UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Departamento de Genética, Ecologia e Evolução
Programa de Pós-Graduação em Ecologia, Conservação
e Manejo da Vida Silvestre

Lucas Santos Pires Dias

CAN PREY FISH (*POECILIA VIVIPARA*) DEVELOP THE ABILITY TO RECOGNIZE INVASIVE PREDATORS THROUGH COEXISTENCE?

Belo Horizonte

2024

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Dissertação apresentada ao Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Mestre em Ecologia, Conservação e Manejo da Vida Silvestre.

Orientador: Prof. Rafael Pereira Leitão

Coorientador: Prof. Paulo Enrique Cardoso Peixoto

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PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA, CONSERVAÇÃO E MANEJO DA VIDA SILVESTRE

Ata da Defesa de Dissertação

Nº 470
Entrada: 2022/2

Lucas Santos Pires Dias

No dia 28 de novembro de 2024, às 09:00 horas, por videoconferência, teve lugar a defesa de dissertação de mestrado no Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, de autoria do(a) mestrando(a) Lucas Santos Pires Dias, orientando(a) do Professor Rafael Pereira Leitão, intitulada: **"Can prey fish (Poecilia vivipara) develop the ability to recognize invasive predators through coexistence?"**. Abrindo a sessão, o(a) Presidente da Comissão, Doutor(a) Rafael Pereira Leitão, após dar a conhecer aos presentes o teor das normas regulamentares do trabalho final, passou a palavra para o(a) candidato(a) para apresentação de seu trabalho. Estiveram presentes a Banca Examinadora composta pelos Doutores: Paula Cabral Eterovick (Leibniz-Institüt DSMZ), Taise Miranda Lopes (UNIOESTE) e demais convidados. Seguiu-se a arguição pelos examinadores, com a respectiva defesa do(a) candidato(a). Após a arguição, apenas os senhores examinadores permaneceram no recinto para avaliação e deliberação acerca do resultado final, sendo a decisão da banca pela:

(X) Aprovação da dissertação, com eventuais correções mínimas e entrega de versão final pelo orientador diretamente à Secretaria do Programa, no prazo máximo de 30 dias;

( ) Reprovação da dissertação (marcar se é a primeira ou segunda reprovação): ( ) *primeira reprovação ( ) segunda reprovação

*Conforme o disposto no Artigo 80 da Resolução Complementar do CEPE/UFMG Nº 02/2017, de 04 de julho de 2017, caso seja a primeira reprovação, poderá ser concedido, a critério do Colegiado de Curso, um prazo para a realização de nova defesa de tese.

Nada mais havendo a tratar, o Presidente da Comissão encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora.

Belo Horizonte, 28 de novembro de 2024.

Assinaturas dos Membros da Banca Examinadora

Documento assinado eletronicamente por **Paula Cabral Eterovick**, **Usuário Externo**, em 20/01/2025, às 14:05, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

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UNIVERSIDADE FEDERAL DE MINAS GERAIS

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

COLEGIADO DO CURSO DE PÓS-GRADUAÇÃO EM ECOLOGIA, CONSERVAÇÃO E MANEJO DA VIDA SILVESTRE

FOLHA DE APROVAÇÃO

"Can prey fish (*Poecilia vivipara*) develop the ability to recognize invasive predators through coexistence?"

LUCAS SANTOS PIRES DIAS

Dissertação de Mestrado defendida e aprovada, no dia **28 de novembro de 2024**, pela Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre da Universidade Federal de Minas Gerais constituída pelos seguintes professores:

Doutor(a) Paula Cabral Eterovick

(LEIBNIZ-INSTITÜT DSMZ)

Doutor(a) Taise Miranda Lopes

(UNIOESTE)

Doutor(a) Rafael Pereira Leitão

(Presidente da Banca)

Belo Horizonte, 28 de novembro de 2024.

Assinatura dos Membros da Banca

Documento assinado eletronicamente por **Paula Cabral Eterovick**, **Usuário Externo**, em 20/01/2025, às 14:05, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

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Aos meus pais, Luiz e Vania, fonte de inspiração e apoio incondicional.

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RESUMO

Através de repetidas interações ao longo do tempo, predadores e presas podem desenvolver características que lhes permitem lidar de forma mais eficaz uns com os outros, seja por meio de aprendizado ou mecanismos evolutivos. Por outro lado, presas podem sofrer altos níveis de predação ao interagir com novos predadores, devido à incapacidade de reconhecê-los e de responder a eles, em um processo conhecido como ingenuidade de presas. Esse cenário pode resultar da introdução de predadores invasores, que podem impactar severamente as populações de presas nativas. Embora a ingenuidade possa ser devastadora para as presas, a coexistência com novos predadores pode levar à aquisição da capacidade de reconhecê-los e de reagir a eles. Para testar essa hipótese, realizamos um experimento em aquário no qual expusemos grupos do peixe nativo *Poecilia vivipara* a pistas visuais de um predador invasor (*Cichla kelberi*) e de um predador nativo (*Hoplias malabaricus*), e mensuramos as respostas antipredatórias de *P. vivipara*. Os indivíduos de *P. vivipara* utilizados no experimento pertenciam a duas populações, sendo uma delas originária da Bacia do Rio Doce e a outra da Bacia do Rio Paraopeba. Ambas as populações coexistem com *H. malabaricus*, enquanto apenas a população da Bacia do Rio Doce coexiste com *C. kelberi*. Esperávamos que ambas as populações de *P. vivipara* respondessem a *H. malabaricus*, e que apenas a população em coexistência com *C. kelberi* respondesse a esse predador invasor. Embora as populações tenham diferido em suas respostas antipredatórias, essas diferenças não se limitaram às pistas visuais do predador invasor. A população em coexistência com *C. kelberi* respondeu a todas as pistas experimentais, incluindo o tratamento controle, sugerindo um medo generalizado de estímulos desconhecidos (neofobia). A neofobia pode ajudar as presas a responder de forma mais eficaz a novos predadores, podendo ser parcialmente responsável pela persistência dessa população após a introdução de predadores invasores. A população que não coexiste com *C. kelberi*, por outro lado, não respondeu a nenhuma pista experimental, o que pode estar relacionado à menor pressão de predação à qual está sujeita, em comparação com a população coexistente, e pode sugerir uma maior vulnerabilidade a predadores não nativos.

Palavras-chave: Ingenuidade de presas; Invasões biológicas; Interações predador-presa; Comportamento antipredatório; Neofobia.

ABSTRACT

Through repeated interactions over time, predators and prey can develop traits that allow them to deal more effectively with each other, either through learning or evolutionary mechanisms. On the other hand, prey can suffer high predation levels when interacting with novel predators, due to the inability to recognize and respond to them, in a process known as prey naiveté. This scenario can result from the introduction of invasive predator species, which can severely impact native prey populations. While naiveté can be devastating for prey, coexistence with novel predators can lead to the acquired ability to recognize and react to them. To test this hypothesis, we conducted an aquarium experiment in which we exposed groups of the native fish *Poecilia vivipara* to visual cues from both an invasive (*Cichla kelberi*) and a native predator (*Hoplias malabaricus*), while measuring the antipredator responses of *P. vivipara*. The tested individuals of *P. vivipara* originated from two populations, one being from the Doce River Basin and the other from the Paraopeba River Basin. Both populations coexist with *H. malabaricus*, while only the Doce River Basin population coexists with *C. kelberi*. We expected both populations of *P. vivipara* to respond to *H. malabaricus* and only the population in coexistence with *C. kelberi* to respond to this invasive predator. While populations differed in their antipredator responses, these differences were not limited to the invasive predator cues. The coexisting population responded to all experimental cues, including the control treatment, suggesting a generalized fear of unfamiliar stimuli (i.e., neophobia). Neophobia can help prey respond more effectively to novel predators and could be partially responsible for this population’s persistence after the introduction of invasive predators. The non-coexisting population, on the other hand, did not respond to any experimental cues, which could be related to the lower predation pressure it is subject to in comparison with the coexisting population, and could suggest a greater vulnerability to non-native predators.

Keywords: Prey naiveté; Biological invasions; Predator-prey interactions; Antipredator behavior; Neophobia.

LISTA DE ILUSTRAÇÕES

SUMÁRIO

INTRODUÇÃO GERAL

A habilidade de reconhecer predadores é essencial para a sobrevivência das presas, dado que o reconhecimento e interpretação de pistas predatórias são fundamentais para a exibição de respostas antipredatórias eficazes (Cox & Lima, 2006; Banks & Dickman, 2007). Quando as presas são incapazes de reconhecer e de reagir a predadores, elas se tornam altamente vulneráveis diante deles, em um processo conhecido como ingenuidade de presas (Schlaepfer *et al*., 2005; Strauss *et al*., 2006; Verhoeven *et al*., 2009; Carthey & Banks, 2014). A ingenuidade de presas está frequentemente associada à introdução de espécies predadoras em áreas fora de sua distribuição original (Cox & Lima, 2006; Carthey & Banks, 2014). As presas nativas, por carecerem de história evolutiva compartilhada e de experiência com os predadores não nativos, podem ser incapazes de reconhecê-los e de reagir a eles, especialmente se tais predadores representarem uma novidade ecológica significativa no sistema em que forem introduzidos (Diamond & Case, 1986; Cox & Lima, 2006; Sih *et al*., 2010; Carthey & Banks, 2014).

