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DIFERENTES SALINIDADES NO DESEMPENHO, HISTOLOGIA DO FÍGADO E BRÂNQUIAS DE JUVENIS DE *Colossoma Macropomum* CRIADOS EM SISTEMAS DE RECICULAÇÃO DE ÁGUA (RAS)

Belo Horizonte, MG

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Dr.Cristiano Campos Mattioli

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Primeiramente a Deus, qeu me abençoou ao longo da caminhada até aqui, aos meus pais por todo apoio, carinho e confiança, aos amigos e colegas que agregaram em minha trajetória.

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RESUMO

O objetivo deste estudo foi avaliar o efeito de diferentes salinidades da água no desempenho e histologia de brânquias e fígado de juvenis de *Colossoma macropomum* cultivados em *recirculating aquaculture system* (RAS). Foram utilizados 448 juvenis (1,37±0,02g) distribuídos em 4 RAS. Cada RAS teve uma salinidade de água: S_0 - água doce (controle); S_2 - 2 g de sal/L; S_4 – 4 g de sal/L e S_6 – 6 g de sal/L de água. Foi utilizada a densidade de 1 juvenil/L, que foram alimentados à vontade com dieta comercial extrusada (45% proteína bruta) as 08:00 e 15:00 h, durante 45 dias. O pH apresentou efeito inversamente proporcional às salinidades testadas, enquanto a condutividade elétrica apresentou relação direta ao aumento das salinidades ($P<0,05$). O oxigênio dissolvido, temperatura e amônia total foram semelhantes entre os tratamentos ($P>0,05$). De maneira geral, até 30 dias de cultivo, o desempenho, sobrevivência e uniformidade do lote foram semelhantes para todas as salinidades ($P>0,05$). Após 45 dias de cultivo a sobrevivência e uniformidade do lote ainda foram semelhantes entre os tratamentos ($P>0,05$). Porém, o peso e a taxa de crescimento específico diária foram maiores para valor estimado pela derivada da equação em 1.98 g de sal/L e 1.58 g de sal/L, respectivamente. O consumo aparente de ração por peixe foi inversamente proporcional ao aumento da salinidade da água. O ganho de peso e a conversão alimentar aparente apresentaram efeito Linear Response Plateau com valor constante até 4.08 g de sal/L e 3.60 g de sal/L, respectivamente, com redução após estas salinidades ($P<0,05$). O índice hepatossomático foi menor para S_6 ($P<0,05$). As análises histológicas revelaram alterações em componentes estruturais e diâmetro lamelar das brânquias e componentes estruturais do fígado e área de hepatócitos dos diferentes tratamentos. O *C. macropomum* pode ser mantido em até 4 g de sal/L sem comprometer o seu desempenho, função hepática e branquial.

Palavras chave: tambaqui; sal; peixe neotropical.

ABSTRACT

This study evaluated the effects of different water salinities on the performance and gill and liver histology of juvenile *Colossoma macropomum* cultured in a recirculating aquaculture system (RAS). A total of 448 juveniles (1.37±0.02g) were distributed across four RAS systems, each with a different water salinity: S_0 = fresh water (control); S_2 = 2 g of salt/L of water; S_4 = 4 g of salt/L and S_6 = 6 g of salt/L. Animals were housed at a density of one juvenile/L and fed an extruded commercial diet (45% crude protein) ad libitum at 08:00 and 15:00 h, for 45 days. pH was inversely proportional to salinity, while electrical conductivity was directly related to it ($P<0.05$). Dissolved oxygen, temperature and total ammonia did not differ significantly among salinities ($P>0.05$). In general, performance, survival and batch uniformity did not differ significantly among salinities at 30 days of cultivation ($P>0.05$), and still at 45 days of cultivation for survival and batch uniformity ($P>0.05$). However, weight and daily specific growth rate were higher than the value estimated by the derivative of the equation at 1.98 g of salt/L and 1.58 g of salt/L, respectively. Apparent feed consumption per fish was inversely proportional to salinity. Weight gain and apparent feed conversion showed a Linear Response Plateau effect, with a constant value up to 4.08 g of salt/L and 3.60 g of salt/L, respectively, and a reduction beyond these salinities ($P<0.05$). The hepatosomatic index was lowest for S6 ($P<0.05$). Histological analyses revealed changes in structural components and lamellar diameter of gills, as well as structural components of the liver and hepatocyte area, for the different treatments. *Colossoma macropomum* can be maintained in salinities of up to 4 g of salt/L without compromising performance and liver and gill function.

Key words: tambaqui, welfare, freshwater neotropical fish

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1. INTRODUÇÃO

Segundo Dawood e Koshio (2020) a obtenção de peixes oriunda do extrativismo tem permanecido estática nas últimas décadas, o que torna a aquicultura importante alternativa para a obtenção de proteína derivada de pescado. Essa proteína representou cerca de 17% de toda proteína de origem animal consumida mundialmente no ano de 2020, segundo dados da FAO (OCDE/FAO, 2022).

A busca por eficiência, produtividade e bem estar animal vem sendo cada vez mais almejados no cultivo de organismos aquáticos, incentivando o desenvolvimento de pesquisas que viabilizem esses requisitos para o sistema produtivo. No cultivo de peixes de água doce, um dos fatores de grande relevância para a produção comercial é um eficaz controle de parâmetros relacionados a qualidade da água, na qual esses animais estão estocados. Dentre esses parâmetros podemos citar a salinidade, onde alterações na concentração salina do meio podem levar a diferentes respostas no organismo animal.

A salinidade pode desencadear respostas significativas em peixes de água doce, sendo comumente utilizada em pisciculturas como agente antiparasitário e mitigador de estresse. Dentre as amplas possibilidades, a manutenção ou melhoria de indicadores relacionados ao desempenho zootécnico pode ser atingida através do uso do sal na água de cultivo de algumas espécies de peixes de água doce.

Um eficiente desempenho zootécnico dos animais no sistema de cultivo pode gerar inúmeros benefícios a cadeia produtiva, economia em insumos, menor ciclo produtivo, taxas de sobrevivência altas, dentre outros. Contudo, a utilização da salinidade no cultivo de espécies estenoalinas deve ser feita com cautela, levando em conta as especificidades do animal utilizado. A utilização do sal na água de maneira inapropriada a espécie pode levar a perdas em desempenho zootécnico e danos morfológicos e fisiológicos aos peixes. Assim, tornam-se necessários estudos para um melhor entendimento do papel da salinidade no cultivo de peixes de água doce.

2. OBJETIVOS

2.1 Objetivo geral

Avaliar os efeitos da salinidade no desempenho, histologia do fígado e brânquias de juvenis Tambaqui (*Colossoma macropomum*) criados em *recirculating aquaculture system* (RAS).

2.2 Objetivos específicos

Avaliar os efeitos da salinidade no desempenho de juvenis de tambaqui cultivado por 45 dias em *recirculating aquaculture system* (RAS);

- Avaliar os efeitos da salinidade no tecido branquial de juvenis de tambaqui cultivados por 45 dias em *recirculating aquaculture system* (RAS);
- Avaliar os efeitos da salinidade no tecido hepático de juvenis de tambaqui cultivados por 45 dias em *recirculating aquaculture system* (RAS),

3. REVISÃO DE LITERATURA

3.1 Tambaqui

O Tambaqui (*Colossoma macropomum*) (Figura 1) é uma espécie de peixe neotropical da família Serrasalmidae, ordem Characiformes (Calcagnotto et al., 2005). Nativo das bacias dos rios Amazonas e Orinoco, já é produzido em outros países da América Latina e vem se mostrado uma espécie cada vez mais presente na aquicultura de alguns países do continente asiático como, Malásia, Mianmar, China e Indonésia (Silva et al., 2003; Woynárovich e Van Anrooy, 2019).

