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Exposure to Predators Induces Phenotypic Plasticity in Cone Opsin Expression Profiles but not in Eye Size of Female Trinidad Guppies

Chia-Hao Chang*

*Department of Science Education, National Taipei University of Education, No.134, Sec.2, Heping E. Rd., Da'an District, Taipei City 10671, Taiwan. *Correspondence: E-mail: chiahao0928@gmail.com; chiahao0928@mail.ntue.edu.tw (Chang). Tel.: +886-2-27321104#53320*

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ORCiD iD:
https://orcid.org/0000-0001-8566-3361

Teleost fish display very diverse visual systems through evolutionary adaptation and phenotypic plasticity to the variable light of aquatic environments. Whether predator presence influences eye size in prey is controversial because though larger eyes endow better optics, they also consume more energy and are more conspicuous. Moreover, whether predation alters the visual spectra of prey is unclear. Predation profoundly affects inter-populational divergence of male coloration and female mating preference in the Trinidad guppy (*Poecilia reticulata*). In this study, newborn guppies were raised under predation or control conditions, which revealed that although eye size did not differ between the treatments, predator presence induced plasticity in cone opsin expression profiles, potentially impacting female mate choice and predator sensitivity, as well as circumventing the costs of having large eyes. This study also lends support to the sensory drive model in guppy by evidencing how predation acts simultaneously on both male coloration and female visual spectra, reducing the survival of bright males, but also making them less attractive to females.

Keywords: Visual systems, Phenotypic plasticity, Female mating preference, Visual spectra, Sensory drive

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BACKGROUND

Vision is a crucial sensory system for most animals, with eyes not only sensing light intensity but also forming visual images. Rod cells in the eye are responsible for scotopic vision, whereas cone cells enable photopic vision, and their signal converting abilities come from their expression of visual pigments comprising a vitamin A-derived light-absorbing chromophore and a light-sensitive G protein-coupled receptor (visual opsin). The maximal absorbance wavelength (λmax) at which a visual pigment absorbs light depends on the amino acid sequence of the visual opsin and the type of chromophore present (Hagen et al. 2023). Rods only express one kind of visual opsin, rhodopsin (*RH*), but cones express up to four distinct visual opsin families: short-wavelength sensitive 1 (*SWS1*, λmax ranges from ultraviolet (UV) to violet); short-wavelength sensitive 2 (*SWS2*, λmax ranges from violet to blue); medium-wavelength sensitive (*RH2*, λmax green); and long-wavelength sensitive (*LWS*, λmax red) (Yokoyama 2000).

Vision profoundly mediates many fitness-related behaviors in vertebrates, including fighting, fleeing, foraging, and reproduction, with various ecological factors promoting genetic-based modifications and/or inducing phenotypic plasticity in optic structures and visual spectra. In the relatively more variable light conditions of aquatic environments, teleosts exhibit diverse interspecific and intraspecific variation in visual systems, rendering them a good model for exploring how visual perception changes in response to external stimuli (Caves et al. 2017; Cortesi et al. 2015; de Busserolles et al. 2013; Dugas and Franssen 2012; Hauser and Chang 2017; Kanazawa et al. 2020; Land and Nisson 2012). Large eyes provide better visual resolution and sensitivity (Land and Nisson 2012), so fishes that live in complex habitats or that are nocturnal, predatory, or migratory all typically have large eyes (Caves et al. 2017; Hulthén et al. 2025; Pankhurst 1989; Schmitz and Wainwright 2011). Teleost visual spectra have evolved by changing the number and/or λmax of visual opsin genes, as well as their expression profiles, thereby facilitating adaptation to distinct habitats (Cortesi et al. 2015; Lin et al. 2017; Musilova et al. 2021; O'Quin et al. 2010; Rennison et al. 2016; Ricci et al. 2023; Torres-Dowdall et al. 2017). Phenotypic plasticity further enables teleosts to adjust their visual systems rapidly, i.e., within a single generation. Water transparency, light properties, and dietary availability all contribute to phenotypic plasticity in eye size (Chang and Fuller 2020; Kröger and Fernald 1994; Kröger and Wagner 1996; McDowall and Pankhurst 2005; Tiarks et al. 2024). Although details of the molecular mechanism are still being investigated, light conditions, thyroid and sex hormones, and dietary carotenoids all influence cone opsin expression profiles to better accomplish various visual tasks, such as foraging and mate procurement (Bertinetti and Torres-Dowdall 2025; Chang 2023; Cheng et al. 2009;

Friesen et al. 2017; Sandkam et al. 2016; Schreiner et al. 2023; Shao et al. 2014; Torres-Dowdall et al. 2024; Wang et al. 2025).

Predation is one of the most influential selection pressures on prey. In teleosts, it can alter morphological characters such as fin and body shape to help prey evade capture (Arnett and Kinnison 2017; Brönmark and Miner 1992; Burns et al. 2009; Hulthén et al. 2024; Meuthen et al. 2019). However, whether having larger eyes is beneficial to prey as an antipredator defense is a controversial topic. Kikuchi et al. (2023) state that "the encounter" is the first stage of a predation sequence, so if prey observe a predator sooner, then their likelihood of survival would increase. Indeed, it has been demonstrated that predation pressure can induce some fishes to have bigger eyes or pupils (Ab Ghani et al. 2016; Meuthen et al. 2019; Vinterstare et al. 2020). Nevertheless, eyes are vital and vulnerable organs that must be protected. For instance, the Ambon damselfish (*Pomacentrus amboinensis*) has developed smaller eyes but larger false eyespots under predation pressure to reduce fatal/eye-damaging predatory attacks (Lönnstedt et al. 2013). Inter-population variation in eye size may also be related indirectly to predation. Predators regulate the population density of Trinidadian killifish (*Rivulus hartii*), with large eyes providing an advantage in intraspecific competition in low-predation populations where the killifish population density is high (Beston et al. 2017; Howell and Walsh 2023; Tran et al. 2024). However, the synergistic or offsetting effects of predation and other ecological factors such as water transparency, diet, and habitat complexity might blur the relationship between predation and eye size when analyzing field data (Andersson et al. 2024; Svanbäck and Johansson 2019).

The single and double cone cells in the teleost retina express different cone opsin genes and they are responsible for different visual tasks (Flamarique and Grebinsky 2025). For example, the elevated expression level of *LWS* genes enables herbivorous damselfishes to more easily detect algae against backgrounds (Stieb et al. 2022). Accordingly, in theory, a shift in the visual spectra could render individuals more sensitive to predators. However, unlike eye size, it has rarely been investigated if predation pressure affects the visual spectra of prey. In fish, increased predation pressure elevates cortisol levels, resulting in many physiological and behavioral changes. The cortisol signaling pathway is tightly linked to other hormones, including the sex hormones and those produced by the thyroid (McGhee et al. 2020; Zwahlen et al. 2025), so it is plausible that predators could induce phenotypic plasticity in the visual spectra of prey.

