UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Departamento de Genética, Ecologia e Evolução
PPG Ecologia, Conservação e Manejo da Vida Silvestre

Clara Massote Pidner

DEUS ESTÁ NOS DETALHES, O DIABO TAMBÉM: a mancha de machos e fêmeas do peixe *Poecilia vivipara* tem função sexual?

Belo Horizonte
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Orientador: Paulo Enrique Cardoso Peixoto
Coorientador: Rafael Pereira Leitão

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Ata da Defesa de Dissertação

Nº 461
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Clara Massote Pidner

No dia 22 de agosto de 2024, às 14:00 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) Clara Massote Pidner, orientando(a) do Professor Paulo Enrique Cardoso Peixoto, intitulada: **“Deus está nos detalhes, o diabo também: a mancha de machos e fêmeas do peixe Poecilia vivipara tem função sexual?”**. Abrindo a sessão, o(a) Presidente da Comissão, Doutor(a) Paulo Enrique Cardoso Peixoto, 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: Renato Chaves Macedo Rego (UFV), Jansen Alfredo Sampaio Zuanon (INPA) 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, 22 de agosto de 2024.

Assinaturas dos Membros da Banca Examinadora

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sei! assinatura eletrônica

Documento assinado eletronicamente por **Renato Chaves de Macedo Rego**, **Usuário Externo**, em 23/08/2024, às 18:23, 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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Em segundo lugar eu quero agradecer ao Paulo. Eu esqueço o quanto tempo estou trabalhando com você porque foram anos tão felizes e divertidos que passaram muito rápido. A sua orientação é realmente fora do comum com o tanto que você é capaz de ensinar de uma forma extremamente didática enquanto sabe tão bem lidar com o lado pessoal e humano das pessoas. Você foi o meu guia para mostrar com o mundo acadêmico da biologia e do comportamento animal funciona (e suas fofocas). Você me fez sentir pertencente do laboratório desde os pequenos atos como dar apelido (o meu, por exemplo, é “Escura”) até a confiança de nos responsabilizar com os projetos. O crescimento profissional e pessoal que eu tive na sua presença é até difícil de mensurar e, com certeza, fico te devendo essa o resto da carreira.

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RESUMO

Traços sexualmente selecionados servem como indicadores da qualidade individual para potenciais parceiros. Grande parte desse entendimento deriva de estudos sobre traços considerados exagerados sob a percepção humana e em espécies sexualmente dimórficas. Um problema com essa abordagem é que os traços podem ser exagerados ou dimórficos para o sistema sensorial humano, mas não para o sistema sensorial da espécie. Para ampliar nossa compreensão sobre a seleção sexual em traços individuais, é essencial investigar traços não exagerados em espécies aparentemente monomórficas. Para isso, investigamos o papel potencial na seleção de parceiros da mancha escura presente na lateral do corpo de machos e fêmeas do peixe *Poecilia vivipara*, uma espécie aparentemente monomórfica. Como a mancha escura se manifesta em indivíduos sexualmente maduros e é custosa de ser produzida, testamos as hipóteses de que a mancha escura 1) indica a qualidade nutricional individual e 2) tem um papel na escolha de parceiros em ambos os sexos. Para isso, investigamos o espectro de reflectância da mancha escura, correlacionamos a área da mancha com medidas morfofisiológicas que podem indicar a qualidade individual e realizamos um experimento de escolha de parceiros. Descobrimos que machos possuem em médias manchas maiores do que fêmeas e que ela indica qualidade individual para fêmeas e machos. Entretando, a correlação para as fêmeas é isométrica enquanto para machos é uma alometria negativa. Com isso podemos concluir que a mancha é na verdade dimórfica. Além disso, a mancha não é usada na escolha de parceiros. Por ser custosa de produzir e por ser sexualmente dimórfica, sugerimos que a mancha escura pode atuar em processos pós-copulatórios.

Palavras-chave

escolha de parceiros, seleção sexual, característica sexualmente selecionada, monomorfismo, sinalização UV

ABSTRACT

Sexually selected traits serve as indicators of individual quality to potential mates. Much of this understanding comes from studies on traits that are perceived as exaggerated by humans and in sexually dimorphic species. A problem with this approach is that traits may appear exaggerated or dimorphic to the human sensory system but not to the sensory system of the species itself. To broaden our understanding of sexual selection on individual traits, it is essential to investigate non-exaggerated traits in apparently monomorphic species. For this purpose, we investigated the potential role in mate selection of the dark spot present on the sides of both males and females of the fish *Poecilia vivípara*, an apparently monomorphic species. Since the dark spot manifests in sexually mature individuals and is costly to produce, we tested the hypotheses that the dark spot 1) indicates individual nutritional quality and 2) plays a role in mate choice in both sexes. To do so, we investigated the reflectance spectrum of the dark spot, correlated the spot area with morphophysiological measures that may indicate individual quality, and conducted a mate choice experiment. We found that males on average have larger spots than females and that it indicates individual quality for both females and males. However, the correlation for females is isometric, while for males it is a negative allometry. From this, we can conclude that the spot is actually dimorphic. Additionally, the spot is not used in mate choice. Given that it is costly to produce and sexually dimorphic, we suggest that the dark spot may play a role in post-copulatory processes.

Key words

Mate choice, sexual selection, sexually selected trait, monomorphism, UV signaling

ÍNDICE

INTRODUÇÃO GERAL

Essa introdução é sobre motivações. Eu quero contar um pouco sobre o que mais me inspirou dentro da área de comportamento. Em particular, duas motivações foram importantes: 1) um viés de gênero: até hoje as contribuições à teoria de comportamento são, em sua maioria, feitas por cientistas homens e 2) viés de objeto de estudo: a espécie que eu investigo, apesar de ser amplamente distribuída no Brasil, ainda é pouco investigada.

Histórico do viés de gênero (o que me motivou para fazer o estudo)

Um dos primeiros contatos que eu tive com a área de comportamento animal foi estudando Charles Darwin na graduação. Darwin foi um cientista que viajou em expedições para o mundo inteiro e, nessas viagens, se interessou por uma miríade de animais e descreveu seus comportamentos e sua morfologia. Um dos seus livros em especial descreve comportamentos que envolvem a reprodução dos animais (Darwin 1871). Nesse livro, Darwin descreve machos com cores muito extravagantes como a cauda grande e colorida dos pavões, ou danças complexas, como os machos das aves do paraíso, que performam um grupo de comportamentos de corte para as fêmeas. Enquanto isso, não se descrevia tanto fêmeas dessas espécies já que elas não exibiam o mesmo tipo de característica (Fritzsche et al. 2021). Por conta dessas diferenças, Darwin sugeriu que as fêmeas preferem machos que apresentavam cores mais fortes e danças mais complexas. Desse jeito, os machos que apresentam essas características se reproduziam e geravam mais filhotes do que machos que não possuem essas características.

Darwin não descreveu só machos com características extravagantes. Ele também se deparou com espécies nas quais as fêmeas apresentavam cores marcantes, enquanto os machos eram pouco chamativos. Uma espécie em particular que chamou a sua atenção foi uma ave asiática chamada toirão-barrado (*Turnix suscitator*). Nessa espécie, as fêmeas são maiores e mais coloridas do que os machos e Darwin as descreveu como “mais vociferantes e certamente mais combativas do que o macho” (Fritzsche et al. 2021). Indivíduos de outra espécie também chamaram a atenção de Darwin pelo mesmo motivo. Ele escreveu sobre as emas: “... essa ema possui uma completa inversão não só do instinto parental e de incubação, mas das qualidades morais usuais dos dois sexos; as fêmeas são selvagens, briguentas e barulhentas, os machos gentis e bons” (tradução livre, Darwin 1871). O toirão-barrado e as emas são exemplos de animais que apresentam o que é chamado de inversão de papel sexual, porque as fêmeas disputam por acesso aos parceiros enquanto os machos as selecionam. Apesar de que ainda encontramos estudos que utilizam esse termo para descrever a dinâmica sexual de uma espécie, ele tem entrado em desuso porque a descrição desse comportamento passa por uma antropomorfização (Ah-King and Ahnesjö 2013; Safari and Goymann 2021). Além disso, o termo reflete uma expectativa estereotipada dos sexos, o que sempre foi algo que me incomodou.

