UNIVERSIDADE FEDERAL DE MINAS GERAIS – UFMG
ESCOLA DE VETERINÁRIA
PÓS-GRADUAÇÃO EM ZOOTECNIA

DENSIDADE DE ESTOCAGEM NA LARVICULTURA E ENGORDA DO TAMBAQUI *Colossoma macropomum* EM SISTEMA DE RECIRCULAÇÃO DE ÁGUA (SRA)

FÁBIO AREMIL COSTA DOS SANTOS

Belo Horizonte
Escola de Veterinária – UFMG
2022

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

Área de concentração: Produção Animal/Aquacultura.

Orientador: Prof. Dr. Ronald Kennedy Luz

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FOLHA DE APROVAÇÃO

DENSIDADE DE ESTOCAGEM NA LARVICULTURA E ENGORDA DO TAMBAQUI *Colossoma macropomum* EM SISTEMA DE RECIRCULAÇÃO DE ÁGUA (SRA)

Fabio Aremil Costa dos Santos

Tese de Doutorado defendida e aprovada, no dia **15 de dezembro de 2022**, pela Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Zootecnia da Universidade Federal de Minas Gerais, constituída pelos seguintes professores:

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Belo Horizonte, 15 de dezembro de 2022.

Dedico esta tese

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RESUMO

Densidade de estocagem na larvicultura e engorda do Tambaqui *Colossoma macropomum* em sistema de recirculação de água (SRA)

O objetivo do presente estudo foi avaliar os efeitos de densidades de estocagem na larvicultura e engorda de *Colossoma macropomum* em SRA. Para tal, realizou-se 3 experimentos. No primeiro foram avaliados o crescimento, sobrevivência e a heterogeneidade do lote durante a larvicultura de *C. macropomum* submetidos as densidades de 10 (D_{10}), 30 (D_{30}) e 50 (D_{50}) larvas L^{-1} com cinco repetições. As larvas foram alimentadas com náuplios de *Artemia* durante os primeiros 15 dias e com dieta comercial do dia 16 ao dia 30 de cultivo. Ao final de 30 dias de larvicultura, a sobrevivência, peso comprimento e taxa de crescimento específico não foram afetados pelas densidades de estocagem ($P<0,05$). A heterogeneidade em todas as densidades testadas apresentou predominância de animais classe Médio (M) (>18,0 mm e <22,2 mm) ($P<0,05$). O segundo experimento investigou as densidades de 60 (D_{60}), 120 (D_{120}) e 180 (D_{180}) larvas L^{-1}, com quatro repetições, avaliando o crescimento, sobrevivência e a heterogeneidade do lote e incluindo uma análise econômica, utilizando os mesmos manejos do primeiro experimento. Ao final de 25 dias de larvicultura, a amônia foi afetada pela densidade de estocagem com o maior valor para D_{180}, entretanto o desempenho e a sobrevivência não foram afetados pelas densidades ($P>0,05$). O número de animais produzidos ao final da larvicultura aumentou com o aumento da densidade de estocagem ($P<0,05$), apresentando predominância da classe M (>17,0 mm e < 22 mm; >0,07 g e < 0,13g) em D_{60} e D_{120}, enquanto não houve diferença entre os P (17,0 mm e < 0,07 g) e M no D_{180} ($P > 0,05$). A receita líquida foi negativa para o tratamento D_{60}, enquanto que para D_{180} teve a melhor receita líquida. No terceiro experimento, foi avaliado os efeitos da densidade de estocagem e da classificação dos animais por tamanho no crescimento e parâmetros fisiológicos de juvenis de *C. macropomum* em RAS. Este experimento foi dividido em três fases. Na Fase I, juvenis (34,88 ± 0,60g) foram estocados nas densidades de $D_{0,5}$= 0,5; $D_{1,0}$= 1,0 e $D_{1,6}$= 1,6 kg m^{-3} por 53 dias. Na Fase II, juvenis (150,61 ± 0,58 g) foram estocados

nas densidades de $D_{1,5}$ = 1,5, $D_{3,0}$ = 3,0 e $D_{4,5}$ = 4,5 kg m^{-3} por 60 dias. Na Fase III, os animais foram classificados quanto ao peso (ou massa) em P = 300–400g; M = 400–500g e G = >500g por 60 dias com biomassa inicial de 3,9 kg m^{-3} para cada classe. Em todas as fases do experimentos, os animais foram alimentados duas vezes ao dia (08h00min e 16h00min), até a saciedade aparente com ração comercial. Após os primeiros 30 dias da Fase I, o peso final (PF) e o ganho de peso diário (GPD) foram maiores para $D_{0,5}$, mas a taxa de conversão alimentar (TCA) foi menor (P<0,05). O consumo diário de ração (CD) foi o menor para $D_{1,6}$ e o maior para $D_{0,5}$ (P<0,05). Aos 53 dias, PF, CD e GPD foram maiores para $D_{0,5}$ e menores para $D_{1,6}$. Do dia 31 ao dia 53 houve diminuição da hemoglobina (Hg) e hematócrito (Htc) e aumento do índice hepatossomático (IHS) para $D_{0,5}$ (P<0,05). Após os primeiros 30 dias da Fase II, PF, GPD e CD foram maiores para $D_{1,5}$ (P<0,05). Após 60 dias, $D_{1,5}$ apresentou os maiores valores de PF e IHS e os menores valores de glicose, triglicerídeos e Htc (P<0,05). Na Fase III, após 30 dias PF, GPD e CD foram maiores para a classe G (P<0,05), e PF permaneceu maior para a classe G após 60 dias. O IHS foi maior para a classe P e menor para a classe G (P<0,05), enquanto os triglicerídeos, colesterol, proteínas totais e Hg foram menores para as classes P e M (P<0,05). Com os resultados obtidos por essa pesquisa concluímos que a larvicultura de *C. macropomum* pode ser realizada em RAS com até 180 larvas L^{-1} com bons resultados de desempenho e sobrevivência. Além disso, foram verificados bons indicadores de viabilidade econômico-financeira para densidades superiores a 120 larvas L^{-1}. Estudos complementares devem ser realizados para otimizar manejos nesta fase de produção, como classificação para produzir lotes mais homogêneos de *C. macropomum*. Já na fase de engorda, a densidade de estocagem deve ser avaliada de acordo com o tamanho dos animais e, apesar do melhor desempenho encontrado para as menores densidades (fases I e II), maiores densidades produziram maior biomassa. A classificação dos animais é uma importante estratégia de manejo para manter a uniformidade para comercialização.

Palavras-chave: espécie amazônica, produção intensiva, RAS, Tambaqui

ABSTRACT

Stocking density in larviculture and fattening of Tambaqui *Colossoma macropomum* in a recirculation aquaculture system (RAS)

The aim of the present study was to evaluate the effects of stocking densities on larviculture and fattening of *Colossoma macropomum* in RAS. For this, 3 experiments were carried out. In the first, the growth, survival and heterogeneity of the batch during larviculture of *C. macropomum* submitted to densities of 10 (D_{10}), 30 (D_{30}) and 50 (D_{50}) L^{-1} larvae with five replications were evaluated. The larvae were fed with Artemia nauplii during the first 15 days and with a commercial diet from day 16 to day 30 of cultivation. At the end of 30 days of larviculture, survival, weight, length and specific growth rate were not affected by stocking densities ($P<0.05$). Heterogeneity in all tested densities showed a predominance of Medium (M) class animals (>18.0 mm and <22.2 mm) ($P<0.05$). The second experiment investigated the densities of 60 (D_{60}), 120 (D_{120}) and 180 (D_{180}) L^{-1} larvae, with four replicates, evaluating the growth, survival and heterogeneity of the batch and including an economic analysis, using the same managements of the first experiment. At the end of 25 days of larviculture, ammonia was affected by stocking density with the highest value for D_{180}, however performance and survival were not affected by densities ($P>0.05$). The number of animals produced at the end of larviculture increased with the increase in stocking density ($P<0.05$), with a predominance of class M (>17.0 mm and < 22 mm; >0.07 g and < 0.13 g) at D_{60} and D_{120}, while there was no difference between P (17.0 mm and < 0.07 g) and M at D_{180} ($P > 0.05$). The net income was negative for the D_{60} treatment, while for D_{180} it had the best net income. In the third experiment, the effects of stocking density and classification of animals by size on the growth and physiological parameters of juveniles of *C. macropomum* in RAS were evaluated. This experiment was divided into three phases. In Phase I, juveniles (34.88 ± 0.60g) were stocked at densities of $D_{0.5}$= 0.5; $D_{1.0}$= 1.0 and $D_{1.6}$= 1.6 kg m^{-3} for 53 days. In Phase II, juveniles (150.61 ± 0.58 g) were stocked at densities of $D_{1.5}$ = 1.5, $D_{3.0}$ = 3.0 and $D_{4.5}$ = 4.5 kg m^{-3} for 60 days. In Phase III, animals were classified according

to weight (or mass) in P = 300–400g; M = 400–500g and G = >500g for 60 days with initial biomass of 3.9 kg m³ for each class. In all phases of the experiments, the animals were fed twice a day (08:00 and 16:00), until apparent satiation with commercial feed. After the first 30 days of Phase I, final weight (FW) and daily weight gain (DWG) were higher for $D_{0.5}$, but feed conversion ratio (FCR) was lower ($P<0.05$). Daily feed intake (DC) was the lowest for $D_{1.6}$ and the highest for $D_{0.5}$ ($P<0.05$). At 53 days, FW, DC and DWG were higher for $D_{0.5}$ and lower for $D_{1.6}$. From day 31 to day 53, there was a decrease in hemoglobin (Hg) and hematocrit (Htc) and an increase in the hepatosomatic index (HSI) to $D_{0.5}$ ($P<0.05$). After the first 30 days of Phase II, FW, DWG and DC were higher for $D_{1.5}$ ($P<0.05$). After 60 days, $D_{1.5}$ showed the highest values of FW and HSI and the lowest values of glucose, triglycerides and Htc ($P<0.05$). In Phase III, after 30 days FW, DWG and DC were higher for class G ($P<0.05$), and FW remained higher for class G after 60 days. The HSI was higher for class P and lower for class G ($P<0.05$), while triglycerides, cholesterol, total proteins and Hg were lower for classes P and M ($P<0.05$). With the results obtained by this research, we conclude that the larviculture of *C. macropomum* can be carried out in RAS with up to 180 larvae L^{-1} with good performance and survival results. In addition, good indicators of economic and financial viability were verified for densities greater than 120 larvae L^{-1}. Complementary studies should be carried out to optimize management in this production phase, such as classification to produce more homogeneous batches of *C. macropomum*. In the fattening phase, the stocking density must be evaluated according to the size of the animals and, despite the better performance found for lower densities (phases I and II), higher densities produced greater biomass. The classification of animals is an important management strategy to maintain uniformity for commercialization.

Keywords: Amazonian species, intensive production, RAS, Tambaqui

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LISTA DE SIGLAS, ABREVIATURAS E SÍMBOLOS

RAS	Recirculating aquaculture system
g	Gramas
m^3	Metro cubico
Kg	Quilograma
S	Small
M	Medium
L	Large
Fw	Peso final
DWG	Ganho de peso diário
FCR	Conversão alimentar
DC	Consumo diário de ração
Hg	Hemoglobina
Htc	Hematócrito
HSI	Índice hepatossomático
Ha	Hectare
L	Litro
m^2	Metro quadrado
TAN	Nitrogênio amoniacal total
NO_2-N	Nitrito

NO_3-N	Nitrato
%	Porcentagem
IBGE	Instituto Brasileiro de Geografia e Estatística
LAQUA	Laboratório de aquacultura
UFMG	Universidade Federal de Minas Gerais
°C	Graus Celsius
mm	Millimeter
mg	Milligram
CEUA	Comissão de Ética no Uso de Animais
SGR	Specific growth rate
ANOVA	Variance analysis
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
h	Hour
OD	Dissolved oxygen
mL	Milliliter
TPP	Total plasma protein
RPM	Rotation per minute
MFI	Mesenteric Fat Index
µS	Micro-siemens

TOC	Total Operating Cost
EOC	Effective Operating Cost
OC	Other Costs
INCC	Índice Nacional de Custo de Construção
CP	Crude protein
KW	Kilowatt
US$	American dollar

SUMÁRIO

1. INTRODUÇÃO

A demanda pela produção global de alimentos provindos do pescado apresenta crescente aumento ao decorrer dos anos. Isso se deve a uma série de fatores como: aumento da população, renda, conscientização em relação aos benefícios que esses produtos podem trazer a saúde, entre outros fatores (Mustapha et al., 2021). Neste contexto, estima-se que o peixe fornece cerca de 20% da ingestão média de proteína animal para cerca de 3,2 bilhões de pessoas em todo o mundo (FAO Statistical Pocketbook, 2015). A produção total de peixes deve atingir 204 milhões de toneladas em 2030 e, esse aumento, corresponde a 15% em relação ao ano de 2018, onde 46% desse valor é decorrente da produção na aquacultura (FAO, 2020). Porém, os impactos gerados pela recente pandemia de COVID-19 diminuíram em geral a produção atual de pescado no mundo. No entanto, no Brasil, a produção de peixes em 2020 aumentou cerca de 5,93% (802.930 toneladas) (PeixeBR, 2021), demonstrando um cenário inverso do que a maioria dos demais países do mundo. Já em 2021, a produção de peixes no Brasil, atingiu a marca de 841.005 toneladas, representando um aumento de 4,7% em relação aos dados de produção de 2020 (PeixeBR, 2022)

Entre os diversos fatores importantes a serem determinados para a otimização da produção em qualquer tipo de prática na aquicultura, a determinação da densidade de estocagem ideal é um desafio. Quanto maior a densidade de estocagem de peixes nos sistemas de cultivo, maior o número de animais produzidos. Porém, este cenário frequentemente resulta em baixo crescimento e produção (Vijayan e Leatherland, 1988; Canario et al., 1998; Roncarati et al., 2006; Nhan et al., 2019). Contudo, o uso de baixas densidades acarreta prejuízos à produção, como a utilização ineficiente do espaço, desempenho ineficiente e prejuízos econômicos (Tolussi et al., 2010; Upadhyay et al.,2022). Relatos na literatura demonstraram que densidades de estocagem inadequadas retardam a taxa de crescimento, reduzem a imunidade e podem afetar negativamente a qualidade da carne e os benefícios econômicos (Ellis, 2002; Lin et al., 2018; Refstie e Kittelsen, 1976; Schram et al.,

2006). Portanto, a determinação da densidade de estocagem é essencial em qualquer tipo de prática na aquicultura intensiva (Upadhyay et al., 2022).

Tambaqui (*Colossoma macropomum*) (Cuvier, 1818) é um peixe Neotropical da família Serrasalmidae, ordem Characiformes (Calcagnotto et al., 2005). É nativa da bacia do rio Amazonas e atualmente é a espécie nativa mais produzida comercialmente no Brasil (PeixeBR, 2021; PeixeBR, 2022), atingindo mais de 25 kg. É também uma das principais espécies de água doce produzidas na aquicultura sul-americana como em vários países asiáticos, incluindo China, Indonésia, Malásia, Mianmar e Vietnã (Woynárovich e Van Anrooy, 2019). Isso deve-se, principalmente, à disponibilidade imediata de juvenis e potencial de crescimento, alta produtividade e robustez (Brandão et al., 2004; Saint-paul, 2017). No Brasil é cultivada nas regiões Norte e Nordeste, onde a temperatura da água é elevada (Baldisserotto e Gomes, 2010).

CAPÍTULO 1

2. REVISÃO DE LITERATURA

2.1. Densidade de estocagem na produção de peixes

Alguns fatores podem influenciar na densidade ideal para a produção como: espécie a ser produzida (Sharma e Chakrabarti, 2003; Jatobá e Silva, 2015; Magouz et al., 2019), sistema de produção (North et al., 2006; Tidwell, 2012) e tamanho ou idade dos animais (North et al., 2006). Esse manejo pode afetar diretamente o comportamento (Manley et al., 2014; Barros et al., 2019), saúde e bem estar (Hasenbein et al., 2016), desempenho zootécnico e sobrevivência (Zarski et al., 2011; Millán-Cubillo et al. 2016; Reis et al., 2021; Santana et al., 2020; Oliveira et al., 2020; Budi et al., 2020; Karnatak et al., 2021), qualidade da água (Luz et al., 2012; Santos et al., 2012) e, consequentemente, a lucratividade da produção (Siddiqui et al., 1989; Lira et al., 2021; Karnatak et al., 2021).

2.1.1 Densidade de estocagem na larvicultura de peixes

A larvicultura é considerada estágio crítico da produção de peixes (Herath e Atapaththu., 2013; Evangelista et al., 2020). O sucesso dessa fase é determinado pela combinação de fatores como nutrição, imunidade, qualidade da água e densidade de estocagem (Herath e Atapaththu, 2013).

A densidade pode afetar o crescimento, sobrevivência, comportamento e heterogeneidade, além de favorecer a ocorrência de canibalismo durante a larvicultura (Zalina et al., 2011; Barros et al., 2018; Arifin et al., 2019; Santos et al., 2021). No entanto, esses efeitos na sobrevivência e no crescimento podem ser variáveis ou até contraditórios (Niazie et al., 2013). Por exemplo, Keer et al. (2018) demonstraram que a taxa de sobrevivência e o crescimento são afetados negativamente pelo aumento na densidade de estocagem de 3, 5 e 7 milhões de larvas ha^{-1}. O uso de um grande número de larvas em um espaço pode levar a um crescimento reduzido, bem como ao aumento da

agressividade, heterogeneidade de tamanho e taxa de mortalidade (Manley et al., 2014; Naumowicz et al., 2017). Uma possível explicação para uma relação positiva entre densidade e agressividade pode ser a maior frequência de encontros com co-específicos (Manley et al., 2014, Souza et al., 2014).

No entanto, aumento na densidade de estocagem pode levar a redução na exibição de comportamento agressivo. Essa relação pode ser justificada porque o aumento da densidade de estocagem causa efeito confuso nas larvas (efeito disruptivo), que não pode selecionar a presa devido ao grande número de indivíduos no grupo (Naumowicz et al., 2017). Santos et al. (2021) em estudo com larvas de *C. macropomun* demonstraram que diferentes densidades de estocagem influenciam na heterogeneidade, sem influenciar no desempenho. A heterogeneidade é considerada um dos problemas centrais da aquacultura, destacando a necessidade de classificações dos animais durante o ciclo. Já, em outros estudos trabalhando com densidade de estocagem de larvas apresentaram resultados onde não houveram diferenças nos parâmetros analisados para algumas espécies como trairão *Hoplias lacerdae* entre 10 e 90 larvas L^{-1} (Luz e Portella, 2005), Goldfish *Carassius auratus* utilizando entre 6 e 15 peixes por aquário (Niazie et al., 2013), Matrinxã *Brycon amazonicus* entre 20 e 60 larvas L^{-1} (Barros et al., 2018), Pacamã *Lophiosilurus alexandri* entre 60 e 300 larvas L^{-1} (Cordeiro et al., 2016), Gurami-gigante *Osphronemus goramy* entre 0,6 e 19,2 larvas L^{-1} (Arifin et al., 2019), Colisa lalia *Trichogaster lalius* entre 5 e 40 larvas L^{-1} e Barbo rosado *Pethia conchonius* entre 20 e 80 larvas L^{-1} (Ramee et al., 2020). Esses trabalhos indicam que o uso de manejos adequados pode favorecer a intensificação da larvicultura. Assim como em juvenis e adultos, em estágios larvais tem que ser levada em consideração, estudando espécies, sistemas de produção e manejos adequados, afim de estipular a densidade de estocagem ideal para a maximização da larvicultura.

