Thus, this thesis aimed to investigate the application of biofloc technology in the rearing of species and developmental stages considered sensitive, with emphasis on rainbow trout (*Oncorhynchus mykiss*). Initially, a review was conducted on the main factors that determine the adaptation of biofloc systems to different species and developmental stages. Based on a literature review, it was proposed that BFT can be managed according to the physiological and ecological characteristics of the species, mainly through the control of the carbon-to-nitrogen ratio and suspended solids. Subsequently, an experiment was conducted with 225 rainbow trout fry, with an initial mean body weight of 0.81 ± 0.06 g, distributed among 15 circular 70-L tanks, with 15 fish per tank, in a completely randomized design with three treatments and five replicates. A control condition in a recirculating aquaculture system and two concentrations of total suspended solids in a biofloc system, 250 and 350 mg L⁻¹, were evaluated for 56 days. At the end of the experimental period, zootechnical performance, intestinal microbiota and histomorphometry, and antioxidant enzyme activity were evaluated. The concentration of 350 mg L⁻¹ reduced final body weight, weight gain, and specific growth rate compared with the control, whereas the concentration of 250 mg L⁻¹ did not differ from the control for these variables. Feed intake, feed conversion ratio, and survival did not differ among treatments. The concentration of lactic acid bacteria in the intestine increased in fish maintained at 350 mg L⁻¹, while the number of goblet cells was lower at this concentration. No differences were observed in intestinal villus height and width or in the activity of the antioxidant enzymes evaluated. The results indicate that rearing rainbow trout fry in a biofloc system is possible at suspended solids concentrations of up to 250 mg L⁻¹, whereas higher concentrations may affect zootechnical performance and some intestinal parameters. Therefore, the management of suspended solids and water quality conditions should be adjusted according to the requirements of the species and developmental stage.

Keywords: Intensive aquaculture; suspended solids; intestinal microbiota; gut health; biofloc technology.

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LISTA DE ABREVIATURAS, SIGLAS E SIMBOLOS

ABRAT	Associação Brasileira de Truticultores
AOB	Ammonia-oxidizing bacteria
BFT	Bioflocos ou Bioflo Thecnology
°C	Grau Celsius
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CEUA	Comitê de Ética no Uso de Animais
CFUs	Colony-forming units
C:N	Relação carbono:nitrogênio
DO	Dissolved oxygen
FAPESC	Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina
FAO	Organização das Nações Unidas para a Alimentação e Agricultura
FCR	Feed conversion ratio
FSH	Hormônio Folículo Estimulante
g	Gramas
GSI	Índice Gonadossomático
GPx	Total Glutathione Peroxidase
GR	Glutathione reductase
IRTA	Institute of Agrifood Research and Technology
LAB	Lactic acid bacteria
LPS	Lipopolysaccharides
L	Litro
MAMPs	Microbial-associated molecular patterns
mg	Miligrama
m ³	Metro cúbico
MRS	De Man, Rogosa, and Sharpe
NH ₃	Amônia
NMDA	N-methyl-D-aspartate
NO ₂	Nitrito
NO ₃	Nitrato
NOB	Nitrite-oxidizing bacteria

O ₂	Oxigênio
OD	Oxigênio dissolvido
PDSE	Programa de Doutorado Sanduíche no Exterior
PRRs	Pattern recognition receptors
RAS	Sistema de Recirculação ou Recirculating Aquaculture Systems
Rh	Rhesus
SOD	Superoxide dismutase
ton	Tonelada
UDESC	Universidade do Estado de Santa Catarina
µm	Micrometro
UV	Radiação Ultravioleta
Kg	Quilograma
TAN	Nitrogênio amoniacal total
TAC	Total antioxidant capacity
TSA agar	Ágar Triptona de Soja ou Tryptic soy agar
TCBS agar	Ágar de tiosulfato, citrato, bÍlis e sacarose
TSS	Sólidos suspensos totais
%	Porcentagem

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1 INTRODUÇÃO

A truta arco-íris é uma das espécies mais cultivadas no mundo, com uma produção de 1,18 milhão de toneladas em 2024 (FAO 2026a), registrando uma taxa de crescimento anual composta (CAGR) entre 2020 e 2024 de 4,41%. Dentre os salmonídeos, *Oncorhynchus mykiss* se destaca como uma das espécies de maior importância comercial, impulsionada pelo aumento do consumo. Essa espécie é amplamente reconhecida por seu valor nutricional e características sensoriais, além do seu alto teor de ácidos graxos. Seu cultivo ocorre tradicionalmente em sistemas de fluxo contínuo, como *raceways*, totalizando em média 70% da produção, porém também é cultivada em sistemas de recirculação (RAS) (10%) e aquaponia (Vasdravanidis et al., 2022). Devido às suas características fisiológicas, a criação da truta arco-íris requer condições específicas de qualidade de água. O fluxo de água contínuo constitui o principal mecanismo para o fornecimento e manutenção do oxigênio dissolvido e para a remoção de compostos nitrogenados, enquanto a concentração de sólidos suspensos é outro fator importante para a manutenção das condições adequadas de criação dessa espécie (Skoronski et al., 2018).

Embora a produção da truta arco-íris já ocorra em sistemas intensivos, a crescente demanda por esse pescado tem impulsionado a busca por sistemas que possibilitem maior eficiência no uso da água, associado à manutenção da qualidade ambiental, biossegurança e estabilidade dos parâmetros de cultivo. Nos sistemas de criação atualmente utilizados para essa espécie, a redução da renovação de água representa um dos principais desafios, uma vez que a manutenção da qualidade da água em sistemas intensivos depende, tradicionalmente, de elevados fluxos e/ou renovação contínua (Vasdravanidis et al., 2022; Pepe-Victoriano et al., 2025). Assim, tecnologias que permitam o controle e a reciclagem dos nutrientes dentro do próprio sistema podem contribuir para reduzir o consumo de água sem comprometer as condições necessárias à criação da espécie.

A tecnologia de bioflocos (BFT) se destaca por reciclar nutrientes por meio de comunidades microbianas que assimilam compostos nitrogenados, reduzindo a necessidade de renovação de água (Nisar et al., 2022). O sistema é caracterizado pela formação complexa de uma comunidade microbiana, na qual microrganismos utilizam compostos nitrogenados e carbono orgânico para a produção de biomassa microbiana (Emerenciano et al., 2017). Além da redução da renovação de água, a manutenção de uma comunidade microbiana ativa pode contribuir para o controle dos compostos nitrogenados e para a estabilidade do sistema, características que podem favorecer a biossegurança e a sustentabilidade da produção. Dessa

forma, o BFT tem demonstrado potencial para melhorar o desempenho zootécnico, a saúde e a sobrevivência dos animais, além de apresentar benefícios econômicos no cultivo (Ogello et al., 2021; Khanjani et al., 2024).

Apesar dessas vantagens, a utilização do BFT ainda está atrelada a espécies consideradas mais rústicas e tolerantes às condições do sistema tradicionalmente conhecido de bioflocos, como as onívoras (McCusker et al., 2022; Yadav et al., 2025), enquanto sua aplicação para espécies fisiologicamente mais exigentes, carnívoras como truta arco-íris, ainda apresenta limitações. A truta possui elevada demanda de oxigênio e é criada em sistemas de fluxo contínuo ou de recirculação (Pepe-Victoriano et al., 2025). Essas características tornam necessário avaliar se os princípios do BFT podem ser adaptados às exigências fisiológicas da espécie, especialmente em relação à manutenção do oxigênio dissolvido e ao acúmulo de sólidos suspensos.

Esta tese tem o objetivo avaliar a viabilidade da utilização da tecnologia de bioflocos na criação de espécies e fases consideradas sensíveis, discutindo estratégias para adaptação e manejo desse sistema de acordo com suas exigências fisiológicas. Posteriormente avaliar a utilização do sistema de bioflocos na criação de truta arco-íris (*Oncorhynchus mykiss*), considerando os efeitos de diferentes concentrações de sólidos suspensos sobre o desempenho zootécnico, e saúde intestinal.

A hipótese desse estudo é de que o sistema de bioflocos não é um sistema engessado, mas sim passível de adaptações para se tornar compatível com as especificidades fisiológicas de diferentes espécies e fases de vida, possibilitando o cultivo de espécies consideradas mais sensíveis.

O estudo foi desenvolvido no laboratório de piscicultura da UDESC Lages e está aqui apresentado em três capítulos:

Capítulo I - Revisão de literatura englobando: Biologia da truta arco-íris (*Oncorhynchus mykiss*), Produção mundial da truta arco-íris, produção no Brasil, Sistemas Intensivos, Sistema de Recirculação e Sistema de Bioflocos.

Capítulo II - Manuscrito em forma de artigo científico com o título: Redefining Species Suitability in Biofloc Technology: A Unified Model of Biophysical Niche Modulation. Está sob as normas do periódico Reviews in Aquaculture. Submissão prevista para setembro.

Capítulo III - Manuscrito elaborado na forma de artigo científico com o título: Feasibility of rainbow trout *Oncorhynchus mykiss* rearing in biofloc system: effect on zootechnical performance, intestinal condition and welfare. Está sob as normas do periódico Animals. Publicado em 28/01/2026.

2 OBJETIVOS

2.1 OBJETIVOS ESPECÍFICOS

Esta tese tem o objetivo geral avaliar a tolerância de diferentes níveis de sólidos suspensos na tecnologia de bioflocos na criação de espécies carnívoras e fases iniciais de desenvolvimento, discutindo estratégias para adaptação e manejo desse sistema de acordo com suas exigências fisiológicas. Posteriormente avaliar a utilização do sistema de bioflocos na criação de truta arco-íris (*Oncorhynchus mykiss*), considerando os efeitos de diferentes concentrações de sólidos suspensos sobre o desempenho zootécnico e saúde intestinal.

2.2 OBJETIVOS ESPECÍFICOS

- a) Delimitar os princípios de funcionamento da tecnologia de bioflocos e os fatores que determinam sua aplicabilidade à criação de espécies carnívoras e consideradas menos tolerantes ao sistema.
- b) Investigar os efeitos de diferentes concentrações de sólidos suspensos em sistema de bioflocos sobre o desempenho zootécnico, condição intestinal e respostas antioxidantes de alevinos de truta arco-íris.
- c) Avaliar os resultados produtivos, intestinais, microbiológicos, antioxidantes e de bem-estar para delimitar faixas de concentração de sólidos suspensos compatíveis com a criação de alevinos de truta arco-íris em sistemas de bioflocos e delimitar critérios para o desenvolvimento de sistemas BFT adaptados às exigências da espécie.

3 CAPÍTULO I - REVISÃO BIBLIOGRÁFICA

3.1 BIOLOGIA DA TRUTA ARCO-ÍRIS (*Oncorhynchus mykiss*)

Nativa da borda do Pacífico no hemisfério norte do sul da Califórnia ao Alasca e Kamchatka (Hardy et al., 2002; Fishbase, 2025), a truta arco-íris *Oncorhynchus mykiss* é uma espécie muito popular e está atualmente difundida em diversos países (De Araújo et al., 2023). Por ser originária de águas frias, tolera temperaturas de 0 a 26°C (Rowe; Chisnall 1995, Monlony, 2001). Apesar disso, há estudos que mostram que seu conforto térmico está em torno de 13 a 20 °C (Nepal et al., 2021), e com temperaturas fora dessa faixa acarretando estresse, redução em crescimento e até mortalidade (X et al., 2024; Lahnsteiner; Dünser, 2024). Em seu habitat natural, a truta possui comportamento de termorregulação para evitar águas quentes (Wilbur et al., 2020). Durante períodos mais quentes, as trutas costumam migrar para locais mais frios ou com maior descarga de água (Wilbur et al., 2020).

Quanto sua morfologia, a truta possui uma grande plasticidade modulada pela genética e ambiente que habita (Keeley et al., 2005). De maneira geral, a truta possui um corpo fusiforme, alongado e hidrodinâmico (Figura 1). Esse formato de corpo está diretamente relacionado a alta performance de hidrodinâmica natatória, necessário para ambientes de águas com alta velocidade. A coloração externa também varia de acordo com seu habitat e ontogenia (Arostegui; Quinn, 2019). Populações que vivem em lagos, apresentam coloração prateada, já as que habitam riachos possuem coloração bandada (Arostegui; Quinn, 2019). Como uma descrição característica da espécie, o dorso apresenta uma coloração azulada a verde oliva. A região caudal há tons prateados e rosados, juntamente com uma faixa avermelhada ao longo do corpo (FAO, 2025a), além de pequenas manchas pretas distribuídas por sua superfície

Os ecótipos de truta são divididos de acordo com seus costumes migratórios (Arostegui; Quinn, 2019). Com três vias principais dessa divisão: As residentes em riacho (fluvial), migrador de lago (adfluvial) e migrador oceânico (anádromo) (Arostegui; Quinn, 2019). A forma migratória difere do ecótipo residente por seu ciclo de vida, características morfológicas, demográficas e ecológicas (Berrebi et al., 2022). Enquanto a anádroma se reproduz em água doce, migra para o oceano onde cresce, e retorna à água doce para desova, a residente permanece em água doce seu ciclo inteiro, realizando migrações de maior altitude (Kurhalux et al., 2020). As diferenças morfológicas que mais se destaca está em seus tamanhos, enquanto a anádroma atinge duas a três vezes maior tamanho que a residente quando adultas (Jonsson et al., 2021).

Figura 1. Truta arco-íris (*Oncorhynchus mykiss*).



Fonte: A. FAO, 2025b B. Arquivo pessoal autora

O dimorfismo sexual da truta é marcado por morfologia corporal, maturação e comportamento (Turgut et al., 2024). A fêmea atinge sua maturidade sexual mais tardiamente (de 2 a 3 anos) do que o macho (de 1 a 2 anos) (Turgut et al., 2024; A. 2022), e após a maturação, há uma estabilização no crescimento. Quanto a características físicas (Figura 2), o macho possui um nariz ante dorsal, nadadeiras dorsais mais elevadas, além da mandíbula inferior ser mais alongada com a ponta curvada para trás em formato de gancho (Turgut et al., 2024). A diferenciação após a maturação de ambos pode ser dada visualmente, onde a fêmea apresentará o formato do ventre mais abaulado e caudas desgastadas pela escavação de ninhos, e os machos terão a característica da mandíbula diferenciada (Zimmerman et al., 2022).

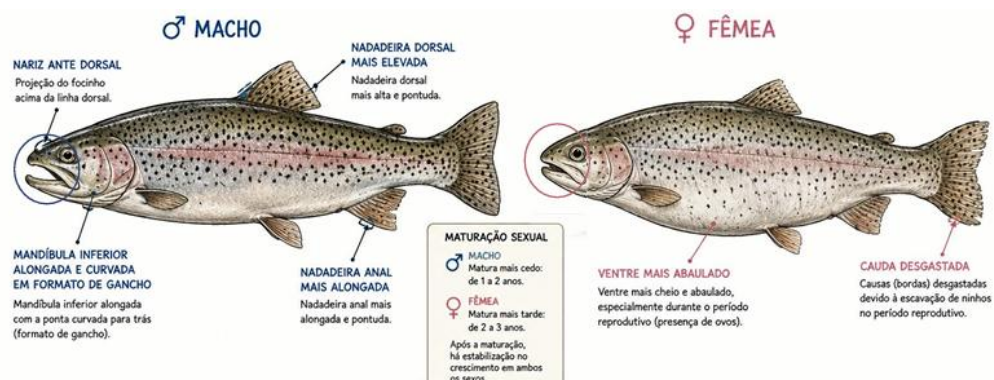
A maturação sexual da truta depende da interação de dois fatores ambientais: luz e temperatura. O fotoperíodo é o primeiro fator que influencia, a redução do comprimento do dia estimula o eixo hipotálamo-hipófise-gônada, desencadeando uma maturação e um aumento no índice gonadosomático (GSI) (Fatima et al., 2018). A temperatura da água modula o processo, elevações de 5 °C na temperatura da água, na troca de estação do inverno e primavera aumentam as taxas de maturação sexual (Wilkinson et al., 2010). O eixo endócrino então responde com picos de liberação de 17 beta-estradiol, testosterona e hormônio folículo-estimulante (FSH), os quais vão conduzir a vitelogênese e o crescimento gonadal (Davies et al., 1999). Para que de fato ocorra a maturação, o peixe necessita realizar um desvio de energia do crescimento

somático para o desenvolvimento das gônadas (Weber et al., 2022). Em peixes que não possuem uma taxa de crescimento adequada, não maturam, já os que possuem, ocorrem altas concentrações de IGF-I na circulação, que sinaliza o eixo reprodutivo que inicie o desenvolvimento gonadal (Wilkinson et al., 2010).