Ainda na graduação, eu comecei a fazer uma iniciação científica em um laboratório cujo foco principal é a seleção sexual (o LaSexIa, laboratório de seleção sexual e interações agonísticas). Para começar o meu projeto, eu comecei a ler os trabalhos clássicos da área. Um trabalho muito importante foi publicado em 1948 na revista Heredity, feito por Angus Bateman. Nesse trabalho, Bateman descreveu que nas moscas da fruta, aquela que a gente encontra na banana (*Drosophila melanogaster*), os machos procuram copular múltiplas vezes enquanto as fêmeas copulam menos e pareciam selecionar machos (Bateman 1948). A partir desse experimento, Bateman propôs que, na natureza, fêmeas em geral são castas e machos são promíscuos (repare aqui que novamente um cientista usou um atributo de cultura humana para descrever um processo no mundo animal). O que ele argumenta é que os machos possuem um gameta muito barato de ser produzido. Por ser barato, machos podem produzir mais gametas que fêmeas. Isso permite que machos possam aumentar o número de filhotes produzidos e com isso a qualidade da parceira não importa. Para as fêmeas, o contrário acontece porque o gameta feminino precisa de mais energia para ser produzido. Assim, fêmeas que copulam sem selecionar parceiros podem gerar filhotes de menor qualidade, o que em uma segunda geração gera menos descendentes ou descendentes de menor qualidade. Além disso, perder um filhote para uma fêmea acarreta um custo maior já que as fêmeas têm menos gametas do que os machos e cada gameta demanda uma quantidade de energia maior para ser produzido.

Nesse momento talvez você pode estar se perguntando como essa ideia de Bateman se encaixa com o caso da ema de Darwin. A ema é um caso clássico de uma “inversão de papéis” que simplesmente não encaixa com a teoria de Bateman. Nela, as fêmeas disputam por machos e não apresentam o comportamento casto. Na realidade, depois de anos de estudo sabemos que isso não acontece só com as emas: há muito mais variação no comportamento de machos e fêmeas para encontrar seus parceiros. O que antes era chamado de papel sexual hoje é uma possibilidade dentro de um espectro de variação comportamental (Wells 1978; Svensson and Petersson 1987; Berglund et al. 1989). Em um grupo peixes, por exemplo, os machos cuidam da prole sozinhos e as fêmeas copulam muito mais do que eles (Ah-King et al. 2005). Fêmeas, inclusive, disputam mais e são mais atraídas por machos que já estão cuidando de alguma prole.

Um cientista chamado Robert Trivers ajudou a desvendar o que pode explicar essa variação comportamental. Ele se intrigou principalmente com o cuidado parental na natureza. Ele observou que espécies nas quais as fêmeas brigavam por machos, os machos eram responsáveis pelo cuidado da prole. Por conta disso, ele argumentou que quando o custo de cuidar da prole é muito alto para o macho, as fêmeas deveriam disputar por eles. Ou seja, as fêmeas, mesmo arcando com um gameta mais custoso, economizam energia ao não cuidar da prole. Isso faz com que, no final do processo de produzir e cuidar da prole, o custo maior é na verdade do macho. A grande conclusão aqui é que o custo reprodutivo total, incluindo cuidado parental, vai ser um peso importante para determinar qual sexo vai procurar copular mais e qual deve selecionar as cópulas.

Mesmo com essa teoria de Trivers ainda havia muitos exemplos bizarros de animais que não seguiam as regras previstas pelo trio Darwin, Bateman e Trivers (Judson, 2003). E, apesar de eles não terem desenvolvido teorias tão generalizáveis, essas teorias não foram questionadas até os meados dos anos 80. Nesse período, começaram a popularizar os primeiros testes de paternidade. Esses testes revelaram que, mesmo em espécies com papéis sexuais “convencionais”, as fêmeas de várias espécies apresentavam filhotes com vários machos diferentes. Além disso, observou-se também que machos não eram capazes de obter tantas cópulas como na previsão do trabalho de Bateman (Judson 2002).

Nos estudos em nosso laboratório, nos deparávamos frequentemente com algum exemplo de um comportamento de espécie que a teoria mais clássica não conseguia explicar. O que a gente sempre dizia é que estudos que focassem em fêmeas eram raros e que no fim, até hoje, pouco se sabe sobre elas. Até que um dia me deparei com um trabalho que quantificou o viés de estudo em machos na área de comportamento animal (Ah-King 2022). O que Ah-King observa é que estudos sobre o comportamento de fêmeas são muito raros e muito recentes, e que eles começaram a crescer em meados dos anos 90. Talvez não coincidentemente a autora principal é uma mulher, e o seu interesse sobre esse viés parte da sua perspectiva feminina. A partir do estudo da Ah-King (2022), encontrei diversos trabalhos que mostraram como o viés humano dos pesquisadores influencia a visão sobre o objeto de estudo (Tobias et al. 2012; Roughgarden 2013; Ah-King 2023).

Basicamente o que Ah-King, Roughgarden e Tobias mostram é que até hoje não sabemos muito sobre fêmeas ou sobre machos com comportamentos inesperados. Eles argumentam que os animais mais estudados são aqueles que despertam mais o interesse do pesquisador. E esse interesse, segundo Hubbard, está inserido em um contexto social. Além disso, a interpretação do pesquisador sobre qualquer fenômeno natural vai refletir aquilo que faz sentido para ele e essa interpretação estará dentro do que é mais socialmente aceito naquele momento (Hubbard 1979). Até recentemente havia, pouca ou nenhuma diversidade entre os tipos de pessoa responsáveis pela produção de conhecimento e hoje, apesar da permanência das dinâmicas sociais de poder sobre quem faz a produção científica, há uma discussão e uma possibilidade maior de inclusão de vozes diferentes. Com tudo isso em mente, uma das minhas maiores inspirações de trabalho é trazer, como mulher, novos olhos e novas perspectivas para a interpretação de fenômenos naturais.

Motivação de percepção: a família *Poeciliidae* e como o meu objeto de estudo é o "primo feio"

Um grupo de animais em especial foi essencial para o desenvolvimento das teorias clássicas de seleção sexual. Esse grupo é fácil de se criar em laboratório e algumas espécies apresentam cores fortes, outras apresentam danças para cortejar o parceiro ou até os dois (Endler 1984; Evans et al. 2011). Além de ser fácil de manter em laboratório, os indivíduos em geral reproduzem facilmente e em abundância. Esse grupo é o dos peixes poecilídeos.

Os peixes mais estudados desse grupo são aqueles que cativaram os pesquisadores por conta das suas cores chamativas, características exacerbadas ou comportamento de corte complexo. Talvez o mais famoso deles seja o *Poecilia reticulata* (Fig. 1, conhecido no Brasil como *guppy*). Essa espécie foi protagonista de uma infinidade de estudos e laboratórios dedicados a ele. Hoje eu não vou falar dele, mas sim do seu "primo feio", *Poecilia vivipara* (Fig. 2). Os indivíduos dessa espécie não têm cores brilhantes e os machos não cortejam as fêmeas. Quando comecei a estudar essa espécie, percebi que, apesar de ser abundante em toda a América do Sul, só encontrei três estudos sobre a sua história natural (Liley 1966; Nascimento and Gurgel 2000; Mendonça and Andreata 2001). Nesses estudos, descobri que essa espécie apresenta uma época reprodutiva marcada, que ocorre no verão. Nessa época, machos perseguem as fêmeas para conseguir cópulas. A única característica morfológica mais marcante dessa espécie é uma mancha preta na lateral do corpo, tanto em machos quanto em fêmeas (Fig. 2). Mas então por que eu resolvi estudar essa espécie?