2.1.2 Densidade de estocagem no desempenho de peixes

A densidade de estocagem é um importante fator ambiental que também pode afetar o crescimento de juvenis (Long et al., 2019). Estudos comprovaram que a densidade de estocagem pode ter efeitos distintos dependendo de uma série de fatores, como espécies de peixes, idade e condições de criação. A alta densidade pode causar redução no consumo de alimentos e supressão do crescimento (Li et al., 2012).

Os efeitos negativos da alta densidade de estocagem no desempenho de juvenis foi relatado para Pargo rosa *Pagrus pagrus* utilizando densidade entre 4-50 kg m^{-3} (Laiz-Carrión et al., 2012), salmão do Atlântico *Salmo salar* entre 21 e 86 kg m^{-3} (Hosfeld et al., 2009), robalo europeu *Dicentrarchus labrax* entre 5,5-36 kg m^{-3} (Lupatsch et al., 2010) , linguado *Scophthalmus maximus* entre 9,3 e 19,1 kg m^{-2} (Jia et al., 2016), bagre americano *Ictalurus punctatus* entre 50 e 300 peixes m^{-3} (Refaey et al., 2018) e esturjão chinês *Acipenser sinensis* entre 4,8 e 12,68 kg m^{-2} (Long et al., 2019). Estes podem estar relacionados a mudanças na competição intraespecífica e no comportamento social (Carvalho et al., 2018), aumento da erosão das nadadeiras (North et al., 2006), liberação de hormônios do estresse (Yadata et al., 2019), alterações imunológicas (Mazur e Iwama,1993), alterações na proteína plasmática total (Tan et al., 2018) e parâmetros metabólicos e hematológicos (Yarahmadi et al., 2015).

Por outro lado, Andrade et al. (2015) demonstraram que a densidade de estocagem não apresentou efeito direto no crescimento do Linguado senegalês *Solea senegalensis* utilizando as densidades de 7, 17 e 24 kg m^{-2}. No entanto, estudos demonstraram que a alta densidade de estocagem tem um impacto positivo no desempenho de crescimento de algumas espécies de peixes, como Pirarucu *Arapaima gigas*, mantidos em 3,6, 6 e 10 peixes m^{-2} (Oliveira et al.,2020), Mulloway, *Argyrosomus japonicus* em 4,08, 8,16 ou 16,32 kg m^{-3} (Pirozzi et al., 2009) e a Corvina-legítima *Argyrosomus regius* em 3, 7, 10 e 13 g l^{-1} (Millán-Cubillo et al., 2016). Estes podem estar relacionados à natureza gregária das espécies citadas (Pirozzi et al., 2009), sugerindo que a ativação crônica do sistema de estresse é realizada nos peixes mantidos em densidades de estocagem mais baixas (Millán-

Cubillo et al., 2016; Pirozzi et al., 2009). Trabalhando com diferentes densidades com *C. macropomum* em viveiros escavados (5, 10 e 15 peixes m^{-2}), Costa et al. (2016) demonstraram que o número final de peixes mostrou um comportamento linear positivo, enquanto os outros parâmetros de desempenho não foram afetados pela densidade. Para as densidades testadas, dos custos de investimento e produção, os itens mais representativos foram construção de viveiro e aquisição de alevinos, respectivamente, o que indica que o aumento da densidade de semeadura afetou positivamente o processo produtivo, melhorando todos os indicadores econômicos

A determinação de densidade de estocagem para juvenis é primordial para otimização da produção em aquicultura. Há uma grande variação entre as espécies, comportamento, sistema de produção entre outros.

2.1.3 Densidade de estocagem no bem-estar animal

O uso de densidades de estocagem inadequadas é um aspecto crítico que pode afetar o bem-estar (Ghozlan et al., 2018; Refaey et al., 2018), considerada um estressor crônico na aquicultura (Zahedi et al., 2019). Durante o estresse crônico induzido por altas densidades de estocagem, o eixo hipotálamo-hipófise-rim (HPI) desempenha papel importante, e esse eixo pode aumentar os níveis séricos de cortisol (Long et al., 2019).

O cortisol desempenha papel essencial na manutenção do crescimento, balanço energético e regulação da imunidade quando os peixes estão em condição de estresse (Hori et al., 2010). Temos que levar em consideração que o estresse é dependente do efeito da densidade de estocagem. Por exemplo, Millán-Cubillo et al. (2016) trabalhando com corvina legítima *Argyrosomus regius* demonstraram que os animais em altas densidades apresentaram uma relação negativa nos níveis de cortisol em relação à densidade de estocagem.

Os hormônios da tireoide podem cooperar com outros hormônios para promover o crescimento de peixes. Fátima et al. (2018) sugeriram que a inibição do crescimento de peixes sob estresse de densidade de estocagem pode estar associada à diminuição das concentrações de tiroxina. Os índices metabólicos séricos, incluindo glicose, proteína total, triglicerídeo total e colesterol total de peixes cultivados sob alta densidade de estocagem também mudaram correspondentemente em resposta ao estresse (Long et al., 2019). O estresse oxidativo ocorre no corpo quando há um desequilíbrio entre o conteúdo de espécies reativas de oxigênio e a atividade da enzima antioxidante (Braun et al., 2010). Isso é especialmente importante na aquicultura, onde os danos oxidativos no tecido dos peixes não afetam apenas o bem-estar dos peixes, mas também a qualidade do produto (Andrade et al., 2015).

Além do citado anteriormente, também pode haver aumento do índice hepatossomatico em peixes com maior densidade de estocagem, como registrado por Bacchetta et al. (2020) em juvenis de Pacu *Piaractus mesopotamicus*. Porém, Pait e Nelson (2003) afirmaram que este é um bom biomarcador para detectar os efeitos perigosos dos estressores ambientais. O catfish (*Ictalurus punctatus*) exposto a 150 e 300 peixes m^{-3} (densidade média e alta, respectivamente) também teve um aumento no índice hepatossomatico como resultado do comprometimento hepático e da indução das atividades das enzimas transaminases (Refaey et al., 2018).

2.1.4 Densidade de estocagem na qualidade de água

A densidade de estocagem é um dos principais fatores que afetam os parâmetros de qualidade da água (Oliveira et al.,2020). É relatado que a alta densidade de estocagem interfere no ambiente aquático devido ao excesso de nitrogênio (Lemos et al., 2018; Yadata et al., 2020).

À medida que a densidade dos peixes aumenta, os parâmetros de qualidade da água podem ser afetados de maneira complexa, produzindo níveis reduzidos de oxigênio dissolvido e maiores níveis de CO_2 e amônia, bem como diminuição do pH (Sundh et al., 2019). Essa deterioração da qualidade da água somado a alta densidade de estocagem em si pode ser estressantes para os peixes e, portanto, uma ameaça ao bem-estar (Sveen et al., 2016). Mesmo em casos citados no item 2.1.2 desta revisão, espécies que não tem efeito no desempenho em altas densidades, podem levar a piora na qualidade da água do sistema de produção. A alta densidade de estocagem interfere no ambiente aquático devido ao excesso de excreção de nitrogênio (Lemos et al., 2018).

2.2. Sistema de recirculação de água

A produção aquícola é caracterizada pela alimentação constante dos organismos que estão em processo produtivo. Com isso o excesso de ração pode prejudicar os ambientes aquáticos, como também inibir a respiração de outros animais aquáticos, possivelmente os levando a morte (Mente et al., 2006). Este processo se deve pelo fato dos microrganismos nativos presentes nos corpos d'água não possuírem a capacidade de decomposição da matéria orgânica com eficiência para que não haja a deterioração da qualidade da água e o crescimento de microrganismos nocivos (Zhou et al., 2017). Nesse sentido, a utilização de sistemas de produção sustentáveis deve ser mais utilizada nos próximos anos. De acordo com as principais características dos animais aquáticos e tipos de equipamentos de tratamento de água, os sistemas de recirculação de aquicultura (SRAs) podem reciclar 90% a 99% da água, reduzindo assim a poluição, que é uma das principais tendências para a aquicultura sustentável (Badiola et al., 2012). Os SRAs podem apresentar diferentes estruturas de montagem entre si, mas os seus objetivos e mecanismos são basicamente iguais. Esse tipo de sistema de produção é composto por biofiltros nitrificantes, nos quais os microorganismos responsáveis pelo tratamento da água são fixados no crescimento na superfície de estruturas conhecidas como "mídia''. Com isso, o nitrogênio

amoniacal total (NAT) e o nitrito (NO_2-N) são oxidados a nitrato (NO_3-N) (Shitu et al., 2020, Shitu et al., 2021). Amônio e nitrito (NO_2-N), que se originam principalmente de sólidos de aquicultura e metabolismo animal, são duas toxinas agudas perigosas para animais aquáticos (Crab et al., 2007 ; Avnimelech, 2012). Além disso, esse tipo de sistema geralmente contém outros componentes, como dispositivos de remoção de sólidos, e alguns são equipados com biofiltros desnitrificantes para remover NO_3-N (Badiola et al., 2012; Badiola et al., 2018; Xiao et al., 2019; Yogev et al., 2020).

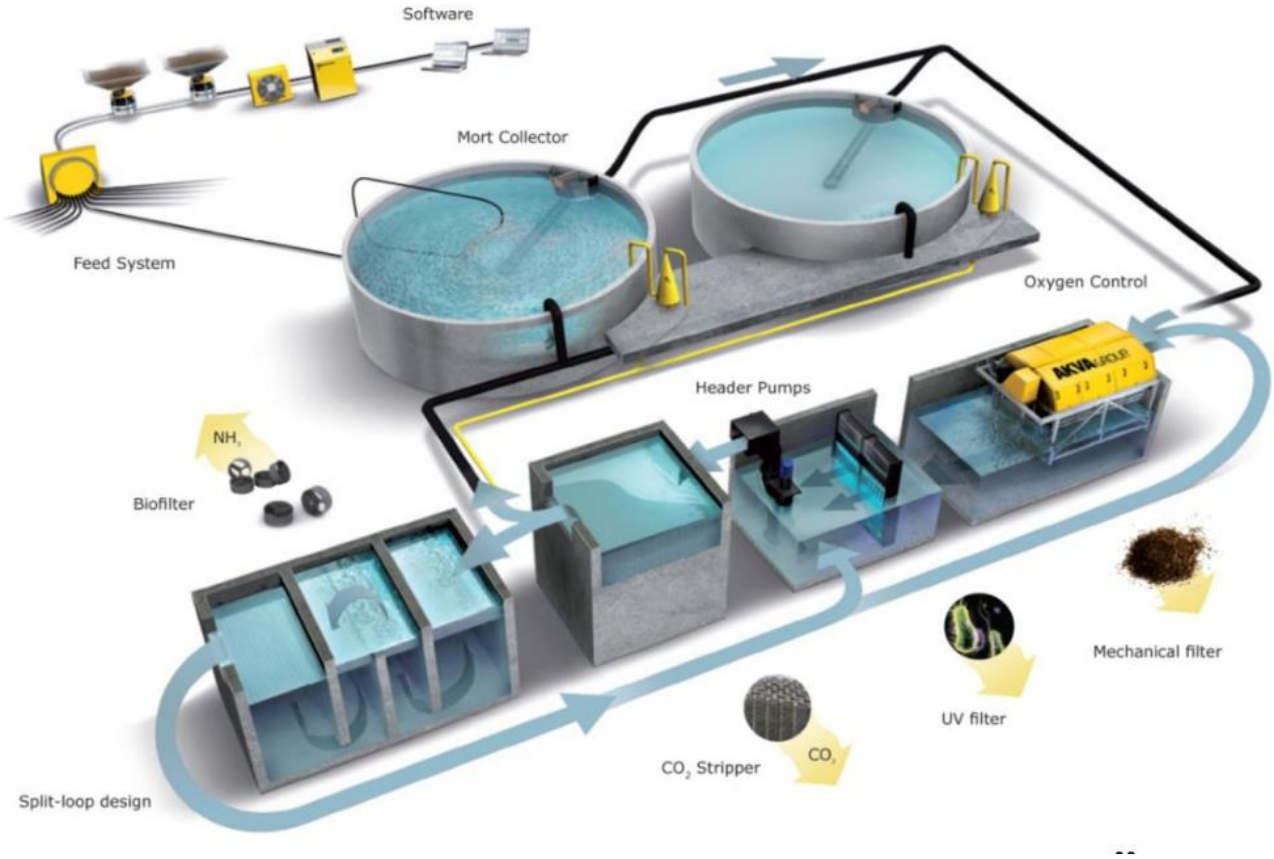

Figura 1 – Exemplo de Sistemas de recirculação de água (SRA). (Fonte: lbaaf.co.nz)

2.3. Marcadores hematológicos e bioquímica sanguínea

A análise sanguínea é um dos métodos comumente usados para avaliar o estado fisiológico e a saúde dos peixes (Grant, 2015; Docan et al., 2018; Fazio, 2019), além de ser uma excelente ferramenta para correlacionar o comportamento dos peixes com qualquer problema ou distúrbio que

possa afetá-los (Guilherme et al., 2021). Esse tipo de análise inclui medições de parâmetros bioquímicos em sangue total, plasma ou soro (Witeska et al., 2022). Algumas análises são comumente utilizadas como nível de glicose e proteína, perfil de colesterol, concentrações de íons, metabólitos ou hormônios, atividades de enzimas e outros parâmetros (Witeska et al., 2022). Os índices hematológicos e bioquímicos fornecem informações extensas sobre a capacidade de transporte de oxigênio dos peixes, potencial imunológico, nível de estresse, doença, intoxicação, estado nutricional entre outros fatores.

2.4. *Análise econômica na produção comercial de peixes*

A aquacultura é vista como uma alternativa para reduzir a lacuna cada vez maior entre a demanda e a oferta de peixes (Flores e Pedroza Filho 2019). Para a otimização desta cadeia produtiva estudos relacionados a viabilidade econômica devem ser levados em consideração, uma vez que atualmente trabalhos na literatura são escassos neste tema. A avaliação da economia da aquicultura, como da agricultura, pode ser realizada de diferentes pontos de vista, dependendo da necessidade e do escopo (Shang et al., 1985).

2.4.1. *Avaliação econômica aliada a estudos com densidade de estocagem para peixes*

As inovações em indústrias como a aquicultura são muitas vezes geradas por múltiplas linhas de pesquisa relacionadas a uma variedade de regimes (por exemplo, dieta, densidade, aplicação de hormônios) e estágios de crescimento (Din et al., 2004). A avaliação de inovações complexas requer ferramentas que avaliem vantagens econômicas e ajudem a dividir os benefícios esperados entre produtores e instituições de transferência de tecnologia e entre as diferentes linhas de pesquisa (Din et al., 2004). Vários fatores podem afetar o retorno econômico do sistema, como rendimento, preço de venda, custo da alimentação, custo das larvas, alevinos ou juvenis, investimento do sistema e custo operacional (Muangkeow et al., 2007).

Nesse sentido recentes trabalhos avaliando a densidade de estocagem foram realizados afim de levar informações sobre a análise financeira de inovações em projetos de pesquisa cientifica como descrito por Oliveira et al. (2012), demonstraram que é economicamente viável produzir juvenis de pirarucu (*Arapaima gigas*) em tanques-rede com densidades moderadas. Em bagre sutchi (*Pangasius sutchi* Fowler 1937) quando produzidos em tanques rede com maior densidade de estocagem resultou em maior rendimento por unidade de custo de produção e menor custo por unidade de rendimento, sendo a receita líquida maior com o aumento da densidade de estocagem (Islam et al.,2006). Yengkokpam et al. (2020) avaliando diferentes densidades de estocagem para juvenis de Labeo bata *Labeo bata* criados em tanques-rede, demonstraram que o desempenho dos peixes criados foi inversamente proporcional à densidade de estocagem. Porém, a biomassa produzida aumentou com o aumento da densidade de estocagem de até 75 alevinos m^{-3}, rendendo uma produção de peixe 50% maior e gerou uma receita líquida 92,5% maior do que a criação na densidade de estocagem mais baixa. Debnath et al. (2016) e Karnatak et al. (2021) relataram maior benefício econômico de densidades de estocagem mais baixas. Este fato está relacionado a maior sobrevivência, melhor crescimento e utilização de ração (Mensah et al. 2013). Com base nestes dados constatamos a aplicação cada vez mais de análises econômicas em estudos na produção comercial de peixes principalmente em trabalhos com densidade de estocagem em foco, para que com isso os produtores comerciais de peixes possam avaliar qual melhor medida econômica a ser adotada em uma produção de grande escala

2.5. Tambaqui

Tambaqui (Figura 2) (*Colossoma macropomum*) (Cuvier, 1818) é um peixe Neotropical da família Serrasalmidae, ordem Characiformes (Calcagnotto et al., 2005). Apresenta destaque e potencial produtivo na piscicultura brasileira, sendo a segunda espécie mais produzida em território nacional e a espécie nativa mais produzida (PeixeBr, 2022). Sua produção em território brasileiro no ano de 2020 atingiu 100,6 mil toneladas/ano, representando um total de 18,2% de toda piscicultura

brasileira. A região norte do país se destaca na produção desta espécie representando cerca de 73% do total produzido em território nacional (IBGE, 2021). Porém outras regiões também vêm se destacando na produção desta espécie como é o caso dos estados do Maranhão e Alagoas (PeixeBR, 2021). Sua produção está se disseminando também em outros locais, como na maioria dos países da America do sul e Central, países do Caribe, e asiáticos como a China, Indonesia, Malasia, Mammar e Vietnã (Woynárovich e Van Anrooy, 2019). Esse sucesso deve-se principalmente à disponibilidade imediata de juvenis e potencial de crescimento, alta produtividade e robustez (Brandão et al., 2004; Saint-paul, 2017).

Figura 2 – Exemplar de Tambaqui (*C. macropomum*) presente no Laboratorio de aquacultura (LAQUA) da UFMG. (Fonte: Fabio Santos)

Esta espécie possui habito alimentar onívoro, com alimentação em ambientes naturais principalmente de frutos, sementes e pequenos organismos naturais (Araujo-Lima e Goulding, 1998). Nativo da bacia amazônica, o Tambaqui pode atingir cerca de 25Kg; porém o peso de abate utilizado pelos produtores pode ser variado (Filho, 2007), devido ao crescimento heterogêneo do lote, necessitando de classificações durante seu período produtivo (Santos et al., 2021). Espécies da bacia amazônica enfrentam barreiras sazonais, durante os períodos de cheias dos rios, os peixes têm alta

disponibilidade de alimento com temperaturas da água mais estáveis, que variam de 25 a 28°C, enquanto nos períodos de vazante, os peixes ficam expostos a disponibilidade de alimentos e temperaturas mais altas da água (34–40°C) (Kramer et al., 1978).

