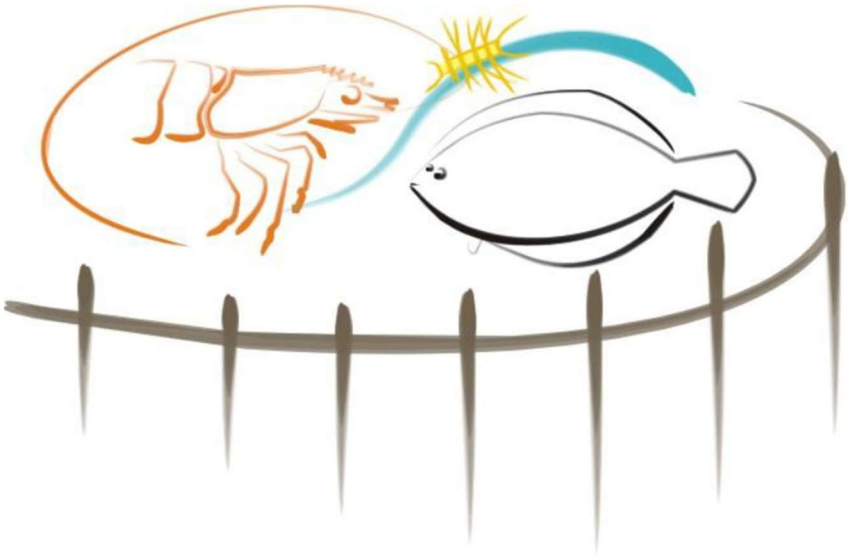
UNIVERSIDADE FEDERAL DO RIO GRANDE – FURG
INSTITUTO DE OCEANOGRAFIA
PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA



DETERMINAÇÃO DA CONCENTRAÇÃO DE SÓLIDOS SUSPENSOS TOTAIS E DENSIDADE DE ESTOCAGEM PARA O CULTIVO DE JUVENIS DE PACU *Piaractus mesopotamicus* EM SISTEMA BFT

LUCAS PELLEGRIN

Rio Grande, RS

2021

UNIVERSIDADE FEDERAL DO RIO GRANDE – FURG
INSTITUTO DE OCEANOGRAFIA
PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA

DETERMINAÇÃO DA CONCENTRAÇÃO DE SÓLIDOS SUSPENSOS TOTAIS E DENSIDADE DE ESTOCAGEM PARA O CULTIVO DE JUVENIS DE PACU *Piaractus mesopotamicus* EM SISTEMA BFT

LUCAS PELLEGRIN

Orientador: Prof. Dr. Luciano Garcia

Co-orientador: Wilson Wasieleky Jr.

Tese apresentada como parte dos requisitos para obtenção do grau de Doutor em Aquicultura pelo Programa de Pós-Graduação em Aquicultura da Universidade Federal do Rio Grande.

Rio Grande, RS

Agosto de 2021

ATA DE APROVAÇÃO

Ficha Catalográfica

P386d Pellegrin, Lucas.
Determinação da concentração de sólidos suspensos totais e densidade de estocagem para o cultivo de juvenis de Pacu *Piaractus mesopotamicus* em sistema BFT / Lucas Pellegrin. – 2021.
144 f.

Tese (doutorado) – Universidade Federal do Rio Grande – FURG, Programa de Pós-Graduação em Aquicultura, Rio Grande/RS, 2021.
Orientador: Dr. Luciano Garcia.
Coorientador: Dr. Wilson Wasieleky Jr.

1. Biofloco 2. Espécies Nativas 3. Altas Densidades 4. SST 5. Parâmetros Hematológicos 6. Desempenho Zootécnico I. Garcia, Luciano II. Wasieleky Jr., Wilson III. Título.

CDU 639.3.05

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

Sumário

Sumário

Dedicatória

Dedico este trabalho para minha esposa Dr. Lilian Fiori Nitz

A meu Pai Luiz e minha mãe Odila!

Agradecimentos

Primeiramente agradeço a Deus e a Nossa Senhora Aparecida, pela luz perpetua que me guiou por todos os caminhos e escolhas que fiz na vida, sempre me protegendo e protegendo a quem eu amo.

A minha esposa Lilian por estar ao meu lado e sempre me mostrar que eu podia mais, que eu era melhor do que eu sentia ser, por acreditar em mim e sempre me motivar. Agradeço por ter aparecido em minha vida como uma grande amiga e assim ser até hoje. Por ser aquela pessoa que mesmo trabalhando o dia inteiro comigo a noite ainda tínhamos milhões de coisas para conversar. Agradeço por ter me motivado a entrar no mestrado e agora depois de tanto esforço e dedicação concluído doutorado. Obrigado por estar sempre ao meu lado, te amo.

Agradeço a minha família por todo incentivo e ajuda durante esse longo período longe de casa, longe da família e longe dos amigos, nada disso parecia tão ruim quando lembrava que sempre tinha para onde voltar.

Agradeço aos amigos que fiz durante toda essa etapa acadêmica, Lucas Maltez que lá no início de tudo me ajudou com minha baixíssima formação em inglês me ajudando a passar na seleção de mestrado. Aos amigos Juan, Juninho, Mari por me ter recebido com grande amizade tornando a adaptação a essa nova cidade muito mais fácil. Aos meus colegas do LAC, Daniel, Gabriel, Luana, Thaline, Lili & Patrick (este último não era membro do LAC, mas se infiltrou por causa da Lili kkkkk) pela grande amizade e companheirismo durante este tempo de mestrado e doutorado. Enfim, um muito obrigado a todos que de um jeito ou de outro me proporcionaram dias incríveis durante esta jornada.

Ao orientador e grande amigo Luciano Garcia, que lá no início, antes mesmo de ser meu orientador, me deu uma baita mão me oferecendo um emprego como serviços gerais no LAC, período este que além de trabalhar serviu para mim aprender com os seus alunos e assim conseguir entrar no mestrado, sem contar com o salário que ajudava em muito em manter as contas com alimentação, moradia e transporte em dia.

Ao meu co-orientador professor Wilson (Mano) pelo auxilio e disponibilidade sempre que precisei, principalmente quando o assunto era BFT, o que foi um desafio para mim e com ajuda dele cosegui desenvolver meus trabalhos nesta área.

A FURG pela oportunidade de desfrutar das instalações dessa excelente Universidade. Ao programa de Pós-Graduação e a todo corpo docente, me ensinaram muito. A CAPES e ao CNPq pelo apoio financeiro.

1

Resumo geral

O pacu *P.mesopotamicus* é uma espécie de peixe nativa da Bacia da Prata, e apresenta ampla distribuição geográfica, além de tolerar ampla faixa térmica, que possibilita seu cultivo em praticamente todas as regiões do Brasil. Esta espécie apresenta uma boa aceitação pelo mercado consumidor devido a qualidade da sua carne, que é comercializada em diferentes formas como, costelinhas, postas, além do peixe inteiro esviscerado. Devido a sua rusticidade apresenta características desejáveis para o cultivo intensivo, como tolerância a baixos níveis de oxigênio, além de ser resistente aos principais compostos nitrogenados gerados no sistema. Dentre os cultivos intensivos existentes o sistema de bioflocos - BFT tem apresentado inúmeras vantagens como, aumento da densidade de estocagem sem comprometimento da qualidade da água, melhora do sistema imunológico dos animais, além de servir como fonte suplementar de alimento, melhorando as taxas de conversão alimentar e reduzindo custos com alimentação. Os bioflocos presentes neste sistema apresentam uma ótima composição nutricional, entretanto, seu acumulo leva ao aumento das concentrações de sólidos suspensos totais (SST) e seu excesso pode se tornar um fator limitante no cultivo, devendo ser mantido em concentrações toleradas pela espécie cultivada. Dessa maneira, testar a adaptação do pacu ao sistema BFT e determinar a sua tolerância aos SST se torna uma forma de garantir o sucesso desta espécie neste meio. Além disso, determinar a melhor densidade de estocagem é uma ferramenta que garante a melhor eficiência nos sistemas de cultivo. Para estas determinações foram realizados 4 experimentos (EXP): EXP 1 - foi realizada uma $CL_{50\text{-}96h}$ a diferentes níveis de SST na água (0, 1500, 3000, 4000, 5000, 6000 e 7000 mg L^{-1}) para determinar o "*safe level*" para a espécie, sendo que a cada 24h foi contabilizada a mortalidade dos animais. EXP 2 - foram testadas 5 concentrações subletais de SST (0, 250. 500, 750 e 1000 mg L^{-1}), avaliandoos parâmetros hematológicos

(glicose, pH, hematócrito, hemoglobina, eritrócitos, e índices hematimetricos) do pacu. O experimento teve duração de 5 dias e no início (dia 1) e fim (dia 5) deste período foram realizadas as coletas de sangue (9 peixes/tratamento). EXP 3 - foram testadas 5 concentrações de SST (0, 250. 500, 750 e 1000 mg L^{-1}) sobre o desempenho zootécnico e sobre os parâmetros hematológicos do pacu. O experimento teve duração de 30 dias e foram realizadas as biometrias no início (dia 0) e fim (dia 30) do experimento, enquanto que a coleta de sangue foi realizada somente no final do período experimental. EXP 4 – neste experimento os juvenis de pacu foram submetidos a 5 diferentes densidades de estocagem (150, 300, 450, 600 e 750 peixes m^{-3}) e foram avaliados os dados de desempenho zootécnico assim como as alterações sobre os parâmetros hematológicos da espécie. As biometrias foram realizadas no início (dia 0) e fim (dia 45) do período experimental, já a coleta de sangue foi realizada ao final do experimento (dia 45). Os resultados demonstraram que o pacu tolera até 5000 mg L^{-1} de SST por 96 horas sem apresentar mortalidades, além disso, concentrações de até 500 mg L^{-1} causam pequenas alterações nos parâmetros hematológicos do pacu e podem ser mantidas por um período curto de tempo de até 5 dias. Entretanto, concentrações de até 250 mg L^{-1} foram as que resultaram nos melhores índices de desempenho zootécnico. Em relação a densidade de estocagem para juvenis de pacu em BFT, as densidades de 150, 300 e 450 peixes m^{-3} não afetaram o desempenho da espécie, contudo, a densidade de 450 peixes m^{-3} é a mais indicada pois permitiu uma maior biomassa final.

Palavras chave: Biofloco, espécies nativas, altas densidades, SST, parâmetros hematológicos, desempenho zootécnico

Abstract

Pacu *P.mesopotamicus* is a native species of the La Plata Basin, and has a wide geographic distribution, in addition to tolerating a wide thermal range, which allows its culture in practically all regions of Brazil. This species has a high acceptance by the consumer market due to the quality of its meat, which is sold in different ways such as ribs, pieces, in addition to gutted whole fish. Due to its rusticity, it presents desirable characteristics for intensive culture, such as tolerance to low oxygen levels, in addition to being resistant to the main nitrogen compounds generated in the system. Among the intensive culture, the biofloc system (BFT) has shown positive results, such as increasing stocking density without compromising water quality, improving the immune system, in addition to serving as an food extra source, increasing feed conversion rates and reducing feed cost. The bioflocs present in this system have an excellent nutritional composition, however, their accumulation leads to an increase in total suspended solids (TSS) and their excess can become a limiting culture factor, and must be maintained at tolerated levels by the culture species. Thus, pacu adaptation to the BFT system and determining its tolerance to SST becomes a way to ensure the success of this species in this environment. Furthermore, determining the best stocking density is a tool that ensures the better efficiency in culture systems. For these determinations, 4 experiments were performed (EXP): EXP 1 - an $LC_{50\text{-}96h}$ was performed at different TSS levels in water (0; 1,500; 3,000; 4,000; 5,000; 6,000 and 7,000 mg L^{-1}) to determine the safety level for the species, and every 24 hours the animal mortality was recorded. EXP 2 - 5 sublethal SST concentrations (0, 250, 500, 750 and 1,000 mg L^{-1}) were tested and their influence on the hematological parameters (glucose, pH, hematocrit, hemoglobin, erythrocytes, and hematimetric indices) of pacu were tested. The experiment lasted 5 days and at the beginning (day 1) and end (day 5) of this period were performed the blood collections (9

fish treatment^{-1}). EXP 3 - 5 SST concentrations (0, 250, 500, 750 and 1,000 mg L^{-1}) were tested on the zootechnical performance and hematological parameters of pacu. The experiment lasted 30 days and were performed as biometrics at the beginning (day 0) and the end (day 30) of the experiment, and blood collection was performed only at the end of the experimental period. EXP 4 - in this experiment pacu juveniles were submitted to 5 different stocking densities (150, 300, 450, 600 and 750 fish m^{-3}) and data on zootechnical performance as well as changes on the hematological parameters of the species were obtained. Biometric measurements were carried at the beginning (day 0) and the end (day 45) of the experimental period, blood collection was performed at the end of the experiment (day 45). The results showed that pacu tolerate up to 5,000 mg L^{-1} of TSS for 96 hours without presenting mortalities, in addition, implying up to 500 mg L^{-1} cause small changes in the hematological parameters of pacu and can be maintained for a short period up to 5 days. However, concentrations up to 250 mg L^{-1} resulted in the better zootechnical performance indices. In relation to stocking density for pacu juveniles in BFT, the densities of 150, 300 and 450 fish m^{-3} did not affect the performance of the species, however, the density of 450 fish m^{-3} is the most indicated as it allowed a better biomass.

Keys Words: Biofloc, native species, high densities, TSS, hematological parameters, zootechnical performance

1. Introdução geral

1.1. Aquicultura

O crescente aumento populacional e a demanda por alimentos têm gerado uma série de esforços a fim de garantir a seguridade alimentar para as próximas gerações. Segundo a FAO (2016), cerca de 9,7 bilhões de pessoas habitarão o planeta terra até o ano de 2050, o que pode levar a uma considerável escassez de alimentos e água potável (FAO, 2020a), a qual já é uma realidade de muitos povos e nações, e tem feito com que o correto uso deste recurso sofra cada vez mais pressão.

Á água é um dos recursos naturais mais importantes para o desenvolvimento da aquicultura, e muitas vezes são utilizados altos volumes durante o cultivo. Além disso, é necessário que a água apresente boa qualidade, para que assim os organismos cultivados manifestem todo seu potencial produtivo. Alterações na qualidade da água podem gerar uma certa negatividade sobre o desenvolvimento da aquicultura, pois ao final de um ciclo de cultivo muitas vezes a água com resíduos é liberada em mananciais causando poluição. Porém, esta atividade vem sendo indicada como um dos setores de produção de proteína animal com capacidade de crescimento e potencial para suprir a crescente demanda de alimentos no mundo. Dessa maneira, ajustes vem sendo realizados a fim de garantir o desenvolvimento desta atividade em concomitância com a conservação dos recursos hídricos e do meio ambiente.

O consumo de carne de pescado vem crescendo cada vez mais, e entre 1961 a 2017 apresentou crescimento superior (3,1% ano^{-1}) ao populacional (1,6% ano^{-1}). No ano de 2018 a produção mundial de peixes atingiu cerca de 179 milhões de toneladas, sendo 156 milhões de toneladas destinadas ao consumo humano direto (FAO, 2020b). Assim a aquicultura de destaca como um dos setores de produção de proteína animal com maiores

índices anuais de crescimento, superando outras fontes como a carne bovina e suína (2,1% ano^{-1}) (FAO, 2020b).

Em relação ao Brasil, a produção aquícola tem apresentado um cenário de constante desenvolvimento, o que é estimulado devido ao imenso potencial hídrico e a crescente demanda por carne e subprodutos do pescado (PEIXE BR, 2021). Dessa maneira, foi possível atingir no ano de 2020 a marca de 802.930 toneladas de peixes de cultivo, resultando em um incremento produtivo de 5,9% em relação ao ano anterior (PEIXE BR, 2021).

A produção brasileira é realizada com espécies como a tilápia-do-Nilo (*Oreochromis niloticus*), responsável por 60,6% da produção, seguida pelos peixes nativos, principalmente os conhecidos como "peixes redondos" como o tambaqui (*Colossoma macropomum*) (2° espécie mais produzida), pacu (*Piaractus mesopotamicus*), a pirapitinga (*Piaractus brachypomus*), e os híbridos tambacu (tambaqui x pacu) e tambatinga (tambaqui x pirapitinga) (PEIXE BR, 2021).

O cultivo de espécies exóticas embora tenha grande potencial, deve ser realizado de forma consciente, organizada e segura a fim de evitar problemas como introdução de patógenos e parasitas, destruição do habitat natural, hibridização e disputas por território (Ross et al., 2008). Dessa forma, o cultivo de espécies nativas tem garantindo a muitos produtores uma fonte de renda, além de apresentar um viés ambiental que cada vez mais deve ser explorado por um mercado consumidor mais consciente e preocupado com o a preservação dos recursos e ambientes naturais.

1.2. Espécie alvo do estudo

Pacu (Piaractus mesopotamicus)

O Brasil apresenta inúmeras espécies nativas com potencial para a aquicultura de água doce e dentre elas o pacu é uma das espécies que se destaca. Representante do grupo conhecido como peixes redondos, apresenta uma vantagem em relação aos demais membros do grupo no que se refere a tolerância a baixas temperaturas, permitindo que seu cultivo seja realizado em todas as regiões do Brasil, desde o Norte até o Sul do país (Valenti et al., 2021).

Popularmente o pacu é conhecido como: pacu, pacu guaçu, pacu do pantanal, pacu caranha ou caranha. No cultivo chegam a atingir cerca de 1,0 a 1,2 Kg em um ano, apresentando coloração cinza-escuro no dorso e amarelo–dourada no ventre, podendo variar conforme o habitat, coloração da água e a época do ano (Urbinati et al., 2013).

Classificação Científica

Reino: Animalia
Filo: Chordata
Superclasse: Actinopterygii
Classe: Actinopteri
Superordem: Characiphysae
Ordem: Characiformes
Família: Characidae
Subfamília: Myleinae
Gênero: *Piaractus*
Espécie: *Piaractus mesopotamicus*

O pacu é uma espécie nativa da Bacia do Prata a qual é composta pelos rios Paraná, Paraguai e Uruguai (Godoy, 1975) e se distribui por vários países da América do Sul como a Argentina, Bolívia, Brasil, Paraguai e Uruguai.

Na Argentina é considerada uma das espécies cultivadas mais importantes (Valladão et al., 2016). No Brasil representa uma boa parcela da produção, constituindo

o grupo de espécies conhecidas como peixes redondos devido ao formato de disco do seu corpo (PEIXE BR, 2021).

No ano de 2019 a produção brasileira desta espécie foi de 11.542 toneladas (IBGE, 2020), sendo que no ano de 2020 o estado da Rondônia liderou a produção (PEIXE BR, 2021). Em termos de valores (US$) em exportações no ano de 2020 houve um aumento de 410,2% em relação ao ano anterior, representando 0,16% do que é exportado pela aquicultura do Brasil (PEIXE BR, 2021).

O pacu é uma espécie que habita preferencialmente águas com temperaturas acima de 22°C (Urbinati et al., 2010), entretanto, estudos recentes têm demonstrado que esta espécie tolera uma ampla variação térmica e sua produção pode ser encontrada desde clima tropical a subtropical (Peel, Finlayson & McMahon, 2007; Neto, Galvani & Vieira, 2015, Pinto et al., 2019; Pinto et al., 2020).

O pacu tem demonstrado tolerância a vários parâmetros de qualidade de água como temperatura (Saint-Paul, 2017; Pinto et al., 2019; Nitz et al., 2020a, b), ampla faixa de pH (Copatti et al., 2019; Pellegrin et al 2020), baixos níveis de oxigênio dissolvido (Bastos et al., 2007; Nitz et al., 2020a) e altos níveis de amônia (Nitz et al., 2019), o que confirma que esta é uma espécie rústica e pode ser bem adaptada ao cultivo intensivo. Além disso, apresenta boa aceitação no mercado consumidor devido a qualidade da sua carne (Jomori et al., 2005).

Em ambiente natural se alimenta de uma grande variedade de alimentos principalmente plantas, caules, sementes e frutos, mas oportunamente se alimenta de insetos, crustáceos e pequenos peixes (Urbinati et al., 2013). Devido a sua dentição adaptada morfologicamente (com dentes molariformes e incisivos, figura 1) está espécie é especializada em triturar alimentos duros como frutos e sementes.

Seu hábito alimentar onívoro com forte tendência a herbívoro permite a substituição parcial e total da farinha de peixe, principal ingrediente oneroso das rações, por fontes vegetais como a soja (Fernandes et al., 2001; Valadão et al., 2016).

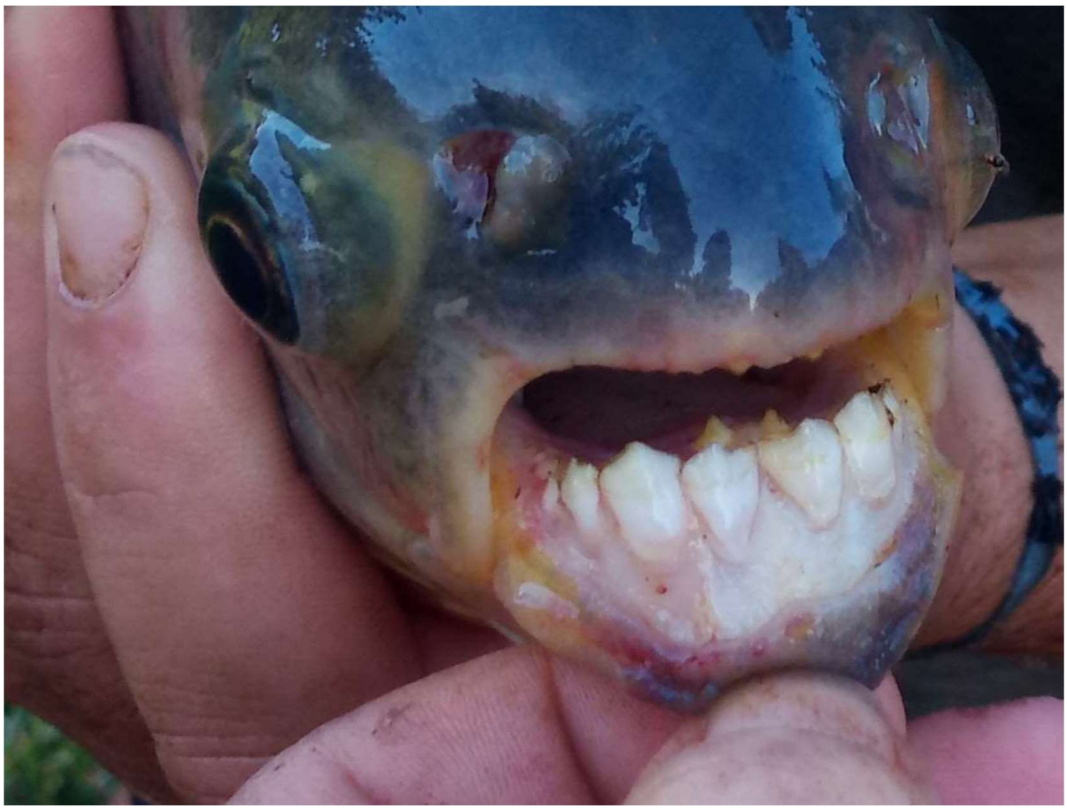


Figura 1: Detalhe dos dentes do pacu *Piaractus mesopotamicus* (Crédito/fonte: Lilian Fiori Nitz).

Na natureza apresenta comportamento migratório e sem alimentação contínua devido a períodos de escassez hídrica. É uma espécie reofílica que apresenta desova total e fecundação externa, atingindo a maturação das gônadas das fêmeas com cerca de 3 anos e 34 cm de comprimento (Urbinati et al., 2013).

Seu consumo é realizado principalmente nos estados da região Centro-Oeste do Brasil, porém, devido à popularização da qualidade da sua carne, seu cultivo vem sendo estimulado em todo o país (Kubitza, 2004; Valenti et al., 2021). Sua produção é realizada principalmente em sistemas extensivos em tanques escavados, entretanto, é possível encontrar o cultivo desta espécie em tanques redes (Silva et al., 2012; Valenti et al., 2021).

O filé apresenta características organolépticas atrativas ao mercado consumidor como teor de cerca de 8% de gordura, considerada uma espécie “meio gordo” (Ackman, 1989), que é apreciada pelos consumidores. Tipicamente seu consumo ocorre através de cortes como a costelinha, a qual apresenta inclusive mercado internacional, postas (ou bandas), petiscos ou inteiro (Valladão, 2016). Outra forma típica de preparar esta espécie é envolver em folhas de bananeiras e assar, o que confere a característica de defumado a sua carne (Figura 2).

Figura 2. Imagem de um pacu (*Piaractus mesopotamicus*) cultivado no noroeste do estado do Rio Grande do Sul, preparado e assado em brasa envolto em folhas de bananeira. (Créditos/fonte: Lucas Pellegrin).

Faria (2003), ao avaliar o rendimento de file do pacu encontrou rendimentos superiores aos encontrados para a tilápia, sendo os valores de 51,60% para filé com pele e 46,73% sem pele, enquanto que para tilápia foram 39,21% e 36,44%, respectivamente.

Para o cultivo em sistemas intensivos está espécie apresenta características positivas como tolerar baixos níveis de oxigênio dissolvido por longos períodos de tempo (Nitz et al., 2020a,b), tolerar o acúmulo de compostos nitrogenados (Nitz et al., 2019; Neves et al comunicação pessoal) a níveis mais elevados que a maioria das espécies cultivadas, garantindo maior margem de segurança durante o cultivo. Além disso, devido ao seu hábito alimentar onívoro alimenta-se de rações com níveis baixos de proteína bruta entre 24 e 32% (Fernandes et al., 2001; Valenti et al., 2021), o que garante rações com menor custo e possibilita uma melhor qualidade da água durante o cultivo. Por conseguinte, adotar o cultivo desta espécie em sistemas intensivos como o sistema BFT em pelo menos umas das fases de cultivo como berçários ou pré engorda poderia ser uma alternativa bastante interessante.

1.3. Sistema BFT

A tecnologia de bioflocos (BFT) é baseada na ciclagem do nitrogênio (N) por uma complexa interação entre matéria orgânica e uma grande variedade de microrganismos que aderidos formam bioflocos na coluna da água (figura 2). Estes bioflocos são compostos principalmente por bactérias, microalgas, fungos, zooplâncton, além de matéria orgânica, coloides, polímeros orgânicos entre outros (De Schryver et al. 2008), os quais podem apresentar diversas formas, tamanhos irregulares, alta porosidade, além de permeabilidade aos fluídos (Chu & Lee 2004; Crab et al. 2012).

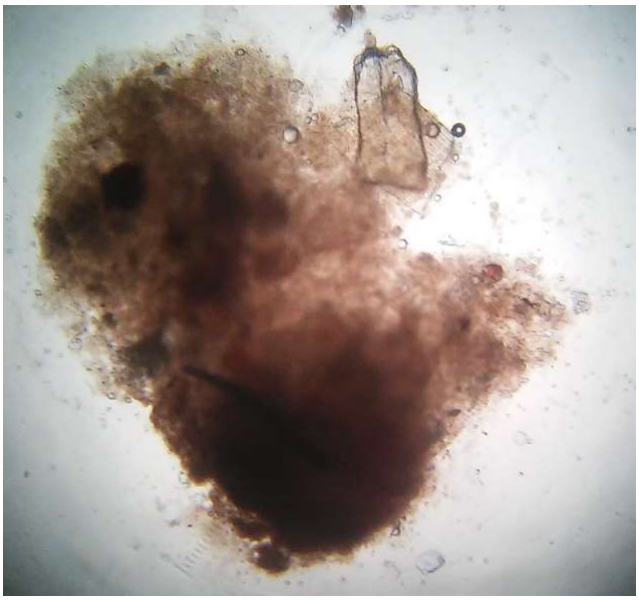


Figura 2. Imagem microscópica de um biofloco do cultivo de pacu (*Piaracatus mesopotamicus*) em sistema BFT (LAC/IO – FURG – Créditos/Fonte: Lucas Pellegrin).

Através da ciclagem do nitrogênio (N) inorgânico que ocorre neste sistema a necessidade de trocas de águas durante o cultivo se tornam mínimas (Azim & Litle, 2008; Ray et al., 2012; Krummenauer et al., 2014). Além disso, é possível a reutilização de águas entre ciclos de cultivo (Avnimelech, 2006; Little et al., 2008; Krummenauer et al., 2014), levando a um melhor aproveitamento da água por vários ciclos de cultivo, o que tem dado a este meio de cultivo o status de sistema ambientalmente amigável (Emereciano et al., 2012; Widanarni et al., 2012).

Durante o cultivo em viveiros convencionais o uso de água por Kg de peixe produzido chega a cerca de 20.000 L Kg^{-1}, já no sistema BFT são usados aproximadamente 200 L Kg^{-1}, um valor bem abaixo do supracitado, podendo ser reduzido ainda mais, através do reuso de água entre ciclos de cultivo (Martínez-Córdova et al., 2016).

Para iniciar um cultivo em sistema BFT a estimulação da comunidade microbiana previamente ao povoamento é uma ferramenta capaz de reduzir os picos de compostos nitrogenados, garantindo uma redução do tempo necessário para atingir as melhores

condições de qualidade de água no cultivo. Na fertilização inicial são utilizadas diversas fontes de nitrogênio como rações comerciais, farelos diversos, entre outros, desde que auxiliem no aumento da relação carbono/nitrogênio (C: N) da água (Jory et al., 2001). O uso de uma parcela da água de um cultivo em sistema BFT anterior denominada como inóculo, também pode auxiliar na aceleração da maturação do meio devido a inserção das bactérias quimioautotróficas ou bactérias autotróficas nitrificantes (Bactérias Amônia Oxidantes e Bactérias Nitrito Oxidantes), garantindo melhores condições de qualidade de água no início do cultivo (Krummenauer et al., 2014; Ferreira et al., 2016).

Para que a ciclagem do nitrogênio ocorra, a utilização de fontes de carbono orgânico (melaço de cana, açúcares, dextrose, farelo de trigo) e inorgânico ($NaHCO_3$, $CaCO_3$) se fazem necessários para manter uma alta relação C: N na água, e resultar na formação de uma comunidade microbiana capaz de metabolizar os principais compostos nitrogenados (Avnimelech e Kochba, 2009; Krummenauer et al., 2014, 2011; Suita et al., 2015). Por meio desta ação é possível anular quase que 100% as trocas de água durante o cultivo (Ray et al. 2011; Vinatea et al. 2010), além de possibilitar o aumento da densidade de estocagem, o que leva ao aumento da produção e maiores lucros ao produtor.

Segundo Avnimelech (1999), manter uma relação C: N de 15 a 25:1 seria o mais indicado para o sistema BFT, pois garante as melhores condições para o crescimento abundante das bactérias heterotróficas (Asaduzzaman et al., 2010) e permite que os compostos nitrogenados sejam convertidos em proteína microbiana. Para cada 1 mg de nitrogênio amoniacal assimilado em biomassa microbiana cerca de 5,7 g de carbono orgânico ou 20 g de carboidratos são gastos (Avnimelech, 1999; Ebeling et al., 2006).

Esta manipulação da relação C: N permite que ocorra a transição de um sistema autotrófico para um sistema predominantemente heterotrófico, embora tenha sido

relatado a existência destes dois meios durante o cultivo. Dessa forma, tem se observado a formação de nitrito em sistemas fechados com alta relação C: N, demonstrando a existência de mais de uma via de remoção de nitrogênio inorgânico no sistema BFT (Ebeling et al., 2006).

Além do uso de fontes orgânicas e inorgânicas de carbono, o uso de um eficiente sistema de aeração se faz necessário para manutenção de duas principais condições: 1) sustentação de níveis de oxigênio dissolvido que supram o consumo dos organismos cultivados e dos microrganismos dos bioflocos; 2) manter em suspensão os sólidos, garantindo a homogeneidade do sistema e permitindo o consumo pelos organismos cultivados, se tornando desta forma fonte extra de nutrientes (De Schrver et al. 2008; Morais et al., 2020; Minaz & Kubilay, 2021).