Além disso, existem condições ambientais para que ocorra a desova. As trutas arco-íris migram para áreas reprodutivas específicas, onde as fêmeas constroem ninhos de cascalho para deposição dos ovos (Roduner et al., 2025). A seleção desse local para o ninho depende de substrato, profundidade e velocidade da água (Roduner et al., 2025). Quando a fêmeas encontra o local ideal, ela começa a organizar seus ninhos. Limpam uma área em torno de 1 metro, onde ocorrerá a deposição (Holecek et al., 2007). Para a fecundação, a fêmea então fica em posição de C, e pressiona em movimentos musculares peristálticos para liberação dos ovos (Fuchs; Caudill, 2019). Assim como as fêmeas, os machos seguem seus movimentos e rapidamente liberam os gametas para fertilização dos ovos (Kholodnyy et al., 2021).

Figura 2. Dimorfismo sexual em truta arco-íris (*Oncorhynchus mykiss*)

A. Desenho esquematizado com diferenças no dimorfismo sexual.



Fonte: Desenho esquematizado com diferenças no dimorfismo sexual elaborado pela autora, com auxílio de inteligência artificial generativa (ChatGPT, OpenAI, 2026).

B. Foto demonstrando dimorfismo sexual



Fonte: Hoitsy, G., Woynarovich, A., & Moth-Poulsen (2012).

3.2 PRODUÇÃO MUNDIAL DA TRUTA ARCO-ÍRIS

A produção global de truta arco-íris em 2020 foi de 739,5 mil toneladas em água doce, e 220,1 mil toneladas em ambientes marinhos e costeiros, posicionando a espécie entre as 15 mais cultivadas no mundo (FAO 2022). Dentre os salmonídeos, a truta arco-íris (*Oncorhynchus mykiss*) se destaca como uma das espécies mais comercializadas no mundo, impulsionada pelo aumento do seu consumo alimentar (FAO, 2021). A espécie é amplamente reconhecida por seu valor nutricional e características sensoriais, com sabor e textura suaves, além do seu alto teor de ácidos graxos essenciais, como ômega-3 (Vranic et al., 2011). Segundo dados do programa informativo da FAO, os principais países produtores de truta arco-íris são Irã, Noruega, França, Itália, Espanha, Dinamarca, Chile, Estados Unidos, Alemanha, e Reino Unido. Sendo o Irã o maior produtor com 20% da produção mundial, seguido pela União europeia com 19% (Eumofa 2023).

A comercialização da truta é realizada principalmente como peixe vivo, fresco ou refrigerado (D'Agaro et al., 2020). Produtos defumados também tem um relevante peso comercial, principalmente na Europa, seguido por filetados, prontos para o consumo, congelados e enlatados (D'Arago et al., 2022; FAO, sd). O tamanho de mercado varia entre os dois sistemas de produção (água doce ou marinha), com o tempo de produção de 10 a 12 meses e tamanho comercial de porção em torno de 250 a 350g (D'Arago et al., 2022; FAO, sd). Nos Estados Unidos o tamanho comercializado exigido é maior que 340 g (Fornshell, 2002), já na União Europeia é realizado segmentação por tamanho, trutas de porção (menores de 500g), trutas médias (em trono de 500 g a 1,2kg) e trutas grandes (passam de 1,2 kg) (D'Arago et al., 2022). Em sistemas Marinhos, como no Chile e Noruega, o tamanho de mercado é de 5 kg com filé avermelhado assemelhando ao salmão (Landazuri-Tveteraas et al., 2021). Quanto a cor do filé, há dois mercados evidentes, um para filés branco-brilhante e outro para filés rosados ou avermelhados (Ahmed et al., 2022). Os filés rosados/ avermelhados costumam atingir maior valorização comercial, apesar de alguns consumidores terem a preferência pelo filé branco (Ahmed et al., 2022).

Os sistemas de cultivo utilizados na produção de truta arco-íris abrangem diferentes tecnologias. Entre os principais, destacam-se os sistemas em gaiola ou tanques-rede, *raceways*, sistemas de recirculação (RAS) e aquaponia (Tabela 1). A produção tradicional de truta ocorre majoritariamente em sistemas de *Raceway*, ainda uma técnica comum em muitos países (Vasdravanidis et al., 2022). Devido ao seu elevado fluxo alto de água, proporciona alta oxigenação e reduz acúmulo de compostos tóxicos, atendendo eficientemente às exigências

fisiológicas as truta arco-íris (Sidoruk et al., 2018). O RAS é um sistema que oferece controle de parâmetros-chave para criação, como oxigênio dissolvido e remoção de resíduos (Pepe-Victoriano et al., 2025), além disso, possibilita altas densidades de estocagem, por possuir mecanismos de filtração eficientes (Becke et al., 2018). Já o sistema de tanques-rede compartilha características semelhantes ao *Raceway*, porém não necessita de um fluxo contínuo de água, mas ainda uma interação direta com o corpo d'água e pode ser instalado em locais de água com menor movimento (Salaro, 2009). Já a aquaponia, é um sistema multitrófico integrado, que une o RAS com o cultivo hidropônico, possuindo eficiente filtração (Vasdravandis et al., 2022). As plantas presentes no sistema contribuem para remoção de compostos nitrogenados, melhorando a qualidade da água (Carneiro et al., 2015).

Tabela 1. Características dos principais sistemas de cultivo de truta arco-íris (*Oncorhynchus mykiss*)

	<i>Raceway</i> / Tanque rede	RAS	Aquaponia
Uso de água	Elevado ¹	90 a 99% reciclagem ²	10% de troca ¹
Densidade de cultivo	< 25kg/m ³ ³	Até 100kg/m ³ ³	Variável ⁴
Controle ambiental	Dependente de fonte ²	Alto controle ³	Controle mediano ¹
Escala de produção	Limitada pela capacidade do corpo d'água ⁵	Exige produção em massa ⁵	Viável apenas com plantas de alto valor ⁶
Custo	Baixo ⁵	Alto ⁵	Médio ⁶

RAS: Sistema de Recirculação. 1 - Vasdravanidis et al., 2022; 2 – Pepe-Victoriano et al., 2025; 3- Laine et al., 2023; 4- Bordignon et al., 2022; 5 – Engle et al., 2024; 6 – Sylver et al., 2025.

Para o cultivo da truta arco-íris, são necessários parâmetros específicos para suprir as exigências fisiológicas da espécie (Tabela 2). O fluxo de água é o mecanismo primário para o fornecimento e manutenção de oxigênio e remoção de compostos nitrogenados do sistema. Em *raceways*, a vazão recomendada é de 37 a 92 L s⁻¹, com velocidade recomendada de 1,5 a 3,5 m s⁻¹ (Bhagat; Barat, 2015), em alguns locais, utilizam 4,8 a 5 L s⁻¹ por tonelada de produção anual de peixe (Boaventura et al., 1997; Ebeling; Timmons, 2010). Além da vazão, a temperatura da água influencia diretamente na concentração de oxigênio dissolvido (OD). À medida que a temperatura da água aumenta, a capacidade de saturação do oxigênio diminui. Além disso, temperaturas elevadas no cultivo, acima do conforto térmico da espécie, impactam negativamente nos índices zootécnicos e na saúde da espécie (Jiang et al., 2019). Por outro lado, temperaturas abaixo do ideal, reduzem o metabolismo do peixe comprometendo seu crescimento (Vinatea, 1997). Níveis de OD inferiores à 5 mg L⁻¹ podem reduzir consumo de ração, crescimento, e provocar mortalidade (Magnoni et al., 2018).

Tabela 2. Valores de referência dos parâmetros de qualidade da água para o cultivo de truta arco-íris (*Oncorhynchus mykiss*).

Temperatura	°C	13-20¹
OD saturação	%	>80²
OD	mg L ⁻¹	7 - 12³
pH	pH	6,5-8,2³
Material em suspensão	mg L ⁻¹	< 25⁴
Alcalinidade	mg L ⁻¹ CaCO ₃	17 - 97²
Amônia não ionizada (NH₃)	mg L ⁻¹	<0,0125³

OD- Oxigênio dissolvido. (Nepal et al., 2021¹; Avkhimovich, 2013²; Pepe-Victoriano et al., 2025³; Seanego et al., 2023⁴)

A concentração de sólidos suspensos no sistema é outro fator que influencia indiretamente a saturação de oxigênio e qualidade da água. O aumento da turbidez causada por esses sólidos provoca uma redução na transparência e penetração da luz, por consequência, redução no OD (Fiordelmondo et al., 2020). Para truta arco-íris a literatura recomenda que os sólidos permaneçam abaixo de 25 mg L⁻¹ (Seanego et al., 2023). No entanto estudos mostram que em sistemas fechados, as trutas toleram sólidos mais altos (Becke et al., 2018). Uma hipótese proposta pelos autores para essa tolerância é a natureza dos sólidos suspensos. Em ambientes naturais os sólidos tendem a ser inorgânicos, com partículas irregulares e abrasivas que podem provocar danos nas brânquias. Em sistemas fechados predominam sólidos de origem orgânica, geralmente de textura macia e pouca abrasividade. Becke et al. (2018) demonstraram que concentrações de sólidos de até 71 mg L⁻¹ não provocaram efeitos crônicos negativos nas trutas cultivadas em RAS.

A demanda nutricional é outra característica importante na criação da truta arco-íris, sendo o principal fator de custo na produção (Cao et al., 2023; Barbosa et al., 2020). Quando em vida livre, sua alimentação é baseada em invertebrados aquáticos e terrestres e peixes pequenos (Fishbase, 2025). O requerimento de proteína bruta para essa espécie varia conforme o estágio de vida, reduzindo a exigência ao longo do crescimento, de 50 até 40%, e lipídeos se mantendo em torno de 16 a 24% (Cao et al., 2023; Kamalam et al., 2020). Por ser considerado um peixe carnívoro, a exigência nutricional da truta eleva os custos de produção (Rahimnejad; Lee, 2013), que já representam 60% do custo total do cultivo (Cortés-Garcia et al., 2025). Essas características de produção resultam em um elevado custo de compra para o consumidor final, tornando a carne desse peixe um produto nobre.

3.3 PRODUÇÃO DA TRUTA NO BRASIL

A introdução da truta arco-íris no Brasil ocorreu oficialmente em 1949, na Serra da Bocaina, em São Paulo, com aproximadamente 5.000 ovos importados pelo Ministério da Agricultura (Porto-Foresti et al., 2007). Esse povoamento foi realizado com o objetivo de povoar ambientes com pouca ictiofauna, além de fomentar turismo e pesca esportiva (Lazzarotto; Caramaschi 2009). Posteriormente, em 1974 iniciou-se a primeira truticultura comercial no Brasil em Campos do Jordão (Proença et al., 2001). Após isso, a truta tornou-se o salmonídeo mais comum e difundido no país (Lazzarotto; Caramaschi 2009).

Somente após algumas décadas, em 1970 iniciaram-se estudos e investimentos voltados aos desafios enfrentados pelos produtores, como fabricação de rações de composição específica rica em proteína e gorduras, e reprodução em tanques (Igarashi, 2023). Para superar esses gargalos, foi criada a Associação Brasileira de Truticultores, ABRAT nos anos 80, com a finalidade de apoiar a pesquisas, realizar reuniões e cursos e difundir novas tecnologias, buscando melhorar a truticultura brasileira (Stempniewski, 2009). O país apresenta grande potencial para o cultivo dessa espécie, uma vez que possui águas de baixa temperatura provenientes de riachos de altitudes elevadas. Além das condições ambientais favoráveis, o aumento do consumo e a crescente demanda por peixes de melhor qualidade têm incentivado os produtores a investirem na truticultura (Igarashi, 2023).

Santa Catarina representa um dos polos de produção de truta no Brasil, concentrado na Serra Catarinense sendo majoritariamente realizada em sistemas de *raceways* (Novaes; Souza, 2024; Igarashi, 2023). Esse sistema consiste no desvio de rios, para garantir uma alta taxa de renovação de água. A vazão deve ser suficiente para que o volume de água se renove ao menos uma vez por hora, com a densidade de produção ajustada conforme o fluxo de água ($1\text{L min}^{-1}\text{ kg}^{-1}$) (Igarashi, 2023; Pereira et al, 2017). Além disso, o formato destes tanques desempenha um papel importante na criação dessa espécie, o qual o mais sugerido é de forma circular. Este design evita áreas de menor concentração de oxigênio dissolvido, como cantos de tanques, e reduz o acúmulo de sujidades, fator crítico para criação de truta que apresenta alta sensibilidade a condições adversas (Tabata; Filho, 2012).

A produção de truta em *raceways* tem duração de até 12 meses, resultando em peixes com peso final de 300 a 500 g (Novaes; Souza, 2024). Esse estilo de truticultura é considerado rentável e lucrativa para pequenos produtores familiares, onde em média conseguem preço de venda de pescado não processado de R\$ 22,10 kg^{-1} e processado como filés, peixes eviscerados e defumados de em média R\$ 40,00 kg^{-1} (Novaes; Souza, 2024), com produtividade anual em

propriedades de média escala de 32,4 ton ano⁻¹ e pequena escala de 10,8 ton ano⁻¹ (Barbosa et al., 2020). Apesar da consistência na criação de trutas nesse sistema, a utilização de sistemas intensivos tem chamado atenção (Grandez-Yoplac et al., 2025). Justificativas como maior biossegurança, maior controle da qualidade da água, viabilidade ambiental, menor uso de água e possibilidade de aumento de densidades na criação (Pepe-Victoriano et al., 2025), estão cada vez mais tomando espaço e tornando isso uma tendência para truticultura no futuro.

3.4 SISTEMAS INTENSIVOS

Na década de 80 a aquicultura sofreu um boom na modernização, apesar de formas antigas de criação existirem há séculos (Teletchea, 2021), porém foi só na virada do século que se consolidou como parte central do sistema alimentar global devido a intensificação na produção (Verdegem et al., 2023). Com o tempo, a pesca extrativista está sofrendo uma estagnação e abrindo espaço à aquicultura (FAO, 2026b). Essa tendência se dá pela dificuldade em suprir a crescente demanda dos consumidores (Hwang et al., 2025; FAO, 2026b). Como reflexo desse aumento no consumo, houve um investimento em sistemas de produção intensiva, que permitem produzir em espaços reduzidos, com maior controle dos fatores ambientais e em altas densidades. Hoje destacam-se os sistemas intensivos como o RAS (*Recirculating Aquaculture System*) e o BFT (*Biofloc Technology*) como alternativas promissoras (Emerenciano et al., 2025), tecnologias modernas que apresentam vantagens e desvantagens na produção aquícola.

3.4.1 Sistema De Recirculação (RAS)

A tecnologia RAS surgiu como resposta à limitação de espaço e água, permitindo altas densidades de estocagem e controle ambiental (Li et al., 2023). O sistema tem como princípio fundamental a reutilização da água permitindo ciclo de 30 a 100% dependendo do desempenho da produção (Gupta et al., 2024). Para garantir a viabilidade do sistema é necessário a remoção contínua dos sólidos suspensos e compostos tóxicos resultantes do metabolismo dos peixes e da decomposição da matéria orgânica (Li et al., 2023). Para que se mantenha viável, a estrutura padrão do RAS é composta geralmente por tanques de cultivo, filtro mecânico, biofiltro, sistema de aeração e ainda podem conter sistemas de desinfecção (como radiação UV ou ozônio) (Li et al., 2023; Gupta et al., 2024). Sistemas auxiliares podem ser acoplados para monitorar e controlar parâmetros físico-químicos essenciais como oxigênio, temperatura (Gupta et al., 2024) (Figura 3).