Estudos recentes vêm demonstrando o avanço de tecnologias de produção para esta espécie, principalmente utilizando RAS, como: cultivo de juvenis em diferentes cores de tanque (Boaventura et al., 2021); diferentes estratégias de manejo alimentar, como a restrição alimentar (Assis et al., 2020) e autoalimentação e alimentação automática com restrição de tempo (Guilherme et al., 2021); crescimento em diferentes densidades de estocagem na larvicultura (Santos et al., 2021a), efeito da densidade de estocagem no crescimento e teste de exposição ao ar de juvenis (Silva et al., 2021), efeitos na hematologia, perfil proteico plasmático e produção de imunoglobulinas em juvenis submetidos a diferentes densidades de estocagem (Costa et al., 2019) e crescimento e bioquímica sanguínea na fase engorda em diferentes densidades de estocagem e na seleção por tamanhos (Santos et al., 2021b); respostas fisiológicas de juvenis após períodos de exposição ao ar (Neves et al., 2022). Através dessas iniciativas de estudo, a viabilidade para produção dessa espécie em sistema controlado como SRA pode trazer novas perspectivas para o futuro da produção da espécie, principalmente em regiões de temperatura mais baixa do que a encontrada na região amazônica.

2. OBJETIVOS

3.1. Objetivos gerais

Avaliar a eficiência de diferentes densidades de estocagem na sobrevivência, fisiologia e produtividade de tambaqui durante as fases de larvicultura e engorda em RAS.

3.2. Objetivos específicos

Avaliar o crescimento, sobrevivência e heterogeneidade de larvas de tambaqui *C. macropomum* mantidos em diferentes densidades de estocagem em RAS.

Avaliar a viabilidade econômica utilizando altas densidades de estocagem na larvicultura de *C. macropomum* em RAS.

Avaliar a importância da classificação durante a larvicultura e engorda de *C. macropomum*

Avaliar o crescimento e respostas fisiológicas de tambaqui *C. macropomum* criados em diferentes densidades de estocagem durante diferentes fases de engorda em RAS.

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CAPÍTULO 2

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Stocking density in *Colossoma macropomum* larviculture, a freshwater fish, in recirculating aquaculture system

Running head: Stocking density in *C. macropomum* larviculture

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Abstract

This study aimed to investigate stocking density during larviculture of *Colossoma macropomum* in recirculating aquaculture system with slightly saline water. Densities of 10 (D_{10}), 30 (D_{30}) and 50 (D_{50}) larvae L^{-1} were tested with five replicates each. Larvae were fed with *Artemia* nauplii during the first 15 days and with commercial diet from day 16 to day 30. Water was maintained at a salinity of 2.01±0.41 g of salt/L and performance and survival were evaluated throughout the experiment. At the end of the experiment, larvae of each tank were classified according to size as small - S (<18.0 mm and <0.08g), medium - M (>18.0 mm and <22.2 mm, >0.08g and <0.15g), and large - L (>22.2 mm and >0.15g), and the size distribution was calculated for each stocking densities evaluated. Larval weight, length and specific growth rate were not affected by the tested stocking densities throughout the experiment ($p>0.05$). Heterogeneity was recorded at 30 days of larviculture for all tested densities with a predominance of class M animals. Survival during feeding with *Artemia* was not affected by the tested densities ($p>0.05$). Survival during feeding with commercial diet was highest for D_{10} and lowest for D_{30} ($p<0.05$). Global survival, considering the experiment as a whole, was similar among the tested stocking densities ($p>0.05$). Larviculture of *C. macropomum* can be successfully carried out at stocking densities of up to 50 larvae L^{-1} during the first 30 days of rearing in slightly saline water in recirculating aquaculture system.

Keywords: tambaqui, intensive larviculture, omnivorous species, RAS.

1. INTRODUCTION

Larviculture is considered a critical stage of fish production (Kolkovski, 2001; Santos and Luz, 2009; Herath and Atapaththu, 2013; Evangelista et al., 2020). The success of this phase is determined by a combination of factors such as nutrition, immunity, water quality, stocking density (Herath and Atapaththu, 2013), and temperature (Kujawa, et al., 1997; Kupren, 2011). The use of an adequate stocking density is commercially beneficial since the use of tanks, water and economic resources is maximized (Fairchild and Howell, 2001). Density can affect growth, survival and behavior, in addition to the occurrence of cannibalism, during larviculture (Melard et al., 1996; Luz and Zaniboni Filho, 2002; Slembrouck et al., 2009; Zalina et al., 2011; Sukumaran et al., 2011; de Barros et al., 2019). However, different densities have been found not to affect growth and survival differently during larviculture for some species in intensive production systems as *Lophiosilurus alexandri* between 20 and 60 larvas L^{-1} (Luz and Santos, 2008), and between 60 and 300 larvae L^{-1} (Cordeiro et al., 2015); *Oreochromis niloticus* between 1 and 30 larvae L^{-1} (Luz et al., 2012); and *Rhinelepis aspera* between 20 and 60 larvae L^{-1} (Santos et al., 2012), indicating that the use of adequate management strategies can favor the intensification of larviculture.

In addition to stocking density, larval feeding plays a significant role with live food being considered more suitable during the first days of exogenous feeding (Conceição et al., 2010), as it provides energy for the growth and development of the digestive tract (Palińska Żarska et al., 2014). In this sense, the use of *Artemia* nauplii beginning with the first feeding has been successful for several neotropical freshwater species such as *Pimelodus maculatus* (Luz and Zaniboni-Filho, 2001), *Hoplias lacerdae* (Luz and Portella, 2002; 2005), *Piaractus mesopotamicus* (Jomori et al., 2012), *Lophiosilurus alexandri*, *Pseudoplatystoma coruscans* and *Prochilodus costatus* (Santos and Luz, 2009), *Rhinelepis aspera* (Luz and Santos, 2010), *Brycon amazonicus*, *Leporinus macrocephalus*, *Astronotus ocellatus* and *Colossoma macropomum* (Jomori et al., 2013), *Betta splendens* (Fabregat

et al., 2017), and *Brycon nattereri* (Maria et al., 2017), among others. However, *Artemia* nauplii have a limited lifetime in fresh water, which can be increased with the use of low salinities (Beux and Zaniboni Filho, 2006; Jomori et al., 2012). Low salinities can also decrease the ionic difference between the animal and the environment (Boeuf and Payan, 2001), reduce the toxicity of nitrogenous compounds (Sampaio et al., 2002) and impede the occurrence of some fish diseases (Altinok and Grizzle, 2001; Puello-Cruz et al., 2010), and thus improve rearing conditions.

However, the restricted use of live food throughout the fish production cycle is not feasible (Person-Le Ruyet, 1989; Faulk and Holt, 2009; Herath and Atapaththu, 2013), making it necessary to use a commercial diet to make it viable fish production. This moment is another critical phase in larviculture due to the high demand for soluble protein and fatty acids, which cannot be obtained from formulated diet (Rønnestad et al., 2013). To date, several approaches, mainly related to the manipulation of the nutritional value of feed, have been evaluated to overcome the delay in the growth of fish that undergo early weaning (Ljubobratovic et al., 2019).

Tambaqui, *Colossoma macropomum*, is an important species in aquaculture in Latin America (Araújo-lima and Goulding, 1997; Sevilla and Günther, 2000), and is the second most produced species in Brazil, second only to *Oreochromis niloticus* (IBGE, 2019). The species is well adapted to cultivation conditions and has excellent growth and food conversion rates (Valladão et al., 2016; Paz and Val, 2018). However, larviculture for the species is still being carried out in fertilized earthen ponds with varying survival rates (Tavares et al., 2007).

The present study aimed to evaluate the effect of stocking density during larviculture of *C. macropomum*, rearing in a recirculating aquaculture system (RAS) with slightly saline water.

2 MATERIALS AND METHODS

The experiment was carried out in Laboratório de Aquacultura (LAQUA) of Universidade Federal de Minas Gerais (UFMG, Brazil). Larvae of *C. macropomum* produced through artificial spawning by hormone induced were acquired form Biofish Aquicultura fish farming located in the city of Porto Velho, state of Rondônia, Brazil. Larvae at 4-days post-hatching were packed in two plastic bags with 5 L of water (approximately 7,000 larvae bag^{-1}) and transported by plane. The time between the closing and opening of the transport bags was 24 hours. At LAQUA, the larvae were acclimatized and stored in four 28-liter circular tanks in RAS and received a feed of *Artemia* nauplii ad libitum to recover from the stress of the trip. During this phase the temperature and dissolved oxygen in the RAS were 28.3±0.86 °C and 6.35±0.59 mg L^{-1}, respectively (measured with a YSI 6920VZ2 multiparameter probe); water pH was 7.32±0.41 measured with a Hanna HI98130 portable multiparameter probe, and total ammonia was 0.30±0.13 mg L^{-1} measured daily by colorimetric method, Teste Labcon.

All procedures described herein were approved by the Committee for Ethics in Animals Use (CEUA - UFMG - nº 94/2020).

2.1 Effect of stocking density on larviculture

A total of 12,600 larvae of *C. macropomum* at 7-days post-hatching (length 5.68±0.47 mm, weight 1.2±0.03 mg) were distributed in 15 28-L tanks in a RAS with a mechanical and biological filters. Densities of 10 (D_{10}), 30 (D_{30}) and 50 (D_{50}) larvae L^{-1} were tested with five replicates each.

Water flow in the tanks was 1.85±0.2 L min^{-1} and larviculture was carried out in slightly saline water at 2.01±0.41 g of salt L^{-1} ($NaCl+Na_4Fe(CN)_6$, Refinaria Sal Garça LTDA, Mossoró, Rio Grande do Norte, Brazil. Ingredients: sodium chloride and sodium ferrocyanide), measured with a Hanna

HI98130 portable multi-parameter probe throughout the 30 days of rearing according to Jomori et al. (2013). The photoperiod was 12 hours with a luminance of 150 lux (Digital Lux Meter, model: ITLD 260) on the water surface. The tanks were siphoned once daily in the afternoon before the last feeding.

Artemia (Bio Artemia A. Ferreira de Melo ME; Grossos - Rio Grande do Norte - Brazil) were offered as food for *C. macropomum* larvae for the first 15 days of larviculture. The daily concentration of prey was 500 *Artemia* nauplii larva^{-1} from day 1 to day 5, 750 *Artemia* nauplii larva^{-1} from day 6 to day 10 and 1,000 *Artemia* nauplii larva^{-1} from day 11 to day 15 (adapted from Jomori et al., 2013). These prey concentrations were divided into three daily meals (08:00, 12:00 and 16:00 h). During this phase the temperature and dissolved oxygen in the RAS were 28.4±0.66 °C (Minimum (Min): 28.3°C and Maximum (Max): 28,4°C) and 7.32±0.47 mg L^{-1} (Min: 6.76 mg L^{-1} and Max: 7.71 mg L^{-1}), respectively; water pH was 7.42±0.57 (Min: 7.40 and Max: 7.43), and total ammonia was 0.31±0.14 mg L^{-1} (Min: 0.27 mg L^{-1} and Max: 0.37 mg L^{-1}).

On day 16 of cultivation, *Artemia* nauplii were completely removed from the larvae's feeding and then for their replacement they started offering a dry commercial diet. Thus, the animals started to be fed exclusively with commercial extruded diet provided three times a day (08:00, 12:00 and 16:00 h) at a rate of 10% of the biomass of each tank, adjusted according to biometrics. The feed used in the experiment was AquaQualy Microextrusada 0.8–1.0mm, containing 450 g Kg^{-1} crude protein, 80 g Kg^{-1} ether extract and 1000 mg Kg^{-1} of vitamin C, and was supplied as mashed diet. During this phase the temperature and dissolved oxygen were 27.86 ± 0.42 °C, and 7.12 ± 0.42 mg L^{-1}, respectively; water pH was 6.85 ± 0.16; and total ammonia was 0.40 ± 0.19 mg L^{-1}, all measured as previously described.

2.2 Growth and survival

Growth was determined using the biometrics of weight (Analytical Balance Ay-220 - 220g X 0,0001g Marte - Brazil) and total length (Starrett electronic caliper measuring tool EC799A-6/150, hardened stainless steel metal, 6-inch range, 0.005" resolution, LCD digital measurement reading, Massachussetts - EUA). Larvae were measured under anesthesia (20 mg L^{-1} eugenol solution) and subsequently returned to culture tanks. Measurements were made on days 8, 15, 23 and 30 of rearing. Larvae of each tank were classified at the end of the experiment according to size as small - S (<18.0 mm and <0.08g), medium - M (>18.0 mm and <22.2 mm, >0.08g and <0.15g), and large - L (>22.2 mm and >0.15g).

Daily specific growth rate (SGR) was calculated using the formula: SGR = 100 x ($\ln W_f - \ln W_i$) / interval between biometrics (days), where Wi is initial weight and Wf is final weight.

Survival was determined after days 15 and 30 of rearing by direct counting of individuals. Survival during the period of *Artemia* feeding (days 1–15), during the period of feeding with commercial diet (days 15–30) and global survival was calculated.

2.3 Statistical analysis

All data were submitted to the Shapiro-Wilk normality test and homoscedasticity of variances by the Leven test. Data for both phases of the experiment were then analyzed by ANOVA followed by Tukey's test at 5% probability.

3 RESULTS

Larvae weight was not affected by the different stocking densities throughout the experiment ($p>0.05$) (Figure 1A), nor was length (Figure 1B)

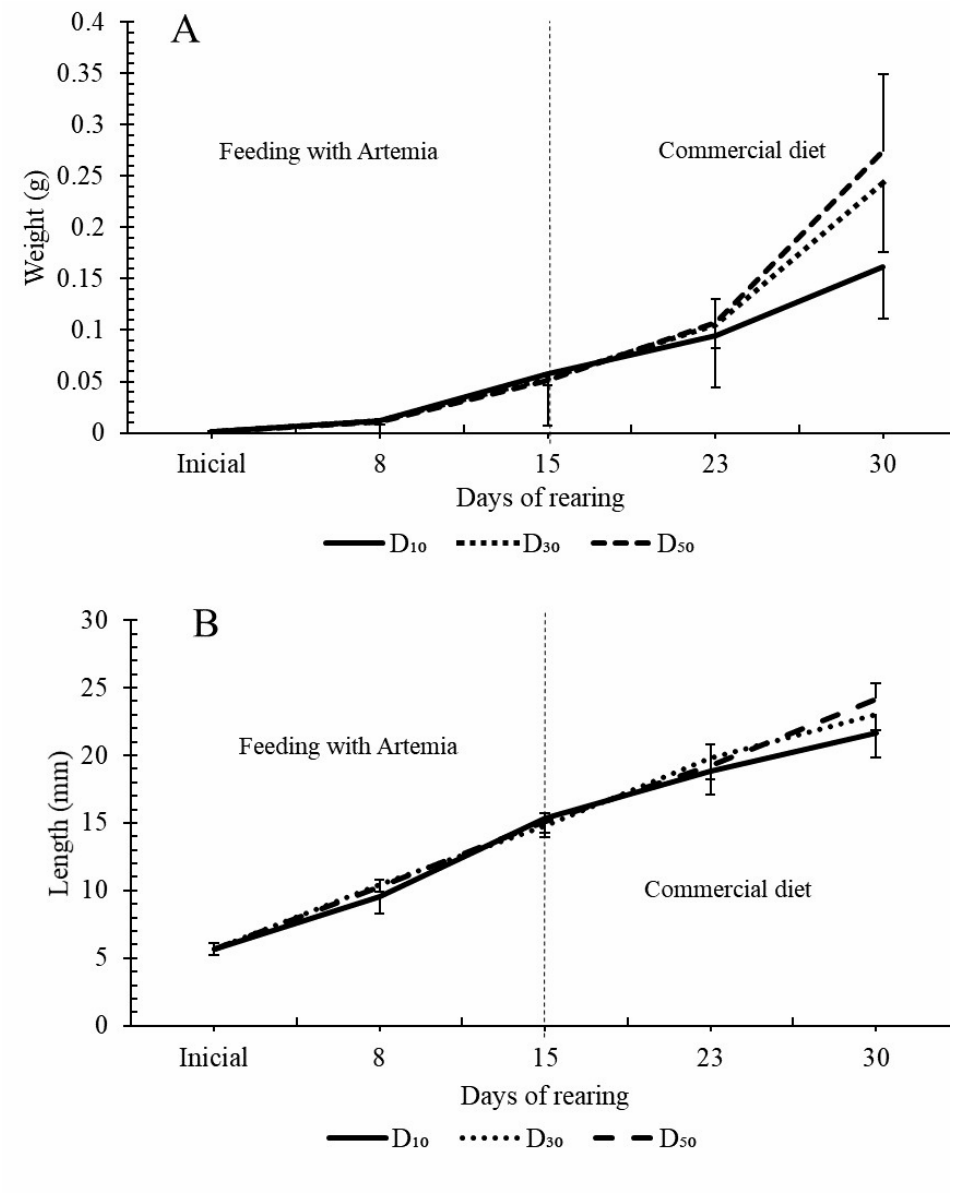

Figure 1 Data for weight (A) and length (B) (mean ± standard deviation) of *C. macropomum* larvae at different stocking densities and fed *Artemia* nauplii (first 15 days of feeding) and commercial

formulated diet (days 16 to 30 of feeding). There were no significant differences for weight and length among the different densities over the 30 days of rearing (Tukey's test at 5% probability).

Daily specific growth rate was also not affected by the different stocking densities during larviculture ($p>0.05$; Figure 2).

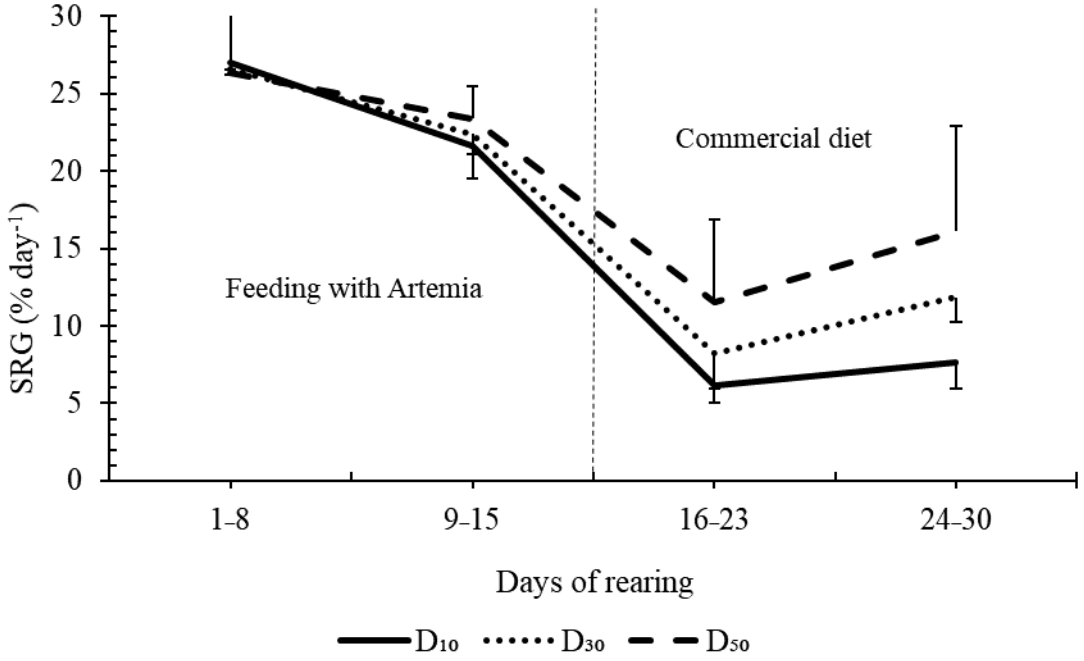

Figure 2 Daily specific growth rate (SGR) of *C. macropomum* larvae at different stocking densities and fed *Artemia* nauplii (first 15 days of feeding) and commercial formulated diet (days 16 to 30 of feeding). There were no significant differences in SGR among the tested densities over the 30 days of rearing (Tukey's test at 5% probability).