Os bioflocos produzidos no sistema BFT têm apresentado diversas características nutricionais interessantes que cada vez mais são exploradas (Crab et al., 2010). De maneira geral, seu consumo ocorre *in situ*, onde os bioflocos estão disponíveis para os organismos cultivados, mas também tem se testado este ingrediente na substituição de fontes convencionais de proteína na formulação de rações (Jung et al., 2020).

Outra vantagem que o sistema BFT apresenta é que através do aumento da diversidade de microrganismos benéficos no meio, ocorre a redução da ação daqueles organismos patogênicos por competição. Além disso, seu consumo estimula o sistema imunológico dos organismos cultivados (Cohen et al., 2005), apresentando efeito similar ao encontrado com o uso de probióticos (Ekasari et al., 2014).

Por estas e outras características, este sistema vem sendo aplicado de forma eficiente, principalmente no cultivo de camarões (Wasielesky et al., 2006; Reis et al., 2019), mas também tem se expandido para o cultivo de peixes (Avnimelech 2006; Da

Rocha et al., 2012; Abad et al., 2014; Poli et al., 2015; Gallardo-Collí et al., 2019a, b) e até mesmo de forma integrada entre as duas espécies (Poli et al., 2018).

No Brasil, além da tilápia, principal espécie testada em cultivos BFT, às espécies nativas como o tambaqui (*Colossoma macropomum*), a piracanjuba (*Brycon orbignyanus*) o pirarucu (*Arapaima gigas*) entre outras, também estão recebendo uma atenção especial das pesquisas em sistema BFT (Dantas, 2018; Sgnaulin et al., 2018; Santos, 2018). Além destas, inúmeras outras espécies também apresentam potencial para serem utilizadas neste sistema, como é o caso do pacu (*Piaractus mesopotamicus*).

1.4. Sólidos suspensos totais - SST

No sistema BFT, com as baixas ou nulas trocas de água, em paralelo ao aumento da densidade de estocagem e das taxas de arraçoamento (Krummenauer et al., 2011), acaba ocorrendo o acúmulo de matéria orgânica proveniente de restos de rações e fezes, o que resulta no aumento das concentrações de sólidos suspensos totais (SST) (Gaona et al., 2016).

O aumento desta quantidade de partículas em suspensão na forma de agregados microbianos é o que dá a coloração marrom café a água, sendo uma das principais características visuais do sistema BFT (Emereciano et al., 2012).

Embora os SST na forma de bioflocos sejam os responsáveis pela manutenção da qualidade da água neste sistema, mesmo quando usada altas densidades de estocagem de peixes ou camarões, seu acúmulo pode causar inúmeros problemas como: dificuldade no estabelecimento da comunidade microbiana (Gaona et al., 2015), aumento da demanda bioquímica de oxigênio (DBO) e, consequente deterioração da qualidade da água (Ray et al., 2010; Arantes et al., 2017). Seu excesso em paralelo com aumento da turbidez e

diminuição da luminosidade também pode inibir o desenvolvimento de algumas espécies de algas (Páez-Osuna, 2003).

Na literatura são citadas concentrações entre 400 e 600 mg L^{-1} de SST, as quais permitem manter uma carga de microrganismos eficiente para a remoção da carga de nitrogênio do sistema com um consumo moderado de oxigênio (Samocha et al., 2007; Gaona et al., 2011; Avnimelech, 2012; Schveitzer et al., 2013), garantindo assim uma boa qualidade da água. Porém, a concentração de SST a ser mantida no cultivo não deve ser definida somente por este fator, mas também pela tolerância da espécie cultivada a este parâmetro e ao aumento da turbidez, o que pode variar de uma espécie para outra (Minaz & Kubilay, 2021).

Dentro da grande diversidade de espécies cultivadas e/ou com potencial de cultivo no mundo é normal que as condições ideais de qualidade de água possam variar. Dessa maneira, a adoção de pacotes tecnológicos já existentes e que apresentam bons resultados para uma determinada espécie ser utilizado para uma outra espécie, sem que ajustes sejam realizados, pode levar a resultados insatisfatórios e desanimadores. De acordo com isso, o verdadeiro potencial da espécie a um determinado meio de cultivo, pode ser subestimado ou mascarado.

Em relação à presença de SST não poderia ser diferente, e isto se destaca no jundiá (*Rhamdia quelen*) que apresenta comportamento mais calmo quando mantidos em ambientes de baixa transparência e menor intensidade luminosa (Gomes et al., 2000), reduzindo por exemplo, o canibalismo nas primeiras fases de vida (Behr et al., 1999). Além disso, períodos de baixa intensidade luminosa são relatados como a condição ideal para o consumo de ração por esta espécie (Baldisserotto et al., 2013), o que é propiciado

constantemente pela presença de sólidos em suspensão (bioflocos) no sistema BFT (Poli et al., 2015; Battisti et al., 2020).

Por outro lado, espécies como o Salmão-Rei (*Oncorhynchus tshawytscha*) habitam locais de águas cristalinas e o aumento da turbidez pode causar estresse a estes animais e até mesmo a morte (Birtwell 1999). Dessa maneira, estudar a biologia das diferentes espécies e as preferencias naturais do organismo cultivado pode auxiliar na escolha do melhor sistema de cultivo a ser utilizado.

A presença ou ausência de SST na água de cultivo pode ser um fator determinante no cultivo de algumas espécies. Schveitzer et al. (2013) demonstraram que o camarão *Litopenaeus vannamei* principal espécie cultivada no sistema BFT por apresentar ótimos resultados produtivos, quando mantida em níveis altos de sólidos apresenta redução na sobrevivência e na biomassa final. Isso demonstra a necessidade de definir concentrações ideais desta variável para cada espécie a fim de garantir as melhores condições de cultivo.

Sabe-se que um dos principais efeitos do excesso de SST é se acumular nas brânquias, o que pode levar a sua obstrução parcial e/ou total desta estrutura, interferindo na captação de oxigênio e nas trocas gasosas (Cordone & Kelley, 1961; Schveitzer et al., 2013), desencadeando diversos distúrbios hematológicos e bioquímicos nos animais.

Além disso, animais mantidos em condições desfavoráveis, como o excesso de SST podem desencadear quadros agudos e até crônicos de estresse, interferindo no bem-estar dos animais (Ray et al., 2010). Tais efeitos do estresse podem desencadear diversas respostas como alterações nos parâmetros hematológicos, alterações comportamentais, redução do crescimento e até mesmo a morte em condições extremas. Dessa forma, determinar a melhor concentração deste parâmetro pode garantir o sucesso do cultivo de uma espécie no sistema BFT.

1.5.Parâmetros hematológicos

1.5.1. Parâmetros hematológicos e estresse em peixes

A determinação das condições de saúde por meio da avaliação hematológica vem sendo aplicada há anos em humanos, e foi adaptada para o uso na maioria das espécies de animais domesticadas, como bovinos, suínos, aves, cães e gatos (Tavares-Dias & Morais 2006; Fazio, 2019).

Na aquicultura sua utilização é mais recente, sendo o registro mais antigo datado no ano de 1943 por Field et al (1943), segundo Fazio (2019). Devido à grande variedade de espécies que existem, a fixação de valores de referência, para os parâmetros hematológicos de peixes podem variar conforme a espécie, sexo e fase de vida (Fazio, 2019).

Tavares-Dias (2015), estudando os parâmetros hematológicos de peixes, determinou os valores de referência para as principais espécies cultivadas no Brasil (nativas, exóticas ou híbridas), entre elas: pacu (*Piaractus mesopotamicus*), tambaqui (*Colossoma macropomum*), matrinxã (*Brycon amazonicus*), piracanjuba (*Brycon orbignyanus*), piavuçu (*Leporinus macrocephalus*), curimbatá (*Prochilodus lineatus*), bagre-americano (*Ictalurus punctatus*), carpa-comum (*Cyprinus carpio*), tilápia-do-nilo (*Oreochromis niloticus*) e os híbridos tambacu (*Piaractus mesopotamicus x Colossoma macropomum*) e tilápia-vermelha (*Oreochromis niloticus x Oreochromuis aureus*). Os resultados apresentados por Tavares-Dias (2015) auxiliam nos demais estudos em diferentes condições ambientais ou de cultivo.

Da mesma maneira, outros autores vêm elaborando trabalhos científicos acerca da determinação de valores referência para algumas espécies em diferentes meios de cultivo e/ou regiões como, por exemplo, para a tilápia-do-nilo (*Oreochromis niloticus*) em

tanque-rede no estado da Bahia (Azevedo et al., 2016) e para a carpa-comum (*Cyprinus carpio*) criadas em viveiros escavados na Polônia (Witeska et al., 2016) ou em sistema de recirculação na Romênia (Bocioc et al., 2015).

Uma das vantagens da avaliação hematológica é o fato de ser uma alternativa não letal de coleta, que pode ser realizada durante todo o período de cultivo, permitindo um diagnóstico confiável do estado de saúde dos animais (Satheeshkumar et al., 2012). As coletas podem ser realizadas com uso de seringas e/ou tubos micro capilares, por meio de uma diversidade de técnicas como: punção da veia caudal (figura 3), coleta por punção de um vaso situado na base das brânquias, punção intracardíaca e coleta por meio do corte completo do pedúnculo caudal com auxílio de bisturi (Ranzani-Paiva et al., 2013).

Figura 3. Coleta de Sangue de juvenis de pacu (*Piaractus mesopotamicus*) via punção da veia caudal (LAC/IO – FURG – Crédito/Fonte: Lucas Pellegrin).

Através das amostras de sangue obtidas é possível avaliar uma série de parâmetros, dentre eles o hematócrito (figura 4A), glicose (figura 4C), pH sanguíneo, hemoglobina, eritrócitos (figura 5), índices hematimétricos, entre outros. A partir dos resultados obtidos com os diferentes parâmetros analisados podemos caracterizar as

diferentes respostas dos animais as condições ambientais, sanitárias e nutricionais, o que auxilia na compreensão do estado nutricional e de higidez dos animais (Tavares-Dias, 2015; Fazio, 2019).

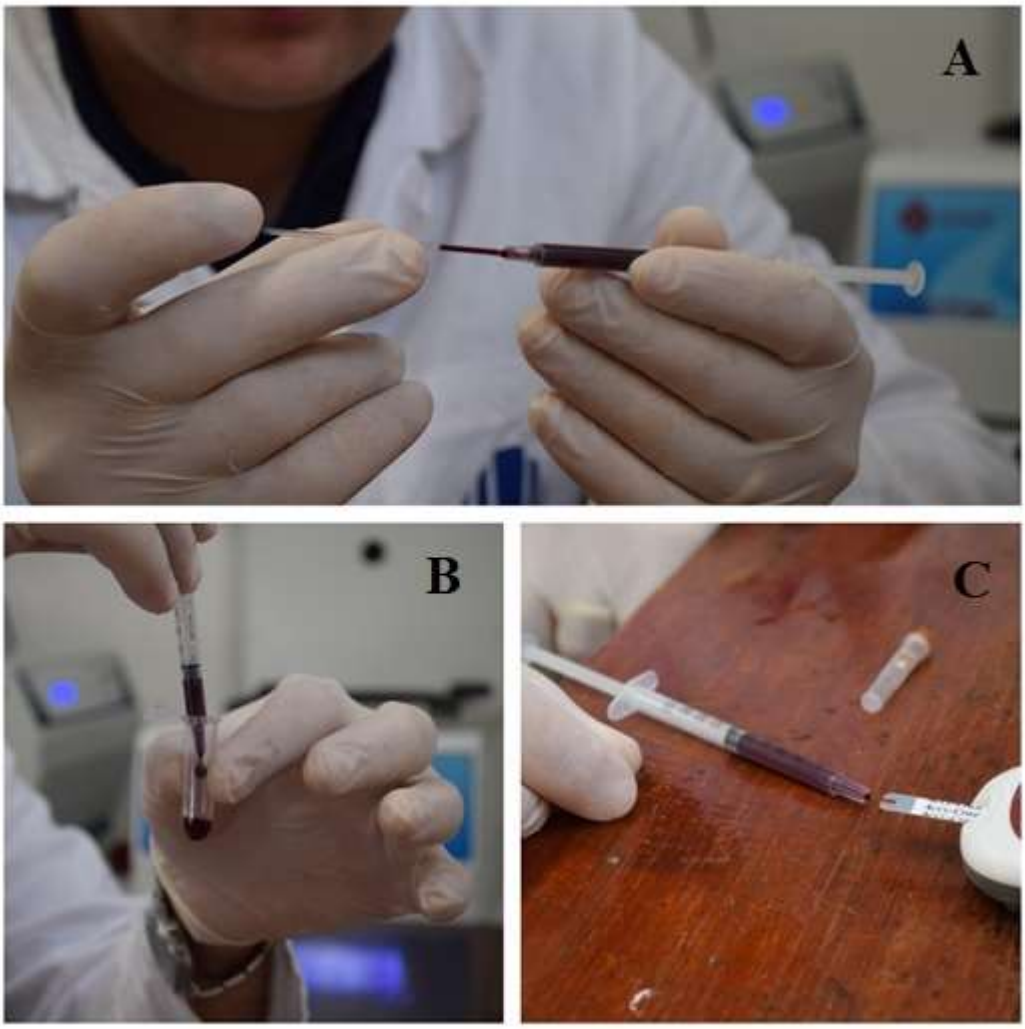


Figura 4. Amostras de sangue de juvenis de pacu (*Piaractus mesopotamicus*) coletadas e analisadas no Laboratório de Aquicultura Continental (LAC/IO-FURG – Créditos/Fonte: Lucas Pellegrin). **A**) Preenchimento de microcapilar para análise de hematócrito; **B**) Amostra de sangue acondicionada em microtubos de 2 mL; **C**) determinação da glicose sanguínea em glicosímetro.

A complexa interação entre os peixes e o meio onde habitam faz com que os parâmetros sanguíneos sejam regulados conforme as condições ambientais (Tavares-Dias & Moraes, 2004; Ranzani-Paiva et al., 2013), ocorrendo tanto de forma interespecífica ou intraespecífica (Clauss et al., 2008). Aspectos relacionados à nutrição, habitat, clima e comportamento também exercem influência sobre as variáveis hematológicas, podendo

desta forma ser avaliadas por meio da hematologia (Tavares-Dias & Moraes, 2004). Dessa maneira, a aplicação de diagnósticos de rotina sobre os parâmetros hematológicos na aquicultura, pode auxiliar na compreensão de condições causadas por doenças, manejos alimentares incorretos e situações estressantes (Tavares-Dias & Moraes, 2006; Tavares-Dias & Moraes, 2007; Pavlidis et al., 2007).

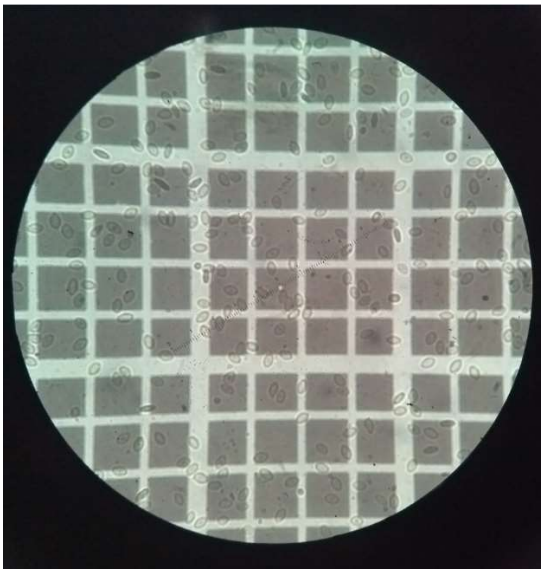


Figura 5. Eritrócitos do sangue de juvenis de pacu (*Piaractus mesopotamicus*) mantidos em sistema BFT e visualizado em câmara de Neubauer com auxílio de microscópio em 400X de aumento. (Créditos/Fonte: Lucas Pellegrin).

O estresse é caracterizado como uma condição, onde a homeostase é afetada por inúmeros motivos. Durante uma condição estressante uma cadeia de reações é desencadeada a fim de garantir ao animal condições de suportar tais condições adversas durante um certo período. Essas reações podem ser subdivididas em respostas primárias, secundárias e terciárias do estresse (Goos & Costen, 2002).

Como resposta primária frente a uma situação estressante ocorre à ativação dos eixos neuroendócrinos, o Hipotálamo -Sistema Nervoso Simpático - Células Cromafins (HSC) que liberam as catecolaminas, adrenalina e noradrenalina como produto final; e o Hipotálamo – Hipófise - Interrenal (HHI) que liberam os corticosteroides (cortisol e cortisona) (Wendelaar Bonga, 1997; Barton 2002; Oba et al., 2009; Gorissen & Flick,

2016). A partir disso, ocorrem várias alterações a nível bioquímico e fisiológico que são espelhadas por alterações nos parâmetros hematológicos, as quais são caracterizadas como respostas secundárias ao estresse.

Durante a resposta secundária vários parâmetros como a glicose, lactato, eritrócitos, trombóticos entre outros sofrem alterações (Barton & Iwama, 1991; Davis, 2006). Por exemplo, durante uma condição estressante ocorre um aumento da glicose circulante em peixes (hiperglicemia), induzida pelo cortisol. Isto acontece através de dois mecanismos, os quais são responsáveis pela sintetização da glicose a partir de percursores não-carboidratos à piruvato, e compostos relacionados entre três e quatro carbonos em glicose (gliconeogênese) (Pickering, 1981; Vijayan et al., 1990; Wendelaar Bonga, 1997). Além disso, também ocorre através da hidrólise das reservas de glicogênio no fígado (glicogenólise) (Pinkering, 1981).

O aumento da glicose circulante acaba agindo como um mecanismo fisiológico de defesa, uma vez que serve como preparação do animal a fuga ou enfrentamento a condição estressante imposta. Dessa maneira, a avaliação da glicose tem sido empregada rotineiramente na avaliação de condições estressantes em peixes (Silva et al.,2009).

Muitas vezes uma condição estressante acontece de forma aguda tendo um curto período de duração, sendo causada por exemplo, por atividades de manejo, biometrias, transportes ou ação de predadores (Inoue et al., 2004). Com a interrupção da condição estressante a homeostase é recuperada (Dhabhar, 2009). Entretanto, algumas condições estressantes tendem a se prolongar se tornando crônicas (Dhabhar, 2014). Quando isto ocorre a energia obtida através da alimentação é direcionada quase que exclusivamente para manutenção de funções vitais à sobrevivência. Dessa maneira, condições como reprodução, crescimento e resistência a doenças são afetadas (Montero et al., 1999; Oba

et al., 2009), e em casos mais graves a permanência em tal condição pode levar a morte, sendo esta considerada como resposta terciária ao estresse.

1.5.2. Parâmetros hematológicos e o sistema BFT

A utilização do sistema BFT no cultivo de organismos aquáticos tem apresentado uma série de vantagens quando comparado a sistemas de cultivo tradicionais (Emereciano et al., 2013; Valente et al., 2020). Dentre estas vantagens a estimulação do sistema imunológico é uma delas, o que tem levado os animais a apresentarem melhores respostas frente a ação de parasitos e doenças (Xu et al., 2013; Vazquez et al., 2009; Najdegerami et al., 2016; Bakhshi et al., 2017).

A melhora do sistema imune dos organismos cultivados no sistema BFT é relacionada ao consumo "*in situ"* dos bioflocos, que devido à grande diversidade de microrganismos ricos em carotenóides (antioxidantes) tem causado o estímulo do sistema imune inato dos animais. Este estímulo ao sistema inato dos animais fornece proteção contra agentes patogênicos (Ekasari et al., 2014; Vazquez et al., 2009; Bakhshi et al., 2017), sendo uma resposta similar ao encontrado com o uso de probióticos na aquicultura (Ekasari et al., 2014).

Embora o cultivo em sistema BFT apresente estas e inúmeras outras vantagens (melhora do sistema imunológico, melhora da conversão alimentar, redução da ação de patógenos), seu manejo apresenta certa complexidade e quando realizado de forma incorreta pode resultar em uma pobre qualidade da água.

Ao longo do cultivo no sistema BFT a tendência é que ocorra o acúmulo de SST, acarretando em uma demanda bioquímica de oxigênio (DBO) maior, o que pode causar a redução dos níveis de oxigênio dissolvido no sistema. Além disso, este excesso de sólidos

também pode exercer ação direta sobre o organismo cultivado e, dessa forma deve ser evitado (Gaona et al., 2016).

Um dos principais efeitos do excesso de sólidos nos animais é causar a oclusão parcial ou total das brânquias, o que já foi reportado para o camarão branco (*Litopenaeus vannamei*) (Schveitzer et al. 2013). Com isto, a capacidade respiratória fica comprometida (Schveitzer et al., 2013), e a tendência é que ocorra o aumento da frequência respiratória (hiperventilar) na tentativa de melhorar a eficiência na captação de oxigênio, mas nem sempre isto é eficiente e o que pode acontecer é um aumento involuntário na excreção de CO_2.

Com o aumento da excreção de CO_2 e a menor eficiência na captação de O_2 a tendência é que ocorra um desequilíbrio na relação gás carbônico/bicarbonato (CO_2/HCO_3) no sangue, levando a quadros de alcalose metabólica (Gilmour, 2001; Perry & Gilmour, 2006). Este aumento do pH sanguíneo pode desencadear vários outros problemas como a desnaturação de proteínas, afetar a atividades de enzimas (Aboagye & Allen, 2018), além de alterar a atividade das ATPases e causar estresse oxidativo nos rins e brânquias (Copatti et al., 2019).

Outro efeito de uma menor capacidade respiratória, que o excesso de SST, pode ocasionar são alterações nas variáveis hematológicas como o hematócrito, hemoglobina, eritrócitos e nos índices hematimétricos. Durante períodos de baixa absorção de oxigênio, a tendência é que ocorram ajustes fisiológicos para que o aporte de oxigênio seja mantido nos tecidos. Dentre estes ajustes, o aumento da quantidade de hemoglobina circulante, responsável pelo transporte de oxigênio no sangue para os tecidos é esperado (Wells, 2009; Luo et al., 2014). A quantidade de eritrócitos também tende a apresentar aumento, mas muitas vezes o que acontece é o aumento do volume do eritrócito (VCM)

aumentando a capacidade de acondicionamento de hemoglobinas no seu interior, o que aumenta a capacidade de transporte de oxigênio para os tecidos (Wells, 2009).

De maneira geral, determinar a melhor concentração de SST a ser mantido no sistema BFT, pode evitar que uma série de problemas ocorram e afetem tantos os animais como resultem em menores índices produtivos.

1.6. Densidade de estocagem

O termo densidade de estocagem refere-se ao número de animais por metro quadrado (m^2) (da Silveira et al., 2020) ou metro cubico (m^3) (AftabUddin et al., 2020; da Silveira et al., 2020) que são utilizados no início de um cultivo (Chakraborty et al., 2010), podendo ser demonstrado por meio de unidades de medida como gramas (g) e/ou quilos (Kg) (Battisti et al., 2020). Além disso, o termo densidade muitas vezes vem sendo empregado na descrição da biomassa ao longo do cultivo (Chakraborty et al., 2010).

Na aquicultura o correto dimensionamento da densidade de estocagem é uma forma de alcançar melhores índices produtivos (Montero et al., 1999) e sua manipulação busca aumentar a produção em um espaço físico menor, reduzir o tempo de produção, além de utilizar menores quantidades de água por Kg produzido (Suresh & Lin, 1992).

Para que uma densidade de estocagem possa ser considerada como ideal em um cultivo ela deve permitir o uso do maior número possível de peixes em um determinado volume de água, sem que parâmetros como o desempenho sejam afetados (Gomes & Schlindwein, 2000), melhorando o retorno do capital investido em estrutura (Hengsawat et al., 1997).

Por outro lado, densidades inadequadas podem resultar em diversos prejuízos, tanto econômicos como ambientais, além do desequilíbrio do sistema de cultivo (Cyrino et al., 2010; Bueno et al., 2011). Com o aumento da densidade um dos primeiros

parâmetros afetados é a disponibilidade de oxigênio dissolvido no sistema, devido ao maior consumo, tornando-se necessário o emprego de fontes mecânicas para suprir tal demanda (da Silveira et al., 2020).

Além da disponibilidade de oxigênio, outros parâmetros também podem ser afetados, como é o caso do acúmulo de matéria orgânica e de compostos nitrogenados oriundos de restos de ração e fezes (Azim & Little, 2008; Crab et al., 2012). Durante um cultivo intensivo apenas 20 a 25% da proteína fornecida aos animais é retida e se transforma em tecidos e músculos resultando no crescimento dos animais (Avnimelech, 2006), o restante acaba sendo excretado na forma de amônia, através das fezes ou gerada pelo processo de decomposição de restos de ração (Azim & Litle, 2008).

As atividades metabólicas dos microrganismos envolvidos na decomposição da matéria orgânica, assim como a respiração dos animais cultivados, resultam na liberação de CO_2 para o sistema. Em baixas densidades de estocagem o CO_2 não traz grandes preocupações, pois acaba volatilizando para a atmosfera. Porém, com o emprego de densidades de estocagem mais elevadas nos sistemas intensivos, o CO_2 acaba sendo excretado em grandes volumes (Aslam et al., 2019), e seu acúmulo pode levar a problemas como hiperventilação (Smith & Jones, 1982), acidose metabólica (Ultsch, 1996) e redução no crescimento dos animais (Danley et al., 2005; Mota et al., 2019).

Dessa forma, o incorreto dimensionamento do sistema em relação a biomassa presente, pode levar ao acúmulo de altos níveis de compostos nitrogenados e resultar em inúmeros fatores negativos que afetam o crescimento, a resistência a doenças, causem estresse (Ellis et al., 2002) e em casos mais extremos, altos níveis de mortalidade.

Além das variáveis químicas e biológicas da água que podem ser afetadas, os parâmetros de produção como hierarquia, territorialismo e estresse, também podem sofrer

influência da densidade, levando a alterações no consumo de alimentos e no crescimento (Lambert & Dutil, 2001; El-Sayed, 2002; Kohli et al., 2002; de Oliveira et al., 2012).

Muitas espécies têm apresentando inibição de certos comportamentos quando são utilizadas densidades elevadas, podendo ser tanto benéficas como maléficas. Por exemplo, espécies como a *Perca fluviatilis*, e a *Seriola dumerili* quando mantidas em altas densidades de estocagem apresentaram comportamento menos agressivo (Baras et al., 2003; Miki et al., 2011). Kucharczyk et al. (1998) observaram que com o aumento da densidade de estocagem no cultivo da Northern pike (*Esox lucius*) houve uma redução no canibalismo, sugerindo que o grande número de animais possa ter causado confusão visual aos animais e assim reduzido a predação. Da mesma maneira, Baras et al. (2003), encontraram reduções no canibalismo da *Perca fluviatilis* com o aumento da densidade, e sugeriram que possa ser resultado de uma interação de fatores como menor proporção de canibais e surgimento retardado do canibalismo.

Por outro lado, o comportamento da tilápia do Nilo (*Oreochromis niloticus*), se tornou mais agressivo com o aumento da densidade de estocagem (Fessehaye et al., 2006), possivelmente devido a disputas por território.

O aumento da densidade de estocagem em níveis muito elevados pode causar o aparecimento de lesões nos animais, as quais podem ser causadas por inúmeros fatores, como o aumento da agressividade, choques entre animais ou com os locais de cultivo, dentre outros (Winfree et al., 1998; North et al., 2006), podendo indicar uma condição de baixo bem-estar aos animais (FSBI, 2002).

O sistema imunológico também pode ser comprometido com o aumento da densidade de estocagem, deixando os animais mais susceptíveis a doenças e maiores mortalidades (Rotllant et al., 1997; Long et al., 2019).

Alguns trabalhos têm mostrado que a permanência de certas espécies a elevadas densidades de estocagem tem causado a elevação e permanência de níveis altos de cortisol no sangue, indicado uma situação crônica de estresse (Pottinger & Moran, 1993; FSBI, 2002; Andrade et al., 2015; Long et al., 2019), resultando em quedas no desempenho zootécnico (Irwin et al., 1999; Lambert & Dutil, 2001; Long et al., 2019). Chakraborty et al. (2010), relataram que existe uma correlação entre o aumento ou redução da densidade de estocagem e a redução do crescimento. Zhao et al, (2019) reportaram que a carpa (*Ctenopharyngodon idellus*) apresenta uma redução na qualidade do músculo quando mantida em altas densidades de estocagem, o que pode afetar diretamente o mercado consumidor, causando menores lucros ao produtor.

Pode-se observar que o aumento da densidade pode afetar vários outros parâmetros, devendo ser realizada de forma consciente e inteligente a fim de garantir os melhores resultados durante o cultivo. A realização de ajustes nos sistemas de cultivos também pode auxiliar no aumento da densidade, principalmente ao que se refere a manutenção dos parâmetros de qualidade da água.

Dentro dos inúmeros sistemas de cultivo existentes o sistema BFT se apresenta como uma ótima opção para o aumento da densidade de estocagem devido a excelente capacidade de assimilação dos compostos nitrogenados pela comunidade microbiana e/ou conversão destes compostos em compostos menos tóxicos como o nitrato (NO_3). Outra premissa que este sistema tem cumprido é o fornecimento de oxigênio em quantidades suficientes para a biomassa utilizada, sendo cada vez mais desenvolvido e testados novos equipamentos e formas eficientes de oxigenação e incorporação de oxigênio na água (Pasco et al., 2017; Lim et al., 2021).

Este sistema tem permitido o uso de densidades bem acima daquelas utilizadas nos sistemas convencionais. Por exemplo, para pós-larvas de camarão branco (*Litopenaeus vanammei*) em sistema BFT, Esparza-Leal et al. (2015), testaram densidades de até 9.000 organismos por m^3 na fase de berçário. Já para o cultivo e engorda da mesma espécie em sistema BFT foi reportado que densidades de até 450 camarões por m^2 resultam em boa sobrevivência e crescimento (Silva et al., 2013). Da mesma maneira, densidades de até 500 camarões por m^2 podem ser utilizadas, desde que bem planejadas, principalmente no que se refere ao fornecimento de oxigênio dissolvido ao sistema (Da Silveira et al., 2020).

Na utilização do sistema BFT para criação de peixes foram sugeridas densidades de até 800 peixes por m^3, as quais podem ser utilizadas tanto para a tilápia do Nilo (*Oreochromis niloticus* – linhagem GIFT) como para a tilápia vermelha (*Oreochromis sp*.) sem comprometer o desempenho zootécnico. Além disso, o uso desse sistema pode ser realizado em pelo menos uma das fazes de cultivo, como em berçários ou períodos de pré engorda, tanto de camarões como de peixes a fim de melhorar os índices produtivos de uma fazenda (Foés et al., 2011; Esparza-Leal et al., 2015).

Os berçários são unidades onde as pós larvas, alevinos ou juvenis são estocados nas primeiras semanas de vida, passando por um primeiro estágio de cultivo. Devido ao pequeno tamanho durante esta fase é possível utilizar altíssimas densidades de estocagem em ambientes mais controlados e de menor dimensão. Após apresentarem uma evolução no seu tamanho e peso, estes podem ser redistribuídos nas unidades de engorda em novas densidades, respeitando o limite de densidade da espécie.

Esta prática apresenta algumas vantagens em relação a estocagem direta, como garantir aos animais melhores condições de sanidade e resistência aos parâmetros físico-

químicos da água; maior controle sobre o manejo, por serem consideradas unidades biosseguras (Apud et al., 1983); permitem aumentar o número de ciclos de cultivo por ano em uma fazenda, devido a estocagem de animais de tamanho maior nas unidades de engorda os quais alcançam o peso comercial em menos tempo (Lourenço et al., 2009).

De maneira geral, aumentar a densidade de estocagem pode auxiliar na obtenção de melhores índices de produção em uma piscicultura. Entretanto, esta deve ser realizada de forma consciente, levando em consideração a capacidade suporte do sistema e as características da espécie cultivada.

Dessa forma, a definição da melhor densidade de estocagem assim como a concentração de sólidos suspensos totais-SST a ser utilizada durante o cultivo de juvenis de pacu, pode servir como elemento norteador no uso desta tecnologia para esta espécie.