Por outro lado, caso as presas sejam capazes de resistir aos efeitos iniciais das invasões biológicas, as intensas pressões seletivas impostas pela convivência com os predadores invasores podem levar a rápidas mudanças comportamentais, seja por meio de aprendizado ou de mecanismos evolutivos (Schlaepfer *et al*., 2005; Banks & Dickman, 2007; Nunes *et al*., 2014; Melotto *et al*., 2020). Diante desse contexto, testamos, nesse trabalho, a hipótese de que presas nativas podem adquirir a capacidade de reconhecer e de responder a predadores invasores através da coexistência com eles. Para testar essa hipótese, conduzimos um experimento no qual mensuramos as respostas antipredatórias de duas populações do peixe nativo guaru (*Poecilia vivipara*) diante de três tratamentos experimentais: pistas visuais do predador nativo traíra (*Hoplias malabaricus*) (Fig. I1A), pistas visuais do predador invasor tucunaré (*Cichla kelberi*) (Fig. I1B), e ausência de pistas predatórias (controle) (Fig. I1C).

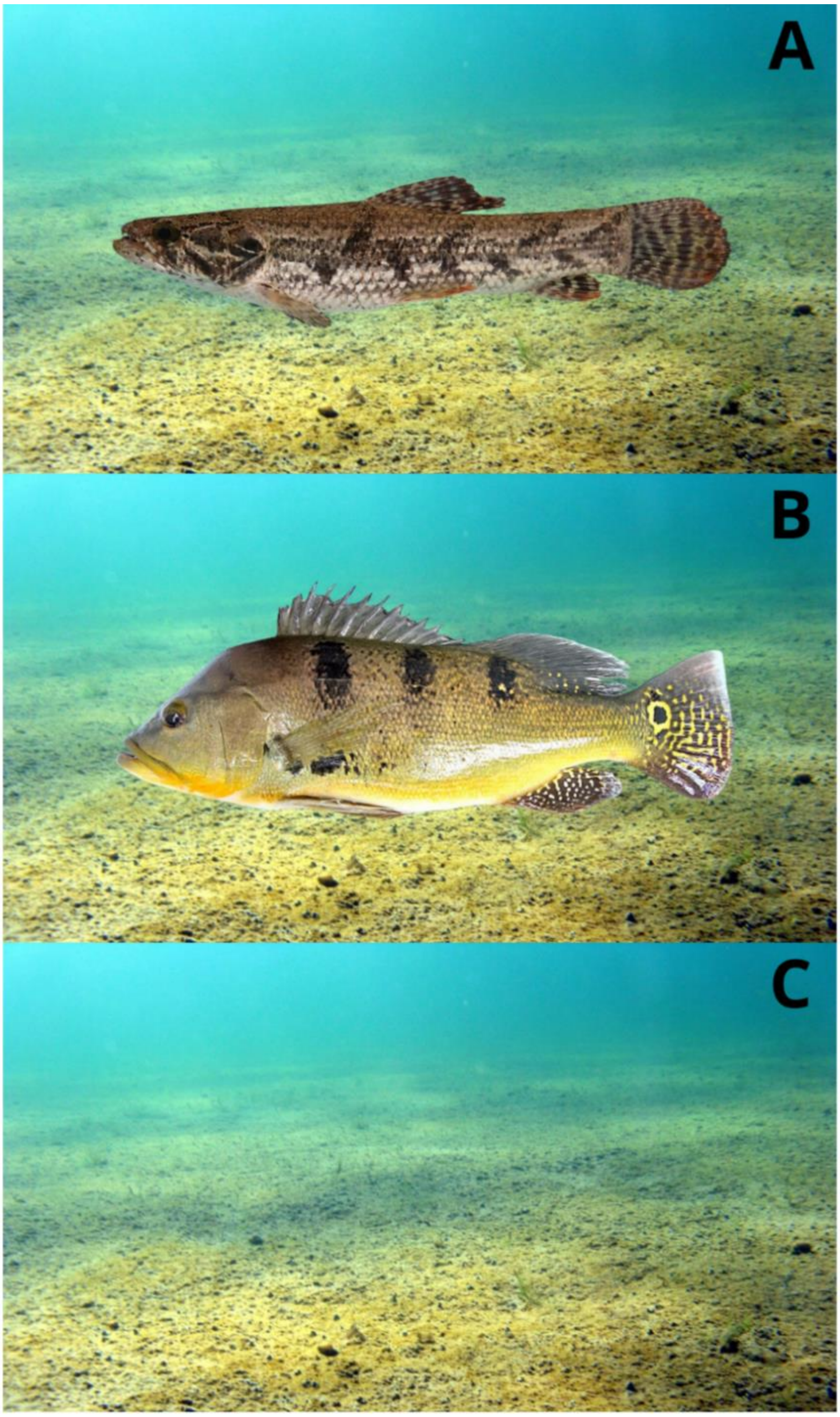

Figura I1: Exemplos de imagens utilizadas no experimento de respostas antipredatórias. As imagens são compostas por fotografias de predadores (predador nativo, *Hoplias malabaricus* - A; predador invasor, *Cichla kelberi* - B), sobrepostas a um cenário subaquático, ou esse mesmo cenário sem imagens de predadores (C). Para os tratamentos de predador nativo e predador invasor, utilizamos fotografias de diferentes indivíduos por rodada experimental dentro de cada população. Fotos: Indivíduos de *Hoplias malabaricus* e *Cichla kelberi* – Gilberto Nepomuceno Salvador; Cenário subaquático – Travis, via Flickr. Disponível em: https://www.onlyinyourstate.com/nature/alaska/underwater-phenomenon-ak. Acesso em: 27 set. 2024.

O guaru (*Poecilia vivipara*) (Fig. I2) é uma espécie de peixe de pequeno porte amplamente distribuída na região Neotropical, ocorrendo em sistemas dulcícolas e estuarinos (Parenti & Rauchemberger, 1989; Gomes-Jr & Monteiro, 2007). Essa espécie constitui um modelo de estudo adequado para testar nossa hipótese, já que pode ser encontrada em ambientes altamente heterogêneos em relação à pressão de predação, tanto na presença quanto na ausência de predadores invasores (Alves & Vono, 1998; Neves & Monteiro, 2003; Gomes-Jr & Monteiro, 2007; Gomes-Jr & Monteiro, 2008; Vieira, 2009; Oporto, 2013).

Figura I2: O guaru (*Poecilia vivipara*) é uma espécie de peixe de pequeno porte amplamente distribuída na região Neotropical. Esta espécie ocorre em habitats altamente heterogêneos em relação à pressão de predação, sendo encontrada tanto na presença quanto na ausência de predadores invasores. Foto: Gilberto Nepomuceno Salvador (adaptada).

Nesse estudo, coletamos indivíduos de *P. vivipara* na Lagoa Dom Helvécio (Fig. I3), no Parque Estadual do Rio Doce (PERD) e em uma das lagoas da Universidade Federal de Viçosa - Campus Florestal (UFV - CAF) (Fig. I4), ambas em Minas Gerais, Brasil. Ambas as populações de guaru testadas apresentam um histórico de coexistência com a traíra, um predador nativo, enquanto apenas a população do PERD apresenta um histórico de coexistência com o tucunaré, desde a introdução desse predador invasor no sistema de lagos do Médio Rio Doce em meados dos anos 1980 (Sunaga & Verani, 1991; Godinho & Formagio, 1992; Pompeu & Godinho, 2001). A introdução do tucunaré teve efeitos devastadores nesse sistema, contribuindo para uma drástica redução na riqueza taxonômica e funcional da ictiofauna local (Fragoso-Moura *et al*., 2016; Godinho & Formagio, 1992; Latini, 2001; Latini & Petrere, 2004; Oporto, 2013; Souza *et al*., 2021; Sunaga & Verani, 1991).

Figura I3: Lagoa Dom Helvécio, no Parque Estadual do Rio Doce, Minas Gerais. Essa lagoa está inserida na Bacia do Rio Doce, onde *Poecilia vivipara* ocorre naturalmente. O predador nativo traíra (*Hoplias malabaricus*) está presente nesse ambiente, assim como o predador invasor tucunaré (*Cichla kelberi*), que foi introduzido há cerca de quatro décadas e teve efeitos devastadores sobre a ictiofauna local. Foto: Valuare.engenharia. Disponível em: https://commons.wikimedia.org/wiki/File:Lagoa_Dom_Helv%C3%A9cio-Lagoa_do_Bispo_4.jpg. Acesso em: 27 set. 2024.