Fig. 1 Uma imagem do peixe *Poecilia reticulata.* Na parte superior da imagem se encontra um macho com coloração laranja e amarela (como percebida pelo nosso sistema visual). Os outros dois indivíduos são provavelmente fêmeas.

Fig. 2 Dois indivíduos de *Poecilia vivipara.* A imagem superior é de uma fêmea e a inferior é de um macho. Fotos de Gilberto Salvador.

Para ajudar a explicar o meu interesse, eu vou contar por alto o conteúdo de um livro muito interessante chamado “An Immense World: How Animal Senses Reveal the Hidden Realms Around Us”, que foi escrito pelo biólogo Ed Young (2022) que estuda sistemas sensoriais. É um relato de como nós estudamos cores e padrões na natureza, muitas vezes sem considerar como os animais de fato veem essas características. Um bom exemplo disso foi que pesquisadores sugeriram que borboletas selecionavam seus parceiros baseados no padrão de coloração presente em suas asas (exemplo de padrão nas asas na Fig. 3). O padrão pareceu muito conspícuo para os pesquisadores e, por isso, parecia lógico pensar que havia alguma seleção baseada nessas cores. Entretanto, os pesquisadores descobriram posteriormente que o sistema visual das borboletas não possui a acuidade para reconhecer a padronagem de cores nas asas. Há várias situações como essa nas quais enxergamos padrões que são invisíveis para outros animais, mas, tentamos entendê-los por chamarem a nossa atenção. Da mesma forma, uma característica de alguma espécie pode passar despercebida para nós, mas ser muito importante e conspícua para os indivíduos daquela espécie. A grande conclusão é que para tentar entender uma espécie precisamos fazer o exercício de tentar enxergar, ouvir e sentir como os indivíduos dessa espécie.

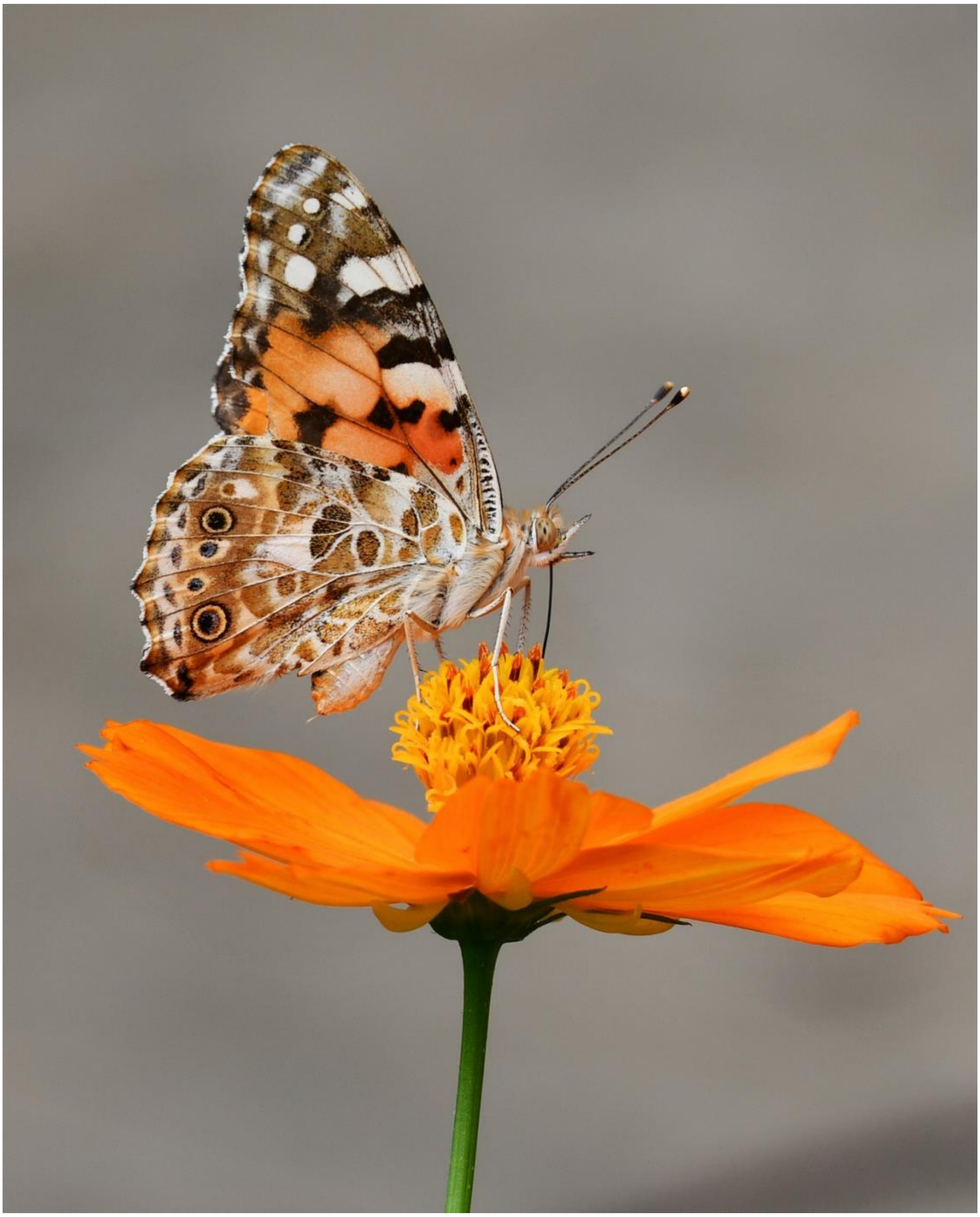

Fig. 3 Exemplo de uma borboleta que apresenta um padrão de coloração no seu par de asas, os quais pesquisadores estudaram como uma possível característica usada na seleção sexual.

Voltando para o meu objeto de estudo, eu sinto que ele é um patinho feio da sua família. Quase não encontramos estudos de comportamento e análise morfológica sobre ele, principalmente ao comparar com *P. reticulata* (Liley 1966). Eu comecei a investigar essa espécie analisando se os indivíduos apresentam cores que não somos capazes de ver, ou seja, que estão fora do espectro de luz visível. Fiz um teste usando um aparelho que é capaz de medir a intensidade da reflexão das cores do seu corpo e vi que praticamente toda a parte lateral desses peixes reflete cores fora do espectro de luz visível (ultravioleta). Depois disso, eu quis investigar se a mancha preta, que pode ser considerada inconspícua para o sistema sensorial humano é uma característica importante para a seleção de parceiros dessa espécie. Para investigar isso, eu parti da lógica de que, se indivíduos com manchas maiores forem também mais saudáveis, talvez eles também sejam mais atrativos como parceiros sexuais. Com isso, um parceiro em potencial pode ganhar um benefício indireto de escolher indivíduos de mancha maior. Como a mancha está presente em ambos os sexos, talvez tanto machos quanto fêmeas selecionem seus parceiros com base nessa característica.na mancha. Com este trabalho, eu quero atacar dois seguintes vieses: o viés de que a teoria por trás do comportamento sexual foi criada na perspectiva androcêntrica, então há uma transferência do conceito de papéis sexuais humanos para as espécies. Para atacar esse viés eu parto da hipótese da escolha mútua de parceiros, que é uma hipótese normalmente desconsiderada. Em segundo lugar, o viés de grupo de estudo, já que se trata de uma espécie sobre a qual se sabe pouco sobre a história natural.