2. Referencias

Abad, D., Rincón, D., Poleo, G. 2014. Carcass yield índex in red belly pacu *Piaractus brachypomus* cultivated in a biofloc system. Zootecnia Tropical, 32, 119–130.

Aboagye, D.L., Allen, P.J. 2018. Effects of acute and chronic hypoxia on acid–base regulation, hematology, ion, and osmoregulation of juvenile American paddlefish. Journal Comparative Physiology B, 188, 77–88. doi:10.1007/s00360-017-1104-7.

Ackman, R. 1989. Marine Biogenic Lipids, Fats and Oils. Vol. II, CRC Press Inc., Boca Raton.

AftabUddin, S., Siddique, M.A.M., Aung Sein, A., Dey, P.K., Rashed-Un-Nabi, M., Haque, M.A. 2020. First use of biofloc technology for *Penaeus monodon* culture in Bangladesh: Effects of stocking density on growth performance of shrimp, water quality and bacterial growth. Aquaculture Reports, 18, 100518. https://doi.org/10.1016/j.aqrep.2020.100518

Andrade, T., Afonso, A., Pérez-Jiménez, A., Oliva-Teles, A., Heras, V., Mancera, J.M., Serradeiro, R., Costas, B. 2015. Evaluation of different stocking densities in a Senegalese sole (*Solea senegalensis*) farm: implications for growth, humoral immune parameters and oxidative status. Aquaculture, 438, 6-11. doi: 10.1016/j.aquaculture.2014.12.034

Apud, F., Primavera, J.H., Torres, P.L. 1983. Farming of prawns and shrimps. Extension Manual, 05. SEAFDEC Aquaculture Department, Iloilo, Philippines, 67p.

Arantes, R., Schveitzer, R., Seiffert, W.Q., Lapa, K.R., Vinatea, L. 2017. Nutrient discharge, sludge quantity and characteristicis in biofloco shrimp culture using two methods of carbohydrate fertilization. Aquacultural Engineering, 76, 1-8. DOI: http://dx.doi.org/doi:10.1016/j.aquaeng.2016.11.002

Asaduzzaman, M.M., Rahman, M.M., Azim, M.E., Ashraful Islam, M., Wahab, M.A., Verdegem, M.C.J., Verreth, J.A.J. 2010. Effects of C/N ratio and substrate addition on natural food communities in freshwater prawn monoculture ponds. Aquaculture, 306, 127–136 p. https://doi.org/10.1016/j.aquaculture.2010.05.035

Aslam, S.N., Navada, S., Bye, G.R., Mota, V.C., Terjesen, B.F., Mikkelsen, O. 2019. Effect of CO_2 on elemental concentrations in recirculating aquaculture system tanks. Aquaculture, 511, 734254. https://doi.org/10.1016/j.aquaculture.2019.734254

Avnimelech, Y. 1999. Carbon: nitrogen ratio as a control element in aquaculture systems. Aquaculture, 176, 227-235. https://doi.org/10.1016/S0044-8486(99)00085-X

Avnimelech, Y. 2006. Bio-filters: The need for a new comprehensive approach. Aquacultural Engineering, 34, 172–178. https://doi.org/10.1016/j.aquaeng.2005.04.001

Avnimelech, Y., Kochba, M. 2009. Evaluation of nitrogen uptake and excretion by tilapia in bio floc tanks, using 15N tracing. Aquaculture, 287, 163–168. https://doi.org/10.1016/j.aquaculture.2008.10.009

Avnimelech, Y. 2012. Biofloc Technology A Practical Guide Book. The Word Aquaculture Society, 271.

Azevedo, T.M.P., Albinati, R.C.B., Guerra-Santos, B., Pinto, L.F.B., Lira, A.D., Medeiros, S.D.C., Ayres, M.C.C. 2016. Valores de referência dos parâmetros hematológicos de *Oreochromis niloticus* (Linaeus, 1758) cultivados em tanques-rede em paulo afonso, no estado da Bahia, Brasil. Brazilian Journal of Aquatic Science and Technology, 20(2). Doi: https://doi.org/10.14210/bjast.v20n2.4588

Azim, M.E., Little, D.C. 2008. The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture, 283, 29-35. doi:10.1016/j.aquaculture.2008.06.036

Bakhshi, F., Najdegerami, E.H., Manaffar, R., Tokmechi, A., Farah, A.K.R., Jalali, A.J. 2017. Growth performance, haematology, antioxidant status, immune response and histology of common carp (*Cyprinus carpio* L.) fed biofloc grown on different carbon sources. Aquaculture Research, 1–11. DOI: 10.1111/are.13469.

Baras, E., Kestemont, P., Melard, C. 2003. Effect of stocking density on the dynamics of cannibalism in sibling larvae of *Perca fluviatilis* under controlled conditions. Aquaculture, 219 (1–4), 241–255. https://doi.org/10.1016/S0044-8486(02)00349-6

Barton, B.A., Iwama, G.K. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Diseases, 1, 3-26. https://doi.org/10.1016/0959-8030(91)90019-G

Barton, B.A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42:517–525. https://doi.org/10.1093/icb/42.3.517

Bastos, V.C., Salles, J.B., Valente, R.H., Léon, I.R., Perales, J., Dantas, R.F., Albano, R.M., Bastos, F.F., Bastos, J.C. 2007. Cytosolic glutathione peroxidase from liver of pacu (*Piaractus mesopotamicus*), a hypoxia-tolerant fish of the Pantanal. Biochimie, 89, 1332–1342. https://doi.org/10.1016/j.biochi.2007.04.003.

Battisti, E.K., Rabaioli, A., Uczay, J., Sutili, F.J., Lazzari, R. 2020. Effect of stocking density on growth, hematological and biochemical parameters and antioxidant status of silver catfish (*Rhamdia quelen*) cultured in a biofloc system. Aquaculture, 524, 735213. https://doi.org/10.1016/j.aquaculture.2020.735213

Becerril-Cortés, D., Monroy-Dosta, M.D.C., Emerenciano, M.G.C., Castro-Mejía, G., Bermúdez, B.S.S., Correa, G.V. 2018. Effect on nutritional composition of produced bioflocs with different carbon sources (Molasses, coffee waste and rice bran) in Biofloc system. International Journal of Fisheries and Aquatic Studies, 6(2), 541-547.

Behr, E.R., Radünz-Neto, J., Tronco, A.P., Fontana, A.P. 1999. Influência de diferentes níveis de luminosidade sobre o desempenho de larvas de Jundiá (*Rhamdia quelen*) (Quoy e Gaimard, 1824) (Pisces: Pimelodidae). Acta Scientiarum, 21, 325–330. DOI: https://doi.org/10.4025/actascibiolsci.v21i0.4446

Birtwell, I.A. 1999. The Effects of Sediment on Fish and their Habitat. Canadian Stock Assessment Secretariat. Research Document 99/139.

Bocioc, E., Cristea, V., Patriche, N., Grecu I., Antache, A., Mocanu, M. 2015. Hematological Profile of the Juvenile Carp (*Cyprinus carpio*, L. 1758) Reared into a Recirculating Aquaculture System with Probiotics Supplement. Bull. UASVM Animal Science and Biotechnology, 72, 8-13. DOI: http://dx.doi.org/10.15835/buasvmcn-asb:10739

Braga, A., Magalhães, V., Hanson, T., Morris, T.C., Samocha, T.M. 2016. The effects of feeding commercial feed formulated for semi-intensive systems on *Litopenaeus vannamei* production and its profitability in a hyper-intensive biofloc-dominated system. Aquaculture Reports, 3, 172–177. https://doi.org/10.1016/j.aqrep.2016.03.002

Bueno, G.W. 2011. Impacto ambiental do fósforo em rações para tilápia do Nilo (*Oreochromis niloticus*). Universidade Estadual do Oeste do Paraná – Unioeste, Toledo, 2011. [Dissertação de Mestrado em Recursos Pesqueiros e Engenharia de Pesca] 64p.

Chakraborty, S.B., Mazumdar, D., Banerjee, S. 2010. Determination of ideal stocking density for cage culture of monosex Nile tilapia (*Oreochromis niloticus*) in India. Proceedings of the Zoological Society, 63, 53–59. https://doi.org/10.1007/s12595-010-0007-3.

Chu, C.P., Lee, D.J. 2004. Multiscale structures of biological flocs. Chemical Engineering Science, 59, 1875–1883. DOI: 10.1016/j.ces.2004.01.040

Cohen, J.M., Samocha, T.M., Fox, J.M., Gandy, R.L., Lawrence, A.L. 2005. Characterization of water quality factors during intensive raceway production of juvenile *L. vannamei* using limited discharge and biosecure management tools. Aquacultural Engineering, 32, 425-442. https://doi.org/10.1016/j.aquaeng.2004.09.005

Clauss, T.M., Dove, A.D.M., Arnold, J.E. 2008. Hematologic disorders of fish. Veterinary Clinics of North America: Exotic Animal Practice, 11, 445–462. doi: 10.1016/j.cvex.2008.03.007.

Copatti, C.E., Baldisserotto, B., Souza, C.F., Monserrat, J.M., Garcia, L. 2019. Water pH and hardness alter ATPases and oxidative stress in the gills and kidney of pacu (*Piaractus mesopotamicus*). Neotropical Ichthyology, 17(4): e190032. DOI: 10.1590/1982-0224-20190032

Cordone, A.J., Kelley, D.W. 1961. The influences of inorganic sediment on the aquatic life of streams. California Department of Fish and Game.

Correia, E.S., Wilkenfeld, J.S., Morris, T.C., Wei, L., Prangnell, D.I., Samocha, T.M. 2014. Intensive nursery production of the Pacific white shrimp *Litopenaeus vannamei* using two commercial feeds with high and low protein content in a biofloc-dominated system. Aquaculturural Engineering, 59, 48–54. https://doi.org/10.1016/j.aquaeng.2014.02.002

Crab, R., Chielens, B., Wille, M., Bossier, P., Verstraete, W. 2010. The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquaculture Research, 41, 559-567. https://doi.org/10.1111/j.1365-2109.2009.02353.x

Crab, R., Defoirdt, T., Bossier, P., Verstraete, W. 2012. Biofloc technology in aquaculture: Beneficial effects and future challenges. Aquaculture, 356-357: 351–56. https://doi.org/10.1016/j.aquaculture.2012.04.046

Cyrino, J.E.P., Bicudo, A.J.A., Sado, R.Y., Borguesi, R., Dairiki, J.K. 2010. A piscicultura e o ambiente – O uso de alimentos ambientalmente corretos em piscicultura. Revista Brasileira de Zootecnia, v. 39 (supl. Especial), p. 68-87. https://doi.org/10.1590/S1516-35982010001300009

Da Rocha, A.F., Abreu, P.C., Wasielesky, W., Tesser, M.B. 2012. Avaliação da formação de bioflocos na criação de juvenis de tainha *Mugil* cf. *hospes* sem renovação de água. Atlântica, Rio Grande, 34(1) 63-74. doi: 10.5088/atl.2012.34.1.63

Da Silveira, L.G.P., Krummenauer, D., Poersch, L.H., Rosas, V.T., Wasielesky, W. 2020. Hyperintensive stocking densities for *Litopenaeus vannamei* grow-out in biofloc technology culture system. Journal World Aquaculture Society, 1–11. DOI: 10.1111/jwas.12718

Danley, M.L., Kenney, P.B., Mazik, P.M., Kiser, R., Hankins, J.A. 2005. Effect of carbon dioxide exposure on intensively cultured rainbow trout *Oncorhynchus mykiss*: physiological responses and fillet attributes. Journal of the World Aquaculture Society, 36, 249–261. https://doi.org/10.1111/j.1749-7345.2005.tb00329.x

Dantas, N.S.M. 2018. Larvicultura do pirarucu em sistema de bioflocos. 64 f. Dissertação (Mestrado em Ciência Animal) – Universidade Federal do Amazonas, Manaus.

Davis, K.B. 2006. Management of physiological stress in finfish aquaculture. North America Journal of Aquaculture, 68, 116-121. doi: 10.1577/A05-007.1.

De Oliveira, E.G., Pinheiro, A.B., de Oliveira, V.Q., da Silva, A.R.M., de Moraes, M.G., Rocha, I.R.C.B., de Sousa, R.R., Costa, F.H.F. 2012. Effects of stocking density on the performance of juvenile pirarucu (*Arapaima gigas*) in cages. Aquaculture, 370-371, pp. 96-101. https://doi.org/10.1016/j.aquaculture.2012.09.027

De Schryver, P., Crab, R., Defoirdt, T., Boon, N., Verstraete, W. 2008. The basics of bio-flocs technology: The added value for aquaculture. Aquaculture, 277, 125–137. https://doi.org/10.1016/j.aquaculture.2008.02.019

Dhabhar, F.S. 2009. Enhancing versus suppressive effects of stress on immune function: Implications for immunoprotection and immunopathology. Neuroimmunomodulation, 16(5), 300–317. doi: 10.1159/000216188

Dhabhar, F.S. 2014. Effects of stress on immune function: the good, the bad, and the beautiful. Immunologic Research, 58 (2), 193–210. doi: 10.1007/s12026-014-8517-0

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

Ekasari, J., Azhar, M.H., Surawidjaja, E.H., Nuryati, S., De Schryver, P., Bossier, P. 2014. Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. Fish & shellfish immunology, 41, 332–339. https://doi.org/10.1016/j.fsi.2014.09.004

Ellis, T., North, B., Scott, A.P., Bromage, N.R., Porter, M., Gadd, D. 2002. The relationships between stocking density and welfare in farmed rainbow trout. Journal of Fish Biology, 61, 493–531. doi:10.1006/jfbi.2002.2057

El-Sayed, A.F.M. 2002. Effects of stocking density and feeding levels on growth and feed efficiency of Nile tilapia (*Oreochromis nilotucus* L) fry. Aquaculture research, 33, 621-626. https://doi.org/10.1046/j.1365-2109.2002.00700.x

Emerenciano, M., Ballester, E.L.C., Cavalli, R.O., Wasielesky, W. 2012. Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). Aquaculture Research, 43, 447–457. https://doi.org/10.1111/j.1365-2109.2011.02848.x

Emereciano, M., Gaxiola, G., Cuzon, G. 2013. Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry. Em Matovic, M.D. 2013. Biomass Now – Cultivation and Utilization. Janeza Trdine 9, 51000 Rijeka, Croatia.

Esparza-Leal, H.M., Cardozo, A.P., Wasielesky Jr., W. 2015. Performance of *Litopenaeus vannamei* postlarvae reared in indoor nursery tanks at high stocking density in clear-water versus biofloc system. Aquacultural Engineering, 68, 28-34. http://dx.doi.org/10.1016/j.aquaeng.2015.07.004

FAO. 2016. The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200 pp.

FAO. 2020a. The State of Food and Agriculture 2020. Overcoming water challenges in agriculture. Rome. https://doi.org/10.4060/cb1447en

FAO. 2020b. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. https://doi.org/10.4060/ca9229en

Faria, R.H.S., Souza, M.L.R., Wagner, P.M., Povh, J.A., Ribeiro, R.P. 2003. Rendimento do processamento da tilápia do Nilo (*Oreochroms niloticus Linnaeus,* 1757) e do pacu (*Piaractus mesopotamicus Holmberg*, 1887). Acta Scientiarum. Animal Sciences, 25, 21-24. DOI: https://doi.org/10.4025/actascianimsci.v25i1.2068

Fazio, F. 2019. Fish hematology analysis as an important tool of aquaculture: A review. Aquaculture, 500, 237-242. doi:10.1016/j.aquaculture.2018.10.030

Ferreira, L.M.H., Lara G., Wasielesky Jr., W., Abreu, P.C. 2016. Biofilm versus biofloc: Are artificial substrates for biofilm production necessary in the BFT system? Aquaculture international, 24(4), 921-930. DOI: 10.1007/s10499-015-9961-0

Fernandes, J.B.K., Carneiro, D.J., Sakomura, N.K. 2001. Fontes e Níveis de Proteína Bruta em Dietas para Alevinos de Pacu (*Piaractus mesopotamicus*). Revista brasileira de zootecnia, 30(3), 617-626. https://doi.org/10.1590/S1516-35982000000300002

Fessehaye, Y., Kabir, A., Bovenhuis, H., Komen, H. 2006. Prediction of cannibalism in juvenile *Oreochromis niloticus* based on predator to prey weight ratio, and effects of age and stocking density. Aquaculture, 255 (1), 314–322. https://doi.org/10.1016/j.aquaculture.2005.11.033

Field, J.B., Elvehjem, C.A., Juday, C. 1943. A study of the blood constituents of carp and trout. Journal of Biologiacl Chemistry, 148, 261–269. https://doi.org/10.1016/S0021-9258(18)72280-1

Fóes, G.K., Fróes, C., Krummenauer, D., Poersch, L., Wasielesky, W. 2011. Nursery of pink shrimp *Farfantepenaeus paulensis* in biofloco technology culture system: survival and growth at different stocking densities. Journal of Shellfish Research, 30 (2), 367–373. DOI: 10.2983/035.030.0224

FSBI, 2002. Fish Welfare. Briefing Paper 2. Fisheries Society of the British Isles, Grant Information Systems, 82A High Street, Sawston, Cambridge.

Gallardo-Collí, A., Pérez-Rostro, C.I., Hernández-Vergara, M.P., Pérez-Legaspi, I.A. 2019a. Microeukaryote community and the nutritional composition of the biofloc during Nile tilapia culture in water-reusing biofloc systems. Aquaculture International, 27, 381–398. https://doi.org/10.1007/s10499-018-0335-2

Gallardo-Collí, A., Pérez-Rostro, C.I., Hernández-Vergara, M.P. 2019b. Reuse of water from biofloc technology for intensive culture of Nile tilapia (*Oreochromis niloticus*): Effects on productive performance, organosomatic indices and body composition. International Aquatic Research, 11, 43–55. https://doi.org/10.1007/s40071-019-0218-9

Gaona, C.A.P., Poersch, L.H., Krummenauer, D., Foes, G.K., Wasielesky, W.J. 2011. The effect of solids removal on water quality, growth and survival of *Litopenaeus vannamei* in a biofloc technology culture system. International Journal of Recirculating Aquaculture, 12, 54–73. http://hdl.handle.net/10919/90645

Gaona, C.A.P., de Almeida, M.S., Viau, V., Poersch, L.H., Wasielesky, W. 2015. Effect of different total suspended solids levels on a *Litopenaeus vannamei* (Boone, 1931) BFT culture system during biofloc formation. Aquaculture Research, 1–10. https://doi.org/10.1111/are.12949

Gaona, C.A.P., Serra, F.P., Furtado, P.S., Poersch, L.H., Wasielesky, W. 2016. Effect of different total suspended solids concentrations on the growth performance of litopenaeus vannamei in a bft system. Aquacultural Engineering, 72-73, 65-69. http://dx.doi.org/10.1016/j.aquaeng.2016.03.004

Godoy, M.P. 1975. Peixes Do Brasil: Subordem Characoidei: Bacia Do Rio Mogi-Guassu. Piracicaba, SP: Franciscana.

Gomes, S.Z., Schlindwein, A.P. 2000. Efeito de períodos de cultivo e densidades de estocagem sobre o desempenho do catfish (*Ictalurus punctatus*) nas condições climáticas do litoral de Santa Catarina. Revista Brasileira de Zootecnia, 29, 1266-1272. https://doi.org/10.1590/S1516-35982000000500002

Goos, H.J.T., Consten, D. 2002. Stress adaptation, cortisol and pubertal development in the male common carp, *Cyprinus carpio*. Molecular and Cellular. Endocrinology, 197, 105–116. doi: 10.1016/s0303-7207(02)00284-8

Gilmour, K.M. 2001. The CO_2/pH ventilatory drive in fish. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 130(2), 219–240. https://doi.org/10.1016/S1095–6433(01)00391–9

Gorissen, M., Flik, G. 2016. The endocrinology of the stress response in fish: An Adaptation-Physiological View. Editor(s): Schreck, C.B., Tort, L., Farrell, A.P., Brauner, C.J. Fish Physiology, Academic Press, 35, 75-111. https://doi.org/10.1016/B978-0-12-802728-8.00003-5.

Hengsawat, K., Ward, F.J., Jaruratjamorn, P. 1997. The effect of stocking density on yield, growth and mortality of African catfish (*Clarias gariepinus* Burchell 1822) cultured in cages. Aquaculture, 152, 67-76. https://doi.org/10.1016/S0044-8486(97)00008-2

IBGE - Instituto Brasileiro de Geografia e Estatística. 2020. Sistema IBGE de Recuperação Automática – SIDRA. Disponível em: https://sidra.ibge.gov.br/.

Inoue, L.A.K.A., Hackbarth, A., Moraes, G. 2004. Assessment of 2-phenoxyethanol and benzocaine as anesthetics for field procedures in matrinxa (*Brycon cephalus*). Biodiversidade Pampeana, Uruguaiana, 2, 10-15.

Irwin, S., O'Halloran, J., Fitzgerald, R.D. 1999. Stocking density, growth and growth variation in juvenile turbot, *Scophthalmus maximus* (Rafinesque). Aquaculture, 178, 77–88. https://doi.org/10.1016/S0044-8486(99)00122-2

Jomori, R.K., Carneiro, D.J., Martins, M.I.E.G., Portella, M.C. 2005. Economic evaluation of *Piaractus mesopotamicus* juvenile production in different rearing systems. Aquaculture, 243, 175– 183. Disponível em: <http://hdl.handle.net/11449/68113>.

Jory, D.E., Cabrera, T.R., Dugger, D.M., Fegan, D., Lee, P.G., Lawrence, A.L., Jackson, C.J., McIntosh, R.P., Castañeda, J. 2001. A global review of shrimp feed management: Status and perspectives. In: Browdy, CL and Jory, DE (Eds.), The New Wave, Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture. The World Aquaculture Society, Baton Rouge, USA, 104-152.

Jung, J.Y., Hur, J.W., Kim, K., Han, H.S. 2020. Evaluation of floc-harvesting technologies in biofloc technology (BFT) system for aquaculture. Bioresource Technology, 314, 123719. https://doi.org/10.1016/j.biortech.2020.123719

Kohli, M.P.S., Ayyappan, S., Langer, R.K., Dubey, K., Prakash, C., Reddy, A.K., Deshmukhe. G. 2002. Cage culture of carps, *Labeo rohita* and *Cyprinus carpio* at Powai lake, Mumbai, Maharashtra. Applied Fisheries and Aquaculture, 2, 1-4.

Krummenauer, D., Cavalli, R.O., Poersch, L.H., Wasielesky, W. 2011. Superintensive culture of white shrimp, *Litopenaeus vannamei*, in a biofloc technology system in southern Brazil at different stocking densities. Journal of the World Aquaculture Society, 42, 726–733. http://repositorio.furg.br/handle/1/3859

Krummenauer, D., Samocha, T., Poersch, L., Lara, G., Wasielesky, W., 2014. The reuse of water on the culture of pacific white shrimp, *Litopenaeus vannamei*, in BFT

system. Journal of the World Aquaculture Society, 45, 3–14. DOI: 10.1111/jwas.12093

Kubitza, F. 2004. Tambaqui, Pacu e Híbridos. Panorama da Aquicultura, 14,(82), 39.

Kucharczyk, D., Mamcarz, A., Kujawa, R., Skrzypczask, A. 1998. Development of cannibalism in larval northern pike, *Esox lucius* (Esocidae). Italian Journal of Zoology, 65, 261– 263. Suppl. https://doi.org/10.1080/11250009809386829

Lambert, Y., Dutil, J.D. 2001. Food intake and growth of adult Atlantic cod (*Gadus moruhua* L.) reared under different conditions of stocking density, feeding frequency and sizegrading. Aquaculture, 192, 133-147. DOI: 10.1016/s0044-8486(00)00448-8

Lim, Y.S., Ganesan, P., Varman, M., Hamad, F.A., Krishnasamy, S. 2021. Effects of microbubble aeration on water quality and growth performance of *Litopenaeus vannamei* in biofloc system. Aquacultural Engineering, 93(1), 102159. https://doi.org/10.1016/j.aquaeng.2021.102159

Long, L., Zhang, H., Ni, Q., Liu, H., Wu, F., Wang, X. 2019. Effects of stocking density on growth, stress, and immune responses of juvenile Chinese sturgeon (*Acipenser sinensis*) in a recirculating aquaculture system. Comparative Biochemistry and Physiology, Part C, 219, 25–34. https://doi.org/10.1016/j.cbpc.2019.02.002

Lourenço, J.A., dos Santos, C.H.A., Neto, F.H.F.B., Arena, M.L., Igarashi, M.A. 2009. Influência de diferentes dietas no desenvolvimento do camarão *Litopenaeus vannamei* (Boone, 1931) em berçários intensivos (Boone, 1931). Acta Scientiarum. Biological Sciences. Maringá, 31 (1), 1-7. DOI: 10.4025/actascibiolsci.v31i1.454

Luo, G., Gaoa, Q., Wanga, C., Liu, W., Suna, D., Li, L., Tan, H. 2014. Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed

tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. Aquaculture, 422–423, 1–7. DOI: 10.1016/j.aquaculture.2013.11.023

Martínez-Córdova, L.R., Martínez-Porchas, M., Emerenciano, M.G.C., Miranda-Baeza, A., Gollas-Galván, T. 2016. From microbes to fish the next revolution in food production. Critical Reviews in Biotechnology, 1-9, 287-295. https://doi.org/10.3109/07388551.2016.1144043

Miki, T., Nakatsukasa, H., Takahashi, N., Murata, O., Ishibashi, Y. 2011. Aggressive behaviour and cannibalism in greater amberjack, *Seriola dumerili*: effects of stocking density, feeding conditions and size differences. Aquaculture Research, 42 (9), 1339–1349. https://doi.org/10.1111/j.1365-2109.2010.02722.x

Minaz, M & Kubilay, A. 2021. Operating parameters affecting biofloc technology: carbon source, carbon/nitrogen ratio, feeding regime, stocking density, salinity, aeration, and microbial community manipulation. Aquaculture International, 29, 1121–1140. https://doi.org/10.1007/s10499-021-00681-x

Montero, D., Izquierdo, M., Tort, L., Robaina, L., Vergara, J. 1999. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. Fish Physiology and Biochemistry, 20, 53-60. DOI: 10.1023/A:1007719928905

Morais, A.P.M., Abreu, P.C., Wasielesky, W., Krummenauer, D. 2020. Effect of aeration intensity on the biofilm nitrification process during the production of the white shrimp *Litopenaeus vannamei* (Boone, 1931) in Biofloc and clear water systems. Aquaculture, 514, 734516. https://doi.org/10.1016/j.aquaculture.2019.734516

Mota, V.C., Nilsen, T.O., Gerwins, J., Gallo, M., Ytteborg, E., Baeverfjord, G., Kolarevic, J., Summerfelt, S.T., Terjesen, B.F. 2019. The effects of carbon dioxide

on growth performance, welfare, and health of Atlantic salmon post-smolt (*Salmo salar*) in recirculating aquaculture systems. Aquaculture, 498, 578–586. https://doi.org/10.1016/j.aquaculture.2018.08.075

Najdegerami, E.H., Bakhshi, F., Lakani, F.B. 2016. Effects of biofloc on growth performance, digestive enzyme activities and liver histology of common carp (*Cyprinus carpio* L.) fingerlings in zero-water exchange system. Fish physiology and biochemistry, 42, 457–465. doi: 10.1007/s10695-015-0151-9

Neto, J.L.S., Galvani, E., Vieira, B.C. 2015. Climates of Brazil: past and present. In B. C. Vieira, A. Salgado, & L. Santos (Eds.), Landscapes and landforms of Brazil (pp. 33–41). Dordrecht, the Netherlands: Springer.

Nitz, L.F., Maltez, L.C., Pellegrin, L., Garcia, L.O., Barbas, L.A.L., Prentice-Hernández, C. 2019. Flesh quality and stress responses of *Piaractus mesopotamicus* after exposure to sublethal levels of ammonia and subsequent recovery. Boletin Instituto de Pesca, 45(1), e.325. https://doi.org/10.20950/1678-2305.2019.45.1.325

Nitz, L.F., Pellegrin, L., Pinto, D.S.B., Maltez, L.C., Copatti, C.E., Garcia, L.O. 2020a. Secondary stress responses to hypoxia and re-oxygenation at different temperatures in pacu (*Piaractus mesopotamicus*) juveniles. Aquaculture Research, 51, 1–11. https://doi.org/10.1111/are.14792

Nitz, L.F., Pellegrin, L., Maltez, L.C., Pinto, D., Sampaio, L.A., Monserrat, J.M., Garcia, L.O. 2020b. Temperature and hypoxia on oxidative stress responses in pacu *Piaractus mesopotamicus*. Journal of Thermal Biology, 92, 102682. https://doi.org/10.1016/j.jtherbio.2020.102682

North, B.P., Turnbull, J.F., Ellis, T., Porter, M.J., Migaud, H., Bron, J., Bromage, N.R. 2006. The impact of stocking density on the welfare of rainbow trout

(*Oncorhynchus mykiss*). Aquaculture, 255, 466-479. https://doi.org/10.1016/j.aquaculture.2006.01.004

Oba, E.T., Mariano, W.S., Santos, L.R.B. 2009. Estresse em peixes cultivados: Agravantes e atenuantes para o manejo rentável. In: Tavares-Dias, M. (organizador) Manejo e sanidade de peixes em cultivo. Embrapa, Amapá, Macapá.

Páez-Osuna, F., Garcia, A., Flores-Verdugo, F., Lyle-Fritch, L.P., Alonso-Rodríguez, R., Roque, A., Ruiz-Fernández, A.C. 2003. Shrimp aquaculture and the environment in the Gulf of California ecoregion. Marine Pollution Bulletin, 46, 806-815. DOI: 10.1016/S0025-326X(03)00107-3

Pasco, J.J.M., Filho, J.W.C., Espirito Santo, C.M., Vinatea, L. 2017. Production of Nile tilapia *Oreochromis niloticus* grown in BFT using two aeration systems. Aquaculture Research, 49 (1), 22-231. DOI: 10.1111/are.13451

Pavlidis, M., Futter, W.C., Kathario, P., Divanach, P. 2007. Blood cells of six Mediterranean mariculture fish species. Journal of Applied Ichthyology, 23, 70–73. https://doi.org/10.1111/j.1439-0426.2006.00771.x

Peel, M. C., Finlayson, B. L., & McMahon, T. A. 2007. Updated world map of the Köppen-Geiger climate classification. Hydrology and Earth System Sciences Discussions, 11, 1633–1644. https://doi.org/10.5194/hess-11-1633-2007

Pellegrin, L., Nitz, L.F., Maltez, L.C., Copatti, C.E., Garcia, L. 2020. Alkaline water improves the growth and antioxidant responses of pacu juveniles (*Piaractus mesopotamicus*). Aquaculture, 519, 734713. https://doi.org/10.1016/j.aquaculture.2019.734713

PEIXE BR. 2021. Anuário Peixe BR da Piscicultura 2021. São Paulo: Associação Brasileira de Piscicultura, 2020. 140p.

Perry, S.F., Gilmour, K.M. 2006. Acid–base balance and CO_2 excretion in fish: Unanswered questions and emerging models. Respiratory Physiology & Neurobiology, 154(1–2), 199–215. https ://doi. org/10.1016/j.resp.2006.04.010

Pickering, A.D. 1981. Stress and fish. London: Academic Press, 1981.