Figura I4: Lagoa da Universidade Federal de Viçosa – Campus Florestal, Minas Gerais. Essa lagoa está inserida na Bacia do Rio Paraopeba, onde *Poecilia vivipara* ocorre naturalmente e coexiste com o predador nativo traíra (*Hoplias malabaricus*). Foto: UFV/CAF. (adaptada). Disponível em: https://spr.caf.ufv.br/wp-content/uploads/2017/02/2-Fevereiro.jpg. Acesso em: 27 set. 2024.

Durante o experimento, expusemos grupos de *P. vivipara* de ambas as populações aos estímulos experimentais (pistas visuais de traíra, tucunaré ou controle) e mensuramos duas respostas antipredatórias comportamentais antes e depois da apresentação dos estímulos: distanciamento (Fig. I5) e agregação (Fig. I6). Ambas as respostas representam comportamentos comumente exibidos por peixes diante de predadores e são frequentemente utilizados como métricas antipredatórias em estudos comportamentais (Welty, 1934; Brock & Riffenburgh, 1960; Cerri, 1983; Magurran *et al*., 1992; Kelley & Magurran, 2003; Botham *et al*., 2008). Nós esperávamos que ambas as populações respondessem às imagens do predador nativo (traíra) e não apresentassem respostas ao tratamento controle (ausência de imagens de predadores). Além disso, esperávamos que apenas a população em coexistência com o predador invasor (tucunaré) respondesse às suas imagens.

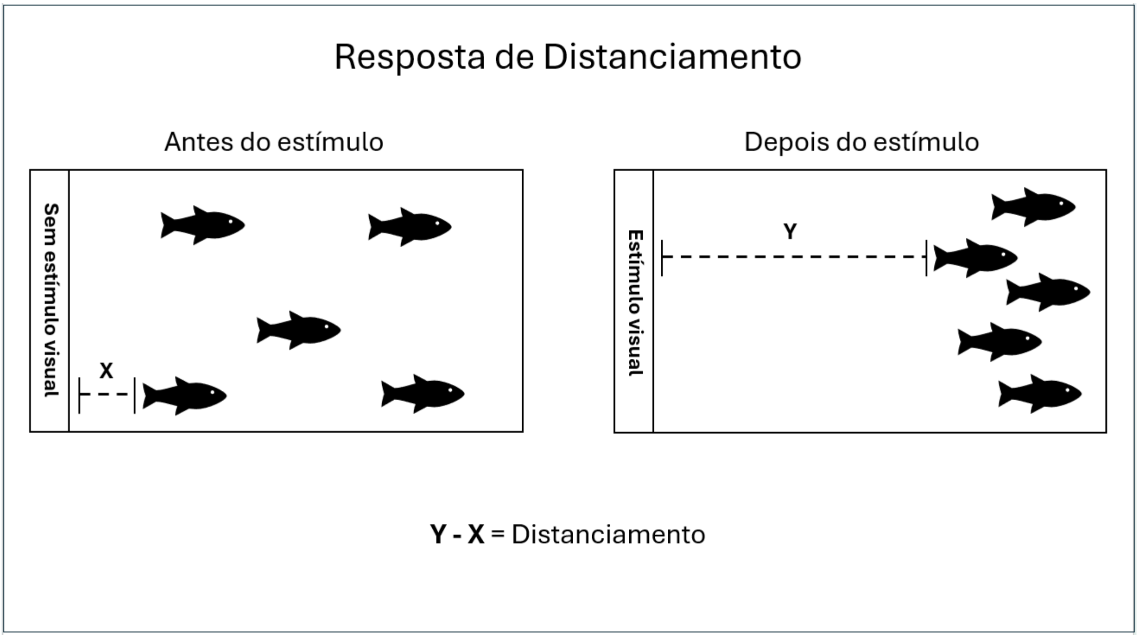

Figura I5: A resposta de distanciamento foi definida como a diferença entre a distância mínima do estímulo (distância entre o lado do aquário onde o estímulo visual foi apresentado e o indivíduo de *Poecilia vivipara* mais próximo a ele) antes e depois da apresentação do estímulo visual (depois - antes). Essa métrica nos informou se os peixes tenderam a se distanciar do estímulo ou não, e, em caso afirmativo, qual foi a intensidade desse comportamento.

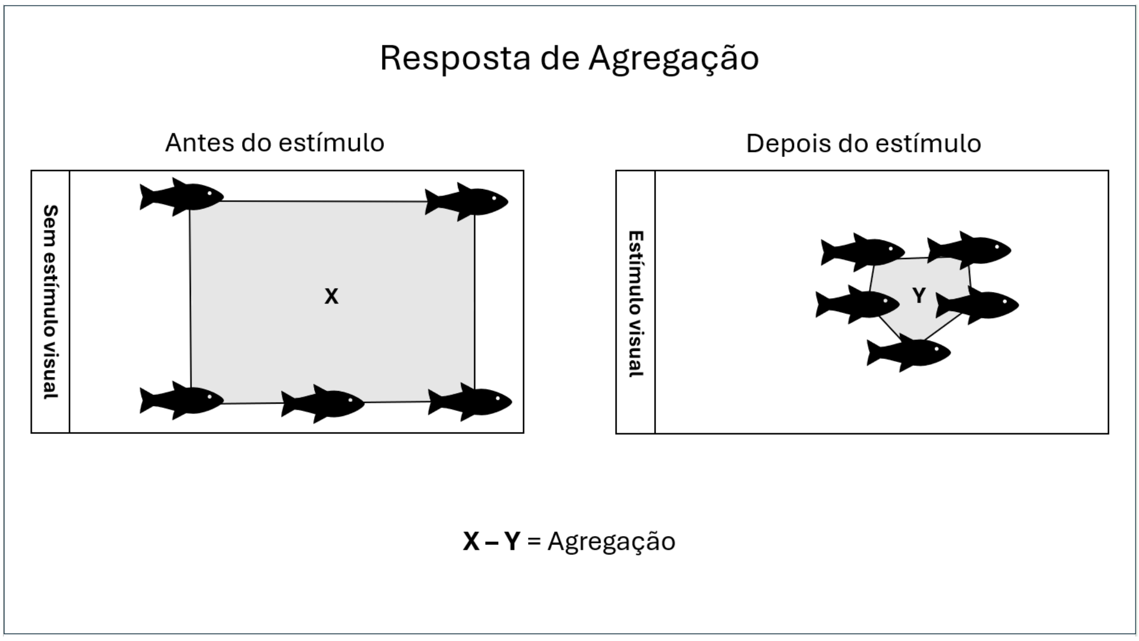

Figura I6: A resposta de agregação foi calculada como a diferença entre a área de agregação do grupo de *Poecilia vivipara* (área do menor polígono convexo formado pelos indivíduos do grupo) antes e depois da apresentação do estímulo visual (antes - depois). Essa métrica nos informou se os peixes tenderam a se aproximar uns dos outros após a exposição ao estímulo e, em caso afirmativo, qual foi a intensidade desse comportamento.

Através deste estudo, buscamos contribuir para uma melhor compreensão de como atributos comportamentais das presas podem influenciar a sua resiliência a predadores invasores e afetar o desfecho das invasões biológicas. Diante do presente cenário, em que novas interações ecológicas tendem a se tornar cada vez mais prevalentes no Antropoceno (Wood & Shepard, 2022), tal compreensão é crucial para um manejo mais eficaz de populações de presas nativas e para a mitigação dos impactos causados pelas invasões biológicas. A presente dissertação é composta por um capítulo e foi elaborada no formato de artigo científico, no idioma inglês.

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Can prey fish (*Poecilia vivipara*) develop the ability to recognize invasive predators through coexistence?

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CAPÍTULO 1

1. INTRODUCTION

Predator-prey interactions play a critical role in the fitness of organisms, especially for prey, as failure to avoid predation often leads to death (Dawkins & Krebs, 1979; Lima & Dill, 1990). Therefore, traits related to the ability to recognize and avoid predators are expected to be developed under strong evolutionary and ecological pressures (Edmunds, 1974; Langerhans, 2007). While defense against predation is of utmost importance for prey species, defensive mechanisms come at a cost, and there are often trade-offs between them and other key aspects of an organism's fitness, such as feeding and reproduction (Sih, 1980; Milinski, 1986; Abrams, 1986; Gilliam & Fraser, 1987; Lima & Dill, 1990; Brown *et al*., 1999). Because of this, the evolutionary value of antipredator adaptations is context-dependent, and mediated by the predation pressure exerted over prey (Vermeij, 1994; Kavaliers & Choleris, 2001; Schmitz, 2017). In other words, a species' suite of antipredator traits is influenced by the predatory landscape in which it evolved, equipping prey to interact with the predators they regularly encounter in their environments (Williams & Nichols, 1984; Helfman, 1989; Helfman & Winkelman, 1997; Abrams, 2000). Given that predators vary greatly in morphology and behavior, there is far from a one-size-fits-all antipredator adaptation. Because of this, what could be an effective trait to deal with a given predator, might prove to be virtually useless against another (Kotler *et al*., 1992; Sih *et al*., 1998; Macdonald & Harrington, 2003; Carthey & Banks, 2014).

