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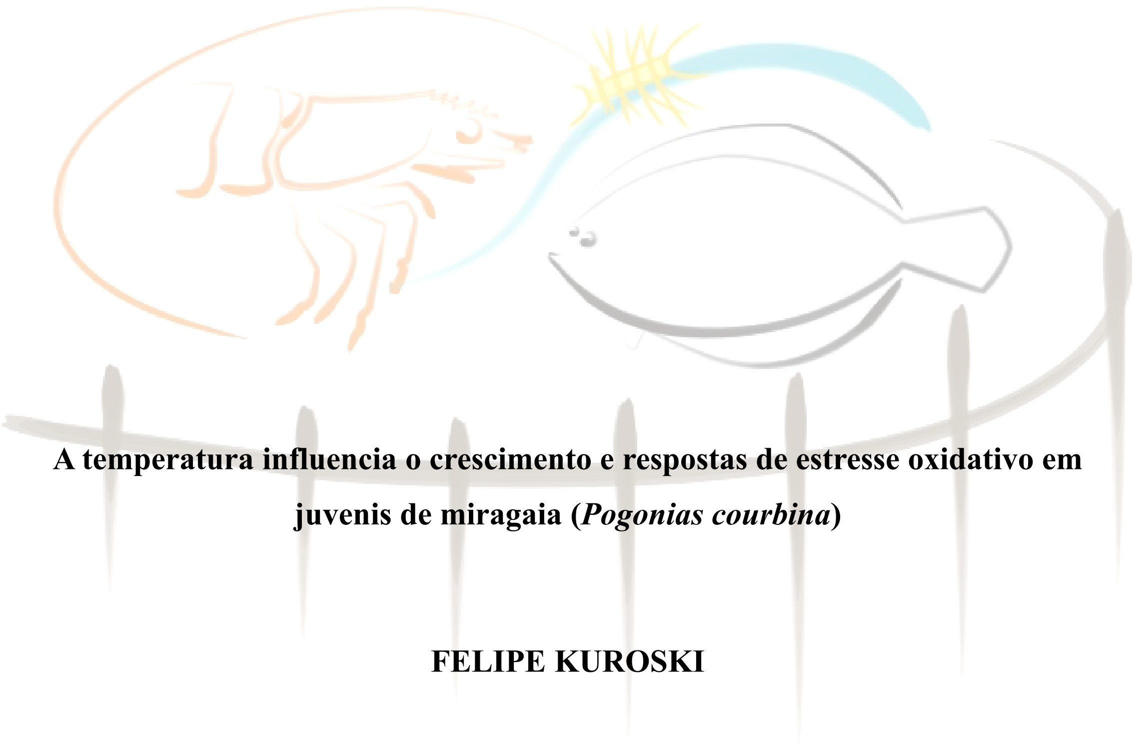

A temperatura influencia o crescimento e respostas de estresse oxidativo em juvenis de miragaia (*Pogonias courbina*)

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A temperatura influencia o crescimento e respostas de estresse oxidativo em juvenis de miragaia (*Pogonias courbina*)

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Orientador: Dr. Luís André Sampaio

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Resumo geral

A temperatura tem forte influência sobre o metabolismo de peixes e nas atividades de produção aquícola. Para entender como esse parâmetro afeta o desempenho, sobrevivência e respostas ao estresse oxidativo de juvenis da miragaia *Pogonias courbina*, 450 indivíduos (0,58 ± 0,19 g) foram criados durante 25 dias em triplicata a: 23, 26 e 29°C, em tanques circulares de 300 L acoplados a sistemas de recirculação de água. Durante o experimento, os peixes foram alimentados quatro vezes ao dia, com dieta formulada contendo 55% de proteína bruta e 13% de lipídios. Os parâmetros de crescimento da miragaia melhoraram significativamente à medida que a temperatura foi aumentada. O peso final foi maior a 29°C, onde os peixes atingiram 8,94 ± 0,56 g, sendo 2,9 vezes maior que o peso final dos peixes criados em 23°C. A taxa de crescimento específico dos peixes mantidos em 29°C foi igual a 10,94 ± 0,25%/dia, resultado significativamente maior do que em qualquer outra temperatura testada. A temperatura também influenciou o consumo total de ração e a conversão alimentar aparente, uma vez que os peixes criados a 26 e 29°C ingeriram maiores quantidades de ração e tiveram melhor conversão alimentar aparente. As temperaturas testadas não afetaram a sobrevivência dos peixes, as quais ficaram acima de 98% em todos os tratamentos. Em relação às respostas sobre os parâmetros de estresse oxidativo no músculo, brânquia e fígado, a exposição a menor temperatura (23°C) levou os peixes a uma maior peroxidação lipídica (LPO) em todos os tecidos testados. Além disso, a exposição a 23°C desencadeou uma regulação positiva da capacidade antioxidante total contra radicais peroxil (ACAP) nas brânquias e no fígado se comparada a 29°C. Os tióis proteicos dos peixes (P-SH) foram maiores no fígado dos indivíduos mantidos a 29 °C do que a 23°C. O melhor desempenho dos peixes mantidos a 29°C pode estar relacionado ao aumento do consumo total de ração, aliado à melhor conversão alimentar aparente, juntamente com menor dano lipídico e proteico, além de menor investimento em capacidade antioxidante. Com base nos resultados obtidos, concluímos que dentre as temperaturas testadas, 29°C é a melhor temperatura para a produção de juvenis de *P. courbina*, pois promove melhor taxa de crescimento, conversão alimentar e menor dano oxidativo.

Palavras-chave: Produção de peixes marinhos, Sciaenidae, antioxidante, dano oxidativo, estresse térmico.

Abstract

The temperature has a strong influence on fish metabolism and aquaculture production activities. To understand how this environmental variable affects growth performance, survival, and oxidative stress responses of juvenile Southern black drum *Pogonias courbina*, 450 individuals (0.58±0.19g) were exposed for 25 days in triplicate to: 23, 26, and 29°C, in 300L circular tanks attached to recirculating aquaculture systems. During the experiment, fish were fed four times a day, with a formulated diet containing 55% crude protein and 13% lipids. Southern black drum growth parameters significantly improved as the temperature was raised. The final weight was higher at 29°C, fish reached 8.94±0.56 g, it was 2.9-fold higher than the final weight of fish reared at 23°C. Specific growth rate of fish reared at 29°C was equal to 10.94±0.25 %/day, significantly higher than at any other temperature tested. Temperature also influenced the total feed intake and apparent feed conversion, since fish reared at 26 and 29°C ingested higher amounts of food and had a better apparent feed conversion. Tested temperatures did not affect fish survival, it was above 98% in all treatments. Regarding responses on oxidative stress parameters on muscle, gill, and liver, the lowest temperature (23°C) induced higher lipid peroxidation (LPO) in all tested tissues. In addition, the exposure to 23°C also triggered upper regulation on the total antioxidant capacity against peroxyl radicals (ACAP) in gill and liver if compared to 29°C. Fish protein thiols (P-SH) were higher in the liver of fish kept at 29°C than 23°C. The best performance of fish kept at 29°C may be related to the increased total feed intake, coupled to better apparent feed conversion, along with lower lipid and protein damage, plus lower investment in antioxidant capacity. Based on the present results, we concluded that among the tested temperatures, 29°C is the best temperature for production of juvenile *P. courbina*, since it promotes better growth rate and feed conversion, along with less oxidative damage.

Keywords: Marine fish culture, Sciaenidae, antioxidant, oxidative damage, thermal stress.