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The devil is in the details: does the male and female dark spot in the fish *Poecilia vivipara* has a sexual role?

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PECP and CMP designed the study. CMP collected the data and, together with PECP, analyzed the data and wrote the manuscript. RPL reviewed the text.

Data availability statement

Data available on Open Science Framework Repository:
https://osf.io/qkfz5/?view_only=303b8bb360f344b7a8a6a03066cb34b7

INTRODUCTION

Two of the main paradigms in intersexual selection is that exaggerated traits are favored in the process of mate choice because they define more attractive partners without additional benefits (i.e. runaway selection) – (Pomiankowski and Iwasa 1997) or that, in addition of defining attractiveness, also indicate direct or indirect benefits to potential mates (Andersson and Iwasa 1996). For direct and indirect benefits, the exaggerated trait often signalizes individual condition that is correlated with the potential benefits that mating partners should gain by mating (Jennions and Kokko 2010). Several studies investigated the evolution of exaggerated traits such as bright colors (e.g. Weiss 2006) or elaborated vocal calling (Drăgănoiu et al. 2002). However, there is a problem in defining what constitutes an exaggerated trait. Apart from traits that grow hyper-allometrically with body size, the criteria to define traits such as individual color or song as exaggerated relies on a grey area (Emlen et al. 2012). Usually, traits that are exaggerated to the human sensory system are widely investigated (Yong 2022). For example, many butterflies have intricate patterns on their wings that have called the attention of researchers. Although researchers investigated several potential functions for these traits, they later discovered that individuals of the species itself do not have a visual system capable of capturing this pattern, and therefore these traits are not used as any type of intraspecific signaling (Yong 2022). Therefore, it may be that the interest on traits that are exaggerated to the human sensory system has created a bias that ignores trait's functions and exaggeration under the species sensory systems (Tarvin and Murphy 2012; Yong 2022).

Traits considered non-exaggerated under the human sensory system can be particularly ignored in sexual selection studies of (apparently or supposedly) sexually monomorphic species. This occurs because traits in sexually monomorphic species are seldom inconspicuous to human sensory system and, because they are present in both males and females, they are usually seen as anti-predatory adaptations (e.g. Van Bergen and Beldade 2019; Mariotto et al. 2022). However, signaling traits in monomorphic species may be used in mate choice under two different scenarios (Tarvin and Murphy 2012): i) when individuals of one sex chooses their mates based on the trait, while individuals of the opposite sex present the trait due to genetic correlation (Lande 1980; Lande and Arnold 1985; Lande, Russell 1987) or ii) when individuals show mutual mate choice based on the trait (Kraaijeveld et al. 2007). Therefore, investigating potential sexual roles of traits in monomorphic species can bring further insights on the role of sexual selection on morphological evolution.

Even in sexually monomorphic species, if a trait is used as a mate choice mechanism associated with some type of benefit, it should be correlated with signals of individual quality. Thus, a way to investigate if a certain trait is associated with a mate choice mechanism is to access the correlation of the trait with proxies of individual quality. One widely reliable proxy for individual quality is body size (e.g. Howard et al. 1998; Beauplet and Guinet 2007; Blomquist and Turnquist 2011). In general, individuals with greater body size have higher quality and may provide greater benefits for their partners (e.g. Ryan 1980; Côte and Hunte 1989; Howard et al. 1998; Aquiloni and Gherardi 2008). For instance, in the lizard *Uta stansburiana*, male body size is positively correlated to the egg mass of their daughters, indicating that females that choose bigger males may receive indirect benefits to the future offspring (Calsbeek and Sinervo 2004). Another commonly reliable proxy for individual quality is body fat (Reznick 1983; Koskimäki et al. 2004). A larger amount of body fat can provide several benefits to the individual since fat is an important source of energy and may affect important aspects of the individual condition (e.g. Arrese and Soulages 2010; Labocha and Hayes 2012; Price 2017). In the damselfly *Hetaerina americana*, for instance, males with higher fat reserves also have a better immune system (Koskimäki et al. 2004). Therefore, traits used during mate choice to indicate individual quality can be correlated with body size and body fat.

In poecilids, there is a wide range of traits used during mate choice due to their role in indicating individual quality. There are species with colorful sexual dimorphism (Milinski 2014) and mating displays (Sargent et al. 1998; Wong and Hopkins 2007) that signal individual quality and enhance individual mating chances. There are also monomorphic species that use their color to signal social status and courtship interactions, such as color displays (Ziegelbecker et al. 2018).

A monomorphic poecilid species with an apparently non-exaggerated trait is *Poecilia vivipara* (Cyprinodontiformes: Poeciliidae). In this species, both males and females present a dark coloration on the top of their heads and a dark melanin-based circular spot on their body sides that only manifests when individuals reach sexual maturity (Liley 1966; Zerulla and Stoddard 2021). Dark spots in poecilids are usually produced by melanin deposition (Zerulla and Stoddard 2021). The melanin-based spots are costly to be produced (Ducrest et al. 2008), and in vertebrates, individuals that produce more melanin usually have a more efficient immune system and increased fertility, due to the pleiotropic effects of the gene responsible for the production of melanin (Ducrest et al. 2008). Thus, there is strong evidence that individuals that produce larger spots of melanin have higher reproductive quality. In addition, we believe that both males and females in *P. vivipara* may show some level of mate choice. Populations of *P.*

vivipara typically present a female biased adult sex ratio (3:1, Mendonça & Andreata 2001). Thus, because males are the least frequent sex, we should expect females to compete for mating opportunities with males (Westneat and Fox 2010). Therefore, males can benefit from selecting females in this species. For females, mate choice is expected because females of *P. vivipara* are viviparous and lecithotrophic and give birth after 30 days of fecundation (Liley 1966). Since females once pregnant are unable to gestate new concomitant offspring and that they spend a long period investing in their offspring (Furness et al. 2019), this period causes females to loose copulation opportunities. Therefore, females that choose their mates based on cues of individual quality may reduce the costs of bearing an offspring of low quality. For these reasons, in this study we investigated how individuals of *P. vivipara* perceive the ir conspecific lateral spot's according to their visual system, whether the dark lateral spot contain cues of individual quality and if the lateral dark spot is used by males and

females to select potential mates. We proposed two hypotheses: 1) the dark spot is a honest indicator of individual quality for males and females; and 2) both sexes choose their mating partners based on the dark spot size. Under hypothesis 1, we expect that males or females should present a positive correlation between the dark spot area and their body fat and/or body length, while under hypothesis 2, we expect that, when allowed to choose between two distinct individuals, both males and females should spend more time swimming towards potential partners with larger dark spots. The outcomes of each scenario of corroborating or not the hypothesis is in Table 1.

Table 1.

Potential theoretical outcomes of each hypothesis

Scenarios	Interpretation
Corroborating hypothesis 1 and 2	The dark spot is an honest signal of quality and individuals use this trait as a sexual cue
Corroborating only hypothesis 1	The dark spot is na honest signal of individual quality, but it is not used is mate choice

Corroborating only hypothesis 2	The dark spot is not an honest signal of individual quality, however, it is used in mate choice. This would probably be explained as a runaway selection proccess, in which, altough the trait is not na honest signal it indicates attractiveness and, therefore, it is favored
Refuting both hypothesis	The dark spot is not a sexual cue nor does it indicates individual quality

METHODS

Field sampling

We sampled individuals in Florestal campus of Federal University of Viçosa (UFV), Minas Gerais state, Brazil (19.845708 S, 44.504342 W). The campus has several artificial lakes connected to each other through channels. According to Köppen classification, the climate is Cwa, characterized by hot and rainy summers (mean temperature above 22 °C) and dry and cold winters (mean temperature around 18 °C, (de Sá Júnior et al. 2012). We observed and sampled the individuals in the littoral zone of one lake, which was easily accessible with few vegetation around. All sampling was done between the months October and December of 2023, thus, during the summer. This was specifically thought so that the sampling and experiments was done during reproductive season (Mendonça and Andreata 2001).