Esse peixe se destaca como a segunda espécie mais produzida no Brasil e dentre as nativas ocupa o primeiro lugar (PeixeBR, 2023). Devido a sua fácil adaptação em diversos sistemas de cultivo, bom desempenho zootécnico, robustez, produtividade, domínio nas técnicas de reprodução e larvicultura, além de boa aceitação do mercado consumidor, a espécie tem se consolidado no setor comercial. (Lopera-Barrero et al., 2015; Saint-paul, 2017).

Figura 1: Tambaqui (*Colossoma macropomum*)- acervo pessoal

O cultivo do tambaqui é comumente realizado em viveiros escavados (Ferreira et al., 2021; Souza et al., 2016), mas a espécie vem apresentando bons resultados em outros sistemas de cultivo como o *recirculating aquaculture system* (RAS). Para o cultivo em sistemas de RAS o tambaqui vem se mostrando um animal promissor, sem perdas em indicadores de desempenho zootécnico e taxas de sobrevivência (Ananias et al., 2023; Santos et al., 2022; Santos et al., 2021; Santos et al., 2021; Boaventura et al., 2021; Silva et al., 2021; Assis et al., 2020), características que viabilizam a produção dessa espécie em escala comercial nesse sistema de cultivo.

Contudo, o cultivo da espécie em água salinizada precisa ser mais elucidado, visto que a utilização de sal para várias espécies de peixes de água doce pode levar a benefícios ao animal estocado e o sistema produtivo, como aumento na produtividade.

3.2 Utilização do sal no cultivo de peixes de água doce

A busca por altos índices de produtividade acaba implicando em densidades elevadas e sistemas de cultivo cada vez mais intensivos, o que pode acarretar em quedas no desempenho e surgimento de problemas relacionados a sanidade animal (Uehara et al., 2021).

O sal comum é um produto de fácil aquisição e valor relativamente baixo, se tornado uma alternativa recorrente para os produtores. Sua utilização é uma prática já observada em diversos segmentos da aquicultura (Takata et al., 2021Rodrigues et al., 2019; Jomori et al., 2013;). Alterações nas concentrações salinas da água podem ser utilizadas em diferentes fases de cultivo, buscando diferentes propósitos, desde melhores resultados relacionados ao

desempenho zootécnico (Jomori et al., 2012; Kombat et al., 2021; Zidan et al., 2022), a tratamentos profiláticos relacionados a salinidade (Nass et al., 2024., Dewi et al., 2018) e estresse desses animais (Fiuza et al., 2015). Como o sal comum, apresenta baixos índices de toxicidade ao ambiente, pode ser utilizado na piscicultura para combater ou amenizar doenças parasitarias, fúngicas ou bacterianas em ovos e em peixes de água doce (Rodrigues et al., 2019; Oladosu e Oladosu., 2019; El-Gawad et al., 2016; Lahnsteiner e Weismann, 2007).

A larvicultura é uma fase crítica na produção comercial de peixes de água doce, onde resultados satisfatórios podem levar a benefícios nas decorrentes etapas da cadeia produtiva (Santos et al., 2021). A utilização de baixas salinidades nessa fase de cultivo pode levar a melhores índices no cultivo de larvas. Para a larvicultura de tambaqui (*C. macropomum*) salinidades próximas a 2 g sal/L podem ser utilizadas sem comprometer o desempenho e a sobrevivência dos animais (Santos et al., 2022; Santos et al., 2021), o que corrobora com o encontrado para larvas de cascudo preto (*Rhinelepis aspera*) (Luz e Santos, 2010), larvas de pacamã (*Lophiosilurus alexandri*) (Luz e Santos, 2008), larvas de tilápia (*Oreochromis niloticus*) (Luz et al., 2013) e juvenis de kilifish (*Hypsolebias radiseriatus*) (Araújo et al., 2020). Além da sobrevivência, o desempenho zootécnico de larvas de peixes de água doce pode ser alterado com a utilização de baixas salinidades. Para Jomori et al. (2012) larvas de pacu (*Piaractus mesopotamicus*) estocados em salinidades de 2 e 4g sal/L apresentaram crescimento melhor do que aquelas que permaneceram em água doce. Segundo os autores, esse desempenho pode estar associado com a relação entre a salinidade da água e o maior tempo de vida de náuplios de artêmia, utilizados para a alimentação desses animais, o que corrobora com resultados encontrados para tambaqui (*C. macropomum*) por (Jomori et al., 2013), para curimbatá (*Prochilodus costatus*) e pacamã (*L. alexandri*) (Santos e Luz, 2009).

Água levemente salinizada pode trazer vantagens a larvicultura de algumas espécies, podendo ser uma estratégia interessante levando em consideração benefícios a sanidade das larvas além de melhorias em indicadores zootécnicos. Para uma melhor eficácia na utilização de baixas salinidades na larvicultura de peixes de água doce é necessário, adequações quanto as concentrações salinas a partir do animal trabalhado, levando em conta os diferentes níveis de tolerância a salinidade entre as espécies de peixes.

3.3 Salinidade no desempenho de juvenis de peixes de água doce

Visando melhores índices produtivos para o cultivo de juvenis de peixes de água doce, a adoção de sistemas intensivos como o RAS, favoreçem melhor controle dos parâmetros da água pode ser uma alternativa interessante para a estocagem desses animais.

Dentre esses parâmetros a salinidade pode ser alterada buscando melhores índices de desempenho e sobrevivência em juvenis de peixe de água doce. A salinidade inicial usada pelos autores de 5g sal/L, não apresentou efeitos prejudiciais para desempenho no cultivo de juvenis de tambaquis (*C. macropomum*) durante 84 dias (Fiuza et al., 2015). Segundo os mesmos autores, o aumento dos níveis de salinidade apresenta relação direta com a queda de desempenho dos animais, e salinidades entre 20 a 22g de sal/L apresentaram letalidade total. Porém, não há informação para salinidades inferiores a 5 g de sal/L. Para carpa comum (*C. carpio*) salinidades de até 12 g sal/L de sal se mostraram toleráveis, mas quando esses gradientes ultrapassaram as 6 g sal/L ocorreram impactos em importantes tecidos do animal, como brânquias e rins (Salati et al., 2011). Para juvenis de tilápia do nilo (*O. niloticus*), salinidade de até 10 g sal/L não afetou de forma negativa seu desempenho de crescimento e taxas de sobrevivência (Kombat et al., 2021), semelhante ao encontrado para bagre africano (*Clarias gariepinus*) (Zidan et al., 2022). Já, para juvenis de pacamã (*L. alexandri*) cultivados durante 28 dias em salinidades em torno de 2,5 g sal/l apresentaram resultados semelhantes para indicadores de desempenho em relação aos estocados em água doce e mortalidade total na salinidade de 10 g de sal/L ao 21º dia, e parcial na salinidade de 7,5 g de sal/L atingindo um percentual de 18% de taxa de sobrevivência ao final dos 28 dias (Mattioli et al., 2017).

3.4 Alterações morfofisiológicas em peixes de água doce decorrentes de altas salinidades

Alterações morfofisiológicas em peixes podem estar relacionadas a problemas apresentados devido a mudanças em fatores bióticos e abióticos no meio de cultivo (Person et al., 2021). Em algumas espécies de peixes de água doce, a salinidade em níveis inadequados pode levar a essas alterações que podem ser constatadas a partir de análises histológicas em tecidos desses animais (Takata et al., 2021: Mohamed et al., 2021: Zidan et al., 2022).

Para o pacamã (*L. alexandri*) cultivados por 28 dias, salinidades superiores a 2,5g sal/L levaram a alterações nos tecidos branquiais como, congestão vascular, hiperplasia do epitélio dos filamentos branquiais, fusão lamelar, aumento da hiperplasia das células da mucosa e perda

da integridade estrutural das células pilares e células cloradas (Takata et al., 2021). Nesse sentido, tilápias do nilo (*O. niloticus*) cultivadas por 10 dias em salinidades entre 10 a 15 g sal/L apresentaram danos aos tecidos branquiais como a adesão das lamelas secundárias, lesões degenerativas e necróticas no tecido hepático além de inflamações renais (Mohamed et al., 2021).