Logo, é importante além dos tanques de cultivo as estruturas acopladas: O filtro mecânico tem a finalidade de remoção de partículas sólidas, como fezes e ração não consumida, com o objetivo também de não entupir os biofiltros, que são acoplados em sequência no sistema (Gupta et al., 2024). Para remoção de amônia tóxica, nitrito e nitrato, realizada por bactérias nitrificantes, são utilizados os biofiltros (Mugwanya et al., 2022). O próximo passo é a desinfecção, que é facultativa em um sistema, porém recomendada. É realizada através de UV e ozônio, para reduzir a carga microbiana e patogênica da água recirculada (Mugwanya et al., 2022). Por último, o compressor e difusor de ar, para suprir o oxigênio dissolvido, visto que em densidades altas, é uma demanda de extrema importância (Mugwanya et al., 2022).

No RAS a água passa pelos tanques de cultivo e é conduzida para o sistema de filtragem, com o primeiro componente para remoção de matéria orgânica particulada, para evitar entupimento do biofiltro subsequente e garantir a eficiência da biofiltração (Lindholm-Lehto et al., 2021). Diferentes tecnologias operam em faixas distintas de tamanho de partículas, como separadores sólido-líquido, tambores rotativos, filtros de tela parabólica, filtro de areia e equipamentos que separam por espuma como *skimmer* (Xiao et al., 2019). Cada uma dessas tecnologias pode ser utilizada de forma complementar para melhorar sua eficiência de filtragem. Os separadores físico mecânicos como filtros de tambor, peneira parabólica, e separadores radial separam partículas maiores de 60 μm (Tawfik et al., 2023). Para remoção de partículas finas e dissolvidas, é necessário equipamentos mais específicos, como Skimmers, que removem matéria orgânica fina e dissolvida, filtro de areia para remoção de matéria particulada e dissolvida de forma passiva e econômica, membranas de ultrafiltração que capturam frações coloidais e malhas de tambor finas (Pfeiffer et al., 2024; Lindholm-Lehto et al., 2021; Fossmark et al., 2020). De maneira sintetizada, a filtração física no RAS é uma barreira sequencial e complementar, podendo utilizar equipamentos gravitacionais e mecânicos para remover sólidos grossos, e skimmers, filtro de areia e membranas para frações finas e dissolvidas.

O segundo elemento de filtragem são os biofiltros. Esse filtro é composto por substratos que permitem a fixação bacteriana, normalmente sendo *bobos*, cerâmicas porosas ou até fibras sintéticas (Da Costa et al., 2026). O principal objetivo dele é a retirada dos compostos nitrogenados, sendo eles amônia (NH_4^+), nitrito (NO_2^-) e nitrato (NO_3^-). Após maturação do biofilme (desenvolvimento e colonização de bactérias), inicia a remoção desses compostos ocorre em duas etapas (Garcia et al., 2022). A primeira consiste na conversão de amônia a nitrito, por bactérias oxidantes de amônia (AOB), como *Nitrosomonas* (Zhao et al., 2025). Em seguida o nitrito é oxidado por bactérias oxidantes de nitrito (NOB), como *Nitrobacter* ou

Nitrospira para nitrato (Garcia et al., 2022). O resultado da nitrificação é o nitrato, um composto menos tóxico que pode ser cumulativo no sistema. O controle desse composto é normalmente realizado por trocas de água ou por desnitrificação (Yogev; Gross, 2019). Durante a desnitrificação, o nitrato por fim é convertido em nitrogênio gasoso (N₂) ou transformado em biomassa vegetal, dependendo da rota microbiana ou assimiladora utilizada (Gupta et al., 2024; Xu et al., 2025).

Após o tratamento no filtro biológico, a água pode ser submetida a um processo de desinfecção. Nesse processo pode ser utilizado radiação ultravioleta (UV), ozonização, ou a combinação de ambos para controlar a microbiota da água recirculada. O UV é amplamente utilizado nas pisciniculturas para inativar microrganismos sem adicionar química à água (Summerfelt et al., 2008). A aplicação do mesmo se dá com lâmpadas de baixa pressão com pico de 254 nm (Semenov et al., 2025) e atua na remoção de patógenos sem causar efeitos danosos ao cultivo, com eficiência de 89 a 99% (Huyben et al., 2018). Porém sua eficiência pode ser comprometida em águas com alta turbidez e exige manutenção periódica, como a substituição das lâmpadas (Gupta et al., 2024). A ozonização também atua como desinfecção, mas tem um segundo papel importante no RAS que é a oxidação de material orgânico (Gonçalves et al., 2011). O risco da utilização do mesmo se dá pela toxicidade residual de ozônio e subprodutos (Gonçalves et al., 2011), portanto a dose precisa ser controlada cuidadosamente. A aplicação sequencial dessas tecnologias, sendo ozonização seguido de UV, tende a superar o uso isolado de cada tecnologia (Teitge et al., 2020). A aplicação da desinfecção é recomendada em dois momentos: na entrada do sistema antes dos biofiltros para inativar patógenos, e dentro do sistema para controlar microbiota (Gupta et al., 2024). A utilização desse método é importante e reflete na sanidade dos organismos cultivados (Xiao et al., 2019), impactando positivamente no desempenho zootécnico. Dentro desse sistema de recirculação, cada componente é desenvolvido de forma a integrar com os demais (Gupta et al., 2024).

Figura 3. Esquema com etapas do sistema RAS



Fonte: Imagem elaborada pela autora esquematizando RAS com auxílio de Inteligência Artificial generativa

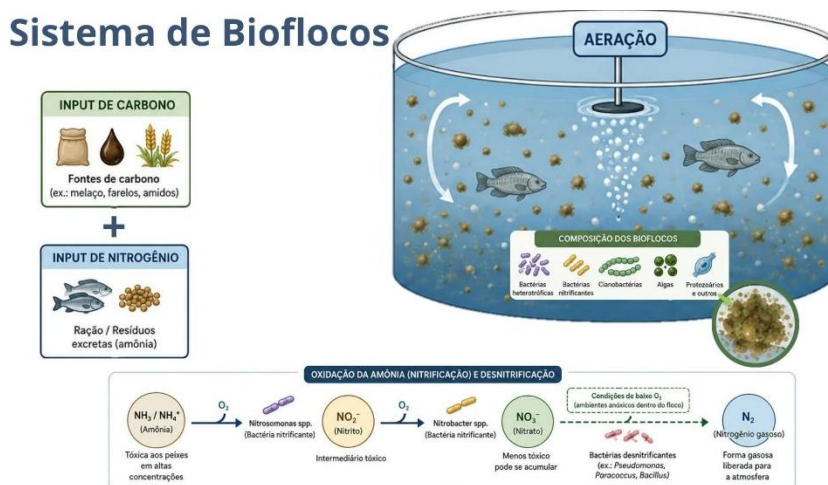
3.4.2 Sistema de bioflocos (BFT)

A tecnologia de bioflocos (BFT do inglês *Biofloc Technology*) foi inicialmente desenvolvida na França, pela *French Institute of Exploration of the Sea* (IFREMER) no início dos anos 70, com bases na pesquisa com camarões peneídeos pelo grupo (Nisar et al., 2022). A partir disso, diversos estudos subsequentes foram realizados, com as espécies tilápia e camarão branco (*Litopenaeus vannamei*) como destaque de cultivo (Li et al., 2025). O BFT é um sistema considerado ambientalmente sustentável (Yu et al., 2023), uma vez que promove reuso da água, controle de componentes nitrogenados e ainda favoreceu o crescimento e a saúde de algumas espécies cultivadas (Emerenciano et al., 2017). Em função dessas características e diante dos desafios atuais enfrentados, como aumento na demanda de pescados, escassez de recursos hídricos e limitação de espaço, o BFT tem se consolidado como uma alternativa promissora e estratégica para a intensificação aquícola.

O BFT é uma tecnologia aplicada em sistemas intensivos de aquicultura caracterizada por mínima ou nula renovação de água, reduzindo o uso em até 90% (Khanjani et al., 2024). Sua principal particularidade reside na presença de uma comunidade microbiana diversa, capaz de reciclar compostos nitrogenados tóxicos, como amônia, convertendo-os em biomassa microbiana (Khanjani et al., 2020) (Figura 4). As bactérias presentes utilizam o nitrogênio

inorgânico, principalmente amônia (TAN) como fonte de nitrogênio para crescimento celular, em associação com uma fonte de carbono orgânico, promovendo a formação de agregados microbianos denominados flocos (Ahmad et al., 2017). Essa dinâmica demanda elevada taxa de aeração, tanto para manter os flocos em suspensão, quanto para garantir níveis adequados de oxigênio dissolvido (Han et al., 2024). Além disso, é necessário o fornecimento controlado de carbono orgânico para manter a relação carbono:nitrogênio (C:N) dentro do equilíbrio necessário (Bossier; Ekasari, 2017). A formação na água do floco ocorre por diversos fatores físicos, químicos e biológicos complexos, entre nutrientes orgânicos, substratos e a variedade de microrganismos presentes (Khanjani et al., 2023).

Figura 4. Esquema geral de funcionamento de bioflocos



Fonte: Desenho esquematizado o sistema de bioflocos, com auxílio de inteligência artificial generativa (ChatGPT, OpenAI, 2026).

Para implementação de um sistema de bioflocos convencional, existem três pontos centrais que precisam estar claros: fontes de nitrogênio e carbono, função da aeração e relação entre biofloco e qualidade da água. A utilização de fonte de nitrogênio e de carbono, e um sistema de aeração eficiente. Como já citado, a formação de bioflocos depende do balanço entre nitrogênio residual e adição externa de carbono para estimular bactérias heterotróficas inicialmente (Emerenciano et al., 2017). As principais fontes de nitrogênio do sistema são a ração, excretas e outros resíduos liberados no cultivo (Khanjani et al., 2024). Quanto à adição do carbono orgânico, possui o objetivo de promover o crescimento microbiano e conversão desses resíduos nitrogenados (Ahmad et al., 2017). Diferentes fontes de carbono estimulam diferentes comunidades microbianas (Khanjani et al., 2021). A aeração possui a função de oxigenar a água, manter os flocos em suspensão e sustentar o processo metabólico das bactérias

presentes no cultivo (Ahmad et al., 2014), ou seja, sua intensidade deve ser alta e contínua, quando falamos em sistemas de bioflocos convencionais (majoritariamente compostos por bactérias heterotróficas). Por último, a qualidade da água está diretamente relacionada à funcionalidade do biofoco (Khanjani et al., 2024) (Tabela 3).

Tabela 3. Relação entre parâmetros de qualidade da água e a dinâmica do floco microbiano em sistemas de bioflocos

Parâmetro	Relação com o floco
OD	Sustenta metabolismo bacteriano e organismos ¹
NAT/ NO ₂ / NO ₃	Indicam eficiência da conversão microbiana do nitrogênio ²
SST/ Sólidos	Excesso favorece acúmulo e eleva demanda de oxigênio no sistema ³
pH/Alcalinidade	Afetam nitrificação e atividade microbiana ²
Temperatura	Modula crescimento microbiano e desempenho do sistema ²

OD: Oxigênio dissolvido; NAT: Nitrogênio amoniacal total; NO₂: Nitrito; NO₃: Nitrato; SST: Sólidos suspensos totais. Minaz et al., 2023¹; Khanjani et al., 2024²; Emerenciano et al., 2017³

O ciclo de um sistema de bioflocos inicia com a colonização por microalgas e bactérias, formando inicialmente uma comunidade de fitoplânctons (Kumar et al., 2021). Clorofitas e cianobactérias atuam como espécies filtradoras, convertendo compostos nitrogenados em formas assimiláveis (Roberto, 2017). Com a adição de carbono orgânico no sistema (C:N de 10 a 15:1), ocorre um favorecimento de bactérias heterotróficas, que se proliferam rapidamente e são consideradas as principais responsáveis inicialmente pela remoção de nitrogênio do sistema (Kumar et al., 2021; Padeniya et al., 2022). Essas bactérias por sua vez, assimilam amônia diretamente em biomassa celular, e pode se notar sua proliferação no sistema pela coloração marrom da água e altos níveis de sólidos suspensos (Kumar et al., 2021). Após a estabilização das bactérias heterotróficas, ocorre uma redução na adição de carbono, permitindo então a coexistência de bactérias heterotróficas e nitrificantes. É nessa etapa que ocorre a assimilação heterotrófica e nitrificação autotrófica simultaneamente, equilibrando a remoção de nitrogênio com a geração de nitrato (Khanjani et al., 2023). Após a fase mixotrófica, com a interrupção de fornecimento de carbono orgânico, bactérias nitrificantes se tornam predominantes no controle do nitrogênio (Carvalho et al., 2024; Emerenciano et al., 2025).

Na primeira etapa de maturação do BFT, o carbono orgânico é fornecido externamente e sua disponibilidade varia conforme a fonte utilizada. Açúcares simples como glicose e melaço apresentam maior biodisponibilidade de carbono, promovendo uma resposta metabólica bacteriana mais rápida, e por consequência uma redução mais rápida na amônia livre (El-Dahhar et al., 2024). Já carboidratos mais complexos, polissacarídeos como em farelos vegetais e amido resultam em uma resposta mais lenta na redução na amônia (Avimelech, 2012). El-shafiey et al. (2018) avaliaram 4 diferentes fontes de carbono, sendo glicose, amido, melaço e

celulose e observaram a eficiência na redução do NAT em todos os tratamentos. Entretanto a celulose obteve uma média de TAN superior as outras fontes. Esse estudo evidenciou que resíduos agrícolas também podem ser utilizados de forma eficaz como fonte de carbono em sistemas de bioflocos. A proporção de C:N também influencia na composição microbiana, Wei et al. (2020) identificaram as famílias bacterianas *Flavobacteriaceae* e *Rhodobacteraceae* como predominantes em um sistema de bioflocos. No entanto em flocos mantidos com relação C:N de 15 a 20:1, apresentam mais *Lactobacillus* spp e *Enterococcus* spp (Islam et al., 2021; Kumar et al., 2021).

O BFT apresenta diversas vantagens econômicas e ambientais, sendo alguma delas já citadas, como a redução no consumo de água, menores riscos de disseminação de doenças e ainda o favorecimento de saúde e índices zootécnicos de organismos cultivados (Khanjani et al., 2020; Raza et al., 2024). A redução na utilização de água está atrelada a eficiência do sistema em reciclar compostos tóxicos. Por não haver fluxo de água constante, também há uma prevenção de disseminação de patógenos, como pode ocorrer em sistemas abertos como *raceways* ou em gaiolas (Raza et al., 2024). O floco ingerido pelos peixes também é uma fonte de suplemento alimentar, com sua composição rica em bactérias, biocompostos, proteínas, lipídeos, vitaminas, carotenoides, enzimas digestivas e outros biocompostos, também atua como probiótico e aditivo, favorecendo o crescimento, a digestão e a saúde dos peixes (Khanjani et al., 2023; Pandeniya et al., 2022) (Tabela 4).