1. Introdução Geral

1.1 Miragaia, *Pogonias courbina*

A família Sciaenidae é composta por 283 espécies de peixes, comumente encontrados em águas costeiras e estuários de todo mundo, sendo um importante recurso para pesca e alimentação humana (Nelson et al., 2016). A miragaia, *Pogonias courbina* (Lacepède, 1803) é o maior representante da família Sciaenidae a habitar águas brasileiras, podendo viver 41 anos e atingir 117 cm de comprimento total (Urtega and Perrotta, 2001). Entretanto, até pouco tempo, essa espécie era conhecida como *Pogonias cromis*. O reconhecimento como uma nova espécie foi apontado por (Azpelicueta et al., 2019) devido a diferenças morfológicas e genéticas entre os grupos do hemisfério norte (*P. cromis*) e aqueles do hemisfério sul (*P. courbina*). A miragaia possui distribuição que se estende desde o estado do Rio de Janeiro no Brasil, até o Golfo de San Matías na Argentina (Haimovici et al., 2020) (Fig. 2).

Figura 1 Indivíduo de miragaia *Pogonias courbina* (CT, 63cm). Imagem cedida por: Rodrigo Oliveira.

A miragaia possui hábito demersal, e boca sub-terminal, bem adaptada para alimentação de fundo. É uma espécie carnívora, que se alimenta principalmente de invertebrados bentônicos, como gastrópodes, bivalves e crustáceos braquiúros (Haimovici, 1997). São organismos estuarino-dependentes, encontrados em regiões estuarinas e costeiras, em profundidade de até 50 m (Haimovici et al., 2020). Devido a sua distribuição e habitat, caracterizado por grandes variações nos parâmetros físico-químicos de água e sazonalidade, esta espécie de peixe apresenta adaptações a um amplo espectro de salinidades e temperaturas (Machado et al., 2020).

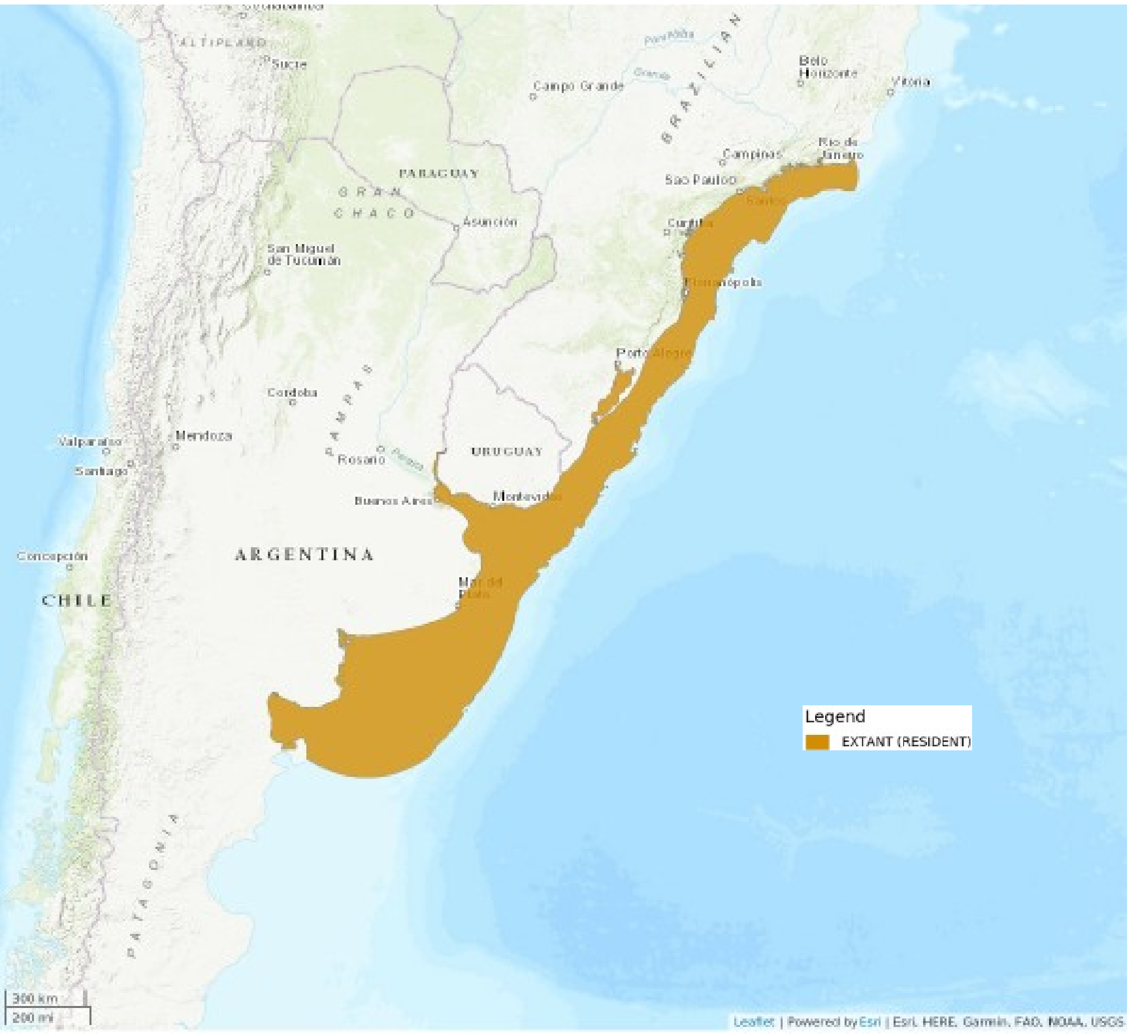

Figura 2 Distribuição geográfica da miragaia *Pogonias courbina*. Fonte: (Haimovici et al., 2020).

A miragaia é um importante recurso para a pesca artesanal, comercial e recreativa na América do Sul (Azpelicueta et al., 2019). Devido a sobrepesca, no Rio Grande do Sul suas capturas passaram de 1.450 toneladas em 1976 para 0 em 2009 (Chao et al., 2015). Isto levou a espécie ao status de “vulnerável” na lista vermelha de espécies da União Internacional para a Conservação da Natureza e dos Recursos Naturais (IUCN) (Haimovici et al., 2020). Apesar da diminuição de seus estoques, ainda há demanda pela captura de *P. courbina*, sendo a produção destes organismos em cativeiro uma alternativa para diminuição da pressão pesqueira sobre os estoques naturais, estabelecimento de ações de repovoamento, o que poderá favorecer o seu reestabelecimento no ambiente natural. Atualmente, no Laboratório de Piscicultura Estuarina e Marinha (LAPEM) da Universidade Federal do Rio Grande - FURG, tem se desenvolvido pesquisa com a espécie. Estes esforços vêm proporcionaram avanços na larvicultura, manejo alimentar e produção destes peixes, as quais proporcionaram a obtenção de juvenis, que hoje destinam-se à pesquisa e ações de repovoamento (L. A. Sampaio comunicação pessoal).

Os scianídeos são bastante apreciados, o que torna sua produção em cativeiro uma atividade com bom potencial para aquicultura. Diversas espécies já são produzidas comercialmente, com destaque para: *Larimichthys crocea*, com produção anual de 177.600 toneladas, sendo a espécie de peixe marinho mais cultivada no mundo (Chen et al., 2020); *Sciaenops ocellatus*, com produção de 88 mil toneladas em 2020 (FAO, 2022); e *Argyrosomus regius*, que tem se tornado cada vez mais representativa na aquicultura de organismos marinhos do mediterrâneo, com uma produção anual de 14.000 toneladas em 2014 (Soares et al., 2018). Entretanto, para que uma espécie seja produzida em escala comercial, primariamente deve-se conhecer sobre suas características e avaliar como estes animais irão comportar-se frente a sua manutenção em instalações aquícolas. A produção de diversos Scianídeos vem sendo estudada, entre eles *P. cromis*, *A. regius*, *Micropogonias furnieri* e *Totoaba macdonaldi* (Holt and Strawn, 1976; Kounna et al., 2021; Ojeda and Strawn, 1980; Sampaio et al., 2008; Yen Ortega et al., 2021). Entre os resultados apresentados nesses trabalhos, pode-se destacar a influência da temperatura sobre seu desempenho zootécnico e sobrevivência. Isto demonstra a necessidade da obtenção de informações acerca da influência da temperatura sobre *P. courbina*, com o objetivo de otimizar a sua produção.