Além do tecido branquial, o tecido hepático dos peixes também pode sofrer alterações a partir da utilização de salinidades inadequadas (Dawood et al., 2022; Dawood et al., 2021). Segundo Dawood et al. (2022), juvenis de tílapia (*O. niloticus*) cultivada por um período de 30 dias, apresentaram alterações no tecido hepático em salinidades iguais a 10 g de sal/L, sendo estas mais intensas quando utilizaram 20 g de sal/L. O aumento na incidência de degenerações diretamente proporcional a elevação da salinidade na água de cultivo também foi observado para juvenis de bagre africano (*C. gariepinus*) cultivados por um período de 30 dias (Dawood et al., 2022).

Portanto, tornam se relevantes as pesquisas que elucidem o efeito da salinidade na morfologia de tecidos de função vital para os peixes como brânquias e fígado, onde as informações disponíveis na literatura são escassas.

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CAPÍTULO 2

Artigo

PERFORMANCE AND LIVER AND GILL HISTOLOGY OF JUVENILE *COLOSSOMA MACROPOMUM* RAISED IN DIFFERENT SALINITIES IN A RECIRCULATING AQUACULTURE SYSTEM (RAS)

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ABSTRACT

This study evaluated the effects of different water salinities on the performance and gill and liver histology of juvenile *Colossoma macropomum* cultured in a recirculating aquaculture system (RAS). A total of 448 juveniles (1.37±0.02g) were distributed across four RAS systems, each with a different water salinity: S_0 = fresh water (control); S_2 = 2 g of salt/L of water; S_4 = 4 g of salt/L and S_6 = 6 g of salt/L. Animals were housed at a density of one juvenile/L and fed an extruded commercial diet (45% crude protein) ad libitum at 08:00 and 15:00 h, for 45 days. pH was inversely proportional to salinity, while electrical conductivity was directly related to it ($P<0.05$). Dissolved oxygen, temperature and total ammonia did not differ significantly among salinities ($P>0.05$). In general, performance, survival and batch uniformity did not differ significantly among salinities at 30 days of cultivation ($P>0.05$), and still at 45 days of cultivation for survival and batch uniformity ($P>0.05$). However, weight and daily specific growth rate were higher than the value estimated by the derivative of the equation at 1.98 g of salt/L and 1.58 g of salt/L, respectively. Apparent feed consumption per fish was inversely proportional to salinity. Weight gain and apparent feed conversion showed a Linear Response Plateau effect, with a constant value up to 4.08 g of salt/L and 3.60 g of salt/L, respectively, and a reduction beyond these salinities ($P<0.05$). The hepatosomatic index was lowest for S6 ($P<0.05$). Histological analyses revealed changes in structural components and lamellar diameter of gills, as well as structural components of the liver and hepatocyte area, for the different treatments. *Colossoma macropomum* can be maintained in salinities of up to 4 g of salt/L without compromising performance and liver and gill function.

Key words: tambaqui, welfare, freshwater neotropical fish

INTRODUCTION

Saline water has been applied in aquaculture for different purposes and a variety of freshwater species. Studies have demonstrated that low salinities can improve zootechnical performance and survival rates in the larviculture of freshwater fish, such as Pacu (*Piaractus mesopotamicus*) (Jomori et al., 2012), black pleco (*Rhinelepis aspera*) (Luz & Santos, 2010), pintado (*Pseudoplatystoma corruscans*), curimatã pioa (*Prochilodus costatus*), pacamã (*Lophiosilurus alexandri*) (Santos & Luz, 2009), matrinxã (*Brycon amazonicus*), apaiari (*Astronotus ocellatus*), piau (*Leporinus macrocephalus*) (Jomori et al., 2013) and Nile tilapia (*Oreochromis niloticus*) (Luz et al., 2013). Low salinities have also been shown to be efficient for, or similar to, freshwater cultivation for the production of juveniles and adults of freshwater fish such as pacamã (*L. alexandri*) (Mattioli et al., 2017, Takata et al., 2021), common carp (*Cyprinos carpio*) (Salati et al., 2011), goldfish (*Carassius auratus*) (Luz et al., 2008) and African catfish (*Clarias gariepinus*) (Dawood et al., 2022).

Salinity can alter the physiology of animals, leading to morphological changes in tissues and in the zootechnical performance of fish such as tambaqui (*Colossoma macropomum*) (Fiuza et al., 2015) and pacamã (*L. alexandri*) (Takata et al., 2021; Mattioli et al., 2017). Tissue changes related to exposure to saline water must be taken into consideration. Juvenile pangasius (*Pangasionodon hypophthalmus*) maintained in salinities of up to 4 g of salt/L did not exhibit histopathological changes in the gills; however, changes were recorded at 8 g salt/L and higher (Hossain et al., 2022). Salinities of up to 2.5 g of salt/L also maintained the integrity of the gill tissue in pacamã (*L. alexandri*), while higher salinities resulted in hyperplasia of gill filament epithelium (Takata et al., 2021). The liver may also experience changes resulting from increased water salinity, as seen in pangasius (*P. hypophthalmus*) for salinities of 8 g of salt/L and higher (Hossain et al., 2022). Therefore, due to the importance of using salt, and for the best well-being of the animal's, responses of fish to this management must be understood.

Tambaqui (*C. macropomum*), a native fish of the Amazon and Orinoco river basins (Lopes et al., 2009), is the most produced native species in Brazil (Peixe Br 2023) and is important in other countries in Latin America and Asia (Woynárovich & Anrooy 2019). Recent studies have shown the species to have good adaptation to cultivation in recirculating aquaculture systems (RASs) during larviculture, (Santos et al., 2021 & Santos et al., 2022), juvenile production (Silva et al., 2021) and fattening (Santos et al., 2021), and a salinity of 2 g of salt/L has been routinely used during larviculture (Jomori et al., 2013; Santos et al., 2021;

Santos et al., 2022). However, there are still no data on the cultivation of juveniles in RAS using saline water.

Thus, the present study aimed to evaluate the effects of different water salinities on performance and gill and liver histology of juvenile *C. macropomum* cultivated in RAS.

MATERIAL AND METHODS

2.1. Ethical approval

All procedures described herein were approved by the Committee for Ethics in Animals Use (CEUA/UFMG – no. 07/2021).

2.2. Animal housing and management

A total of 448 juvenile *C. macropomum*, weighing 1.37±0.02g and measuring 4.54±0.17 cm in length, were distributed across four RAS. Each RAS was composed of a water tank with a mechanical and biological filter and water temperature control and four 28-L cultivation tanks. Each RAS had a different water salinity treatment, namely: S_0 = fresh water (control); S_2 = 2 g of salt/L of water; S_4 = 4 g of salt/L and S_6 = 6 g of salt/L. The salt used was from the Sal Garça LTFA Refinery, Mossoró, Rio Grande do Norte, Brazil (ingredients: sodium chloride). Juveniles were stocked directly in the different salinities at a density of 1 juvenile/L of water (28 animals per tank). The following water quality parameters were measured daily in the morning: temperature, pH, salinity and conductivity, using a model HI9146 Hanna Instruments probe; dissolved oxygen, using a YSI 6920VZ2 multiparameter probe; and total ammonia using a Labcon kit. The photoperiod was 12L:12D (digital timer, Key West DNI group).

The juveniles were fed ad libitum two times a day (08:00 and 15:00 h) with an extruded commercial feed: Nanolis 45% - Socil 0.8 mm in diameter, containing 450 g/kg of crude protein, 80–90 g/kg of ether extract, 150 g/kg of mineral matter, 35–40 g/kg of crude fiber, 20–30 g/Kg of calcium, 10 g/Kg of phosphorus, 300–480 mg/Kg and 1000–1800 mg/Kg of vitamin C (manufacturer's data). Upon reaching satiety (on average 30 minutes post-feeding), the leftovers were collected and then dried in an oven (Nova Ética/Ethink) at 55ºC, to calculate consumption. Tank cleaning procedures were employed daily in the morning to remove feces and the water volume was partially changed in each tank every 15 days, with replacement using water of the same salinity and temperature as each RAS.