Predation by pike cichlids (*Crenicichla alta*) is a significant driving force promoting the evolution of the Trinidad guppy (*Poecilia reticulata*). The varying abundance of pike cichlids among guppy habitats in Trinidadian rivers is not only associated with the evolution of male coloration but also influences female preference. Transplanting male guppies from downstream to upstream areas results in a shift from dull to bright colors, indicating that predation shapes male guppy ornamentation (Endler 1980; Kemp et al. 2009; Magurran 1998). The co-evolution of male coloration and female mate preference has been reported previously for guppies (Magurran 2005; Schwartz and Hendry 2007). Guppies possess one *SWS1* (λmax = 353 ± 2 nm), two *SWS2* (*SWS2A*, λmax = 438 ± 0.7 nm; *SWS2B*, λmax = 408 ± 1.3 nm), two *RH2* (*RH2-1*, λmax = 516 ± 1 nm; *RH2-2*, λmax = 476 ± 2 nm), and four *LWS* (*LWS-1*/180Ser, λmax = 571 ± 0.6 nm; *LWS-1*/180Ala, λmax = 562 ± 0.6 nm; *LWS-2*, λmax = 516 ± 6.9 nm; *LWS-3*, λmax = 519 ± 2 nm; *LWS-R*, λmax = ? nm) opsin genes (Kawamura et al. 2016). The two *LWS-1* alleles in guppy, *LWS-1*/180Ser and *LWS-1*/180Ala, display distinct λmax values, and Sandkam et al. (2015) uncovered how mate preference of female guppies coevolved with male coloration based on these two *LWS-1* alleles, with the higher frequencies of *LWS-1*/180Ser in low-predation populations making females view males with more red-orange coloration as being more attractive. Sakai et al. (2018) further verified that transcription levels of *LWS-1*/180Ser are boosted, supporting the idea that females with the *LWS-1*/180Ser allele (λmax = 571) are more sensitive to long wavelengths. Overall, co-evolution of nuptial coloration and female preference in guppy represents a good example of the sensory drive model, which interprets how environmental characteristics shape the evolution trajectory of signal communication between signalers and receivers (Cummings and Endler 2018). Besides shaping the evolution trajectory, the predation pressure also evokes the phenotypic plasticity in guppies. It has been demonstrated previously that the behavior, gestational period, offspring number and size, metabolism and growth rate, size at maturity, and brain size development of guppies are all impacted by sympatry with predators (Dzikowski et al. 2004; Evans et al. 2007; Fox et al. 2024; Gosline and Rodd 2008; Handelsman et al. 2013; Ord et al. 2020; Reddon et al. 2018; Ruell et al. 2013). Collectively, these studies support that predator presence influences the hormonal system of guppies. Indeed, cortisol levels in guppies subjected to predation vary depending on sex and a given population's evolutionary history and early life experiences (Chouinard-Thuly et al. 2018; Fischer et al. 2014).

Thus, the visual system is critical to both anti-predator defense in fishes and mate choice in guppies, with predators likely affecting the guppy visual system by influencing its hormone systems. Although Chang (2025) reported that the visual spectra of female guppies do not change under predation pressure, that study did not measure eye size and the test guppies had not been reared from the larval stage. As previously discussed, the issue of whether to possess larger eyes under predation pressure remains controversial. However, the matador-like divertive antipredator strategy exhibited by female guppies suggests that the darker pigmented regions of larger eyes are more likely to attract the attention of predators (Heathcote et al. 2020). Therefore, the initial hypothesis of this study is that female guppies are unlikely to develop larger eyes when reared under conditions of predation pressure. Female guppies would also significantly benefit in terms of their own fitness if they were able to perceive predators with greater sensitivity and if they viewed bright males as being less attractive in high-predation environments, which is possible by adjusting cone opsin expression profiles. Therefore, the second hypothesis of this study is that female guppies exhibit predator-induced phenotypic plasticity in their visual spectra to enhance fitness.

MATERIALS AND METHODS

Fish collection and maintenance

Pike cichlids (*Crenicichla* sp.) of ~23 cm standard length (SL) were purchased from an aquarium store in New Taipei City. The source wild guppies (~200 adults) were caught by trap from two feral populations at Zhudong Riverside Ecological Park, Hsinchu County and Academia Sinica, Taipei City, Taiwan. Approximately 60 adult guppy or one pike cichlid were hosted in a glass rearing aquarium (60 × 30 × 36 cm, $l \times w \times h$) with an air-powered filter for water circulation. A 120-cm long full-spectrum LED tube light (380–780 nm) was placed ~28 cm above the water surface. A spectrometer (MK350N, Gamma Scientific) was used to measure the spectra of the photic conditions (Fig. S1). The temperature was kept at 22–28°C and the photoperiod was 12:12 h light:dark. Artificial guppy flakes (Tetra GmbH, Germany), frozen artemia, frozen bloodworms, freeze-dried krill (Tetra GmbH, Germany), and fresh-frozen guppy were purchased from a pet store. The pike cichlids were fed a few freeze-dried krill and 2~3 fresh-frozen guppies once per day. The guppies were allowed to breed in the aquaria and were fed *ad libitum* with guppy flakes, as well as frozen artemia and bloodworms, twice a day. Once females went into labor, they were separated into floating fish-breeding cages within the aquaria so that newborns could be collected. The parental guppy specimens in the study have been lab-raised, and at least the second generation of hybrids of the source populations. These parental guppies were housed in two glass rearing aquaria, each containing approximately 20 females and 10 males, for the purpose of breeding larvae for the experiments. Experimental protocols and specimen handling were performed with approval (112034) from the Institutional Animal Care and Use Committee (IACUC) of National Taiwan Ocean University.

Alarm substance extraction

Due to the scarcity of lab-raised guppies, the alarm substance was extracted from about 25~30 adult females (standard length (SL) = 30 to 38 mm) purchased from pet stores. Females were quickly euthanized by means of immersion in iced water and then flayed on both sides of the skin. The removed guppy skin samples were placed in 60 mL chilled deionized water and then homogenized using a mortar and pestle. Finally, the homogeneous liquid was filtered through polyester filter floss, resulting in ~45 mL alarm cue. Aliquots (1 mL) of the alarm substance were kept in 1.5 mL Eppendorf tubes and stored at -20 ˚C until use.