Tabela 4. Exemplificação de composição nutricional média de biofloco reportada na literatura

% Matéria Seca	Biofloco
Proteína bruta	12 a 58 ^{1,2}
Lípídeo bruto	3 a 7,75 ^{2,3}
Energia	19 kJ g ⁻¹ ¹
Minerais (Ca, P e Zn)	1,2 a 24 mg g ⁻¹ ³
Cinzas	39,2 ¹
Fibras	8,3 ¹

Ahmad et al., 2017¹; Paul et al., 2023²; Zaki et al., 2024³

Sarsangi et al. (2022) mostraram a viabilidade de reduzir 15% da alimentação diária de tilápias do Nilo criadas em sistema BFT sem comprometer seu desempenho zootécnico. Porém Vinatea et al. (2018) em seu estudo com *Tinca tinca* e *Mugil cephalus*, criados em sistema BFT, demonstraram que a composição do biofloco e a diversidade microbiana no mesmo são influenciadas pela espécie cultivada no sistema. Sendo assim, a composição do biofloco pode variar amplamente, a proteína pode chegar a 53.65%, lipídeos a 24.33%, cinzas a 61,70%, assim como os compostos bioativos como carotenoides, clorofilas, bromofenol e outros (Khanjani et

al., 2023). Apesar da variação da composição dos flocos, há diversos estudos que mostram a possibilidade na redução de fornecimento de ração ou de porcentagem de proteína na mesma para os peixes. Um exemplo disso é o estudo de Hisano et al. (2019), que demonstraram a possibilidade de reduzir a proteína bruta da ração em 8% sem prejudicar o desempenho zootécnico de tilápias do Nilo e reduzindo impacto ambiental e custos de produção.

3.4.3 BFT Dados de produção

Uma das espécies mais estudada e cultivadas no sistema de BFT é a tilápia do Nilo *Oreochromis niloticus*, por ser um peixe amplamente consumido e de considerável rusticidade (Ogello et al., 2014). Ensaio já foram realizados com essa espécie e observaram melhora na sobrevivência, desempenho e conversão alimentar (Mirzakhani et al., 2019; Azim; Little, 2008). Além da melhora no desempenho, também foi observada uma melhora na função imunológica dessa espécie cultivada no sistema (Sarasagi et al., 2022). Widanarni, Ekasari e Maryam (2012) demonstraram também uma possibilidade de maior densidade de estocagem no cultivo de tilápia vermelha (*Oreochromis* sp.), contribuindo para um sistema de cultivo mais eficiente e sustentável. Parâmetros relacionados a saúde intestinal também foram avaliados, como o aumento de enzimas digestivas amilase e protease quando comparados aos cultivados em água clara, indicando uma melhora na digestão e absorção dos nutrientes (Khanjani et al., 2021).

O sistema BFT também tem sido recentemente aplicado com sucesso no cultivo de peixes ornamentais. Estudos com kinguio (*Carassius auratus*), uma das espécies ornamentais mais populares mundialmente, demonstraram boa adaptação ao cultivo nesse sistema (Besen et al., 2021; Cunha et al., 2020). Essa mesma espécie apresentou um bom desempenho zootécnico e uma coloração mais intensa quando comparada aos sistemas de água clara (Cunha et al., 2020). Resultados positivos também foram apresentados na fase de larvicultura de kinguio, com melhorias significativas nas taxas de crescimento e sobrevivência (Minaz et al., 2024; Besen, et al., 2021). O bioflocos tem se tornado também uma alternativa para espécies sensíveis, porém como fornecimento do flocos como suplemento alimentar. Stringhetta et al., 2024 mostraram em seu estudo que o fornecimento de bioflocos como suplementação alimentar melhorou significativamente o desempenho zootécnico do betta (*Betta splendens*).

O cultivo aquícola em sistema de bioflocos não só demonstra melhora no crescimento, como também melhor conversão alimentar, sobrevivência e coloração dos peixes (Yu et al., 2023; Besen et al., 2021). Outras espécies como carpa comum (*Cyprinus carpio*) (Adineh et al.,

2019; Bakhshi et al., 2018) Kingio (*Carassius auratus*) (Yu et al., 2022), Curimã (*Mugil cephalus*) (Garcés et al., 2025) e bagre asiático (*Heteropneustes fossilis*) (Zafar et al., 2021) já possuem estudos com resultados positivos na criação dentro deste sistema.

Embora as espécies mais tolerantes à criação em sistema de BFT sejam onívoras ou detritívoras (Azim; Little, 2008), já existem alguns estudos de espécies carnívoras nesse sistema. Brasileiro et al. (2024) evidenciaram a possibilidade da criação de peixe dourado (*Salminus brasiliensis*) no sistema BFT. Ainda observaram que os peixes criados no sistema de bioflocos apresentaram melhora nos parâmetros zootécnicos como conversão alimentar, ganho de peso e sobrevivência. Por outro lado, Romano et al. (2020) investigaram a criação do achigã (*Micropterus salmoides*), e observaram uma melhora na qualidade de água, porém os peixes apresentaram sinais de estresse, o que comprometeu o desempenho zootécnico. Nesse estudo, os autores sugerem que o uso do sistema por um curto período na criação da espécie é possível, visto terem um melhor controle da qualidade da água. Outra espécie carnívora avaliada nesse sistema é o pirarucu (*Arapaima gigas*). Aguiar et al. (2025) não observaram diferença nos índices zootécnicos do pirarucu em comparação àqueles criados em sistema de água clara. No entanto o sistema BFT também se mostrou mais eficiente quanto ao controle de qualidade da água, fazendo-os concluir viabilidade na criação da espécie no sistema.

De forma geral, os resultados encontrados mostram que a resposta ao sistema de bioflocos pode variar entre as espécies, sendo principalmente condicionada pelos hábitos alimentares e tolerância a sólidos suspensos (Banu et al., 2024; Das et al., 2024). Enquanto algumas espécies carnívoras apresentam desempenho satisfatório no sistema (Banu et al., 2024), outras podem apresentar respostas negativas, principalmente quando as condições do sistema não são adequadas às suas exigências (Romano et al., 2020). Dessa forma, a utilização do BFT para espécies carnívoras depende da adaptação das condições do sistema às características de cada espécie. Nesse contexto, a truta arco-íris (*Oncorhynchus mykiss*) apresenta-se como uma espécie de interesse para avaliar os limites da utilização do BFT, considerando suas exigências em relação à qualidade da água e sua sensibilidade às condições de cultivo.

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4 CAPÍTULO II - ARTIGO 1: REDEFINING SPECIES SUITABILITY IN BIOFLOC TECHNOLOGY: A UNIFIED MODEL OF BIOPHYSICAL NICHE MODULATION

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4.1 ABSTRACT

Biofloc Technology has emerged as a promising approach to ecological aquaculture engineering, enabling nutrient recycling and minimal water discharge. However, its application has historically focused on hardy, omnivorous, and microphagous species, contributing to the perception of biofloc as high-solids, heterotrophic production system, unsuitable for sensitive life stages or carnivorous fish. This review proposes that biofloc should instead be considered an adaptive production in which operational conditions can be adjusted to ecological and physiological requirements of the cultured species. By examining how some key variables, including the carbon-to-nitrogen ratio, total suspended solids, and dominant microbial pathways, influence the suitability of this system for different biological niches, this work examines four aspects of BFT application beyond conventional hardy species: (1) the role of biofloc in larval ontogeny and early developmental barriers; (2) the challenges of applying this system in carnivorous fish and top predators, focusing on how the system variables interacts with metabolic requirements of these species; (3) the adjustment of biofloc configurations according to species-specific physiological and ecological traits, from nitrifying-dominated systems for sensitive species to conventional heterotrophic systems for more hardy organisms; and (4) the transition from standardized biofloc protocols to a species-specific system design, in which carbon input, suspended solids, and microbial pathways can be adjusted to meet the biological requirements of the cultured species. This paper provides a perspective framework for adapting biofloc system to different species and life stages requirements, while maintaining conditions compatible with animal performance, welfare and system stability.

Keywords: Microbial management; Physiological homeostasis; Total suspended solids;

4.2 INTRODUCTION

The global expansion of aquaculture has intensified the search for sustainable production systems that optimize water use, maximize nutrient recycling, and minimize environmental discharge [1]. Within this emerging scenario, Biofloc Technology (BFT) is an innovator strategy in ecological aquaculture engineering [2,3]. Manipulating the carbon-to-nitrogen (C:N) ratio stimulates the development of heterotrophic and chemoautotrophic microbial communities in BFT systems [4]. These microorganisms assimilate toxic nitrogenous compounds while concurrently generating macroaggregates that serve as a continuous supplemental nutrient-rich supplemental feed source [5, 1].

Historically, commercial application and academic literature on BFT have focused on omnivorous species [5,1,2]. The high stocking-density tolerance, trophic plasticity and physiological robustness of species such as Nile tilapia (*Oreochromis niloticus*) and the Pacific white shrimp (*Penaeus vannamei*) have made them the main models for biofloc research [5]. However, this narrow focus has reinforced the perception of BFT as a less flexible system with high turbidity, exclusively suited for resilient, low-trophic organisms. Consequently, contributing to the view that its implementation is limited for sensitive life stages, such as larvae and post-larvae, or for carnivorous aquatic organisms.

Current understanding of BFT often overlooks the ecological complexity of biofloc environment. From a physiological perspective, species suitability in BFT are not determined by the system technology itself, but by the capacity of the fish to maintain homeostasis under specific culture dynamics [2,1]. During early ontogeny, a turbid heterotrophic environment and high-water turbulence can challenge underdeveloped structures, especially the gills [6, 7]. In carnivorous species, which require high-protein diets, elevated branchial ammonia excretion [8] combined with high total suspended solids (TSS) can impose metabolic and mechanical stress on delicate gill lamellae [6]. When poorly managed, these cumulative stressors impair gas exchange, triggers excessive mucus production, and induce chronic physiological stress [6].

Nevertheless, recent advances in environmental engineering and microbial ecology indicate that BFT can be adapted and managed as a highly modular production system [2, 9]. The biochemical and physical characteristics can be tailored to align with the distinct physiological profile of the target species. Through microbiome manipulation, such as the promotion of chemoautotrophic (nitrifying) pathways, and strategic water clarification to regulate TSS, the biophysical composition of biofloc can be dynamically modulated [2, 1].

This review aims to challenge the traditional boundaries of biofloc technology by demonstrating its adaptability across diverse and demanding aquaculture systems. By systematically evaluating how managed system characteristics influence larval ontogeny, branchial histopathology, and the performance of carnivorous fish, this paper indicates specific engineering and biological protocols required to extend BFT applicability. Ultimately, this work proposes a conceptual shift from viewing BFT as a species-specific strategy to recognizing it as a versatile tool for ecological aquaculture management.

4.3 LARVAL ONTOGENY AND EARLY DEVELOPMENTAL BARRIERS – OVERCOMING INITIAL VULNERABILITY

4.3.1 Hydrodynamic Stress and Ammonia Toxicity: Physical and Chemical Bottlenecks

The operational management of Biofloc Technology can be challenging during the early stages of fish life, like altricial larvae, which have limited swimming capacity and underdeveloped feeding structures [10, 11]. During this life stage, their sensitivity can be increased to both hydrodynamic conditions of the system and fluctuations in water quality. To maintain microbial aggregates in suspension and adequate dissolved oxygen levels, the system requires an intensive and continuous aeration [12], which inevitably generates water turbulence within the rearing units. For altricial larvae, excessive water turbulence can increase risk of physical collisions and stress. Research with early developmental stages of Largemouth bass (*Micropterus salmoides*), showed inflammation signals and gill damage when raised in this system [6]. In contrast, more robust, omnivorous species with relatively large and precocial larvae, such as Nile tilapia (*Oreochromis niloticus*) and goldfish (*Carassius auratus*), demonstrated mechanical resilience in early BFT cultures [13, 14].

Beyond the hydrodynamic conditions, the high concentration of organic loads presents an additional challenge. The accumulation of suspended organic matter increases microbial oxygen demands, making the dissolved oxygen highly depend on continuous aeration. This characteristic causes rapid oxygen depletion when aeration failure, leading to high mortality rates of the cultured fish [15]. Fluctuations in ammonia concentrations can further constrain the use of this system for sensitive larvae, as change in microbial activity can affect the availability of nitrogenous compounds [16, 15]. Thus, one key to the successful use of BFT during the early life stage development of fish is paying close attention to balancing aeration intensity, water movement, microbial activity, and the load of suspended organic solids in the water.

These challenges can be overcome through modifications in tank design and operational management in BFT (Figure 1). Hydrodynamic shelters or low-velocity resting zones can mitigate hydrodynamic stress and swimming effort by strategically providing larvae areas where they are less exposed to high-velocity currents and turbulent flow [17]. Additionally, temporary reduction of aeration intensity during the critical post-hatching period may prevent premature physical exhaustion and enhance early survival rates, while still maintaining adequate oxygen availability [18]. However, this last strategy should be carefully controlled because insufficient mixing may promote biofloc sedimentation and localized oxygen depletion. Tank form can also contribute to this balance. Cylindric-conical tanks optimize hydrodynamic circulation and maintain bioflocs in suspension, potentially reducing the need for excessive aeration rates [14]. These approaches should be cautiously chosen, hydraulically and operationally adjustment can be sufficient to minimize unnecessary turbulence and physical stress.

During the first BFT system maturation phase, ammonia fluctuation commonly occurs because of the not well microbial establishment [19]. Heterotrophic bacteria are in their lag phase and yet not totally developed to immobilize nitrogenous waste - total ammonia nitrogen (TAN) and un-ionized ammonia (NH_3) - in the water [19]. During the inception of biofloc development, the slow-grow total unity of chemoautotrophic nitrifying bacteria – including ammonia-oxidizing bacteria (AOB) such as *Nitrosomonas spp* and nitrite-oxidizing bacteria (NOB) such as *Nitrobacter spp.* - are frequently unestablished, creating a window of nitrogenous compounds instability [20]. Fish larvae are critically vulnerable to the chemical instability inherent to this initial phase of maturation [16].

Unlike juveniles or adults, teleost larvae lack functional gills at hatch, with branchial maturation developing through days to weeks, they depend on cutaneous surface for ion exchange and ammonia excretion [21]. When peaks of ammonia happen, it can oppose outward excretion, due to the NH_3 partial pressure gradients [22], which compromises the capacity for ammonia excretion via epithelial cells by acid-trapping mechanism, linked to H^+ secretion and Rh transporters [23, 24]. Consequently, the exposure to sub-lethal ammonia concentrations disrupts neurotransmitter expressions, induces coordinated neurobehavioral deficits, and causes systemic oxidative stress, damaging branchia and hepatic tissues [25, 26], ultimately depressing larval survival and disrupting ontogenetic growth performance [16, 27]. However, ammonia tolerance is not consistent among fish species and may vary according to life stage, trophic habit, and the form of ammonia evaluated. Reported concentrations associated with acute toxicity or physiological impairment differ considerably among species, highlighting the need

to consider species-specific thresholds when assessing the feasibility of BFT, particularly for sensitive species (Table 1). Therefore, modulating aeration dynamics, load of organic matter and pre-stabilizing the nitrifying microbial community are mandatory eco-engineering prerequisites to mitigate these early developmental bottlenecks in BFT larviculture.

Table 1. Reported ammonia thresholds associated with acute toxicity and physiological effects in fish species in Biofloc Technology.

Species	Trophic habit	Life stage	Culture System	96 h Metric*	Physiological or survival impact	DOI
<i>Oreochromis niloticus</i>	Omnivorous	Fry	Pilot BFT nursery (28 days)	>1 to >2 mg L ⁻¹ NO ₂ -N	TAN remained <1 mg L ⁻¹ , but nitrite peaks >2 mg L ⁻¹ reduced survival at high stocking densities	https://doi.org/10.3390/ani15202942
<i>Ompok bimaculatus</i>	Carnivorous	Larvae	45 – day BFT (C:N 10:25)	96h LC50: 12,19 mg L	Highest post-challenge SOD in C/N 20; lowest glucose and cortisol in C/N 15–20	https://doi.org/10.1016/j.aquaculture.2021.737184
<i>Rhynchocypris lagowski</i>	Omnivorous	Juveniles	56-day BFT (C/N 10.8–25)	96 h acute challenge	C:N 20 significantly boosted SOD, CAT, TAOC, and GR	https://doi.org/10.1016/j.etap.2020.103380
<i>Tinca tinca</i> & <i>Mugil cephalus</i>	Omnivorous	Fry	BFT vs RAS (50 days)	6 mg L ⁻¹ TAN intervention limit	When total ammonium reached >6 mg L ⁻¹ , a 50% water exchange was triggered	https://doi.org/10.1016/j.aquaculture.2017.09.041
<i>Carassius auratus</i>	Omnivorous	Larvae	BFT vs clear water (15 days)	Ambient toxic ammonia threshold	High ammonia reduced growth despite BFT enhancing survival and goblet cells	https://doi.org/10.1007/s10499-023-01068-w

*96 h Ammonia Thresholds in Biofloc. BFT, biofloc technology; C:N, carbon-to-nitrogen ratio; CAT, catalase; GR, glutathione reductase; LC50, median lethal concentration; NO₂-N, nitrite-nitrogen; RAS, recirculating aquaculture system; SOD, superoxide dismutase; TAOC, total antioxidant capacity; TAN, total ammonia nitrogen.