2.3 Survival and zootechnical performance

Growth was determined through weight biometrics using a semi-analytical scale (Ay-220–220 g × 0.0001 g Marte - Brazil analytical balance) and measurement with a digital caliper (Starrett electronic, Massachusetts - USA). Biometrics took place after 15, 30 and 45 days of cultivation. The following were calculated using the obtained data:

- Weight (W) (g) = Biomass (g) / number of animals per tank;
- Weight gain (WG (g)) = Weight (W) (g) - Initial weight (IW) (g);
- Total apparent feed consumption (AFC) (g) = Total feed consumption (g) / number of animals per tank;
- feed conversion rate (FCR) = Total apparent feed consumption (g) / weight gain (g).
- Daily specific growth rate (SGR) (%/day) = 100 x ($\ln W_f - \ln W_i$) / interval between biometrics (days), where W_i is initial weight, W_f is final weight;

Survival was determined at each biometric by the direct count of individuals.

Thirty-six animals (n = 9 per treatment) were euthanized at the end of the experiment using a solution containing 285 mg/L of eugenol (Mattioli et al., 2017). The liver of each animal was then collected and weighed to determine the Hepatosomatic Index (HSI) = 100 x (Liver weight/Animal body weight).

2.4. Gill and liver morphometry

For histological and morphometry analysis, of euthanized fish, the 2nd gill arch and liver fragment of each animal was fixed in Bouin solution for 24 h, embedded in paraffin, sectioned at 5-µm thickness, and stained with PAS-hematoxylin (Bancroft & Gamble, 2007). The main structures of these organs were quantified using 40 histological fields (8 histological fields from each animal at 40x magnification) from five individuals analyzed per sample group (0, 2, 4 and 6 g of salt/L salinity treatments). The relative proportions of components of the gill (epithelial cells, pillar cells, mucous cells, ionocytes, erythrocytes/blood capillaries, undifferentiated cells and cartilaginous tissue) and liver (hepatocytes, glycogen granules, blood capillaries and Kupffer cells/ immune cells) were determined using a grid containing 609 intersection points overlaying each field and ImageJ 1.52 software. Blank spaces and artifacts were excluded from the counts. To measure the diameter of the primary and secondary lamellae of the gills and the hepatocytes area of the liver were used 50 measurements extracted from five fish per group (10 measurements from each animal). This measurement was performed using the AxioVision SE64 software coupled to an Axioplan 2 microscope (Zeiss).

2.5 Statistical Analysis

All data were subjected to Levene's homoscedasticity test and Shapiro-Wilk's normality test, followed by ANOVA and the Tukey's test ($p<0.05$) or regression analysis to verify the best model to be used. The Kruskal-Wallis test followed by the Dunn's *post hoc* test were used to compare the morphometric data for gill and liver between the samples. Analyses were performed using the software Infostat Versiom 2014, Cordoba, Argentina.

RESULTS

pH was inversely proportional to salinity (Figure 1A), while electrical conductivity was directly related to it (Figure 1B) ($P<0.05$). The other water quality parameters were not affected by salinity (Table 1)

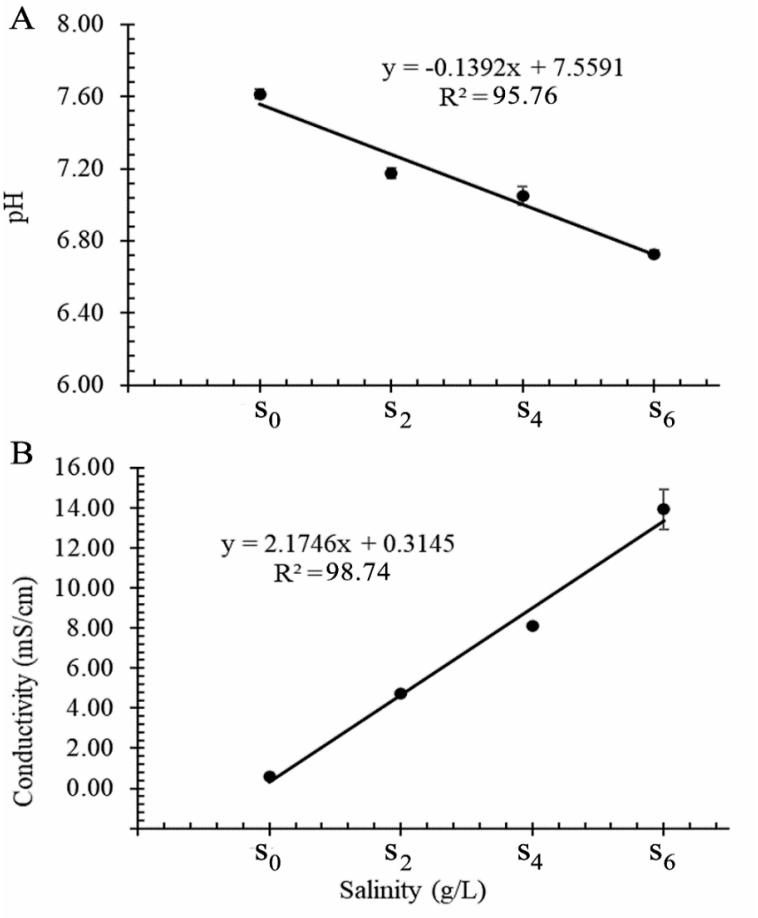

Figure 1. Values for pH (1A) and water conductivity (1B) during the cultivation of juvenile *C. macropomum* under different salinities in a recirculating aquaculture system (RAS) for 45 days. S_0 = fresh water (control); S_2 = 2 g of salt/L; S_4 = 4 g of salt/L; and S_6 = 6 g of salt/L of water.

Table 1. Water quality parameters (mean ± standard deviation) during 45 days of cultivation of juvenile *C. macropomum* in different salinities.

Treatment	Salinity (g of salt/L)	Temperature (°C)	Total ammonia (mg/L)	Dissolved oxygen (mg/L)
S_0	0.15±0.04d	28.35±0.06	0.17±0.02	6.89±0.16
S_2	2.17±0.01c	28.34±0.06	0.17±0.01	6.81±0.09
S_4	4.19±0.03b	28.41±0.11	0.17±0.2	6.85±0.10
S_6	6.39±0.03a	28.27±0.13	0.17±0.1	6.88±0.12
P-value	<0.0001	>0.05	>0.05	>0.05

Different letters in the same column indicate significant differences by Tukey's test (5%).
S_0 = fresh water (control); S_2 = 2 g of salt/L of water; S_4 = 4 g of salt/L; and S_6 = 6 g of salt/L.

After 15 days of cultivation, W, WG, SGR and FCR did not differ significantly among the salinities (P>0.05) (Table 2), while AFC was highest in S_6 and lowest in S_0 (P<0.05).

After 30 days of cultivation, none of the evaluated differed significantly among the tested salinities (Table 2).

Table 2. Zootechnical performance (mean ± standard deviation) of juvenile *C. macropomum* after 15 and 30 days of cultivation in different salinities in a water recirculation system (RAS).

	Salinity (g of salt/L)				
15 days of cultivation	S_0	S_2	S_4	S_6	*P*-value
W (g)	2.78±0,03	2.93±0.28	3.10±0.41	3.11±0.36	0.4911
WG (g)	1.40±0,08	1.56±0.28	1.73±0.42	1.75±0.35	0.4617
SGR (%/dia)	4.66±0.31	5.02±0.65	5.41±0.94	5.49±0.79	0.4608
AFC (g)	1.21±0.06b	1.24±0.06ab	1.25±0.07ab	1.38±0.10a	0.0455
FCR	0.87±0.08	0.82±0.16	0.75±0.20	0.82±0.22	0.8712
Survival (%)	100	100	100	100	

30 days of cultivation

W (g)	5.57±1.11	6.34±0.99	5.68±0.27	5.38±0.92	0.4774
WG (g)	2.99±0.87	3.41±0.74	2.58±0.38	2.27±0.98	0.2352
SGR (%/dia)	5.10±0.92	5.10±0.50	4.07±0.80	3.61±1.37	0.1099
AFC (g)	2.21±0.30	2.32±0.44	2.01±0.05	2.30±0.37	0.5461
FCR	0.79±0.25	0.70±0.21	0.79±0.13	1.20±0.59	0.224
Survival (%)	95.24±4.12	94.64±4.61	98.21±2.06	89.29±2.92	>0.05

Different letters in the same row indicate significant differences by Tukey's test (5%).