Experimental setup

The experimental aquaria included one large glass aquarium (130 × 30 × 36 cm) and two smaller ones (60 x 30 x 36 cm). The large aquarium is approximately twice as long as the small one. They were arranged as shown in figure 1. The large glass aquarium was positioned in front of the two smaller ones, with opaque foam-board sheets between them to block visual communication among the fish in the aquaria. A transparent acrylic sheet with 33 gaps (80 x 1 mm, length x width for each gap) was positioned in the middle of the large aquarium to create two separate zones. Thus, water flow and visibility of fish between the two zones was not restricted. All aquaria were equipped with air-powered filters for water circulation. The light conditions, as well as temperature settings, were identical to those described above for the glass rearing aquarium. During the experiment, two pike cichlids were hosted separately in one of the small aquariums and in one zone of the large aquarium. Newborn guppies from a given female were divided equally and released into the other small aquarium and the non-cichlid zone of the large aquarium. Numbers of newborn guppies involved in the experiment were recorded. The pike cichlids were fed a small amount of freeze-dried krill and 2 or 3 fresh-frozen guppies once a day. Newborn guppies were fed *ad libitum* with guppy flakes, as well as frozen artemia and bloodworms, several times a day until they reached sexual maturity. A few submergent plant and algae shrimps (*Neocaridina* sp.) were also placed in the aquaria with guppies to maintain water quality. In addition to kairomones excreted by the pike cichlids that could spread from the predator-hosting zone to the guppy-hosting zone, 1 mL of the above-described alarm substance was added to the guppy-hosting zone on Monday, Wednesday, and Friday of each week. Guppies in the small aquarium could neither see nor smell the pike cichlid. Moreover, as a control for the alarm substance treatment, 1 mL of aged tap water was added to the small aquarium at the same time as alarm substance was released into the guppy-hosting zone of the large aquarium. Overall, the guppies subjected to the predation treatment (under predation pressure) could receive both visual and olfactory stimulation from the pike cichlid, as well as olfactory stimulation from the conspecific alarm cue. In contrast, those under the control treatment (without predation pressure) sensed no predatory stimulation or injured conspecifics. The aquaria were cleaned every weekday, when ~20% of the water was replaced with aged tap water, and an ultraviolet sterilizer was operated once a week to control algal growth. The raised guppies in the experimental aquaria were checked frequently to ensure that adult males (identifiable by the possession of a gonopodium) could be removed immediately, guaranteeing that the female guppies were virgin. Due to space limitations, it is difficult to conduct multiple replicated experiments at once. Three experimental trials were conducted from 5 November 2023 to 13 June 2024, 20 June 2024 to 10 February 2025, and 10 February to 2 October 2025, respectively.

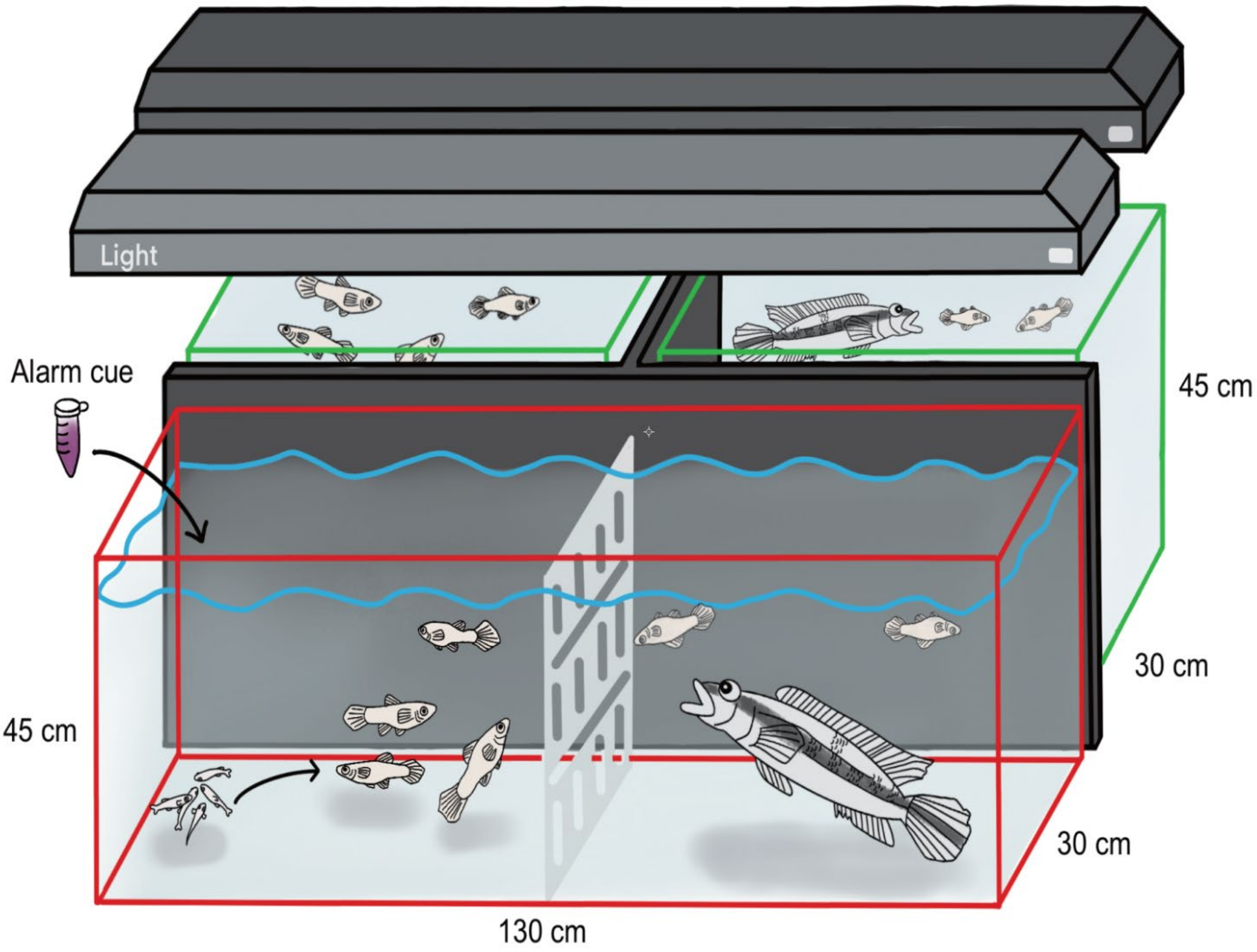

Fig. 1. The experimental apparatus included one 130-cm-long aquarium and two 60-cm-long aquaria. The large aquarium was separated equally into two zones by using a transparent acrylic sheet fitted with 33 gaps that allowed water to flow freely between the two zones. A single pike cichlid was placed in one zone of the large aquarium and in one of the small aquaria. Newborn guppies raised until adulthood in the other zone of the large aquarium, outlined in red, represented the experimental group, and those raised in the small aquarium without cichlid, outlined in green, represented the control group. Male guppies were removed when sexual characters appeared. Guppies of the experimental group could receive both visual and chemical stimulation from the predator, as well as a conspecific alarm cue, whereas those of the control group were neither subjected to visual stimulation from the predator, which was blocked from view by an opaque shield, nor chemical signals from either the predator or conspecifics.

Measurement of eye and pupil sizes and statistical analysis

In order to limit the effect of diurnal variations in opsin expression, female guppies were euthanized (0.025% MS-222) between 12:00 and 14:00 PM. After each specimen was rendered comatose, it was placed on laminated graph paper (to 1 mm) and then digital pictures were captured from above using a camera (Sony α6400) mounted on a stand. After imaging, both eyeballs of each specimen from the first two trials were removed and stored in RNAlater solution (AM7020, Invitrogen), and their caudal peduncle tissues were also removed and preserved in 100% ethanol. The specimens from the third trial were preserved in 10% formalin for future morphometric study. These eyeball and muscle tissue samples were maintained at 4˚C for 24 hours before being transferred to -80˚C until RNA/DNA extraction. Eye and pupil 2D area, as well as the SL of each specimen, were quantified from the digital images using the freeware ImageJ. Eye area, pupil area, and SL for a given specimen were measured in triplicate, with respective mean values used in subsequent analyses. Eye and pupil area relative to SL were determined to account for correlations. Two linear regressions were conducted to determine the extent to which SL predicts eye or pupil area, with the residuals from these regressions acting as measures of relative eye and pupil areas. Finally, a linear mixed regression model with a type III sum of squares using the “lmer” function of the “lme4” package in R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) was employed to test if predation treatment affected guppy visual structure. The predation treatment represents fixed effects, with experimental trial acting as a random effect. The model can be described as: relative eye area or pupil area ~ predation treatment + (1|trial).