Figure 1. Mitigation strategies for Biofloc system operational challenge



Mitigation strategies for operational challenges in biofloc systems. (A) Conventional high-aeration tank: Unmodified rearing units operated under continuous, high-intensity aeration, resulting in strong water movement and turbulence that may increase physical stress and compromise larval survival. (B) Targeted structural modifications and management: Three approaches to improve the hydrodynamic conditions experienced by larvae. (1) Hydrodynamic shelters [17]: Strategic placement of internal baffles and screens to create low-velocity resting zones and reduce exposure to turbulent water movement. (2) Temporary aeration reduction [18]: Reduction of aeration intensity during the critical post-hatching period (days 3–6), while maintaining sufficient water circulation and biofloc suspension. (3) Optimized tank design [16]: Use of cylindric-conical tanks to promote more controlled, shape-driven water circulation, allowing bioflocs to remain suspended with lower hydrodynamic energy.

4.3.2 Enzymatic Transition and the Autochthonous Microbiome

The transition from endogenous yolk sac reserves to external feeding represents a critical metabolic and digestive event during early teleost development [28]. At the beginning of gastric ontogenetic, the gastrointestinal tract is a straight undifferentiated tube [29], morphologically underdeveloped lacking functional gastric glands and differentiated acid-secreting stomach [30, 31]. The pancreas, liver and gall bladder become functional just before the first exogenous feeding, having then an alkaline environment using pancreatic enzymes [29, 28]. Consequently, this late gastric development limits the capacity to digest complex nutrients [30], and it is completed by intense pinocytosis followed by lysosomal hydrolysis within posterior enterocytes [32; 31].

In parallel, protein digestion also occurs incipient with pancreatic proteases (e.g. trypsin) as an enzymatic luminal digestion, but at a low activity level [33]. Due to larvae

immature digestive tract, it creates a reliance on live feed that partially hydrolyzed nutrients [34, 35]. BFT system mitigates this limitation by functioning as a suspended bioreactor of nutritional and enzymatic components [36]. Microflocs are densely colonized by bacteria as well as protozoa, rotifers, and microalgae, forming a complex, nutrient-dense trophic matrix [37, 38]. This microbially-derived matrix can continuously provide stable extracellular enzymes, including alkaline proteases (trypsin and chymotrypsin), alpha-amylases, and lipases [39, 40]. Upon ingestion, these exogenous microbial enzymes can supplement the endogenous digestive capacity of the larvae [39, 41], significantly upregulating digestive enzyme activity within the larval gut lumen, thereby accelerating nutrient assimilation and intestine maturation [42].

The continuous intake of biofloc-associated nutrients improves gut morphology, digestive enzymes activity and goblet cell density [15, 16, 43], these parameters can show an accelerated differentiation and maturation of the digestive tract [43]. Intestinal histological morphometry reveals a significant increase in villus height, crypt depth, and mucosal area of larvae reared under optimized BFT conditions [16, 42, 43], accompanied by enhancing structural protection and local innate immune defenses [16]. These biofloc-induced changes supported by feed supplementation, can result in a more efficient ontogenetic development from intracellular pinocytosis to an extracellular luminal digestion [31, 45]. This functional transition optimizes feed digestion and mitigates reliance on live feeds, such as *Artemia* nauplii [16].

4.3.3 The Biofloc Microbiome as an Immune-Modulating Environment

During the early ontogenetic stages, teleost fish have an immature adaptive immune system [46]. Consequently, larvae rely mostly on innate immune mechanisms to survive environmental pathogen challenges [46]. Traditional clear-water larviculture can favor opportunistic bacteria, such as r-strategist pathogens (e.g., *Vibrio* spp. and *Aeromonas* spp.) [47]. BFT system suppresses this vulnerability by sustaining a mature microbial community that outcompetes pathogens and provides continuous antigenic stimulation [47, 48, 49]. Biofloc contains a diverse microbial compound that acts like microbial-associated molecular patterns (MAMPs), such as beta-glucans, lipopolysaccharides (LPS), and peptidoglycans [41, 49].

Continuous exposure to non-pathogenic microorganisms and their structural MAMPs via microbial flocs can activate their non-specific immune system, triggering pattern recognition receptors (PRRs) on mucosal surfaces [49]. Microbial BFT, like heterotrophic

bacteria, form aggregates whose cell walls possess structural components like lipopolysaccharide, peptidoglycan and others, that when ingested act like immunostimulants [49, 50]. Thin-lipped mullet (*Liza ramada*) reared in BFT showed higher phagocytic activity, lysozyme, IgM, goblet cells and other immunological parameters, demonstrating an immune activation when reared under biofloc conditions [42]. The interaction of these microorganisms and their MAMPs induces a state of systemic immunological priming, enhancing readiness against potential pathogens before the onset of disease [50, 51, 52]. Although direct immunological assays are less explored in early life-stage studies, there are some, such as improvements in humoral immune responses of Nile tilapia fry reared in biofloc system [53], and yet this phenomenon can be empirically supported, like higher survival rates of goldfish (*Carassius auratus*) larvae reared under optimized food management in BFT system [16].

4.4 CARNIVOROUS FISH AND TOP PREDATORS: RESPIRATORY, OSMOREGULATORY AND METABOLIC BOTTLENECKS

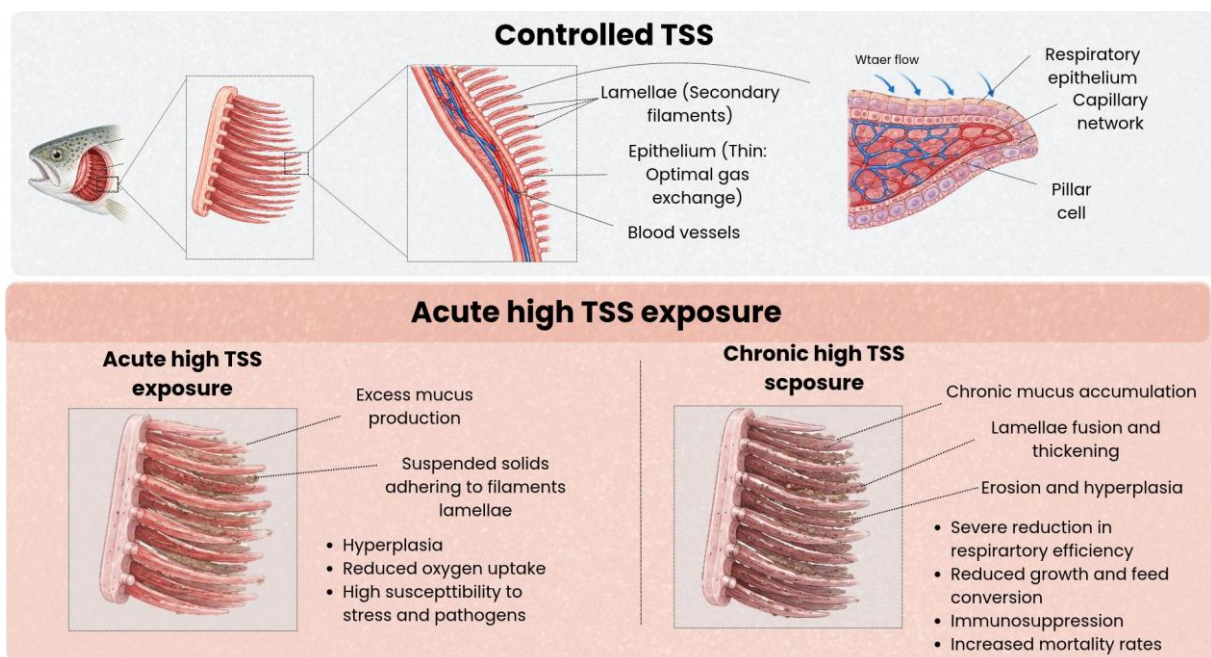
4.4.1 The Gill Interface: Mechanical Interactions, Mucosal Remodeling, and Functional Hypoxia

Carnivorous teleost fish, particularly rheophilic and pelagic apex predators, have evolved highly specialized gill architectures to meet high metabolic oxygen demands [54, 55]. These high-performance teleosts have high total filament length due to the presence of numerous long gill filaments and a high lamellar frequency, maximizing respiratory surface area [55]. In intensive systems, this delicate respiratory interface becomes highly susceptible to suspended particles [56]. Unlike omnivorous, iliophagous species such as *Oreochromis niloticus*, which have well-developed branchial rakers that filter large particles [57], carnivorous fish can be highly vulnerable to high concentrations of TSS due to their gills being optimized for gas exchange rather than particle handling. Importantly, the physiological impact of suspended particles depends strongly on their composition [58]. Organic micro flocs typical of BFT system are soft, cellular aggregates with lower abrasive potential than inorganic particles [58]. As a result, sensitive carnivorous fry, such as rainbow trout (*Oncorhynchus mykiss*), can maintain normal growth performance, baseline antioxidant enzyme activity, and overall welfare under moderate biofloc concentrations ($\leq 250 \text{ mg L}^{-1}$) [59].

However, when TSS accumulation is not properly controlled and exceeds critical thresholds, the high load of suspended particles can induce severe branchial pathology [60]

(Figure 2), with consequent reductions in growth performance [59]. Although the organic nature of bioflocs provokes lower acute mechanical damage than inorganic ones [58], the continuous exposure to hyper-turbid conditions can trigger a pronounced localized inflammatory response [6, 7]. Histomorphology analyses showed shorter secondary lamellae, reducing ions and gas exchange [6]. Another common consequence of high suspended solids is the accumulation of mucus in the body's surface and gill, which is increased during times of stress [6, 61]. While this response initially serves as an immediate physical shield, it also thickens the boundary layer, impairing gas exchange [6]. In parallel, exposure to elevated solids can promote hyperplasia of the branchial epithelium, and partial or complete fusion of lamellae (lamellar fusion) [61]. These structural changes reduce the effective respiratory surface area, ultimately compromising branchial gas exchange efficiency.

Figure 2. Gill structural responses to controlled and excessive total suspended solids (TSS) exposure.



Representation of the effects of total suspended solids (TSS) on fish gill structure and respiratory function. Under controlled TSS conditions, the gills maintain intact secondary lamellae, a thin respiratory epithelium, and an efficient capillary network that supports gas exchange. Acute exposure to high TSS concentrations can promote excessive mucus production and the adhesion of suspended particles to the lamellar filaments, potentially causing epithelial hyperplasia, reduced oxygen uptake, and increased susceptibility to stress and pathogens. Chronic exposure can result in persistent mucus accumulation, lamellar fusion and thickening, epithelial erosion and hyperplasia, leading to severe impairment of respiratory efficiency, reduced growth and feed conversion, immunosuppression, and increased mortality.

4.4.2 Nitrogenous Overload: High-Protein Catabolism and Branchial Diffusion Failure

Due to their strictly carnivorous evolutionary history, carnivorous fish often require high-protein diets [62]. Under physiological conditions, most of the nitrogenous waste is excreted across the gill epithelium into the surrounding water, primarily as un-ionized ammonia through passive transcellular diffusion and facilitated transport mediated by Rhesus (Rh) glycoproteins [63, 64]. When biofloc systems operate with carbon-to-nitrogen (C:N) ratios (above 10:1) to promote heterotrophic assimilation, this balance becomes highly sensitive to biofilter stability [65, 66]. Even short-term disruptions can lead to rapid increases in ambient NH_3 concentrations [1].

When exposed to high concentration of NH_3 , the trans-branchial diffusion gradient is reduced or reversed in fish, preventing ammonia excretion and causing its accumulation in plasma and tissues, possibly causing systemic hyperammonemia [64]. Excess NH_3 readily crosses the blood–brain barrier, compromising neural homeostasis [64, 67]. This condition disrupts the glutamate–glutamine cycle, as astrocytes intensify the conversion of ammonia and glutamate into glutamine via glutamine synthetase [68]. The intracellular buildup of glutamine creates an osmotic imbalance that leads to water influx, causing cerebral edema [69]. In addition, hyperammonemia promotes the overactivation of N-methyl-D-aspartate (NMDA) receptors, triggering oxidative stress, neural damage and ultimately neural death [70].

4.4.3 Sensory Modulation: Turbidity as a Behavioral Shield Against Aggression and Cannibalism

While high turbidity and elevated TSS can impose severe respiratory constraints [6], the optical properties of BFT systems provide a distinct behavioral advantage for intensive culture of carnivorous fish. Predatory and aggressive species often rely on visual cues to locate prey, establish dominance hierarchies, and defend territories [71]. In clear-water systems, high stocking densities during larval and nursery stages can intensify agonistic interactions, resulting in fin damage, size variability, and cannibalism [72, 73]. In contrast, suspended micro flocs in BFT scatter and attenuate light, reducing visual range [74]. This diminished visibility disrupts predator-prey detection among conspecifics, limiting the ability of larger individuals to identify and capture smaller ones [75, 76]. Additionally, the low-visibility environment weakens territorial behavior by preventing dominant fish from establishing and maintaining spatial boundaries [77].

Studies in larviculture and nursery phase of aggressive species indicate that the moderate turbidity characteristic of BFT reduces cannibalism rates, improves survival consistency, and promotes more uniform size distribution [78, 79]. From a systems engineering perspective, TSS management in carnivorous BFT requires balancing two competing objectives: maintaining sufficient turbidity to reduce visually mediated aggression, while preventing excessive TSS accumulation that impairs respiratory function and gill health.

4.5 BIOPHYSICAL NICHE MODULATION IN BFT SYSTEMS

4.5.1 Conceptual Framework: Adaptive BFT Management Across Species and Developmental Stages

This review proposes a paradigm shift: BFT should be regarded not as a rigid production protocol, but as a flexible, eco-engineered system. The failures reported when standard BFT has been applied to non-traditional species, such as larvae and apex predators, do not reflect intrinsic limitations of technology. Instead, they arise from a mismatch between system parameters and biological adaptations of the target organism. The performance of BFT depends on the precise adjustment of three key factors: the carbon-to-nitrogen (C:N) ratio, total suspended solids (TSS) dynamics, and the dominant microbial pathways (Figure 3) [3, 80]. By modulating these variables, the physicochemical and biological conditions of the system can be tailored to meet species-specific requirements (Figure 3, Table 2). This allows the culture environment to operate within the appropriate homeostatic, sensory, and osmoregulatory limits of fish and crustaceans, regardless of trophic level or developmental stage. Therefore, the effectiveness of BFT lies not in strict adherence to a standardized protocol, but in its capacity for dynamic adaptation to the physiological and ecological requirements of the cultured species.

Figure 3. Conceptual framework of fish niches along the suspended-solids gradient in biofloc aquaculture.