W - Weight; WG - Weight gain; SGR – Daily specific growth rate; AFC – total apparent feed consumption; FCR - Feed conversion rate. S_0 = fresh water (control); S_2 = 2 g of salt/L of water; S_4 = 4 g of salt/L; S_6 = 6 g of salt/L.

After 45 days of cultivation, W showed a Linear Response Plateau effect with a constant value up to 4.03 g of salt/L, followed by a reduction ($P<0.05$) (Figure 2A). AFC was inversely proportional to salinity ($P<0.05$) (Figure 2B). WG also showed a Linear Response Plateau effect, with a constant value up to 4.08 g of salt/L, followed by a reduction ($P<0.05$) (Figure 2C). FCR showed a Linear Response Plateau effect with a constant value up to 3.60 g of salt/L, followed by a reduction ($P<0.05$) (Figure 2D). SGR showed a quadratic effect with the highest value estimated by the derivative of the equation at 1.58 g of salt/L ($P<0.05$) (Figure 2E). Survival and U did not differ significantly among the tested salinities during this period ($P>0.05$) (Table 3).

At the end of the experiment, HSI was lowest for S_6 (Fig. 3).

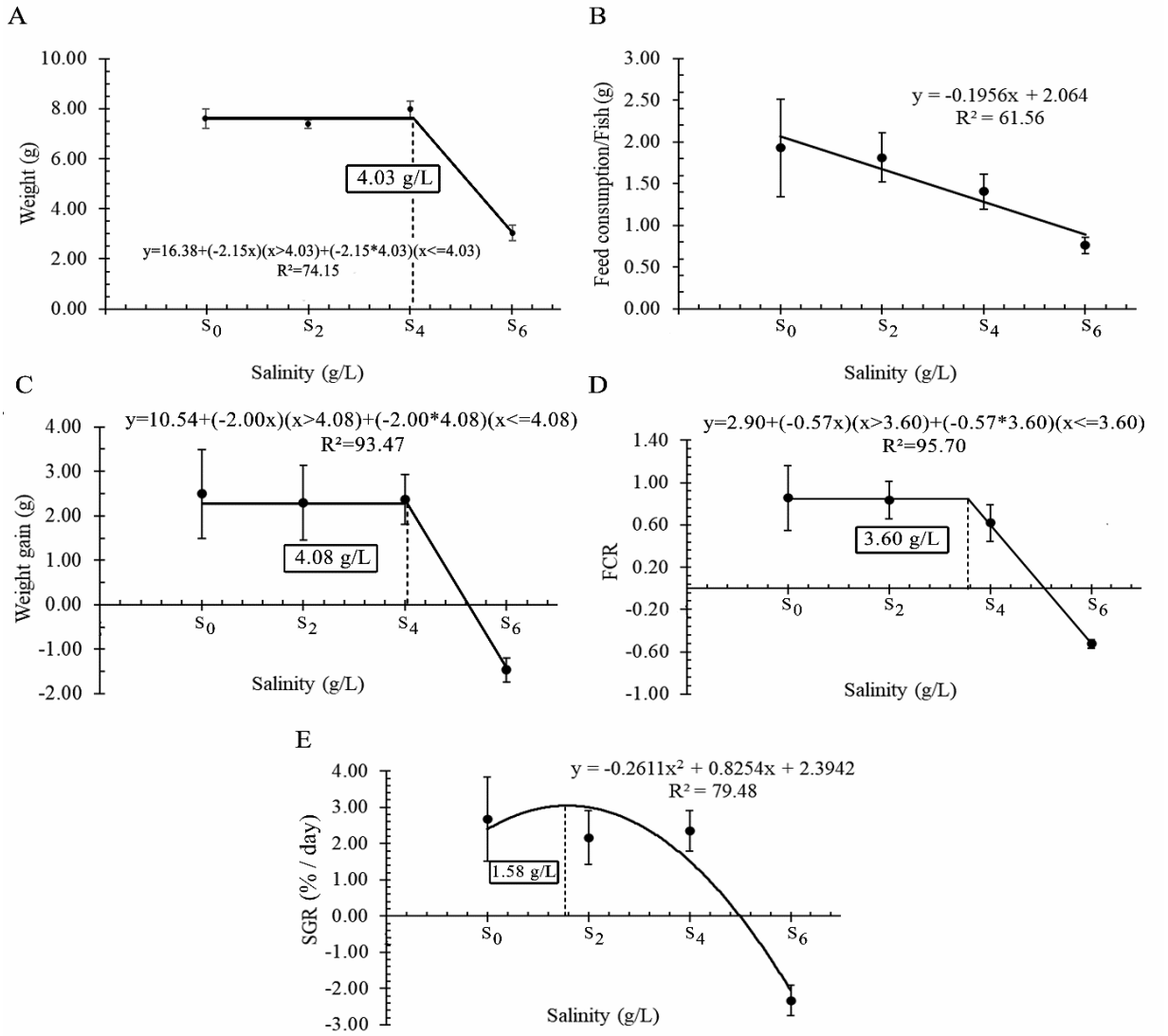

Figure 2. Mean values (± standard deviation) for A - Average weight (g) (W), B - Total apparent feed intake (g), C - Average weight gain (g) (WG), D - Apparent feed conversion (FCR); and E – daily specific growth rate (SGR) from 30 to 45 days of cultivation for juvenile *C. macropomum* cultivated in different salinities in a water recirculation system (RAS). S_0 = fresh water (control); S_2 = 2 g of salt/L of water; S_4 = 4 g of salt/L; and S_6 = 6 g of salt/L.

Table 3. Survival (mean ± standard deviation) of juvenile *C. macropomum* after 45 days of cultivation in different salinities in a water recirculation system (RAS).

	Salinity (g of salt/L)				
	S_0	S_2	S_4	S_6	*P*-value
Survival (%)	78.57±21.43	78.57±16.37	89.29±9.45	78.57±10.71	>0.05

Submitted to Anova (5% significant)

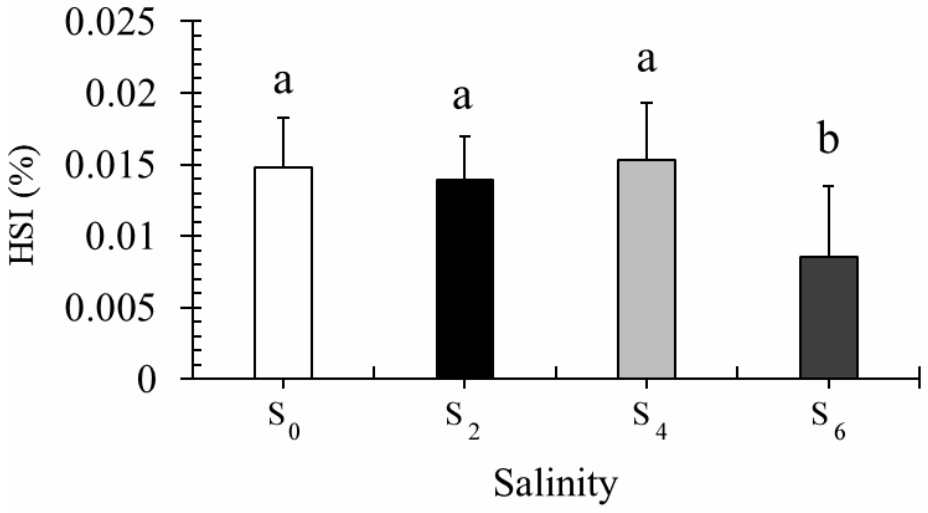

Figure 3. The Hepatosomatic Index (HSI) for juvenile *C. macropomum* after 45 days of cultivation in different salinities in a water recirculating system (RAS). Different letters in a column indicate significant differences by Tukey's test (5%). S_0 = fresh water (control); S_2 = 2 g of salt/L of water; S_4 = 4 g of salt/L; and S_6 = 6 g of salt/L.