DNA extraction and *LWS-1* genotype identification

DNA samples were extracted from caudal peduncle using a DNA extraction kit (Cat. No. GS100, Geneaid). There are two alleles of the *LWS-1* gene in the guppy genome, *LWS-1*/180Ala and *LWS-1*/180Ser, each having distinct λ_{max} values. The *LWS-1* genotype of each specimen was determined by polymerase chain reaction (PCR), which was performed in a final reaction volume of 25 µL containing 20 ng DNA, 1 µl each of the GuppyLWS1_genoF and GuppyLWS1_genoR primers (10 µM) (Table 1), 12.5 µl of Fast-Run^{TM} Advanced Taq Master Mix (ProTech, Taipei, Taiwan), and distilled water. The thermal cycling protocol was as follows: one cycle at 94°C for 4 min; 35 cycles of denaturation at 94°C for 30 sec, 56°C for 30 sec, and 72°C for 1 min; and a final single extension step at 72°C for 5 min. Sequencing was performed using the GuppyLWS1_seq primer (Table 1) by Genomics Biotech Inc., Taipei, Taiwan. After trimming, sequencing reads were aligned with human long wave sensitive opsin (NM_020061) to identify the *LWS-1* genotypes (AA, homozygous for *LWS-1*/180Ala, SS, homozygous for *LWS-1*/180Ser, or AS, heterozygous for *LWS-*

1/180Ala and *LWS-1*/180Ser) of specimens based on Ala/Ser polymorphism at the 180th amino acid residue.

Table 1. Primer sequences used to amplify β-actin, to verify *LWS-1* genotype, and to quantify expression levels of cone opsin genes in Trinidad guppy

Gene	Forward (5'-3')	Reverse (5'-3')	Reference
Gene amplification			
β-actin	CCTGTACGCTTCTGGTCGTA	CCTCCAATCCAGACAGAGTA	Chang et al. 2020
LWS-1	GuppyLWS1_genoF: TGGCACACGGTGTCTGTAAT	GuppyLWS1_genoR: ACAGGTCACAGGTCCAAAACA	Sakai et al. 2016
	GuppyLWS1_seq: GATGGCCAACCACACAGCC		
qPCR			
SWS1 (*Ei* = 94.9%)	GAGCTCCTGCGTCTACAACC	TTCCCAAACACCATTTCCAT	Sakai et al. 2016
SWS2A (*Ei* = 90.2%)	CCAAGTTTCACGGTTCAGAA	ATGAGATGTTGTCTGTGTCC	Chang 2025
SWS2B (*Ei* = 98.7%)	TCGGGAACTTTGCCTTCAAG	TCCGCATGAACACTGCATTC	Sakai et al. 2016
RH2-1 (*Ei* = 94.3%)	GCCTCGTCATGACAGTCAAA	AAGCCCAACACCATCAAGAC	Sakai et al. 2016
RH2-2 (*Ei* = 101.3%)	TGGCCATTCCTGCCTTTTTC	TCCAATTGTTGCCAGCATGC	Sakai et al. 2016
LWS-1 (*Ei* = 98.0%)	AAACAGGTGGATGATGGTTCT	CAATAAATAGTTTCTGTACAGGTCAC	Sakai et al. 2016
LWS-2 (*Ei* = 99.1%)	TGCAGCAGCTAGATAGTGAATCAA	AAAGCAGGCGAAAGTGGCA	Sakai et al. 2016
LWS-3 (*Ei* = 90.9%)	CACCATCTACAACCCTGTTAT	CTGTGCAGGTGACAGTAGTTTA	Sakai et al. 2016
LWS-R (*Ei* = 96.6%)	ACCCAGAACGCCCAGAAG	GCCGTCATCCACCTCTTTC	Sakai et al. 2016

Ei – amplification efficiency of qPCR primers.

RNA extraction, and cDNA preparation

An RNeasy Plus Universal Mini Kit (Cat. No. 73404, QIAGEN) was used to isolate total RNA from eyeballs according to the manufacturer's protocol. Four eyeballs from two sampled females were placed together in a 2-ml microcentrifuge tube containing two stainless steel beads and then homogenized using a TissueLyser II apparatus (QIAGEN). Total RNA content and quality were measured using a NanoDrop 1000 system (Thermo Fisher Scientific). RNA samples were first treated with TURBO™ DNase (Thermo Fisher Scientific), as per the manufacturer's protocol. RNase inhibitor (E0126-40D6, Lucigen) was then added, before preserving the samples at -80°C. Reverse transcription polymerase chain reaction (RT-PCR) was conducted with ~1 µg of total RNA using a Verso cDNA Synthesis Kit (Cat No. 00764129, Thermo Fisher Scientific) and Anchored Oligo-dT in a final volume of 20 µl. Quality of the resulting cDNA was checked by means of PCR with β-actin primers (Chang et al. 2020).

Cone opsin expression profiling

Expression profiles of cone opsin genes were determined by quantitative PCR (qPCR) in a Roche LightCycler480 system (Roche). Each 20 µl reaction contained 10 µl of Roche LightCycler480 SYBR Green I Master mix (Roche Applied Science), 20 ng of cDNA, and 1 µl of paired primers (10 µM) (detailed in Table 1) for the different cone opsin genes. The qPCR reactions were performed in a LightCycler 480 Multiwell Plate system (Roche) with optical adhesive film (Cat. No. 4360954, Applied Biosystems,) and the following thermal cycle: one cycle of 50°C for 2 min and 95°C for 10 min; 45 cycles of 95°C for 10 sec, 60°C for 10 sec, and 72°C for 10 sec; and then one cycle of 95°C for 5 sec and 65°C for 1 min. A melting-curve analysis was used to verify that qPCR only generated a single pure amplicon. RNA-free water was used as a template in control reactions to determine background levels of non-specific primer amplification. Three replicates were performed for each target gene for each specimen. Raw data have been submitted to Figshare (https://doi.org/ 10.6084/m9.figshare.29918462).

The visual properties of guppy specimens were determined based on proportional expression of each cone opsin gene to reveal relative abundances among transcripts, which was calculated as:

$$\frac{T_i}{T_{all}} = \frac{(1/(1+E_i)^{Ct_i})}{\sum(1/(1+E_i)^{Ct_i})}$$

where T_i/T_{all} is the proportional expression of a given cone opsin gene i, and E_i is the amplification efficiency for each pair of cone opsin primers (Fuller et al. 2004) (see Table 1).