1.2 Influência da temperatura em peixes e sua produção

A temperatura é uma das principais variáveis ambientais para a produção de organismos aquáticos. Peixes são de maneira geral ectotérmicos termo-conformantes, sendo a temperatura ambiental crucial para a manutenção de sua temperatura corporal, já que estes organismos não possuem mecanismos fisiológicos para sua estabilização (Jobling, 1996; Volkoff and Rønnestad, 2020). Mudanças nessa variável podem afetar seu crescimento e sobrevivência (Boltaña et al., 2017). O metabolismo basal de peixes, responsável pela manutenção da vida, varia de acordo com a temperatura, tamanho e espécie estudada (Brett and Groves, 1979; Neubauer and Andersen, 2019; Volkoff and Rønnestad, 2020). Sendo assim, esses animais desenvolveram diversas adaptações que vão desde alterações bioquímicas a comportamentais para lidar com mudanças térmicas (Guderley and St-Pierre, 2002). Se não compensados, os processos metabólicos tendem a aumentar de duas à três vezes a cada aumento de 10°C na temperatura ambiental, sendo a influência da temperatura sobre a taxa metabólica expressa pelo coeficiente “Q_{10}” (taxa na qual uma resposta fisiológica muda com um aumento de 10°C na temperatura) (Volkoff and Rønnestad, 2020). O metabolismo de organismos pode se adaptar de diversas maneiras a mudanças térmicas: (1) diminuir seus processos fisiológicos e se submeter aos efeitos da temperatura, (2) aumentar o efeito da temperatura sobre seus processos metabólicos, (3) diminuir os efeitos da temperatura sobre seus processos metabólicos (Guderley and St-Pierre, 2002). Estas modulações são possíveis graças a alterações fisiológicas, que vão desde alterações enzimáticas até mudanças na regulação da expressão gênica (Hazel and Ladd, 1974).

Os organismos ectotérmicos, incluindo os peixes, apresentam uma curva de performance de crescimento com formato de sino, onde há maior crescimento na temperatura ótima, e decréscimo à medida que a temperatura se distancia deste ponto, até se tornar zero na temperatura crítica máxima e mínima (Volkoff and Rønnestad, 2020). O aumento da temperatura, dentro da faixa ótima para determinada espécie, possui dois efeitos sobre o crescimento, um negativo, decorrente do maior custo energético resultante do aumento do metabolismo, e um positivo, resultado do maior consumo de alimento e capacidade de transformar alimento em energia livre (Fang et al., 2010; Xiao-Jun et al., 1992).

A temperatura é o principal fator para a definição da quantidade de alimento ingerida por um peixe (Jobling, 1993). Esta variável está diretamente correlacionada com a busca por alimento, quimio-detecção (Volkoff and Rønnestad, 2020) e capacidade digestiva dos organismos (Azevedo et al., 1998; Xiao-Jun et al., 1992). Dentro da faixa ótima, a quantidade de alimento ingerida é positivamente correlacionada com o aumento moderado da temperatura (Volkoff and Rønnestad, 2020). Além de sua influência sobre a saciedade e busca por alimento, deve-se destacar a importância da temperatura sobre a capacidade de processamento e obtenção de energia proveniente do alimento. A expressão e atividade de enzimas digestivas está diretamente correlacionada com a temperatura, podendo alterar a capacidade dos organismos em digerir matéria seca, proteínas, etc, resultado em menor digestão aparente do alimento ofertado, promovendo menor aproveitamento da ração e crescimento (Bureau et al., 2003; Kofuji et al., 2005). Além da influência das enzimas digestivas sobre o aproveitamento de energia, a velocidade do trânsito intestinal e capacidade de processamento de alimento, pode ser alterada pelo aumento do metabolismo e maior necessidade de aporte energético gerado pelo aumento da temperatura (Kounna et al., 2021). Entretanto, apesar dos efeitos positivos da temperatura sobre o aporte de alimento e processamento deste para a obtenção de energia, é importante frisar que um acréscimo indiscriminado desta variável, extrapolando o limite térmico ideal para a espécie, leva a um aumento do aporte energético em manutenção, reduzindo a quantidade de energia livre para crescimento (Volkoff and Rønnestad, 2020).

Sabendo disso, é de fundamental importância conhecer o efeito das variáveis ambientais sobre uma espécie que se deseja produzir. A temperatura ótima de criação de uma determinada espécie, é determinada pela temperatura onde haja maior crescimento dos peixes, melhor eficiência alimentar e condições ambientais para o bem-estar animal (Cavrois-Rogacki et al., 2019). Diversos estudos avaliaram a influência da temperatura sobre a produção de espécies marinhas. Estes trabalhos têm encontrado diversas faixas ideais para crescimento e conversão alimentar, as quais são determinadas pela espécie estudada. Em experimentos realizados na Grécia com *A. regius*, sua taxa de crescimento específico foi 2,5 vezes maior em 26°C que em peixes mantidos a 17°C. Padrão semelhante ocorreu com a taxa de conversão alimentar, que foi maior na temperatura mais baixa se comparada as superiores (Kounna et al., 2021). Yen Ortega et al. (2021), estudando *T. macdonaldi* no México, encontraram maior crescimento em peixes mantidos à 26°C que organismos expostos a 23°C. Ojeda e Strawn (1980) avaliaram o crescimento de *P. cromis* em tanques rede instalados na Baía de Upper Galveston, Texas, encontrando maiores taxas de crescimento durante os períodos do ano onde a temperatura foi maior (26-28°C) do que em temperaturas mais baixas (14-15°C). Além de alterações na taxa de crescimento e conversão alimentar, a escolha da temperatura de cultivo é fundamental para a manutenção da sanidade, bem-estar e sobrevivência de peixes, uma vez que pode atuar diretamente na sua imunidade, desencadear situações de estresse, aumentar sua susceptibilidade à doenças, e em casos extremos, podendo desencadear a sua morte (Wen et al., 2021). Isto determina com que a temperatura seja um dos fatores a ser considerado na escolha da localidade de instalação de empreendimentos aquícolas, e no manejo da temperatura em sistemas onde exista esta possibilidade, como é o caso dos sistemas de recirculação de água (SRA), onde é viável a instalação de resfriadores e aquecedores para o controle da temperatura da água. Adotar um regime de temperatura ideal auxilia no sucesso produtivo, pois pode favorecer ganhos, tanto no crescimento dos peixes, como na maior assimilação dos nutrientes disponibilizados através da ração ofertada, evitando perdas financeiras e decréscimo na qualidade de água do cultivo.

1.3 Estresse oxidativo

Em organismos aeróbicos como é o caso dos peixes, o oxigênio molecular (O_2) é normalmente reduzido via transformação tetravalente pela cadeia transportadora de elétrons para a produção de ATP e termina com a formação de água. Entretanto, mesmo em condições normais, parte deste oxigênio é reduzido parcialmente pela adição de elétrons livres, levando à formação de espécies reativas de oxigênio (EROs) como o ânion superóxido ($O_2^{\bullet -}$), o peróxido de hidrogênio (H_2O_2) e o radical hidroxila ($^{\bullet}OH$) (Lushchak, 2011). Estas EROs têm a capacidade de oxidar macromoléculas incluindo proteínas, lipídios e DNA, as quais podem ter sua função biológica comprometida ou perdida. Dessa forma, os organismos aeróbicos necessitam um sistema de defesa antioxidante, composto por defesas enzimáticas e não enzimáticas para combater o acúmulo e ação das EROS nas células (Carneiro et al., 2021). Um antioxidante é qualquer substância que quando presente em baixas concentrações, se comparadas às de um substrato oxidável, retarda ou impede significativamente a sua oxidação. Dentre os princípios gerais dos antioxidantes podem ser citados alguns exemplos como: (1) agentes que removem EROs cataliticamente, como a superóxido desmutase (SOD) e calatase, (2) agentes que controlam a formação de EROs, como proteínas de desacoplamento (3) “agentes de sacrifício”, que preferencialmente reagem com EROs e impedem ataques a biomoléculas, exemplo GSH (Halliwell and Gutteridge, 2015).