Measurements of P. vivipara’s *lateral body* vivipara reflectance

To investigate whether the reflectance spectra of the lateral dark spot differ from the rest of the body, we investigated the reflectance pattern of males and females adults of *P. vivipara* using a spectrophotometer. We were segure that the individuals were adults because they all had the dark spot, a trait that only appears with sexual maturity (Liley, 1966). Although there are no studies of *P. vivipara*’s visual system, it is known that guppies (*P. reticulata*) have ocular media with UV (300-400 nm) transmittance (Douglas and McGuigan 1989; see also Douglas and Hawryshyn 1990) and cone photoreceptors sensitive to UV (Archer et al. 1987; Archer and Lythgoe 1990). They also present at least three other types of cone photoreceptors (Sandkam et al. 2018) that are sensitive to blue, green and red light (400-700nm). Therefore, it is likely that individuals of *P. vivipara* are also able to see in the whole 300 – 700 nm (UV-visible) spectra. Perhaps, because this species potentially accesses UV cues, the black spot may be exaggerated to the individuals due to a reflectance in the UV wavelengths. We evaluated UV-visible reflectance the dark spot region, however, because the spot was smaller than the equipment was able to measure, we could not be sure that the area evaluated was exactly on the dark spot (Fig.1s). We then decided to evaluate reflectance of the body side outside de dark spot to test the difference in both measures (Fig. 2s). Furthermore, the top of the individuals’ heads also presented a dark coloration, so we decided to also measure the reflectance in this area to test it was different from the dark spot area (Fig.3s). Finally, we also measured the reflectance of the caudal region since we found similar results between the body side and dark spot reflectance (see Supplementary material), and we wished to test if the whole-body side had similar reflectances (Fig. 4s). We measured the reflectance of 10 individuals to build the mean spectral curves of the four *P. vivipara*’s body regions.

We used a spectrophotometer (USB2000+UVVIS-ES, Ocean Optics Inc.) radiometrically calibrated between 250 and 750 nm with a deuterium/tungsten light (DH-2000-BAL, Ocean Optics Inc). Such calibrations allow a precise evaluation of spectral reflectance parameters in the UV-visible spectra. The first step was to calibrate the equipment with a UV-reflective white standard made of Spectralon® (WS-1-SL, Ocean Optics Inc.) and then calibrate with a black background to establish the minimum and the maximum light reflectance. Following calibration, we measured relative spectral reflectance (%/nm) of the four body surfaces with a bifurcated optical fiber probe (R400-7-UV-VIS, Ocean Optics Inc.) connected to the spectrophotometer and to a pulsed xenon light (PX-2, 220 Hz, 220–750 nm, Ocean Optics Inc.). We mounted the optical fiber light sensor on a black opaque support (RPH-1, Ocean

Optics Inc.) with 45° angle and 0.5 cm distance between the own equipment's light source and the sample to be measured. We used the software SpectraSuite (Ocean Optics Inc.) for reflectance curve acquisition and analysis. We euthanized all fishes used for body reflectance measurements using a solution of Eugenol 0,5 ml/L diluted into the water (ICMBio permit nº 307/2022).

Fig. 1 Two individuals of adults of the fish *Poecilia vivipara*. Pictures taken by Gilberto Salvador.

Body measures and fat extraction

To investigate if the dark spot indicates individual quality, we tested for correlations between the size of the dark spot with individual size and body fat mass. To estimate individual traits, we collected and immediately euthanized 80 individuals, 37 males and 43 females, with an overdose of Eugenol 0,5 ml/L diluted in water. After this, we photographed (Canon EOS Rebel

T5) the lateral side of each individual to extract five morphometric measures (Karachle & Stergiou 2012). Morphometrics and allometry in fishes. *morphometrics*, 65-86.): dark spot area, body area without the fins, caudal-peduncle minimal depth (CPd), body depth at the level of the pectoral-fin insertion (PFb) and body length (mouth to caudal-peduncle). We took the measures using the ImageJ software (Abràmoff *et al.* 2004).

To measure the amount of body fat, we first dried the individuals for 48 h at 60 °C (Reznick 1983) using a digital drying oven (Lucadema Inc.) and then weighted the individuals using a precision scale (0.00001 g, Marte Inc.). Since we did not know the amount of time necessary to completely dry individuals, we placed 10 individuals in the drying oven for additional 72 h. Because the weight difference of each individual between 48 h and 72 h drying was lower than 0.001 g, we determined that the time necessary to eliminate the water in each individual was 48 h. After drying, we submersed each individual into a mixture of chloroform and methanol (1:1) (Bligh & Dyer 2016), which is capable of dissolving body fat. We kept the individuals submerged for 48 h, then dried them for 48 h on a digital drying oven and weighted each one (Reznick & Braun 1987). After this, we submerged the individuals in chloroform and methanol for another 24 h to investigate whether they continued to lose fat. At this point the differences in weights between the two fat extractions were ranging between - 0.001 g and 0.002 g. Therefore, we decided to stop the fat extraction since negative values indicated that individuals could be gaining weight due to humidity accumulation and, at the same time, no individual with positive values lost a high amount of fat in the second extraction. To calculate the amount of body fat, we used the dry weight difference before and after immersion in chloroform. Some studies remove the somatic and reproductive tissue before performing these extractions (Reznick & Braun 1987), however, since we are interested in evaluating the correlation between the dark spot and individual quality in males and females, the amount of fat in the somatic tissue could be a signal of the total fat accumulated by the individual and therefore, could indicate its overall nutritional quality. Therefore, we choose to not remove the somatic tissue before fat extractions.

Mate choice experiment

To investigate if individuals of *P. vivipara* show a preference for mates with larger lateral dark spots, we performed mate choice experiments between November and December of 2023, months of reproductive activity of this species (Mendonça and Andreata 2001). In order to maintain most of the natural behavior all individuals used in the experiment were collected and put though the experiment on the same day. Each experiment involved three individuals that consisted in one focal individual that could select between two possible mating partners. We carried out a total of 35 experiments, in which 17 had a focal male that could choose between two females and 18 had a focal female that could choose between two males. In each experiment, we positioned an aquarium (22 cm length, 16 cm width, 10 cm high) in a sunny area next to the lake in which the individuals were collected. To remove any influence from the surrounding landscape and to remove any influence of potential predator cues in the individual's behavior, we placed a cardboard box around the aquarium that avoided the individual from observing the local landscape. The aquarium has three compartments divided by glass that avoided water exchange among them (Fig. 5s). The central compartment (12 cm length, 16 cm width, 10 cm high) contained the focal individual, which was able to visually access both other compartments. The focal individual positioned on the central compartment could be either a male or a female. The other two compartments in the side of the aquarium (5 cm length, 16 cm width, 10 cm high) were occupied by one individual each, representing the options (individuals of the opposite sex) for the focal individual to choose. We filled the aquarium with approximately 10 cm depth of water. We took the water from the same lake in which the individuals were collected to avoid behavioral changes due to distinct water conditions. After each experiment, we discarded the water and collected a new set from the same lake for the next round. We did this to avoid that chemical cues left from the previous individuals could affect the behavior of the new individuals. We did not, however, washed the tanks between experiments.