Through repeated interactions over time, predators and prey can develop traits that allow them to deal more effectively with each other (Curio, 1993; Grubb, 2006). On the other hand, during novel predator-prey interactions, such as the ones produced by biological invasions, the species involved might lack the needed traits to deal with each other's pressures, resulting in ecological mismatches (Schlaepfer *et al*., 2005; Strauss *et al*., 2006; Verhoeven *et al*., 2009; Carthey & Banks, 2014). If non-native predators represent a significant ecological novelty in the system into which they are introduced, native prey may be unable to defend themselves against them, or even recognize them as a threat, in a process known as prey naiveté (Diamond & Case, 1986; Cox & Lima, 2006;

Sih *et al*. 2010; Carthey & Banks, 2014). Failing to recognize novel predators constitutes the most impactful form of naiveté, as the ability to detect and interpret predatory cues is essential for performing effective antipredator behaviors (Cox & Lima, 2006; Banks & Dickman, 2007). On the other hand, selective pressures imposed by novel predators can drive rapid behavioral changes, either through evolution or learning (Schlaepfer *et al*., 2005; Banks & Dickman, 2007; Nunes *et al*., 2014; Melotto *et al*., 2020). Therefore, if native prey populations can resist the initial effects of biological invasions, the coexistence with invasive predators could lead to the acquired ability to recognize and react to them (Maloney & McLean, 1995; Kiesecker & Blaustein, 1997; Anson & Dickman, 2013; Polo-Cavia & Gomez-Mestre, 2014; Steindler *et al*., 2020).

In one particularly impactful example of invasive predators in freshwater systems, the introduction of the piscivorous fish peacock bass (*Cichla kelberi*) in the Middle Rio Doce Lake System, Minas Gerais, Brazil, contributed to a drastic reduction of the taxonomic and functional diversity of the local ichthyofauna (Fragoso-Moura *et al*., 2016; Godinho & Formagio, 1992; Latini, 2001; Latini & Petrere, 2004; Oporto, 2013; Souza *et al*., 2021; Sunaga & Verani, 1991). There are marked morphological and behavioral differences between the peacock bass and the primary native piscivorous fish in the area, the wolf fish (*Hoplias malabaricus*) (Gilliam & Fraser, 1987; Montenegro *et al*., 2013), indicating *C. kelberi* might have represented a significant ecological novelty in this system upon its arrival. While many of the native species were locally extinct after the introduction of *C. kelberi*, some managed to persist in the Middle Rio Doce Lake System (Sunaga and Verani, 1991; Gomes *et al*., 2007; Fragoso-Moura *et al*., 2016). One example of this is the guaru (*Poecilia vivipara*), a native fish that has been coexisting with *C. kelberi* for decades (~40 years) (Fragoso-Moura *et al*., 2016; Gomes *et al*., 2007; Lopes Bueno *et al*., 2016; Oporto, 2013). The period during which *P. vivipara* has coexisted and interacted with *C. kelberi* could have led to the development of the ability to recognize and react to this invasive predator.

In this study, we tested the hypothesis that prey can develop the ability to recognize and respond to invasive predators through coexistence. To test this hypothesis, we conducted an experiment to measure the antipredator behavioral responses of individuals belonging to two populations of the native fish *P. vivipara*: one that has coexisted with *C. kelberi* for approximately 40 years and another that has never coexisted with this invasive predator.

2. METHODS

2.1 Model species and study populations

Our model prey species, the guaru (*P. vivipara*), is a small fish species belonging to the Poeciliidae family (Fig. 1). *Poecilia vivipara* is widely distributed in freshwater and estuarine systems across the Neotropical region (Parenti & Rauchemberger, 1989; Gomes-Jr & Monteiro, 2007) and inhabits highly heterogeneous habitats regarding predation pressure (Alves & Vono, 1998; Neves & Monteiro, 2003; Gomes-Jr & Monteiro, 2007, 2008; Vieira, 2009; Oporto, 2013). Given that the geographic range of *P. vivipara* includes both invaded and uninvaded ecosystems, this species constitutes a suitable model to investigate the effect of coexistence on prey's ability to recognize and react to invasive predators.

We conducted an experiment using individuals from two populations of *P. vivipara* that differ in whether they have coexisted with the invasive predator *C. kelberi*. The coexisting population came from the Dom Helvécio Lake, which is part of the Middle Rio Doce Lake System, in the Rio Doce State Park (PERD), Minas Gerais, Brazil. The presence of the invasive predator *C. kelberi* in the Dom Helvécio Lake has been reported since the 1980s (Sunaga & Verani, 1991; Godinho & Formagio, 1992; Pompeu & Godinho 2001). The non-coexisting *P. vivipara* population came from a lake on the Florestal Campus of the Federal University of Viçosa (UFV), Minas Gerais, Brazil. The Florestal lake is part of the Paraopeba River Basin, where *P. vivipara* naturally occurs (Alves & Vono, 1998). The primary native predator in both lakes, *H. malabaricus*, is known to use *P. vivipara* as one of its main prey items (Mazzoni & Iglesias-Rios, 2002). *Cichla kelberi* is also a potential predator to *P. vivipara* since it preys on a wide variety of small fish (Gomiero *et al*., 2010; dos Santos *et al*., 2011). Additionally, *Cichla* species have been recorded preying on members of the Poeciliidae family, even leading to local extirpation of native poeciliid species (Zaret & Paine, 1973; Wainwright *et al*., 2001).

Figure 1: The guaru (*Poecilia vivipara*) is a small fish species belonging to the Poeciliidae family. This species is widely distributed in freshwater and estuarine systems across the Neotropical region and inhabits highly heterogeneous habitats regarding predation pressure. Photo: Gilberto Nepomuceno Salvador (adapted).

2.2 Fish collection and housing

We used semicircular sieves (80 cm in diameter, 0.5 mm mesh) and seines (8 m long x 1.5 m high, 0.5 cm mesh) in the littoral zones of the Dom Helvécio and Florestal lakes to capture *P. vivipara* individuals. We transported the fish in plastic bags containing water from their respective collection sites and housed them at the Fish Ecology Laboratory Bioterium, in the Federal University of Minas Gerais (UFMG), in tanks equipped with external filters and thermostats. We housed fish in three 72 L (~25 individuals), two 54 L (~20 individuals), and four 10 L (~5 individuals) tanks, and fed them *ad libitum* with commercial flake food. We housed *P. vivipara* individuals from different populations in separate tanks, and they did not come in contact with each other. Since the two lakes differ in water temperature (Maia-Barbosa *et al*., 2003; Souza *et al*., 2008; Rocha *et al*., 2015) we kept individuals from different populations at temperatures according to their respective lake of origin (PERD: ~28ºC, Florestal: ~22ºC), and in a pH of approximately 7.0, which is under the range observed in both lakes (Rocha *et al*., 2015; Souza *et al*., 2008). We kept fish from PERD and Florestal populations in quarantine for 35 and 22 days, respectively, before the start of the experiment. We isolated animals showing signs of illness in hospital tanks and treated them with commercial medication. After treatment, fish that recovered from illness were used in the experiment. Fish collection, transportation and experimental procedures were authorized by the Instituto Estadual de Florestas – IEF (061/2023), Instituto Chico Mendes de Conservação da Biodiversidade - ICMBIO/SISBIO (58000-1) and the Ethics Committee of the Federal University of Minas Gerais – CEUA/UFMG (307/2022; 339/2022).

2.3 Antipredator responses experiment

For the antipredator responses experiment, we separated fish into groups of five individuals, since we observed *P. vivipara* in the PERD and Florestal lakes congregating in shoals. Poeciliid fish typically live in social groups, which, among other things, help reduce the risks of predation (Earley & Dugatkin, 2005). In accordance with the biased sex ratio in favor of females commonly found in natural populations of *P. vivipara* (e.g.: ~1:4 males to females ratio) (Nascimento & Gurgel, 2008), we established groups of four females and one male. We formed seven groups of *P. vivipara* from the PERD population (n=35 individuals) and eight groups from the Florestal population (n=40 individuals), with individuals from each population randomly distributed among groups.