Apesar da constante produção de EROs durante o metabolismo celular, sua concentração é finamente controlada, mantendo-se usualmente inferior a 10^{-8} M (Lushchak, 2011). Entretanto, quando há um aumento transitório ou crônico na concentração de EROs, seja pelo aumento na sua produção, e/ou deficiência no sistema antioxidante, resultando em perturbação do metabolismo, sinalização redox, ou danos aos constituintes celulares, o organismo tende a passar por um estado denominado de “estresse oxidativo” (Halliwell and Gutteridge, 2015; Lushchak, 2011; Nitz et al., 2020).

Dentre as variáveis ambientais que podem levar um organismo a um cenário de estresse oxidativo está a temperatura (Nitz et al., 2020). O seu aumento, em concordância com as leis da termodinâmica, tende a estimular todos os processos metabólicos, podendo aumentar a formação de EROs como um subproduto do aumento da taxa metabólica (Lushchak, 2011). A elevação da temperatura ambiental tem sido correlacionada com aumento da peroxidação lipídica, alterações nos níveis de tióis e enzimas antioxidantes (Bagnyukova et al., 2007).

Por outro lado, é de se esperar que a redução da temperatura ambiental resulte em menor risco de indução de estresse oxidativo, entretanto isto não é uma verdade absoluta (Malek et al., 2004; Nitz et al., 2020). Isto se dá pois a temperatura tem outros efeitos sobre os organismos, que podem aumentar sua susceptibilidade a dano oxidativo (Vinagre et al., 2012), diminuir a capacidade antioxidante, ou até mesmo promover a formação de EROs (Lushchak, 2011). Um dos efeitos da diminuição da temperatura sobre os organismos é a alteração na fluidez de membrana, a qual deve ser precisamente regulada, uma vez que o transporte de moléculas e alguns processos enzimáticos podem ser afetados com o aumento de sua viscosidade. Esta característica pode ser alterada pela composição lipídica e pela temperatura a qual determinada membrana esteja exposta (Alberts et al., 2017; Guderley and St-Pierre, 2002). Na tentativa de manter a fluidez de membrana durante situações de frio, os organismos tendem a sintetizar maiores concentrações de ácidos graxos poli-insaturados, característicos por seu maior número de duplas ligações (Wen et al., 2021). Esta exposição ao frio, pode aumentar a proporção de ácidos graxos altamente insaturados (HUFA) como: ácido docosapentaenóico (22:5), ácido docosahexanóico (22:6) e ácido araquidônico (20:4) (Hazel and Ladd, 1974). O aumento na proporção de HUFA aumenta a susceptibilidade das membranas a sofrer peroxidação lipídica, uma vez que suas ligações duplas tornam essa classe de lipídios 20-40 vezes mais susceptível a sofrer este tipo de reação do que os ácidos graxos mono-insaturados (Crockett, 2008). A diminuição da temperatura e a necessidade de manutenção de atividades motoras, podem levar os peixes a compensação térmica do metabolismo. Este processo se dá principalmente pelo aumento da capacidade oxidativa do musculo esquelético, visando a produção de ATP, resultado do aumento da densidade mitocondrial, capacidade oxidativa mitocondrial, ou uma mistura destes, sendo o segundo, resultado das alterações de membrana em decorrência ao frio anteriormente citadas (Guderley and St-Pierre, 2002). Este aumento na atividade e número de mitocôndrias em situações de frio, pode desencadear o aumento na formação de espécies reativas de oxigênio (Nitz et al., 2020), que associado ao maior número de ácidos graxos poli-insaturados, mais susceptíveis a peroxidação lipídica, pode levar os organismos a sofrer maior dano oxidativo.

Além de alterar metabolismo, atividade mitocondrial e perfil de ácidos graxos, a temperatura também modifica a maneira com que os indivíduos são capazes de lidar com EROs. Isto pode ocorre através de alterações no seu sistema antioxidante, seja por mudanças sobre a expressão e atividade de enzimas antioxidantes (Abele and Puntarulo, 2004; Malek et al., 2004), ou em decorrência de alterações na quantidade de antioxidantes exógenos, provenientes da ração, uma vez que alterações na temperatura também modificam a quantidade de alimento consumido e assimilado por peixes (Volkoff and Rønnestad, 2020). Sendo assim, evitar situações onde haja estresse térmico na aquicultura é importante para seu sucesso, uma vez que a produção de enzimas antioxidantes, indispensáveis para combater os efeitos de EROs, leva a um aumento do custo energético para manutenção dos organismos, diminuindo o investimento desta energia para crescimento somático (Sokolova et al., 2012), podendo em alguns casos, desencadear maior custo para a engorda dos peixes. Além disso, o estímulo de um cenário próoxidante em decorrência de estresse térmico, pode levar ao aumento na concentração de EROs, que por sua vez, irão oxidar macromoléculas, trazendo danos às estruturas celulares, proporcionar susceptibilidade às doenças, e podendo levar à morte dos organismos em casos mais graves. Estas perdas podem se refletir para além do período produtivo, uma vez que a peroxidação lipídica acarreta em alterações organolépticas ao filé, causando a diminuição da qualidade do produto (Conde-Guerrero et al., 2021).

Entretanto, apesar da importância da avaliação de respostas de estresse oxidativo em peixes para o desenvolvimento da aquicultura, ainda não existem trabalhos que avaliem a viabilidade produtiva de *P. courbina*, nem a influência da temperatura sobre suas respostas de estresse. Sendo assim, os resultados deste trabalho poderão contribuir para o seu entendimento. Isto poderá proporcionar informações úteis para o desenvolvimento de protocolos comerciais, uma vez que permitirão o manejo adequado da temperatura nos tanques de cultivo.

2. Objetivos

Objetivo Geral

- Determinar a influência da temperatura na sobrevivência, desempenho zootécnico e respostas de estresse oxidativo em juvenis de miragaia.

Objetivos específicos

- Determinar o efeito da temperatura sobre o crescimento e sobrevivência de juvenis de miragaia;
- Avaliar os efeitos da temperatura sobre a capacidade antioxidante contra radicais peroxil (ACAP);
- Avaliar os efeitos da temperatura sobre o dano proteico (Tióis proteicos, P-SH) e dano lipídico (substâncias reativas ao ácido tiobarbitúrico, TBARS) no músculo, fígado e brânquias de juvenis de miragaia.

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Capítulo I

Temperature influences growth and oxidative stress responses of juvenile Southern black drum (*Pogonias courbina*)

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Abstract

The temperature has a strong influence on fish metabolism and aquaculture production activities. To understand how this environmental variable affects growth performance, survival, and oxidative stress responses of juvenile Southern black drum *Pogonias courbina*, 450 individuals (0.58±0.19g) were exposed for 25 days in triplicate to: 23, 26, and 29°C, in 300L circular tanks attached to recirculating aquaculture systems. During the experiment, fish were fed four times a day, with a formulated diet containing 55% crude protein and 13% lipids. Southern black drum growth parameters significantly improved as the temperature was raised. The final weight was higher at 29°C, fish reached 8.94±0.56 g, it was 2.9-fold higher than the final weight of fish reared at 23°C. Specific growth rate of fish reared at 29°C was equal to 10.94±0.25 %/day, significantly higher than at any other temperature tested. Temperature also influenced the total feed intake and apparent feed conversion, since fish reared at 26 and 29°C ingested higher amounts of food and had a better apparent feed conversion. Tested temperatures did not affect fish survival, it was above 98% in all treatments. Regarding responses on oxidative stress parameters on muscle, gill, and liver, the lowest temperature (23°C) induced higher lipid peroxidation (LPO) in all tested tissues. In addition, the exposure to 23°C also triggered upper regulation on the total antioxidant capacity against peroxyl radicals (ACAP) in gill and liver if compared to 29°C. Fish protein thiols (P-SH) were higher in the liver of fish kept at 29°C than 23°C. The best performance of fish kept at 29°C may be related to the increased total feed intake, coupled to better apparent feed conversion, along with lower lipid and protein damage, plus lower investment in antioxidant capacity. Based on the present results, we concluded that among the tested temperatures, 29°C is the best temperature for production of juvenile *P. courbina*, since it promotes better growth rate and feed conversion, along with less oxidative damage.