When choosing the individuals to put in the lateral compartments (individuals to be chosen by the focal one), we opted to select individuals with similar body size (maximum difference of 10% in body length) but with greater differences in spot areas (minimum difference of 10%) to avoid confounding effects of body size on mate choice. To measure the individuals in the field we photographed each live individual over a millimeter sheet. By analyzing the photo, we were able to pair de individuals using their body length. The dark spot, however, was too small to analyze in the field by looking at the photo. Therefore, we performed the experiments by pairing only by body length and, using the measurements made posteriorly, we discarded the experiments in which the individuals had a difference on spot area smaller than 10%. During the placement of the individuals on lateral compartments, we changed the side to insert the larger individuals in each trial (left or right), to avoid that an eventual bias of focal individuals to a particular side could affect the result. After adding the individuals (the two individuals to be chosen and the focal individual) to the aquarium, we waited for 5 min for acclimation and then recorded the focal individual behavior for additional 5 min to access how much time each focal individual spent choosing each option. To determine if the focal individual was choosing one of the options on the lateral compartments, we divided the central part of the aquarium in three strips (4 cm width, 16 cm length, 10 cm high). The mean length of the individuals was 2.1 cm, therefore, the 4 cm width was enough for the individuals to swim in this compartment. We considered the central part of this division as the neutral zone that represented an area in which no choice was being made by the focal individual. Conversely, we considered the time the focal individual spent in the left or right zones (choosing zones) as the time in which the focal individual spent choosing the individual on that side. We considered that the individual entered the specific zone if their whole body passed through the line that divided the zones. This analysis was done by checking the video footage or the audio recording of each experiment.

Because guppies have ultraviolet vision and their body sides reflect UV cues (see results), it may be that mate choice is based on information provided by the lateral black spot in UV length. For this reason, we decided to investigate if UV cues were important for mate choice in this species (Smith 2002). For this, we repeated the same experiment with other group of individuals: 31 focal individuals, 13 with a male as the focal one and 18 with a female. However, for these trials we used an aquarium that avoided the focal individuals to access potential UV cues of the individuals in the choosing zones. For this, we added a layer of UV blocker (*Insufilm*) over the glass of the compartments. By doing so, focal individuals were able to visually assess the two potential mates only through the visual light spectrum. If they presented a clear pattern of choice when UV cues were present, but changed their pattern in the absence of UV cues we would thus consider that the UV cues are important in their mate choice.

Statistical analysis

First, we aimed to test if the dark spot area could be a signal of individual quality. To do so, we fitted a general least squares model in which we used, sex (male or female), scaled values of body length and scaled values of body fat and their interaction as predictor variables. For body length we used the *varPower* option to model the residuals while for body fat we used *varExp*. The response variable was the scaled values of spot area. We constructed separated models for body fat and body length because they are highly correlated (81%).

Secondly, we aimed to understand if the focal individuals presented a choice for either of the individuals in the lateral aquarium zone. To do so, we first tested if individuals spent more time outside then inside the central zone. Considering that we filmed each individual for 300 s, spending less than 100 s in the central zone would indicate that they preferentially stayed near the other individuals. To evaluate this, we performed a one-way t-test to check whether the mean value of time spent in the neutral zone was smaller than 100 s.

The third step was to test if the focal individuals spent more time in a specific side of the aquarium, independent of the fish placed at the side. We tested this to be sure that the time spent in the choosing zones was not affected by individuals' preferences for a given aquarium side (a potential confounding factor). To check this, we fitted a generalized linear model with beta binomial error distribution in which the side of the aquarium (left or right) was the predictor variable. The response variable was the proportion of time the focal individual spent swimming on the either side over the total time of the trial, not considering the time spent in the neutral zone. The proportion values included 0 and 1. Because the model did not accept the inclusion of these values, we performed a recommended transformation (Douma and Weedon 2019) of the response variable using the formula $\frac{x*(n-1)+0.5}{n}$ in which x is each ratio of time value and n is the number of samples. We used the *betareg* function of the *betareg* R package to construct a model that follows the beta binomial distribution (Cribari-Neto and Zeileis 2010; Douma and Weedon 2019).

Finally, we aimed to understand if the focal individuals spent more time close to individuals with the larger spot area. To analyze this, we also fitted a generalized linear model with beta binomial error distribution in which the gender of the focal individual (male or female) and the presence or absence of the UV filter were predictor variables. The response variable was the proportion of time the focal individual spent swimming close to the individual with the larger spot area, disconsidering the time spent in the neutral zone. We also transformed this proportion data according to the formula mentioned before.

Results

In the reflectance tests, the individuals presented a peak of UV reflectance on their body side, caudal fin and dark spot, but not on the top of their heads (Fig. 1s, 2s, 3s and 4s). Female mean weight was 0.17 g (sd = 0.13 g) and male mean weight was 0.26 g (sd = 0.11 g). Female average body length was 23.51 mm (n = 43, sd = 3.32 mm) and male body length was 19.61 mm (n = 37, sd = 4.92 mm). Finally, average spot area for females was 1.45 mm^2 (sd = 0.82 mm^2); and

2.50 mm^2 (sd = 1.19 mm^2) for males. Spot area was positively correlated to body length ($F_{(1,75)} = 199.33$, $p < 0.001$, Fig. 2), gender ($F_{(1,75)} = 3.96$, $p = 0.05$) and their interaction ($F_{(1,75)} = 5.24$, $p = 0.02$). The relationship between body length and spot area was steeper in females than in males (Fig. 2). The 95% confidence interval for the slope of the relationship between spot area and body length for females ranged from 0.79 to 1.01 while for males ranged from 0.44 to 0.60. Spot area was also positively correlated to body fat (Fig. 3, $F_{(1,75)} = 24.14$, $p < 0.001$) and was greater in males when compared to females ($F_{(1,75)} = 7.28$, $p = 0.007$), but was unrelated to the interaction between fat mass and gender ($F_{(1,75)} = 0.26$, $p = 0.61$). The 95% confidence interval for the slope of the relationship between spot area and body fat for females ranged from 0.72 to 1.33 while for males ranged from 0.26 to 0.84.