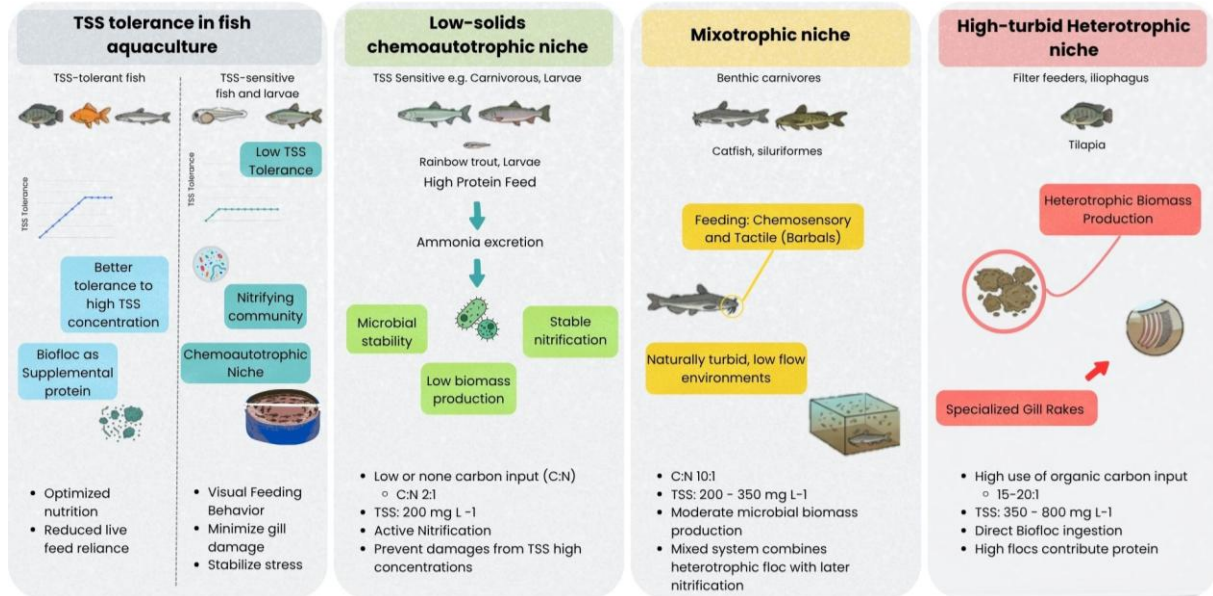


Illustration of four conceptual niches modulation in Biofloc Technology (BFT) systems. The evolutionary and ontogenetic spectrum outlines the clear transition from a strictly chemoautotrophic, low-solids autotrophic environment (TSS < 200 mg L⁻¹, minimized C:N ratio) engineered for visual, hydrodynamic-sensitive fish and larvae, mixotrophic environment for benthic carnivorous, (Intermediate TSS 200 – 350 mg L⁻¹ and C:N) to a hyper-turbid heterotrophic matrix (TSS 350 - 800 mg L⁻¹, elevated C:N ratio) tailored for downstream filter-feeders and omnivorous models. Specific system engineering controls—such as inline clarifiers, automated purging, and carbon dosing dynamics—allow the modulation of system architecture to map directly onto target physiological, sensory, and osmoregulatory characteristics, overcoming historic phenotypic constraints across diverse trophic groups.

4.6 MECHANISTIC BREAKDOWN OF THE MODULATED NICHES

4.6.1 The Ontogenetic Window of Total Suspended Solids (TSS) Tolerance in Fish Larviculture

The application of BFT system in larviculture phase requires a precise modulation of the microbial community according to the specific physiological plasticity and ontogenetic development of the raised species [81]. Research has primarily focused on hardy freshwater species, such as Nile tilapia [13, 44], goldfish (*Carassius auratus*) [14, 16], and South American catfish (*Rhamdia quelen*) [18] which exhibit a consider tolerance to TSS. Some species that hatch at a more advanced developmental stage with a larger size, more developed digestive systems, have greater tolerance to suspended solids [44, 82]. Studies indicate that goldfish and silver catfish (*Rhamdia quelen*) larvae can tolerate TSS concentrations up to 300 mg L⁻¹ and 200 mg L⁻¹ without significant impacts on survival, growth performance, or final zootechnical yields [14, 18]. Under these tolerance possibilities, strict chemoautotrophic control is not required. Instead, BFT system may operate at a mixotrophic state level where the bioflocs act

both as a protective microbial barrier and as a supplementary in situ protein source [80, 83]. This high tolerance to TSS tolerance directly optimizes nutrition and reduces reliance on costly live feeds [16, 83].

In contrast, marine fish larvae and freshwater rheophilic fish (such as migratory Neotropical characiforms) exhibit physiological traits that requires a highly controlled, low turbidity autotrophic environment [84, 85]. Marine larvae are characterized by underdeveloped organs, low osmotic resilience, and reliance on visual predation [85, 86]. Similarly, rheophilic larvae, adapted to slow flowing, well oxygenated and high food availability river systems [85]. During these early developmental stages, a limitation in space capacity can be reflected in a low dissolved oxygen (DO), which affects larvae growth and survival [84], who displays high metabolic demands and delicate gill structures [87]. For fish species with greater physiological sensitivity, BFT system should be maintained in a mixotrophic system, with majority of chemoautotrophic nitrifying bacteria. Chemoautotrophic BFT management minimizes heterotrophic solids accumulation, which preserves low-turbid, high-oxygen conditions required for these sensitive species [60, 88, 89]. A lower C:N ratio limits heterotrophic bacterial growth and reduces oxygen consumption associated with organic carbon degradation [90, 91]. Instead, the BFT system matures in a way that promotes the development of nitrifying communities, including ammonia-oxidizing bacteria (*Nitrosomonas*) and nitrite-oxidizing bacteria (*Nitrospira*) [90, 92]. This configuration may support visual feeding behavior, stabilize physiological stress, and minimize the risk of gill damage caused by particle abrasion, clogging, or mechanical stress.

4.6.2 The Low-Solids Quimoautotrophic Niche (Carnivores)

Pelagic, fast-swimming fish such as salmonids have high metabolic demands and are commonly fed high-protein diets, often exceeding 45% crude protein [93]. The resulting high nitrogen intake can increase post-feeding ammonia excretion, and at the same time, their thin lamellar membranes remain highly vulnerable to physical fouling, placing additional demands on the system's capacity for nitrogen removal [93, 94]. To address these constraints, biofloc system may operate with predominantly chemoautotrophic or balanced mixotrophic configurations. With these adjustments, the system has the capacity to maintain water quality with a lower suspended solid [20, 95], utilizing integrated inline clarifiers if needed.

In this configuration the carbon input should be carefully controlled to limit excessive heterotrophic biomass accumulation while maintaining nitrification [95, 96]. Although some relatively resilient life stages, including rainbow trout, can tolerate suspended solids at moderate levels above traditional salmonid thresholds [59], maintaining TSS proximately 150 mg L^{-1} may provide a conservative operational safety margin for early and sensitive life stages. Keeping suspended solids at moderate levels can support microbial nutrient cycling while reducing the potential for branchial damage and particle accumulation, particularly when suspended particles are predominantly soft and organic [58, 60]. By efficiently oxidizing ammonia to nitrate, the nitrifying community lowers the external ammonia concentration, thereby maintaining the diffusion gradient across the gills and limiting ammonia accumulation in the blood [97].

4.6.3 *The Mixed Standard Niche (Benthic Carnivores and Siluriformes)*

Benthic carnivores, such as catfish, exhibit a distinct adaptation to environments where visual cues are often limited. Their feeding behavior relies substantially on chemosensory and tactile mechanisms, including the use of barbels, reflecting adaptation to turbid and low-flow benthic habitats [98]. These species can be reared under biofloc conditions with moderate TSS concentrations, with values of approximately $200\text{-}400 \text{ mg L}^{-1}$ without detrimental effects on performance [18, 99]. Studies showed improvement in growth, immunity and water quality of catfish reared in this system [18, 100, 101]. The branchial morphology of Catfish also reflects adaptation to particle-rich environments, including mucous structures that may contribute to the protection of respiratory surface [102, 103]. Because these species rely less on visual stimuli for feeding, elevated turbidity levels may have a smaller effect on feeding than in visually oriented predators [103, 98]. Therefore, biofloc system for benthic carnivores may accommodate a greater contribution of heterotrophic processes, provided that TSS and oxygen demand remain within species-specific physiological limits.

4.6.4 *The Hyper-Turbid Heterotrophic Niche (Classic Omnivorous Model)*

The conventional biofloc model is particularly well suited to filter-feeding and iliophagous species, such as *Oreochromis niloticus*. Typically operated at relatively high C:N ratio (10-20:1), this approach favors heterotrophic biomass formation, resulting in relatively

high TSS concentration, often around 300-500 mg L⁻¹ [105, 106]. These species possess well-developed gill rakers that facilitate the retention and processing of suspended particles [57], allowing bioflocs to contribute directly to nutrient intake [107]. Within this framework, biofloc can provide an additional nutrition source, and can stimulate digestive activity, including upregulation of endogenous digestive enzymes [37, 80]. This complementary nutrient supply can partially reduce dietary nutrient requirements and, under appropriate conditions, reduce the dependence on high protein formulated feeds [107].

Table 2. Different Biofloc systems characteristics

Characteristic	Heterotrophic BFT	Mixotrophic or mature BFT	Chemoautotrophic-Oriented BFT
Principal microbial pathway	Dominated by heterotrophic bacteria	Mixed system combines heterotrophic floc formation with later nitrification	Dominated by nitrifying chemoautotrophs
Carbon input	High, use organic carbon (e.g. molasses, sucrose, glycerol). C:N 15-20:1	Intermediate, often added early and then reduced or stopped. C:N 10-15:1	Low or none during routine operation. C:N >2:1
Ammonia control	Usually, fast ammonia assimilation, but performance can include TAN or nitrite peaks	Appears intermediate, balancing floc assimilation and nitrification	Provides stable nitrification-based control once established
Microbial biomass production	High biomass and suspended solids production	Moderate biomass, generally less than fully heterotrophic systems	Lower biomass and TSS because nitrifiers grow more slowly
Supplementary feed value	High, flocs contribute protein and can replace part of formulated feed	Important, especially where fish are encouraged to consume flocs	Lower, direct feed contribution is likely because biomass production is lower
Immune or health effects	Improved hematology, immunity, antioxidant status, intestine conditions	Mature systems are intended to stabilize microbial communities, but fish specific immune comparisons are limited	Better microbial stability and lower pathogenic pressure are plausible, but direct fish immune data are limited
Best-suited fish context	Species that utilize solids, such as omnivorous or filter-feeding species, such as tilapia and carp	Situations needing a compromise between floc nutrition and solids control, such as benthic species	Situations prioritizing nitrogen stability and lower reliance on dense suspended flocs such as Larval stage and carnivorous fish

Comparative characterization of the three biofloc types and their associated microbial niches, highlighting differences in microbial composition, carbon input, ammonia control, supplementary feed value, immune or health effects and possible species cultivation [20, 74, 108, 109, 110, 111, 112, 113, 114; 115]].

4.7 CONCLUSION AND PARADIGM ASSESSMENT

The central focus of this review is that BFT should not be regarded as a production system inherently restricted to hardy species. Rather, its operational limits are determined by interaction between system conditions and the physiological and ecological requirements of the cultured organisms. This perspective provides a framework for understanding how BFT can be adapted to a larger range of teleost species, including larvae and carnivorous fish. These applications, however, may require modification of conventional high-solids protocols, including greater control of microbial community, management of TSS concentrations, and greater reliance on nitrifying microbial communities when low-turbid conditions are required. At the same time, feed and system management should rely on species-specific demands and characteristics, such as digestive development, oxygen demand, and ammonia excretion capacity.

In this context, chemoautotrophic biofloc systems, with TSS lower than 200 mg L⁻¹ and none or minimum input of carbon (C:N 2:1), may be particularly suitable for fish and life stages sensitive to high suspended solids and turbidity, whereas multitrophic systems, with TSS 200 till approximately 350 mg L⁻¹, and C:N around 10:1, may provide an alternative environment for benthic species by maintaining water quality stability at lower solids concentrations while still providing organic matter that can serve as a complementary food source. Overall, these approaches support a broader view of BFT as an adaptable production technology, in which system configuration and operational conditions can be tailored to the physiological requirements of different cultured organisms.

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5 CAPÍTULO III - ARTIGO 2: FEASIBILITY OF RAINBOW TROUT *Oncorhynchus mykiss* REARING IN BIOFLOC SYSTEM: EFFECT ON ZOOTECHNICAL PERFORMANCE, INTESTINAL CONDITION AND WELFARE

Article

Feasibility of rainbow trout (*Oncorhynchus mykiss*) fry rearing in biofloc system: effect of total suspended solid levels on zootechnical performance, intestinal condition and antioxidant enzyme activity

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5.1 SIMPLE SUMMARY

This study evaluated the effects of different biofloc total suspended solid (SST) levels (0, 250, and 350 mg L⁻¹) on the productive performance, intestinal condition and antioxidant enzyme activity of rainbow trout (*Oncorhynchus mykiss*) fry reared in a biofloc technology (BFT) system. Rearing rainbow trout fry in a BFT system with TSS levels up to 250 mg L⁻¹ proved feasible, maintaining zootechnical performance, intestinal condition and antioxidant enzyme activity during the early stages of development. In contrast, the TSS concentration of 350 mg L⁻¹ negatively affected fry growth performance. Despite this increased gut colonization by lactic acid bacteria was observed, indicating that biofloc may serve as a source of probiotic bacteria for rainbow trout. BFT system is recommended as an alternative system for rearing rainbow trout fry, offering enhanced biosecurity and reduced water consumption.

5.2 ABSTRACT

This study evaluated the effects of different total suspended solid (SST) levels (0, 250, and 350 mg L⁻¹) on the productive performance, intestinal condition and antioxidant enzyme activity of rainbow trout (*Oncorhynchus mykiss*) fry reared in a biofloc technology (BFT) system. Experimental design was completely randomized with three treatments and five replicates. A total of 225 rainbow trout fry (0.81 ± 0.06 g) were distributed in 15 tanks (70 L working volume), at a stocking density of 15 fish per tank.

Tanks designated for the control treatment (clear water) were connected to a recirculating aquaculture system, whereas BFT treatments were maintained in independent units. Performance analyses were conducted and fish were sampled for intestinal histomorphometry, intestinal microbial counting and antioxidant enzymatic activity. Rearing rainbow trout fry in BFT systems with TSS levels up to 250 mg L⁻¹ proved feasible and preserved zootechnical performance, intestinal condition and antioxidant enzyme activity during the early stages of development. The higher TSS concentration negatively affected fish growth performance. However, increased colonization by lactic acid bacteria was observed, indicating that biofloc may serve as a source of probiotic bacteria. BFT system is a viable alternative for rearing rainbow trout fry, enhancing biosecurity and reduced water consumption.

Keywords: Growth; Salmonid; Total suspended solids; Sustainability

5.3 INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is a carnivorous fish that inhabits clear cold waters [1]. This fish meat is highly appreciated, presenting a strong market demand. Therefore, it is one of the most produced fish in world aquaculture [2] with an average annual growth rate of 2.5% over the last decade [3]. The most recent data [2], inland rainbow trout production reached 739,500 tonnes, accounting for 1.5% of total global aquaculture. Traditional trout farming relies on flow-through raceway systems that require the use of vast water volumes and specific topographical conditions [4-6]. Furthermore, these flow-through system are susceptible to the introduction of external pathogens and unwished factors into the farming, what can be a biosafety issue [7,8]. Given the increasing concern regarding water scarcity [9], there is a need for more sustainable and environmentally responsible practices in trout farming. In this context, trout production in a biofloc technology (BFT) system emerges as promising key-alternative.

The BFT systems enable intensive fish production with minimal water exchange by using carbon sources to stimulate nitrogen cycling and maintain water quality [10]. During this process, biofloc aggregates are formed comprising microorganisms and organic residues such as zooplankton and phytoplankton [11]. Furthermore, these particles are rich in protein, lipids, vitamins and micronutrients [12], being a possible supplementary feed source for farmed animals [13]. Flocs may also be a source of beneficial probiotic bacteria with benefits to the intestine [14]. On the other hand, some factors can negatively impact the health of fish in BFT system. Decomposition of organic matter such as the oxidation of ammonia to nitrite, is an oxidative process that can generate reactive oxygen species (ROS) [15]. To counteract oxidative stress, fish rely on the production of antioxidant enzymes [16]. However, during early development, fish display intensified growth and high metabolic activity, leading to mitochondrial working at maximum rate and increasing generation of ROS [4]. Several aquatic species have been already successfully produced in BFT systems, but mainly omnivorous organisms were evaluated, such as *Oreochromis niloticus* [17], *Rhamdia quelen* [18], *Pimephales promelas* [19] and *Cyprinus carpio* [20]. More recently, feasibility of rearing carnivorous fish such as *Salminus brasiliensis* and *Arapaima gigas* were also demonstrated [21,22]. Nonetheless, there remains a demand for studies focusing on the fry and early stages, which are more sensitive and require more stringent rearing control.