The heterogeneity of the animals was recorded for all tested densities at the end of the experiment. The size classes varied within each stocking density ($p<0.05$) (Figure 3). There was a predominance of size class M in the three tested densities, however, for each size class there was no difference among the densities ($p>0.05$).

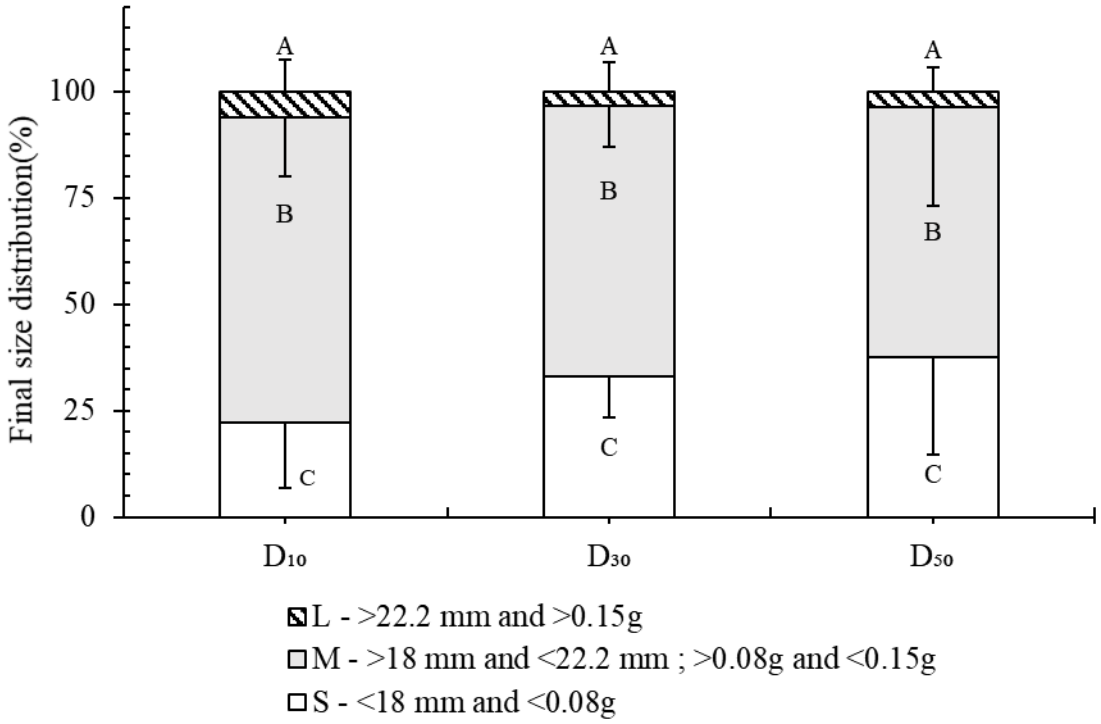

Figure 3 Distribution of size classes (%) of *C. macropomum* larvae at different stocking densities at 30 days of rearing. For each density, capital letters indicate a significant difference among the percentages of size classes (Tukey's test at 5% probability).

Survival during feeding with *Artemia* (days 1–15 of cultivation) was not affected by the stocking densities ($p>0.05$) (Figure 4). During feeding with commercial diet (days 15–30) survival was highest for D_{10} and lowest for D_{30} ($p<0.05$). Considering the experiment as a whole, global survival was similar among the different stocking densities ($p>0.05$).

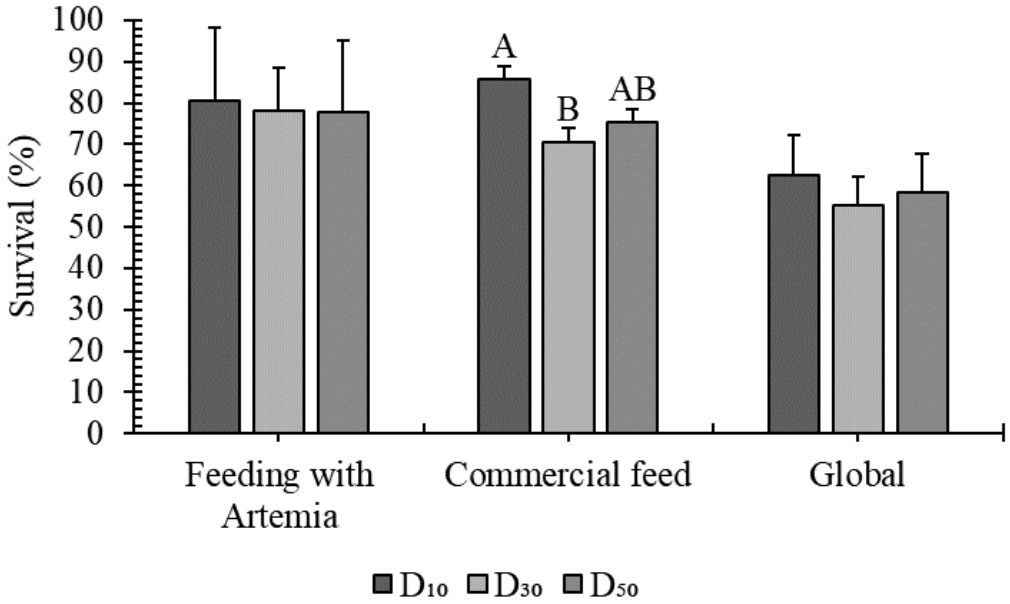

Figure 4 Survival (mean ± standard deviation) of *C. macropomum* larvae at different stocking densities and fed *Artemia* nauplii (first 15 days of feeding) and commercial formulated diet (days 16 to 30 of feeding). Different letters indicate a statistical difference between stocking densities (Tukey's test at 5% probability).

4 DISCUSSION

Larviculture of *C. macropomum* was carried out successfully in slightly saline water in a RAS during 30 days, which represents the first work done for this species in this type of rearing system. A salinity of 2 g of salt L^{-1} had been previously used for the species for the first 10 days of feeding with *Artemia* (Jomori et al., 2013). This salinity also has been beneficial in the larviculture of other freshwater species such as *H. lacerdae* (Luz and Portella, 2002), *L. alexandri* (Luz and Santos, 2008; Santos and Luz, 2009; Nascimento et al., 2019), *P. coruscans* and *P. costatus* (Santos and Luz, 2009), *R. aspera* (Luz and Santos, 2010), *P. mesopotamicus* (Jomori et al., 2012), *A. ocellatus* and *B. amazonicus* (Jomori et al., 2013), *O. niloticus* (Luz et al., 2013), *Pterophyllum scalare* and *Heros severus* (Eiras et al., 2019), *B. splendens* (Fabregat et al., 2017), and *B. vonoi* (Coraspe-Amaral et al., 2017), showing it to be a great alternative for intensive larviculture of freshwater fish.

The different stocking densities of the present study did not affect growth in weight and length of *C. macropomum* larvae during the larviculture. However, there are no data on different stocking densities in the larviculture of this species in controlled conditions. Santos et al. (2007) found that the density of 50 post-larvae m^{-3} led to better performance compared to a higher density of 100 post-larvae m^{-3} in larviculture of *C. macropomum* in tanks. This suggesting that the rearing system and management adopted are important for intensifying larviculture of *C. macropomum*. Results similar, without differences on growth to the present study for performance at different densities in controlled conditions were also registered for the larviculture of *O. niloticus* at densities between 1 and 30 larvae L^{-1} (Luz et al., 2012), *R. aspera* at densities between 20 and 60 larvae L^{-1} (Santos et al., 2012), *B. amazonicus* at densities 20 and 60 larvae L^{-1} (de Barros et al., 2019), *L. alexandri* at densities between 20 and 60 larvae L^{-1} (Luz and Santos, 2008) and between 60 and 300 larvae L^{-1} (Cordeiro et al., 2015), *Trichogaster lalius* at densities between 5 and 40 larvae L^{-1} and *Pethia conchonius* at densities between 20 and 80 larvae L^{-1} (Ramee et al., 2020). These results show the importance of larviculture for different species under controlled conditions for intensification production on this critical phase.

Daily specific growth rate decreased during the experiment until the period of day 15 to day 23 of rearing. Decreased SGR during larviculture is common, as seen for larvae of *P. mesopotamicus*, round fish, as well as *C. macropomum* (Honorato et al., 2016) and larvae of other species such as *H. lacerdae* (Luz and Portella, 2015; Luz and Portella, 2005), *L. alexandri* (Santos et al., 2015) and *B. orthotaenia* (Pedreira et al., 2008). The lowest values for SGR were soon after exchanging inert food for live food. Working with intensive larviculture of *P. mesopotamicus*, Leitão et al. (2011) demonstrated a continuous decrease in SGR for larvae that were subjected to exchanging inert food for live food. This phenomenon is due to stress in adapting to a new food (Duray and Bagarinao, 1984). Some authors have reported that low utilization of dry diets may be related to the digestive capacity of fish larvae due to the ongoing formation of the digestive system (Menossi et al., 2012,

Hachero-Cruzado et al., 2009), or to larvae not possessing enzymes in sufficient quantities to digest inert diets (Kolkovski, 2001). However, in the present study, there was an increase in SGR for the different densities of *C. macropomum* from day 23 to day 30, indicating the acceptance of inert food and recovery of SGR, a finding also reported for *L. alexandri*, a carnivorous fish (Santos et al., 2016). It is necessary to look for other diets that maintain SGR during food exchange or even to test co-feendig management during the weanig of *C. macropomum*.

Exchanging inert food for live food after 15 days of food was successfully performed for *C. macropomum*, since growth in weight and length were maintained by the larvae of the different densities and there was recovery of SGR. These results are similar to those reported for *P. mesopotamicus*, which had its best performance, when submitted to different forms of weaning, with 12 and 15 days of feeding with *Artemia* nauplii (Jomori et al., 2013). In contrast, Lombardi and Gomes (2008) found that change of live food for inert food was not recommended during intensive larviculture of tambacu (♀ *C. macropomum* X ♂ *P. mesopotamicus*), indicating the use of *Artemia* nauplii for at least 22 days. Other neotropical freshwater species have also been successfully submitted to change of live food for inert food after 15 days of feeding with *Artemia,* such as *H. lacerdae* (Luz and Portella, 2015) and *L. alexandri* (Luz et al., 2011). There are, however, different forms of exchanging inert food for live food weaning. According to Honorato et al. (2016), the success of to *P. mesopotamicus* may be related to the type of diet used, its nutritional quality and the requirements of the larvae. Thus, it is important that further studies be carried out related to these aspects in the larviculture of *C. macropomum*.

The different stocking densities influenced the heterogeneity of *C. macropomum* larvae at the end of larviculture. This heterogeneity was also recorded at the end of larviculture of *P. mesopotamicus* in different rearing systems and may have direct implications on the commercial value of animals (Jomori et al., 2005). This information can be important since it indicates the need for classifications during larviculture of *C. macropomum.* It is also important because, in spite of *C.*

macropomum being an omnivorous species, larger animals were found attacking and eating smaller animals. Heterogeneity in size is considered one of the central problems in aquaculture (Carvalho et al., 2018) when smaller fish are consumed by larger fish (DeAngelis et al., 1980; Hecht and Appelbaum, 1988; Baras, 1998). Studying *B. amazonicus*, Carvalho et al. (2018) highlighted the importance of understanding social behavior in order to propose effective measures to manipulate biotic and abiotic factors to reduce aggression and mortality in larval stages. Another factor to be considered may be nutrition, and it is important to search for diets that meet the requirements of larvae in order to avoid this problem (Toledo-Solís et al., 2019), since the tested densities did not affect survival.

The tested stocking densities had high survival rates during the first 15 days with live feed. With larvae of the same species, Jomori et al. (2013) obtained 99.0%±1.4 survival after the first ten days with food containing *Artemia* nauplii. For larvae of *P. mesopotamicus*, Jomori et al. (2012) recorded survival above 83% after the ninth day of life when fed *Artemia* nauplii. Working with productive performance of larvae of tambacu (♀ *C. macropomum* X ♂ *P. mesopotamicus*) submitted to different diets, Lombardi and Gomes (2008) recorded an average survival of 92.72 ± 3.63% in the first 12 days of rearing when fed *Artemia* nauplii. Working with 50 and 100 larvae/m³ in masonry tanks, Santos et al. (2007) obtained survival values below those found in the present study, with 46.86±16.05% and 37.06±17.19%, respectively, for feeding with plankton during the first 15 days and subsequent offering of ration up to 40 days. These data confirm the possibility of greater production of juveniles in different intensive rearing systems.

5 CONCLUSIONS

We conclude that larviculture of *C. macropomum* can be performed in slightly saline water in RAS at a density of up to 50 larvae L^{-1} with good results for performance and survival. However,

other studies must be carried out to maximize production through the use of higher densities with attention to nutritional requirements, types of diets and different forms of weaning.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY

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

ETHICAL APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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CAPÍTULO 3

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Growth performance and physiological parameters of

Colossoma macropomum in a recirculating aquaculture system (RAS):

importance of stocking density and classification

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Abstract

Tambaqui (*Colossoma macropomum*) is the main freshwater species produced in South American aquaculture and is also produced in several Asian countries. The objective of the present study was to evaluate the growth and physiology of juvenile *Colossoma macropomum* submitted to different stocking densities and classified by size in a recirculating aquaculture system (RAS) to maximize the production and enable the fish rearing in colder regions. In Phase I, juveniles (34.88 ± 0.60g) were stocked at densities of $D_{0.5}$ = 0.5, $D_{1.0}$ = 1.0 and $D_{1.6}$ = 1.6 kg/m³ for 53 days, and in Phase II, juveniles (150.61 ± 0.58 g) were stocked at densities of $D_{1.5}$ = 1.5, $D_{3.0}$ = 3.0 and $D_{4.5}$ = 4.5 kg/m³ for 60 days. In Phase III, the animals were classified according to size as S = 300–400g; M = 400–500g and L = >500g for 60 days with an initial biomass of 3.9 kg/m³ for each class. After the first 30 days of Phase I, final weight (FW) and daily weight gain (DWG) were highest for $D_{0.5}$, but feed conversion rate (FCR) was lowest. Daily feed intake (DC) was the lowest for $D_{1.6}$ and the highest for $D_{0.5}$. At 53 days, FW, DC and DWG were highest for $D_{0.5}$ and lowest for $D_{1.6.}$ From day 31 to day 53 there was a decrease in hemoglobin (Hg) and hematocrit (Htc) and an increase in the hepatosomatic index (HSI) for $D_{0.5.}$ After the first 30 days of Phase II, FW, DWG and DC were highest for $D_{1.5}$. After 60 days, $D_{1.5}$ had the highest FW and HSI and lowest values for glucose, triglycerides and Htc ($P<0.05$). In Phase III, after 30 days FW, DWG and DC were highest for class L ($P<0.05$), and FW remained highest for class L after 60 days. The HSI was highest for class S and lowest for class L ($P<0.05$) while triglycerides, cholesterol, total proteins and Hg were lowest for classes S and M ($P<0.05$). Stocking density must be evaluated according to the size of the individuals and, despite the best performance found for the lowest densities (phases I and II), higher densities produced greater biomass. The classification of animals is an important management strategy to maintain uniformity for commercialization because different densities and sizes of animals can interfere with some hematological and biochemical parameters, which can be used as indicators of animal welfare.

Keywords: intensive production, welfare, blood parameters.

1. Introduction

Stocking density is one of the main factors that determines the productivity of aquaculture systems (Bacchetta et al., 2020). This aspect of management is defined as the quantity or biomass of fish per unit area or volume (Sousa et al., 2020), and is specific to each species, production system, age and weight (Merino et al., 2007). Stocking density can interfere with the performance of tambatinga (*Colossoma macropomum* × *Piaractus brachypomus*) (Sousa et al., 2020), sea bass (*Dicentrarchus labrax*) (Sammouth et al., 2009), pirarucu (*Arapaima gigas*) (Oliveira et al., 2012), rainbow trout (*Oncorhynchus mykiss*) (Bayir and Bayir, 2017) and grass carp (*Ctenopharyngodon idellus*) (Zhao et al., 2019), among other species. High stocking density can also change fish behavior (Martins et al., 2012), such as the swimming speed of rainbow trout (*O. mykiss*) (Cooke et al. 2000) and Atlantic halibut (*Hippoglossus hippoglossus*) (Kristiansen et al. 2004), and affect fish welfare and production (Costa et al., 2019; Ghozlan et al., 2018; Refaey et al., 2018; Kpundeh, 2013), being considered a stress factor (Bacchetta et al., 2020). It can also lead to physiological changes (Refaey et al., 2018), fin erosion (North et al., 2006), alteration of immune factors (increased susceptibility to diseases) and modifications of metabolic and hematological parameters and in the aggressiveness of fish (Kelley, 2000).

The use of low stocking densities can lead to hierarchical problems in a lot, resulting in size heterogeneity of stocked animals, which is considered one of the central problems in aquaculture (Kestemont et al., 2003; Carvalho et al., 2018). Heterogeneous size distributions usually determine the social domain, where larger animals are generally dominant while smaller animals are subordinate (Huntingford and Turner 1987). The establishment and maintenance of such a hierarchy unleashes a potentially stressful situation in which dominant individuals show less social stress than subordinates (Fernandes and Volpato, 1993).

Thus, it is evident that determining the ideal density in fish production is essential not only for designing a better intensive system, but also for ideal cultivation practices (Ghozlan et al., 2018;

Chambel et al., 2015; Aksungur et al., 2007). In this sense, testing the growth of different species in recirculating aquaculture systems (RAS) may lead to alternatives for production intensification. Recirculating aquaculture systems are gaining more attention and interest in intensive aquaculture production due to the potential to reduce water and energy consumption and create a stable cultivation environment (Dalsgaard et al., 2013; Shao et al., 2019). These systems are often operated intensively in a semi-closed manner, with effective treatment of waste flows (Summerfelt and Vinci, 2009; Dalsgaard et al., 2013; de Farias Lima et al., 2019), enabling the cultivation of species in regions outside their comfort zone due to the control of water temperature.

Tambaqui (*Colossoma macropomum*) (Cuvier, 1818) is a Neotropical fish of the family Serrasalmidae, order Characiformes (Calcagnotto et al., 2005). It is native to the Amazon River basin and is currently the most commercially produced native species in Brazil (PeixeBR, 2020), reaching over 25 kg. It is also one of the main freshwater species produced in South American aquaculture and is also produced in several Asian countries including China, Indonesia, Malaysia, Myanmar and Vietnam (Woynárovich and Van Anrooy, 2019). Is mainly due to the immediate availability of juveniles and the potential for growth, high productivity and robustness (Brandão et al., 2004; Saint-paul, 2017). In Brazil it is cultivated in the North and Northeast regions, where the water temperature is high (Baldisserotto and Gomes, 2010). According to de Farias Lima et al. (2019), the production of tambaqui has been carried out with low levels of technology, mainly in extensive and semi-intensive systems in earth ponds. According to these same authors, an initial study in RAS showed that initial stocking densities of 1.4 and 2.5 kg/m³ for this species did not affect performance, indicating that studies with the use of higher densities in the system are needed.