A linear mixed regression model with type III sum of squares using the "lmer" function of the "lme4" package in R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) was employed to test if the predation treatment and *LWS-1* genotype affected the visual properties of the guppy. Both predation treatment and *LWS-1* genotype were considered fixed effects and experimental trial acted as a random effect. The model can be described as follows: proportional expression of a cone opsin gene ~ predation treatment + *LWS-1* genotype + predation treatment: *LWS-1* genotype + (1|trial). Pair-wise post-hoc tests were performed using the "emmeans" package in R.

RESULTS

In the first experimental trial, a total of 102 and 123 guppy larvae from multiple females were released into the control and predation treatment aquaria, respectively, with 51 and 22 females collected at the end of the experimental period. In the second trial, 159 and 167 larvae from multiple females were released, with 60 and 52 females then gathered from the control and predation treatment aquaria at the end of this trial. In the third trial, 133 and 137 larvae from multiple females were released, with 50 and 48 females then gathered from the control and predation treatment aquaria, respectively, at the end of this trial. *LWS-1* genotyping of 51 and 54 females raised in the control treatment of the first and second trials revealed that 1 and 5 females had genotype AA, 25 and 20 possessed genotype AS, and 25 and 29 had the SS genotype, respectively. *LWS-1* genotyping of 22 and 48 females raised in the predation treatment of the first and second trials showed that 2 and 5 females had genotype AA, 8 and 16 had genotype AS, and 12 and 27 displayed the SS genotype, respectively.

Relative eye/pupil area in response to predation pressure

After excluding specimens with spinal deformities or unclear photographs, a total of 261 females (48 specimens from the control treatment and 18 from the predation treatment in the first trial, 54 specimens from the control treatment and 46 from the predation treatment in the second trial, and 50 specimens from the control treatment and 45 from the predation treatment in the third trial) gathered from the three trials were included in the analysis to determine if predation pressure impacts eye or pupil size. Individual standard lengths (SL, mean ± SD) did not differ significantly between the control (24.40 ± 2.15 mm) and predation (24.00 ± 2.90 mm) treatments ($t = 1.0968$, $P = 0.2741$). However, SL varied significantly among the three trials ($F = 1.1234$, $P = 0.0054$), with means of 27.10 ± 2.11 mm, 24.10 ± 1.55 mm, and 22.40 ± 1.51 mm for the first, second, and third trials, respectively. Mean (plus/minus standard deviation) eye area for specimens in the control treatment were 3.28 ± 0.36 mm^2, and for the predation treatment it was 3.34 ± 0.41 mm^2. Mean pupil area of specimens in the control treatment were 0.87 ± 0.13 mm^2, and those in the predation treatment were 0.90 ± 0.16 mm^2. After standardizing eye and pupil area against SL values using residual analysis, linear regression models showed that neither the relative eye nor pupil area of female guppies is influenced by predation pressure (Table 2). Both mean relative eye area and mean pupil area were larger for individuals from the predation treatment group than the control group, but the differences are not statistically significant (relative eye area: $F = 2.7242$, $P = 0.1001$; relative pupil area: $F = 1.4922$, $P = 0.2230$) (Figs. 2, 3).

Table 2. Proportional expression levels of cone opsin genes and relative eye and pupil area as a function of predation treatment, *LWS-1* genotype, and their interaction

A: Relative eye area			
Term	*F*	*DF* (num, denom)	*P*
(Intercept)	0.0231	1, 164	0.9040
Predation	2.0267	1, 164	0.3157
B: Relative pupil area			
Term	*F*	*DF* (num, denom)	*P*
(Intercept)	0.0500	1, 164	0.8599
Predation	0.6756	1, 164	0.4123
C: Proportional expression level of *SWS1*			
Term	*F*	*DF* (num, denom)	*P*
(Intercept)	360.735	1, 44	0.019162
Predation	7.547	1, 44	0.009051
LWS-1 genotype	8.007	2, 43	0.001211
Predation × *LWS-1* genotype	2.3274	2, 43	0.110948
D: Proportional expression level of *SWS2A*			
Term	*F*	*DF* (num, denom)	*P*
(Intercept)	17.9626	1, 44	0.1125
Predation	0.5306	1, 44	0.4707
LWS-1 genotype	0.1539	2, 43	0.8579
Predation × *LWS-1* genotype	1.4564	2, 43	0.2455
E: Proportional expression level of *SWS2B*			
Term	*F*	*DF* (num, denom)	*P*
(Intercept)	210.145	1, 44	0.008101
Predation	3.9527	1, 44	0.053835
LWS-1 genotype	1.7775	2, 43	0.182488
Predation × *LWS-1* genotype	0.373	2, 43	0.691117
F: Proportional expression level of *RH2-1*			
Term	*F*	*DF* (num, denom)	*P*
(Intercept)	136.8839	1, 44	0.02921
Predation	23.8494	1, 44	<0.0001
LWS-1 genotype	1.0645	2, 43	0.35461
Predation × *LWS-1* genotype	0.2832	2, 43	0.75488
G: Proportional expression level of *RH2-2*			
Term	*F*	*DF* (num, denom)	*P*
(Intercept)	433.9807	1, 44	0.008679
Predation	1.4458	1, 44	0.236455
LWS-1 genotype	14.5892	2, 43	<0.0001
Predation × *LWS-1* genotype	0.5897	2, 43	0.559362
H: Proportional expression level of *LWS-1*			
Term	*F*	*DF* (num, denom)	*P*
(Intercept)	89.6905	1, 44	0.017147
Predation	3.6246	1, 44	0.064313
LWS-1 genotype	7.7905	2, 43	0.001429
Predation × *LWS-1* genotype	0.5452	2, 43	0.584091

I: Proportional expression level of *LWS-2*

Term	*F*	*DF* (num, denom)	*P*
(Intercept)	74.9296	1, 44	0.05166
Predation	0.3803	1, 44	0.54104
LWS-1 genotype	20.0649	2, 43	<0.0001
Predation × *LWS-1* genotype	0.427	2, 43	0.65549

J: Proportional expression level of *LWS-3*

Term	*F*	*DF* (num, denom)	*P*
(Intercept)	8.7235	1, 44	0.1971481
Predation	15.189	1, 44	<0.0001
LWS-1 genotype	19.9438	2, 43	<0.0001
Predation × *LWS-1* genotype	0.8356	2, 43	0.4412263

K: Proportional expression level of *LWS-R*

Term	*F*	*DF* (num, denom)	*P*
(Intercept)	1.4997	1, 44	0.43399
Predation	3.1334	1, 44	0.08452
LWS-1 genotype	0.1677	2, 43	0.84621
Predation × *LWS-1* genotype	1.2414	2, 43	0.30014