Pinto, D., Pellegrin, L., Nitz, L.F., Costa, S.T., Monserrat, J.M. Garcia, L. 2019. Haematological and oxidative stress responses in *Piaractus mesopotamicus* under temperature variations in water. Aquaculture Research, 50, 3017–3027. https://doi.org/10.1111/are.14260

Pinto, D., Pellegrin, L., Nitz, L.F., Monserrat, J.M., Garcia, L. 2020. Influence of temperature on growth, feed consumption and chemical composition in the muscle of Pacu *Piaractus mesopotamicus*. Aquaculture Research, 51, 3760-3767. DOI: 10.1111/are.14726

Poli, M.A., Schveitzer, R., Nuñer, A.P.O. 2015. The use of biofloc technology in a South American catfish (*Rhamdia quelen*) hatchery: Effect of suspended solids in the performance of larvae. Aquacultural Engineering, 66, 17–21. http://dx.doi.org/10.1016/j.aquaeng. 2015.01.004

Poli, M.A., Legarda, E.C., Lorenzo, M.A., Martins, M.A., Vieira, F.N. 2018. Pacific white shrimp and Nile tilapia integrated in a biofloc system under different fish-stocking densities. Aquaculture, 498, 83–89. http://dx.doi.org/10.1016/j.aquaculture.2018.08.045

Pottinger, T.G., Moran, T.A. 1993. Differences in plasma cortisol and cortisone dynamics during stress in two strains of rainbow trout (*Oncorhynchus mykiss*). Journal of Fish Biology 43, 121-130. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1095-8649.1993.tb00415.x

Ranzani-Paiva, M.J.T., Pádua, S.B., Tavares-Dias, M.; Egami, M.I. 2013. Métodos para análises hematológicas em peixes. Maringá: Eduem. 2013.

Ray, A.J., Seaborn, G., Leffler, J.W., Wilde, S.B., Lawson, A., Browdy, C.L. 2010. Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management. Aquaculture, 310, 130–138. https://doi.org/10.1016/j.aquaculture.2010.10.019

Ray, A.J., Dillon, K.S., Lotz, J.M. 2011. Water quality dynamics and shrimp (*Litopenaeus vannamei*) production in intensive, mesohaline culture systems with two levels of biofloc management. Aquaculture Engineering, 45, 127–136. https://doi.org/10.1016/j.aquaeng.2011.09.001

Ray, A.J., Seaborn, G., Vinatea, L., Browdy, C.L., Leffler, J.W. 2012. Effects of biofloc reduction on microbial dynamics in minimal-exchange, superintensive shrimp, *Litopenaeus vannamei*, culture systems. Journal of the World Aquaculture Society, 43 (6), 790–801. https://doi.org/10.1111/j.1749-7345.2012.00607.x

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

Ross, L.G., Palacios, C.A.M., Morales, E.J. 2008. Developing native fish species for aquaculture: the interacting demands of biodiversity, sustainable aquaculture and livelihoods. Aquaculture Research, 39, 675-683. doi:10.1111/j.1365-2109.2008.01920.x

Rotllant, J., Pavlidis, M., Kentouri, M., Abad, M.E., Tort, L. 1997. Non-specific immune responses in the red porgy *Pagrus pagrus* after crowding stress. Aquaculture, 156, 279-290. https://doi.org/10.1016/S0044-8486(97)00075-6

Saint-Paul, U. 2017. Native fish species boosting Brazilian's aquaculture development. Acta of Fisheries and Aquatic Resources, 5(1): 1-9. DOI 10.2312/ActaFish.2017.5.1.1-9

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

Santos, D.K.M. 2018. *Bacillus subtilis* na criação de tambaqui em sistema de bioflocos. 2018. 51 f. Dissertação (Mestrado em Ciência Animal) -Universidade Federal do Amazonas, Manaus, 2018.

Satheeshkumar, P., Ananthan, G., Senthilkumar, D., Khan, A.B., Jeevanantham, K. 2012. Comparative investigation on haematological and biochemical studies on wild marine teleost fihes from Vellar estuary, southeast coast of India. Comparative Clinical Pathology, 21, 275–281. DOI 10.1007/s00580-010-1091-5

Schveitzer, R., Arantes, R., Costódio, P.F.S; Espirito Santo, C.M., Arana, L.V., Seiffert, W.Q., Andreatta, E.R. 2013. Effect of different biofloc levels on microbial activity, water quality and performance of *Litopnaeus vannamei* in a tank system operated with no water exchange. Aquacultural Engineering, 56, 59-70. http://dx.doi.org/10.1016/j.aquaeng.2013.04.006

Sgnaulin, T., Lemos de Mello, G., Thomas, M.C., Garcia, J.R.E., Montes de Oca, G.A.R., Emerenciano, M.G.C. 2018. Biofloc technology (BFT): An alternative

aquaculture system for piracanjuba *Brycon orbignyanus*? Aquaculture, 485, 119-123. https://doi.org/10.1016/j.aquaculture.2017.11.043

Silva, R.D., Rocha, L.O., Fortes, B.D.A., Rodrigues, C.P.F., Lobo, J.R., Faleiro, M.B.R., De Paula, F.G., Vieira, D. 2009. Determinação de glicose plasmática em exemplares adultos de Tilápia (*Oreochromis niloticus*) por glicosímetro digital portátil e por método enzimático. In: Congresso de Ensino Pesquisa e Extensão, 6., 2009, Goiânia. Anais... Goiânia: UFG, 2009, p. 5914-5919.

Silva, J.R., Rabenschlag, D.R., Feiden, A., Boscolo, W.R., Signor, A.A., Bueno, G.W. 2012. Economic return from cage production of pacu in Itaipu reservoir, Brazil. Archivos de Zootecinia, 61 (234), 245-254. DOI: 10.4321/S0004-05922012000200009

Silva, A.F., Lara, G.R., Ballester, E.C., Krummenauer, D., Abreu, P.C., Wasielesky, W. 2013. Efeito das altas densidades de estocagem no crescimento e sobrevivência de *Litopenaeus vannamei* na fase final de engorda, cultivados em sistemas de bioflocos (BFT). Ciência animal brasileira, Goiânia, 14, (3), 279-287. https://www.revistas.ufg.br/vet/article/view/10419

Smith, F.M., Jones, D.R. 1982. The effect of changes in blood oxygen carrying capacity on ventilation volume in the rainbow trout (*Salmo gairdneri*). Journal of Experimental Biology, 97, 325-334.

Suita, S.M., Ballester, E.L.C., Abreu, P.C., Wasielesky, W. 2015. Dextrose as carbono source in the culture of *Litopenaeus vannamei* (Boone, 1931) in a zero Exchange system. Latin American Journal of Aquatic Research, 43, 526–533. DOI: 10.3856/vol43-issue3-fulltext-13

Suresh, A.V., Lin, C.K. 1992. Effect of Stocking Density on Water Quality and Production of Red Tilapia in a Recirculated Water System. Aquacultural Engineering, 11, 1-22. https://doi.org/10.1016/0144-8609(92)90017-R

Tavares-Dias, M., Moraes, F.R. 2004. Hematologia de peixes teleósteos. Ribeirão Preto, Villimpress Complexo Gráfico.

Tavares-Dias, M.; Moraes, F.R. 2006. Hematological parameters for the *Brycon orbignyanus* Valenciennes, 1850 (Osteichthyes: Characidae) intensively bred. Hidrobiológica, 16, 271–274.

Tavares-Dias, M., Moraes, F.R. 2007. Leukocyte and thrombocyte reference values for channel catfih (*Ictalurus punctatus* Raf.), with an assessment of morphological, cytochemical, and ultrastructural features. Veterinary Clinical Pathology, 36, 49–54. DOI: 10.1111/j.1939-165X.2007.tb00181.x

Tavares-Dias, M., Mariano, W.S. 2015. Aquicultura no Brasil: novas Perspectivas. [Vol.1]. São Carlos: Pedro & João Editores, 2015. 429p.

Ultsch, G.R. 1996. Gas exchange, hypercarbia and acid-base balance, paleoecology, and the evolutionary transition from water-breathing to air-breathing among vertebrates. Palaeogeogr. Palaeoclimatol. Palaeoecol, 123, 1–27. https://doi.org/10.1016/0031-0182(96)00121-6

Urbinati, E.C., Gonçalves, F.D., Takahashi, L.S. 2010. Pacu (*Piaractus mesopotamicus*). In B. Baldisserotto, & L. C. Gomes (Eds.), Espécies nativas para piscicultura no Brasil (pp. 205-231). Santa Maria: UFSM.

Urbinati, E.C., Goncalves, F.D., Takahashi, L.S. 2013. Pacu *Piaractus mesopotamicus*. In: Baldisseroto, B., Gomes, L.C., Orgs (Eds.), Espécies Nativas para piscicultura no Brasil. UFSM, Santa Maria, RS, pp. 1–606.

Valente, C.S., Ortiz, K.O., Depperschmidt, R., Fraga, A.P.M., Vieira, F.N., Marques, M.R.F. 2020. Transcription of defense related genes in Pacific white shrimp, *Litopenaeus vannamei*, kept in biofloc and in clear seawater and challenged with the white spot syndrome virus. Aquaculture International, 28, 293–307. https://doi.org/10.1007/s10499-019-00461-8

Valenti, W.C., Barros, H.P., Moraes-Valenti, P., Bueno, G.W., Cavalli, R.O. 2021. Aquaculture in Brazil: past, present and future. Aquaculture Reports, 19, 100611. https://doi.org/10.1016/j.aqrep.2021.100611

Valladão, G.M.R., Gallani, S.U., Pilarski, F. 2016. South American fish for continental aquaculture. Reviews in Aquaculture, 0, 1–19. doi: 10.1111/raq.12164

Vazquez, L., Alpuche, J., Maldonado, G., Agundis, C., Pereyra-Morales, A., Zenteno, E. 2009. Review: Immunity mechanisms in crustaceans. Innate immunity, 15, 179–188. DOI: 10.1177/1753425909102876

Vijayan, M.M. J.S., Ballantyne, J.S., Leatherland, J.F. 1990. High stocking density alters the energy metabolism of brook charr, *Salvelinus fontinalis*. Aquaculture, 88, 371-381. https://doi.org/10.1016/0044-8486(90)90162-G

Vinatea, L., Gálvez, A.O., Browdy, C.L., Stokes, A., Venero, J., Haveman, J., Lewis, B.L., Lawson, A., Shuler, A., Leffler, J.W. 2010. Photosynthesis, water respiration and growth performance of *Litopenaeus vannamei* in a super-intensive raceway culture with zero water exchange: Interaction of water quality variables. Aquacultural Engineering, 42, 17–24. DOI: 10.1016/j.aquaeng.2009.09.001

Wasielesky, W., Atwood, H., Stokes, a, Browdy, C. 2006. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. Aquaculture, 258, 396–403. https://doi.org/10.1016/j.aquaculture.2006.04.030

Wells, R.M.G. 2009. Blood-gas transport and hemoglobin function: Adaptations for functional and environmental hypoxia. Fish physiology, 27, 255-299. DOI: 10.1016/S1546-5098(08)00006-X

Wendelaar Bonga, S.E. 1997. The stress response in fish. Physiology Reviews, 77, 591–625. https://doi.org/10.1152/physr ev.1997.77.3.591.

Widanarni, Ekasari, J., Maryam, S. 2012. Evaluation of Biofloc Technology Application on Water Quality and Production Performance of Red Tilapia *Oreochromis sp*. Cultured at Different Stocking Densities. HAYATI Journal of Biosciences, 19, 73–80. https://doi.org/10.4308/hjb.19.2.73

Winfree, R.A., Kindschi, G.A., Shaw, H.T. 1998. Elevated water temperature, crowding, and food deprivation accelerate fin erosion in juvenile steelhead. The Progressive Fish-Culturist, 60, 192-199. https://doi.org/10.1577/1548-8640(1998)060<0192:EWTCAF>2.0.CO;2

Witeska, M., Lugowska, K., Kondera, E. 2016. Reference values of hematological parameters for juvenile *Cyprinus carpio*. Bulletin of the European Association of Fish Pathologists. 36, 169-180.

Xu, W.J., Pan, L.Q., Sun, X.H., Huan, J. 2013. Effects of bioflocs on water quality, and survival, growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zero-water exchange culture tanks. Aquaculture Research, 44, 1093–1102. https://doi.org/10.1111/j.1365-2109.2012.03115.x

Zhao, H., Soufan, O., Xia, J., Tang, R., Li, L., Li, D. 2019. Transcriptome and physiological analysis reveal alterations in muscle metabolisms and immune responses of grass carp (*Ctenopharyngodon idellus*) cultured at different stocking densities. Aquaculture, 503, 186–197. https://doi.org/10.1016/j.aquaculture.2019.01.003

3. Objetivos

3.1. Objetivo Geral

O objetivo deste trabalho foi avaliar a adaptação do pacu ao sistema BFT e determinar a melhor concentração de SST a ser utilizada para o cultivo, assim como, determinar a melhor densidade de estocagem a ser utilizada para juvenis de pacu na pré engorda em sistema BFT.

3.1. Objetivos específicos

- Avaliar a concentração letal de sólidos suspensos totais para o pacu;

- Analisar os efeitos agudos de diferentes concentrações subletais de sólidos suspensos totais nos parâmetros hematológicos de juvenis de pacu em sistema BFT;

- Determinar os efeitos crônicos de diferentes concentrações de sólidos suspensos totais sobre os parâmetros hematológicos e o desempenho zootécnico de juvenis de pacu em sistema BFT;

- Testar diferentes densidades de estocagem de juvenis de pacu em sistema BFT a fim de determinar a melhor e assim garantir os melhores índices de desempenho zootécnico.

Capitulo I

Effects of suspended solids in the survival and hematological parameters of pacu juveniles (*Piaractus mesopotamicus*) in a biofloc technology culture system

Short running title: biofloc technology culture system in pacu

Lucas Pellegrin[a], Lilian Fiori Nitz[a], Daniel de Sá Britto Pinto[a], Carlos Eduardo Copatti[c], Wilson Wasielesky [b], Luciano Garcia[a*].

[a]Laboratório de Aquacultura Continental, Instituto de Oceanografia, Universidade Federal do Rio Grande - FURG, Rio Grande, RS, Brazil.
[b]Laboratório de Carcinocultura, Instituto de Oceanografia, Universidade Federal do Rio Grande - FURG, Rio Grande, RS, Brazil.
[c]Instituto de Biologia, Universidade Federal da Bahia, Av. Adhemar de Barros, s/n, Salvador, BA, 40170-290, Brazil.

*Corresponding author: Laboratório de Aquacultura Continental, Universidade Federal do Rio Grande – FURG, 96201-900, Rio Grande, RS, Brazil. Phone: +55 53 3503-2474; E-mail address: garcia_log@hotmail.com

Artigo aceito na revista *Aquaculture Research*

ABSTRACT

This study determined the effects of total suspended solids (TSS) on pacu (*Piaractus mesopotamicus*) culture in a biofloc technology (BFT) system by performing two experiments. Experiment 1 evaluated the LC50-96h of pacu at seven TSS levels (0, 1,500, 3,000, 4,000, 5,000, 6,000, and 7,000 mg L^{-1}). Experiment 2 evaluated hematological variables (glucose, pH, hematocrit, hemoglobin, erythrocytes, and hematimetric indices) in juveniles exposed to five different sublethal TSS concentrations (0, 250, 500, 750, and 1,000 mg L^{-1}) for five days. Treatments below 5,000 mg L^{-1} did not cause mortality, and the LC50-96h of TSS was estimated at 5,477 mg L^{-1}. The TSS concentrations that caused mortality in pacu juveniles were extremely high and rarely maintained in culture systems. Exposure to high TSS concentrations (mainly 750 and 1,000 mg L^{-1}) increased blood glucose, pH, hematocrit, erythrocytes, hemoglobin values, and haematimetric indices on the first day. On the fifth day, most of the evaluated parameters stabilized at different TSS concentrations. In conclusion, pacu exposed to high TSS concentrations for short periods may undergo physiological changes, and TSS concentrations below 250 mg L^{-1} are recommended for its culture in a BFT system.

Keywords: BFT system, Blood parameters, Hematimetric indices, Total suspended solid, Physiological parameters.

1. Introduction

Pacu (*Piaractus mesopotamicus*) is a viable fish species for culture in temperate climate regions due to its tolerance to low temperatures (Pinto, Pellegrin, Nitz, Costa, Monserrat, & Garcia, 2019; Nitz, Pellegrin, Pinto, Maltez, Copatti, & Garcia, 2020a).

Pacu cultivation is of great importance in South American countries such as Argentina and Paraguay (Valladão, Gallani, & Pilarski, 2016). In Brazil, it is part of a group known as round fish that represent the second largest group of farmed fish (Valenti, Barros, Moraes-Valenti, Bueno, & Cavalli, 2021). In addition, this species is resistant to low oxygen levels (Cunha Bastos, Salles, Valente, León, Perales, Dantas, Albamo, Bastos, & Cunha Bastos, 2007; Nitz, Pellegrin, Maltez, Pinto, Sampaio, Monserrat, & Garcia, 2020b) and tolerates medium and high ammonia levels (Nitz, Maltez, Pellegrin, Garcia, Barbas, & Prentice-Hernández, 2019) and adverse conditions of acid and alkaline pH in water (Copatti, Baldisserotto, Souza, & Garcia, 2019; Pellegrin, Nitz, Maltez, Copatti, & Garcia, 2020). Moreover, pacu are omnivorous and have a low-protein diet based on vegetables, consequently reducing production costs (Urbinati, Gonçalves, & Takahashi, 2010; Valladão, Gallani, & Pilarski, 2016). The sum of these characteristics shows promising potential for the culture of this species in intensive systems.

Biofloc technology (BFT) uses inorganic ($NaHCO_3$ and $CaCO_3$) and organic (alcohols, sugars, starches, and fibers) carbon sources. Additionally, it employs an efficient and constant aeration system to promote the growth of microorganisms (heterotrophic or chemoautotrophic microbial communities), which are transformed into microbial protein (biofloc), and convert toxic forms of nitrogen into non-toxic forms from feed leftovers and fish excretions (Azim & Little, 2008; Avnimelech, 2015). High C: N ratios are commonly used in biofloc production and ammonia removal (Hargreaves, 2006; Avnimelech, 2015). The BFT is an intensive culture system that has gained prominence due to increasing sustainable aquaculture production using highly controlled culture systems with low or zero effluent release, even in zero-water exchange systems (Azim & Litle, 2008). Since the BFT system is an economical and sustainable alternative to

decrease the use of commercial diets for many aquaculture species, it has been efficiently applied worldwide (Crab, Defoirdt, Bossier, & Verstraete, 2012; Dauda, 2020).

The use of BFT for pacu in juvenile phase can be economically advantageous due water renewal and artificial diet supply, because pacu is omnivorous, which indicates that it could consume microbial flocs. The current consensus is that BFT systems can promote fish growth with typical filter-feeding habits, improving feed conversion rates (Avnimelech & Kochba, 2009). Omnivorous species can consume these microbial flocs, which become a complementary source of food (Poli et al., 2018; Dauda et al., 2020; Sandoval-Vargas, Jiménez-Amaya, Rodríguez-Pulido, Guaje-Ramírez, Ramírez-Merlano, & Medina-Robles, 2020). Furthermore, in southern Brazil, winter temperatures are commonly below 10 ºC (Garcia et al., 2008), affecting the development of pacu juveniles (Urbinati et al., 2010). Therefore, this system could contribute to the increase of growth and survival of pacu in juvenile phase, mainly during the coldest seasons of the year, where the cultivation in acclimatized BFT system would minimize the negative effects of low temperatures. We are not aware of BFT application in the pacu species, although previous studies have reported its effectiveness in the same genus, e.g., pirapatinga (*Piaractus brachypomus*) (Abad et al., 2014; Garcés, González, & Carrasco, 2017; Sandoval-Vargas et al., 2020).

The BFT system has been used for many fish farming (Avnimelech & Kochba, 2009; Abad, Rincón, & Poleo, 2014; Poli, Schveitzer, & Nuñer, 2015; Gallardo-Collí, Pérez-Rostro, Hernández-Vergara, & Pérez-Legaspi, 2019a; Gallardo-Collí, Pérez-Rostro, & Hernández-Vergara, 2019b) and integrated fish and shrimp systems (Poli, Legarda, Lorenzo, Martins, Vieira, 2018; Holanda, Santana, Furtado, Rodrigues, Cerqueira, Sampaio, Wasielesky, & Poersch, 2020). Thus, considering that the BFT system needs more technological and financial support (Crab et al., 2012; Hargreaves,

2013), the production of juvenile pacu could be indicated mainly for fish farms where this system is already implemented for the cultivation of shrimp and other fish species.

Total suspended solids (TSS) are evaluated to quantify biofloc levels in the culture, maintaining the water quality through the direct assimilation of nitrogenous compounds (Poli et al., 2015). Furthermore, very high TSS concentrations in water can be a harmful factor because they can directly compromise the respiratory activity of fish by fusion of gill lamellae (Crab et al., 2012; Romano et al., 2020), leading to physiological and biochemical imbalances in fish. However, Azim and Little (2008) did not verified differences in the occurrence of gill lesions of Nile tilapia reared in BFT and water recirculation system (RAS).

In addition, increased TSS results from increased microbial biomass, which takes advantage of the C: N ratio from organic fertilization and excreta of fish (Holanda et al., 2020). Avnimelech (2015) and Poli et al. (2015) studied Nile tilapia (*Oreochromis niloticus*) and silver catfish (*Rhamdia quelen*) and recommended TSS values of 200 and 200-400 mg L^{-1}, respectively. High TSS concentrations appeared to restrict daily feed intake and, at a maximum of 2,100 mg L^{-1}, caused fish distress and limited mortality (Green et al., 2014). In BFT systems, TSS presence is a crucial part of the system, and the tolerance to this parameter must be considered when choosing the species to be farmed (Crab et al., 2012; Dauda, 2020). Nevertheless, there are no known studies on the optimal concentrations for pacu production.

Therefore, to determine the best TSS concentration to be used in the pacu culture, this study evaluated the survival and hematological parameters of pacu juveniles exposed to different TSS concentrations in the water of the BFT system.

2. Materials and methods

1

2 *2.1. Animals*

3 The experimental protocol was approved by the Committee on Animal
4 Experimentation of the Federal University of Rio Grande (FURG) (No. Pq004/2020). The
5 pacu juveniles (~ 5 g) were obtained from a commercial fish farm in Ajuricaba (Rio
6 Grande do Sul State, Brazil) and transported to the Continental Aquaculture Laboratory
7 (LAC) of the FURG, where they were kept in RAS until analysis.

8

9 *2.2. Biofloc technology system*

10 The TSS concentrations were obtained by maintaining a BFT culture system of
11 pacu and stimulating the microbial community using organic (cane molasses in the
12 proportion of 15:1 C: N ratio) and inorganic (sodium bicarbonate) carbon sources. The
13 system consisted of three 310-L tanks (250 L of useful volume), where 100 juveniles
14 (70.90 ± 0.48 g) were stored per tank. The TSS concentration maintained in these systems
15 was 512.20 ± 8.12 mg L^{-1}. The TSS was composed of feces, feed pellets, and microbial
16 growth. The bioflocs (~100-150 μm) were then collected and concentrated and/or diluted
17 according to each treatment. The TSS concentrations of each treatment in both
18 experiments were previously adjusted before fish storage.

19 Two experiments were carried out with pacu juveniles not used in the
20 acclimatization period in order to evaluate the effects of TSS on survival and health. In
21 experiment 1, lethal and sublethal TSS concentrations for pacu in water were determined
22 (n = 84). In experiment 2, the effects of sublethal TSS concentrations on the
23 hematological and blood parameters of pacu (n = 126) were determined.

24

25 *2.3.Water quality*

The following water physicochemical variables were monitored daily: pH (pH meter; HI 8424, HANNA®), temperature, dissolved oxygen meter (DO; 200A EcoSense®), nitrite (Boyd & Tucker, 2014), alkalinity, total ammonia (Eaton, Clesceri, Rice, & Greenberg, 2005), un-ionized ammonia (calculated from a conversion table specific for fresh water), TSS (Strickland & Parsons, 1972), and suspended solids (SS; cone Imhoff-30 min). A 12:12-h light/dark cycle was used.

2.4. Experiment 1

A tolerance test was performed to determine which TSS concentrations are lethal to pacu (20.70 ± 0.31 g) in the BFT system. Seven TSS levels were tested in the water for 96 h: 0 (T0; control), 1,500 (T1500), 3,000 (T3000), 4,000 (T4000), 5,000 (T5000), 6,000 (T6000), and 7,000 mg L^{-1} (T7000). Each treatment was carried out in triplicate using four fish per tank (12 fish per treatment). The treatments were kept in a static system in 8 L-tanks (6 L of useful volume) with aeration where only the water collected for water quality analysis was replaced. Dead individuals were counted twice a day (09:00 AM and 04:00 PM).

2.5. Experiment 2

The effects of different sublethal TSS concentrations were evaluated using the hematological parameters of pacu (28.41 ± 0.30 g), and a second 5-day experiment was performed. In addition to the control group, four sublethal TSS concentrations in water were used based on the results of experiment 1, where two sublethal concentrations were defined both below and above the safety levels found. Safe levels were estimated after 96 h of exposure and based on Sprague (1971) and the National Academy of Sciences/National Academy of Engineering (NAS/NAE, 1973). Five TSS levels were tested in five days: 0 (T0; control), 250 (T250), 500 (T500), 750 (T750), and 1,000 mg L^{-}

[1] (T1000). The control treatment was maintained in a water recirculation system, and the other treatments were kept in a static system similar to experiment 1, although 80 L-tanks (60 L of useful volume) with six fish per tank (18 fish per treatment) were used.

2.6. Sample collection and analysis

Three fish from each tank (9 fish per treatment) were randomly collected for blood sampling. The animals were removed from the tank, anesthetized with benzocaine hydrochloride (50 ppm), and blood was collected (1 mL) from the caudal vasculature using heparinized syringes. Blood glucose was recorded using a digital glucometer (Accu-Chek Performa®). Blood pH was measured by a pH meter (HI2210/HANNA®) with a sensor (HI1083/HANNA®).

The samples were then transferred to heparinized capillaries and centrifuged at 4 °C and 12000×*g* for 5 min to determine hematocrit (Hct) values using the microhematocrit method (Goldenfarb, Bowyer, Hall, & Brosious 1971). Erythrocytes (Ery) were counted in a Neubauer chamber using a binocular optical microscope after diluting 0.02 mL blood in a 4.0 mL Natt and Herrick solution. The hemoglobin concentration (Hb) was quantified using a colorimetric kit (Bioclin®) and read on a spectrophotometer (540 nm).

Calculations of the haematimetric indices were estimated according to the following equations: mean corpuscular volume (MCV, fL) = Hct×10/Ery(×10^6 µL), mean corpuscular hemoglobin (MCH, pg) = Hb×10/Ery(×10^6 µL), and mean corpuscular hemoglobin concentration (MCHC, g dL^{-1}) = Hb × 100/Hct.

2.7. Statistical analysis

The results are expressed as the mean ± SEM, and Levene's test was performed to evaluate the homogeneity of data variances. Survival data from experiment 1 were analyzed using the trimmed Spearman-Karber method (Hamilton, Russo, & Thurston, 1977) to determine the LC50-96h. Water quality variables were compared using a one-way analysis of variance (ANOVA), and hematological variables were compared using a one-way ANOVA (day versus TSS concentration) followed by Tukey's pair-wise comparisons. Comparisons with the control group were made using Dunnett's method. Differences were considered significant at $p<0.05$.

3. Results

3.1. Experiment 1

The LC50-96 h of TSS was estimated in 5,477 mg L^{-1}, with a 10% pacu security level of 548 mg L^{-1}. Treatments below T5000 did not cause mortality, although T6000 caused 25% mortality, and all fish in T7000 died (Fig. 1).

The TSS and SS differed significantly between treatments (T7000>T6000>T5000>T4000>T3000>T1500>T0; $p<0.05$). The other water quality variables did not differ significantly between treatments (Table 1).

3.2.Experiment 2

The TSS and SS differed significantly between treatments (T1000>T750>T500>T250>T0; $p<0.05$). The other water quality variables did not differ significantly between treatments (Table 2).

Blood glucose levels were significantly higher on day 1 than on day 5 in all treatments ($p<0.05$). On day 1, blood glucose levels in T0 were significantly higher than

in T250, T500, and T1000. On the same day, blood glucose levels were significantly higher in T750 than T250 ($p<0.05$). On day 5, T500 had blood glucose values significantly lower than the other treatments ($p<0.05$; Fig. 2a).

Blood pH values were higher and lower in T250 and T750, respectively, on day 5 than the same treatments on day 1 ($p<0.05$). On day 1, blood pH values were significantly lower in T0 and T250 than in the other treatments ($p<0.05$). In general, blood pH values were significantly lower in T0 on day 5 compared to the other treatments and significantly higher in T250 and T500 than in the other treatments ($p<0.05$; Fig. 2b).

Hematocrit values were significantly lower in T250 and significantly higher in T500 and T750 on day 1 compared to the same treatments on day 5 ($p<0.05$). On day 1, hematocrit values were significantly higher in T7500 than in the other treatments except for T500 ($p<0.05$). Additionally, T0 and T250 showed hematocrit percentages significantly lower than the other treatments on day 1 ($p<0.05$; Fig. 2c).

Erythrocyte values were significantly lower in T0 and T500 on day 1 and notably higher in T750 and T1000 than the same treatments on day 5 ($p<0.05$). Moreover, erythrocyte values were significantly higher in T1000 on day 1 compared to the other treatments except for T750, which presented much higher erythrocyte values than T0 ($p<0.05$). On day 5, erythrocyte values were significantly higher in T0, T250, and T500 than in T750 and T1000 ($p<0.05$). In addition, on this same day, erythrocyte values were notably higher in T1000 than T750 ($p<0.05$; Fig. 2d).

Hemoglobin values were significantly lower on day 1 compared to day 5 for T250 and T500 ($p<0.05$). Furthermore, hemoglobin values were significantly higher in T750 and T1000 on the same day than the other treatments ($p<0.05$). On day 5, hemoglobin values were significantly higher in T500 than in T0 or T1000 ($p<0.05$; Fig. 2e).

The MCV values were significantly higher on day 1 than day 5 in T250 and T500 ($p<0.05$). On day 1, MCV values were higher in T500 and T750 compared to the other treatments ($p<0.05$). On day 5, T500 and T750 had, respectively, the lowest and highest MCV values ($p<0.05$; Fig. 2f). On day 1, MCH values were significantly higher than on day 5 in T500 ($p<0.05$). On day 1, MCH values were lower in T0 and T250 compared to the other treatments ($p<0.05$; Fig. 2g).

On day 1, MCHC values were significantly lower than on day 5 in T250 and T750 ($p<0.05$). On day 1, T500 showed significantly lower MCHC values than the other treatments ($p<0.05$). In addition, even on day 1, MCHC values were notably lower in T750 than T1000 ($p<0.05$). On day 5, the highest MCHC values occurred in T500. In addition, even on day 5, MCHC values were significantly higher in T250 than in T0, T750, and T100 ($p<0.05$; Fig. 2h).

4. Discussion

4.1. Experiment 1

In the present study, the TSS concentrations up to 5,000 mg L^{-1} did not cause fish mortality by 96 h, although fish survival decreased significantly in T6000 and T7000. High TSS concentrations in the water are lethal to aquatic organisms. Thus, such concentrations must be known for different fish species in aquaculture systems. The main effects of high TSS concentrations are skin irritations, fin erosion, blockage of the opercular cavity, gas diffusion inhibition, nitrogen compound excretion, and changes in ion exchange (Holanda et al., 2020; Schumann & Brinker, 2020). In our study, the TSS concentrations that caused mortality in pacu juveniles were extremely high (6,000 and 7,000 mg L^{-1}), which is rarely maintained in a culture system. Moreover, BFT systems

are typically operated at SS concentrations below 1,000 mg L^{-1} and most often less than 500 mg L^{-1} (Hargreaves, 2013).

Previous research has also verified the LC50-96h of TSS in other species. In sockeye salmon (*Oncorhynchus nerka*) and coho salmon (*Oncorhynchus kisutch*), there was increased mortality with 9,850 and 40,000 mg L^{-1}, respectively (Servizi & Martens, 1987; Lake & Hinch, 1999). For rainbow trout (*Oncorhynchus mykiss*), 100% mortality occurred with 100,000 mg L^{-1} (Chapman, Popham, Griffin, Leslie, & Michaelson, 1987). Nonetheless, Poli et al. (2015) exposed silver catfish to TSS concentrations up to 1,000 mg L^{-1} and found no mortality, as also reported for Nile tilapia in the BFT system (Azim & Littlle, 2008; Luo, Gao, Wang, Liu, Sun, Li, & Tan, 2014).