Thus, the aim of the present study was to evaluate the effects of stocking density and the classification of animals by size on growth and physiological parameters of juvenile *C. macropomum* in RAS.

2. Material and methods

The experiment was carried out in the Laboratório de Aquacultura (LAQUA) at the Universidade Federal de Minas Gerais (UFMG, Brazil). All procedures herein described were approved by the Committee for Ethics in Animals Use (CEUA / UFMG - nº 107/2020).

2.1 Experimental design and management

The experiments were carried out using a completely randomized design with three treatments with three replicates each for a total of nine polyethylene tanks, with a total volume of 1m³ and a useful volume of 0.8m³, assembled in a RAS (Figure 1). Each tank was equipped with an "air-lift" system with an attached mechanical and biological filter. The air-lift system had an average flow of 0.89 m³/h and so the total volume of water in the tank passed through the filtration system every 54 min 33 seg, or 26.5 times a day.

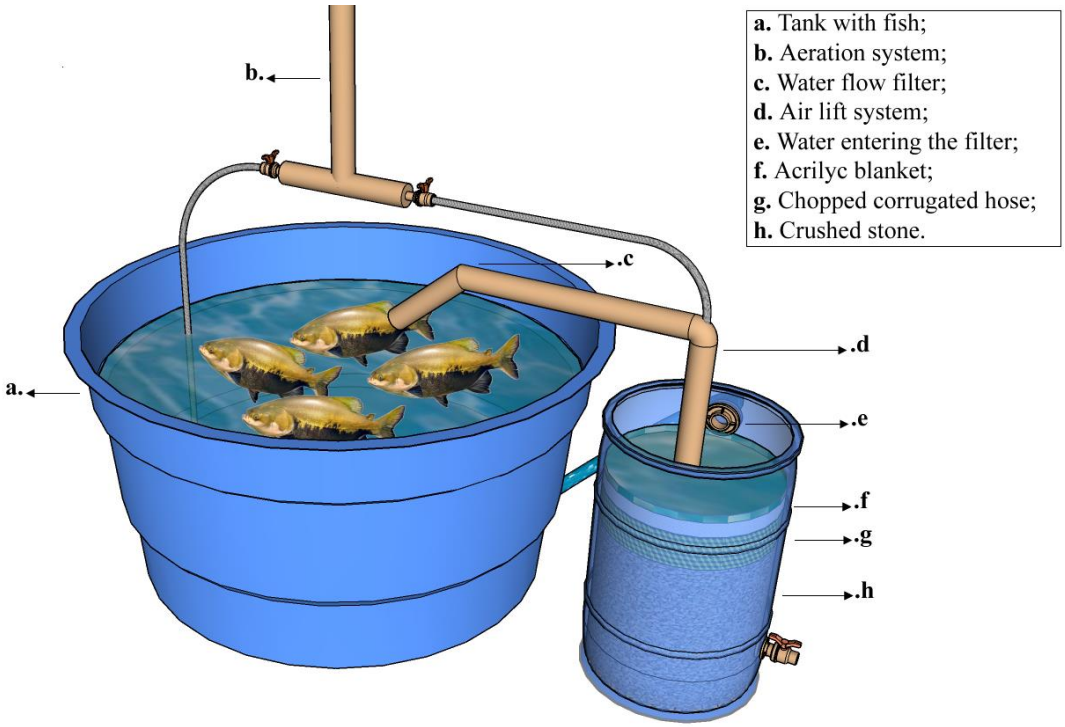

Figure 1. Recirculating aquaculture system (RAS) experimental unit used during the experiments.

During all experiment phases, the animals were fed manually twice a day (at 08h00min and 16h00min), until apparent satiety with commercial feed (pellet with diameter of 4 mm and 32% crude protein - Socil, linha Laguna). Upon reaching satiety, leftover rations were collected, dried in an oven (Nova Ética/Ethink) at 55ºC, and weighed to calculate consumption.

The following water quality parameters were measured daily in the morning: temperature, pH and conductivity using a model HI9146 Hanna instruments probe; dissolved oxygen (OD) with a YSI 6920VZ2 multiparameter probe; and total ammonia with a kit (Labcon). The photoperiod was 12L: 12D (digital timer, group Key West DNI).

The following water exchange management methods were adopted in the three experimental phases: during the first two weeks of each experimental phase, there was no water exchange; in the third week, 20% of the volume of each tank was changed once a week; in the fourth week, 10% of the volume of each tank was changed three times a week; beginning on the 30th day of each experimental phase, water changes of 10% of the volume of each tank were performed daily. These exchanges were intensified during each phase based on the monitoring of total ammonia levels.

2.1.1 Phase I: effects of stocking density

Phase I used 216 juvenile *C. macropomum* of 34.88 ± 0.60 g and 13.70 ± 0.09 cm. The animals were distributed in the nine tanks of the RAS in three stocking densities: $D_{0.5}$ = 0.5 kg/m³ (15 animals/0.8m³); $D_{1.0}$ = 1.0 kg/m³ (24 animals/0.8m³); and $D_{1.6}$ = 1.6 kg/m³ (36 animals/0.8m³). Biometrics were performed after 30 and 53 days of the experiment and survival was evaluated after 53 days.

2.1.2 Phase II: effects of stocking density

Phase II used 144 juvenile *C. macropomum* selected from Phase I of 150.61 ± 0.58 g and 20.94 ± 1.55 cm. The animals were redistributed them in the nine tanks of the RAS in three stocking densities: $D_{1.5}$ = 1.5 kg/m³ (8 animals/0.8m³); $D_{3.0}$ = 3.0 kg/m³ (16 animals/0.8m³); and $D_{4.5}$ = 4.5 kg/m³ (24 animals/0.8m³). Biometrics were performed after 30 and 60 days of the experiment and survival determined after 60 days.

2.1.3 Phase III: effects of animal size on performance under same stocked biomass

Phase IIII used 63 juvenile *C. macropomum* from Phase II. Due to heterogeneous growth, the animals were divided in to three size classes: class S (small) = nine animals with weights within 300 – 400g; class M (medium) = seven animals with weights within 400 – 500g; and L (large) = five animals with weights above 500g. Thus, each tank had the same initial total biomass (3.9 kg/m³) for all treatments. Growth was evaluated by performing biometrics after 30 and 60 days of the experiment and survival was determined at the end.

2.2 Biometric index

The animals were weighed at each biometry (Balança Wellmix 10Kg Digital 82674/wx502) and the data used to calculate. The final weight (FW) in grams was calculated by the final biomass (g) divided by the number of animals per tank. Daily weight gain (GPD) in grams by weight gain (g) over time in days of experiment. Daily feed consumption by fish (DC) in grams was measured by the total feed consumption divided by the days of the experiment and the number of animals per tank. Apparent feed conversion (FCR) was caused by total apparent feed intake (g) by weight gain (g). Survival (%) was measured by the number of fishes at the end of each phase divided by the initial

number of fish multiplied by 100. Finally, uniformity (%) was calculated by the number of animals with total weight ± 20% around the average of the tank by the total number of fish in each tank multiplied by 100.

2.3 Blood samples

The animals were fasted for 24 hours at the end of each of the three experimental phases and then blood was collected from five animals of each replicate (n = 15 animals per treatment). Each fish was restrained in a damp cloth and an approximately 1 mL sample of blood sample was collected by caudal venipuncture using syringes containing EDTA. The blood was then placed in microtubes containing 10% EDTA anticoagulant for subsequent use to determine hematocrit (Htc) and hemoglobin (Hg). Hematocrit was determined. By the microhematocrit method (Goldenfarb et al., 1971) using capillary tubes. Total plasma protein (TPP) was determined with an analog refractometer (0 to 90% Brix) - RHB0-90 after breaking the microhematocrit tube. To determine hemoglobin concentrations, 4 μL of blood was homogenized in 1 mL of color reagent (Bioclin®), followed by reading on a Term Plate Analyzer Basic® (Tonks, 1983). The rest of the blood was centrifuged at 5000 RPM for 10 minutes, and the biochemical parameters of glucose, cholesterol and triglycerides were determined using commercial kits (Bioclin®) and read using light spectrophotometry (Spectrophotometer Biochrom Libra S21 - S22).

2.4 Mesenteric fat index and hepatosomatic index

After blood collection, the animals were euthanized with a solution containing 285mg/L of eugenol (Mattioli et al., 2017). The mesenteric fat and the liver of each animal was then collected to determine the following indices:

Mesenteric Fat Index (MFI) = 100 × (mesenteric fat weight (g) / body weight (g))

Hepatosomatic Index (HSI) = 100 x (liver weight (g) / body weight(g))

2.5 Data analysis

All data were subjected to Levene's homoscedasticity test and the Shapiro-Wilk normality test, followed by analysis of variance ANOVA and Tukey test ($p<0.05$). Nonparametric data were submitted to the Kruskal-Wallis test ($p<0.05$).

3. Results

3.1 Water parameters

The water quality parameters were affected by the different treatments (stocking densities in phases I and II and size classes stocked at the same biomass in Phase III) in each experimental phase.

Table 1. Water quality parameters (mean ± standard deviation) for each experimental stage of

cultivation of juvenile *C. macropomum*.

Experimental phase	Total ammonia (mg / L)	OD (mg/L)	pH	Temperature (ºC)	Conductivity (µS/cm)
I	0.019±0.004	5.03±0.31	8.01±0.09	28.43±0.27	315.31±12.97
P value	>0.05	0.08	0.16	0.49	0.16
CV (%)	21.45	4.71	0.99	0.98	2.37
II	0.018±0.003	5.25±0.18	7.99±0.04	29.53±0.19	266.42±23.96
P value	0.30	0.06	0.39	0.33	0.13
CV (%)	16.23	2.30	0.45	0.59	7.36
III	0.016±0.003	6.21±0.16	7.98±0.025	29.83±0.12	276.05±11.64
P value	0.49	0.09	0.59	0.25	0.26
CV (%)	16.77	4.71	0.34	0.36	3.90

Data were submitted to ANOVA.

Stocking density tested for 53 days in Phase I: $D_{0.5}$ 15 animals/0.8m³ = 0.5 kg/m³; $D_{1.0}$ 24 animals/0.8m³ = 1.0 kg/m³; and $D_{1.6}$ 36 animals/0.8m³ = 1.6 kg/m³.

Stocking density tested for 60 days in Phase II: $D_{1.5}$ 8 animals/0.8m³ = 1.5 kg/m³; $D_{3.0}$ 16 animals/0.8m³ = 3.0 kg/m³; and $D_{4.5}$ 24 animals/0.8m³ = 4.5 kg/m³.

Treatments tested for 60 days in Phase III: S = nine animals with weights within 300 – 400g; M = seven animals with weights within 400 – 500g; and L = five animals with weights above 500g. Each tank had the same total initial biomass (3.9 kg/m³).

*DO - dissolved oxygen, CV - coefficient of variation

3.2 Phase I

3.2.1 Zootechnical performance

The performance results for juvenile *C. macropomum* are shown in Table 2. At 30 days, the FW and DWG were highest for treatment $D_{0.5}$ ($P<0.05$), while FCR showed a worse average compared to the other treatments. DC was lowest for treatment $D_{1.6}$ and highest for $D_{0.5}$ ($P<0.05$). Survival and lot uniformity were similar among the tested densities ($P>0.05$) and the final stocking density was highest for treatment $D_{1.6}$ ($P<0.05$).

Table 2. Growth performance (mean ± standard deviation) for juvenile *C. macropomum* cultivated in different stocking densities in a recirculating aquaculture system for 53 days in Phase I.

Growth performance - *Phase I*							
1-30rd day							
Treatment (Kg/m³)	FW (g)	DWG (g/day)	DC (g)	FCR	Survival (%)	Uniformity (%)	Final stocking density (kg/m³)
$\mathbf{D}_{0.5}$	108.94±7.66^{A}	2.46±0.23^{A}	3.13±0.15^{A}	1.28±0.09^{A}	100.00	77.78±4.81	1.64±0.11^{C}
$\mathbf{D}_{1.0}$	98.90±2.39^{AB}	2.14±0.06^{AB}	2.46±0.04^{B}	1.15±0.03^{AB}	100.00	72.22±12.73	2.97±0.08^{B}
$\mathbf{D}_{1.6}$	91.03±1.63^{B}	1.87±0.04^{B}	2.14±0.07^{C}	1.14±0.01^{B}	98.15±1.61	74.07±3.21	4.10±0.08^{A}
p-value	0.0102	0.0068	0.0001	0.043	0.0787	0.7065	<0.0001
CV (%)	4.74	6.65	3.9	4.68	0.93	10.81	3.10
31-53rd day							
$\mathbf{D}_{0.5}$	189.06±2.29^{A}	3.48±0.38^{A}	3.28±0.06^{A}	0.95±0.10	97.22±4.81	72.22±12.73	2.76±0.14^{B}
$\mathbf{D}_{1.0}$	164.32±5.17^{B}	2.84±0.30^{AB}	2.47±0.21^{B}	0.87±0.07	100.00	71.2±18.37	3.92±1.03^{AB}
$\mathbf{D}_{1.6}$	149.24±4.19^{C}	2.53±0.24^{B}	1.99±0.12^{C}	0.79±0.09	97.11±2.94	69.52±16.34	5.55±1.27^{A}
p-valor	0.008	0.0254	0.0001	0.1628	0.5105	0.9785	0.0307
CV (%)	6.24	10.58	5.44	9.88	3.29	22.52	23.26

Different letters indicate significant differences by the Tukey test (5%).

FW - Final weight; DWG - Daily weight gain; DC - Daily consumption; FCR - Feed conversion rate

Between 31 and 53 days, FW, DC and DWG were highest for treatment $D_{0.5}$ and lowest for $D_{1.6}$ (Table 2) ($P<0.05$). There was no significant difference among treatments for FCR, survival and lot uniformity ($P>0.05$). The final stocking density remained highest for $D_{1.6}$ and lowest for $D_{0.5}$ ($P<0.05$).

Figure 2 shows HSI and MFI at 53 days. The highest HIS in Phase I was for treatment $D_{0.5}$ ($P<0.05$). There was no difference in MFI among the tested densities ($P>0.05$).

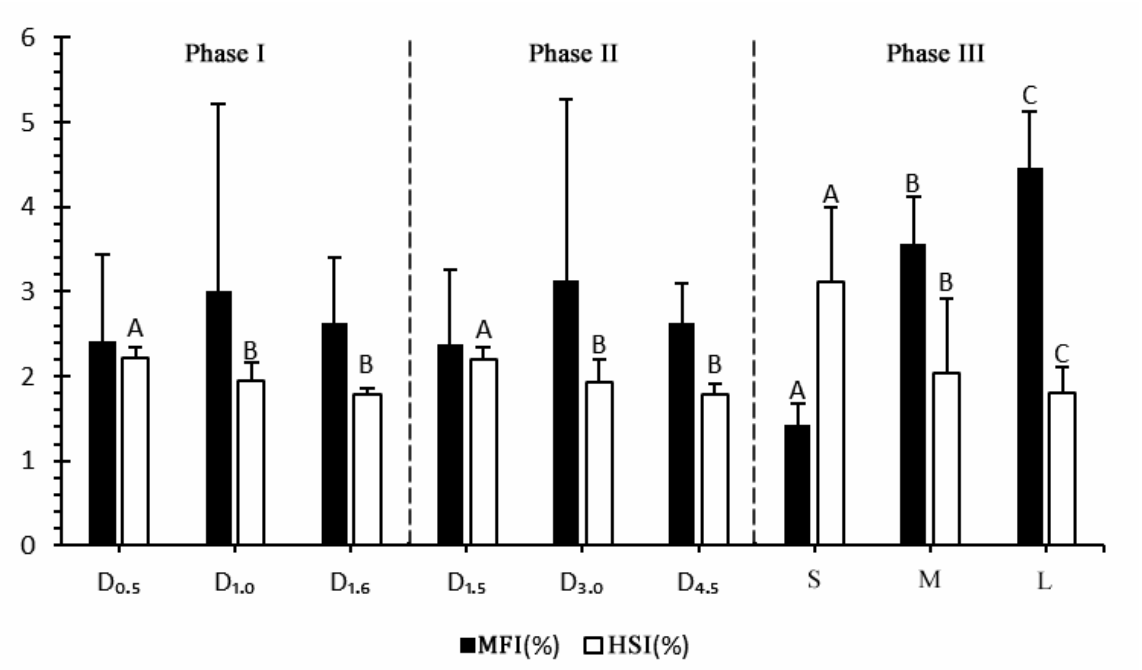

Figure 2. Hepatosomatic Index (HSI) and Mesenteric Fat Index (MFI) (mean ± standard deviation) of juvenile *Colossoma macropomum* in three experimental phases. Different letters indicate differences among treatments within in each phase separately and for each factor analyzed.

3.2.2 Blood samples

After 53 days of cultivation, the biochemical parameters were similar among treatments ($P>0.05$) and the concentrations of Hg and Htc were lower for the lowest stocking density tested ($D_{0.5}$) ($P<0.05$) (Table 3).

Table 3. Blood parameters (mean ± standard deviation) for juvenile *C. macropomum* in Phase I, after 53 days of cultivation in different stocking densities water recirculation aquaculture system (RAS).

	Biochemical analysis			**Hematological analysis**		
Treatment (Kg/m³)	Glucose (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	TPP (mg/dl)	Hg (g/dl)	Htc (%)
$\mathbf{D}_{0.5}$	51.19±6.11	198.95±38.49	93.51±20.42	5.54±0.47	$6.56±0.78^{B}$	$24.5±2.39^{B}$
$\mathbf{D}_{1.0}$	59.42±16.29	205.93±53.16	91.73±15.96	5.77±0.40	$7.68±1.18^{A}$	$28.00±3.00^{A}$
$\mathbf{D}_{1.6}$	56.18±12.66	226.93±68.18	125.39±89.41	5.87±0.43	$8.04±0.92^{A}$	$29.64±1.90^{A}$
p-valor	0.3032	0.4203	0.1644	0.2069	0.0004	0.0001
CV (%)	22.34	25.81	52.35	7.66	13.06	9.06

Glucose, triglycerides, cholesterol and TPP were submitted to the Kruskal-Wallis test (5%). Hg and Htc were compared by the Tukey test (5%).

TPP - Total protein; Hg - Hemoglobin; Htc - Hematocrit

3.3 Phase II

3.3.1. Zootechnical performance

At 30 days, FW, DWG and DC were highest for treatment $D_{1.5}$ ($P<0.05$) (Table 4). FCR, survival and lot uniformity were similar among the tested densities ($P>0.05$). The final stocking density was highest for $D_{4.5}$ ($P<0.05$).

After 60 days, FW was highest for $D_{1.5}$ and lowest for $D_{4.5}$ ($P<0.05$) (Table 4). The parameters DWG, FCR, DC, survival and lot uniformity were similar among treatments ($P>0.05$). The final density remained highest for $D_{4.5}$ ($P<0.05$).