Despite its potential, BFT systems have not yet been evaluated for rainbow trout production. The accumulation of total suspended solids (TSS) in the BFT system can be problematic, as excessive concentrations may cause gill damage impairing gas exchange and osmoregulation [23]. For efficient nitrogen cycling without compromising productive performance, the recommended total suspended solids levels range are from 200 to 600 mg L⁻¹ [23,24]. In contrast, tolerance limits for salmonids in flow-through systems are significantly lower, around 25 mg L⁻¹ [25]. However, previous studies have demonstrated that juvenile rainbow trout reared in closed systems can tolerate higher concentrations due to differences in the physicochemical characteristics of the TSS [26]. We hypothesized that rainbow trout fry can adapt to BFT system rearing and tolerate up to 350 mg L⁻¹ of TSS without adverse effect on productive performance. As an alternative production system, it may offer both economic and

environmental benefits, increasing the sustainability of the activity. Furthermore, possible ingestion of bioflocs may improve nutrients' absorption and be a source of probiotics to increase intestinal health [27]. Thus, this study aimed to evaluate the effect of different concentration of total suspended solids in the BFT system on productive performance, intestinal condition and oxidative enzyme response of rainbow trout fry, as well as validating the feasibility of BFT for early life stages of this salmonid species.

5.4 METHODOLOGY

5.4.1. Experimental Design

This study was approved by the Ethics Committee on Animal Use (CEUA) of UDESC with the number 9440250324 on August 30, 2024. The experimental design was completely randomized with three treatments with five replicates. A recirculating aquaculture system RAS served as a control treatment, while two levels of total suspended solids (250 and 350 mg L⁻¹) were evaluated in the BFT system. The 56-day experiment was conducted at the Fish Farming Laboratory of the State University of Santa Catarina (UDESC) located in Lages, SC, Brazil.

5.4.2 Animals and facilities

Rainbow trout fry were purchased from a commercial hatchery (Recanto das Trutas, Bocaina do Sul, SC, Brazil) and acclimated to laboratory conditions for 30 days. After the acclimatization period, fish with an initial average body weight of 0.80 ± 0.06 g (mean $\pm$ standard deviation) were randomly distributed in 15 circular tanks (70 L working volume) at the initial density of 15 fish per tank (0.21 fish L⁻¹). This value is below the recommendations for rainbow trout farming, which would be at least 13 fish L⁻¹ [1]. A lower density was selected due to technical facility limitations and to comply with Animal Ethics Committee's guidelines to minimize the number of experimental animals to meet the principles of the 3Rs in animal experimentation.

Control tanks were integrated into a RAS with UV system, mechanical and biological filter, and individual aeration via microporous hoses. The RAS was selected over the flow-through system as a control group due to the precise controlling of the experimental conditions. BFT tanks were equipped only with an intensive aeration system with microporous hoses to maintain floc in suspension. Fish were fed a commercial diet (Laguna Carnívoros Crescimento, ADM, Primavera do Leste, MT, Brazil) containing 45% crude protein, 4,435 kcal kg⁻¹ of gross energy and crude lipid levels of 12% (data provided by feed manufacturer). The feed was ground and sieved to obtain particles of approximately 2 mm in diameter, size suitable for mouth gap fish. Feeding was performed twice daily, at 09:00 am and 05:00 pm, to apparent satiety. Since the feed pellets were crushed, they lost their buoyancy. During feeding, aeration system was temporally suspended for about 10 min so the fish could identify the pellets entering the water. Since turbid BFT system made visualization of the ingestion difficult, this technique was necessary for the fish to learn to feed on the surface before the pellets sank, thus allowing for a quantification of satiety. Apparent satiety was considered reached when the fish no longer ascended into the water column to capture the pellets.

Two months prior the trial, 20 L of matured biofloc inoculum previously cultured with *Oreochromis niloticus* were added in each BFT tank. Clear water from a artesian well was added until the working volume was completed. The same commercial feed used during the experiment was used as a nitrogen source. Molasses (38% carbon) was added to maintain a C:N ratio of 20:1 [28] in each BFT tank from the experiment, assuming that 75% of total ammonia nitrogen from the feed is released into water [29] to mature the system. Water quality was monitored, and when nitrate levels began to rise, the system was considered mature, indicating established nitrification and the fish were introduced. Throughout the experimental period, the addition of molasses was not necessary because, due to the use of mature

biofloc, the concentrations of total ammonia nitrogen and nitrite were maintained consistently low and stable. When total suspended solids concentration exceeded the predetermined treatment thresholds (250 and 350 mg L⁻¹), as measured using an Imhoff cone, clarification were performed to maintain the levels within treatment specifications. Water from an artesian well was used in the RAS as well. In both systems low concentrations of sodium chloride were also used (4-6 ppt) as a prophylactic measure [30].

5.4.3 Water Quality

Water parameters were evaluated weekly (Table 1) and maintained within the optimal range for the rainbow trout. Temperature, pH, salinity, and dissolved oxygen were measured using a multiparameter device (AK88, AKSO® Produtos Eletrônicos, São Leopoldo, RS, Brazil). There was a temperature discrepancy between systems due to RAS operational heat generation, maintaining it 2°C higher than the BFT systems tanks. Photoperiod was standardized across treatment (12h light, 12h dark). Total suspended solids were determined according to the method described by Rice et al. [31] (Figure1) . Total ammonia nitrogen, nitrite and nitrate levels [31] were measured using spectrometer (Spectro kit, Alfakit, Florianópolis SC, Brazil) according to the method of Rice et al. [31]. There were no ammonia peaks, and the presence of nitrate indicated complete nitrogen cycling in the systems. Settleable solids were measured twice a week following adapted settleable solids methodology, using an Imhoff cone [32]. One liter of water was collected in the cone and allowed to settle for 10 min. Partial water exchanges via sedimentation were performed when the levels of settleable solids exceeded the established thresholds (20 and 30 mL L⁻¹). Accumulated solids in the RAS tanks were removed weekly by siphoning.

Table 1. Water quality analysis values for rainbow trout after 56 days of the experiment.

	RAS	BFT 250	BFT 350
Temperature (T°C)	20.9 ± 1.53	18.7 ± 0.96	18.8 ± 0.97
Total suspended solids (mg L ⁻¹)	4.10 ± 1.55	274.72 ± 31.34	365.35 ± 36.67
Settleable solids (mL L ⁻¹)	0.00 ± 0.00	24.32 ± 4.40	33.38 ± 4.03
Ammonia (mg L ⁻¹)	0.03 ± 0.08	0.00 ± 0.00	0.00 ± 0.00
Nitrite (mg L ⁻¹)	0.07 ± 0.12	0.00 ± 0.03	0.00 ± 0.00
Nitrate (mg L ⁻¹)	64.77 ± 47.83	228.8 ± 31.6	327.6 ± 50.2
pH	8.89 ± 0.10	8.82 ± 0.91	8.81 ± 0.10
O ₂ (mg L ⁻¹)	7.11 ± 0.73	6.87 ± 0.67	6.91 ± 0.62
Salinity (mg L ⁻¹)	4.42 ± 0.77	6.38 ± 1.27	6.14 ± 0.95

RAS: Recirculation aquaculture system; BFT250: Biofloc 250 mg L⁻¹; BFT350: Biofloc 350 mg L⁻¹.

Figure 1. Total suspended solids. Top: suspended solids collected from the 250 mg L⁻¹ treatment; bottom: suspended solids collected from the 350 mg L⁻¹ treatment.



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5.4.4 Fish Performance

At the beginning (September 7th, 2024) and end (November 2nd, 2024) of the 56-day experiment, the fish were weighed on an analytical balance (Marte; AY220; Minas Gerais, Brazil) to monitor fish growth. Prior to weighing, all fish were submitted to a 24-hour fasting period, anesthetized with eugenol with dose of 60 mg L⁻¹ [33], and individually weighed. Productive performance was carried out by analyzing the following parameters:

$$\text{Weight gain (WG, g)} = \text{final average weight} - \text{initial average} \quad (1)$$

$$\text{Specific growth rate (\% day}^{-1}\text{)} = \frac{\text{Ln}\left(\frac{\text{final average weight}}{\text{initial average weight}}\right)}{\text{experimental period}} \times 10 \quad (2)$$

$$\text{Feed intake (FI, g)} = \frac{\text{total dry feed intake}}{\text{number of surviving fish}} \quad (3)$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{feed intake}}{\text{total weight gain}} \quad (4)$$

$$\text{Survival rate (S, \%)} = \frac{\text{total fish at the end}}{\text{total fish at the beginning}} \times 100 \quad (5)$$

Two fish per tank (n = 10 per treatment) were anesthetized (eugenol at a dose of 60 mg L⁻¹) [33] and euthanized by spinal sectioning for intestinal microorganism counting and intestinal histomorphometry. Additionally, eight fish per treatment were sampled for antioxidant enzyme. Intestinal samples destined for enzymatic analysis were immediately stored at -80 °C and subsequently lyophilization (Terroni; Liofilizador LS, São Paulo, Brazil). In contrast, the samples reserved for antioxidant enzymes were lyophilized and stored in dry place until the analysis were performed at IRTA (Spain) research facilities.

5.4.5 Intestinal Microorganism Counting

Intestines were carefully aseptically removed, weighed and homogenized. The homogenized tissue was diluted (1:10) in tubes containing sterile saline solutions (0.65%). The diluted homogenate was plated on Petri dishes with MRS agar (de Man, Rogosa, and Sharpe) for lactic acid bacteria (LAB) quantification, TSA agar (tryptic soy agar) for total heterotrophic bacteria quantification, and TCBS agar for *Vibrio* sp. Bacterial quantification was performed as described by Kaktcham et al. [34]. Colony-forming units (CFUs) were counted after incubation under optimized conditions (TSA and TCBS media were incubated at 35 °C for 24 h, while MRS medium was incubated at 35 °C for 48 h).

5.4.6 Intestinal Histomorphometry

Sections of approximately 5 cm from the proximal intestine were carefully collected and immediately fixed in 10% buffered formalin for 24 h to prevent tissue degradation. After fixation, the samples were dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin for sectioning. Thin tissue sections (5 µm thick) were prepared using a microtome according to the methodology adapted from [35]. Samples (2 slides per fish) were stained using the periodic acid-Schiff (PAS) method to highlight structural components and goblet cells [35]. The slides were examined under an optical microscope (Opticam Microscopy Technology, 10× magnification; São Paulo, Brazil) and with a digital camera (Moticam 2300, 3 MP, resolution of 3264 × 2448, Germany, RML 5). One histological section per slide was selected for morphometric analysis. The total h photographed eight and width of the villi were measured using ToupTek ToupView – x64 image analysis software. Goblet cells were quantified in five representative villus per section and were examined per slide.

5.4.7 Antioxidant Enzyme Activity

Prior to analysis, the intestines were sectioned into small fragments and diluted in phosphate buffer 100 mM (pH 7.4) (1:20, w/v). Samples were homogenized using a Turrax homogenizer (in ice bath to prevent thermal degradation), and after that, put it in a sonicator for 30 s (pulses of 5 s on and 1 s off) to ensure complete cell lysis. Resulting homogenates were centrifuged at 10,000 rpm for 30 min at 4°C. The supernatants were then collected and stored for enzymatic activity determination.

Total antioxidant capacity (TAC) was determined using a commercial kit (Sigma-Aldrich; Darmstadt, Germany), based on the reduction of Cu^{+2} to Cu^{+} , with absorbance measured at $\lambda = 570$ nm [36]. Total glutathione Peroxidase (GPx ; EC 1.11.1.9) activity was measured at $\lambda = 340$ nm, and it was determined by the NADPH oxidation [37] using glutathione 75 mM and NADPH 8.75 mM as a substrate ($\epsilon = 6.22 \text{ mM}^{-1} \text{ cm}^{-1}$). Glutathione reductase (GR, E.C. 1.8.1.7) activity was determined by measuring NADPH oxidation at $\lambda = 340$ nm using glutathione disulfide 20 mM and NADPH 2 mM as substrate [38]. Superoxide dismutase (SOD, E.C. 1.15.1.1) activity were measured at $\lambda = 450$ nm by assessing the inhibition of cytochrome c reduction by O_2 generated by the xanthine oxidase/hypoxanthine system using a commercial kit (19160 SOD determination kit Sigma-Aldrich; Darmstadt, Germany) [39]. Soluble protein in tissue homogenates was determined using Bradford's method using bovine serum albumin as standard [40].

Enzyme activities were expressed as specific activity: nmol substrate catalyzed per minute per mg of protein ($\text{nmol min}^{-1} \text{ mg}^{-1} \text{ protein}^{-1}$) for GPx, GR, Superoxide dismutase (SOD) activity was expressed as the percentage of inhibition, and total antioxidant capacity (TAC) was expressed in µM for TAC. All assays were performed in triplicate (methodological replicates) at 25 °C using a spectrophotometer (TecanTM infinite M200, Switzerland).

5.4.8 Statistical Analyses

For the productive performance analyses, each tank was considered an experimental replicate. For the remaining variables, individual fish were used as replicates. The data were expressed as the mean $\pm$ standard deviation (SD). Statistical analyses were performed using SAS® software (SAS Institute Inc., Cary, NC, USA), version 9.4. All the data were submitted to test to verify the normality of errors with Shapiro-Wilk and the homoscedasticity of variances using Levene's test. When normality and homoscedasticity were verified, the differences between the results were determined with analysis of variance ANOVA and Tukey's test was used at $P < 0.05$ probability. When the normality was not met, the treatments were compared using the non-parametric Kruskal-Wallis test ($P < 0.05$).

5.5 RESULTS

Fish Performance at the end of the trial, rainbow trout fry from the BFT 350 mg L⁻¹ treatment showed lower ($P < 0.05$) final weight and weight gain compared to control treatment RAS (Table 2) (Figure 2). However, feed intake, feed conversion, and survival were not affected ($P > 0.05$) regardless of the production systems and levels of suspended solids in the BFT tanks. Biofloc ingestion was not quantified; however, its presence in the stomachs of fish reared in the BFT system was qualitatively confirmed.

Table 2. Average values ($\pm$ standard deviation; $n = 5$) of performance variables of rainbow trout (*Oncorhynchus mykiss*) fry reared in different levels of suspended solids in water after 56 days.

	RAS	BFT 250	BFT 350	P values
Initial Weight (g)	0.83 $\pm$ 0.05	0.77 $\pm$ 0.02	0.84 $\pm$ 0.06	0.079
Final Weight (g)	3.13 $\pm$ 0.18 ^a	2.74 $\pm$ 0.26 ^{ab}	2.41 $\pm$ 0.49 ^b	0.017
Weight Gain (g)	2.29 $\pm$ 0.19 ^a	1.98 $\pm$ 0.24 ^{ab}	1.57 $\pm$ 0.54 ^b	0.024
Specific growth rate (% day ⁻¹)	2.36 $\pm$ 0.14 ^a	2.34 $\pm$ 0.10 ^{ab}	1.85 $\pm$ 0.47 ^b	0.041
Feed Intake (g fish ⁻¹)	2.86 $\pm$ 0.80	3.06 $\pm$ 0.74	3.11 $\pm$ 0.54	0.855
Feed Conversion ratio	1.34 $\pm$ 0.12	1.55 $\pm$ 0.39	1.98 $\pm$ 0.65	0.166
Survival (%)	89.33 $\pm$ 7.60	88.33 $\pm$ 11.39	78.33 $\pm$ 15.75	0.336

Different letters in the same row indicate a significant difference ($P < 0.05$) within the factors (biofloc concentration levels) by the Tukey test. RAS: Recirculation aquaculture system; BFT250: Biofloc 250 mg L⁻¹; BFT350: Biofloc 350 mg L⁻¹.