4.2. Experiment 2

On day 1, the fish had higher blood glucose levels than on day 5, mainly in the control group. Furthermore, the fish (on day 1) were transferred from the maintenance tank directly to their respective treatments, and the capture management may have likely stressed them. The higher blood glucose levels in stressed fish are directly associated with increased carbohydrate metabolism (Lemos, Chung, Ribeiro, & Copatti, 2018), which was an expected result in this study. Similar evidence has also been reported in different stressful situations for pacu in other studies (Nitz et al., 2019; Copatti et al., 2019; Pinto et al., 2019).

In general, fish submitted to treatments containing different TSS concentrations had lower glucose levels than in fish kept in clear water on day 1, which was maintained at T500 on day 5. This response demonstrates that the low water transparency caused by TSS probably reduced the fish stress in the period immediately after changing tanks, which has already been suggested by Poli et al. (2015) for silver catfish larvae. Kim, Kim,

& Kim (2018) reported that flatfish (*Paralichthys olivaceus*) kept in a BFT system had lower glucose levels than those raised in a system without the presence of solids, thus corroborating our hypothesis. After five days of exposure to different treatments, blood glucose levels decreased in all treatments, indicating a possible recovery.

Maintaining blood pH homeostasis is crucial for protein stability and enzyme function (Aboagye & Allen, 2018). In our study, the blood alkalosis found in treatments T500, T750, and T1000 on day 1 and T250 and T500 on day 5 was possibly related to TSS presence, which may hinder oxygen uptake in the fish (Schumann & Brinker, 2020). As the dissolved oxygen levels in the water remained similar between treatments, its direct effects on blood alkalosis were ruled out, leaving the hypothesis of possible functional hypoxia caused by TSS presence in the water, which would have interfered with the O_2 uptake.

In this type of situation, respiratory rates (hyperventilation) usually increase. However, when O_2 uptake is not efficient even at higher respiratory rates, CO_2 excretion may increase, leading to a CO_2/HCO_3 imbalance in the blood (Gilmour, 2001; Palmer, 2012; Johnson, 2017), which may explain the respiratory alkalosis described herein for fish in the BFT system. Nevertheless, this alkalosis was surprisingly reduced in treatments with the highest TSS levels (T750 and T1000) on day 5, suggesting that fish may show a compensatory response in a situation with a more significant TSS presence, reducing the ionic exchanges with water.

A change in ionic exchanges with water can trigger a series of disturbances in physiological parameters (Copatti et al., 2015), as verified in our study for juveniles kept in the BFT system and worsening at higher TSS concentrations. Exposure to high TSS concentrations (T750 and T1000) resulted in high hematocrit, erythrocytes, and hemoglobin values and haematimetric indices in a short period (day 1). On the same day,

T0 and T250 showed lower hematological values. The fish in the T500 group, nonetheless, presented intermediate situations and physiological alterations on day 1 by increasing mainly hematocrit, MCH, and MCV, which decreased hemoglobin concentration in the erythrocyte, as demonstrated by the MCH reduction.

In the T750, the effects were even more evident on day 1, with increased hematocrit values caused by two main factors: 1) higher number of erythrocytes and; 2) larger red blood cells (MCV). Therefore, there may have been an increase in the number of hemoglobins due to more significant quantities of erythrocytes. This same increase in hemoglobin was also observed in T1000 on day 1, although this occurred through an increase in the number of available erythrocytes, which increased hematocrit levels.

The alterations triggered in the BFT system treatments indicate that TSS presence in the water affected the O_2 uptake in pacu juveniles, resulting in a series of hematological changes to supply this deficiency. The increase in erythrocyte and hemoglobin values, for example, allow improving the O_2 transport capacity, resulting in an increase in the percentage of hematocrit (Witeska, 2013). With the increased hemoglobin values, there is a tendency to maintain the demand for O_2 in organs and tissues, even under hypoxia conditions (Wells, 2009; Witeska, 2013). However, the amount of O_2 transported and delivered to tissues per unit volume of blood depends on other factors such as the number of erythrocytes per unit volume, the amount of functional hemoglobin within the cell, and the affinity of hemoglobin with oxygen (Nikinmaa, 2001).

In our study, all treatments where TSS was present on day 5 showed hematocrit stabilization, corroborating the results found for Nile tilapia and flatfish maintained in BFT systems (Long, Yang, Li, Guana, & Wu, 2015; Kim et al., 2018). In addition, there was a reduction in erythrocytes compared to day 1 for fish in T750 and T1000. Nonetheless, even on day 5, juveniles in T500 had higher hemoglobin levels than those

found in T0, which may indicate that lower TSS concentrations affect the hematological parameters of pacu juveniles less significantly. Given the findings mentioned above, we provided the first study for pacu culture in the BFT system employing different TSS concentrations. Despite our data, further research efforts are necessary to validate the BFT system for pacu in intensive culture.

5. Conclusion

Pacu juveniles showed resistance to TSS concentrations up to 5,000 mg L^{-1}, which are not commonly used in BFT systems. Nevertheless, high TSS concentrations can cause short-term physiological changes in pacu as verified in our study, and TSS concentrations of up to 250 mg L^{-1} are recommended for BFT system production. Further research is recommended to verify the ability to adjust pacu to BFT systems at different TSS concentrations in long-term exposure.

Credit authorship contribution statement

Lucas Pellegrin: Main author. He was responsible for the planning and execution of this experiment. He participated in blood analysis. He carried out the statistical analysis of the results. Responsible by writing and review of manuscript. Lilian F. Nitz: This author was helpful during the experiment. Participated in blood and statistical analysis. Collaborated in literature review and writing and review manuscript. Daniel Pinto: This author was helpful during the experiment, water quality, statistical analysis and collaborated with the manuscript discussion. Carlos Eduardo Copatti: This author collaborated in the statistical analysis and preparation and review of this manuscript. Wilson Wasielesky: Collaborated with the planning of the experiment and preparation of this manuscript. Luciano Garcia:

Collaborated with the planning and financial support to the experiment. Participated in literature review, preparation, and review of the manuscript.

Declaration of Competing Interest

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

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

This study was supported by research funds from the Fundação de Amparo a Pesquisa do Rio Grande do Sul (FAPERGS – PQG - 19/2551- 0001890-8), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The authors are gratefull to CNPq for productivity research fellowships provide for Garcia, L. (311352/2020-0), Wasielesky, W. (310652/2017-0) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Finance Code 001 for a PhD scholarship provided for MSc. Pellegrin, L (process number 88882.182342/2018-01).

References

Abad, D., Rincón, D., & Poleo, G. (2014). Carcass yield índex in red belly pacu *Piaractus brachypomus* cultivated in a biofloc system. Zootecnia Tropical, 32, 119–130.

Aboagye, D. L., & Allen, P. J. (2018). Effects of acute and chronic hypoxia on acid–base regulation, hematology, ion, and osmoregulation of juvenile American paddlefish. Journal of Comparative Physiology B, 188, 77–88. https://doi.org/10.1007/s00360-017-1104-7

Avnimelech, Y., & Kochba, M. (2009). Evaluation of nitrogen uptake and excretion by tilapia in biofloc tanks, using 15N tracing. Aquaculture, 287, 163–168. https://doi.org/10.1016/j.aquaculture.2008.10.009

Avnimelech, Y. (2015). Biofloc technology – A practical guide book (3rd ed.). Baton Rouge, LA: The World Aquaculture Society.

Azim, M. E., & Little, D. C. (2008). The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture, 283, 29–35. https://doi.org/10.1016/j.aquaculture.2008.06.036

Boyd, C. E., & Tucker, C. S. (2014). Handbook for aquaculture water quality. Auburn, AL: Craftmaster Printers Inc.

Chapman, P., Popham, J. D., Griffin, J., Leslie, D., & Michaelson, J. (1987). Differentiation of physical from chemical toxicity in solid waste fish bioassays. Water, Air, and Soil Pollution, 33, 295–308. https://doi.org/10.1007/BF00294198

Copatti, C. E., Baldisserotto, B., Souza, C. F., & Garcia, L. (2019). Protective effect of high hardness in pacu juveniles (*Piaractus mesopotamicus*) under acidic or alkaline pH: Biochemical and haematological variables. Aquaculture, 502, 250–257. https://doi.org/10.1016/j.aquaculture.2018.12.028

Copatti, C. E., Bolner, K. C. S., De Rosso, F. L., Loro, V. L., & Baldisserotto, B. (2015). Tolerance of piava juveniles to different ammonia concentrations. Semina: Ciências Agrárias, 36, 3991–4002. https://10.5433/1679-0359.2015v36n6p3991

Crab, R., Defoirdt, T., Bossier, P., & Verstraete, W. (2012). Biofloc technology in aquaculture: Beneficial effects and future challenges. Aquaculture, 356–357, 351–356. https://doi.org/10.1016/j.aquaculture.2012.04.046

Cunha Bastos, V. L. F., Salles, J. B., Valente, R. H., León, I. R., Perales, J., Dantas, R. F., Albamo, R. M., Bastos, F. F., & Cunha Bastos, J. (2007). Cytosolic glutathione peroxidase from liver of pacu (*Piaractus mesopotamicus*), a hypoxia-tolerant fish of the Pantanal. Biochimie, 89, 1332–1342. https://doi.org/10.1016/j.biochi.2007.04.003

Dauda, A. B. (2020). Biofloc technology: A review on the microbial interactions, operational parameters and implications to disease and health management of cultured aquatic animals. Reviews in Aquaculture, 12, 1193–1210. https://doi.org/10.1111/raq.12379

Eaton, A. D., Clesceri, L. S., Rice, E. W., & Greenberg, A. E. (2005). Standard methods for the examination of water and wastewater (21st ed.) Washington, DC: APHA.

Gallardo-Collí, A., Pérez-Rostro, C. I., Hernández-Vergara, M. P., & Pérez-Legaspi, I. A. (2019a). Microeukaryote community and the nutritional composition of the biofloc during Nile tilapia culture in water-reusing biofloc systems. Aquaculture International, 27, 381–398. https://doi.org/10.1007/s10499-018-0335-2

Gallardo-Collí, A., Pérez-Rostro, C. I., & Hernández-Vergara, M. P. (2019b). Reuse of water from biofloc technology for intensive culture of Nile tilapia (*Oreochromis niloticus*): Effects on productive performance, organosomatic indices and body

composition. International Aquatic Research, 11, 43–55. https://doi.org/10.1007/s40071-019-0218-9

Garcés, S. C. C., González, J. J. G., & Carrasco, S. C. P. (2017). Biofloc effect on juvenils cachama blanca *Piaractus brachypomus* growth parameters. Medicina Veterinaria y Zootecnia, 12, 170–180. https://dx.doi.org/10.21615/cesmvz.12.3.1

Garcia, L. O., Copatti, C. E., Wachholz, F., Pereira Filho, W., & Baldisserotto, B. (2008). Freshwater temperature in the state of Rio Grande do Sul, Southern Brazil, and its implication for fish culture. Neotropical Ichthyology, 6, 275–281. http://doi.org/10.1590/S1679-62252008000200016

Gilmour, K. M. (2001). The CO_2/pH ventilatory drive in fish. Comparative Biochemistry and Physiology A, 130, 219–240. https://doi.org/10.1016/S1095-6433(01)00391-9

Goldenfarb, P. B., Bowyer, F. P., Hall, E., & Brosious, E. (1971). Reproducibility in the hematology laboratory: The microhematocrit determination. The American Journal of Clinical Pathology, 56, 35–39. https://doi.org/10.1093/ajcp/56.1.35

Green, B. W., Schrader, K. K., & Perschbacher, P. (2014). Effect of stocking biomass on solids, phytoplankton communities, common off-flavors, and production parameters in channel catfish biofloc technology production system. Aquaculture Research, 45, 1442–1458. https://doi.org/10.1111/are.12096

Hamilton, M. A., Russo, R. C., & Thurston, R. V. (1977). Trimmed Sperman-Karber: Method for estimating median lethal concentrations in toxicity biossays. Environmental Science Technology, 11, 714-719. https://doi.org/10.1021/es60130a004

Hargreaves, J. A. (2006). Photosynthetic suspended-growth systems in aquaculture. Aquacultural Engeneering, 34, 344–363. https://doi.org/10.1016/j.aquaeng.2005.08.009

Hargreaves, J. A. (2013). Biofloc production systems for aquaculture. SRAC Southern Regional Aquaculture Center. 4503:1–12.Holanda, M., Santana, G., Furtado, P., Rodrigues, R. V., Cerqueira, V. R., Sampaio, L. A., Wasielesky Jr., W., & Poersch, L. H. (2020). Evidence of total suspended solids control by *Mugil liza* reared in an integrated system with pacific white shrimp *Litopenaeus vannamei* using biofloc technology. Aquaculture Reports, 18, 100479. https://doi.org/10.1016/j.aqrep.2020.100479

Johnson, R. A. (2017). A quick reference on respiratory alkalosis. Veterinary Clinics: Small Animal Practice, 47, 181–184. https://doi.org/10.1016/j.cvsm.2016.10.005

Kim, J. H., Kim, S. K., & Kim, J. H. (2018). Bio-floc technology application in flatfish *Paralichthys olivaceus* culture: Effects on water quality, growth, hematological parameters, and immune responses. Aquaculture, 495, 703–709. https://doi.org/10.1016/j.aquaculture.2018.06.034

Lake, R. G., & Hinch, S. G. (1999). Acute effects of suspended sediment angularity on juvenile coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences, 56, 862–867. https://doi.org/10.1016/10.1139/f99-024

Lemos, C. H. da P., Chung, S., Ribeiro, C. V. di M., & Copatti, C. E. (2018). Growth and biochemical variables in Amazon catfish (*Pseudoplatystoma reticulatum*♀ x *Leiarius marmoratus*♂) under different water pH. Anais da Academia Brasileira de Ciências, 90, 3573–3581. http://dx.doi.org/10.1590/0001-3765201820180241

Long, L., Yang, J., Li, Y., Guana, C., & Wu, F. (2015). Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*). Aquaculture, 448, 135–141. https://doi.org/10.1016/j.aquaculture.2015.05.017

Luo, G., Gao, Q., Wang, C., Liu, W., Sun, D., Li, L., & Tan, H. (2014). Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. Aquaculture, 422–423, 1–7. http://dx.doi.org/10.1016/*j.aquaculture*.2013.11.023

NAS/NAE. (1973). Water Quality Criteria. Technical Report. Washington, DC: National Academy Press.

Nikinmaa, M. (2001). Haemoglobin function in vertebrates: Evolutionary changes in cellular regulation in hypoxia. Respiration Physiology 128, 317–329. https://doi.org/10.1016/s0034-5687(01)00309-7

Nitz, L. F., Maltez, L. C., Pellegrin, L., Garcia, L. O., Barbas, L. A. L., & Prentice-Hernández, C. (2019). Flesh quality and stress responses of *Piaractus mesopotamicus* after exposure to sublethal levels of ammonia and subsequent recovery. Boletim do Instituto de Pesca, 45~~(1):~~ e.325. https://doi.org/10.20950/1678-2305.2019.45.1.325

Nitz, L. F., Pellegrin, L., Pinto, D. S. B., Maltez, L. C., Copatti, C. E., & Garcia, L. O. (2020) a. Secondary stress responses to hypoxia and re-oxygenation at different temperatures in pacu (*Piaractus mesopotamicus*) juveniles. Aquaculture Research, 51, 4471–4481. https://doi.org/10.1111/are.14792

Nitz, L. F., Pellegrin, L., Maltez, L. C., Pinto, D., Sampaio, L. A., Monserrat, J. M., & Garcia, L. O. (2020) b. Temperature and hypoxia on oxidative stress responses in pacu *Piaractus mesopotamicus.* Journal of Thermal Biology, 92, 102682. https://doi.org/10.1016/j.jtherbio.2020.102682

Palmer, B. F. (2012). Evaluation and treatment of respiratory alkalosis. American Journal of Kidney Diseases, 60, 834–838. https://doi.org/10.1053/j.ajkd.2012.03.025

Pellegrin, L., Nitz, L. F., Maltez, L. C., Copatti, C. E., & Garcia, L. (2020). Alkaline water improves the growth and antioxidant responses of pacu juveniles (*Piaractus mesopotamicus*). Aquaculture, https://doi.org/10.1016/j.aquaculture.2019.734713

Pinto, D., Pellegrin, L., Nitz, L. F., Costa, S. T., Monserrat, J. M. & Garcia, L. (2019). Haematological and oxidative stress responses in *Piaractus mesopotamicus* under temperature variations in water. Aquaculture Research, 50, 3017–3027. https://doi.org/10.1111/are.14260

Poli, M. A., Legarda, E. C., Lorenzo, M. A., Martins, M. A., & Vieira, F. N. (2018). Pacific white shrimp and Nile tilapia integrated in a biofloc system under different fish-stocking densities. Aquaculture, 498, 83–89. http://dx.doi.org/10.1016/j.aquaculture.2018.08.045

Poli, M. A., Schveitzer, R., & Nuñer, A. P. O. (2015). The use of biofloc technology in a South American catfish (*Rhamdia quelen*) hatchery: Effect of suspended solids in the performance of larvae. Aquacultural Engineering, 66, 17–21. http://dx.doi.org/10.1016/j.aquaeng. 2015.01.004

Romano, N., Surratt, A., Renukdas, N., Monico, J., Egnew, N., Sinha, A.H. (2020). Assessing the feasibility of biofloc technology to largemouth bass *Micropterus salmoides* juveniles: Insights into their welfare and physiology. Aquaculture, 520, 735008. https://doi.org/10.1016/j.aquaculture.2020.735008

Schumann, M. & Brinker, A. (2020). Understanding and managing suspended solids in intensive salmonid aquaculture: A review. Reviews in Aquaculture, 12, 2109–2139. https://doi.org/10.1111/raq.12425

Sandoval-Vargas, L. Y., Jiménez-Amaya, M. N., Rodríguez-Pulido, J., Guaje-Ramírez, D. N., Ramírez-Merlano, J. A., & Medina-Robles, V. M. (2020). Applying biofloc technology in the culture of juvenile of *Piaractus brachypomus* (Cuvier, 1818):

Effects on zootechnical performance and water quality. Aquaculture Research, 51, 3865–3878. https://doi.org/10.1111/are.14734

Servizi, J. A., & Martens, D. W. (1987). Some effects of suspended Fraser River sediments on sockeye salmon (*Oncorhynchus nerka*). In H. D. Smith, L. Margolis & C. C. Wood (Eds.), Sockeye Salmon (*Oncorhynchus nerka*) population biology and future management (pp. 254-264). Ottawa: Canadian Government Publishing Centre.

Sprague J. B. (1971). Measurement of pollutant toxicity to fish: III. Sublethal effects and "SAFE" concentrations. Water Research, 5, 245–266. https://doi.org/10.1016/0043-1354(71)90171-0

Strickland, J. D. H., & Parsons, T. R. (1972). A practical handbook of seawater analysis. (2nd ed.). Ottawa: Fisheries Research Board of Canada.

Urbinati, E. C., Gonçalves, F. D., & Takahashi, L. S. (2010). Pacu (*Piaractus mesopotamicus*). In B. Baldisserotto, & L. C. Gomes (Eds.), Espécies nativas para piscicultura no Brasil (pp. 205-231). Santa Maria: UFSM.

Valenti, W. C., Barros, H. P., Moraes-Valenti, P., Bueno, G. W., & Cavalli, R.O. (2021). Aquaculture in Brazil: past, present and future. Aquaculture Reports, 19, 100611. https://doi.org/10.1016/j.aqrep.2021.100611

Valladão, G. M. R., Gallani, S. U., & Pilarski, F. (2016). South American fish for continental aquaculture. Reviews in Aquaculture, 10, 351–369. https://doi.org/10.1111/raq.12164

Wells, R. M. (2009). Blood-gas transport and hemoglobin function: Adaptations for functional and environmental hypoxia. In A. P. Farrell, J. G. Richards, & C. J. Brauner (Eds.), Fish physiology (pp. 255–299). London: Academic Press.

Witeska, M. (2013). Erythrocytes in teleost fishes: A review. Zoology and Ecology, 23, 275–281. https://doi.org/10.1080/21658 005.2013.846963

FIGURE 1. Survival (%) of pacu juveniles (*Piaractus mesopotamicus*) under different total suspended solids concentration (0; 1,500; 3,000; 4,000; 5,000; 6,000, and 7,000 mg L^{-1}) in BFT system by 96 h.

FIGURE 2. Blood variables of pacu juveniles (*Piaractus mesopotamicus*) under different total suspended solids concentrations (0; 250; 500; 750, and 1,000 mg L^{-1}) in BFT system. A = Glucose (mg dL^{-1}). B = Blood pH. C = Haematocrit (%). D = Erytrocytes (106 μL^{-1}). E = Hemoglobin (g dL^{-1}). Data are presented as mean ± SE (n = 9 fish per treatment). Different uppercase letters indicate statistically significant differences between same total suspended solids concentration in the different time (days) ($p < 0.05$). Different lowercase letters indicate statistically significant differences between treatment in the same time (days) ($p < 0.05$).

FIGURE 3. Blood variables of pacu juveniles (*Piaractus mesopotamicus*) under different total suspended solids concentrations (0; 250; 500; 750, and 1,000 mg L^{-1}) in BFT system. A = Mean Corpuscular Volume (MCV). B = Mean Corpuscular Haemoglobin (MCH). C = Mean Corpuscular Haemoglobin Concentration (MCHC). Data are presented as mean ± SE (n = 9 fish per treatment). Different uppercase letters indicate statistically significant differences between same total suspended solids concentration in the different time (days) ($p < 0.05$). Different lowercase letters indicate statistically significant differences between treatment in the same time (days) ($p < 0.05$).

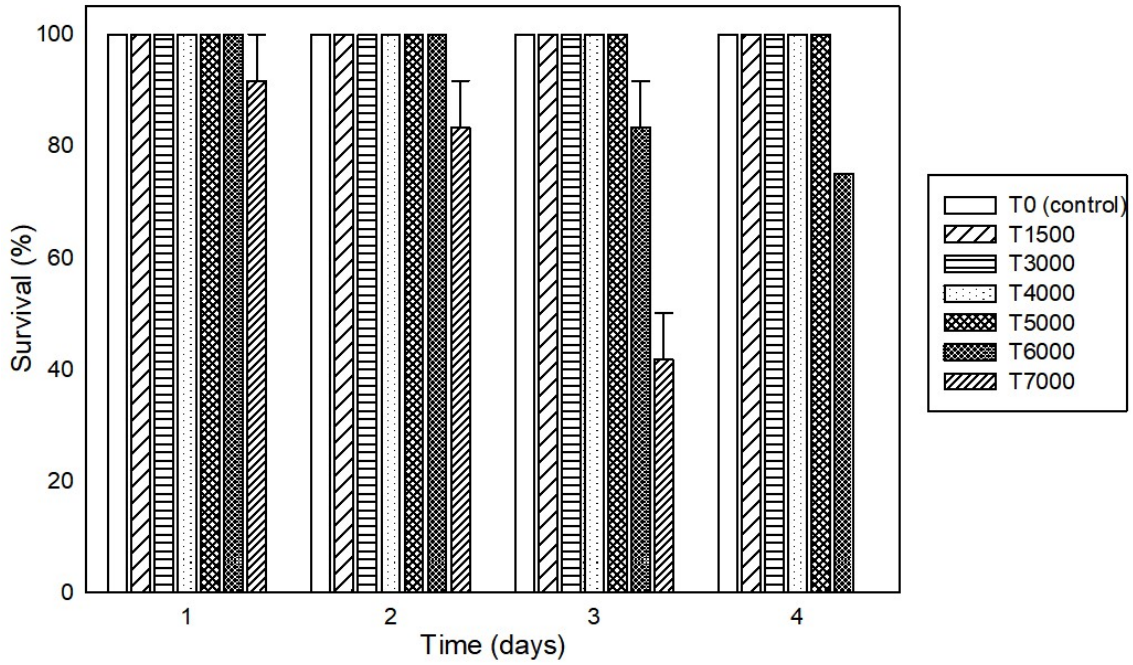
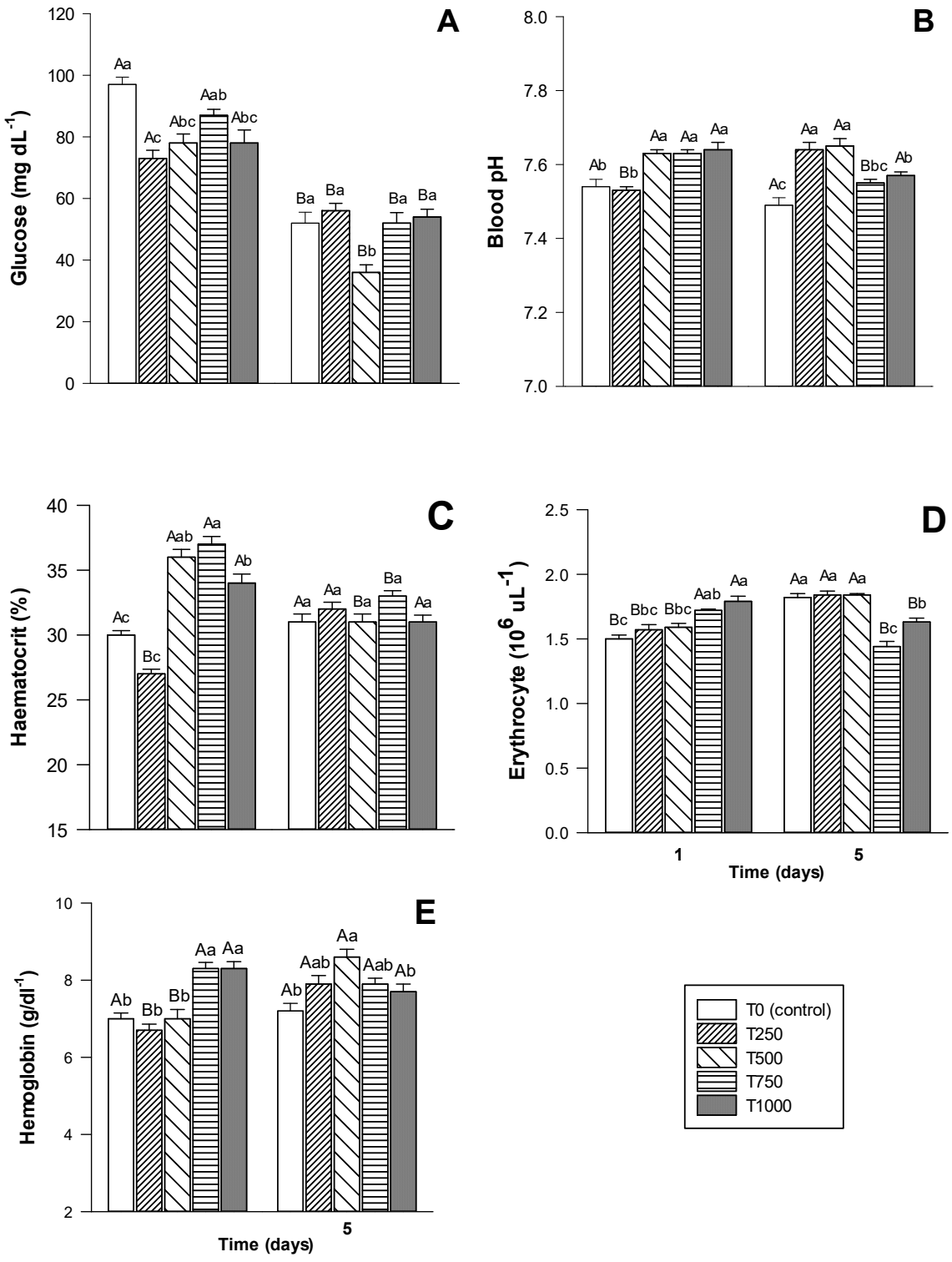
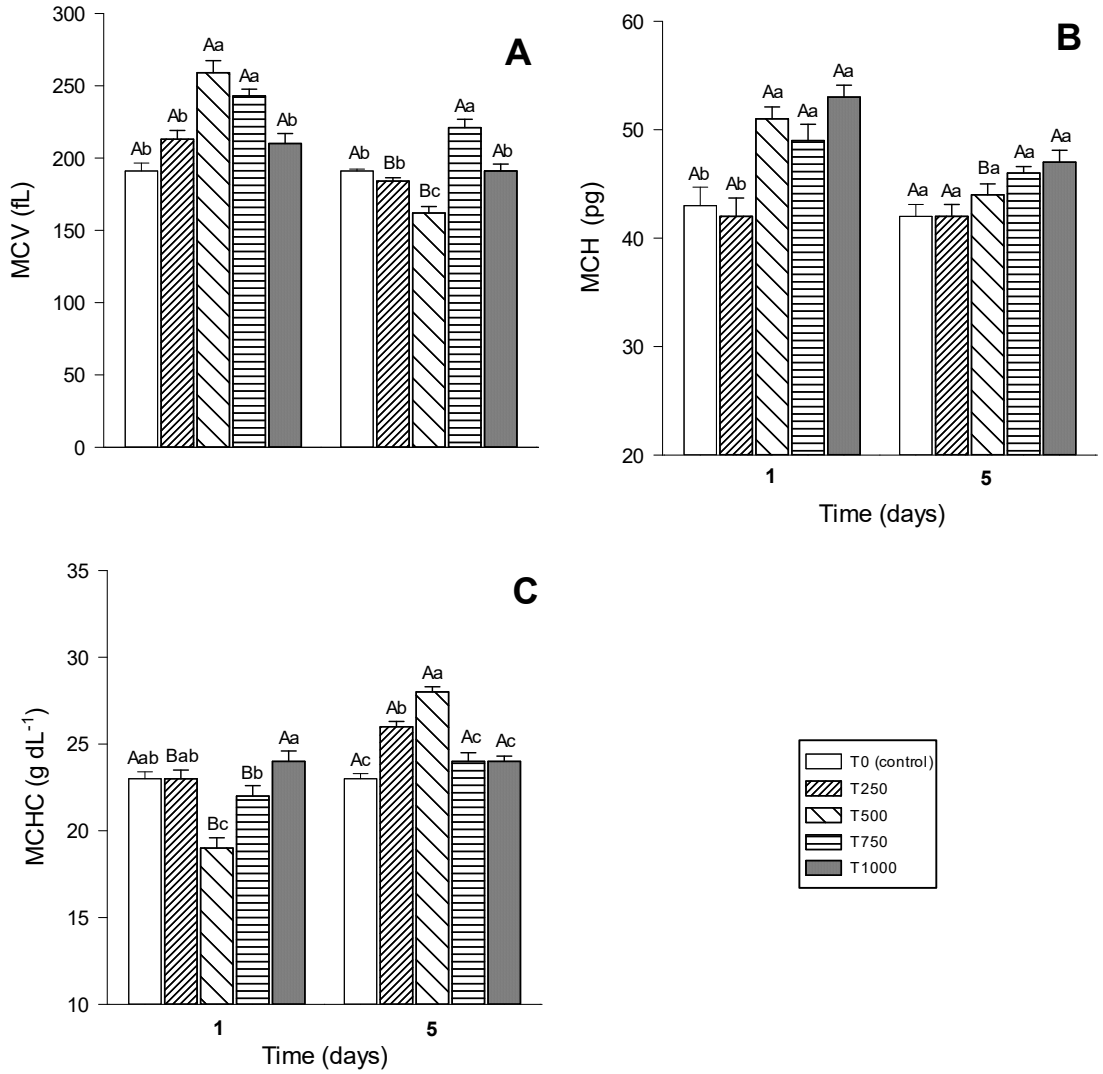

100
80
60
40
20
0
Survival (%)
1
2
3
4
Time (days)
T0 (control)
T1500
T3000
T4000
T5000
T6000
T7000

Figure 1


A
Glucose (mg dL-1)
120
100
80
60
40
20
0
Aa
Ac
Abc
Aab
Abc
Ba
Ba
Bb
Ba
Ba
B
Blood pH
8.0
7.8
7.6
7.4
7.2
7.0
Ab
Bb
Aa
Aa
Aa
Ac
Aa
Aa
Bbc
Ab
C
Haematocrit (%)
40
35
30
25
20
15
Ac
Bc
Aab
Aa
Ab
Aa
Aa
Ba
Ba
Aa
D
Erythrocyte (10^6 uL^-1)
2.5
2.0
1.5
1.0
0.5
0.0
Bc
Bbc
Bbc
Aab
Aa
Aa
Aa
Aa
Bc
Bb
1
5
Time (days)
E
Hemoglobin (g/dl-1)
10
8
6
4
2
Ab
Bb
Bb
Aa
Aa
Ab
Aab
Aa
Aab
Ab
5
Time (days)
T0 (control)
T250
T500
T750
T1000

1 Figure 2


A
MCV (fL)
300
250
200
150
100
50
0
Ab
Ab
Aa
Aa
Ab
Ab
Bb
Bc
Aa
Ab
B
MCH (pg)
60
50
40
30
20
Ab
Ab
Aa
Aa
Aa
Aa
Aa
Ba
Aa
Aa
1
5
Time (days)
C
MCHC (g dL-1)
35
30
25
20
15
10
Aab
Bab
Bc
Bb
Aa
Ac
Ab
Aa
Ac
Ac
1
5
Time (days)
T0 (control)
T250
T500
T750
T1000

1 Figure 3

Table 1. Physical-chemical parameters of water in pacu juveniles (*Piaractus mesopotamicus*) culture under different total suspended solids concentrations in BFT system by 96 h.