At the end of the Phase II, HIS was highest for $D_{1.5}$ ($P<0.05$), while MFI did not differ among the tested densities ($P<0.05$) (Figure 2).

Table 4. Zootechnical performance (mean ± standard deviation) for juvenile *C. macropomum* cultivated in different stocking densities in a recirculating aquaculture system (RAS) for 60 days in Phase II.

Growth performance - *Phase II*							
1-30rd day							
Treatment (Kg/m³)	FW (g)	DWG (g/day)	DC (g)	FCR	Survival (%)	Uniformity (%)	Final stocking density (kg/m³)
$D_{1.5}$	329.21±17.25^{A}	5.96±0.56^{A}	7.58±0.99^{A}	1.27±0.09	100.00	54.17±12.50	3.29±0.17^{C}
$D_{3.0}$	282.21±14.50^{B}	4.38±0.47^{B}	5.64±0.59^{B}	1.29±0.03	97.92±3.61	62.50±14.43	5.53±0.45^{B}
$D_{4.5}$	257.52±19.50^{B}	3.56±0.66^{B}	4.72±0.47^{B}	1.34±0.01	94.44±2.41	64.69±8.74	7.29±0.42^{A}
p-valor	0.0058	0.0058	0.0075	0.7413	2.57	0.5649	<0.001
CV (%)	12.34	12.33	12.08	8.9	0.087	20.06	6.85
31-60rd day							
$D_{1.5}$	521.67±43.61 ^{A}	6.42±1.62	6.70±1.14	1.08±0.18	100.00	58.33±28.87	5.22±0.44 ^{C}
$D_{3.0}$	473.46±27.01^{AB}	6.38±1.07	6.42±1.50	1.00±0.12	100.00	61.94±9.86	9.47±0.54 ^{B}
$D_{4.5}$	426.67±16.99 ^{B}	5.64±1.20	5.24±0.42	0.95±0.12	89.86±9.05	72.27±13.63	11.91±0.84 ^{A}
p-valor	0.0274	0.7315	0.3071	0.5857	0.087	0.674	<0.001
CV (%)	6.58	21.47	18.27	13.88	5.41	30.06	7.09

Different letters indicate significant differences by the Tukey test (5%).

FW - Final weight; DWG - Daily weight gain; DC - Daily consumption; FCR - Feed conversion rate

3.3.2 Blood samples

After 60 days of Phase II, the concentrations of glucose, triglycerides and Htc were lowest for treatment $D_{1.5}$ ($P<0.05$) (Table 5). Cholesterol, TPP and Hg levels were similar among treatments ($P>0.05$).

Table 5. Blood parameters (mean ± standard deviation) for juvenile *C. macropomum* in Phase II, after 60 days of cultivation in different stocking densities in recirculating aquaculture system (RAS).

	Biochemical analysis			**Hematological analysis**		
Treatment (Kg/m³)	Glucose (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	TPP (mg/dl)	Hg (g/dl)	Htc (%)
$D_{1.5}$	57.03±10.17^{B}	223.79±61.58^{B}	100.71±11.13	5.60±0.32	8.05±1.25	24.00±1.41^{B}
$D_{3.0}$	65.38±15.42^{AB}	278.17±49.17^{A}	111.66±16.93	5.68±0.33	7.41±1.50	26.55±2.46^{A}
$D_{4.5}$	74.93±18.80^{A}	298.57±113.74^{A}	99.35±10.35	5.61±0.20	7.20±0.78	25.20±1.40^{AB}
p-valor	0.0293	0.0341	0.0905	0.7819	0.1817	0.0187
CV (%)	25.13	19.28	17.25	16.98	16.4	14.56

Glucose, triglycerides, cholesterol and TPP were submitted to the Kruskal-Wallis test (5%). Hg and Htc were compared by the Tukey test (5%).

TPP - Total protein; Hg - Hemoglobin; Htc – Hematocrit

3.4 Phase III

3.4.1 Zootechnical performance

At 30 days, class L had the highest FW, DWG and DC ($P<0.05$) (Table 6), while class S had the lowest DC ($P<0.05$). FCR, survival, lot uniformity and final stocking density were similar among the different size classes ($P>0.05$).

At 60 days, class L had the highest FW ($P<0.05$) (Table 6). DWG, FCR, DC, survival, lot uniformity and final stocking density were similar among treatments ($P>0.05$). HSI was highest for class S and lowest for L ($P<0.05$) (Figure 2), while MFI was lowest for class S and highest for class L ($P<0.05$) (Figure 2).

Table 6. Zootechnical performance (mean ± standard deviation) for juvenile *C. macropomum* separated into different size classes and stocked at the same initial biomass in a recirculating aquaculture system (RAS) for 60 days in Phase III.

Growth performance - *Phase III*							
1-30rd day							
Class	FW (g)	DWG (g/day)	DC (g)	FCR	Survival (%)	Uniformity (%)	Final stocking density (kg/m³)
S	521.41±17.74^{C}	5.74±0.62^{B}	7.55±0.54^{C}	1.32±0.09	100.00	96.30±6.42	5.86±0.20
M	658.14±47.66^{B}	7.08±1.46^{AB}	9.75±0.89^{B}	1.40±0.16	100.00	95.24±8.25	5.76±0.42
L	878.33±4.44^{A}	8.67±002^{A}	12.38±0.08^{A}	1.43±0.01	100.00	93.33±11.55	5.49±0.03
p-valor	<0.0001	0.0227	0.0002	0.4792	-	0.9208	0.2826
CV (%)	4.3	12.85	6.07	7.56	-	9.47	4.67
31-60rd day							
S	729.44±46.08^{B}	6.93±1.08	10.09±1.88	1.45±0.05	100.00	81.48±23.13	8.21±0.52
M	891.19±125.02^{B}	7.77±2.58	12.13±3.00	1.59±0.16	100.00	95.24±8.25	7.80±1.10
L	1129.13±70.23^{A}	8.36±2.23	14.47±3.07	1.76±0.21	100.00	80.00±20.00	7.06±0.44
p-valor	0.0039	0.7111	0.2192	9.48	-	0.5629	0.2384
CV (%)	9.49	26.85	22.12	0.1206	-	21.37	9.69

Different letters indicate significant differences by the Tukey test (5%). Class S (small) = nine animals with weights within 300 – 400g; class M (medium) = seven animals with weights within 400 – 500g; and L (large) = five animals with weights above 500g.

FW - Final weight; DWG - Daily weight gain; DC - Daily consumption; FCR - Feed conversion rate

FW - Final weight; DWG - Daily weight gain; DC - Daily consumption; FCR - Feed conversion rate. class S (small) = nine animals with weights within 300 – 400g; class M (medium) = seven animals with weights within 400 – 500g; and L (large) = five animals with weights above 500g.

3.4.2 Blood samples

After 60 days, the concentrations of triglycerides, cholesterol, TPP and Hg were lowest for classes S and M ($P<0.05$) (Table 7), while there was no significant difference for glucose and Htc among treatments ($P>0.05$).

Table 7. Blood parameters (mean ± standard deviation) for juvenile *C. macropomum* in Phase III, after 60 days of cultivation in different size classes and stocked at the same initial biomass in water recirculation aquaculture system (RAS).

	Biochemical analysis				**Hematological analysis**	
Class	Glucose (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	TPP (mg/dl)	Hg (g/dl)	Htc (%)
S	86.22±9.78	348.67±111.82^{B}	131.74±27.76^{B}	5.83±0.44^{B}	7.16±0.66^{B}	24.69±6.98
M	86.76±17.24	294.02±51.97^{B}	135.56±27.31^{B}	5.90±0.32^{AB}	7.20±0.53^{B}	25.38±3.34
L	89.88±13.29	432.12±133.23^{A}	199.73±79.29^{A}	6.31±0.35^{A}	8.02±0.75^{A}	27.56±3.21
p-valor	0.7348	0.0044	0.0008	0.0222	0.0028	0.2283
CV (%)	15.61	15.32	9.56	6.47	5.25	22.23

Glucose, triglycerides, cholesterol and TPP were submitted to the Kruskal-Wallis test (5%). Hg and Htc were compared by the Tukey test (5%). TPP - Total protein; Hg - Hemoglobin; Htc – Hematocrit; Class S (small) = nine animals with weights within 300 – 400g; class M (medium) = seven animals with weights within 400 – 500g; and L (large) = five animals with weights above 500g.

4. Discussion

The present study found that juvenile *C. macropomum* adapted to cultivation in RAS because they had high survival rates and individuals with an average initial weight of 34.8g reached 729 to 1129 g at the end of 173 days of cultivation. This adaptation to RAS corroborates what was reported by López and Anzoátegui (2015) and de Farias Lima et al. (2019) for the same species.

Water quality remained similar among treatments in the three experimental phases and within the ideal range for cultivation of *C. macropomum* (Souza and Teixeira Filho 1986; Morais and O´Sullivan, 2017; Wood et al., 2018; Woynárovich and Van Anrooy, 2019). This finding demonstrates the efficiency of the RAS used in the present study for the tested biomasses, producing up to 11.9 kg/m^3 at the end of Phase II. This biomass was similar to that recorded by López and Anzoátegui (2013), who reached a final biomass of 11.24 kg/m³. de Farias Lima et al. (2019) obtained biomass values of between 5.31 and 6.58 kg/m³ in RAS after 90 days of cultivation. In a study with juvenile *Piaractus brachypomus*, a species of the same family as *C. macropomum*, Poleo et al. (2011) obtained final biomass of 12.13 and 12.96 kg/m³, at the end of 192 days of cultivation in RAS, which are values close to those of the present study. However, these values are still low compared to RAS cultivation of species that have already been studied and produced commercially, which can even produce 40 kg/m³ or more, such as for Atlantic salmon (*Salmo salar*) (Liu et al., 2014), Nile tilapia (*Oreochromis niloticus*) and European eel (*Anguilla anguilla*) (Dalsgaard et al., 2013). Thus, studies are still needed to further optimize the productivity of *C. macropomum* in RAS without affecting animal welfare.

A high survival rate was recorded during the experiment, with values greater than 97% at the end of Phase I (densities of 0.5 to 1.6 kg/m^3), 89% at the end of Phase II (densities of 1.5 to 4.5 kg/m^3) and 100% at the end of Phase 3 (different size classes at a density of 3.9 kg/m^3). These survival rates are in accordance with other findings for *C. macropomum* in RAS (de Farias Lima et al., 2019; de Azevedo et al., 2016). High survival (mean of 99%) was also recorded for cultivation in concrete

tanks testing densities of 15 and 24 kg/m³ (Frisso et al., 2020) and in excavated tanks (mean above 87%) with densities between 5 and 15 fish/m² (Costa et al. 2016). These results, along with those of the present study, show that *C. macropomum* adapts well to different production systems and when stored in different stocking densities.

In relation to the FCR, in the first 30 days of Phase I, the worst FCR was 1.28 for $D_{0.5}$. Between 30 and 60 days there was no significant difference in FCR among densities, which ranged from 0.79 to 0.95. In phases II and III, there were also no differences among treatments with FCR ranging from 0.95 to 1.34 and 1.32 to 1.76, respectively. This zootechnical variable can be influence by the species, size and activity of the fish, as well as changes in the environmental parameters and cultivation system used (Craig et al., 2017). The increase in FCR in our study over the course of the experimental phases may be related to the increase in the size of the animals since smaller fish have a higher relationship of growth rate with the maintenance requirement compared to larger fish (Venugopal et al., 2004). López and Anzoátegui (2015) obtained a FCR of 1.72. For the same species cultivated in RAS for 303 days, with it ranging from 1.46 to 1.96 for different densities (de Farias Lima et al., 2019). However, cultivation of the hybrid tambatinga (*C. macropomum* × *P. brachypomus*) in RAS for 50 days at stocking densities between 5 and 15 fish/0.08m³ obtained FCR values of 1.08 and 1.16, respectively, which are similar to the present study. On the other hand, in small volume net tanks, *C. macropomum* obtained FCR values between 1.8 to 3.5, which are much higher than in the present study (Inoue et al., 2014), suggesting a better use of food in RAS.

The best responses for performance in Phase I and at 30 days of Phase II were for juveniles grown in the lowest densities tested. This result may have been due to the lower energy expenditure by fish during competition for space and food. In addition, increased stocking density can also influence the physiological and metabolic processes of animals (Vijayan et al., 1990). Similar performance was observed among densities at 31 days and 60 days of Phase II, with only FW being

higher for animals grown at the lowest density tested in this phase ($D_{1.5}$). Da Silva and Fujimoto (2015) and Brandão et al. (2004) also found the best performance for juvenile *C. macropomum* grown in the lowest stocking densities tested in net tanks.

Despite the better performance of animals grown at lower densities, the highest final biomass (11.91 kg/m³) was achieved at the highest density. Biomass production is an important factor for aquaculture, as it allows economic evaluation of the production system as productivity per area (Ali et al., 2006). Generally, the highest productivity is achieved with the highest densities, which is due to the greater number of animals in tanks (Sousa et al. 2020). However, increased stocking density can be limited by the carrying capacity of the system due to reduced in oxygen levels and increased organic matter and nitrogen compounds (Ali et al., 2006; Allen and Backer, 1974; Sousa et al. 2020). However, in the present study, there was no significant change in water quality among treatments, which indicates the possibility of carrying out experiments with even higher stocking densities.

After the classification of the animals by size (Phase III – 60 days), FW was found to be the highest for class L, but without differences in the final density achieved among size classes. This classification is important for the commercial production of *C. macropomum*, a practice that is carried out in commercial fish farms that work with this species. The classification system (double cycle), aims to optimize the use of production spaces for breeding (Filho, 2007), where smaller animals are separated from larger ones, which is a management practice that can lead to compensatory growth. According to the same author, another management practice used by producers of this species is the partial removal of fish during cultivation with different sizes to increase the availability of space for the growth of the lots that will stay longer, which allows several removals in the same production cycle.

Lot uniformity was similar among densities during Phase I (mean value of 70.98%) and Phase II (mean value of 73.09%). Lot uniformity was also similar in Phase III after the animals

were classified, but with higher values (mean value of 85.57%) than those for phases I and II. Several production management practices have been taken into account as they can increase the growth rates of fish while decreasing variation in size and intraspecific competition, with classification being important for improving uniformity in growth rates and decreasing the cultivation time required to reach market size (Brett, 1979; Irwin et al., 1999; Filho, 2007).

Hematology and blood biochemistry are important indicators of the health status of fish and serve to assess their physiological status, which is of importance to the aquaculture industry (Ahmed et al., 2020; Costa et al., 2019; Fazio, 2019). At the end of Phase I, only Hg and Htc were lowest at the lowest density tested ($D_{0.5}$). At the end of Phase II, density affected the concentrations of glucose, triglycerides and Htc, which were also lowest in the lowest density tested ($D_{1.5}$). The increase in Hg concentrations in the two highest stocking densities in Phase I may be related to greater stress causing splenic contraction and the release of red blood cells into circulation, which may have caused an increase in hemoglobin to improve oxygen transport (Li et al., 2018; Ruane et al., 2000; Wells and Pankhurst, 1999). The pattern of variation in hemoglobin was similar to that for Htc, since red blood cells contain hemoglobin, as described in other studies for different species of fish (Pirbeigi et al., 2016; Abdel - Tawwab et al., 2014). In addition, Hct values can vary with sex, age, water quality, photoperiod, diet and season (Hrubec et al. 2001; Hrubec and Smith 2000; Lane 1979; Ram-Bhaskar and Srinivasa-Rao, 1989). The increased plasma glucose and triglyceride levels in fish reared in the highest densities in Phase II may have been due to energy metabolism via gluconeogenesis, glycogenolysis and lipolysis (Barton and Iwama, 1991; Montero et al. 1999) causing an increase in the available energy to face stressful situations such as high stocking density (Refaey et al., 2018).

At the end of Phase III, the levels of triglycerides, cholesterol, TPP and Hg were lowest for classes S and M. The higher levels of triglycerides and cholesterol for fish the class L can be explained by the lower metabolic rate of these animals, which can lead to the accumulation of fat in the tissues.

The concentrations of these metabolites in plasma may indicate a migration of extracellular fluids to active muscles (Wang et al., 1994) or increased mobilization of proteins and lipids for intense muscle contraction (hillart and Van Raaij, 1995). Levels of lipids in the bloodstream may come from newly consumed food or from the mobilization of energy reserves (Li et al., 2018; Sheridan, 1988). Therefore, the higher concentrations of triglycerides in the bloodstream for animals of the L class may be indicative of the mobilization of energy reserves. The mobilization of energy reserves in the bloodstream can also cause several biochemical and hematological changes, which may explain the higher levels of PPT and Hg (Aride et al., 2018). Another point that should be taken into account is that the biochemical and hematological parameters may vary depending on the size, age and sexual maturation stage of the animals (Ahmed et al., 2020), which has already been reported for *Mugil platanusr* (Ranzani-Paiva and Ishikawa, 1996), *Salmo trutta caspius* (Jamalzadeh and Ghomi, 2009) and *Tenualosa ilisha* (Jawad et al., 2004).

The HSI was highest for the lowest densities tested in Phase I ($D_{0.5}$) and Phase II ($D_{1.5}$), while there was no change in MFI in both phases. HIS is related to the nutritional status and growth rate of fish (Luckenbach et al., 2007). Increases in HIS values may indicate variation in the amount of fat stored in the liver, and thus allow inferences to be made about health status (Everaarts et al. 1993). HIS is often used to estimate the energy status and metabolic activity of fish (Janssens and Waterman, 1988). In the present study, this index indicated greater demand for these energy reserves at the highest density, probably due to the increased mobilization of lipids at high stocking densities (Ni et al., 2016). Our findings are in agreement with those found for *Salvelinus fontinalis* in high stocking densities and in RAS (Vijayan et al.,1990). However, for *Solea senegalensis*, a species with more sedentary behavior than *C. macropomum*, Costas et al. (2008) did not record an effect of stocking density on HIS, suggesting different responses depending on the behavior of each species.

In Phase III, HSI was highest for class S and lowest for class L, while MFI was lowest for class S and highest for class L. Visceral fat content is an indicator of the nutritional status of fish (Ng and Hanim, 2007). The reduction in fat content may the result of increased lipid metabolism to meet the growing demand for energy under stress from high stocking density, for which lipids are a valuable source of energy for fish. In the present study, larger liver size was recorded for smaller fish and less fat accumulation in the mesenteric cavity for fish of the S class. Studies to assess nutrition during the fattening of *C. macropomum* in RAS are essential to improve the physiological and metabolic state of animals when compared to larger fish.

5. Conclusions

Juvenile *C. mocropomum* present excellent adaptation to RAS with good results for zootechnical performance.

Stocking density must be evaluated according to the size of the animals, and the best performance was for the lowest densities in Phase I (0.5kg/m³ - initial weight of 34.88±0.60g) and in Phase II (1.5 kg/m³ - initial weight of 150.61 ± 0.58g).

Classification of animals is an important management to maintain uniformity for commercialization.

Different stocking densities and animal sizes can interfere with some hematological and biochemical parameters, which can be used as indicators of animal welfare.