Keywords: Marine fish culture, Sciaenidae, antioxidant, oxidative damage, thermal stress.

1. Introduction

Temperature is one of the most important abiotic parameters for aquaculture, since it directly influences fish metabolism, feeding, digestion (Volkoff and Rønnestad, 2020), and health (Wen et al., 2021), which are finally correlated with fish growth and survival (Boltaña et al., 2017; Kounna et al., 2021). Optimal rearing temperature may be defined as the temperature where growth and feeding efficiency are maximized, while it preserves health and welfare of the organisms (Cavrois-Rogacki et al., 2019). This knowledge is determinant for defining the appropriate sites for aquaculture facilities when operating in open water systems, and management of temperature in closed water systems.

Temperatures outside the optimum range of a species can induce oxidative stress in fish (Nitz et al., 2020). This condition is caused by the imbalance between prooxidant and antioxidant defenses (Halliwell and Gutteridge, 2015). During the regular cell function, reactive oxygen, nitrogen, and sulfur species are normally produced, but they are known to cause oxidative damage to biomolecules as proteins, lipids, and DNA, which could compromise their biological functions (Paulsen and Carroll, 2013). In order to neutralize the possible harmful effects of accumulation of these molecules, aerobic organisms, including fish, have an antioxidant defense system composed by enzymes and other molecules with antioxidant properties (Lushchak, 2011). However, inappropriate rearing temperatures can lead fish to an oxidative stress scenario through its influence on metabolism and respiration rate, which can increase reactive oxygen species (ROS) formation (Christen et al., 2018), and/or by reducing the antioxidant defenses (Wen et al., 2021). The energetic cost for maintenance of an organism tends to increase due to damage caused by ROS during environmental stress situations, since it is necessary higher investment in protection against oxidative damage and repair, thus reducing energy available for growth, once maintenance costs are priority (Sokolova et al., 2012). Many biomarkers are useful to assess the oxidative responses of an organism. Protein thiols (P-SH) are important to cellular redox homeostasis, through acting as a redox buffer during oxidation (Hansen et al., 2009). Low levels of P-SH may be interpreted as a protein indicator of oxidant conditions in cells (Mitton et al., 2016; Nitz et al., 2020). Due to the complexity of the antioxidant systems, holistic methods to measure the antioxidant competence, such as the antioxidant capacity against peroxyl radicals (ACAP), are desirable, since they can provide a better understanding of the general resistance of organisms to oxidative stress (Amado et al., 2009). Lipid peroxidation (LPO) is a well-known pathway of cellular damage caused by ROS that can be induced at suboptimal temperatures. The higher solubility of oxygen at lower temperatures, and the need to increase the content of polyunsaturated fatty acids (PUFA) in order to maintain the fluidity of cell membrane when fish are exposed to low temperatures, make these organisms more susceptible to LPO (Wen et al., 2021).

Fish of the Family Sciaenidae have a worldwide spread distribution, and they are known as important items of the fisheries market. In South America, these fish live in warmer tropical, subtropical, and temperate waters, inhabiting marine, brackish, or freshwater environments (Azpelicueta et al., 2019). The Southern black drum *Pogonias courbina* is an estuarine-dependent species, distributed along coastal marine areas through the State of Rio de Janeiro, Brazil, to the Gulf of San Matías, Argentina (Azpelicueta et al., 2019). As a result of their distribution, these fish are adapted to a wide range of salinities and temperatures (Costa Machado et al., 2020). This species can reach 117 cm and is an important resource to artisanal, recreational, and commercial fisheries in South America (Azpelicueta et al., 2019). It is now classified as a vulnerable species in the IUCN red list (Haimovici et al., 2020), due to over exploitation of its fishing stocks during the 20th century (Chao et al., 2015). The production of these organisms in captivity could be an alternative to fulfill the demand of the market and reestablish the wild population through reduction of fisheries efforts and stock enhancement programs.

The knowledge of adequate temperatures for fish production is important to optimize growth and feed efficiency (Cavrois-Rogacki et al., 2019). Some studies carried out in net cages at Upper Galveston Bay (Texas, USA) with the congeneric *Pogonias cromis* have demonstrated that higher summer temperature (26-28°C) and average salinity (15‰) favored fish growth, while during winter rainy conditions (temperature 14-15°C, salinity 5‰) feed intake and growth are reduced (Ojeda and Strawn, 1980). However, there is still no research focused on the influence of temperature for *P. courbina* production.

This study aimed to investigate the influence of different rearing water temperatures on *P. courbina* survival, growth performance and oxidative stress responses. Influence of temperature extremes on fish performance and oxidative stress was not within the scope of the present investigation. The selected temperatures for this study were based on previous information of the best temperature for growth and survival of other Sciaenidae species (Fontaine et al., 2007; Kounna et al., 2021; Yen Ortega et al., 2021) inhabiting subtropical waters.

2. Material and methods

2.1 Experimental fish production

Juvenile *P. courbina* were produced at the Laboratory of Marine Fish Culture (LAPEM) of the Federal University of Rio Grande (Brazil). Larvae were obtained after natural spawning of the wild broodstock kept at LAPEM. Standard larviculture protocols for marine fish farming were used, which includes the use of rotifer *Brachionus plicatilis* as first food, followed by *Artemia*, before they are fully weaned into dry commercial larval diets. During this process, temperature was kept at 25.2±0.4°C (average ± standard deviation).

All the experiment procedures were approved by the Ethics Committee on Animal Use of FURG (P071/2017).

2.2 *Experimental design*

A total of 450 fish (0.58±0.19 g) were randomly distributed among nine tanks (300 L) placed in three recirculating aquaculture systems (RAS). Each RAS was equipped with skimmer, biological filter, sand filter, bag filter, aeration, and a settling tank. Water temperature was maintained either by a chiller, or a heater, equipped with temperature controllers. During the trial, fish were fed on a formulated diet (INVE O.range, 0.5-0.8mm) containing 55% crude protein and 13% crude lipid, which were offered four times a day (9, 12, 15, and 18h) until apparent satiety. The photoperiod was fixed at 12h light/12 h dark.

Fish were kept at three temperature levels (23, 26, and 29°C) in triplicates for 25 days. Temperatures were increased or reduced at 1°C/day (from 25.2±0.4°C) until the desired temperatures were achieved. Mean actual temperatures in each treatment during the experiment were equal to: 23.1±0.1, 26.2±0.3, and 29.3 ±0.2°C. Throughout the experiment water quality was monitored daily and when needed it was corrected to fit the desired levels. Apart from the pre-determined temperature treatments, the other water quality parameters measured did not differ significantly among treatments: mean dissolved oxygen saturation was higher than 88% independent of temperature, and it was measured with a handheld oxymeter (YSI 550A); pH was kept at 8.01±0.1 (pHmeter Seven2Go) and salinity at 30±1‰ (Multi-parameter Hach HQ40D). Nitrogen compounds were measured with colorimetric methods: total ammonia 0.40±0.70 mg NH_3-N.L^{-1} (UNESCO, 1983); nitrite 0.77±1.09 mg NO_2-N.L^{-1} (Bendschneider and Robinson, 1952), and nitrate 10.93±7.12 NO_3^-.L^{-1} (García-Robledo et al., 2004). Alkalinity was measured by titrimetry (Eaton et al., 2005) and it averaged 137.4±16.0 mg $CaCO_3$.L^{-1}.