Figure 2. Rainbow trout at the end of the experiment



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5.5.1 Intestinal Microorganism Counting

Results in terms of counting viable heterotrophic, LAB and *Vibrio* sp. bacteria are shown in Table 3. The BFT350 treatment increased ($P < 0.05$) the concentration of lactic acid bacteria in the intestines of rainbow trout fry (Table 3). Heterotrophic bacteria and *Vibrio* sp. concentration were not affected ($P > 0.05$) regardless of the production system considered (RAS or BFT).

Table 3. Average values ($\pm$ standard deviation; $n = 5$) of intestinal counts of heterotrophic bacteria, lactic acid bacteria, and *Vibrio* spp. ($\log_{10}$ CFU g^{-1}) in rainbow trout (*Oncorhynchus mykiss*) fry reared under different levels of suspended solids in the water after 56 days.

	RAS	BFT 250	BFT 350	P values
Heterotrophic*	7.19 ± 0.65	7.28 ± 0.77	6.40 ± 0.88	0.202
Lactic Acid bacteria*	4.0 ± 0.08^b	3.84 ± 0.13^b	4.86 ± 0.33^a	0.001
<i>Vibrio</i> sp.**	4.35 ± 0.70	ND	3.95 ± 0.88	0.201

*Means followed by different letters differ ($P < 0.05$) by the Tukey test. **Treatments don't differ ($P < 0.05$) by Kruskal-Wallis test. RAS: Recirculation aquaculture System; BFT250: Biofloc 250 $mg\ L^{-1}$; BFT350: Biofloc 350 $mg\ L^{-1}$; ND: Not detected.

5.5.2 Intestinal Histomorphometry

In the RAS group, the intestinal mucosa of rainbow trout displayed the typical architecture, with well-defined, elongated, and uniformly arranged intestinal folds. A continuous epithelial lining was visible along the folds, and goblet cells were regularly distributed. The *lamina propria* appeared thin, without evident thickening or disorganization. The submucosa and muscular layers were distinguishable as intact and well-defined structures, maintaining the expected arrangement. Overall, tissue integrity was preserved, with no detectable structural alterations at this magnification. No significant differences ($P > 0.05$) were observed among treatments for the intestinal histomorphometry of rainbow trout fry regarding villi height and width (Table 4). In contrast, fry from the BFT 350 group exhibited a significant lower count of goblet cells ($P < 0.05$) compared to RAS group, while BFT250 treatment showed intermediate values.

Table 4. Average values ($\pm$ standard deviation; $n = 5$) of histological analysis of intestinal villi of rainbow trout (*Oncorhynchus mykiss*) fry reared under different levels of suspended solids in the water after 56 days.

	RAS	BFT250	BFT350	P values
Height (μm)	211.73 ± 43.29	221.51 ± 29.43	182.81 ± 23.32	0.332
Width (μm)	94.50 ± 19.54	82.81 ± 4.28	74.94 ± 14.52	0.185
Goblet Cells (nb)	18.42 ± 3.19^a	18.94 ± 5.96^{ab}	12.39 ± 3.72^b	0.031

Means followed by different letters differ ($P < 0.05$) by the Tukey test. RAS: Recirculation aquaculture system; BFT250: Biofloc 250 $mg\ L^{-1}$; BFT350: Biofloc 350 $mg\ L^{-1}$; u: each goblet cell was contacted individually, and the value is expressed per villus.

5.5.3 Antioxidant Enzyme Activity

The levels of total antioxidant capacity (TAC) and activity of antioxidant enzymes did not show any difference ($P > 0.05$) between the treatments (Table 5).

Table 5. Average values of antioxidant enzyme analysis ($\pm$ standard deviation; $n = 21$) of the intestines of rainbow trout (*Oncorhynchus mykiss*) fry reared under different levels of total suspended solids in the water after 56 days.

	RAS	BFT250	BFT350	P values
TAC (μM)	902.18 $\pm$ 130.37	960.87 $\pm$ 192.66	923.36 $\pm$ 157.89	0.072
GR (nmol.min ⁻¹ .mg prot ⁻¹)	225.07 $\pm$ 39.61	292.29 $\pm$ 125.46	233.09 $\pm$ 36.92	0.593
SOD (%)	56.57 $\pm$ 12.48	59.02 $\pm$ 6.19	65.14 $\pm$ 7.89	0.144
GPx (nmol.min ⁻¹ .mg prot ⁻¹)	121.67 $\pm$ 42.99	99.76 $\pm$ 33.40	103.07 $\pm$ 26.38	0.392

Means do not differ by the ANOVA test ($P > 0.05$). RAS: Recirculation aquaculture system; BFT250: Biofloc 250 mg L⁻¹; BFT350: Biofloc 350 mg L⁻¹. TAC- Total Antioxidant Capacity; GPx- Total Glutathione Peroxidase; GR - Glutathione reductase; SOD- Superoxide dismutase.

5.6 DISCUSSION

Results from the current study indicated that the use of concentrations up to 250 mg L⁻¹ of total suspended solids in the BFT system did not affect the productive performance of rainbow trout fry. To our knowledge, this is the first study to demonstrate the feasibility of utilizing BFT system for production of this species. The total suspended solids levels of 250 mg L⁻¹ was sufficient to maintain adequate water quality, results that were in accordance with those used in the literature for different fish species [23,41]. Biofloc system reduces water exchange at minimal rate and can contribute to the sustainability of trout farming. In the present study, water consumption was not measured in the different systems for rainbow trout. However, for Nile tilapia farming in a BFT system, the minimum daily exchange rate is 5% [42]. In contrast, conventional raceway rainbow trout farming systems require much higher turnover rates, with a total water exchange every hour [43]. Excessive total suspended solids reduces water transparency, compromises feed detection capabilities, and reduces feed efficiency and growth rates [44]. Furthermore, TSS accumulation on gill surface may also hinder oxygen uptake and trigger physiological stress responses [44-46]. Recommended concentration of total suspended solids for rainbow trout fry extrapolated from other salmonids is 25 mg L⁻¹ [47]. However, it has already been demonstrated that rainbow trout juveniles tolerate higher levels of total suspended solids in RAS system, achieving 71 mg L⁻¹ [26]. According to the authors, total suspended solids found in RAS are predominantly of organic origin (i.e., fish feces, rest of uneaten feed pellets) and less abrasive, potentially causing less damage to the gills [26]. Since the solids in BFT system have similar organic composition and physical characteristics [12,48], these findings support a greater tolerance to higher solids levels than previously reported, like the one shown in the present study.

Under current experimental conditions, the higher concentration of total suspended solids (350 mg L⁻¹) negatively affected the growth of rainbow trout fry. For other carnivorous fish species reared in BFT systems, total suspended solids were not measured for validation and results are restricted to juveniles [21,22,49]. In contrast, omnivorous species typically tolerate TSS loads, such as Nile tilapia fry (200 - 600 mg L⁻¹) [24], Prussian carp fry (600–800 mg L⁻¹) [50], red-bellied pacu fry (200-300mg L⁻¹) [45]. The adverse effects of excessive TSS loads may be associated with stress and metabolic changes such as hepatic inflammation and higher cortisol levels [49]. Furthermore, in BFT systems, high concentrations of total suspended solids are linked to decreased visibility and reduced feed intake potential, which can impair growth performance and feed conversion efficiency [51]. Rainbow trout fry are visual feeders [52,53], managing TSS is critical for maintaining feed visibility and fish performance. Therefore, the impact of TSS on ingestion may have been further amplified by using ground feed, which exhibited reduced buoyancy.

Lactic Acid bacteria are recognized probiotic that modulate intestinal health and are frequently identified in BFT systems [54,55]. There was an increase in the lactic acid bacteria concentration in the BFT350 treatment. Fish can ingest bioflocs and use it as a food source and supply bioactive compounds [51]. Bioflocs were observed within digestive tract of rainbow trout fry confirming ingestion, suggesting these TSS as a potential source of bioactive compounds and probiotics. Lactic acid bacteria have the ability to acidify intestinal pH by producing organic acids, thereby modulating the microbiota [56]. This group of bacteria can synthesize bioactive compounds like bacteriocins and peptides, which can inhibit the growth of other bacteria and potential pathogens [57, 58]. Probiotic bacteria can also lead to improved feed conversion [55]. However, the ingestion of bioflocs did not lead to an enhancement of

growth performance in rainbow trout fry. The impaired growth performance in the BFT350 treatment may have masked the potential benefits in terms of feed efficiency parameters. No significant differences were detected in *Vibrio* sp. counts, but no count was observed in the BFT250 treatment, indicating a possible modulation of pathogenic microorganisms by the BFT system when 250 mg L⁻¹ of total suspended solids were used.

Rainbow trout rearing in BFT system has not affected the height and width of intestinal villus. Probiotic bacteria present in the BFT system can lead to improvements in the villi characteristics [59]. In other species, such as Nile tilapia [59] and thin lip grey mullet [60] have been observed under BFT conditions. High variability in the data may have hindered the detection of differences. Also, it is already known that intestinal histomorphometry does not always show significant changes when microbial-based strategies (e.g., probiotics or biofloc) are applied [61], it can have masked the beneficial effects of beneficial bacteria. Furthermore, in the BFT350 treatment, there was a reduction in goblet cells in intestinal epithelium. Goblet cells play a role in mucus secretion and intestinal immunity [62] while such changes in goblet cell density may be related to poor growth performance in this treatment.

BFT system rearing did not alter the antioxidant enzyme activity in the intestine of rainbow trout fry. Antioxidant enzymes play a role against oxidative stress and may be also used as a welfare indicator [12]. Studies show that tissues with higher mitochondrial density and elevated aerobic metabolic capacity have an increase in ROS generation and oxidative damage [63]. Similarly, during early development, fish display intensified growth and high metabolic activity, leading to mitochondrial working at maximum rate and increasing generation of ROS. In response to ROS accumulation, the antioxidant system activates specific enzymes to neutralize these radicals, preventing cellular damage or apoptosis [16]. The absence of differences between treatments suggests that BFT system did not affect the intestinal antioxidant response of the fish. This demonstrates that BFT system is safe for rainbow trout with no indication of compromised welfare. Notably, even in the BFT350 treatment, which showed the lowest growth performance, antioxidant enzymes activity were not affected.

5.7 CONCLUSION

Rearing rainbow trout fry in BFT systems with total suspended solids levels up to 250 mg L⁻¹ was feasible as it maintains zootechnical performance, intestinal condition and antioxidant enzyme activity during the early development stages. Conversely, a concentration of 350 mg L⁻¹ negatively affected fish growth. Despite the reduced growth at higher solids levels, the increased colonization by lactic acid bacteria suggests that biofloc serves as a source of probiotic bacteria for rainbow trout. Therefore, BFT systems managed with TSS levels up to 250 mg L⁻¹ are recommended as a viable alternative for rearing rainbow trout fry, offering enhanced biosecurity and water saving.

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Data availability statement: The data that supports the findings of this study are available from the corresponding author upon request.

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6. CONSIDERAÇÕES FINAIS

Após delimitar os princípios de funcionalidade da tecnologia de bioflocos e avaliar os principais fatores que limitam sua aplicação para criação de espécies e fases sensíveis, a presente tese demonstrou a possibilidade de ampliar o uso dessa tecnologia para além das espécies tradicionalmente associadas ao sistema. Propõe-se no primeiro artigo que peixes e fases de vida mais sensíveis à presença de flocos possam ser cultivados em sistemas de biofloco quimioautotróficos, caracterizados por menores concentrações de sólidos suspensos, menor turbidez e maior estabilidade de parâmetros de qualidade da água. Para espécies bentônicas, sistemas multitróficos podem representar uma alternativa, por apresentarem menor concentração de sólidos em comparação ao biofloco heterotrófico, mantendo a estabilidade na qualidade da água, e, simultaneamente, disponibilizando matéria orgânica que pode atuar como fonte complementar de alimento. Além disso, foi demonstrada a possibilidade de criação de alevinos de truta arco-íris (*Oncorhynchus mykiss*) no BFT. Os resultados obtidos no segundo artigo evidenciam que essa aplicação está diretamente relacionada ao controle da concentração de sólidos suspensos e à adequação das condições de cultivo às características fisiológicas da espécie.

A revisão apresentada nesta tese amplia essa interpretação ao propor que a adequação do BFT não seja definida por um único modelo operacional. Diferentes configurações microbianas podem ser estabelecidas de acordo com o objetivo do sistema, desde configurações predominantemente heterotróficas, caracterizadas por maior produção de biomassa microbiana, até sistemas com maior participação de microrganismos nitrificantes e menor dependência de elevados níveis de sólidos suspensos. Essa abordagem permite compreender o BFT como uma tecnologia modulável, na qual os processos microbianos e as características físico-químicas do ambiente podem ser ajustados às necessidades da espécie cultivada.

No segundo artigo foram avaliados os efeitos de diferentes concentrações de sólidos suspensos no sistema de bioflocos para alevinos de truta arco-íris. O experimento demonstrou que níveis moderados de sólidos suspensos em BFT são compatíveis com a criação da truta, enquanto o aumento excessivo da concentração de sólidos pode representar uma piora no desempenho produtivo. A manutenção de SST em aproximadamente 250 mg L⁻¹ não comprometeu o desempenho zootécnico, condição intestinal ou a resposta antioxidante, enquanto 350 mg L⁻¹ resultou em redução do peso final, ganho de peso e taxa de crescimento específico e parâmetros intestinais (altura e largura de vilosidades e contagem de células

caliciformes). Esse resultado reforça a importância da utilização de indicadores complementares ao desempenho zootécnico na avaliação da adequação de novos sistemas de cultivo. Em conjunto, esses resultados permitem propor que a concentração de sólidos suspensos totais deve ser considerada uma variável de controle central na adaptação do BFT para espécies sensíveis, e não apenas uma consequência da formação dos bioflocos.

Portanto, a principal contribuição desta tese não está apenas em demonstrar que a truta arco-íris pode ser criada em sistema de bioflocos, mas em ampliar a compreensão sobre como essa tecnologia pode ser adaptada a uma espécie fisiologicamente exigente. Os resultados indicam que a sensibilidade da espécie não constitui, por si só, uma barreira à utilização do BFT, o desafio está em estabelecer uma configuração que mantenha o equilíbrio entre os benefícios da atividade microbiana e os limites fisiológicos do peixe. Nesse sentido, o BFT deve ser compreendido menos como um protocolo fixo de produção e mais como uma estratégia ambiental e microbiana, cuja configuração pode ser ajustada conforme espécie, estágio de desenvolvimento e objetivo produtivo.

Por fim, os resultados desta tese constituem uma evidência inicial da utilização do BFT para a truta arco-íris, mas não permitem estabelecer um protocolo universal para a espécie. Estudos futuros deverão ser aplicados para avaliar períodos produtivos mais longos, diferentes fases ontogenéticas, composição e funcionalidade do biofoco, consumo e aproveitamento nutricional dos flocos, dinâmica detalhada da microbiota, respostas imunológicas e parâmetros econômicos e ambientais do sistema.

6.1 DECLARAÇÃO DE USO DE INTELIGÊNCIA ARTIFICIAL GENERATIVA

Durante a elaboração desta tese, ferramentas de inteligência artificial generativa, ChatGPT (OpenAI) e Microsoft Copilot, foram utilizadas como recursos auxiliares na revisão da redação em inglês, correção gramatical e aprimoramento de algumas ilustrações e esquemas apresentados no trabalho. O conteúdo científico, a interpretação dos resultados, os conceitos apresentados e as informações utilizadas na tese foram definidos, avaliados e verificados pela autora com base na literatura científica pertinente. Todo o conteúdo produzido com auxílio dessas ferramentas foi revisado pela autora, que assume total responsabilidade pelo conteúdo, precisão e apresentação final da tese.

6.2 COMITÊ DE ÉTICA

O presente estudo foi aprovado pelo Comitê de Ética no Uso de Animais (CEUA), sob o protocolo nº 9440250324.

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