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CAPÍTULO 4

Artigo publicado na Aquaculture (2022)

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High stocking densities in the larviculture of *Colossoma macropomum* in a recirculating aquaculture system: performance, survival and economic viability

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Abstract

This study investigated the use of high stocking densities and in the larviculture of *Colossoma macropomum* in a recirculating aquaculture system (RAS), including an economic analysis. Densities of 60 (D_{60}), 120 (D_{120}) and 180 (D_{180}) larvae L^{-1} were tested, with four replicates each. The larvae were fed with *Artemia* nauplii during the first 15 days of cultivation and with ration from the 16th to the 25th day, through the direct exchange of commercial diet for live food. Ammonia was affected by stocking density with the highest value for D_{180} ($P<0.05$). Performance and survival were not affected by stocking density throughout the experiment ($P>0.05$). The number of animals produced at the end of the experimental period increased with the increase in density ($P<0.05$). At the end of larviculture, animals classified as medium predominated in D_{60} and D_{120} ($P<0.05$), while there was no difference between small and medium classes in D_{180} ($P>0.05$). Net revenue was negative for treatment D_{60}, leading to a financial loss in production, while treatment D_{180} had the best net revenue. In conclusion, intensive larviculture of *C. macropomum* can be carried out in RAS with up to 180 larvae L^{-1}, with good results in terms of performance and survival, as well as good indicators of economic and financial viability.

Key words: tambaqui, RAS, economic viability, intensive larviculture

1. Introduction

Stocking density is a critical aspect of management as it directly influences the economic viability of aquaculture production (Osofero et al., 2009; Baldwin, 2010). Its optimization is a prerequisite for the development of production of any fish species (Karnatak et al., 2021) to maximize yield and economic benefits (Zhu et al., 2011). Stocking densities that result in higher yields and, subsequently, higher economic returns for a farming enterprise are ideal for catalyzing aquaculture development (Abaho et al., 2020).

Factors that can influence the ideal density for production include the species to be produced (Sharma and Chakrabarti, 2003; Jatobá and Silva, 2015; Magouz et al., 2019), the production system (North et al., 2006; Tidwell, 2012) and the size or age of the animals (North et al., 2006). Density management can directly affect behavior (Manley et al., 2014; Barros et al., 2019), health and well-being (Hasenbein et al., 2016), zootechnical performance and survival (Zarski et al., 2011; Millán-Cubillo et al. 2016; Reis et al. 2020; Santana et al. 2020; Oliveira et al. 2020; Budi et al. 2020; Karnatak et al., 2021), water quality (Luz et al. , 2012; Santos et al., 2012) and, consequently, the profitability of production (Siddiqui et al., 1989; Lira et al., 2021; Karnatak et al., 2021). Density management is also important in larviculture and its intensification has been possible under controlled conditions. High stocking densities were tested in the larviculture of *Lophiosilurus alexandri* (60 to 300 larvae L^{-1}) (Cordeiro et al., 2015); *Carassius carassius* (50 to 600 larvae L^{-1}) (Zarski et al., 2011); *Hoplias lacerdae* (10 to 90 larvae L^{-1}) (Luz and Portella, 2005); *Aspius aspius*, *Leuciscus idus* and *Leuciscus cephalus* (50 to 400 larvae L^{-1}) (Kupren et al., 2011) and *Tinca tinca* (20 to 320 larvae L^{-1}) (Celada et al., 2007) indicating the possibility of intensifying the larviculture of different species of freshwater fish.

Colossoma macropomum, known as tambaqui, is the second most produced fish species in Brazil (PeixeBR, 2021; Woynárovich and Van Anrooy, 2019), with territorial expansion of its production, mainly in tropical countries, due to its favorable zootechnical characteristics (Guimarães

et al., 2014). The larviculture of this species is commonly carried out in nurseries (Santos et al., 2007; Sipaúba-Tavares and Braga, 2007). However, Santos et al. (2021) described the possibility of intensive larviculture in a recirculating aquaculture system (RAS) at a density of up to 50 larvae L^{-1} during the first 30 days of feeding, without reduction in growth and survival relative to lower densities. Thus, higher densities must be evaluated.

This study aimed to evaluate the effects of high stocking densities during larviculture of *C. macropomum* in RAS and their economic viability for production.

2. Material and methods

The experiment was carried out at Universidade Federal de Minas Gerais (UFMG, Brazil), in the Laboratório de Aquacultura (LAQUA). Larvae of *C. macropomum* were acquired from the Biofish Aquicultura fish farm located in the city of Porto Velho in the state of Rondônia, Brazil. Larvae, 4-days post-hatching, were packed in four plastic bags with 5 L of water each (approximately 15,000 larvae bag^{-1}) and transported by plane. The time between closing and opening the transport bags was 24 hours. After acclimatization at LAQUA, the larvae were stored in eight 28-L tanks mounted in a RAS and fed *ad libitum Artemia* nauplii to recover from the stress of the trip.

All procedures herein described were approved by the Committee for Ethics in Animals Use (CEUA / UFMG - nº 275/2020).

2.1 Effects of stocking density on larviculture

Initially, 40,320 6-days post-hatching larvae of *C. macropomum* (length 4.23±0.01 mm, weight of 0.0011±0.03 mg) were distributed in 12 28-L tanks kept in a RAS. The RAS consisted of a mechanical and biological filter, temperature control and supplementary aeration. Densities of 60

(D_{60}), 120 (D_{120}) and 180 (D_{180}) larvae L^{-1} were tested, with four replicates each, based on Santos et al. (2021).

The water flow in the tanks was 2.05±0.2 L min^{-1} and larviculture was carried out in slightly saline water of 2.14±0.38g of salt L^{-1} (Refinaria Sal Garça LTDA, Mossoró, Rio Grande do Norte, Brazil; ingredients: sodium chloride and sodium ferrocyanide) (Jomori et al., 2013; Santos et al., 2021), measured by a Hanna-HI98130 portable multiparameter probe during the 25 days of cultivation. The photoperiod was 12 hours with a luminosity of 150 lux on the water surface. The tanks were siphoned once a day, in the afternoon before the last feeding.

Artemia nauplii (Bio Artemia A. Ferreira de Melo ME) were offered as food in the daily prey concentration of 500 nauplii $larva^{-1}$ from the first to fifth day of larviculture, 750 nauplii $larva^{-1}$ from the sixth to the tenth day and 1000 nauplii $larva^{-1}$ from the 11th to the 15th day (Jomori et al., 2013; Santos et al., 2021). These concentrations were divided into three daily meals (08:00, 12:00 and 16:00 h).

From the 16th to the 25th day of cultivation, an extruded commercial diet was exclusively offered four times a day (08:00, 11:00, 13:00 and 16:00 h) at a rate of 10% of the biomass of each tank (Santos et al., 2021). The ration used in the experiment was Qualis Acqua fingerlings 46% 1.5mm, containing 460 g Kg^{-1} of crude protein, 80 g Kg^{-1} of ether extract and 1030 mg Kg^{-1} of vitamin C. However, due to the small size of the fish, the diet was mashed.

2.2 Water quality

During the experimental period, temperature, dissolved oxygen, pH and conductivity were measured daily in the RAS reservoir and every four days in all tanks using a YSI 6920VZ2 multiparameter probe. Total ammonia was measured daily in the RAS reservoir and every four days

in all tanks and in the reservoir by colorimetric kit (Alcon / LabconTest). Toxic ammonia (NH_3) was obtained by converting total ammonia using the table accompanying the kit. During the experimental period, the water quality parameters of the RAS reservoir were: temperature 28.05 ± 0.84 °C, dissolved oxygen 5.18 ± 0.59 mg L^{-1}, water pH 7.25 ± 0.32, electrical conductivity 4.47 ± 0.82 μS cm^{-1} and NH_3 of 0.004 ± 0.02 mg L^{-1}.

2.3 Growth and survival

Growth was determined by weight biometrics, using a Marte digital scale with 0.0001 g precision, and total length, using a Starret digital caliper with 0.01 mm precision. The larvae were anesthetized with a 20 mg L^{-1}eugenol solution for biometrics (Santos et al., 2021), after which they were returned to the culture tanks. Biometrics took place after 5, 10, 15, 20 and 25 days of cultivation. At the end of the experiment the larvae of each tank were classified into the following sizes, adapted from Jomori et al. (2003) and Santos et al. (2021): small = S (<17.0 mm and <0.07g), medium = M (>17.0 mm and <22 mm; >0.07g and <0.13g), and large = L (>22 mm and >0.13g).

The daily specific growth rate (SGR) was calculated using the weight data and the following formula:

$$SGR = 100 \times (\ln Pf - \ln Pi) / \text{interval between biometrics (days)}$$

where *Pi* is initial weight, *Pf* is final weight.

Survival was determined after 15 and 25 days of culture by the direct counting of individuals. Survival was calculated for the period of feeding with *Artemia* (1–15th day); the period of feeding with commercial diet (15–25th day), considering the final number of larvae (25 days) / number of larvae at 15 days in each (*100); and the final survival considering the number of larvae at 25 days / initial number of larvae in each tank (*100) (1–25th day).

2.4 Economic analysis

Total Operating Cost (TOC), Average Total Operating Cost (average TOC), gross revenue and net revenue were calculated. TOC is composed of the Effective Operating Cost (EOC) and Other Costs (OC). The following items were considered for EOC: acquisition of tambaqui larvae, inputs and food (*Artemia* nauplii, feed and salt), laboratory maintenance (costs of maintaining the tanks used in each treatment suitable for a period of 25 days), electricity, cost of water use and labor (which included the time for cleaning the tanks, producing food, offering food and handling the hatching of *Artemia* nauplii used in the first 15 days until the replacement of live food by inert food).

Depreciation was computed for OC considering that the productive period of the laboratory is 365 days/year. Fish with a total length of 4.23±0.01 mm were used for the beginning of the experiment, whose price was considered in the average TOC. The calculation of cost, maintenance and depreciation of the laboratory used the laboratory value calculated by Jomori et al. (2005) updated for June 2021 by the Índice Nacional de Custo de Construção (INCC).

To calculate the costs of inputs and food spent on *Artemia* nauplii (US$ 0.072 g^{-1} *Artemia* cysts at an average hatch rate of 200,000 nauplii g^{-1} cysts), commercial diet 46% CP (US$ 2.50/kg, with an average consumption of about 16 g day^{-1} in tanks) prices were calculated based on sales values in Brazil. The worker's monthly salary mode in Brazil was used to calculate the hourly cost of labor, resulting in a monthly cost of R$ 1100.00 (US$ 196.78).

The electrical energy used by the equipment was calculated considering the capacity of the laboratory and appropriating the value corresponding to the use in each treatment. The value used for the calculation of electrical energy was US$ 0.078 for each KW/h, a price used in Brazil in June 2021. The calculation of electrical energy used by the equipment considered the following: air blower (four outlets for each treatment), as the amount needed to supply a 1-hp Ibram model CJ2/1 air compressor with a consumption of 0.736 kWh; freezer, for which the average cost of a vertical/horizontal freezer

is 0.069 KWh; heaters used for 15 days for the hatching of *Artemia* and in the experimental system, considering the use of 10 hours for heating and temperature correction with an average of 0.2 kWh; and 1/3cv pump for water recirculation with a consumption of 0.474 kWh. Assuming equal energy expenditure for all treatments over a 25-day period at a rate of $0.15 per kWh, the amount of electrical energy spent was US$ 61.20 divided by three treatments, resulting in US$ 20.40 for each treatment. The cost of expenses with the use of water by the system was calculated considering the system's capacity and adapting it to the corresponding value for each treatment. The amount used to calculate the use of water was US$ 3,949 for the 0 to 10 m^3 spending range, a price in Brazil in June 2021. In addition, we considered the sewage fee charged, which was 90% of the amount of water spent in the month. The consumption of water considered the amount used for the total filling of the system (~1.25m^3), the amount used in the partial water changes and cleaning (about 10% of the volume of each tank per day or ~0.045m^3 day^{-1}) and that used for hatching *Artemia* during the first 15 days of cultivation (0.9m^3). The average amount of water used was 3.268m^3 in 25 days, so the cost of water was about US$24.52 divided by three treatments, resulting in US$8.17 for each treatment.

The maintenance cost of the laboratory with 64 tanks (facilities and equipment) was the same for all treatments, considering the value of US$6,917.84, which resulted in a daily maintenance/tank cost of US$0.25 (7% of the laboratory value/25 days/64 tanks). Four tanks were used for a period of 25 days for each treatment, resulting in a value of US$ 30.00 for the maintenance of the laboratory during the experiment. The sale of juveniles at US$ 0.04 per individual was determined for the calculation of gross revenue.

Thus, TOC, average TOC, gross revenue and net revenue of the production of *C. macropomun* larvae with 25 days of intensive larviculture were determined. Survival data were used to obtain gross revenue for each treatment.

2.5 Statistical analysis

The Shapiro-Wilk normality test and Levene's homoscedasticity test were used for data analysis. Data of both phases of the experiment were analyzed by ANOVA followed by Tukey's test with 5% probability.

3.Results

3.1 Water parameters

NH_3 was affected by stocking density, with the highest value being for D_{180} ($P<0.05$) (Table 1). Salinity, conductivity, temperature, pH and DO were not affected by stocking density ($P>0.05$).

Table 1. Water quality parameters (mean ± standard deviation) during 25 days of intensive larviculture of *C. macropomum* in RAS at different stocking densities.

Density (Larva L^{-1})	NH_3 (mg L^{-1})	pH	Salinity (g L^{-1})	Conductivity (µS cm^{-1})	Temperature (◦C)	OD (mg L^{-1})
D_{60}	0.0043±0.001c	7.22±0.03	2.06±0.02	4.29±0.19	28.00±0.12	5.24±0.22
D_{120}	0.0069±0.001b	7.25±0.04	2.06±0.06	4.22±0.11	28.07±0.09	5.21±0.04
D_{180}	0.0130±0.0027a	7.24±0.13	2.06±0.07	4.15±0.16	28.06±0.15	5.08±0.22
P – value	<0.0001	0.9297	0.9806	0.5014	0.696	0.4845
CV (%)	13.32	1.14	2.64	3.71	0.45	3.48

Data were submitted to ANOVA, followed by Tukey test at 5%.

Stocking densities tested for 25 days in *C. macropomum* larviculture: D_{60} = 60 larvae L^{-1}, D_{120} = 120 larvae L^{-1}, D_{180} = 180 larvae L^{-1}. * DO – dissolved oxygen.

-3.2 Growth and survival

Weight (Figure 1A), length (Figure 1B) and SGR (Figure 1C) were not affected by stocking density throughout the experiment (P>0.05).

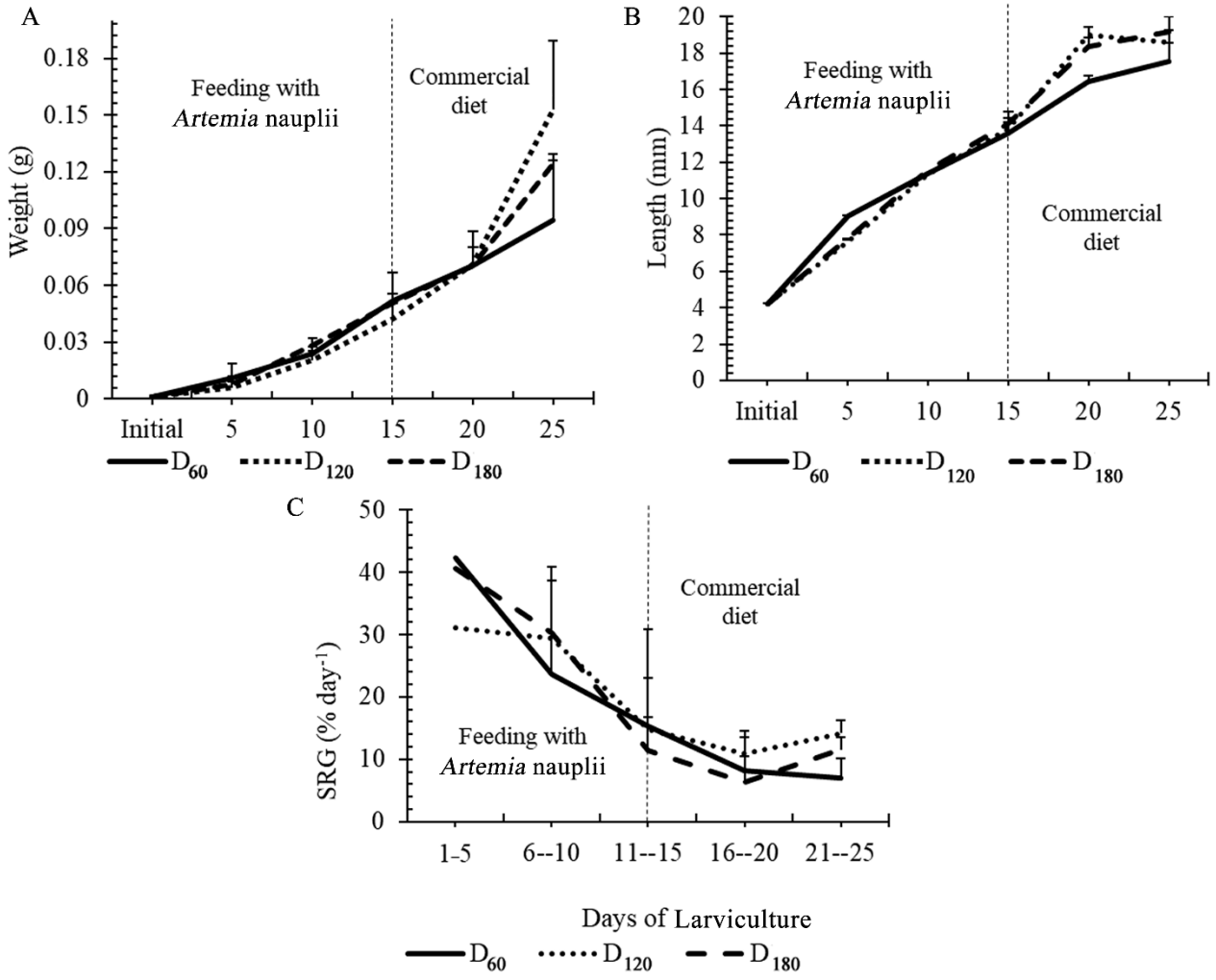

Fig 1. Data on weight (a), length (b) and specific growth rate SGR (c) (mean ± standard deviation) during larviculture of *C. macropomum* cultivated in different stocking densities and fed with *Artemia* nauplii (first 15 days of feeding) and formulated commercial diet (days 16–25 of feeding) in RAS. There were no significant differences for the analyzed parameters among the different densities over the 25 days of rearing (Tukey test at 5% probability).

Survival after the first 15 days of larviculture (feeding with *Artemia*) was lower for D_{180} (P<0.05) and similar between D_{60} and D_{120} (P>0.05) (Figure 2A). Survival during the second phase,

between 16 and 25 days of cultivation with commercial food, did not differ among treatments (P>0.05). On the other hand, overall survival, considering the experiment as a whole (1 to 25 days of cultivation), was similar among the different stocking densities (P> 0.05). The number of animals produced increased with increasing density with lower production for D_{60} and higher for D_{180} (P<0.05) (Figure 2B).