2.3 Tissue sampling and homogenization

At the end of the experiment, six fish were randomly sampled from each tank (n=18 per treatment) and euthanized using a lethal concentration of benzocaine (500 mg/l) followed by cervical transection. Muscle, gill, and liver of each fish were collected, stored in microtubes, and immediately frozen in liquid nitrogen. All samples were stored in an ultra-freezer at -80°C.

Tissue samples were weighed and then homogenized with sonicator in buffer (1/5; w/v) containing Tris-HCL (100µl), EDTA (2µl), and $MgCl_2$ (5mM) proposed by Gallagher et al. (1992). Finally, they were centrifuged for 20 minutes at 10,000 x g 4°C to obtain supernatants, which were also kept at -80°C until the oxidative stress analysis.

2.4 Oxidative stress responses assay

Protein concentration was determined using a commercial kit (Doles®, Brazil) by the biuret method. Antioxidant capacity against peroxyl radicals (ACAP) was performed according to Amado et al. (2009). All samples were previously standardized to 2mg/mL of protein to perform ACAP analysis. A higher ACAP relative area means lower antioxidant capacity and vice-versa. Content of protein thiols was assayed based on the reaction with 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) with tissue sample, according to the method proposed by Sedlak and Lindsay (1968). Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) (Oakes and Van Der Kraak, 2003). All analyses were performed on 96-well microplates and read in a spectrofluorometer (Biotek®, Synergy HT).

2.5 Fish performance

Fish (n=40 per tank) were randomly collected at the end of the trial in order to evaluate the influence of temperature on growth performance, apparent feed conversion, and survival of juvenile *P. courbina*. Juveniles were anesthetized in benzocaine (50 mg/L), measured (total length, cm), and weighed (g). The following formulas were used to estimate performance parameters:

- *Weight gain (WG) = final weight - initial weight;*
- *Specific growth rate (SGR) = (ln final weight – ln initial weight) /days) x 100;*
- *Daily feed intake (DFI) = (mean dry feed consumed daily / (Initial fish biomass + final fish biomass)/2)) x 100;*
- *Total feed intake (TFI) = The total amount of feed consumed by fish.*
- *Apparent feed conversion (AFC) = total feed intake (g) / weight gain (g);*
- *Fulton's condition factor (K) = (final weight / total length^3) x 100;*
- *Survival = (final number of fish / initial number of fish) x 100.*

2.6 Statistical analysis

Mean values of each tank were utilized to analyze data, three replicas per treatment. Data normality and homoscedasticity were verified using the Shapiro-Wilk and Levene's tests, respectively. The Rank transformation was applied when data did not have a normal distribution, and/or were not homoscedastic. Then, One-Way ANOVA test was conducted, followed by Tukey's post-hoc test. The level of significance adopted was 5% (p<0.05). All results were expressed as mean ± standard deviation.

3. Results

3.1 Growth performance and survival

Fish growth and final weight were positively influenced by increasing temperature. The fastest growing fish were those reared at 29°C. Their final weight (8.94±0.56 g) was significantly higher (P<0.05) than fish maintained in any other treatment. Even tough fish reared at 23°C had the lowest SGR (7.38±0.30 % day^{-1}), their final weight was 6.3 times larger than the initial weight. Furthermore, weight gain of fish reared at 29°C was 2.7-fold higher than fish reared at 23°C. Besides the lower growth performance of fish reared at 23°C, they also showed the higher AFC. The total amount of food consumed was higher for fish kept at the highest temperature. Independent of the rearing temperature, survival was always above 98% (P<0.05) (Table 1).

Table 1 Growth performance of P. courbina (Mean ±SD) exposed to three different temperatures for 25 days.

	Temperature °C		
	23	26	29
Initial weight (g)	0.58±0.19	0.58±0.19	0.58±0.19
Final weight (g)	3.68±0.28^{c}	5.93±0.43^{b}	8.94±0.56^{a}
Weight gain (g)	3.10±0.28^{c}	5.35±0.43^{b}	8.36±0.56^{a}
SGR (% day^{-1})	7.38±0.30^{c}	9.29±0.28^{b}	10.94±0.25^{a}
TFI (g)	212.8±8.9^{c}	304.9±9.6^{b}	456.4±4.0^{a}
AFC	1.38±0.08^{b}	1.14±0.05^{a}	1.09±0.08^{a}
Survival (%)	98±2^{a}	99±3^{a}	98±2^{a}

Means in the same row with different superscript letters are significantly different (Tukey's test. $p < 0.05$). Specific growth rate (SGR), total feed intake (TFI), apparent feed conversion (AFC).

3.2 Oxidative Stress responses

The temperature did not trigger changes in muscle ACAP. However, among all tested tissue, muscle obtained the higher levels of ACAP irrespective of the temperature (Fig. 1a). The gill and liver of fish exposed to 23°C showed 1.5 and 1.7-fold higher antioxidant competence (smaller ACAP relative area) when compared to 29°C ($P<0.05$) (Fig. 1 b, c). Likewise, fish exposed to the lower temperature, showed poorer protein thiol in the liver. In this tissue, fish reared at 29°C had higher levels of P-SH than those exposed to 23°C ($P<0.05$) (Fig. 2). No statistical differences ($p>0.05$) were observed in muscle and gill P-SH. Fish exposed to 23°C had higher LPO in muscle in comparison with the other temperatures (Fig. 3). In gill and liver, TBARS levels decreased as the temperature increased, with statistical difference among all treatments ($P<0.05$).

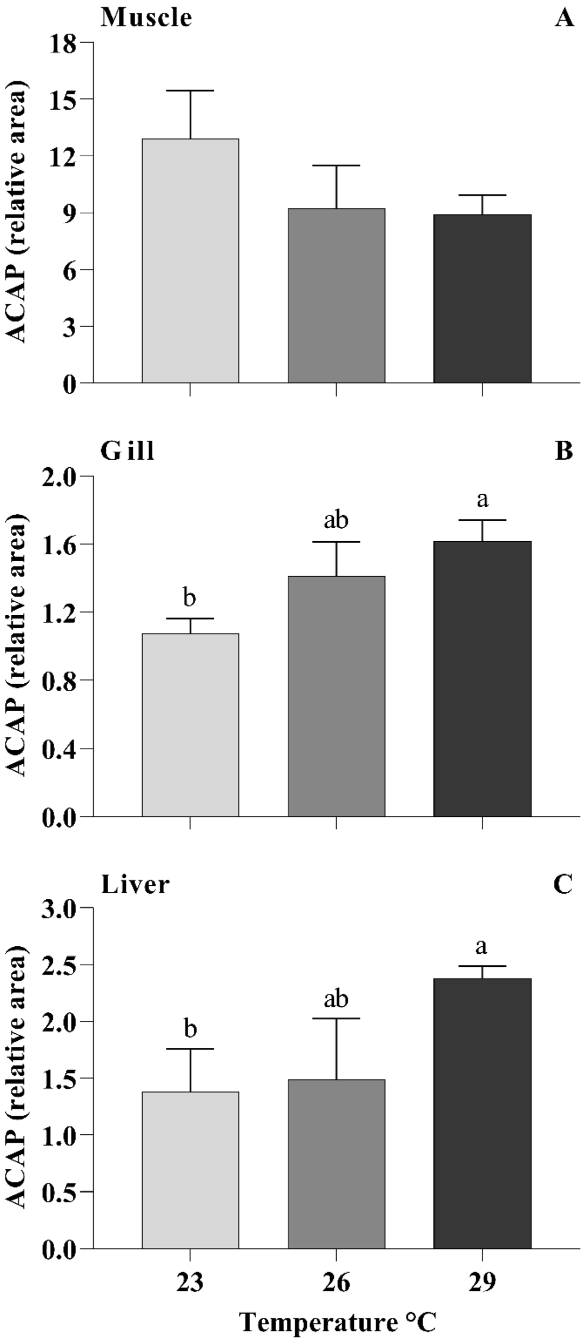

Fig 1 Antioxidant capacity against peroxyl radicals (ACAP) (Mean ± SD) in muscle (A), gill (B), and liver (C) of juvenile *Pogonias courbina* (n= 16-18) reared at different temperatures. Different letters in each column indicate significant differences among treatments (Tukey's test. $p < 0.05$).