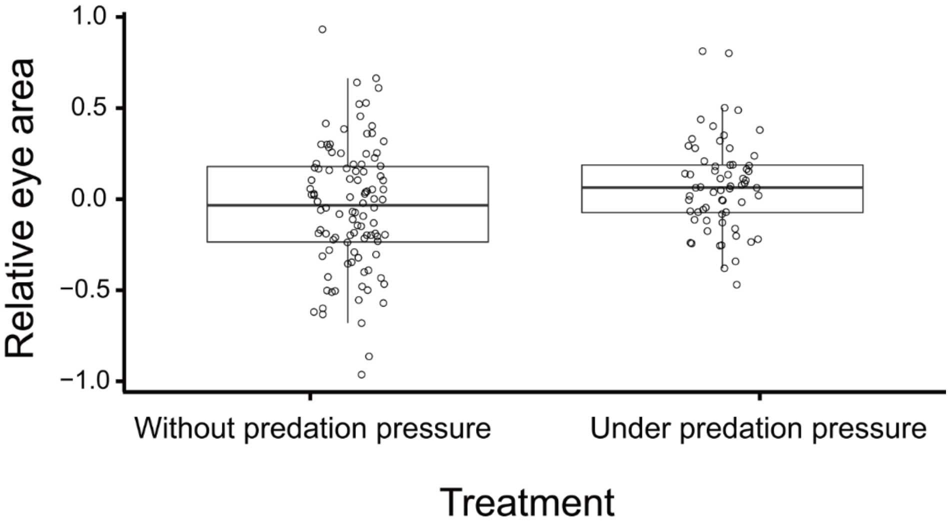

Fig. 2. Box plot showing the median (—), the 25th and 75th percentiles (box), the 95% range (|), and outliers (•) of relative eye area values for the control (without predation pressure) and predation (under predation pressure) treatments.

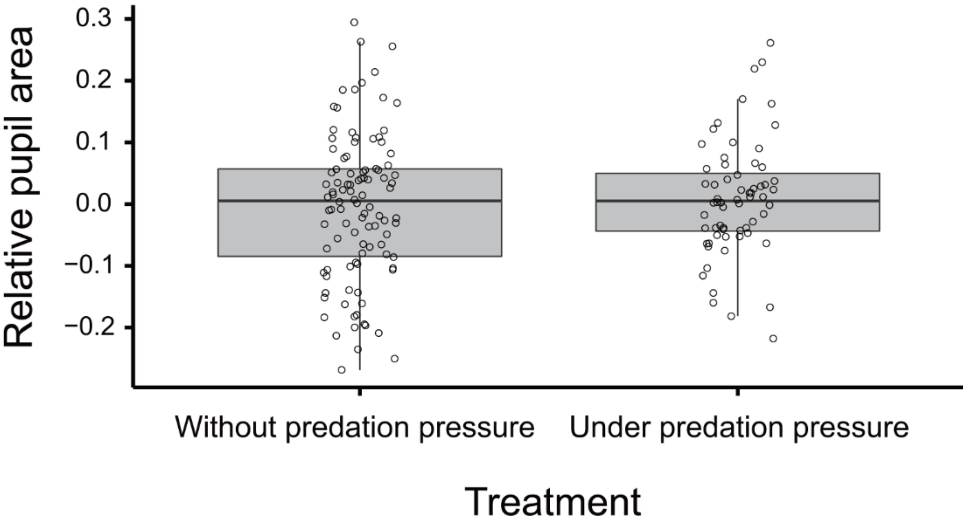

Fig. 3. Box plot showing the median (—), the 25th and 75th percentiles (box), the 95% range (|), and outliers (•) of relative pupil area values for the control (without predation pressure) and predation (under predation pressure) treatments.

Proportional cone opsin expression profiles in response to predation pressure

There were much fewer females of the AA genotype than the AS and SS genotypes. Regrettably, a problem during RNA extraction meant that many of the AA genotype specimens could not be included in the analysis. Overall, a total of 46 RNA samples were collected, including 2 samples of the AA genotype from the control treatment (0 from trial 1; 2 from trial 2), 2 samples of the AA genotype from the predation treatment (0 from trial 1; 2 from trial 2), 9 samples of the AS genotype from the control treatment (3 from trial 1; 6 from trial 2), 11 samples of the AS genotype from the predation treatment (5 from trial 1; 6 from trial 2), 11 samples of the SS genotype from the control treatment (5 from trial 1; 6 from trial 2), and 11 samples of the SS genotype from the predation treatment (5 from trial 1; 6 from trial 2).

Statistical results for mean and standard deviation (SD) of proportional expression of each cone opsin gene under the predation or control treatments are presented in Supplementary Table S1. Regardless of *LWS-1* genotypes, the top five most highly expressed cone opsins in the female guppies were *SWS1* (35.70 ± 5.29% (mean ± SD)), *LWS-1* (22.90 ± 9.28%), *RH2-1* (16.90 ± 6.05%), *RH2-2* (11.90 ± 2.53%), *SWS2B* (11.20 ± 4.04%), and *LWS-3* (1.35 ± 1.06%) in the control treatment, but they were *SWS1* (32.10 ± 4.66%), *RH2-1* (26.30 ± 4.11%), *LWS-1* (15.40 ± 8.84%), *SWS2B* (14.3 ± 2.64%), *RH2-2* (11.20 ± 2.39%), and *LWS-3* (0.57 ± 0.49%) in the predation treatment. Mean expression levels of *SWS2A*, *LWS-2*, and *LWS-R* were all less than 0.10% in both treatments (Fig. 4). The linear regression model revealed that both *LWS-1* genotype and predation pressure have an effect on cone opsin transcription. In particular, *LWS-1* genotypes affected the transcript abundances of *SWS1*, *RH2-2*, *LWS-1*, *LWS-2*, and *LWS-3*, while predation pressure significantly altered the expression levels of *SWS1*, *RH2-1*, and *LWS-3* (Table 2).