Salinity did not affect gill cartilaginous tissue (P>0.05) (Table 4) (Figure 1). Percentage of epithelial cells (%) was higher for S_4 and S_6 (Figure 1 C-D) (P<0.05) and lowest in S_0 (Figure 1 A) (P>0.05). Percentage of pillar cells (%) was highest for S_4 (Figure 1 C), which was similar to S_6 (Figure 1 D), and lowest for S_0 (Figure 1 A). Percentages of mucous cells (%) and ionocytes (%) were similar between S_0 and S_2 (Figure 1 A-B) (P>0.05), and lower for S_4 and S_6 (Figure 1 C-D) (P<0.05), which were also similar to each other. Percentage erythrocytes/blood capillaries (%) was highest for S_6 (Figure 1 D) and lowest for S_2 (Figure 1 B), with intermediate values for S_0 and S_4 (Figure 1 A-C) (P<0.05). Percentage of undifferentiated cells (%) was highest for S_0 (Figure 1 A) (P<0.05). Percentage primary lamellae (%) (Figure 1 A) was highest for S_2 (Figure 1 B) and lower for S_4 and S_6 (Figure 1

C-D) (P<0.05). Percentage secondary lamellae (%) (Figure 1 A) was highest for S_0 (Figure 1 A) and lower for S_2 and S_4 (Figure 1 B-C) (P<0.05).

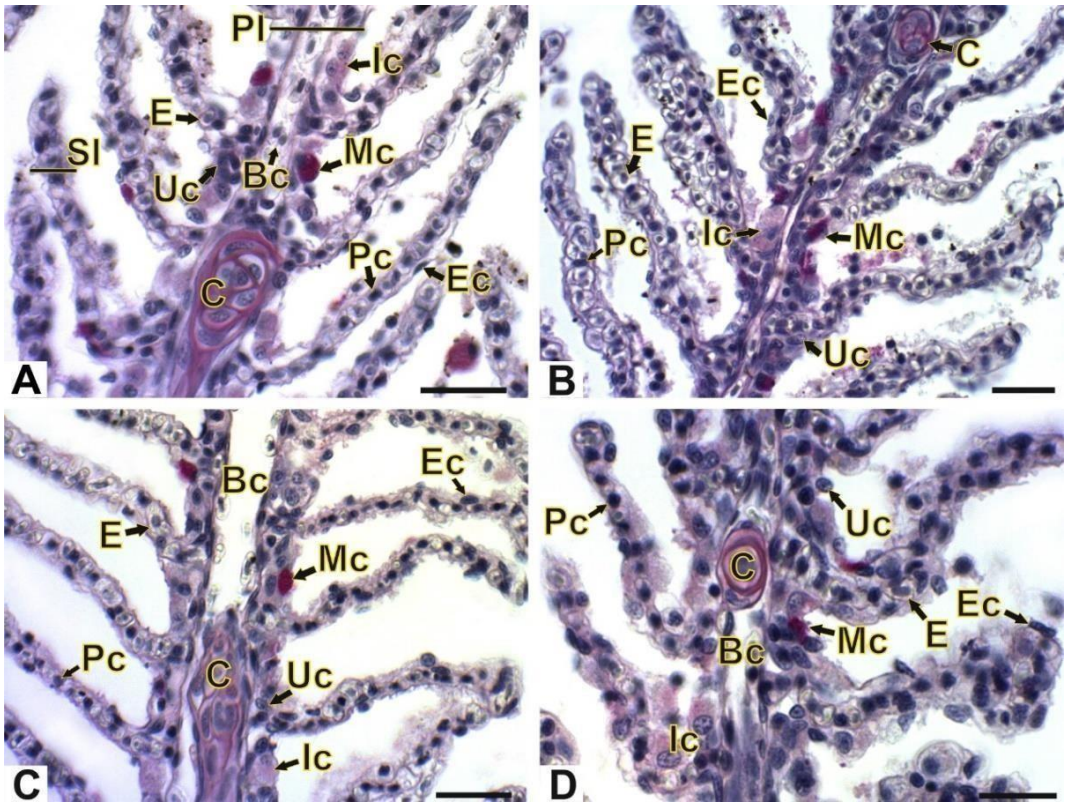

Figure. 4. Longitudinal histological sections stained with PAS-hematoxylin of gills of juveniles of *C. macropomum* submitted to salinity regimes. (A) salinity 0% (control), (B) salinity treatment at 2%, (C) salinity treatment at 4%, (D) salinity treatment at 6%. Primary (Pl) and secondary (Sl) lamellae, epithelial cell (Ec), pillar cell (Pc), mucous cell (Mc), ionocyte (Ic), erythrocyte (E), blood capillary (Bc), undifferentiated cell (Uc), cartilaginous tissue (C). Scale bars (A–D) = 50 μm.

Table 4. Relative proportion (%) of structural components and lamellar diameter (μm) of gills in juveniles of *C. macropomum* submitted to different salinity regimes.

Gill structures	Salinity (g of salt/L)				
	S_0	S_2	S_4	S_6	*P*-value
Epithelial cells	17.1 ± 0.6^{c}	21.1 ± 0.5^{b}	25.0 ± 0.6^{a}	25.2 ± 0.7^{a}	< 0.0001
Pillar cells	23.5 ± 0.9^{c}	26.4 ± 0.7^{bc}	30.8 ± 0.7^{a}	29.6 ± 0.7^{ab}	< 0.0001
Mucous cells	7.2 ± 0.4^{a}	9.9 ± 0.5^{a}	3.7 ± 0.2^{b}	3.6 ± 0.2^{b}	< 0.0001
Ionocytes	7.1 ± 0.3^{a}	8.0 ± 0.4^{a}	4.2 ± 0.3^{b}	4.7 ± 0.2^{b}	< 0.0001
Erythrocytes/ blood	12.2 ± 0.7^{ab}	10.0 ± 0.6^{b}	11.8 ± 0.6^{ab}	12.8 ± 0.7^{a}	= 0.0243

capillaries					
Undifferentiated cells	30.7 ± 1.1[a]	22.2 ± 0.6[b]	22.2 ± 0.8[b]	21.4 ± 0.7[b]	< 0.0001
Cartilaginous tissue	2.2 ± 0.2[a]	2.4 ± 0.3[a]	2.3 ± 0.2[a]	2.7 ± 0.3[a]	= 0.7031
Primary lamellae	29.09 ± 0.56[b]	32.97 ± 0.51[a]	20.05 ± 0.59[c]	21.50 ± 0.45[c]	< 0.0001
Secondary lamellae	7.84 ± 0.15[a]	7.14 ± 0.17[b]	6.63 ± 0.20[b]	7.24 ± 0.20[ab]	< 0.0001

Different letters in a row indicate significant differences by Dunn test ($P < 0.05$) among sampling groups. Values are expressed as mean ± standard error (SE).

For the liver, percentage of hepatocytes (%) was higher for S_4 and S_6 (Figure 2 C-D) and lower for S_0 and S_2 (Figure 2 A-B) (P<0.05) (Table 5). Percentage of glycogen granules (%) was higher for S_0 and S_2 (Figure 2 A-B) and lower for S_4 and S_6 (Figure 2 C-D) (P<0.05). Percentage of blood capillaries (%) was highest for S_0 (Figure 2 A) and lowest for S_6 (Figure 2 D) (P<0.05). Percentage of Kupffer cells/immune cells (%) was highest for S_6 (Figure 2 D) and lower for the other treatments (P<0.05). Percentage hepatocyte area (%) was highest for S_0 (Figure 2 A) (P<0.05).