Parameters	Treatments						
	T0	**T1500**	**T3000**	**T4000**	**T5000**	**T6000**	**T7000**
TSS	0.01 ±0.01^{g}	1,358.33 ±73.50^{f}	3,150.00 ±68.62^{e}	4,300.00 ±46.55^{d}	4,900.00 ±198.33^{c}	6,067.67 ±176.38^{b}	7,092.67 ±87.96^{a}
SS	0.00± 0.01^{g}	60.00 ±2.89^{f}	180.00 ±5.00^{e}	250.67 ±4.63^{d}	300.67 ±6.23^{c}	375.33 ±7.69^{b}	415.67 ±2.96^{a}
DO	6.79 ±0.01	6.55 ±0.01	6.59 ±0.02	6.72 ±0.01	6.58 ±0.01	6.62 ±0.03	6.73 ±0.03
Temp	23.4 ±0.10	23.5 ±0.10	23.5 ±0.10	23.4 ±0.10	23.4 ±0.10	23.4 ±0.10	23.5 ±0.10
pH	7.50 ±0.05	7.59 ±0.01	7.57 ±0.10	7.68 ±0.01	7.57 ±0.01	7.58 ±0.10	7.63 ±0.10
TAN	0.00 ±0.00	0.08 ±0.01	0.12 ±0.02	0.10 ±0.01	0.11 ±0.01	0.13 ±0.02	0.10 ±0.01
UIA	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Nitrite	0.00 ±0.01	0.03 ±0.01	0.05 ±0.01	0.04 ±0.01	0.05 ±0.01	0.03 ±0.01	0.03 ±0.01
Alk	145.00 ±5.00	130.00 ±10.00	140.00 ±3.00	130.00 ±5.00	125.00 ±15.00	135.00 ±7.50	140.00 ±20.00

Total suspended solids (SST - mg L^{-1}), Suspended solids (SS - mL L^{-1}), temperature (Temp - °C), dissolved oxygen (DO – mg L^{-1} O_2), total ammonia (TAN - mg L^{-1} $N\text{-}NH_3$), un-ionized ammonia (UIA - µg L^{-1} $N\text{-}NH_3$), nitrite (mg L^{-1} $N\text{-}NO_2$) and alkalinity (Alk - mg $CaCO_3$ L^{-1}). Data are presented as mean ± SEM (n = 3 tanks per treatment). Different letters indicate statistically significant differences between treatments ($p < 0.05$).

Table 2. Physical-chemical parameters of water in pacu juveniles (*Piaractus mesopotamicus*) culture under different total suspended solids concentrations in BFT system by 5 days.

Parameters	Treatments				
	T0	**T250**	**T500**	**T750**	**T1000**
TSS	0.03±0.03^{e}	275.00±52.83^{d}	541.67±35.16^{c}	891.67±39.61^{b}	1152.67±42.20^{a}
SS	0.02±0.02^{e}	7.67±0.42^{d}	20.00±2.26^{c}	30.67±0.88^{b}	49.00±2.46^{a}
DO	6.44±0.09	6.41±0.08	6.24±0.07	6.18±0.06	6.07±0.04
Temp	23.61±0.25	22.87±0.23	23.39±0.31	23.48±0.21	23.43±0.16
pH	7.69±0.02	7.65±0.02	7.31±0.11	7.64±0.04	6.51±0.16
TAN	0.50±0.22	0.02±0.01	0.01±0.01	0.005±0.01	0.01±0.01
UIA	0.01±0.01	0.00±0.0	0.00±0.00	0.00±0.00	0.00±0.00
Nitrite	0.05±0.002	0.02±0.01	0.03±0.01	0.02±0.01	0.06±0.01
Alk	130.83±3.96	100.00±2.88	103.33±3.33	96.67±2.47	108.33±1.18

Total suspended solids (SST - mg L^{-1}), Suspended solids (SS - mL L^{-1}), temperature (Temp - °C), dissolved oxygen (DO – mg L^{-1} O_2), total ammonia (TAN - mg L^{-1} $N\text{-}NH_3$), un-ionized ammonia (UIA - µg L^{-1} $N\text{-}NH_3$), nitrite (mg L^{-1} $N\text{-}NO_2$) and alkalinity (Alk - mg $CaCO_3$ L^{-1}). Data are presented as mean ± SEM (n = 3 tanks per treatment). Different letters indicate statistically significant differences between treatments ($p < 0.05$).

Capitulo II

Can pacu juveniles (*Piaractus mesopotamicus*) cope with high concentrations of total suspended solids in the BFT system?

Running title: Pacu coping with total suspended solids

Lucas Pellegrin[a], Lilian Fiori Nitz[a], Daniel de Sá Brito Pinto[a], Carlos Eduardo Copatti[b], Wilson Wasielesky[c], Luciano Garcia[a*].

[a]Laboratório de Aquacultura Continental, Instituto de Oceanografia, Universidade Federal do Rio Grande - FURG, Rio Grande, RS, Brazil

[b]Instituto de Biologia, Universidade Federal da Bahia, Av. Adhemar de Barros, s/n, Salvador, BA, 40170-290, Brazil

[c]Laboratório de Carcinocultura, Instituto de Oceanografia, Universidade Federal do Rio Grande - FURG, Rio Grande, RS, Brazil

*Corresponding author: Laboratório de Aquacultura Continental, Universidade Federal do Rio Grande – FURG, 96201-900, Rio Grande, RS, Brazil. Phone: +55 53 3503-2474; E-mail address: garcia_log@hotmail.com

Artigo nas normas da revista"*Aquaculture*"

ABSTRACT

Biofloc technology (BFT) is a widely available system for aquaculture production, reducing water and artificial diet consumption. This study evaluated the survival, growth performance, and hematological parameters of pacu juveniles subjected to different total suspended solids (TSS) concentrations of the BFT system. Juveniles were exposed to five different TSS concentrations: 0, 250, 500, 750, and 1,000 mg L^{-1} (or control, BFT250, BFT500, BFT750 and BFT1000) for 30 days. The experimental design was completely randomized in five treatments and three replicates (9 fish per tank). Except for TSS and suspended solids, the water quality variables did not differ between treatments. The survival rate was 100%, and the growth performance was higher in the control group, followed by BFT1000. The hepatosomatic index was significantly lower in the control group compared to the BFT1000. In general, the highest blood glucose and pH levels were found in BFT500 and BFT750, respectively. The treatments with TSS showed several hematological changes (except for erythrocytes), with the greatest changes occurring in juveniles exposed to values above 500 mg L^{-1} TSS. In conclusion, although the treatments with TSS can reduce growth and/or cause hematological changes, it is a promising alternative for the pacu juveniles' production, mainly BFT1000.

Keywords: Biofloc Technology, glucose, growth performance, hematological parameters, survival.

1. Introduction

Biofloc technology (BFT) is a cultivation system that allows greater production of animals in a smaller area and with low environmental impacts in the aquaculture

(Avnimelech, 2006). This system generally uses a high stocking density of fish and/or shrimp, reduces the diseases and allows the water reuse between various cultivation cycles (Avnimelech, 2006; Little et al., 2008). To make this possible, organic ($NaHCO_3$ and $CaCO_3$) and inorganic (alcohols, acetate, sugars, glycerol, starches, and fibers) carbon sources are used, which stimulate heterotrophic bacteria and other microorganisms to convert ammonia and other nitrogenous compounds from feed leftovers, feces and even animals killed in microbial protein (bioflocin) (Azim and Little, 2008; Ray et al., 2012; Avnimelech, 2015). Therefore, these bioflocs are a complementary source in feeding aquaculture organisms (Sandoval-Vargas et al., 2020), reducing the accumulation of nitrogenous waste and improving feed conversion and use of protein from artificial diets (Hisano et al., 2020).

The BFT is a widely available system for shrimp production (Wasielesky Jr. et al., 2006; El-Sayed, 2020). Recently, it has also been applied to the cultivation of fish, such as Nile tilapia (*Oreochromis niloticus*) (Hisano et al., 2021; Shourbela et al., 2021), silver catfish (*Rhamdia quelen*) (Poli et al., 2015), piracanjuba (*Brycon orbignyanus*) (Sgnaulin et al., 2018) and cachama blanca (*Piaractus brachypomus*) (Garcés et al., 2017). Furthermore, a partial analysis of the cost-effectiveness revealed that the BFT model was more effective than the recirculation aquaculture system (RAS) in Nile tilapia culture (Luo et al., 2014).

In South America, where Brazil is the most prominent for continental fish farming, the cultivation of native fish species has been encouraged due to their importance in the sustainable development of aquaculture. Among neotropical species, the round fish (i.e., fish with a discoid body), composed of species such as tambaqui (*Colossoma macropomum*), cachama blanca, pacu (*Piaractus mesopotamicus*), and their hybrids, are the most produced (Valladão et al., 2018; Valenti et al., 2021). Pacu is a fish species that

has an omnivorous eating habit, which could be reared on a BFT system at least in its juvenile phase as a strategy to increase its growth performance, reducing production costs. In addition, its fillet is highly appreciated by the consumer market (Nitz et al., 2019) and, during cultivation, it adjusts to different environmental conditions, supporting changes in water quality parameters such as ammonia (Nitz et al., 2019), temperature (Pinto et al., 2020; Nitz et al., 2020), dissolved oxygen (Nitz et al., 2020) and pH (Copatti et al., 2019, Pellegrin et al., 2020).

During the cultivation of animals in the BFT system, there is usually an increase in total suspended solids (TSS) concentrations, composed of microbial aggregates from the assimilation of nitrogen compounds. Therefore, the TSS evaluation can quantify the level of biofloc in in this system, maintaining it in adequate conditions for fish (Poli et al., 2015). Nevertheless, the decomposition performed by microbial forms can increase the oxygen demand and, consequently, reduce the dissolved oxygen levels, which would hinder the development of cultivated species (Gaona et al., 2011; 2016). Moreover, the excess of these solids can also cause gill obstruction, as previously reported for white shrimp (*Litopenaeus vannamei*) (Schveitzer et al., 2013; Gaona et al., 2016), impairing growth and causing osmoregulatory and respiratory changes (with hematological alterations), which may affect even the animals' survival.

Before adopting pacu cultivation in the BFT system, the adjustment of this species to this system must be considered. Therefore, determining the best TSS concentrations that must be maintained for pacu to express its full productive potential is necessary. Thus, not only the evaluation of productive performance is crucial, but also the evaluation of hematological parameters, since it may show the effects of different TSS concentrations on health (Kim et al., 2018). Furthermore, the use of BFT in experimental conditions for pacu can also be advantageous from an economic point of view, mainly

concerning water renewal and artificial diet supply. Therefore, this study aimed to evaluate the survival, growth performance, and hematological parameters of pacu juveniles subjected to different TSS concentrations in the BFT system.

2. Material and methods

2.1 Ethic animal

The experimental protocol was approved by the Committee on Animal Experimentation of the Federal University of Rio Grande (FURG), process number Pq004/2020.

2.2 Biofloc technology system

The TSS concentrations were obtained by maintaining a BFT culture system of pacu and stimulating the microbial community using organic (cane molasses in the proportion of 15:1 C/N ratio) and inorganic ($NaHCO_3$) carbon sources. This system consisted of three 310 L tanks (250 L of useful volume), where about 100 juveniles (70.91 ± 0.47 g) were stored per tank. The TSS concentration maintained in these systems was 512.87 ± 8.11 mg L^{-1}. The TSS was composed of feces, feed pellets, and microbial growth. The bioflocs (~110-140 μm) were then collected and concentrated, and/or diluted according to each treatment. TSS concentrations according to each treatment were previously adjusted before fish storage.

2.3 Fish and experimental conditions

Pacu juveniles (135 fish, ~5 g) were obtained from a commercial fish farm located in Ajuricaba, RS, Brazil, and they were transported to the Continental Aquaculture Laboratory (LAC) of the Federal University of Rio Grande (FURG). They were kept in five RAS (each system with 3 tanks with 210 L of useful volume) until the experiments were carried out.

The laboratory conditions maintained during acclimation were constant aeration, pH > 7, dissolved oxygen > 7, total suspended solids (TSS) of 0.0 mg L^{-1}, and a natural photoperiod (12L:12D). During the experimental period, water quality parameters were kept similar to the acclimatization period except for the TSS concentrations, adjusted according to the determinations of each treatment. In addition, the following water physicochemical variables were monitored daily: TSS (Strickland and Parsons, 1972), suspended solids (SS) (cone Imhoff-30 min), temperature, dissolved oxygen (oxygen meter; DO 200A EcoSense®), pH (pH meter; HI 8424/HANNA®), alkalinity, hardness, total ammonia (Eaton et al., 2005), nitrite (Boyd & Tucker, 2014) and un-ionized ammonia (calculated from a conversion table specific for freshwater).

Juveniles were fed commercial food (32% crude protein, 3,200 kcal digestible energy: Supra Acqua Line®, São Leopoldo, RS, Brazil) twice daily (08:00 AM and 04:00 PM) at 3% of the biomass. The experimental design was completely randomized in five treatments and three replicates (9 fish per tank or 27 fish per treatment). The experiment lasted 30 days.

In this study, five TSS concentrations were tested: 0, 250, 500, 750, and 1,000 mg L^{-1}, which were here called control, BFT250, BFT500, BFT750, and BFT1000. The control treatment was maintained in a RAS. The other treatments were kept in a static

system in 310 L tanks (80 L of useful volume) with aeration. The water collected for water quality analysis was replaced.

2.4 Sample collection

Three fish from each tank (9 fish per treatment) were randomly sampled for biometric evaluations and blood collection on days 1 and 30. The juveniles were removed from the tank, anesthetized with benzocaine hydrochloride (50 ppm), and blood was collected (1 mL) from the caudal vasculature using heparinized syringes. The fish collected on day 1 did not return to the tanks. Thus, 6 fish per tank remained until the end of the experiment (day 30).

2.5 Hematological parameters

Blood glucose was recorded using a digital glucometer (Accu-Chek Performa/Roche ®). A 0.5 mL aliquot was placed in 1 mL microtubes and measured by a pH meter (HI2210/Hanna®) with a sensor (HI1083/Hanna®) to determine the blood pH.

Samples were then transferred to heparinized capillaries and centrifuged at 4 °C and 12000×*g* for 5 min to determine hematocrit (Hct) values using the microhematocrit method (Goldenfarb et al., 1971). Erythrocytes (Ery) were counted in a Neubauer chamber using a binocular optical microscope after diluting 0.02 mL blood in a 4.0 mL Natt and Herrick solution. The hemoglobin concentration (Hb) was quantified using a colorimetric kit (Bioclin®) and read on a spectrophotometer (540 nm).

Calculations of the hematimetric indices were estimated according to the following equations: mean corpuscular volume (MCV, fL) = Hct×10/Ery(×10^6 µL), mean

corpuscular hemoglobin (MCH, pg) = Hb×10/Ery(×10^6 μL), and mean corpuscular hemoglobin concentration (MCHC, g dL^{-1}) = Hb × 100/Hct.

2.6 Zootechnical performance

The biometric values served to calculate the following productive performance variables:

Weight gain (WG, g) = final body weight (g) − initial body weight (g);

Specific growth rate (SGR, % per day) = 100 × (Ln final weight (g) − Ln initial weight (g))/time (days);

Condition factor (CF, g cm^{-3}) = 100 × (body weight (g)/body length (cm))^3;

Feed conversion rate (FCR) = (feed intake(g)/weight gain(g));

Hepatosomatic index (HSI, %) = 100 × (liver weight/whole body weight);

Survival (%) = 100 × (final fish number/initial fish number).

2.7 Statistical analysis

Results are expressed as the mean ± Standard error of the mean (SEM). Levene's test was performed to evaluate the homogeneity of data variances. The variables were compared using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Comparisons with the control group were made using Dunnett's method. Differences were considered significant at $p<.05$.

3. Results

3.1 Water quality parameters

The TSS and SS differed significantly between treatments (BFT1000>BFT750>BFT500>BFT250>control; $p<.05$). However, the other water quality variables did not differ significantly between treatments (Table 1).

3.2 Zootechnical parameters

The survival rate was 100% in all treatments. The control group had final weight, weight gain, final length, and SGR significantly higher and FCR significantly lower than fish belonging to BFT250, BFT500, and BFT750 (except for SGR to BFT250) ($p<.05$). In addition, weight gain and final length were significantly higher, and HSI was significantly lower in the control group compared to the BFT1000 ($p<.05$). The weight gain of juveniles exposed to the treatment with the highest TSS concentration was significantly greater than in the other TSS concentrations ($p<.05$). Initial weight and condition factors did not differ between treatments ($p>.05$; Table 2).

3.3 Blood glucose and pH levels

Blood glucose levels decreased significantly at the end of the experiment (day 30) compared to the beginning of the experiment (day 1) ($p<.05$), except for BFT1000. On day 1, blood glucose levels were significantly higher and lower, respectively, in the BFT500 and BFT1000 than in the other treatments ($p<.05$). On day 30, blood glucose levels of BFT500 remained significantly higher than fish in control and BFT750 groups ($p<.05$; Fig. 1a). In addition, the BFT750 had blood pH values significantly higher than

in the other treatments on day 30 and in relation to the same treatment (BFT750) on day 1 ($p<.05$; Fig. 1b).

3.4 Hematological parameters

The hematocrit values of fish belonging to BFT500 and BFT750 were significantly higher than in other treatments on day 1 ($p<.05$). However, on day 30, these same treatments (BFT500 and BFT750) had an inverse effect and were significantly lower than the control group ($p<.05$). Additionally, on day 1, animals in the control and BFT750 groups had significantly lower and higher hematocrit values than on day 30 ($p<.05$; Fig. 2a). However, the erythrocyte counts did not differ significantly between treatments ($p>.05$; Fig. 2b).

Hemoglobin levels decreased significantly on day 30 compared to day 1 ($p<.05$), except for BFT1000. On day 1, BFT500 and BFT750 had hemoglobin levels significantly higher than BFT250 ($p<.05$). On day 30, hemoglobin levels were significantly higher in juveniles exposed to the treatment with the highest TSS concentration than in the other treatments ($p<.05$; Fig. 2c).

On day 1, the MCV and MCH values were significantly lower in the control and BFT250 groups than in the other treatments ($p<.05$). In general, the MCV and MCH values were significantly higher in animals exposed to BFT500 and BFT750 on day 1 than on day 30 ($p<.05$). Conversely, on day 30, the highest MCV and MCH values occurred in control and BFT1000 groups (except for MCH values between BFT750 and BFT1000) ($p<.05$; Fig. 2d and 2e).

On day 1, the CHCM values were significantly higher in BFT250 and BFT1000 than in the control and BFT750 groups, while the BFT500 had CHCM values

significantly higher than the BFT750 ($p<.05$). On day 30, the CHCM values were significantly higher in BFT750 and BFT1000 than in the other treatments. In addition, for BFT500, CHCM was significantly higher than the control group ($p<.05$). On day 1, CHCM values were significantly higher in the control and BFT250 groups and significantly lower in the BFT750 than the same treatments on day 30 ($p<.05$; Fig. 2f).

4. Discussion

Findings demonstrated that different TSS concentrations did not affect the fish survival, which indicates that values up to 1,000 mg L^{-1} TSS can be used in the cultivation of pacu juveniles. Hisano et al., (2021) also reported 100% survival for Nile tilapia maintained in the BFT system (732.13–1,389.75 mg L^{-1} TSS) for 60 days. On the other hand, the survival of piracanjuba (*Brycon orbignuanus*) was 77.80% in the BFT system with 4.29 mL L^{-1} of settling solids (TSS not informed) (Sgnaulin et al., 2018). The absence of mortality in concentrations up to 1,000 mg L^{-1} TSS for an extended period is crucial for choosing potential species for cultivation in the BFT system, and concentrations higher than this are not recommended due to the high biochemical oxygen demand reported in aquaculture organisms (Avnimelech, 2015; Hargreaves, 2013). In general, recommended TSS concentrations for fish and shrimp farming in this system range from 200-400 (Avnimelech, 2015) and 500-600 mg L^{-1} TSS (Gaona et al., 2011; Schveitzer et al., 2013), respectively. However, this study found that pacu juveniles can cope with higher TSS concentrations.

In our study, the most significant weight gain and final length occurred for the control group. Nevertheless, the evaluation of the other zootechnical parameters showed that the control group was superior to BFT250, BFT500, and BFT750. However,

compared to BFT1000, there were no statistical differences for final weight, SGR, and FCR. Furthermore, unlike the other treatments, the fish in the control group were reared in RAS, a system that has already proved efficient in previous research for pacu cultivated under different water quality conditions (Pinto et al., 2020; Nitz et al., 2020; Copatti et al., 2019; Pellegrin et al., 2020). Despite this, the current study proposed to verify the effectiveness of the BFT system given its economic advantages (e.g., reducing the use of water and artificial diets) to produce pacu juveniles (Luo et al., 2014).

Therefore, in the current study, BFT1000 was the treatment that achieved the best growth performance compared to other treatments in the BFT system. Moreover, there was also an increase in HSI in BFT1000 compared to the control group. The HSI is an analysis that can quantify the energy supply in the form of glycogen present in the liver (Cyrino et al., 2000). In short, we believe that the fish belonging to BFT1000 consumed a greater amount of bioflocin, which contributed to their better growth performance and caused a greater accumulation of energy in the liver. Although some differences were observed for growth performance in the control and BFT1000 groups, we did not find significant differences concerning the CF. The CF is an indicator of animal welfare related to the environment and nutrition and can help determine stressful situations and their effects on fish (Tavares-Dias et al., 2008).

In addition to the CF analysis, the assessment of the fish health status must also consider the analysis of the hematological parameters (Fazio, 2019). For example, blood glycemia can indicate both the animal's nutritional status and its ability to cope with stressful conditions (Oliveira et al., 2019). Blood glucose homeostasis involves the coordinated regulation of many metabolic pathways, as gluconeogenesis and glycolysis (Walker et al., 2020). In our study, hyperglycemia occurred in juveniles in BFT500 at the beginning and end of the experimental period, which could cause stress to the animals.

This would be possible since the increase in blood glucose levels may result from an increase in circulating blood cortisol and catecholamines (Lemos et al., 2018), which are hormones antagonistic to insulin and, therefore, inhibit blood glucose uptake in the cells and tissues of the organism (Walker et al., 2020). The BFT1000, in turn, showed an inverse behavior where blood glucose levels were the lowest at the beginning of the experiment and remained in homeostasis at the end, indicating that possibly the animals immediately adjusted to this experimental condition without triggering harmful changes to the body's use of energy.

Changes in blood pH levels may also indicate physiological imbalances attributed to changes in water parameters, such as TSS, and the maintenance of blood pH homeostasis is crucial for the proper functioning of body enzymes (Aboagye and Allen, 2018). In our study, blood pH levels remained stable in most treatments, except for fish in BFT750 at the end of the experimental period, which showed metabolic alkalosis. Furthermore, an increase in blood pH levels can alter ATPases and cause oxidative stress in the gills and kidneys of pacu (Copatti et al., 2019).

In our study, hematocrit values increased at the beginning of the experimental period for BFT500 and BFT750 and the end in the control group. This increase must have occurred due to the increase in VCM and/or MCH values. The modification of these two hematimetric parameters result in a change in hemoglobin levels (Pinto et al., 2019), which was reported for the BFT500 and BFT750, but not for the control group. The increase in hemoglobin levels can be a compensatory response of fish to partial or total deprivation of both supply and uptake of O_2 by fish (Wells, 2009). Therefore, in BFT500 and BFT750, the increased MCV and MCH values may be related to an increase in erythrocytes volume to improve oxygen supply to maintain physiological homeostasis.

Moreover, the blood hyperglycemia and alkalosis that occurred respectively in BFT500 and BFT750 may have caused stress in fish, which may have released catecholamines into the bloodstream that stimulate the functioning of Na^+/H^+. The increase in the sodium concentration in the erythrocytes may stimulate water entrance by osmosis, altering its volume (Thomas & Perry, 1992). In the control group, the higher hematocrit levels at the end of the experiment were caused by the increase in MCV values; however, no changes in hemoglobin were observed, which suggests a possible macrocytosis caused by the presence of older cells and/or cells with greater volume of water (Verde et al., 2011).

In the current study, the BFT1000 had higher hemoglobin values at the end of the experimental period, caused by increased MCH and MCHC values. The increase in hemoglobin levels reported in this treatment may suggest that the highest TSS concentration could negatively interfere with the uptake of O_2 by fish (Wells, 2009; Luo et al., 2014), causing a compensatory response from juveniles which resulted in increased hemoglobin concentration. Finally, despite several hematological changes observed in fish from this study, the number of erythrocytes did not change significantly, and thus it would be possible to avoid increasing blood viscosity (Verde et al., 2011).

5. Conclusion

Concentrations up to 1,000 mg L^{-1} TSS can be used in pacu production without causing mortality; nevertheless, animals kept in the absence of TSS showed the best growth performance. Concerning groups exposed to different TSS concentrations, the best results for zootechnical parameters occurred in the BFT1000. In addition, many alterations were observed in the hematological parameters of fish exposed to

concentrations above 500 mg L^{-1} TSS. Finally, although the treatments with TSS have reduced growth and/or caused hematological changes, it is a promising alternative for the pacu juveniles' production. Since the pacu did not present mortality and had an excellent ability to cope with the conditions tested in our study, we encourage further studies to analyze the cost-effectiveness of the BFT system for pacu farming.

Credit authorship contribution statement

LP and LFN: carried out the experiments, biometric and metabolic analyses and contribution in the discussion. CEC: statistical analysis, collaboration on discussion and final text. DSBP: collaboration on data analysis and discussion. WW: maintaining of BFT culture system and collaboration on discussion. LG: conception and design, supervised the findings and discussion of the results. All the authors have read and approved the manuscript.

Declaration of Competing Interest

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

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

This study was supported by research funds from the Fundação de Amparo a Pesquisa do Rio Grande do Sul (FAPERGS – PQG – 19/2551-0001890-8), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The authors are grate full to CNPq for productivity research fellowships provide for L.G. (311352/2020-0), W.W. (310652/2017-0) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Finance Code 88882.182342/2018-01 for a PhD scholarship provided for L.P.

References

Aboagye, D.L., Allen, P.J., 2018. Effects of acute and chronic hypoxia on acid–base regulation, hematology, ion, and osmoregulation of juvenile American paddlefish. Journal Comparative Physiology B, 188, 77–88. https://doi.org/10.1007/s00360-017-1104-7

Avnimelech, Y., 2006. Bio-filters: The need for a new comprehensive approach. Aquacultural Engineering, 34, 172–178. https://doi.org/10.1016/j.aquaeng.2005.04.001

Avnimelech, Y., 2015. Biofloc technology – A practical guide book (3rd ed.). Baton Rouge, LA: World Aquaculture Society.

Azim, M.E., Little, D.C., 2008. The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture, 283, 29–35. https://doi.org/10.1016/j.aquaculture.2008.06.036.

Boyd, C.E., Tucker, C.S., 2014. Handbook for aquaculture water quality. Handbook for Aquaculture Water Quality. P.439

Copatti, C.E., Baldisserotto, B., Souza, C.F., Monserrat, J.M., Garcia, L., 2019. Water pH and hardness alter ATPases and oxidative stress in the gills and kidney of pacu (*Piaractus mesopotamicus*). Neotropical Ichthyology, 17, e190032. https://doi.org/10.1590/1982-0224-20190032

Cyrino, J.E.P., Portz, L., Martino, R.C., 2000. Retenção de proteína e energia em juvenis de "black bass" *Micropterus salmoides*. Scientia Agricola, 57, 609–616. https://doi.org/10.1590/S0103-90162000000400003

Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E., 2005. Standard methods for the examination of water and wastewater. 21st ed. Washington: APHA.

El-Sayed, A.M., 2020. Use of biofloc technology in shrimp aquaculture: A comprehensive review, with emphasis on the last decade. Reviews in Aquaculture, 1–30. doi: 10.1111/raq.12494

Fazio, F., 2019. Fish hematology analysis as an important tool of aquaculture: A review. Aquaculture, 500, 237–242. https://doi.org/10.1016/j.aquaculture.2018.10.030

Garcés, S.C.C., González, J.J.G., Carrasco, S.C.P., 2017. Biofloc effect on juvenils cachama blanca *Piaractus brachypomus* growth parameters. Medicina Veterinaria y Zootecnia, 12, 170–180. http://dx.doi.org/10.21615/cesmvz.12.3.1

Gaona, C.A.P., Poersch, L.H., Krummenauer, D., Foes, G.K., Wasielesky, W.J., 2011. The effect of solids removal on water quality, growth and survival of *Litopenaeus vannamei* in a biofloc technology culture system. International Journal of Recirculating Aquaculture, 12, 54–73. http://hdl.handle.net/10919/90645

Gaona, C.A.P., Serra, F.P., Furtado, P.S., Poersch, L.H., Wasielesky, W.J., 2016. Effect of different total suspended solids concentrations on the growth performance of

Litopenaeus vannamei in a BFT system. Aquacultural Engineering, 72-73, 65–69. https://doi.org/10.1016/j.aquaeng.2016.03.004

Goldenfarb, P.B., Bowyer, F.P., Hall, E., Brosious, E., 1971. Reproducibility in the hematology laboratory: the microhematocrit determination. American Journal of Clinical Pathology, 56, 35–39. DOI: 10.1093/ajcp/56.1.35

Hargreaves, J.A., 2013. Bioflocs production system for aquaculture. Southern Regional Aquaculture Center (SRAC) Publication No. 4503.