We conducted the experiment in a glass tank (60 cm × 28 cm × 25 cm, ~42 L), with an internal glass wall delimiting a small (15 cm × 28 cm × 25 cm) and a large compartment (45 cm × 28 cm × 25 cm). The large compartment of the tank contained a group of *P. vivipara*, while the smaller compartment contained a video monitor, which was used to display experimental visual cues. The experimental treatments consisted of photographs of either *Cichla kelberi* (invasive predator) or *Hoplias malabaricus* (native predator) individuals superimposed on an underwater lake scene representative of the *P. vivipara* collection sites, or this same underwater scene without any predator images (control). The ability to recognize predators through visual cues has been shown in several aquatic taxa, including poeciliids (Coates, 1980; Kelley & Magurran, 2003; Culumber, 2015; Sommer-Trembo *et al*., 2016; Fischer *et al*., 2017; Landeira-Dabarca *et al*., 2019; Melo *et al*., 2021). Additionally, several studies have shown that fish can discern predators and even conspecifics through static two-dimensional images, such as the photographs we used in this study (Katzir, 1981; Karplus *et al*., 1982; Bando, 1991; Kohda *et al*., 2015; Hotta *et al*., 2019; Sogawa *et al*., 2023). Since prey fish can assess the level of threat based on individual predator cues, such as size (Helfman, 1989; Helfman & Winkelman, 1997; Smith & Belk, 2001), we used photographs of different predator individuals for each experimental trial, and matched all the predator images to a fixed total length (165 mm). The same set of predator photographs was used for both populations. *Cichla kelberi* and *Hoplias malabaricus* individuals with this size would be capable of preying on the *P. vivipara* individuals used in this experiment, based on their standard-length (PERD: ($\bar{x}$) = 3,3 cm ± 0,9 cm; Florestal: ($\bar{x}$) = 2,1 cm ± 0,5 cm) (Paiva, 1974; Winemiller, 1989; de Almeida *et al*., 1997; Hill *et al*., 2004). We exposed *Poecillia vivipara* groups once to each one of the three experimental treatments in varying orders to prevent a habituation effect from influencing the results. We waited at least 24 h before exposing the same group to a different experimental trial. We kept *P. vivipara* groups isolated from other fish until they went through all experimental trials. Additionally, we kept the fish that went through trials separated from those that had not gone through them yet. We performed a full water change in the experimental tank before every trial, to avoid the influence of potential chemical cues originating from the previously tested animals.

We filmed experimental trials with a "Logitech C920s PRO HD" webcam, positioned on a styrofoam structure at a height of 45 cm above the tank, parallel to the water surface. Both the tank and the rest of the structure were covered in black fabric to isolate *P. vivipara* individuals from external visual stimuli. The experimental setup also included an LED light fixture to illuminate the tank during trials. The water temperature and pH of the experimental tank during trials were adjusted according to the parameters of the maintenance tanks. In each trial, acclimatization of the *P. vivipara* groups lasted for 20 min, followed by 10 min in which their actions were filmed. During the first 5 min of filming, the video monitor remained turned off. After this period, it was turned on to display an image corresponding to one of the experimental treatments. *Poecilia vivipara* individuals were free to move inside the larger compartment of the tank and had visual access to the cues displayed on the video monitor.

We took two screenshots of each trial footage, 5 s before and 5 s after the presentation of the visual cues to measure two primary behavioral metrics with the ImageJ software: the aggregation area of *P. vivipara* groups (area of the smallest convex polygon formed by the individuals in the group) and the minimum distance from the stimulus (distance between the side of the tank where visual cues were presented and the closest *P. vivipara* individual to it). To obtain our antipredator responses, we calculated the difference between the primary behavioral metrics before and after the presentation of visual cues in each experimental trial. The selection of screenshots from 5 s before and 5 s after the presentation of visual cues was done to allow sufficient time to observe antipredator responses while minimizing the risk of fish habituating to the stimulus. Prey fish are expected to respond rapidly after detecting visual predatory cues, as the visualization of a predator requires that it is in close proximity to prey, representing an imminent risk, especially in aquatic environments (Arteaga-Torres *et al*., 2020; Brönmark & Hansson, 2000; Brown *et al*., 2000; Hall & Clark, 2016).

2.4 Antipredator responses

Avoidance response

The avoidance response was defined as the difference between the minimum distance from the stimulus before and after the presentation of visual cues (after - before). This metric informed us as to whether fish tended to distance themselves from the cues or not, and if so, what was the intensity of this behavior (Fig. 2). Positive values for the avoidance response indicate *P. vivipara* individuals tended to distance themselves from the visual cues presented, which is a well-known antipredator behavior performed by fish when facing predation threats (Welty, 1934; Brock & Riffenburgh, 1960; Neill & Cullen, 1974; Pitcher & Wyche, 1983; Magurran *et al*., 1985; Morgan & Godin, 1985; Pitcher, 1986). Keeping a safe distance from predators can give prey fish an advantage over them in terms of swimming acceleration and relative maneuverability (Weihs & Webb, 1983). The behavior of distancing from predators has been extensively observed and used as a metric in antipredator studies concerning the Poeciliidae family (Seghers, 1973; Magurran & Seghers, 1990a; Magurran *et al*., 1992, 1995; Dugatkin & Godin, 1992; O'Steen *et al*., 2002; Kelley & Magurran, 2003).

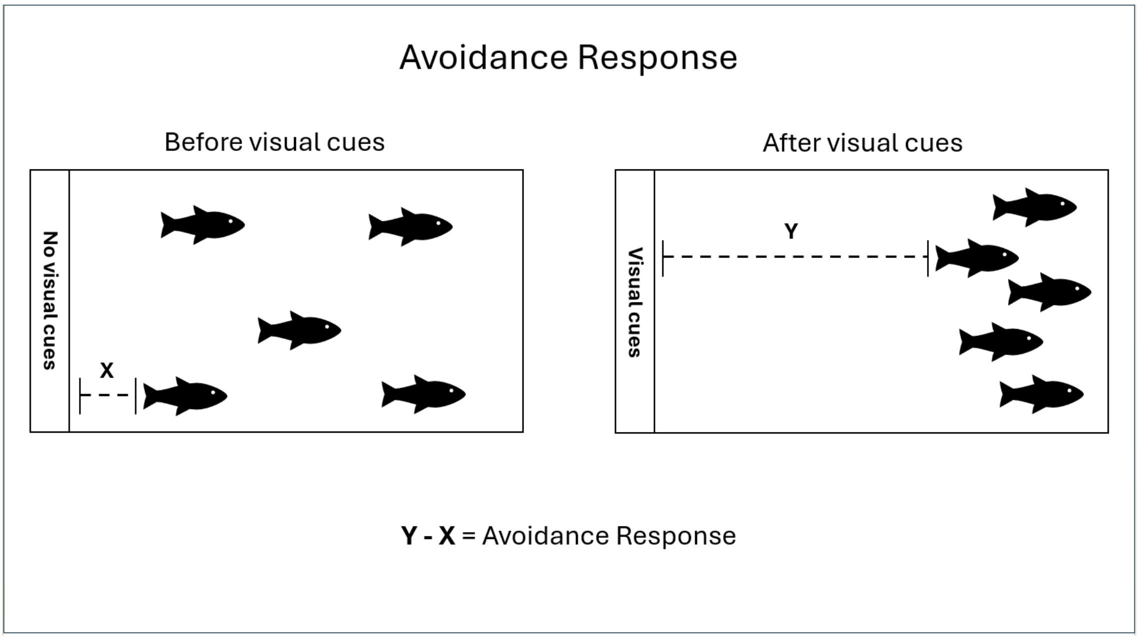

Figure 2: The avoidance response for *Poecilia vivipara* groups was calculated as the difference between the minimum distance from the stimulus (distance between the side of the tank where visual cues were presented and the closest *Poecilia vivipara* individual to it) five seconds before and after the presentation of the visual cues (after - before). Positive values for the avoidance response indicate *Poecilia vivipara* individuals tended to distance themselves from the cues presented.

Shoaling response

The shoaling response was calculated as the difference between the aggregation area of *P. vivipara* groups before and after the presentation of visual cues (before - after). This metric informed us as to whether fish groups tended to get more cohesive (i.e., fish getting closer to each other) after exposure to the cues, and if so, what was the intensity of this behavior (Fig. 3). Positive values indicate fish individuals got closer to each other after exposure to the visual cues, which is a common behavior for prey fish when detecting predators (Andörfer, 1980; Cerri, 1983; Pitcher *et al*., 1983; Lopes *et al*., 2021). The formation of shoals by prey fish is a well-known antipredator behavior that can reduce the risk of predation by aquatic piscivores through multiple mechanisms, such as predation risk dilution, reduced predator efficiency, and increased prey vigilance (Welty, 1934; Brock & Riffenburgh, 1960; Neill & Cullen, 1974; Magurran *et al*., 1985; Morgan & Godin, 1985; Pitcher, 1986). This behavior has been extensively recorded for the family Poeciliidae, and it is frequently used as an antipredator response metric in behavioral studies (Seghers, 1973; Magurran & Seghers, 1990b; Magurran *et al*., 1992; Weetman *et al*., 1998; Kelley & Magurran, 2003; Botham *et al*., 2008).