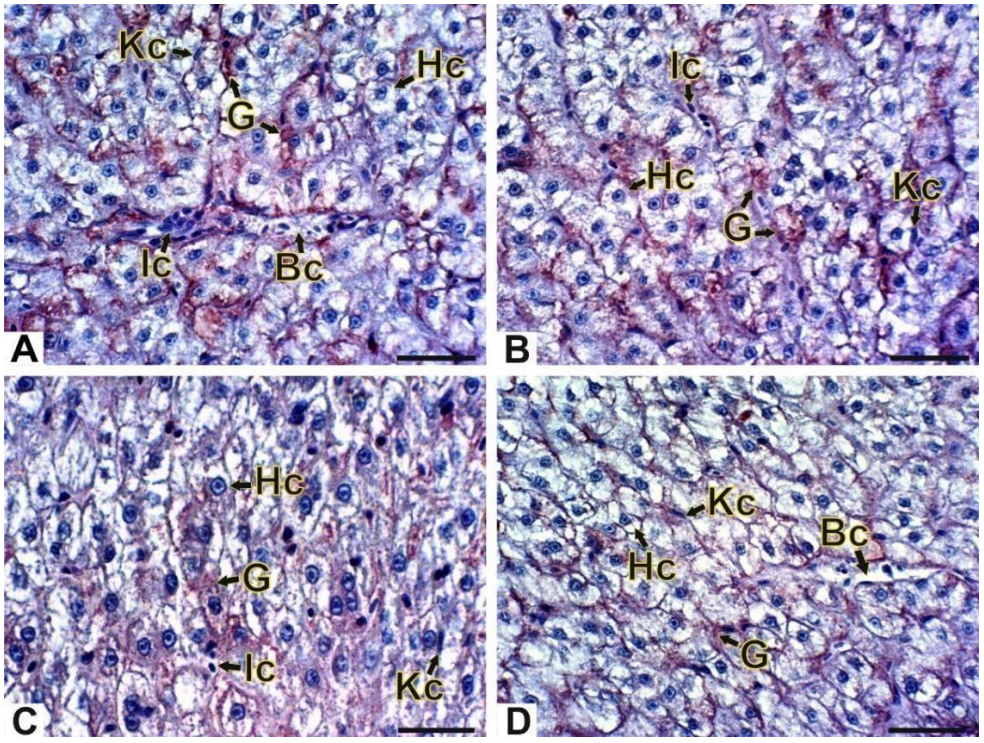

Figure. 5. Transversal histological sections stained with PAS-hematoxylin of liver of juveniles

of *C. macropomum* submitted to salinity regimes. (A) salinity 0% (control), (B) salinity treatment at 2%, (C) salinity treatment at 4%, (D) salinity treatment at 6%. Hepatocyte (Hc), glycogen granules (G), blood capillary (Bc), Kupffer cell (Kc) and immune cells (Ic). Scale bars (A–D) = 20 μm.

Table 5. Relative proportion (%) of structural components of the liver and hepatocyte area (μm²) in juveniles of *C. macropomum* submitted to different salinity regimes.

Liver structures	Salinity (g of salt/L)				
	S_0	S_2	S_4	S_6	*P*-value
Hepatocytes	86.5 ± 0.3^{b}	87.6 ± 0.4^{b}	92.1 ± 0.3^{a}	91.8 ± 0.2^{a}	< 0.0001
Glycogen granules	9.9 ± 0.3^{a}	9.0 ± 0.3^{a}	$4.8 \pm 0,1^{b}$	5.1 ± 0.2^{b}	< 0.0001
Blood capillaries	3.3 ± 0.2^{a}	3.0 ± 0.2^{ab}	2.7 ± 0.1^{bc}	2.5 ± 0.1^{c}	< 0.0001
Kupffer cells/ immune cells	0.3 ± 0.1^{b}	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	0.6 ± 0.1^{a}	< 0.0001
Hepatocyte area	213.55 ± 5.06^{a}	147.34 ± 5.33^{b}	141.21 ± 3.39^{b}	155.91 ± 4.22^{b}	< 0.0001

Different letters in a row indicate significant differences by Dunn test among sampling groups. Values are expressed as mean ± standard error (SE).

DISCUSSION

Colossoma macropomum adapted well to salinities of up to 4 g salt/L in RAS. Salinity has been an important management tool for larvae and juveniles of freshwater fish such as tilapia (*O. niloticus*) (Dewi et al., 2018; Kombat et al., 2021), African catfish (*C. gariepinus*) (Zidan et al., 2022), carp (*C. carpio*) (Salati et al., 2011), pacu (*P. mesopotamicus*) (Jomori et al., 2012), pirapitinga (*P. brachypomus*) (Ferreira et al., 2023), pintado (*P. corruscans*), curimba (*P. costatus*) (Santos and Luz, 2009), and killifish (*Hypsolebias radiseriatus*) (Araujo et al., 2020), among others.

The evaluated salinities led to changes in water pH and conductivity, which were expected with the addition of salt to the system, as verified in other studies (Camargo et al., 2006; Luz et al., 2008; Imanpoor et al., 2012). However, pH values remained within those recommended for the species and conductivity remained close to values that have already

worked for *C. macropomum* (Fiuza et al., 2015).

The juvenile *C. macropomum* were tolerant to the tested salinities during the first 30 days of cultivation, with good FCR values. Salinities of up to 6 g of salt/L did not negatively affect animal performance during this period. An absence of negative effects on performance for up to 6 g salt/L has also been reported for goldfish (*Carassius auratus*) (Luz et al., 2008, Imanpoor et al., 2012) as well as catfish (*Heteropneustes fossilis*) (Ahmmed et al., 2017). This tolerance has also been observed for juveniles of other freshwater species such as tilapia (*O. niloticus*) (Kombat et al., 2021), African catfish (*C. gariepinus*) (Zidan et al., 2022), common carp (*C. carpio*) (Salati et al., 2011) and perch (*A. testudineus*) (Debroy et al., 2022).

However, between 31 and 45 days of cultivation, salinities began to affect the juveniles. The estimated salinity of up to 4.08 g of salt/L proved to be adequate for weight performance and weight gain, although the best SGR was observed at salinities of up to 1.58 g of salt/L. Fiuza et al. (2015) tested salinities of 5 g of salt/L and higher and found negative effects on the performance of juvenile *C. macropomum*. However, these authors did not test concentrations lower than 5 g of salt/L, suggesting there may be a very narrow limit between what can be used without affecting performance and what can be harmful. Similar observations have been made for other species, such as *Tor soro* (Leying et al., 2023), perch (*A. testudineus*) (Debroy et al., 2022) and pacamã (*L. alexendri*) (Takata et al., 2021). The authors reported that pacamã (*L. alexendri*) resisted salinities of up to 10 g of salt/L for 16 days, and of up to 7.5 g of salt/L for 21 days, with the onset of mortality only after these periods (Mattioli et al., 2017). Furthermore, the same authors reported that salinities above 2.5 g of salt/L already started to negatively affect performance.

Colossoma macropomum showed good results for performance in salinities of up to 4 g of salt/L, similar to other freshwater species such as tilapia (*O. niloticus*) (Kombat et al., 2021), African catfish (*C. gariepinus*) (Zidan et al., 2022), common carp (*C. carpio*) (Salati et al., 2011) and perch (*A. testudineus*) (Debroy et al., 2022). However, species such as pacamã (*L. alexendri*) (Takata et al., 2021; Mattioli et al., 2017), goldfish (*Carassius auratus*) (Luz et al., 2008), betta fish larvae (*Betta splendens*) (Dias et al., 2016), pacu (*P. mesopotamicus*) (Jomori et al., 2012), pirapitinga (*P. brachypomus*) (Ferreira et al., 2023), pintado (*P. corruscans*) and curimba (*P. costatus*) (Santos and Luz, 2009) showed good performance in salinities of up to 2 g of salt/L. The results presented here show that *C. macropomum* is more tolerant to cultivation in different salinities for long periods in RAS, reinforcing variable tolerance among species and life stage.