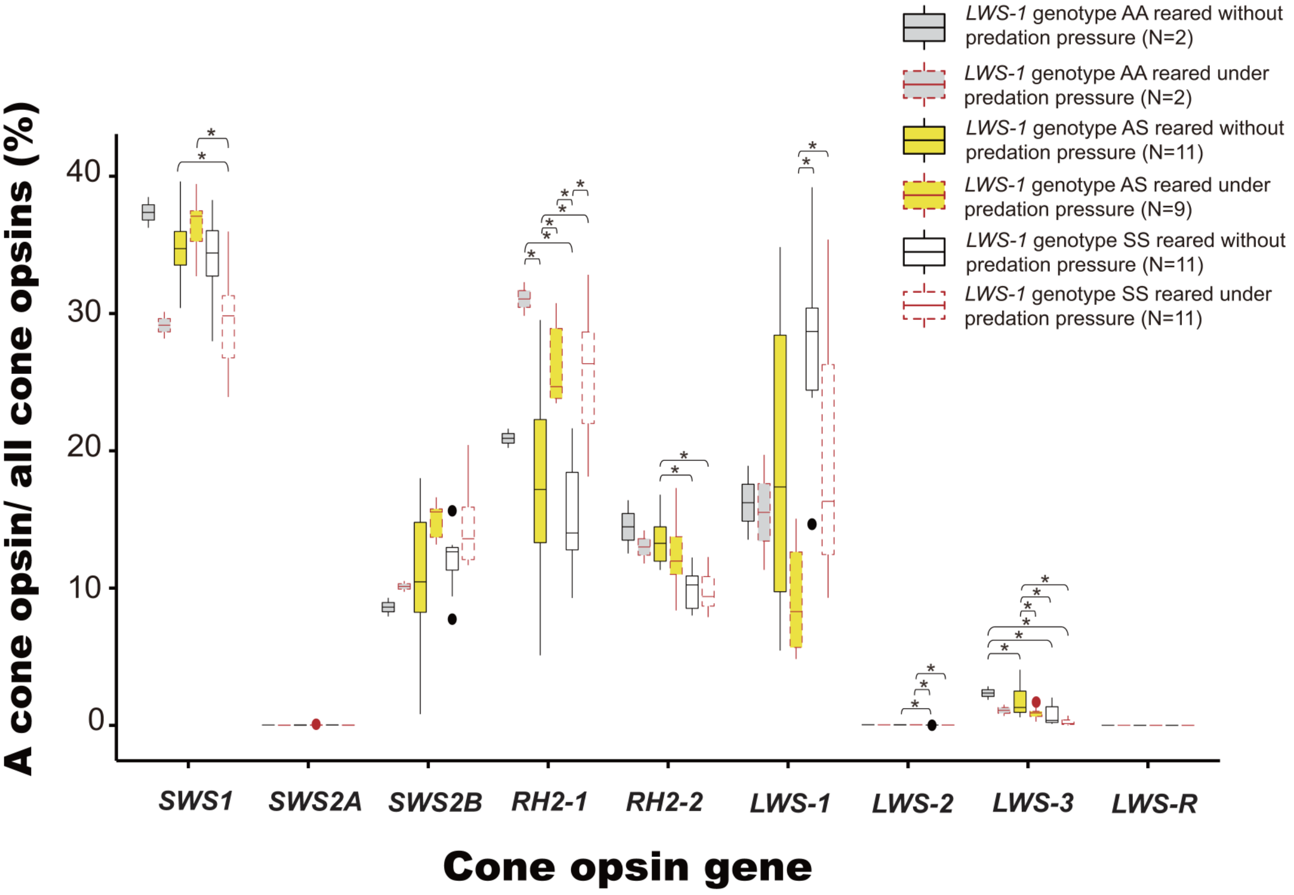

Fig. 4. Box plot showing the median (—), the 25th and 75th percentiles (box), the 95% range (|), and outliers (•) of cone opsin proportional expression for six distinct combinations of the two treatments (control and predation) and the three *LWS-1* genotypes (AA, AS, and SS). Boxes outlined in black represent the control treatment, whereas those outlined in red represent the predation treatment. An asterisk (*) indicates that the pairwise comparison is significantly different ($P < 0.05$).

DISCUSSION

Lowering activity levels and spending more time in shelter is a typical behavioral response to predation stress. Consequently, prey likely suffer from lower food intake and higher exposure to the low-light condition of shelters. Since starvation and available light are strong factors influencing the development of visual systems in fish (Bertinetti and Torres-Dowdall 2025; Bilandžija et al. 2020; Kotrschal et al. 2014; Kröger and Fernald 1994; Tiarks et al. 2024), predator presence could markedly impact the optic features of prey. The SL of female guppies from the two treatments of this study did not differ and no shelter was provided in the aquaria, so the plasticity induced in guppy visual system is directly attributable to predator presence. Although physiological attributes, such as cortisol levels, were not measured, the adult females subjected to the predation treatment exhibited clear anti-predator behavior, such as shoaling and staying near the water surface (Botham et al. 2006), indicating that they indeed sensed the cichlid predator (Supplementary video 1). In contrast, adult females of the control treatment did not display such anti-predator behaviors (Supplementary video 2). The analysis of eye and pupil area support the first hypothesis that predation does not induce female guppies to have bigger eyes. Why do female guppies not grow large eyes under predation risk? First, neural tissue is highly energy consuming and selection favors reductions in sensory modalities when they are not needed (Niven and Laughlin 2008). This “expensive tissue hypothesis” is supported by data from different morphs of Mexican tetra (*Astyanax mexicanus*), showing that a reduction in the visual system could substantially save on metabolic energy (Moran et al. 2015). Large eyes are not affordable for predator-aware guppies since they forage less (Botham et al. 2006). Second, though large eyes theoretically contribute to improved vision, the extent to which size must be increased to elicit significant benefits remains uncertain. Notably, Corral-López et al. (2017) did not detect any difference when they compared the visual acuity of two artificially selected guppy strains having different eye sizes. Predation pressure limits the foraging behavior of female guppies, and having slightly larger eyes may not enhance visual performance, so it is reasonable that female guppies would not exhibit predation-induced plasticity in eye size.

However, some drawbacks of the predation treatment in the current study may have obscured the impact of predation-induced phenotypic plasticity on eye size. The case of Trinidadian killifish demonstrated that selection favors individuals with larger eyes when intraspecific competition is intense (Beston and Walsh 2019; Tran et al. 2024). In all three trials of the study, a higher number of larvae were released into the aquarium of the predation treatment compared to the control treatment. However, at the conclusion of each trial, the number of individuals remaining in the predation treatment was consistently fewer than those in the control treatment, suggesting that the mortality rate was likely higher in the predation condition. Although competition for food may not have been a significant factor in this study, it remains unclear whether other variables, such as social interactions, could influence the fitness of individuals with varying eye or pupil sizes or induce plasticity in eye or pupil development. Notably, the study only assessed the relative eye or pupil size at the end of each trial. If individuals with smaller eyes or pupils perished during the larval or juvenile stages, they would not have been included in the analyses.

Using an alarm cue extracted from specimens of different populations, Brown et al. (2010) revealed population-specific behavioral responses, with guppies displaying a stronger response to intra-population signals. The mismatch between the source populations for specimens used to produce the alarm cue preparation and subsequent experiments in this study might explain the absence of predator-induced plasticity in eye size reported herein. Furthermore, some fishes can decipher the age of donors from which an alarm cue emanates and exhibit a more intensive anti-predator response when an alarm cue comes from individuals of the same age (Mirza and Chivers 2002; Mitchell and McCormick 2013). Guppies start to display anti-predator behavior to an alarm cue extracted from contemporaries 14 days after birth (Xia et al. 2017), but the larvae in the predation treatment of the current study behaved differently to adults, appearing to lack awareness of the predation risk from the pike cichlid (Supplementary video 3). Trinidadian killifish rather than pike cichlids are the major predator of guppy juveniles, but only pike cichlids were used in the predation treatment. In addition to issues with the alarm cue and predator species used in the current study, it is unclear how the response of the feral Taiwanese guppy population raised under artificial conditions represents the wild Trinidad guppy. Phenotypic plasticity evolves rapidly and varying degrees of predator-induced morphological plasticity have been validated in Trinidad guppy populations under distinct predation risks (Torres-Dowdall et al. 2012). The guppy likely invaded Taiwan through the pet trade (Liang et al. 2006), so feral guppies in Taiwan experienced a period of artificial selection. Thus, any predator-induced plasticity in eye size may have been lost due to genetic drift, genetic hitchhiking, and/or a pleiotropic effect during the history of selection in the pet trade. Finally, compared to other predator-induced eye/pupil size plasticity studies, the number of replicated trials in this study is relatively low, which may hinder the elucidation of the true relationship between predation pressure and relative eye/pupil size. Only in the third trial did predation pressure result in significantly larger relative pupil sizes ($F = 1.1234$, $P = 0.0054$); however, the other trials indicated no significant differences in relative eye/pupil sizes between treatments. Interestingly, individuals collected in the third trial were significantly smaller than those from the first two trials. Whether this difference in body size acted as a confounding factor that triggered the observed treatment effect remains elusive. Conducting further replicated trials using wild Trinidadian guppies will be essential to rigorously verify the definitive effects of predation pressure on eye development.