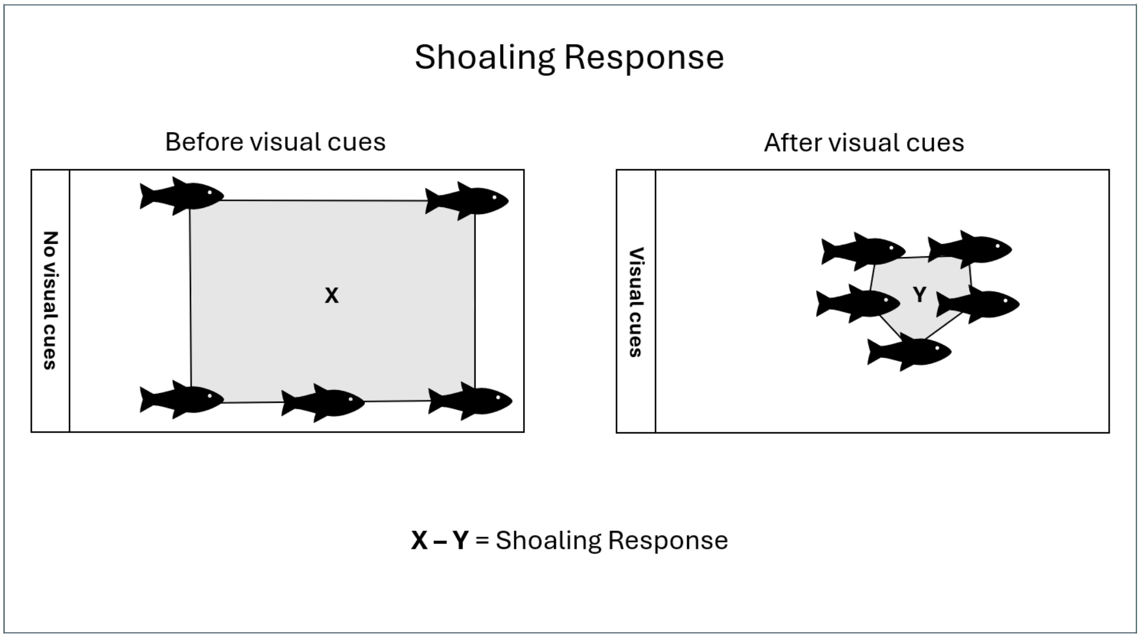

Figure 3: The shoaling response for *Poecilia vivipara* groups was calculated as the difference between the group aggregation area five seconds before and after the presentation of visual cues (before – after). The group aggregation area was defined as the area of the smallest convex polygon formed by the individuals in the group. Positive values for the shoaling response indicate fish got closer to each other after exposure to the cues presented.

We expected both populations to respond to the native predator stimulus (positive values of avoidance and shoaling responses) and not to respond to the control treatment (zero or negative values of shoaling and avoidance responses). Additionally, we expected only the coexisting population (PERD) to respond to the invasive predator cues (positive values of avoidance and shoaling responses).

2.5 Statistical analysis

We used Generalized Least Squares (GLS) models to test if the avoidance and shoaling responses (i.e., response variables) differed according to the experimental treatments (invasive predator, native predator, and control), populations (PERD, Florestal) (i.e., explanatory variables) and the interaction between experimental treatments and populations. We checked for model assumptions of normality and homogeneity of variance using residual plots (Figs. S1, S2). For the analysis of both response variables, we accounted for heteroscedasticity by applying a variance structure in the model, using the “varIdent” function in GLS, to allow different variances across levels of the explanatory variables. We evaluated the significance of the effects using F-tests. We plotted the means of the response variables across different levels of the explanatory variables, associated with 95% confidence intervals, to check if values differed significantly from zero, and if they were positive or negative. We performed all statistical analyses in the R software (R Core Team 2024), using the *nlme* (Pinheiro *et al*., 2024; Pinheiro *et al*., 2000) package. Figures displaying means and confidence intervals were constructed using the *ggplot2* (Wickham, 2016) and *dplyr* (Wickham *et al*., 2023) packages.

3. RESULTS

3.1 Avoidance response

Neither the experimental treatments ($F = 0.284$, $p = 0.75$) (Fig. 4) nor the interaction between populations and experimental treatments ($F = 0.049$, $p = 0.95$) influenced the avoidance response of *Poecilia vivipara* to the visual stimuli. On the other hand, the populations of *P. vivipara* differed in their avoidance response, with the PERD population exhibiting a more intense response than the Florestal population ($F = 6.590$, $p = 0.01$) (Fig. 5). The mean avoidance response of the PERD population was greater than zero, while the mean response of the Florestal population did not differ significantly from zero.

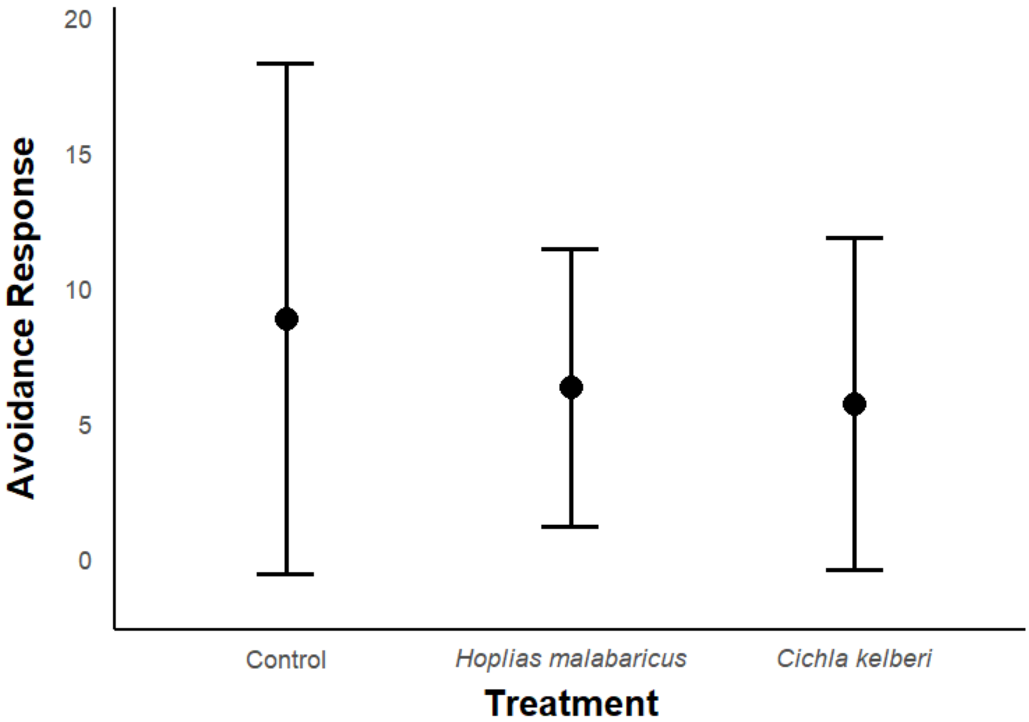

Figure 4: The avoidance response of *Poecilia vivipara* to different experimental treatments: control (no predator), *Hoplias malabaricus* (native predator), and *Cichla kelberi* (invasive predator). Black circles represent the mean value of the avoidance response for each experimental treatment and bars represent the 95% confidence interval.

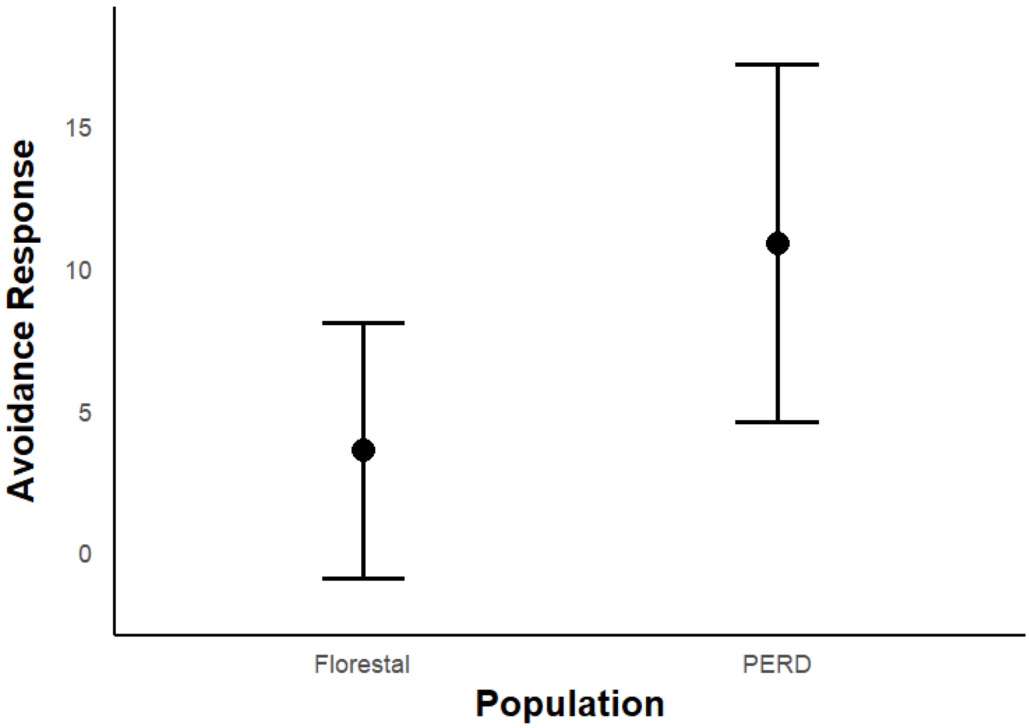

Figure 5: The avoidance response of *Poecilia vivipara* from two populations: one that coexists with the invasive predator *Cichla kelberi* (PERD) and one that does not (Florestal). Black circles

represent the mean value of the avoidance response for each population and bars represent the 95% confidence interval.