Whereas FCR was not affected by salinity during the first 30 days, after this period the estimated salinity of up to 3.60 g of salt/L presented results similar to those for fresh water. Such an absence of a difference for FCR was also recorded for the same species after 84 days in fresh water, 5 g of salt/L and 10 g of salt/L (Fiuza et al., 2015). However, at the end of the 45 days of the present study, animals kept at the highest salinity (6 g of salt/L) showed lower consumption than did those of lower salinities. This relationship was also observed for the same species at the end of 84 days (Fiuza et al., 2015), with the authors attributing this drop in food consumption to a lack of appetite caused by the increase in salinity. Mattioli et al. (2017) observed higher cortisol levels in juvenile pacamã (*L. alexendri*) at higher salinities, suggesting a state of constant stress that could reduce food consumption. Lower food consumption at higher salinities as also been observed for juvenile grass carp (*Ctenopharyngodon idella*) (Al-Khshali et al., 2023), African catfish (*C. gariepinus*) (Zidan et al., 2022) and goldfish (*C. auratus*) (Luz et al., 2008).

Final survival was similar among treatments. Juveniles of the cyprinid *Tor serum* (Leying et al., 2023), goldfish (*C. auratus*) (Imanpoor et al 2012) and catfish (*H. fossilis*) (Ahmmed et al., 2017) cultivated in salinities of up to 6 g of salt/L also maintained survival rates similar to that for animals kept in fresh water. The use of low salinities can help maintain high survival rates and the use of salt in prophylactic management can generate benefits in management situations, such as against ectoparasites of some species of freshwater fish, including *Argullus* sp. (fish lice) parasitizing juvenile Nile tilapia (*O. niloticus*) (Dewi et al., 2018).

At the end of the experiment, HSI was lowest for the salinity of 6 g of salt/L. This can be explained by weight loss and negative SGR from 30 to 45 days. This result was opposite to that found for goldfish (*C. auratus*), for which HSI did not differ among the tested salinities, which reached up to 12 g of salt/L for a period of 30 days (Ningsih et al 2020). This lower HSI for the S_6 treatment may also be related to the changes in in the fish liver tissue observed in histological images, such as smaller hepatocyte area, which may be due to the metabolization of glycogen reserves during prolonged stress.

Gill tissue is an important component of the physiological system of fish and stands out as the main organ tissue responsible for osmoregulation and ionic exchange between the animal and the environment (Evans, 2008; Yang et al., 2017). Cartilaginous tissue has a structural function, supporting the gill filaments (Wilson & Lauret 2002). The salinities tested in the present study did not affect the cartilaginous tissue of the gills of *C. macropomum*.

The incidence of epithelial cells and pillar cells was higher in treatments with higher salt concentrations (S_4 and S_6). These cell groups are composed of rich cells and mitochondria (Sales et al., 2017). According to Ramos et al. (2013), the greater relative proportion of these cells may be related to the greater energy demand for osmotic regulation and maintenance of homeostasis in the animal body. Another factor that may be related to the increase in pillar and epithelial cells is change in the structure of gill filaments at higher salinities, such as fusion and lamellar congestion, evidenced in the present work for *C. macropomum* in the S_6 treatment. This type of degeneration has also been found in other freshwater species such as pacamã (*L. alexandri*) (Takata et al., 2021), tilapia (*O. niloticus*) (Azevedo et al., 2015; Mohamed et al., 2021) and African catfish (*C. gariepinus*) (Dawood et al., 2022), where more pronounced changes accompanied an increase in saline levels.

Mucous cell percentage was higher for treatments S_0 and S_2. These cells play an important role as the first defense barrier of the branchial epithelium through the secretion of mucins (Persson et al., 2020). The higher relative proportion of mucous cells at the lower salinities used in the present work may be related to an improved equilibrium condition of animals stored at salinities lower than 4 g of salt/L. According to Persson et al. (2020), the dynamics of this cell group can be affected by environmental changes, such as physical-chemical factors of the water, as with salinity in the present work.

Ionocytes showed a similar behavior, with a greater relative proportion in treatments S_0 and S_2. According to Dymowska et al. (2012), these cells are related to the transport of ions, acting in the maintenance homeostasis and blood pH. The significance of the greater number of ionocytes in S_0 and S_2 is not clear but it may be related to greater efficiency in ion excretion. According to Hiroi and Mccormick (2012), ionocytes play an important role in the secretion of ions and present particularities regarding their distribution and morphology among different fish species.

The proportions of erythrocytes/blood capillaries were highest for S_6 and lowest for S_2. These cells are directly linked to gas exchange and oxygen transport in gills (Nikinmaa et al., 2019). The degenerations generated in the gills of animals cultivated in S_6 may have compromised these functions. An increase in erythrocyte number may be an adaptation to compensate for deficit in obtaining oxygen. It is likely that the *C. macropomum* cultivated in S_6 experienced a greater need for oxygen for metabolic demands aimed at maintaining homeostasis. Increased oxygen consumption in direct proportional to increased salinity was also observed for labari (*Deuterodon iguape*) (BarbierI et al., 2019).

The number of undifferentiated cells was greater for the S_0 treatment. Cells of this group can modify themselves into other structures as an adaptation to a state of imbalance (Wood and Eom 2021). It is possible that these cells underwent some modification and differentiated into other structures in treatments with salt, which would explain the results of the present study.

Gill lamellae are related to ionic and gas exchange and are in direct contact with water flow (Nilsson et al., 2012). Specific secondary lamellae are smaller structures that further increase the contact surface of the gill epithelium with water (Kumari et al., 2011). The number of primary lamellae was highest for S_2 and lowest for S_4 and S_6. The smaller relative proportion of primary lamellae for these treatments may lessen the gill area in contact with water, which may reduce the effectiveness of their function. The relative proportion of secondary lamellae was highest for S_0 and lowest for S_2 and S_4. This differentiation may be due to structural changes generated by salinity, but without implying greater damage to gill tissue.

Liver tissue plays an important role in physiological functions linked to osmotic regulation by fish, as evidenced by Xiong et al. (2020) for steelhead trout (*Oncorhynchus mykiss*). The present work observed changes in liver tissue in direct proportion to salinity, with them being more present in treatment S_4 and even more so in S_6. This result was also observed for African catfish (*C. gariepinus*) cultured at different salinities for 30 days (Dawood et al., 2022).

Histological analyses of the liver of *C. macropomum* showed higher hepatocyte concentrations for S_4 and S_6. These cells may play a role in the conversion of galactose to glucose in fish (Baron et al., 2012). Despite the greater relative proportion of the number of hepatocytes for treatments S_4 and S_6, the area of these cells was smaller compared to S_0, which may be related to a greater glycogen reserve in hepatocyte cytoplasm of juveniles of this treatment.

Kupffer cells may be related to the immune system in fish. Hussein (et al., 2023) observed a relationship between Kupffer cells and the expression of proteins associated with lymphocyte production in mollies (*Poecilia sphenops*). The same authors also reported a correlation between these cells and autophagic processes directly related to adaptations to stress. The present study observed relatively higher proportions of Kupffer cells for juveniles of treatment S_6, which may be related to an adaptive response of the immune system to possible stress generated by exposure to an unsuitable salinity for the species.

The present work found greater relative proportions of glycogen granules for treatments S_0 and S_2. These structures are characterized as an energy reserve in organs with high energy

demand and perform the same function in the liver and gills of fish (Tseng et al., 2007), which may indicate the maintenance of homeostasis in juvenile *C. macropomum* cultivated in salinities of up to 2 g of salt/L. The incidence of blood vessels was higher for S_0, which may be related to greater vascularization of the liver and the maintenance of energy reserves.

CONCLUSION

Juvenile *C. macropomum* cultured in salinities of up to 4 g of salt/L for 45 days in RAS did not experience impaired performance in relation to those cultured in fresh water. Histological analyses showed a lower incidence of changes in liver and gill tissues of animals cultivated in 2 and 4 g of salt/L and more evident changes for those cultivated in 6 g of salt/L. Therefore, salinities of up to 4 g of salt/L can be used for *C. macropomum* cultivation without compromising performance and liver and gill function.

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