Hisano, H., Parisi, J., Cardoso, I.L., Ferri, G.H., Ferreira, P.M.F., 2020. Dietary protein reduction for Nile tilapia fingerlings reared in biofloco technology. Journal of the World Aquaculture Society, 51, 452–462. DOI: 10.1111/jwas.12670

Hisano, H., Barbosa, P.T.L., Hayd, L.A., Mattioli, C.C., 2021. Comparative study of growth, feed efficiency, and hematological profile of Nile tilapia fingerlings in biofloc technology and recirculating aquaculture system. Tropical Animal Health and Production, 53-60. https://doi.org/10.1007/s11250-020-02523-z

Kim, J.H., Kim, S.K., Kim, J.H., 2018. Bio-floc technology application in flatfish *Paralichthys olivaceus* culture: Effects on water quality, growth, hematological parameters, and immune responses. Aquaculture, 495, 703–709. https://doi.org/10.1016/j.aquaculture.2018.06.034

Lemos, C.H.D.P., Chung, S., Ribeiro, C.V.D.M., Copatti, C.E., 2018. Growth and biochemical variables in Amazon catfish (*Pseudoplatystoma reticulatum*♀ x *Leiarius marmoratus*♂) under different water pH. Anais Academia Brasileira Ciencias, 90, 3573-3581. http://dx.doi.org/10.1590/0001-3765201820180241

Little, D.C., Murray, F.J., Azim, E., Leschen, W., Boyd, K., Watterson, A., Young, J.A., 2008. Options for producing a warm-water fish in the UK: limits to “Green

Growth"? Trends in Food Science & Technology, 19, 255–264. DOI:10.1016/J.TIFS.2007.12.003

Luo, G., Gaoa, Q., Wanga, C., Liu, W., Suna, D., Li, L., Tan, H., 2014. Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. Aquaculture, 422–423, 1–7. https://doi.org/10.1016/j.aquaculture.2013.11.023

Nitz, L.F., Maltez, L.C., Pellegrin, L., Garcia, L.O., Barbas, L.A.L., Prentice-Hernández, C., 2019. Flesh quality and stress responses of *Piaractus mesopotamicus* after exposure to sublethal levels of ammonia and subsequent recovery. Boletim do Instituto de Pesca, 45(1): e.325. https://doi.org/10.20950/1678-2305.2019.45.1.325

Nitz, L.F., Pellegrin, L., Pinto, D.S.B., Maltez, L.C., Copatti, C.E., Garcia, L.O., 2020. Secondary stress responses to hypoxia and re-oxygenation at different temperatures in pacu (*Piaractus mesopotamicus*) juveniles. Aquaculture Research, 51, 4471–4481. https://doi.org/10.1111/are.14792

Oliveira, C.P.B., Lemos, C.H. da P., Vidal, L.V.O., Couto, R.D., Pereira, D.S.P., Copatti, C.E., 2019. Anaesthesia with eugenol in hybrid Amazon catfish (*Pseudoplatystoma reticulatum* × *Leiarius marmoratus*) handling: Biochemical and haematological responses. Aquaculture, 501, 255–259. https://doi.org/10.1016/j.aquaculture.2018.11.046

Pellegrin, L., Nitz, L.F., Maltez, L.C., Copatti, C.E., Garcia, L., 2020. Alkaline water improves the growth and antioxidant responses of pacu juveniles (*Piaractus mesopotamicus*). Aquaculture, 519, 734713. https://doi.org/10.1016/j.aquaculture.2019.734713

Pinto, D., Pellegrin, L., Nitz, L.F., Costa, S.T., Monserrat, J.M. Garcia, L., 2019. Haematological and oxidative stress responses in *Piaractus mesopotamicus* under temperature variations in water. Aquaculture Research, 50, 3017–3027. https://doi.org/10.1111/are.14260

Pinto, D., Pellegrin, L., Nitz, L.F., Monserrat, J.M., Garcia, L., 2020. Influence of temperature on growth, feed consumption and chemical composition in the muscle of Pacu *Piaractus mesopotamicus*. Aquaculture Research, 00, 1–8. DOI: 10.1111/are.14726

Poli, M.A., Schveitzer, R., Nuñer, A.P.O., 2015. The use of biofloc technology in a South American catfish (*Rhamdia quelen*) hatchery: Effect of suspended solids in the performance of larvae. Aquacultural Engineering, 66, 17–21. http://dx.doi.org/10.1016/j.aquaeng. 2015.01.004

Ray, A.J., Seaborn, G., Vinatea, L., Browdy, C.L., Leffler, J.W., 2012. Effects of biofloc reduction on microbial dynamics in minimal-exchange, superintensive shrimp, *Litopenaeus vannamei*, culture systems. Journal of the World Aquaculture Society, 43 (6), 790–801. https://doi.org/10.1111/j.1749-7345.2012.00607.x

Sandoval-Vargas, L.Y., Jiménez-Amaya, M.N., Rodríguez-Pulido, J., Guaje-Ramírez, D.N., Ramírez-Merlano, J.A., Medina-Robles, V.M., 2020. Applying biofloc technology in the culture of juvenile of *Piaractus brachypomus* (Cuvier, 1818): Effects on zootechnical performance and water quality. Aquaculture Research, 51, 3865–3878. https://doi.org/10.1111/are.14734

Schveitzer, R., Arantes, R., Costódio, P.F.S., Santo, C.M.E., Vinatea, L.A., Seiffert, W.Q., Andreatta, E.R., 2013. Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated

with no water exchange. Aquacultural Engineering 56, 59–70. https://doi.org/10.1016/j.aquaeng.2013.04.006

Sgnaulin, T., Lemos de Mello, G., Thomas, M.C., Garcia, J.R.E., Montes de Oca, G.A.R., Emerenciano, M.G.C., 2018. Biofloc technology (BFT): An alternative aquaculture system for piracanjuba *Brycon orbignyanus*? Aquaculture, 485, 119-123.doi:10.1016/j.aquaculture.2017.11.043

Shourbela, R.M., Khatab, S.A., Hassan, M.M., Doan, H.V., Dawood, M.A.O., 2021. The Effect of Stocking Density and Carbon Sources on the Oxidative Status, and Nonspecific Immunity of Nile tilapia (*Oreochromis niloticus*) Reared under Biofloc Conditions. Animals, 11, 184. https://doi.org/10.3390/ani11010184

Tavares-Dias, M., Marcon, J.L., Lemos, J.R.G., Fim, J.D.I., Affonso, E.G., Ono, E.A., 2008. Índices de condição corporal em juvenis de *Brycon amazonicus* (SPIX and AGASSIZ,1829) e *Colossomoma macropomum* (CUVIER, 1818) na Amazônia. Boletimdo Instituto de Pesca, 34, 197–204.

Thomas, S., Perry, S.F., 1992. Control and consequences of adrenergic activation of red blood cell Na^+/H^+ exchange on blood oxygen and carbon dioxide transport in fish. Journal of Experimental Zoology, 263, 160−175. https://doi.org/10.1002/jez.1402630206

Valadão, G.M.R., Gallani, S.U., Pilarski, F., 2018. South American fish for continental aquaculture. Reviews in Aquaculture 10, 351-369. https://doi.org/10.1111/raq.12164

Valenti, W.C., Barros, H.P., Moraes-Valenti, P., Bueno, G.W., Cavalli, R.O., 2021. Aquaculture in Brazil: past, present and future. Aquaculture Reports, 19, 100611. https://doi.org/10.1016/j.aqrep.2021.100611

Verde, C., Giordano, D., Di Prisco, G., 2011. Blood. Erythropoiesis in fishes. In: Farrel, A. P., (Ed.) Encyclopaedia of fish physiology, from genome to environment, 2, 992-997. https://doi.org/10.1016/B978-0−12-374553-8.00268-9.

Walker, A.M., Copatti, C.E., Melo, F.V.S.T., Melo, J.F.B., 2020. Metabolic and physiological responses to intraperitoneal injection of chromium oxide in hyperglycaemic Nile tilapia juveniles. Aquaculture, 517, 734821. https://doi.org/10.1016/j.aquaculture.2019.734821

Wasielesky, J.R., Atwood, W.H., Stokes, A., Browdy, C.L., 2006. Effect of natural production in a zero-exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. Aquaculture, 258, 396-403. https://doi.org/10.1016/j.aquaculture.2006.04.030

Wells, R.M.G., 2009. Blood-gas transport and hemoglobin function: Adaptations for functional and environmental hypoxia. Fish Physiology, 27, 255-299. DOI: 10.1016/S1546-5098(08)00006-X

Table 1. Water physicochemical parameters of pacu juveniles (*Piaractus mesopotamicus*) under different total suspended solids concentrations in the BFT system by 30 days.

	Treatments				
Parameters	**Control**	**BFT250**	**BFT500**	**BFT750**	**BFT1000**
Temp	26.39±0.42	26.34±0.35	26.04±0.23	25.92±0.21	26.03±0.31
DO	6.06±0,07	5.85±0.12	5.86±0.14	5.83±0.10	5.65±0.11
pH	7.30±0.07	7.55±0.08	7.67±0.07	7.71±0.07	7.70±0.09
Nit	0.07±0.00	0.04±0.01	0.05±0.02	0.07±0.03	0.06±0.02
TA-N	0.08±0.02	0.06±0.05	0.06±0.04	0.07±0.03	0.04±0.02
UIA	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01
Alk	136.00±7.23	198.00±29.82	200.00±33.36	190.00±30.00	207.00±44.00
Hard	72.00±12.00	177.00±10.00	166.00±8.00	180.00±9.00	156.00±10.00
SS	0.00±0.00^{e}	19.09±2.48^{d}	26.74±5.12^{c}	42.60±5.61^{b}	63.10±11.70^{a}
TSS	0.00±0.00^{e}	251.75±41.40^{d}	488.00±111.81^{c}	680.59±54.89^{b}	954.76±100.15^{a}

Temperature (Temp) is expressed as °C, dissolved oxygen (DO) is expressed as mg L^{-1} O_2, total ammonia nitrogen (TA-N is expressed as mg L^{-1} NH_3-N, un-ionized ammonia (UIA) is expressed as µg L^{-1} NH_3-N, nitrite (Nit) is expressed as mg L^{-1} NO_2-N, alkalinity (Alk) and hardness (Hard) are expressed as mg $CaCO_3$ L^{-1}, total suspended solids (TSS) is expressed as mg L^{-1} and suspended solids (SS) as mL L^{-1}. Data are presented as mean ± SEM (n = 3 tanks per treatment). Different letters indicate statistically significant differences between treatments ($p<.05$).

Table 2. Growth performance of pacu juveniles (*Piaractus mesopotamicus*) under different total suspended solids concentrations in the BFT system in 30 days.

	Treatments				
Variables	**Control**	**BFT250**	**BFT500**	**BFT750**	**BFT1000**
IW	21.59±0.73	21.47±0.62	21.07±1.18	23.90±0.58	23.09±1.05
FW	44.02±1.75^{a}	34.95±1.53^{b}	35.20±1.75^{b}	37.02±1.17^{b}	41.05±1.52^{ab}
WG	22.43±1.15^{a}	13.49±0.41^{c}	14.14±0.43^{c}	13.33±0.55^{c}	17.96±1.20^{b}
FL	12.52±0.19^{a}	11.56±0.16^{b}	11.33±0.19^{b}	11.75±0.18^{b}	11.67±0.17^{b}
CF	2.25±0.07	2.21±0.06	2.40±0.05	2.37±0.09	2.48±0.07
SGR	2.32±0.21^{a}	1.76±0.08^{ab}	1.68±0.13^{b}	1.39±0.12^{b}	1.86±0.08^{ab}
FCR	1.32±0.14^{b}	2.00±0.11^{a}	2.19±0.006^{a}	2.01±0.16^{a}	1.78±0.21^{ab}
HSI	1.76±0.10^{b}	1.96±0.12^{ab}	2.02±0.09^{ab}	1.84±0.10^{ab}	2.21±0.07^{a}

IW (initial weight), FW (final weight), and WG (weight gain) are expressed in g, FL (final length) is expressed as cm, CF (condition factor) is expressed as g cm^{-3}*100, SGR (specific growth rate) is expressed as % day^{-1}, HSI (hepatosomatic index) is expressed as %. FCR = feed conversion ratio. Data are presented as mean ± SEM (n = 3 tanks per treatment). Different letters indicate statistically significant differences between treatments ($p<.05$).

Legend figures

Fig. 1. Blood glucose (a) and pH (b) levels of pacu juveniles (*Piaractus mesopotamicus*) on days 1 and 30 under different total suspended solids concentrations (0; 250; 500; 750 and; 1000 mg L^{-1} or control, BFT250, BFT500, BFT750 and BFT1000) in BFT system. Data are presented as mean ± SEM (n = 9 fish per treatment). Different uppercase letters indicate statistically significant differences between days ($p<.05$). Different lowercase letters indicate statistically significant differences between treatments ($p<.05$).

Fig. 2. Hematological parameters of pacu juveniles (*Piaractus mesopotamicus*) on days 1 and 30 under different total suspended solids concentrations (0; 250; 500; 750 and; 1000 mg L^{-1} or control, BFT250, BFT500, BFT750 and BFT1000) in BFT system. a = hematocrit; b = erythrocytes; c = hemoglobin; d = MCV (mean corpuscular volume); e = MCH (mean corpuscular hemoglobin) and; f = MCHC (mean corpuscular hemoglobin concentration) and. Data are presented as mean ± SEM (n = 9 fish per treatment). Different uppercase letters indicate statistically significant differences between days ($p<.05$). Different lowercase letters indicate statistically significant differences between treatments ($p<.05$).

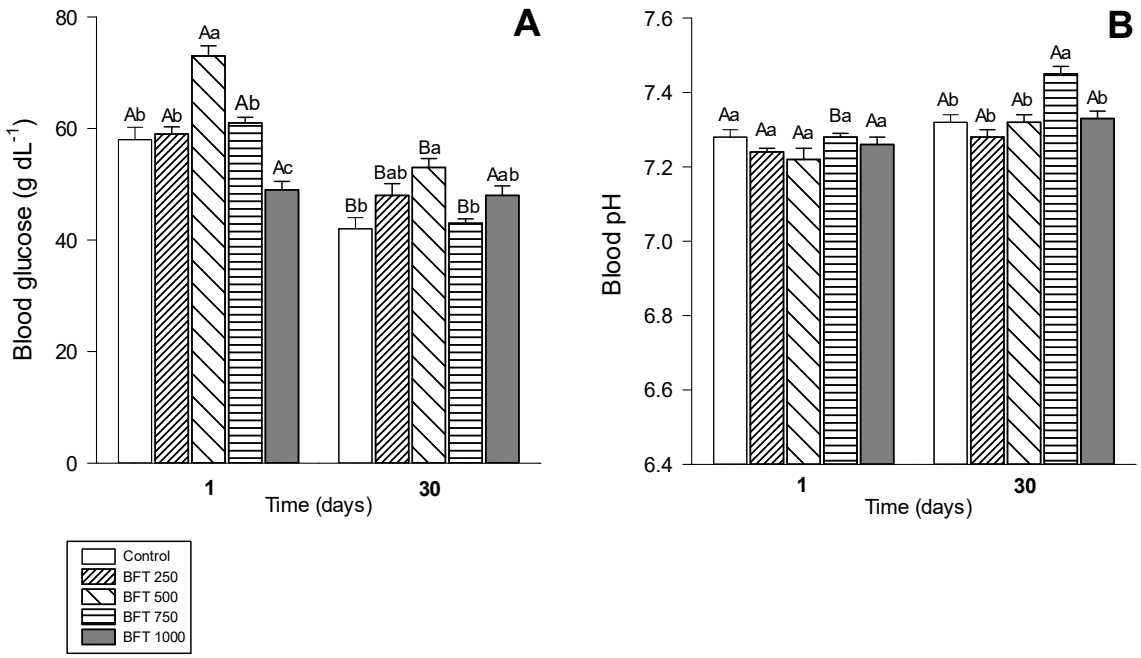

A
B
Blood glucose (g dL-1)
Blood pH
Time (days)
Time (days)
1
30
1
30
Ab
Ab
Aa
Ab
Ac
Bb
Bab
Ba
Bb
Aab
Aa
Aa
Aa
Ba
Aa
Ab
Ab
Ab
Aa
Ab
Control
BFT 250
BFT 500
BFT 750
BFT 1000

Figure 1

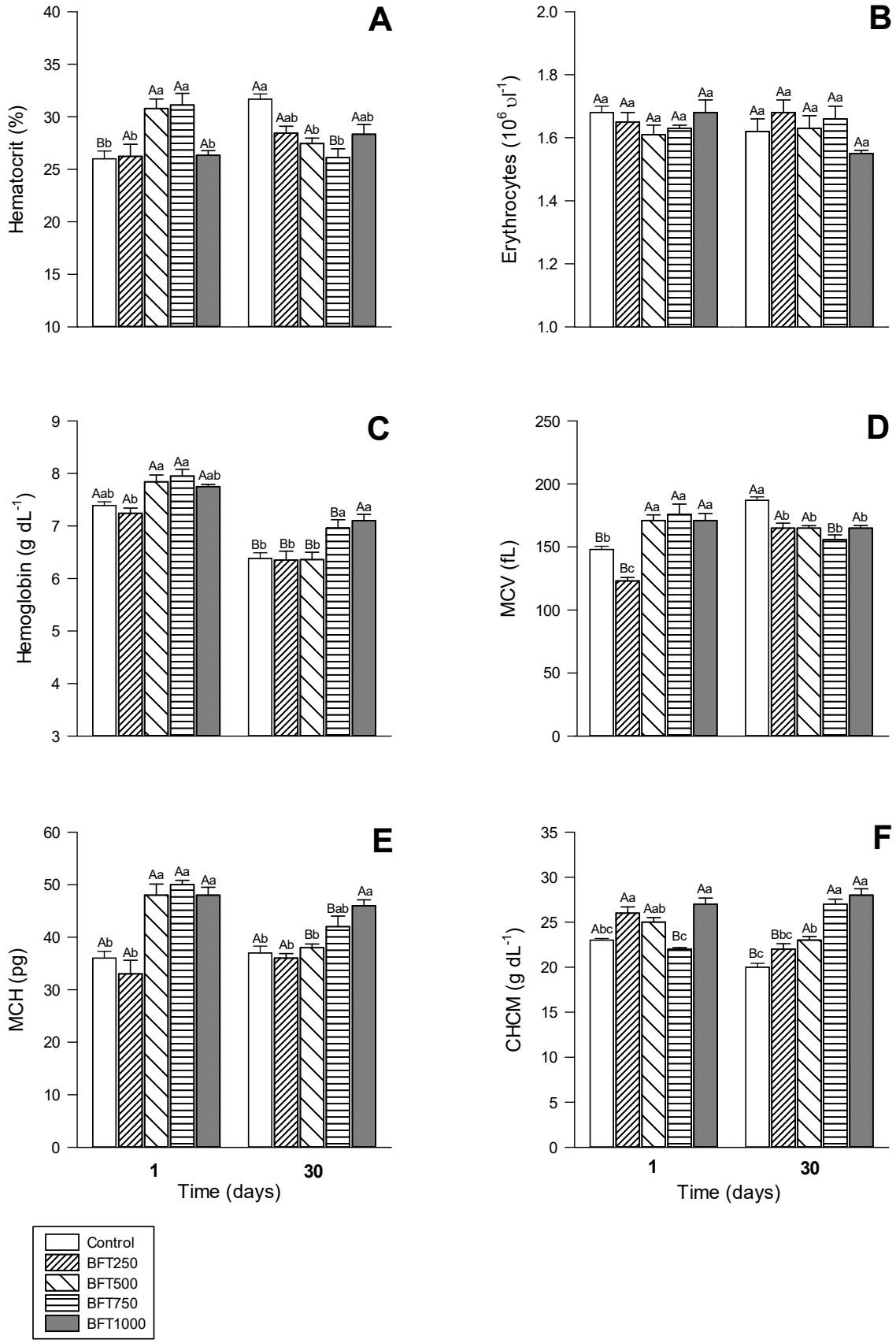

A
Hematocrit (%)
B
Erythrocytes (10^6 ul^{-1})
C
Hemoglobin (g dL^{-1})
D
MCV (fL)
E
MCH (pg)
F
CHCM (g dL^{-1})
1
30
Time (days)
Control
BFT250
BFT500
BFT750
BFT1000

Figure 2

Capitulo III

Effect of different stocking densities on hematological parameters and zootechnical performance of pacu (*Piaractus mesopotamicus*) juveniles in BFT system

Running title: Stocking density of pacu in BFT system

Lucas Pellegrin[a], Lilian Fiori Nitz[a], Daniel de Sá Brito Pinto[a], Carlos Eduardo Copatti[b], Wilson Wasielesky[c], Luciano Garcia[a*].

[a]Laboratório de Aquacultura Continental, Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG, Rio Grande, RS, Brazil.
[b]Laboratório de Carcinocultura, Instituto de Oceanografia, Universidade Federal do Rio Grande – FURG, Rio Grande, RS, Brazil.
[c]Instituto de Biologia, Universidade Federal da Bahia, Av. Adhemar de Barros, s/n, Salvador, BA, 40170–290, Brazil.

*Corresponding author: Laboratório de Aquacultura Continental, Universidade Federal do Rio Grande – FURG, 96201–900, Rio Grande, RS, Brazil. Phone: +55 53 3503–2474; E–mail address: garcia_log@hotmail.com

Artigo formatado nas normas da revista "*Aquaculture*"

Abstract

This study evaluated the growth performance and hematological parameters of pacu (*Piaractus mesopotamicus*) juveniles subjected to different stocking densities (SD) in biofloc technology (BFT) system. The individuals were distributed in five SD: 150, 300, 450, 600 and 750 fish m^{-3} (here called SD150, SD300, SD450, SD600 and SD750) for 45 days. The experimental design was completely randomized in five treatments and three replicates. The physical-chemical parameters of water quality remained stable. Survival was greater than 94% in all treatments. Increasing SD in the diet had a positive linear effect on initial and final biomass and a negative linear effect on final weight, final length, weight gain, specific growth rate and feed intake. The SD600 and SD750 showed the higher values for feed conversion rate. In general, the hepatosomatic index was higher in SD450. The highest SD had blood glucose values significantly higher than the other SD. Increasing SD had a positive linear effect on blood pH and MCV levels and a negative linear effect on hemoglobin and CHCM concentrations. In conclusion, pacu juveniles can be reared in a BFT system with SD of 450 fish m^{-3}, because it presented an increase in final biomass without compromising hematological parameters.

Keywords: biofloc, biomass, feed intake, glucose, hemoglobin, survival.

Introduction

An increase in the contribution of biofloc technology (BFT) system to the production of aquatic organisms, such as shrimp and fish, has been intensified since the decade of 2010 (Liu et al., 2018b; Battisti et al., 2020; Kaya et al., 2020; Almeida et al., 2021; Shourbela et al., 2021). Traditionally, this system depends on stimulating the formation of microbial aggregates (heterotrophic and chemoautotrophic bacteria,

protozoa, metazoans, rotifers, microalgae, feces, and remains of dead organisms) using intense aeration and organic (for example molasses, dextrose and, wheat bran) and inorganic carbon sources (for example $NaHCO_3$ and $CaCO_3$). Thus, nitrogen compounds are transformed into microbial biomass, which functions as a complementary source in the diet of fish and shrimp (Azim and Litle, 2008; De Schryver et al., 2008; Ray et al., 2012; Avnimelech, 2015). The intrinsic characteristics of a BFT system, such as its high amount of nutrients, make water renewal during cultivation unnecessary (Widanarni et al., 2012), which has contributed to the sustainable development of aquaculture.

Due to the greater control over the water quality parameters of a BFT system, it is possible to adopt the use of high stocking density (SD) to improve aquaculture species production (Adineh et al., 2019), including using SD higher than those used in a clear water system (Liu et al., 2018b; Fauji et al., 2018). The increase in SD is an alternative implemented by many aquaculture farmers to optimize spaces, increase productivity, and reduce costs, without the need to increase the size of the production units (Fauji et al., 2018; Chung et al., 2020). For fish, the BFT system could be advantageous in its juvenile phase, using high SD in smaller tanks, with greater control of predators and less water consumption, where microbial flocs become a complementary source of food or filtering and/or omnivorous species (Poli et al., 2018; Sandoval–Vargas et al., 2020).

The ideal SD for each species in each cultivation system must guarantee the best conditions for growth, survival, and welfare (Battisti et al., 2020). This can be achieved under conditions in which organisms are not affected by stressful factors such as: reduced supply of dissolved oxygen, accumulation of nitrogen compounds and confrontations for space and food (MacIntyre et al., 2008). The use of an SD above or even below the tolerance limit of a species, however, can result in stress, causing behavioral changes and physiological imbalance (Lemos et al., 2018). Therefore, there is mobilization of energy

sources for the maintenance of physiological systems and the consequent alteration of metabolic reserves, causing damage to animal health and growth (Barton and Iwama, 1991; Montero et al., 1999).

Stress in fish can be characterized in primary (neuroendocrine), secondary (metabolic and hematological) and tertiary (zootechnical performance) responses. However, such responses often do not follow a logical sequence in the organism, and can occur in an integrated manner (Bonga, 1997; Barton, 2002). In addition, such responses are quantitatively related to the severity and longevity of the stressor's action (Davis, 2006). Both high and low SD activate stress responses in fish (Lemos et al., 2018), which can be evaluated in a non–lethal way, through biometric assessments and blood collection for the performance of analyzes such as glucose, hematocrit, hemoglobin, and erythrocytes (Adam and Agab 2008; Satheeshkumar et al., 2012; Fazio et al. 2017; 2019).

Pacu (*Piaractus mesopotamicus*) is a species native to the Prata Basin, belonging to the group called "round fish", with good acceptance by the consumer market in Brazil, Paraguay, and Argentina (Valadão et al., 2018). Previous studies have already verified its good ability to adjust to several physical–chemical factors of water quality, such as dissolved oxygen (Nitz et al., 2020a,b), pH (Copatti et al., 2019; Pellegrin et al., 2020), temperature (Pinto et al., 2019; Nitz et al., 2020a,b) and nitrogen compounds (Nitz et al., 2019). In addition to its notorious ability to tolerate environmental changes, another important factor that indicates its potential to be cultivated in BFT systems is its omnivorous eating habit, which demonstrates that he could consume microbial flocs.

Therefore, this study aimed to evaluate the growth performance, survival, and hematological parameters of pacu juveniles subjected to different SD concentrations in BFT system.

Material e methods

Ethic animal

The experimental protocol was approved by the Committee on Animal Experimentation of Universidade Federal do Rio Grande – FURG, process number Pq004/2020.

Fish and experimental conditions

The pacu juveniles (n =500, ~5 g) were obtained from a commercial fish farm located in Ajuricaba, RS, Brazil, and they were transported to the Continental Aquaculture Laboratory (LAC) of the Federal University of Rio Grande (FURG). So, they were kept in five RAS (each system with 3 tanks with 250–L) until carrying out the experiments.

The laboratory conditions maintained during acclimation were constant aeration, pH > 7, dissolved oxygen > 7, total suspended solids (TSS) of 0.0 mg L^{-1} and a natural photoperiod (12L:12D). During the experimental period, water quality parameters were kept similar to acclimatization period, except for TSS concentrations, which were adjusted according to the determinations of each treatment. So, the following water physicochemical variables were monitored daily: TSS (Strickland and Parsons, 1972), suspended solids (SS) (cone Imhoff–30 min), temperature, dissolved oxygen (oxygen meter; DO 200A EcoSense®), pH (pH meter; HI 8424/HANNA®), alkalinity, hardness, total ammonia (Eaton et al., 2005), nitrite (Boyd & Tucker, 2014) and un–ionized ammonia (calculated from a conversion table specific for fresh water).

The juveniles were fed commercial food (32% crude protein, 3,200 kcal digestible energy; Supra Acqua Line®, São Leopoldo, RS, Brazil) twice daily (09:00 AM and 04:00

PM) provided until apparent satiety. The food consumption of each experimental unit was measured every three days by the difference in the weight of the diets contained in the containers.

The experimental design was completely randomized in five treatments and three replicates. Were tested five stocking densities: 150, 300, 450, 600 and 750 fish m^{-3} (or 12, 24, 36, 48 and 60 fish $tank^{-1}$ or 7.40, 14.78, 22.34, 29.70 and 37.22g L^{-1}), which were here called SD150, SD300, SD450, SD600 and SD750. The treatments were kept in a static system in 80 L–tanks (60 L of useful volume) with aeration. The water collected for water quality analysis was replaced. The experiment lasted 45 days.

Biofloc Technology system

The BFT system stimulating the microbial community using organic (cane molasses in the proportion of 15:1 C/N ratio) and inorganic ($NaHCO_3$) carbon sources. This system consisted of three 310–L tanks (250 L of useful volume), where about 100 pacu juveniles (70.91 ± 0.47 g) were stored per tank. The bioflocs (~110–140 μm) were then collected and distrutied in tanks before fish storage.

Zootechnical performance

At the end of the experimental period (45 days), the fish were fasted for 24 h before growth performance analysis and collection of blood samples. The total length (cm) and weight (g) of pacu juveniles were measured to calculate the following productive performance variables:

Weight gain (WG, g) = final body weight (g) − initial body weight (g);

Specific growth rate (SGR, % per day) = 100 × (Ln final weight (g) − Ln initial weight (g))/time (days);

Condition factor (CF, g cm^{-3}) = 100 × (body weight (g)/body length (cm))^3;

Feed intake (FI, g) = (total of consumed ration (g)/number of fish per repetition);

Feed conversion rate (FCR) = (feed intake(g)/weight gain(g));

Hepatosomatic index (HSI, %) = 100 × (liver weight/whole body weight)

Survival (%) = 100 × (final fish number/initial fish number).

Sample collection and hematological parameters

Three fish from each tank (9 fish per treatment) were randomly sampled for biometric evaluations and blood collection on day 45. The juveniles were removed from the tank, anesthetized with benzocaine hydrochloride (50 ppm) (Stringhetta et al., 2017), and 1 mL of blood was collected from each fish via a caudal vessel puncture using a sterile syringe containing 10 μL of heparin (5000 UI). After, the fish were killed to perform the HIS analysis.

Blood glucose was recorded using a digital glucometer (Accu–Chek Performa/Roche ®). To determine the blood pH, a 0.5 mL aliquot was placed in 1 mL microtubes and it was measured by a pH meter (HI2210/Hanna®) with sensor (HI1083/Hanna®).

The samples were then transferred to heparinized capillaries and centrifuged at 4 °C and 12000×*g* for 5 min to determine hematocrit (Hct) values using the microhematocrit method (Goldenfarb et al., 1971). Erythrocytes (Ery) were counted in a Neubauer chamber using a binocular optical microscope after diluting 0.02 mL blood in a 4.0 mL Natt and Herrick solution. The hemoglobin concentration (Hb) was quantified using a colorimetric kit (Bioclin®) and read on a spectrophotometer (540 nm). Calculations of the haematimetric indices were estimated according to the following equations: mean corpuscular volume (MCV, fL) = Hct×10/Ery(×106 μL), mean

corpuscular hemoglobin (MCH, pg) = Hb×10/Ery, and mean corpuscular hemoglobin concentration (MCHC, g dL^{-1}) = Hb × 100/Hct.

Statistical analysis

The results are expressed as the mean ± Standard error of the mean (SEM). Levene's test was performed to evaluate the homogeneity of data variances. Orthogonal polynomial contrasts with all values were used to determine the linear and quadratic effects of the different treatment levels. The data were subjected to regression analysis to fit the best model based on p–value. The variables also were compared using a one–way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences were considered significant at $p<0.05$.

Results

Water quality parameters

The water quality variables did not show a linear or quadratic regression, nor a significant difference among the treatments (Table 1).

Zootechnical parameters

The initial biomass differed significantly between treatments (SD750>SD600>SD450>SD300>SD150; $p<0.05$). The SD150 presented final weight, final length, weight gain and, SGR values significantly higher than the other treatments (except SGR for SD300) ($p<0.05$). In addition, these same variables presented values significantly lower in the SD600 and SD750 than the other treatments ($p<0.05$). Final biomass was significantly higher in treatments above 450 fish m^{-3} in comparison with

treatments with 150 or 300 fish m^{-3} ($p < 0.05$). The SD600 and SD750 showed significantly higher values for FCR than the other treatments ($p < 0.05$). The FCR was also significantly higher in SD750 than SD600 ($p < 0.05$). The CF was significantly higher in SD300 than SD150 ($p < 0.05$). Feed intake values were significantly higher in SD150 and SD300 than SD750 ($p < 0.05$). The HSI in SD450 and SD600 were significantly higher than SD150 ($p < 0.05$). In addition, HSI in SD450 also were significantly higher than SD300 and SD750 ($p < 0.05$) (Table 2).

Increasing SD had a positive linear effect on initial and final biomass and a negative linear effect on final weight, final length, weight gain, SGR and feed intake. A quadratic effect was found for FCR, where the highest values were found in the highest SD. The different SD used for pacu production did show a linear or quadratic regression, nor a significant difference among the treatments for initial weight, condition factor, HSI and, survival (Table 2).