3.2 Shoaling response

Neither the experimental treatments (F = 0.385, p = 0.68) (Fig. 6) nor the interaction between populations and experimental treatments (F = 0.765, p = 0.47) influenced the shoaling response of *P. vivipara* to the visual stimuli. On the other hand, the populations of *P. vivipara* differed in their shoaling response, with the PERD population exhibiting a more intense response than the Florestal population (F = 4.677, p = 0.04) (Fig. 7). The mean shoaling response of the PERD population was greater than zero, while the mean response of Florestal population did not differ significantly from zero.

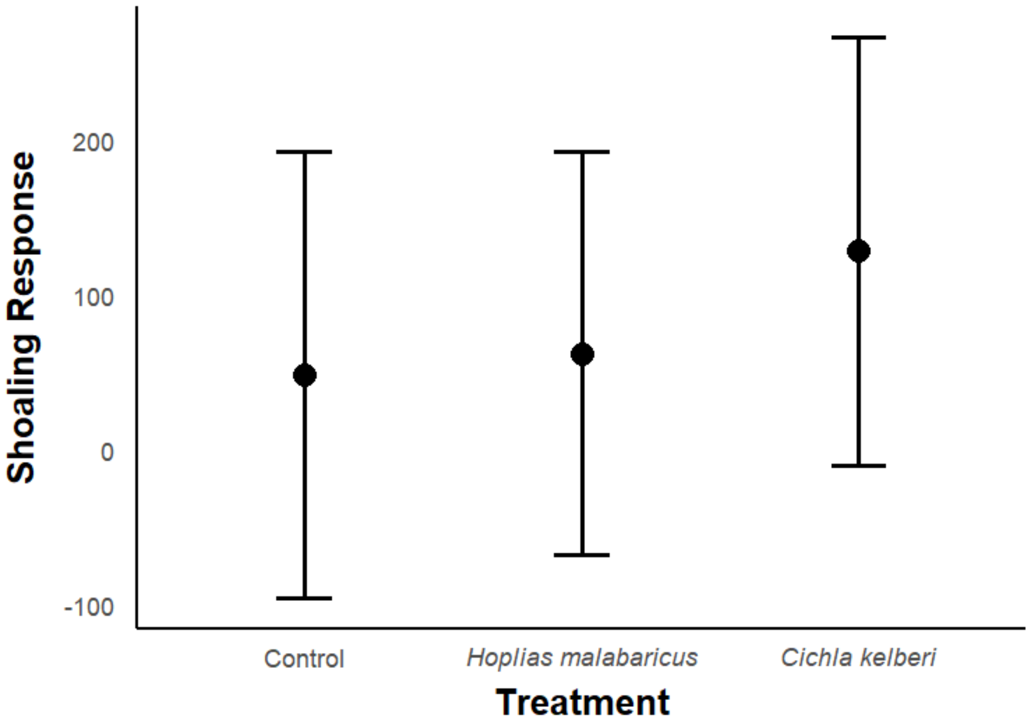

Figure 6: The shoaling response of *Poecilia vivipara* to different experimental treatments: control (no predator), *Hoplias malabaricus* (native predator), and *Cichla kelberi* (invasive predator). Black circles represent the mean value of the shoaling response for each experimental treatment and bars represent the 95% confidence interval.

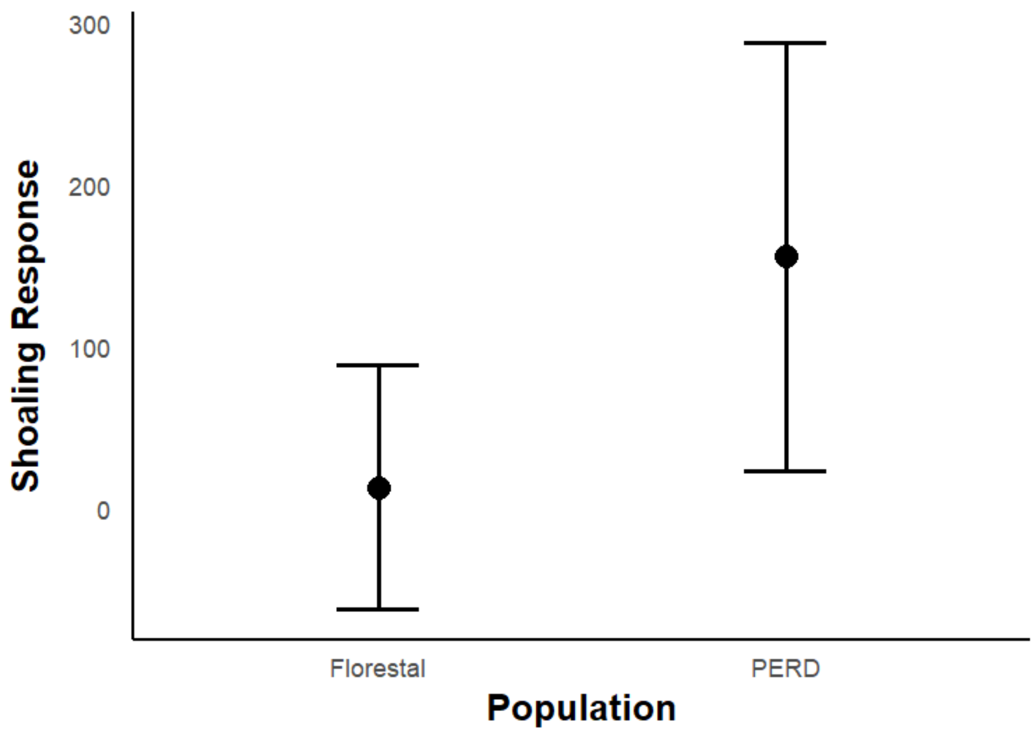

Figure 7: The shoaling response of *Poecilia vivipara* from two populations: one that coexists with the invasive predator *Cichla kelberi* (PERD) and one that does not (Florestal). Black circles represent the mean value of the shoaling response for each population and bars represent the 95% confidence interval.

4. DISCUSSION

In this study, we investigated the effects of coexistence with an invasive predator fish on the ability of *Poecilia vivipara*, a native prey species, to recognize and react to this predator. We expected that *P. vivipara* individuals that have coexisted for approximately 40 years with the invasive predator *C. kelberi* would respond to it, while *P. vivipara* individuals that have never coexisted with this predator would not. Furthermore, we expected that individuals from both populations would respond to the native predator *Hoplias malabaricus*. Contrary to our expectations, differences in antipredator responses between populations were not limited to the visual cues of the invasive predator. For the population in coexistence with *C. kelberi* (PERD), we found a generalized pattern of antipredator responses to all stimuli presented, for both the avoidance and shoaling metrics. Alternatively, for the population that does not coexist with *C. kelberi* (Florestal), our results showed a generalized lack of antipredator responses to all stimuli, for both metrics used in this study.

While we expected the PERD population to respond to both the invasive and native predator cues, we did not expect a response to the control treatment, since it lacked any visual cues of a predator. These results suggest that the mere presentation of images during experimental trials was enough to trigger antipredator responses in *P. vivipara* from PERD, regardless of their ability to discern between the contents of the images. Therefore, we cannot determine if the fish were able to recognize the predators' visual cues. A possible explanation for these results is neophobia, a behavioral trait characterized by a generalized fear of novel stimuli (Greenberg, 1990; Crane & Ferrari, 2017). Performing antipredator responses in non-dangerous situations is a costly behavior, which could reduce the time for other important activities such as feeding and reproduction (Lima & Dill, 1990; Crane & Ferrari, 2017; Crane *et al*., 2020). Because of this, neophobic individuals can be poorer competitors (Ferrari *et al*., 2019) and incur stress-related mortality (Cavigelli & McClintock, 2003). On the other hand, when prey inhabit high-risk environments, where they tend to frequently encounter diverse and dangerous predators, the costs of incorrectly responding to novel non-threatening stimuli could be outweighed by the costs of failing to respond to novel threatening ones (Crane & Ferrari, 2017). Therefore, neophobic responses could present an evolutionary advantage under variable and intense predation landscapes, allowing naive prey to react to novel predators, even without recognizing them (Brown *et al*., 2013; Crane *et al*., 2018; Crane & Ferrari, 2017; Ferrari, Crane, *et al*., 2015; Ferrari, McCormick, *et al*., 2015). Furthermore, the relationship between neophobia and predation pressure has been shown for different fish species, with individuals raised in high-risk environments tending to exhibit more neophobic responses and to maintain such responses for longer than individuals from lower-risk sites (Brown *et al*., 2013, 2015, 2016; Chivers *et al*., 2014; Ferrari, McCormick, *et al*., 2015; Meuthen *et al*., 2016). Prey fish in the Dom Helvécio Lake are under intense levels of predation pressure, due to the high abundance of piscivorous predators (Oporto, 2013; Fragoso-Moura *et al*., 2016; Lopes Bueno *et al*., 2016), such as the native wolf fish (*Hoplias malabaricus*), and the invasives peacock bass (*Cichla kelberi)* and red piranha (*Pygocentrus nattereri*), two voracious predators introduced in the system in the 1980s (Sunaga & Verani, 1991; Godinho & Formagio, 1992; Latini, 2001; Lopes Bueno *et al*., 2016). Therefore, the neophobic responses observed in this population of *P. vivipara* could be advantageous given the intense predation pressure in its environment. Since neophobia could help prey respond more effectively to novel predators, this behavioral trait might have assisted *P. vivipara* in persisting in the Dom