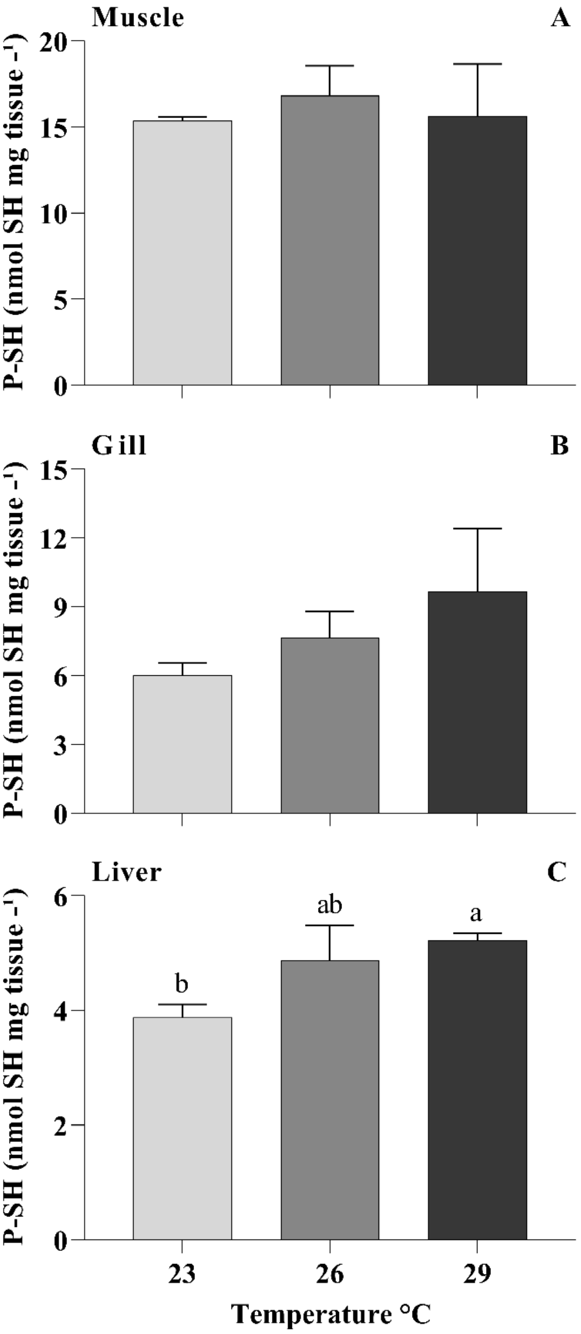

Fig 2 Content of protein thiols (P-SH) (Mean ± SD) in muscle (A), gill (B), and liver (C) of juvenile *Pogonias courbina* (n= 16-18) reared at different temperatures. Different letters in each column indicate significant differences among treatments (Tukey's test. $p < 0.05$).

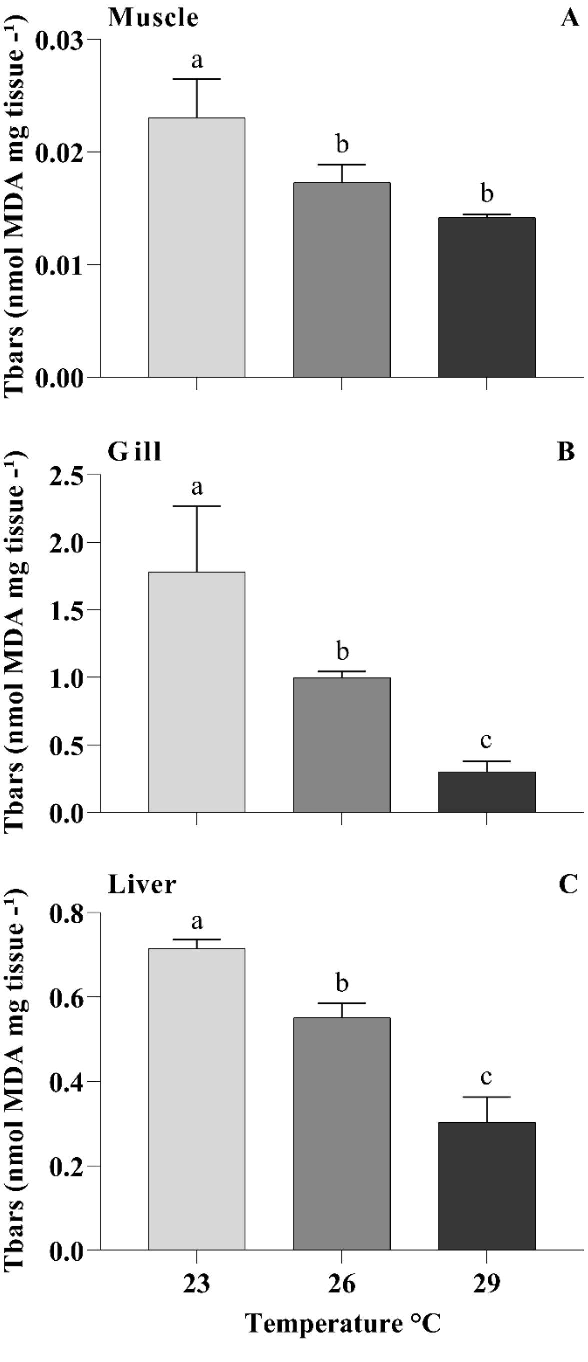

Fig. 3. Thiobarbituric acid reactive substances (TBARS) content (Mean ± SD) in muscle (A), gill (B), and liver (C) of juvenile *Pogonias courbina* (n= 14-18) reared at different temperatures. Different letters in each column indicate significant differences among treatments (Tukey's test. $p < 0.05$).

4. Discussion

The Southern black drum *P. courbina* is a new species being considered for aquaculture. However, there is scarce information regarding the influence of environmental parameters on its production in captivity. In this study, we evaluated the effects of temperature on survival, growth performance, and oxidative stress responses for juvenile reared in RAS. Our results have shown that fish reared at 29°C had higher growth rate and total feed intake, best apparent feed conversion, along with better oxidative status, demonstrated by reduced oxidative damage. On the other hand, fish reared at 23°C had lower growth rates and smaller total feed intake, feed conversion was worst. Despite the larger investment in antioxidant defenses evidenced by ACAP results, these fish had higher LPO and lower levels of protein thiols.

According to Xiao-Jun et al. (1992), the gradual temperature rise has two antagonistic effects on growth: a negative effect due to higher energy demand for metabolism maintenance, and a positive effect due to higher food consumption and digestion rate. The growth rate of *P. courbina* was considerably higher while the temperature increased throughout the tested range. The same pattern was described in other studies, such as for meagre (*Argyrosomus regius*) (Kounna et al., 2021), totoaba (*Totoaba macdonaldi*) (Yen Ortega et al., 2021), and red drum (*Sciaenops ocellatus*) (Fontaine et al., 2007). Volkoff and Rønnestad (2020) described fish thermal performance (growth, reproduction, and locomotion) as a bell chapped curve, which tends to increase while temperature is raised until it reaches an optimal temperature, followed by a decline. Our results did not demonstrate any down regulation on fish growth while temperature was increased, which suggests that temperature for best growth of *P. courbina* could be equal to or even higher than 29°C. These results are similar to those found by Fontaine et al. (2007) when studying red drum (*Sciaenops ocellatus*), where fish had better growth at 29°C if compared to 19 and 25°C.