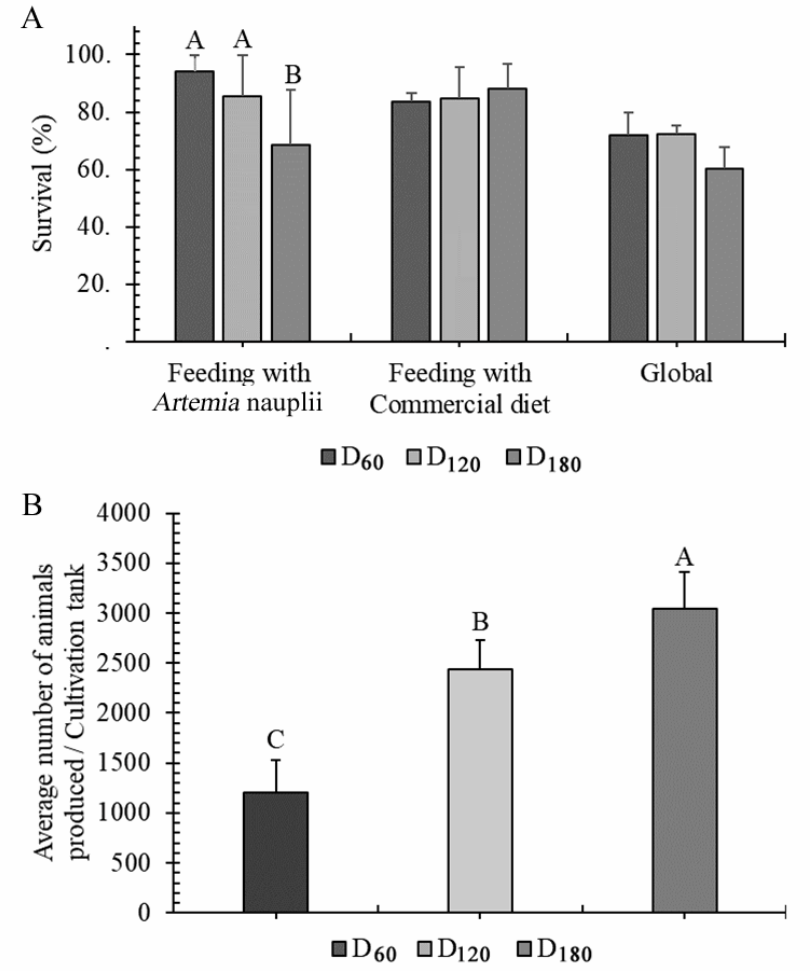

Fig 2. Survival (A) (mean ± standard deviation) during larviculture of *C. macropomum* in different stocking densities and fed with *Artemia* nauplii (first 15 days of feeding) and formulated commercial diet (days 16–25 of feeding) in RAS. (B) Number (mean ± standard deviation) of *C. macropomum* juveniles produced in different stocking densities after 25 days of cultivation in RAS. Different letters indicate statistical difference between stocking densities (Tukey's test at 5% probability).

Animal heterogeneity was recorded at the end of the experiment for all densities tested (Figure 3). Size classes varied within each stocking density ($P<0.05$). There was a predominance of the M size class in densities D_{60} and D_{120}, while there was no difference between size classes S and M for D_{180} ($P>0.05$). When comparing each size class among the different densities, the S class predominated in D_{180}, and M in D_{60} and D_{120} ($P<0.05$); the percentage of class L animals was similar among treatments ($P>0.05$).

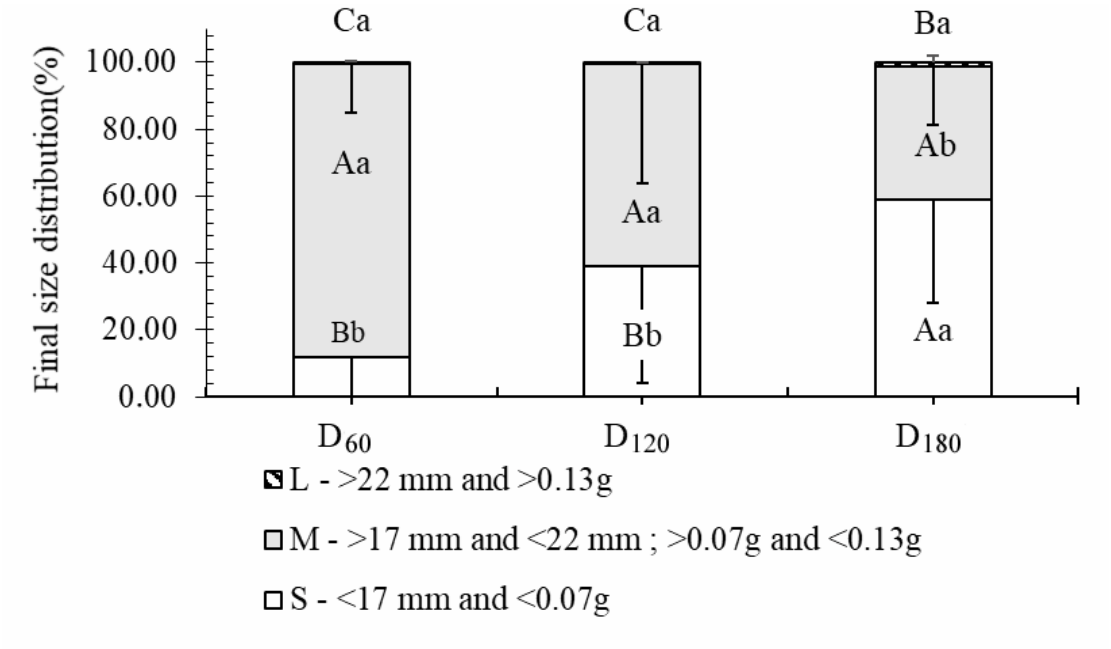

Fig 3. Distribution of size classes (%) of *C. macropomum* in different stocking densities after 25 days of larviculture in RAS. For each density, capital letters indicate a significant difference between size class percentages. Lowercase letters indicate significant. difference between size classes at the different densities tested (Tukey's test at 5% probability).

3.3 Economic analysis

The item in the inputs with the greatest impact was related to the acquisition value of *C. macropomun* larvae, with the D_{180} treatment having the highest cost. As the other inputs are directly related to density, they had higher values for the D_{180} treatment as well (Table 2).

Table 2. Quantity and values (US$) of inputs used during intensive larviculture of *C. macropomum* in different stocking densities in RAS.

Inputs	**Unitary value (US$)**		**D_{60}**	**D_{120}**	**D_{180}**
			6,720 (Larvae)	**13,440 (Larvae)**	**20,160 (Larvae)**
Larvae 0.0011g	$ 0.0057	(unity)	$ 38.47	$ 76.94	$ 115.41
Artemia Nauplii (cyst)	$ 2.75	(Kg)	$ 36.67	$ 73.33	$ 110.00
Commercial extruded diet (mash)	$ 2.50	(Kg)	$ 1.03	$ 1.91	$ 3.06
Total (US$)			**76.16**	**152.17**	**228.46**

* US$ = American dollar

Stocking densities tested for 25 days in *C. macropomum* larviculture: D_{60} = 60 larvae L^{-1}, D_{120} = 120 larvae L^{-1}, D_{180} = 180 larvae L^{-1}.

Labor costs differed in terms of work times for cleaning the tanks and producing food, with D_{60} requiring the least service time (45.83 hours of work) (Table 3).

Table 3. Technical coefficients and labor cost of items that varied during intensive larviculture of *C. macropomum* at different stocking densities in RAS.

Items/Density	**D_{60}**		**D_{120}**		**D_{180}**	
	minutes/day	**25 days**	**minutes/day**	**25 days**	**minutes/day**	**25 days**
Cleaning	15	375	25	625	35	875

Food production	15	375	25	625	35	875
Feeding	10	250	10	250	10	250
Artemia management	70	1750	70	1750	70	1750
Time worked (minutes)	110	2750	130	3250	150	3750
Worked hours		45.83		54.17		62.50
Labor benefits (US$)		$24.28		$28.70		$33.11
Hour worked value (US$)		$40.79		$48.21		$55.63
Total value (US$)		**$65.07**		**$76.91**		**$88.74**

* US$ = American dollar

Stocking densities tested for 25 days in *C. macropomum* larviculture: D_{60} = 60 larvae L^{-1}, D_{120} = 120 larvae L^{-1}, D_{180} = 180 larvae L^{-1}.

The highest depreciation amount for materials and equipment was related to the laboratory (US$2.14/25 days) (Table 4).

Table 4. Value and depreciation of the laboratory and equipment and materials in the trial period in 25 days of laboratory use per year.

Items	Quantity	Total amount (US$)	Useful life (years)	Production days	Depreciation US$/day	Days of use in the experiment	Use in the experiment	Depreciation/25 days
Artemia nauplii incubators	4	195.31	5	25	0.006	15	100%	0.09
Larvae culture tank	4	39.06	15	25	0.002	25	100%	0.05
Filtering Reservoir	4	35.16	15	25	0.002	25	100%	0.05
Heater (200w)	5	13.17	2	25	0.003	25	100%	0.07
Heater (50w) *Artemia* incubators	4	12.66	2	25	0.003	15	100%	0.04
*Balance	1	664.06	15	25	0.164	*1	100%	0.16
Freezer	1	332.03	15	25	0.078	25	5%	0.12
Other materials	1	58.59	1	25	0.012	25	100%	0.29
**Laboratory	1	6917.84	40	25	0.695	25	10.24%	2.14
***Air blower	1	453.46	10	25	0.139	25	10.24%	0.43
RAS pump	1	136.72	10	25	0.109	25	100%	2.73
Total							US$	6.18

US$ = American dollar

*Scale for 6 hours a day

**Laboratory contains 64 tanks / 4 used / 3 treatments

***Blower capacity 64 tanks / 4 used / 3 treatments

The highest average TOC was for D_{180} (US$381.95) and the lowest for D_{60} (US$205.99) (Table 5). However, the D_{60} treatment presented a negative net income, leading to a financial loss in production. The D_{180} treatment showed the best net revenue value (US$ 104.47) even with lower survival (60.32%), with 12,160 juveniles produced after 25 days.

Table 5. Cost and profitability of the production of larviculture of *C. macropomum* in a recirculation aquaculture system for 25 days at different stocking densities.

Items/Treatment	**D_{60}**	**D_{120}**	**D_{180}**
Inputs and food (US$)	$ 76.16	$ 152.17	$ 228.46
Labor	$ 65.07	$ 76.91	$ 88.74
Electricity	$ 20.40	$ 20.40	$ 20.40
Water	$ 8.17	$ 8.17	$ 8.17
Laboratory maintenance	$ 30.00	$ 30.00	$ 30.00
Effective operating cost	**$ 199.81**	**$ 287.65**	**$ 375.77**
Other costs			
Depreciation	$ 6.18	$ 6.18	$ 6.18
Total operating cost	$ 205.99	$ 293.84	$ 381.95
Final survival 25 days larviculture	72.01%	72.46%	60.32%
Number of larvae produced (unit)	4839.072	9738.624	12160.512
Average operating cost	$ 0.04	$ 0.03	$ 0.03
Gross Revenue (US$ 0.04 unit)	$ 193.56	$ 389.54	$ 486.42
Net Revenue (US$)	**$ -12.43**	**$ 95.71**	**$ 104.47**
Net revenue 64 tanks	$ -198.84	$1,531.32	$ 1,671.51

* US$ = American dollar

Stocking densities tested for 25 days in *C. macropomum* larviculture: D_{60} = 60 larvae L^{-1}, D_{120} = 120 larvae L^{-1}, D_{180} = 180 larvae L^{-1}.

4. Discussion

The intensive larviculture of *C. macropomum* in RAS presented relevant and optimistic results for the use of high stocking densities — higher than demonstrated by Santos et al. (2021) who tested up to 50 larvae L^{-1}.

Regarding water quality, NH_3 increased with increasing density. The same was demonstrated in the larviculture of *H. lacerdae* (Luz and Portella, 2005) *L. alexandri* (Luz and Santos, 2008), *Oreochromis niloticus* (Luz et al., 2012), *Rhinelepis aspera* (Santos et al., 2012), and L333 king tiger pleco *Hipancistrus* sp. (Reis et al., 2020), indicating the need for greater care with water quality at higher densities. However, the highest NH_3 value, which was for D_{180} (0.0130±0.0027 mg L^{-1}), remained below reported lethal levels (1.63 mg L^{-1}) for juveniles of tambacu (*Colossoma macropomum* x *Piaractus mesopotamicus*) (Quaresma et al., 2020). The other water quality parameters remained similar among stocking densities and within recommended levels for fish farming (Boyd and Tucker, 1992).

The weight and length of *C. macropomum* larvae were not influenced by the increase in stocking density during 25 days of culture. These results optimize those reported by Santos et al. (2021), who also did not find differences in performance with densities between 10 and 50 larvae L^{-1} during 30 days in a RAS. High stocking densities also did not influence the performance of *L. alexandri* (60–300 larvae L^{-1}; Cordeiro et al., 2015), *H. lacerdae* (10–90 larvae L^{-1}; Luz and Portella, 2005), *T. Tinca* (20–320 larvae L^{-1}; Celada et al., 2007), or *Barbus barbus* (20–200 larvae L^{-1}; Zarski et al., 2011). There was a reduction in SGR over the experimental period, but with no difference among the different stocking densities. Reduction of SGR during larviculture has been reported previously for *C. macropomum* (Santos et al., 2021), as well as in the larviculture of *H. lacerdae* (Luz and Portella, 2005).

The lowest survival value after 15 days of larviculture, during which the larvae were fed with *Artemia* nauplii, was for D_{180} (68.57%), which may be related to intraspecific competition at higher

stocking densities, which can lead to cannibalism (Hecht and Pienaar 1993). Cannibalism was observed in the present study despite *C. macrompomum* being an omnivorous species. However, after feeding with the commercial diet, D_{180} showed low mortality, resulting in a final survival value of 60.32%, which was similar to that for the lower densities tested. The effects of stocking density on survival and growth can be variable or even contradictory (Niazie et al., 2013), depending on the species, rearing conditions and age of the fish (Saoud et al., 2008). Survival rates in this phase can range from 0 to 66%, with a lack of knowledge of the causes of mortality, according to Gomes et al. (2018). The average survival values in the present study were higher than those recorded by Santos et al. (2021) of between 62.35 and 55.15%, in RAS (10–50 larvae L^{-1}), Santos et al. (2007) of 46.86 and 37.06% in masonry tanks (50 and 100 post-larva L^{-1}, respectively) and Atencio-Garcia (2001) with values greater than 50% in nurseries with natural food production (50–150 post-larvae m^2) for *C. macropomum*. Woynarovich and Woynarovich, (1998) highlight average survival rates of 55% in the larviculture of *C. macropomum*, provided that the management is adequate for 30 days in smaller nurseries.

The average number of larvae produced increased with increasing density. Similar results were recorded for *H. lacerdae* (Luz et al., 2005), thus optimizing the production system with higher densities without compromising performance and survival. In intensive larviculture of *L. alexandri* in RAS, there was better production of juveniles at higher densities even with reduced survival, with four times more individuals at the highest density tested (Cordeiro et al., 2015). Thus, density studies, must consider final production of animals in addition to survival.

Size heterogeneity was common at the end of larviculture. Density treatments D_{60} and D_{120} had a predominance of individuals from class M. In larviculture of the same species, Santos et al. (2021) also recorded a predominance of class M animals at the different densities tested. However, in the present study the D_{180} lot was more heterogeneous, with no predominance between animals

classified as M and S. Heterogeneous lots can be considered a problem (Carvalho et al., 2018), and may be influenced by the imposed stocking density (Jomori et al., 2003), where the accepted storage limit can result in greater size heterogeneity (Holm et al., 1990; Ruzzante and Doyle, 1990; Canario et al., 1998). Size heterogeneity can even result in losses due to cannibalism as a consequence of animals with different sizes being within the same lot (Fox and Aldrich, 1990; Baras et al., 2000a,b; Szkudlarek and Zakes, 2002; Baras et al., 2003), as discussed above. Heterogeneity can vary according to species and life stages (Kestemont et al., 2003). Such heterogeneity requires some management to be performed, such as animal classification, which can increase fish performance while increasing homogeneity and decreasing intraspecific competition (Brett, 1979; Irwin et al., 1999; Filho, 2007; Santos et al., 2021).

Economic evaluation in production is fundamental for its success. In intensive larviculture, this parameter provides important information to help fish farmers increase juvenile production and profitability by adopting more efficient techniques (Jomori et al., 2005). The use of an intensive larval rearing system could increase the availability of quality juveniles (Cestarolli and Portella, 1994), which could reduce the larviculture period (Weirich et al., 2001) and increase the number of animals before being moved to stages in another production system. The main input cost in the present study was the acquisition of *C. macropomum* larvae. This finding was also reported in the larviculture of tambatinga *Colossoma macropomum* x *Piaractus brachypomus*, (Lira et al., 2021) and pacu *P. mesopotamicus*, (Jomori et al., 2005). Our economic assessment demonstrated that the total operating cost increased with increasing density. However, the average operating cost decreased with increasing density, demonstrating that the cost tends to decrease as the production level increases, a finding also reported by Lira et al. (2021) for tambatinga larviculture. Consequently, the highest net revenue was found for D_{180}, making it the most economic stocking density for *C. macropomum* larvae production in RAS. With larviculture of *P. mesopotamicus*, Jomori et al. (2005) demonstrated that production costs increased with intensive rearing, however, gross income was higher, resulting in greater profits

in the economic evaluation. With the results presented here, we emphasize the importance of more studies that include economic feasibility analyses, which are still scarce in the literature, because such data can be a crucial factor in decision making in intensive fish larviculture.

5. Conclusions

The present study concludes that intensive larviculture of *C. macropomum* can be carried out in RAS with up to 180 larvae L^{-1} with good results in terms of performance and survival. In addition, good indicators of economic and financial viability were verified for the highest density tested. Complementary studies must be carried out to optimize managements in this production phase, such as classification to produce more homogeneous lots of *C. macropomum*.

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Conclusões

Concluímos que a larvicultura de *C. macropomum* pode ser realizada em água levemente salina em RAS com até 180 larvas L^{-1} com bons resultados de desempenho e sobrevivência.

Além disso, foram verificados bons indicadores de viabilidade econômico-financeira para densidades superiores a 120 larvas L^{-1}. Estudos complementares devem ser realizados para otimizar manejos nesta fase de produção, como classificação para produzir lotes mais homogêneos de *C. macropomum*, assim como testar densidades ainda mais altas.

Já, os juvenis de *C. macropomum* apresentaram excelente adaptação ao RAS com bons resultados para desempenho zootécnico.

A densidade de estocagem na recria e engorda deve ser avaliada de acordo com o tamanho dos animais, e o melhor desempenho foi para as menores densidades na Fase I (0,5kg m^{-3} - peso inicial de 34,88±0,60g) e na Fase II (1,5 kg m^3 - inicial peso de 150,61 ± 0,58g), porem menor produção em biomassa. Estudos complementares devem ser realizados para maximizar a produção através de analises de viabilidade econômica.

A classificação dos animais é um manejo importante para manter a uniformidade para comercialização.

Diferentes densidades de estocagem e tamanhos de animais podem interferir em alguns parâmetros hematológicos e bioquímicos, que podem ser utilizados como indicadores de bem-estar animal.