Helvécio Lake after the introduction of invasive predators, while many other native species were locally extinct (Sunaga and Verani, 1991; Gomes *et al*., 2007; Fragoso-Moura *et al*., 2016). An interesting question that remains unclear is whether the neophobia observed in this population was a pre-existing trait, which emerged before the introduction of invasive predators, or if it was selected by the pressures exerted by these novel predation threats.

In contrast to the PERD population, *P. vivipara* from Florestal did not respond to any stimuli, including images of *Hoplias malabaricus*, a native predator it coexists with. This lack of behavioral responses could mean fish were either unable to discern between the images presented or they did not perceive the predators' visual cues as threatening enough to elicit discernible antipredator responses. In either scenario, our results show *P. vivipara* from Florestal lack the neophobic responses observed in the PERD population. This behavioral difference could be related to lower levels of predation pressure experienced by the Florestal population in comparison with the PERD one, and the absence of invasive predators in the Florestal lake, which are highly abundant in the Dom Helvécio Lake (Oporto, 2013; Fragoso-Moura *et al*., 2016; Lopes Bueno *et al*., 2016). Assuming that *P. vivipara* individuals were able to recognize the visual predator cues presented, the lack of response towards them could be related to the level of predation pressure the Florestal population is subject to in its environment. For instance, for the Trinidadian Guppy (*Poecilia reticulata*), a close relative to *P. vivipara*, it is well known that fish from populations under reduced predation risk exhibit a higher alarm threshold and less intense antipredator responses (i.e., shoaling, keeping distance from predators) when compared to those from areas with greater predation pressure (Seghers, 1973, 1974; Magurran *et al*., 1992; Magurran & Seghers, 1994; Kelley & Magurran, 2003). Additionally, similar results to ours have also been observed for the prey fish *Astyanax ruberrimus*, in which individuals from an uninvaded site (under lower predation pressure) failed to respond to chemical cues from an invasive (*Cichla monoculus*) and a native predator (*Hoplias microlepis*), while individuals from an invaded site (under higher predation pressure) responded to both predators' cues (Sharpe *et al*., 2021). Therefore, the lower predation pressure experienced by *P. vivipara* in the Florestal lake could be responsible for weaker antipredator responses, a higher response threshold, and consequently, a greater vulnerability to invasive predators in this population than in the PERD one. Additionally, this threat could be imminent for *P. vivipara* and other prey species in the Florestal region, given that *Cichla* has already been recorded in the Paraopeba River Basin (Alves, 2012).

In conclusion, even though the tested *Poecilia vivipara* populations differed in their responses to *C. kelberi*, we cannot attribute the observed differences to coexistence or lack thereof with this invasive predator. This is due to the fact that the studied *P. vivipara* populations also differed in their responses to *Hoplias malabaricus*, a predator species with which both coexist, and to the control conditions, that lacked any predator cues. Therefore, our results suggest that there are factors other than coexistence with predators influencing prey's responses to experimental cues. Additionally, *P. vivipara* from the PERD population exhibited neophobic responses, while those from the Florestal population did not. Neophobic responses are usually associated with high levels of background predation risk and could be favorable when dealing with variable and intense predation threats, like those stemming from invasive predators (Brown *et al*., 2013; Crane & Ferrari, 2017). Because of this, the neophobic responses of the PERD population could be related to its resistance to the introduction of *C. kelberi*, while many other prey species were locally extinct (Sunaga and Verani, 1991; Gomes *et al*., 2007; Fragoso-Moura *et al*., 2016). Moreover, given that the Florestal population did not exhibit neophobia or pronounced antipredator responses, we suggest this population could be highly vulnerable to the introduction of novel predators. Finally, our study aimed to contribute to a better understanding of how behavioral traits of prey can influence the outcome of biological invasions, which is crucial for the effective management of native prey populations and to mitigate the impacts caused by biological invasions. The need for such an understanding is crucial in the present times, seeing as novel interactions are becoming increasingly prevalent (Wood & Shepard, 2022). Additional research would be valuable to further elucidate how neophobia and the background predation risk of prey can influence their antipredator responses and resilience to invasive predators.

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SUPPLEMENTARY MATERIAL

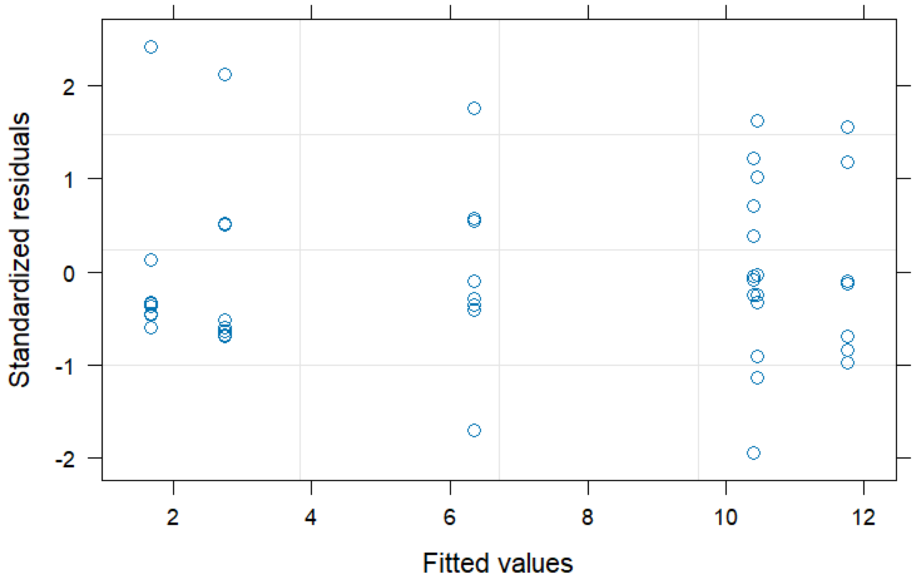

Figure S1: Residual plot of the GLS model with the avoidance response as a function of population, experimental treatment, and their interaction, to check for model assumptions of normality and homogeneity of variance.

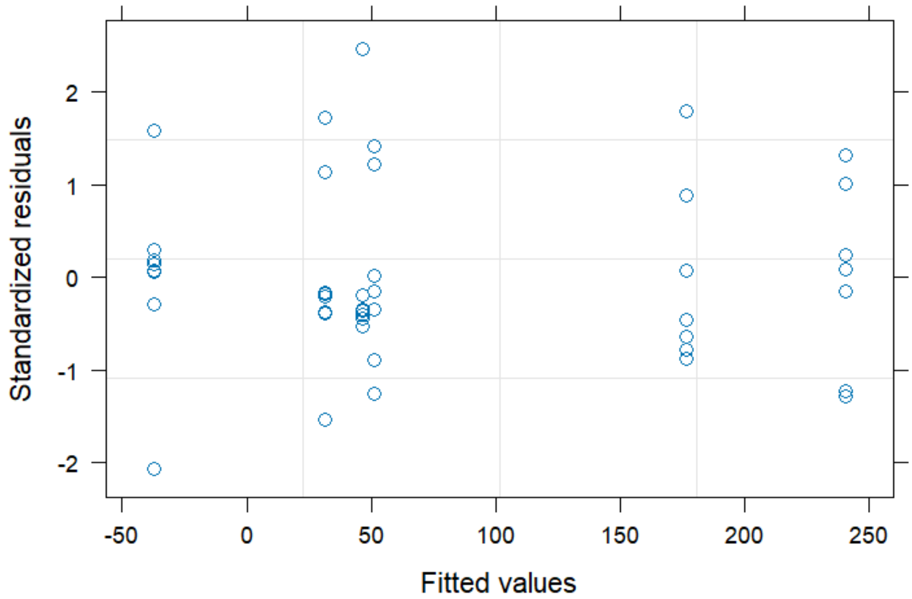

Figure S2: Residual plot of the GLS model with the shoaling response as a function of population, experimental treatment, and their interaction, to check for model assumptions of normality and homogeneity of variance.