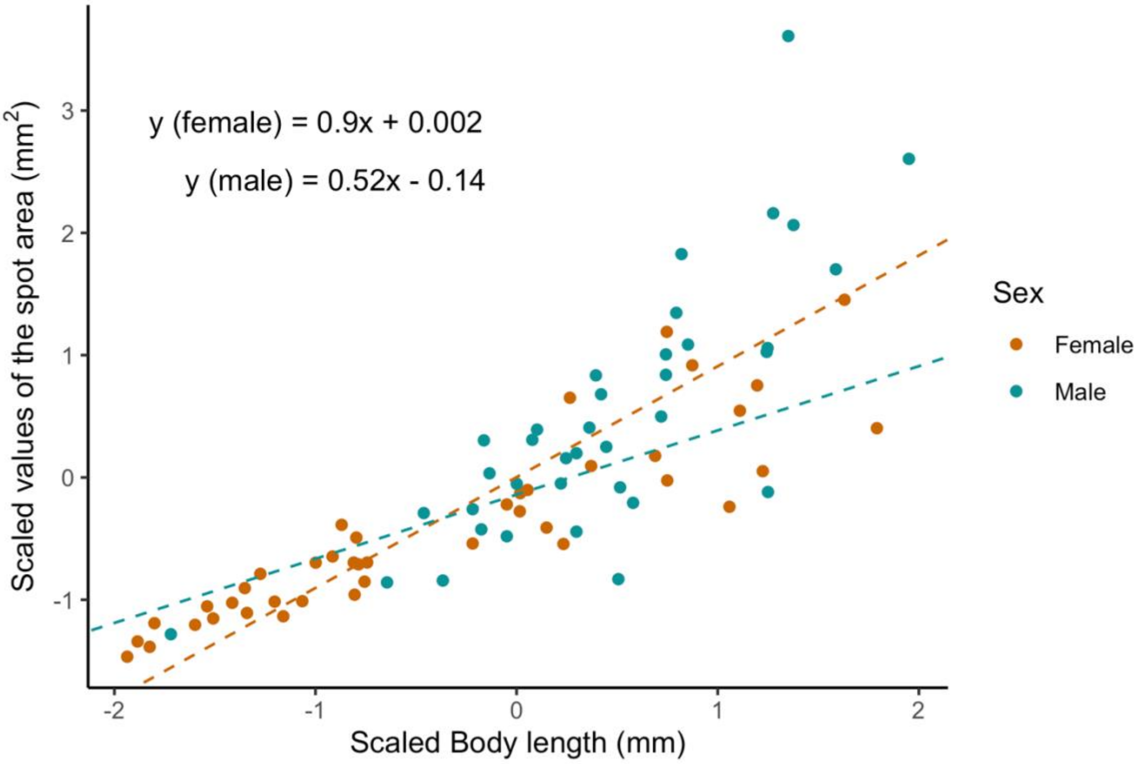

Fig. 2 Relationship between the scaled values of the spot area (mm) and scaled values of individual body length (mm) for males and females of the fish *P. vivipara*. Each point represents an individual measure, and the dashed line represents the data tendency using the linear regression method. Orange is for the measures and predicted relationship for females and blue is for the measures and predicted relationship for males.

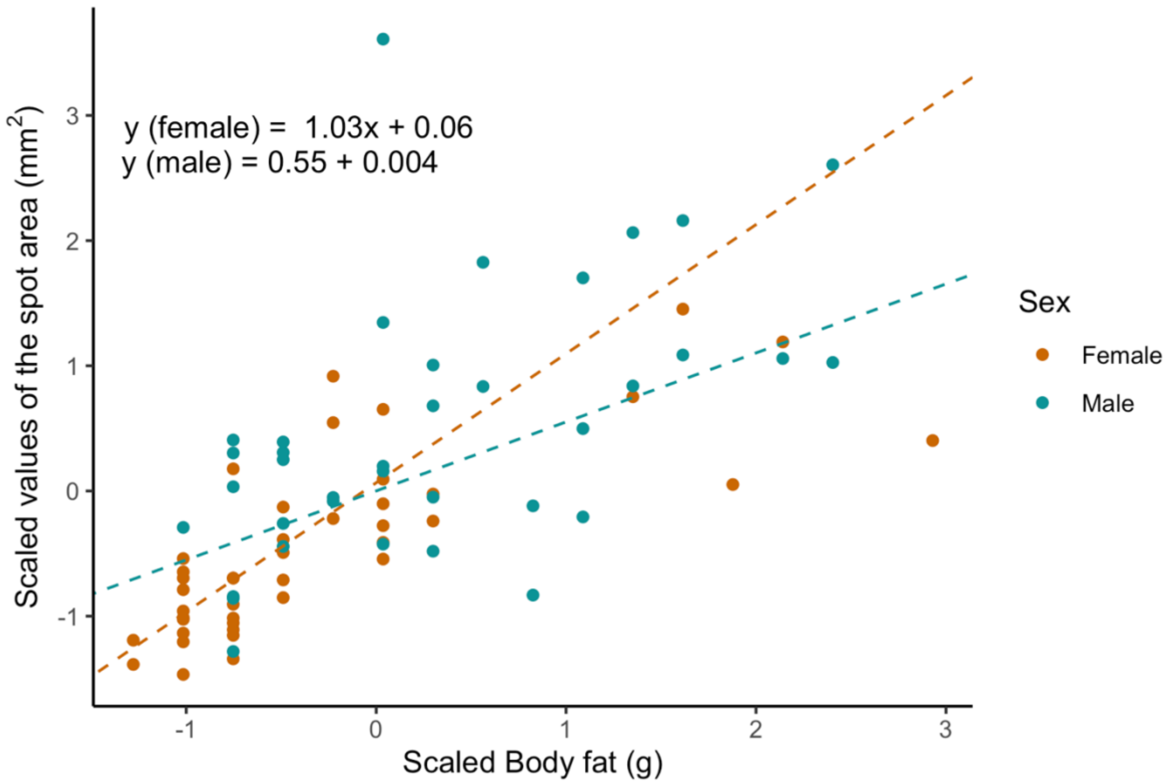

Fig. 3 Relationship between scaled values of the spot area and the scaled values of the individual body fat for females and males. Each point represents an individual measure, and the dashed line represents the data tendency using the linear regression method. Orange is for the measures and predicted relationship in females and blue is for the measures and predicted relationship in males.

Females spent on average 266.22 s in the choosing zones without the UV filter and 270.2 s in the choosing zones with the UV filter. Males spent on average 259.6 s in the choosing zones without the UV filter and 275.2 s in the choosing zones with the UV filter. The individuals spent less time in the neutral zone than expected by chance (Fig. 5s, $t = 14.71$, $df = 69$, $p < 0.001$) and the choice was not consistent with a specific side of the aquarium ($\chi^2_{(1)} = 0.08$, $p = 0.77$, Fig. 6s).

Out of the 17 focal males, 7.14% spent more time closer to the female with the bigger spot area of the pair, 82.15% switched between the choosing zones repeatedly throughout the experiment, 7.14% spent more time closer to the female with smaller spot area and 3.57% first swam closer to the female with smaller spot area, but once it changed to the side of the female with bigger spot area it stayed there for the rest of the trial. As for the females, out of the 18 focal females, 33.34% spent more time closer to the male with the bigger spot area of the pair, 54.54% switched between the choosing zones repeatedly throughout the experiment and 12.12% spent more time closer to the male with smaller spot area. Individuals of both genders did not spend more time close to the individual with the larger spot area, independent of the presence or absence of the UV filter ($\chi^2_{(3)} = 0.12$, p = 0.99, Fig. 4).