Hematological parameters

The highest SD showed blood glucose values significantly higher than the other SD ($p<0.05$). In contrast, fish submitted to the SD600 or SD750 treatments showed significantly lower values than SD150 and SD300 treatments for CHCM and others SD for hemoglobin ($p<0.05$). Increasing SD had a positive linear effect on blood pH and MCV levels and a negative linear effect on hemoglobin and, than CHCM concentrations. The different SD used for pacu production did show a regression, nor a significant difference among the treatments for hematocrit, erythrocytes and, MCH (Table 3).

Discussion

Stressful situations triggered by improper SD can compromise water quality and, consequently, fish survival (Ni et al., 2014; Fauji et al., 2018; Liu et al., 2018a). Despite this, in the present study, water quality parameters and survival were not influenced by different treatments, where survival rates were high in all treatments (> 94%). However, growth performance and hematological parameters differed between treatments, where adverse effects on growth and health of pacu juveniles were verified mainly for the two largest SD (600 and 750 fish m^{-3}).

Chronic effect of stress caused by an inadequate SD can cause damage to fish farming, such as reduced growth, changes in food and reduced immune defenses in fish (Suárez et al., 2015, Lemos et al., 2018; Liu et al., 2018a; Chung et al., 2021). Our study found that a low SD resulted in higher final weight, final length, weight gain and SGR. However, to obtain greater profitability in fish farming, it is often more important to assess the final biomass, especially for fish in juvenile phase, before they are transferred to the farm, where they are grown to harvest size. Additionally, low SD can contribute to the occurrence of territorial behavior due to the underutilization of space with consequent unequal fish growth (Millán–Cubillo et al., 2016). Therefore, in the present study, the treatment that stood out was SD450, as it had a higher final biomass compared to treatments SD150 and SD300, with a weight gain that was higher than the treatments SD600 and SD750, which had a higher initial biomass. Generally, the increase in SD is carried out to increase production, however even with the increase in dietary supply, production capacity is limited, and an exceedingly high SD tends to reduce fish growth (Kpundeh et al., 2013; Chung et al., 2020). In the current study, when SD exceeded 600 fish m^{-3}, the negative effects of an increase in the number of fish per experimental unit were evident, since SD600 and SD750 treatments showed the worst results for growth performance.

In short, increasing SD is a strategy that allows increasing productivity by area or volume of water used and reducing production and investment costs, however, when it is done incorrectly it can compromise the growth of animals (Fauji et al., 2018). Similarly to our results, Lemos et al. (2018) found in a clear water experiment that it is preferable to keep Nile tilapia juveniles (*Oreochromis niloticus*) in an intermediate SD to achieve the best cost–benefit ratio in relation to their growth. Nile tilapia juveniles raised in high SD (600 fish m^{-3}) in BFT system showed reduced SGR (Liu et al., 2018a). In contrast, Battisti et al. (2020) found that the highest final weight of silver catfish (*Rhamdia quelen*) cultured in a BFT system occurred in the highest SD (30 g L^{-1}).

The increase in the final biomass found in the SD450 treatment in this study seems to be related to the increase in HSI verified in the same treatment. The HSI is an analysis that can quantify the energy supply in the form of glycogen present in the liver (Chellappa et al., 2006). Possibly, the fish belonging to SD450 treatment consumed a greater amount of bioflocin, which contributed for their growth and caused a greater accumulation of energy in the liver. Additionally, this treatment did not differ in relation to the other treatments in relation to the CF – an indicator of animal welfare related to nutrition. This demonstrates that, although CF values were higher in the SD300 treatment than the SD150 treatment, in general, the nutritional status of the fish in the SD450 treatment was similar to the other treatments.

The present study also found that a high SD was related to the worst results found for FCR (SD600 and SD750) and feed intake (SD750). This may indicate that not all animals were sufficiently fed during feeding due to the stress triggered by the smaller space available to fish (Van de Nieuwegiessen et al., 2009; Fauji et al., 2018). In addition, during a stressful situation to maintain homeostasis, there is a greater directing of energy towards priority organs and functions, decreasing the contribution to long–term anabolic

activities such as growth (Barcellos et al., 2000). The reduction in FCR with the increase in SD has already been reported for other fish species, such as Atlantic salmon (*Salmo salar*) (Liu et al., 2017) and golden pompano (*Trachinotus ovatus*) (Yang et al., 2020). This result demonstrates that juveniles used the energy of food for other physiological functions and not for growth, which is a tertiary response to stressful conditions (Goos & Costen 2002; Das et al., 2006).

In addition to a reduction in growth, the treatments with the highest SD of the current study also resulted in greater hematological changes and in the pH and blood glucose levels. Such parameters are causally related to both the nutritional conditions of cultivation and to stressful conditions (Pinto et al., 2019; Chung et al., 2020; Nitz et al., 2020a). In the present study, animals exposed to the SD750 treatment had the highest glucose levels. The increase in glucose would be triggered by the metabolism of carbohydrates and energy reserves during a stressful situation (Barton and Iwama, 1991; Wu et al., 2018; Walker et al., 2020), and has already been reported for pacu exposed to stressful conditions of temperature, ammonia and water oxygenation (Pinto et al., 2019; Nitz et al., 2020a,b). In the present study, possibly the fish submitted to the two major SD may have increased the respiratory rates (hyperventilation) to supply the greater oxygen demand necessary to maintain physiological homeostasis. In this situation, there is commonly CO_2/HCO_3 imbalance in the blood caused by increased CO_2 excretion and consequent metabolic alkalosis (Gilmour, 2001; Perry & Gilmour, 2006).

There is another factor that reinforces the hypothesis that blood alkalosis in the SD600 and SD750 treatments was caused by hyperventilation. This is due to such treatments having a lower amount of hemoglobin in the blood, reducing blood O_2 transport by decreasing the carrying capacity (Copatti et al., 2019), where hyperventilation could be a strategy to enhance Hb–O_2 binding affinity. The reduction in

hemoglobin values in fish caused by the increase in SD has been reported in previous studies for Nile tilapia juveniles (Kpundeh et al., 2013; Zaki et al., 2020).

The increase in SD also increased MCV values. Considering that HCM was not influenced by SD and CHCM decreased with increasing SD, the increase in MCV values does not seem to be related to the size of red blood cells (to support a higher hemoglobin concentration), but it must have been triggered by disorders ionic cells responsible for the accumulation of water within erythrocytes (Mcdonald and Milligan, 1997). In an integrative way, our study found that several hematological disorders occurred in SD600 and SD750 treatments and such changes help to explain the worst zootechnical performance found in these treatments. In contrast, in the treatments with 150 to 450 fish m^{-3}, the hematological parameters were more stable, and they are in accordance with the higher growth performance verified in the SD150, SD300 and SD450 treatments.

Conclusion

Pacu juveniles can be reared in a BFT system with a SD of 450 fish m^{-3}. Although this SD has presented a lower weight gain compared to the lowest SD (150 fish m^{-3}), the SD450 treatment allows the best use of the water volume without harming the fish health, because it presented an increase in final biomass without compromising hematological parameters. However, SD above 600 fish m^{-3} should be avoided, as fish subjected to this condition had several hematological disorders and reduced growth performance.

References

Adam, H.M., Agab, H., 2008. Haematological and biochemical indices of *Clarias garipeinus* collected from River Nile, Sudan. Suda Aca Sci J 2:91–97

Adineh, H., Naderi, M., Hamidi, M.K., Harsij, M., 2019. Biofloc technology improves growth, innate immune responses, oxidative status, and resistance to acute stress in common carp (*Cyprinus carpio*) under high stocking density. Fish and Shellfish Immunology. doi: https:// doi.org/10.1016/j.fsi.2019.10.057.

Azim, M.E., Little, D.C., 2008. The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture, 283, 29–35. https://doi.org/10.1016/j.aquaculture.2008.06.036.

Almeida, M.S., Carrijo–Mauad, J.R., Gimenes, R.M.T., Gaona, C.A.P., Furtado, P.S., Poersch, L.H., Wasielesky, W., Fóes, G.K., 2021. Bioeconomic analysis of the production of marine shrimp in greenhouses using the biofloco technology system. Aquaculture International. https://doi.org/10.1007/s10499–021–00653–1

Avnimelech, Y., 2015. Biofloc technology – A practical guide book (3rd ed.). Baton Rouge, LA: The World Aquaculture. Society.

Barcellos, L.J.G., Souza, S.M.G., Woehl, V.M., 2000. Estresse em peixes: Fisiologia da Resposta ao Estresse, Causas e Consequências (Revisão). Boletim do Instituto de Pesca, 26 (1): 99–111.

Battisti, E.K., Rabaioli, A., Uczay, J., Sutili, F.J., Lazzari, R., 2020. Effect of stocking density on growth, hematological and biochemical parameters and antioxidant status of silver catfish (*Rhamdia quelen*) cultured in a biofloc system. Aquaculture 524 (2020) 735213

Barton, B.A., Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of cortocosteroids. Annu. Rev. Fish Dis. 3–26. https://doi.org/https://doi.org/10.1016/0959–8030(91)90019–G

Chellappa, S., Huntingford, F.A., Strang, R. H. C., Thomson, R.Y., 2006. Condition factor and hepatosomatic index as estimates of energy status in male three–spined stickleback. Journal of Fish Biology 47, 775–787. DOI: 10.1111/j.1095–8649.1995.tb06002.x

Chung, S., Lemos, C.H.P., Teixeira, D.V., Fortes–Silva, R., Copatti, C.E., 2020. Essential oil from *Ocimum basilicum* improves growth performance and does not alter biochemical variables related to stress in pirarucu (*Arapaima gigas*). An. Acad. Bras. Ciênc. 92, e20181374. http://doi.org/10.1590/0001–3765202020181374

Copatti, C.E., Baldisserotto, B., Souza, C.F., Garcia, L.O. 2019 Protective effect of high hardness in pacu juveniles (*Piaractus mesopotamicus*) under acidic or alkaline pH: Biochemical and haematological variables. Aquaculture, 250–57. https://doi.org/10.1016/j.aquaculture.2018.12.028

Das, P.C., Ayyappan, S., Jena, J.K., 2006. Haematological changes in the three Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) exposed to acidic and alkaline water pH. Aquaculture 256 80–87.

Davis, K.B., 2006. Management of physiological stress in finfish aquaculture. N. Am. J. Aquac. 68, 116–121. https://doi.org/10.1577/A05–007.1.

De Schryver, P., Crab, R., Defoirdt, T., Boon, N., Verstraete, W., 2008. The basics of bioflocs technology: The added value for aquaculture. Aquaculture, 277, 125–137.

Fauji, H., Budiardi, T., Ekasari, J. 2018. Growth performance and robustness of African catfish *Clarias gariepinus* (Burchell) in biofloc–based nursery production with different stocking densities. Aquaculture Research. 49, 1339–1346. DOI: 10.1111/are.13595

Fazio, F., Saoca, C., Vazzana, I., Piccione, G., 2017. Influence of body size on blood hemogram in rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). Vet Med Open J 2:91–94. https://doi.org/10.17140/VMOJ–2–121

Fazio, F., 2019. Fish hematology analysis as an important tool of aquaculture: a review. Aquaculture. 500:237–242. https://doi.org/10.1016/j.aquaculture.2018.10.030

Gilmour, K. M. (2001). The CO_2/pH ventilatory drive in fish. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 130(2), 219–240. https://doi.org/10.1016/S1095–6433(01)00391–9

Goos, H.J.T., Consten, D., 2002. Stress adaptation, cortisol and pubertal development in the male common carp, *Cyprinus carpio*. Molecular and Cellular. Endocrinology.197: 105–116.

Kaya, D., Genc, E., Genc, M.A., Aktas, M., Eroldogan, O. T., Guroy, D., 2020. Biofloc technology in recirculating aquaculture system as a culture model for green tiger shrimp, *Penaeus semisulcatus*: Effects of different feeding rates and stocking densities. Aquaculture. https://doi.org/10.1016/j.aquaculture.2020.735526

Kpundeh, M.D., Xu, P., Yang, H., Qiang, J., He, J., 2013. Stocking densities and chronic zero culture water exchange stress' effects on biological performances, hematological and serum biochemical indices of GIFT tilapia juveniles (*Oreochromis niloticus*). J Aquac Res Development 4: 189 doi:10.4172/2155–9546.1000189

Lemos, C.HP., Ribeiro, C.V.M., Oliveira, C.P.B., Couto, R.D., Copatti, C.E., 2018. Effects of interaction between pH and stocking density on the growth, haematological and biochemical responses of Nile tilapia juveniles. Aquaculture 495, 62–67. https://doi.org/10.1016/j.aquaculture.2018.05.037.

Liu, B., Liu, Y., Sun, G., 2017. Effects of stocking density on growth performance and welfare–related physiological parameters of Atlantic salmon *Salmo salar* L. in recirculating aquaculture system. Aquac. Res. 48 (5), 2133–2144. https://doi.org/10.1111/are.13050

Liu, W., Luo, G., Chen, W., Tan, H., Wu, S., Zhang, N., Yu, Y., 2018a. Effect of no carbohydrate addition on water quality, growth performance and microbial community in water–reusing biofloc systems for tilapia production under high–density cultivation. Aquaculture Research. 49, 2446–2454. https://doi.org/10.1111/are.13704

Liu, G., Ye, Z., Liu, D., Zhao, J., Sivaramasamy, E., Deng, Y., Zhu, S., 2018b. Influence of stocking density on growth, digestive enzyme activities, immune responses, antioxidant of *Oreochromis niloticus* fingerlings in biofloc systems. Fish and Shellfish Immunology. DOI: 10.1016/j.fsi.2018.07.047

Mcdonald, G.; Milligan, C.L. 1997. Ionic, osmotic and acid-base regulation in stress. In Iwama, G.W.; Pickering, A.D.; Sumpter, J.P.; Schreck, C.B. (Eds.). Fish stress and health in aquaculture. Cambridge: University Press, p. 119-144.

MacIntyre, C., Ellis, T., North, B.P., Turnbull, J.F., 2008. The influences of water quality on the welfare of farmed trout: a review. In: Branson, E.J. (Ed.), Fish Welfare. Blackwell Publishing, Oxford, pp. 150–184.

Millán–Cubillo, A.F., Martos–Sitcha, J.A., Ruiz–Jarabo, I., Cárdenas, S., Mancera, J.M., 2016. Low stocking density negatively affects growth, metabolism and stress pathways in juvenile specimens of meagre (*Argyrosomus regius*, Asso 1801). Aquaculture 451, 87–92. http://dx.doi.org/10.1016/j.aquaculture.2015.08.034.

Montero, D., Izquierdo, M.S., Tort, L., Robaina, L., Vergara, J.M., 1999. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. Fish Physiol. Biochem. 20, 53–60. https://doi.org/10.1023/A:1007719928905.

Nitz, L. F., Maltez, L. C., Pellegrin, L., Garcia, L. O., Barbas, L. A. L., Prentice–Hernández, C., 2019. Flesh quality and stress responses of *Piaractus*

mesopotamicus after exposure to sublethal levels of ammonia and subsequent recovery. Bol. Inst. Pesca 2019, 45(1): e.325. https://doi.org/10.20950/1678–2305.2019.45.1.325

Nitz, L. F., Pellegrin, L., Pinto, D. S.B., Maltez, L. C., Copatti, C.E., Garcia, L. O. 2020a. Secondary stress responses to hypoxia and re–oxygenation at different temperatures in pacu (*Piaractus mesopotamicus*) juveniles. Aquaculture Research. 2020; 00:1–11.

Nitz, L.F., Pellegrin, L., Maltez, L. C., Pinto, D., Sampaio, L.A., Monserrat, J.M., Garcia, L.O., 2020b. Temperature and hypoxia on oxidative stress responses in pacu *Piaractus mesopotamicus.* Journal of Thermal Biology 92, 102682. https://doi.org/10.1016/j.jtherbio.2020.102682

Pellegrin, L., Nitz, L.F., Maltez, L.C., Copatti, C.E., Garcia, L., 2020. Alkaline water improves the growth and antioxidant responses of pacu juveniles (*Piaractus mesopotamicus*). Aquaculture, https://doi.org/10.1016/j.aquaculture.2019.734713

Perry, S. F., & Gilmour, K. M. (2006). Acid–base balance and CO_2 excretion in fish: Unanswered questions and emerging models. Respiratory Physiology & Neurobiology, 154(1–2), 199–215. https ://doi. org/10.1016/j.resp.2006.04.010

Pinto, D., Pellegrin, L., Nitz, L.F., Costa, S.T., Monserrat, J.M. Garcia, L., 2019. Haematological and oxidative stress responses in *Piaractus mesopotamicus* under temperature variations in water. Aquacult. Res. 50, 3017–3027. https://doi.org/10.1111/are.14260

Poli, M.A., Legarda, E. C., Lorenzo, M. A., Martins, M. A., Vieira, F. N., 2018. Pacific white shrimp and Nile tilapia integrated in a biofloc system under different fish–stocking densities. Aquaculture, 498, 83–89. http://dx.doi.org/10.1016/j.aquaculture.2018.08.045

Ray, A.J., Seaborn, G., Vinatea, L., Browdy, C.L., Leffler, J.W., 2012. Effects of biofloc reduction on microbial dynamics in minimal–exchange, superintensive shrimp, *Litopenaeus vannamei*, culture systems. J. World Aquacult. Soc. 43 (6), 790–801.

Sandoval–Vargas, L.Y., Jiménez–Amaya, M.N., Rodríguez–Pulido, J., Guaje–Ramírez, D.N., Ramírez–Merlano, J.A., Medina–Robles, V.M., 2020. Applying biofloc technology in the culture of juvenile of *Piaractus brachypomus* (Cuvier, 1818): Effects on zootechnical performance and water quality. Aquaculture Research, 51, 3865–3878. https://doi.org/10.1111/are.14734

Shourbela, R.M., Khatab, S.A., Hassan, M.M., Doan, H.V., Dawood, M.A.O., 2021. The effect of stocking density and carbon sources on the oxidative status, and nonspecific immunity of Nile tilapia (*Oreochromis niloticus*) reared under biofloc conditions. Animals, 11, 184. https://doi.org/10.3390/ani11010184

Stringhetta GR, Barbas LAL, Maltez LC, Sampaio LA, Monserrat JM, Garcia LO. Oxidative stress responses of juvenile tambaqui *Colossoma macropomum* after short-term anesthesia with benzocaine and MS-222. An Acad Bras Ciênc. 2017; 89(3 Suppl.):2209-2218.

Suárez, M., Trenzado, C., García–Gallego, M., Furné, M., García–Mesa, S., Domezain, A., Alba, I. & Sanz, A., 2015. Interaction of dietary energy levels and culture density on growth performance and metabolic and oxidative status of rainbow trout (*Oncorhynchus mykiss*). Aquacultural Engineering, 67, 59–66. https://doi.org/10.1016/j.aquaeng.2015.06.001

Valadão, G.M.R., Gallani, S.U., Pilarski, F., 2018. South American fish for continental aquaculture. Reviews in Aquaculture 10: 351–369. https://doi.org/10.1111/raq.12164

Van de Nieuwegiessen, P.G., Olwo, J., Khong, S., Verreth, J.A., Schrama, J. W., 2009. Effects of age and stocking density on the welfare of African catfish, *Clarias gariepinus* Burchell. Aquaculture. 288, 69–75. https://doi.org/10.1016/j.aquaculture.2008.11.009

Widanarni, Ekasari, J. & Maryam, S. (2012) Evaluation of Biofloc Technology Application on Water Quality and Production Performance of Red Tilapia *Oreochromis sp*. Cultured at Different Stocking Densities. HAYATI Journal of Biosciences, 19, 73–80.

Walker, A.M., Copatti, C.E., Melo, F.V.S.T., Melo, J.F.B., 2020. Metabolic and physiological responses to intraperitoneal injection of chromium oxide in hyperglycaemic Nile tilapia juveniles. Aquaculture 517, 734821. https://doi.org/10.1016/j.aquaculture.2019.734821

Wu, F., Wen, H., Tian, J., Jiang, M., Liu, W., Yang, C., Yu, L., Lu, X., 2018. Effect of stocking density on growth performance, serum biochemical parameters, and muscle texture properties of genetically improved farm tilapia, *Oreochromis niloticus*. Aquac. Int. 26, 1247–1259. https://doi.org/10.1007/s10499–018–0281–z

Yanga, Q., Guob, L., Liu, B., Guob, H., Zhub, K., Zhang, N., Jiang, S., Zhang, D., 2020. Effects of stocking density on the growth performance, serum biochemistry, muscle composition and HSP70 gene expression of juvenile golden pompano *Trachinotus ovatus* (Linnaeus, 1758). Aquaculture 518, 734841. https://doi.org/10.1016/j.aquaculture.2019.734841

Table 1. Physical–chemical variables of pacu juveniles (*Piaractus mesopotamicus*) maintained under different stocking densities in BFT system.

	Stocking density (fish m^{-3})				
Variables	**150**	**300**	**450**	**600**	**750**
DO	7.88±0.11	7.69±0.15	7.61±0.17	7.65±0.15	7.39±0.17
Temp	24.03±0.59	23.98±0.59	23.77±0.55	23.61±0.57	23.60±0.56
pH	8.34±0.08	8.09±0.13	7.92±0.17	7.96±0.14	7.88±0.14
TAM	0.05±0.04	0.00±0.00	0.06±0.05	0.01±0.02	0.09±0.09
UIA	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Nitrite	0.01±0.01	0.05±0.03	0.10±0.05	0.08±0.04	0.12±0.03
Alk	177.71±16.19	163.54±18.60	165.00±18.98	152.92±21.54	165.21±21.66
TSS	469.05±84.61	540.48±99.92	588.10±72.05	604.76±3.95	647.22±4.26
SS	30.30±3.89	32.33±3.91	31.47±2.30	30.63±87.48	26.45±90.40

Dissolved oxygen (DO) is expressed as mg L^{-1} O_2, temperature (Temp) is expressed as °C, total ammonia (TAM) is expressed as mg L^{-1} $N–NH_3$, un–ionized ammonia (UIA) is expressed as µg L^{-1} $N–NH_3$, nitrite (Nit) is expressed as mg L^{-1} $N–NO_2$, alkalinity (Alk) is expressed as mg $CaCO_3$ L^{-1}, total suspended solids (TSS) is expressed as mg L^{-1} and suspended solids (SS) as mL L^{-1}. Data are presented as mean ± SEM (n = 3 tanks per treatment). Different letters indicate statistically significant differences between treatments ($p<.05$).

Table 2. Growth performance of pacu (*Piaractus mesopotamicus*) maintained under different stocking densities in BFT system.

	Stocking density (fish m^{-3})				
Variables	**150**	**300**	**450**	**600**	**750**
IW	36.99±0.49	36.94±0.27	37.23±0.22	37.13±0.36	37.22±0.44
IB[1]	443.83±5.89^{e}	886.53±6.52^{d}	1340.13±7.77^{c}	1782.20±17.40^{b}	2233.33±26.56^{a}
FW[2]	65.09±1.40^{a}	58.36±1.75^{b}	57.46±1.60^{b}	42.27±0.82^{c}	38.29±0.30^{c}
FB[3]	738.57±37.66^{c}	1325.09±79.59^{b}	2049.68±63.91^{a}	1974.15±74.48^{a}	2259.64±56.05^{a}
FL[4]	14.32±0.32^{a}	13.10±0.18^{b}	13.21±0.25^{b}	12.03±0.11^{c}	11.62±0.11^{c}
WG[5]	28.10±0.97^{a}	21.42±1.66^{b}	20.24±1.82^{b}	5.14±0.83^{c}	1.07±0.47^{c}
SGR[6]	1.26±0.02^{a}	1.01±0.06^{ab}	0.96±0.07^{b}	0.29±0.04^{c}	0.05±0.03^{c}
CF	2.22±0.10^{b}	2.60±0.11^{a}	2.50±0.08^{ab}	2.43±0.02^{ab}	2.44±0.04^{ab}
FCR[7]	1.10±0.07^{c}	1.38±0.10^{c}	1.27±0.08^{c}	3.88±0.18^{b}	21.23±0.32^{a}
FI[8]	28.75±3.93^{a}	26.39±3.87^{a}	25.22±2.84^{ab}	15.20±5.28^{ab}	9.31±2.30^{b}
HSI	1.18±0.07^{c}	1.37±0.05^{bc}	1.63±0.03^{a}	1.49±0.05^{ab}	1.34±0.04^{bc}
Surv	94.45±2.78	94.45±2.78	99.07±0.93	97.22±1.84	98.34±1.66

IW (initial weight); FW (final weight); IB (initial biomass); FB (final biomass); WG (weight gain) and; FI (feed intake) are expressed in g; FL (final length) is expressed in cm; SGR (specific growth rate) is expressed as % day^{-1}; CF (condition factor) is expressed as g cm^{-3}*100; HSI (hepatosomatic index) and Surv (survival) are expressed in %. FCR = feed conversion ratio. Data are presented as mean ± SEM (n = 3 tanks per treatment). Different letters indicate statistically significant differences between treatments ($p<0.05$). Regression equations: $Y^1 = -5.197 + (2.983x)$, $R^2 = 1.00$; $Y^2 = 73.198 - (0.0464x)$, $R^2 = 0.93$; $Y^3 = 562.071 + (2.461x)$, $R^2 = 0.87$; $Y^4 = 14.796 - (0.0043x)$, $R^2 = 0.92$; $Y^5 = 36.296 - (0.0469x)$, $R^2 = 0.93$; $Y^6 = 1.656 - (0,0021x)$, $R^2 = 0.92$; $Y^7 = 11.378 - (0.0768x) + (0.00012x^2)$, $R^2 = 0.92$; $Y^8 = 35.996 - (0.0334x)$, $R^2 = 0.90$.

Table 3. Blood variables of pacu (*Piaractus mesopotamicus*) maintained under different stocking densities in BFT system.

	Stocking density (fish m^{-3})				
Variables	**150**	**300**	**450**	**600**	**750**
Glucose	49.33±2.19[b]	49.89±1.42[b]	45.75±1.70[b]	48.44±1.64[b]	79.22±2.50[a]
pH[1]	7.58±0.02[b]	7.65±0.02[b]	7.63±0.03[b]	7.81±0.03[a]	7.80±0.03[a]
Hct	30.22±1.14	29.33±0.71	33.11±0.92	31.78±0.80	31.33±1.28
Ery	1.59±0.03	1.54±0.09	1.64±0.09	1.42±0.06	1.45±0.09
Hg[2]	7.03±0.09[a]	7.05±0.07[a]	7.02±0.04[a]	6.63±0.06[b]	6.44±0.03[b]
MCV[3]	190.53±7.59	193.22±9.20	200.46±10.50	225.02±8.88	218.42±10.97
MCH	44.25±0.48	46.41±1.87	43.13±1.61	46.97±1.52	45.00±1.77
CHCM[4]	23.54±1.02[a]	24.11±0.45[a]	21.69±0.59[ab]	20.96±0.50[b]	20.78±0.69[b]

Glucose is expressed as g dL^{-1}; Hct (hematocrit) is expressed as %; Ery (Erythrocytes) is expressed as $\times 10^6$ μL^{-1}; Hg (hemoglobin concentration) and MCHC (mean corpuscular hemoglobin concentration) are expressed as g dL^{-1}; MCV (Mean corpuscular volume) is expressed as fL and; MCH (Mean corpuscular hemoglobin) is expressed as pg. Data are presented as mean ± SEM (n = 9 fish per treatment). Different letters indicate statistically significant differences between treatments ($p<0.05$). Regression equations: $Y^1 = 7.514 + (0{,}0004x)$, $R^2 = 0.80$; $Y^2 = 7.314 - (0{,}0011x)$, $R^2 = 0.81$; $Y^3 = 179{,}260 + (0{,}0584x)$, $R^2 = 0.81$; $Y^4 = 24{,}817 - (0{,}0058x)$, $R^2 = 0.81$.

4. Considerações finais e perspectivas

De acordo com os resultados obtidos em nosso trabalho, ficou evidente que o pacu é uma espécie tolerante a presença de sólidos imposta pelos sistemas de cultivo em BFT. Concentrações elevadas de sólidos podem resultar na obstrução das brânquias e levar a morte, o que aconteceu somente nas concentrações acima de 5000 mg L^{-1}. Manter uma concentração tão elevada de sólidos em um sistema de cultivo não é recomendado, devido a elevada demanda bioquímica de oxigênio que tal condição pode gerar, sem contar os efeitos diretos sobre o organismo cultivado.

Concentrações mais baixas de sólidos na água (0, 250, 500, 750 e 1000 mg L^{-1}) foram testadas por um período de 5 dias e resultaram em distúrbios hematológicos mais evidentes nas concentrações acima de 500 mg L^{-1}. Alterações nos parâmetros hematológicos são usados como ferramenta no diagnóstico de diferentes condições sobre os animais, podendo indicar o estado nutricional e de higidez dos animais, além de indicar situações estressantes.

Foi observado que os animais distribuídos nos tratamentos que continham diferentes concentrações de sólidos (250, 500, 750 e 1000), após o manejo estressante de captura, apresentaram níveis de glicose mais baixos que os distribuídos no tratamento com água clara (controle), o que indica que a presença de sólidos no sistema BFT tem efeito positivo sobre o estresse do manejo desta espécie.

Concentrações de até 1000 mg L^{-1} não causam mortalidades mesmo em períodos mais logos como 30 dias. Entretanto, os parâmetros hematológicos sofreram vários distúrbios quando utilizadas concentrações acima de 250 mg L^{-1} o que indica que esta concentração de sólidos ser mais recomendada para o cultivo de juvenis de pacu em sistema BFT.

O sistema BFT se apresenta como uma ótima opção para o cultivo intensivo de juvenis de pacu. Neste trabalho ficou evidente que densidades de estocagem de até 450 peixes m^3 podem ser utilizadas no cultivo de juvenis de pacu, sem afetar o desempenho da espécie. Densidades acima disto causam redução no crescimento, ganho de peso e conversão alimentar não sendo indicadas, nem mesmo durante um período de pré engorda da espécie.

O cultivo de juvenis de pacu em pelo menos uma das fases de cultivo pode ser realizada com sucesso no sistema BFT sendo este uma alternativa de cultivo intensivo para a espécie. Entretanto, mais trabalhos são necessários a fim de garantir que este sistema seja utilizado para a engorda da espécie, assim como para avaliar se á espécie utiliza ou não o floco como fonte complementar de alimento.

5. Conclusões Gerais

-*P. mesopotamicus* tolera até 5000 mg L^{-1} de sólidos suspensos totais – SST por 96 horas sem apresentar mortalidade.

-Concentrações de 6000 mg L^{-1} causaram 75% de mortalidade em 96h, enquanto que a concentração de 7000 mg L^{-1} foi letal para todos os animais.

-A CL50-96 h de SST para o pacu foi estimada em 5477 mg L^{-1}, com "*safe level*" de 10% estimado em 548 mg L^{-1}.

-Os peixes estocados no sistema BFT após manejo de captura, biometria e transporte apresentaram níveis de glicose mais baixos do que os estocados em água clara, o que indica que este sistema auxilia na redução do estresse pós manejos.

- A curto prazo (5 dias) a concentração de 250 mg L^{-1} de SST foi a que menos causou alterações nos parâmetros hematológicas do pacu.

- A longo prazo (30 dias) os melhores resultados de desempenho zootécnico foram encontrados nos animais mantidos água clara e em 1000 mg L^{-1} de SST no sistema BFT.

- A densidade de 150 peixes por m^3 resultou no maior peso final, comprimento final e ganho de peso, entretanto, a densidade de 450 p/m^3 apresentou uma biomassa final maior o que permite o melhor aproveitamento da área, sendo esta a densidade mais indicada para o cultivo de juvenis de pacu em sistema BFT.

-Densidades acima de 450 p/m^3 não são indicadas pois afetam o desempenho zootécnico da espécie, apresentando baixo crescimento e ganho de peso, aliado a uma baixíssima conversão alimentar.

-O cultivo de pacu pode ser realizado em densidades de até 450 p/m^3 em uma concentração de até 500 mg L^{-1} de SST no sistema BFT.