Usually, along optimal temperature range for a given species, feed intake increases with moderate temperature raise (Volkoff and Rønnestad, 2020). This pattern was found by our results throughout the different tested temperatures, where fish reared at 23°C consumed 2.14-fold less food than those reared at 29°C, which demonstrates that the highest temperature is within the optimal temperature range for *P. courbina*. Apparent feed conversion was affected by temperature, and fish kept at higher temperatures had better assimilation of food into somatic growth. This could have happened for a couple of reasons: i) the increase in temperature could be correlated with better digestibility of the diet offered (Azevedo et al., 1998; Kofuji et al., 2005; Miegel et al., 2010; Xiao-Jun et al., 1992); ii) fish reared at 23°C were in an environmental stressful situation, which resulted in an energy-costly upregulation of antioxidant defenses to preserve organisms' homeostasis, that increases maintenance costs, while reducing food energy allocation on growth (Sokolova et al., 2012). Usually, the most suitable temperature for better feed conversion is lower than optimal temperature for growth (Handeland et al., 2008), thus corroborates that best temperature for *P. courbina* growth may be higher than 29°C, since in the present experiment, the best feed efficiency was found at the same temperature at which growth rate was higher.

Tested temperatures did not affect survival rate, since, in all treatments almost 100% of fish survived. This species is well adapted to live at different temperatures in nature (Costa Machado et al., 2020), and our results confirmed it in the laboratory, demonstrating the feasibility of producing *P. courbina* at different environmental conditions.

Exogenous environmental stressors are known to be responsible to rise ROS production. Fish protect themselves from oxidative damage by ROS with enzymatic and non-enzymatic defenses (Lushchak, 2011). The antioxidant system capacity of an organism can be modulated by environmental stressors conditions. Prooxidant scenarios are known to upper regulate antioxidant enzymes expression (Kaur et al., 2005). Malek et al. (2004), have evidenced upregulation of enzymatic antioxidant genes of fish held in 18°C compared to 28°C, induced by the increased degree of fatty acid unsaturation caused by the upper regulation of desaturase enzymes, in order to maintain cell membrane fluidity. Nitz et al. (2020), studying pacu (*Piaractus mesopotamicus*), showed a positive influence of the highest tested temperature (28°C) in liver antioxidant competence against peroxyl radical, while the opposite was observed in the gills. Our results showed higher levels of antioxidant capacity in the gill and liver of fish reared at 23°C, which could be a result of the increased propensity of cell membranes to suffer oxidative damage due to increased levels of polyunsaturated fatty acids in these membranes. Although liver and gill of fish maintained at 23°C showed higher antioxidant capacity than fish kept at 26 and 29°C, these tissues suffered more LPO, and P-SH oxidation. Which evidenced that despite the increase in antioxidant defense system, measured as ACAP, it was not enough to inhibit oxidative damages caused by ROS at the lowest temperature experienced in this trial.

The sulfhydryl (-SH) groups, linked to cysteine amino acids are sensitive to ROS oxidation. They are established redox sensors of prooxidant condition in proteins (Eaton, 2006; Nitz et al., 2020; Paulsen and Carroll, 2013). Liver of *P. courbina* reared at 23°C had lower amount of protein thiols than those maintained at 29°C. These fish also had highest LPO, which increased lipid radicals, which could have interacted with membrane-bound P-SH, resulting in their oxidation (Kaur et al., 2005). Most oxidative thiols modifications are reversible, being responsible to quickly restoration to the original cell redox state after stress conditions have ceased. But in extended oxidative stress conditions, when "overoxidation" of cysteine occurs, excessive disulfide bonding formation, protein misfolding, and degradation can lead cell to death (Brandes et al., 2009).

Lipid peroxidation is sensitive to environmental temperature. The results found showed that lower temperatures tended to increase LPO in muscle, gill, and liver of *P. courbina*. It may have happened for some reasons: i) The lower rearing temperatures may have acted as an environmental stressor, which is known to rise ROS production (Vinagre et al., 2012), capable of promoting lipid peroxidation. The probable increased level of ROS would also corroborate with the prooxidant conditions expressed by the decreased amounts of protein thiols in liver of fish kept at 23°C. ii) Furthermore, fish under cold stress tend to synthetize higher amounts of unsaturated fatty acids to maintain cell membrane fluidity (Wen et al., 2021), which are 20-40 times more prone to LPO than monounsaturated fatty acids (Crockett, 2008), which could favor peroxidation of cell membranes. Oxidation of membrane phospholipids may be deleterious because it influences membrane physical properties, and function of membrane-associated proteins, such as Na^+/K^+-ATPase activity (Crockett, 2008). Another side effect is that muscle LPO due to alterations in environmental conditions during fish production e.g., temperature and photoperiod, could favor faster development of rancid taste and reduced postmortem quality in fish filet (Nordgarden et al., 2003).

As previously mentioned, temperature has a strong influence on oxidative stress parameters. In our trial, fish kept at 23°C presented worse responses in oxidative stresses parameters among all treatments. Vinagre et al. (2012), investigating the influence of temperature on seabass (*Dicentrarchus* labrax) oxidative stress responses, concluded that temperatures closer to fish thermal optimum (24°C) resulted in lower LPO and catalase activity, while the opposite was observed in temperatures further from seabass thermal optimum. Regarding matrinxã (*Brycon amazonicus*), acute heat stress (32-34°C) inhibited antioxidant enzymes, depressed non-enzymatic antioxidant capacity, and increased ROS production and LPO (Baldissera et al., 2020). For the pufferfish (*Takifugu fasciatus*), which best temperature for growth is at 23-32°C, decrease in temperature from 25 to 13°C, resulted in higher LPO and catalase activity (Wen et al., 2021). It suggests that both, low and high temperatures could lead fish to an oxidative stress scenario, and fish responses will depend on how far the temperature is from the thermal optimum for the species. Knowing this, our oxidative stress parameters findings indicate that 29°C is more suitable for *P. courbina* production than the other tested temperatures.

5. Conclusion

The results of the present trial are important for production of *P. courbina*, since they provide information about the influence of temperature on growth performance and oxidative stress parameters for this species. These results demonstrated that juvenile *P. courbina* can survive and grow at all tested temperatures, showing the possibility to produce this species in locations with different temperature regimes. Increasing temperature from 23 to 29°C promoted increased total feed intake, better apparent feed conversion, and higher growth rate. Fish reared at the lowest temperature showed higher lipid peroxidation and protein thiols oxidation. This scenario induced upper regulation of antioxidant defenses (ACAP), which may have increased the energy budget for fish maintenance. Our research pointed out that among the tested temperatures, 29°C was the best for *P. courbina* production, since at this temperature, fish had larger growth rate, ingested more food, had better feed conversion, and lower oxidative stress.

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7. Conclusões

- Juvenis de miragaia *Pogonias courbina* podem sobreviver e crescer entre 23-29°C. Isto demonstra que a espécie é indicada para cultivo em localidades com diferentes regimes de temperatura.
- O aumento da temperatura, de 23°C para 29°C aumenta o consumo de alimento, melhora a conversão alimentar e o crescimento de juvenis de *P. courbina*.
- A menor temperatura testada (23°C) desencadeou maior peroxidação lipídica e oxidação proteica, acompanhado de maior investimento em defesas antioxidantes contra radicais peroxil.
- A melhor temperatura para a produção de *P. courbina*, dentre aqueles testada nesse trabalho, é 29°C, uma vez que nessa condição foram observadas as maiores taxas de crescimento, maior ingestão de comida, melhor eficiência alimentar e menor estresse oxidativo.