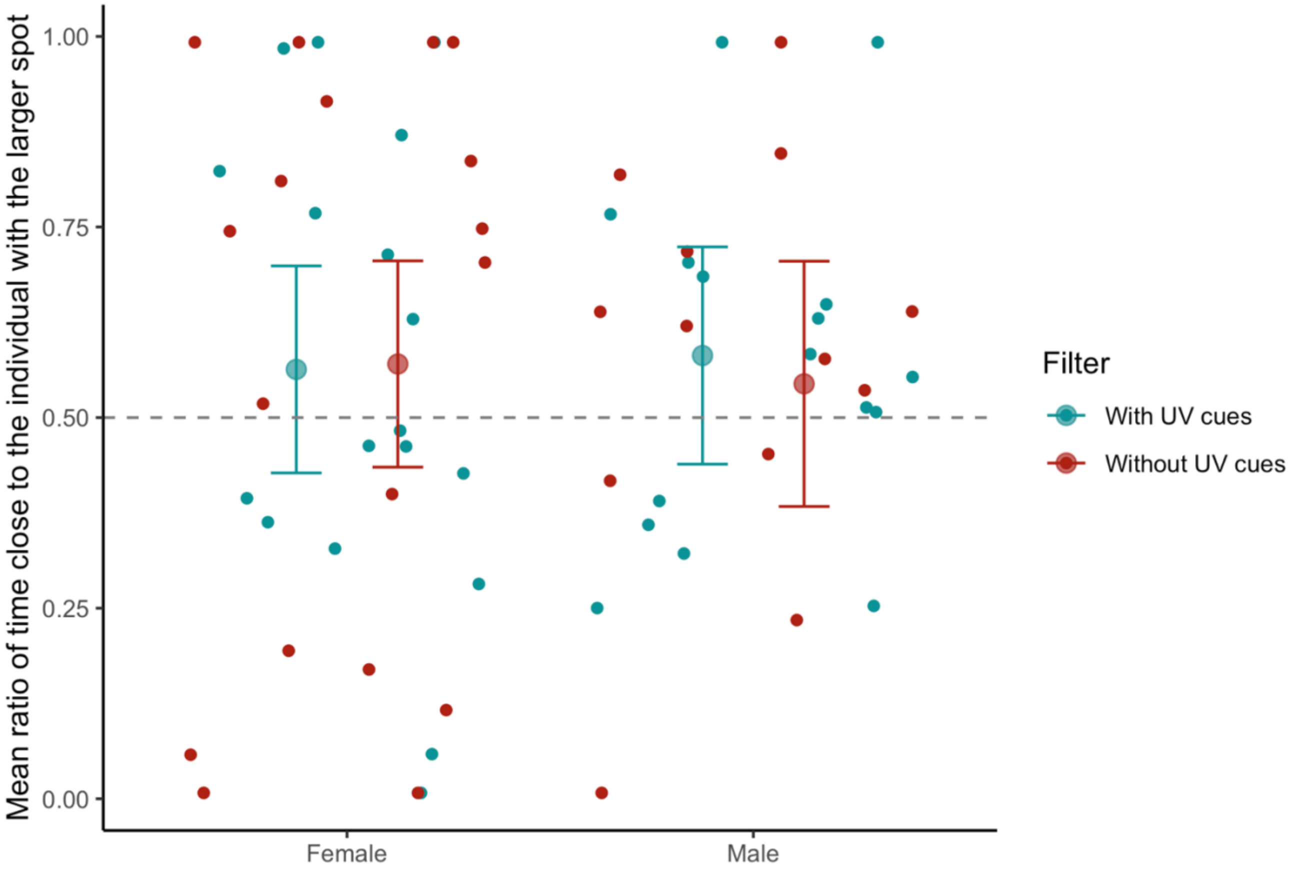

Fig. 4. Relationship between gender and the filter treatment with the amount of time spent close to the individual with the larger spot. Greater dots represent the mean value, and the bars represent the 95% confidence intervals. In the x axis we have the information for females and males. In blue is the data regarding the experiment done with the full light spectrum and in red is the data of the experiment without UV cues. In the y axis is the ratio of time the focal individual spent in the zone close to the individual with the larger spot, out of the total time in the choosing zones.

DISCUSSION

Our main objectives were to investigate if the dark spot could act as an honest signal of individual quality and if this trait was preferred by males and females when choosing their mating partners. The two proxies of individual quality investigated, body length and body fat, were positively correlated to the spot area for both females and males, although the correlation for body length was steeper for females than for males (Fig. 2 and Fig. 3). This indicates that the dark spot is, in fact, an honest signal of quality for both females and males. However, as for the mate choice experiment, we did not find a pattern of focal individuals spending on average a greater time close to individuals with the larger spot area in both treatments, with and without UV cues (Fig. 4). This indicates that the spot area does not increase the chance of being selected for copulation for both females and males.

Regarding the correlation of the dark spot and quality proxies the female correlation was isometric while, for males, it was a negative allometry. Furthermore, males have larger spot areas then females even when considering untransformed data. Since body length is a measure of individual quality, it may be that the smaller slope for the relationship between spot area and body length for males is a result of producing larger dark spots, which are known to be costly (Ducrest et al. 2008). Although the interaction of body fat and gender is not significative, the pattern of their relationship is similar to body length, in which females present a steeper correlation than males. Perhaps we did not find the interaction to be significative because the energy that could represent and increase in body fat can allow males to invest slightly more in the dark spot. As for females, overall, their dark spots are smaller than the male's, therefore and increase in body fat seem to translate into a proportionate increase in dark spot area. Since males and females are sexually dimorphic in relation to the spot area, it may be that this trait has some sexual role.

Although we found that the dark spot is an honest signal of individual quality and that the dark spot is, in fact, dimorphic, we still did not find that the spot was a criterion for mate choice. Considering that the dark spot imposes a high cost of production it is unlikely that the trait would be favored without any sort of function (Zerulla & Stoddard 2021). Also, individuals, although they do not seem to select mates, when we removed the predator pressure in our experiment, they still preferred to stay close to each other and not on the neutral zone. Since this behavior prevailed without the predator pressure, we considered three potential functions for the dark spot. The first potential function would be a role in intrasexual interactions. Since the dark spot in fact signalizes quality, individuals could use this trait to access stronger competitors and perhaps avoid contests with them. However, neither females nor have shown any type of aggressive interactions, therefore, we believe that agonistic interactions are not an important part of this species life story. The second potential function we suggest is that this trait could be used as when trying to identify a conspecific. Copulating with an individual of a different species represents a cost for the individual since it would waste time and gametes without a chance of generating any offspring. We believe that the dark spot could be a cue of conspecific identification since it is a taxonomic character of identifying the species and that fish have been documented to being able to identify conspecifics using traits (Griffiths & Ward 2011).

The third and final potential function for the dark spot is being used as a cue for a post-copulatory process. A recent meta-analysis has shown that, in several groups, the strength of sexual selection might be modulated mostly by postcopulatory processes (Macedo-Rego et al., 2024), and this is no different in poecilids. In poecilids, there are cues that postcopulatory sexual selection occurs through sperm competition (Evans et al. 2011). In sympatric environments, males may adjust their ejaculate expenditure, dispending more sperm with female conspecifics (Evans et al. 2011). For *P. vivipara*, the most common reproductive behavior is expressed by males chasing females to obtain copulas (Pers. Obs). Females run away from males, while males try to insert their gonopodium in the female gonopore, indicating that most copulas in *P. vivipara* are forced by males. Furthermore, individuals are seen in cohesive groups of males and females. Due to these behaviors, females that try to actively avoid males can pay high energetic costs due to male harassment. Therefore, females seem to have little opportunity for pre-copulatory choice and thus, investment in post-copulatory choice mechanisms can be favored in this species. In this sense, some of the pos-copulatory selection may be related to male traits that indicate individual quality, such as the lateral spot size. Since the dark spot also indicate female quality, males can use this cue to harass for longer periods females with larger spots.

An interesting result was the peak of UV reflectance on the individual's body sides and caudal fin (Fig. 1s and Fig. 4s), but not on the top of their heads (Fig. 2s). The lack of coloration on the top of their heads could reduce the chance of being detected by external predators such as birds, especially considering that predators detect UV cues (Cuthill et al. 2000). Thus, the black coloration on the top area could be related to an anti-predatory strategy. As for the lateral body sides, which include the dark spot, it could be an area conspicuous to predators such as odonate larvae and other fishes due to its UV reflection (Losey et al. 1999; Bybee et al. 2012). Furthermore, since the group of *P. vivipara* was seen in the shallow waters in which the sunlight is especially prominent, the UV fish reflectance should be enough to be detected by these potential predators. For this reason, it seems unlikely that the lateral dark spot has any anti-predatory function , thus, increasing the chances that its function relates to other mechanisms, such as post-mating choice.

In this work one of our main goals was to deal with two common biases in sexual selection studies: i) investigate traits that are non-exaggerated to the human sensory system, and ii) investigate if a conspicuous morphological trait has a sexual role on a supposedly monomorphic species. Regarding the first bias, we concluded that the non-exaggerated trait, the dark spot, is sexually dimorphic and correlated to individual quality in unexpected ways for both males and females. As for the second bias, although the black spot was not used directly in mate choice, its size was correlated to body size and body fat. Also, the trait was sexually dimorphic when considering the lateral spot area. Combined with the fact that the production of the melanic spot is costly (Zerulla & Stoddard 2021) it may be that the black spot has a sexual role, maybe in postcopulatory selection or in conspecific recognition. Therefore, by investigating an overlooked trait due to its inconspicuousness to the human sensory system, we found a potentially intricated role during reproduction.

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