Although the high expression levels of the *LWS* and *RH2* cone opsin genes detected in adult guppies of the current study is consistent with previous analyses (Chang 2025; Ehlman et al. 2015; Laver and Taylor 2011; Sakai et al. 2018; Sandkam et al. 2015; Sandkam et al. 2016), the predominance of *SWS1* expression reported herein is novel. One possible explanation for this discrepancy is the light conditions of the experimental setup. Guppies are capable of tuning the sensitivity of their visual spectral to align with the dominant light wavelengths in their rearing environment (Sakai et al. 2018; Sakai et al. 2016). The guppies in the current study were reared under full-spectrum LED tubes. Since *SWS1* is expressed at higher levels under a broad-spectrum light environment (Chang 2025; Sakai et al. 2018), it might account for the high *SWS1* expression detected here. Among the four *LWS* paralogs, *LWS-1* presented the highest expression level, followed by *LWS-3*, with *LWS-R* having the lowest level of expression. The limited transcription of *LWS-R* may be attributable to it being a retrotransposed gene, so its expression is linked to that of its gene, *gephyrin* (*gphn*) (Chang 2022). Previous studies have reported different levels of *RH2-1* and *RH2-2* transcription. For adult guppies under control conditions, some studies have reported higher expression of *RH2-1* than *RH2-2* (Chang 2025; Ehlman et al. 2015; Sandkam et al. 2015), but the opposite has also been observed (Laver and Taylor 2011; Sakai et al. 2018; Sandkam et al. 2015). These disparities might be due to differential sensitivity to turbidity, light conditions, or population origins (Ehlman et al. 2015; Sakai et al. 2018; Sandkam et al. 2015).

Sakai et al. (2018) asserted that guppy *LWS-1* genotypes (a combination of *LWS-1*/180Ser and *LWS-1*/180Ala) are correlated with transcription levels of *LWS-1*, *LWS-2*, and *SWS2B*, likely due to the conserved locus control region (LCR) in the *SWS2-LWS* synteny regulating expression of both *SWS2* and *LWS* (Hagen et al. 2023; Lin et al. 2017). Apart from confirming an influence of *LWS-1* genotype on transcription of *LWS* paralogs in the *SWS2-LWS* synteny, this study also detected that *LWS*1 genotype affects the expression of *RH2*-2 and *SWS1*. Although *RH2* and the *SWS2-LWS* synteny are in the same linkage group, they lie far apart in the guppy genome (~28 Mb), making it almost impossible for the LCR in the *SWS2-LWS* synteny to control *RH2* expression. Furthermore, *SWS1* is not even in the same linkage group as these latter. Consequently, how *LWS-1* genotype affects *RH2-2* and *SWS1* transcription is unclear. One possible mechanism is that they share the same transcription factor, *Tbx2a*, which has binding sequences in *cis*-regulatory elements of both *LWS* and *RH2* in African cichlids (Sandkam et al. 2020), but with opposing regulatory actions, accounting for a switch between these two cone opsin genes. Therefore, an analysis is warranted of the guppy genome to uncover possible binding sequences for transcription factors near the *LWS-1*, *RH2-2*, and *SWS1* loci.

Unlike in Chang (2025) where a change in guppy cone opsin expression was not detected for adults subjected to a predation treatment for 5 weeks, the current study supports the hypothesis that predation can induce phenotypic plasticity in fish visual spectra, boosting *RH2-1* expression, but inhibiting transcription of *SWS1* and *LWS-3*. Alternatively, the distinct visual sensitivities observed between treatments might stem from the reflectance spectrum of the pike cichlid, which potentially altered sidewelling light conditions. Although the study did not quantify the spectral reflectance of the pike cichlid, if its presence alone could alter guppy cone opsin expression profiles by modifying ambient lighting, such differential expression should have been captured by Chang (2025), given that both studies utilized identical pike cichlid individual.

Although *LWS-3* is not the most highly expressed *LWS* paralog, it exhibits transcriptional plasticity in response to ontogenetic stage, sex, turbidity, and rearing light environment (Ehlman et al. 2015; Laver and Taylor 2011; Sakai et al. 2018; Sakai et al. 2016; Sandkam et al. 2015).

Moreover, gene conversion tends to occur between *LWS-1* and *LWS-3* in *Poecilia* fishes due to their genetic architecture, but this gene conversion is inhibited in the subgenus *Lebistes*, that includes guppy, with strong sexual selection for red/orange coloration (Sandkam et al. 2017). Sakai et al. (2018) also positively linked *LWS-3* expression to a female mating preference for strongly orange-colored males. In addition to red-orange male ornaments being attractive to females, UV reflectance patterns (linked to *SWS1*) in male guppies also play a vital role in female mate choice (Kodric-Brown and Johnson 2002; Smith et al. 2002; White et al. 2003). The current study demonstrates that female guppies raised under predation pressure present reduced expression of *SWS1* and *LWS-3*, potentially indicating a diminished attraction to brightly colored males.

The sensory drive model emphasizes how environmental conditions influence both signals and sensory perception, thereby promoting their co-evolution. Previous studies on guppy have focused on how *LWS-1* alleles impact visual spectra to influence female mating preference. However, unlike the sensory drive scenario of Lake Victoria cichlids, *Pundamilia* spp., in which red-shifted light conditions at various water depths are correlated with variations in *LWS* allele frequency (Seehausen et al. 2008), it cannot fully explain the specific environmental conditions responsible for the distribution of *LWS-1* alleles. If predator-induced developmental plasticity in female guppy visual spectra is also observed in Trinidadian rivers, then predation emerges as a definitive environmental factor influencing both guppy coloration and female mate choice. In populations subjected to high predation, bright males likely suffer an increased mortality rate and would be perceived as less attractive by females, thereby reinforcing the principles of the sensory drive model.

Guppies exhibit a mosaic arrangement of single and double cone cells, with a long single cone being surrounded by four pairs of double cones, each separated by a short single cone. *SWS2B* is likely expressed in long single cones, *SWS1* in short single cones, and *RH-1*, *RH2-2*, *LWS-1*, and/or *LWS-3* in double cones (Sandkam et al. 2018). Laver and Taylor (2011) described two types of double cone opsins in guppies. The L/L double cones express *LWS* opsins in both primary and accessory parts of the cells, and are common in the ventral retina. M/L double cones express *RH2* opsins in the accessory parts and *LWS* opsins in the primary parts, and they are predominant in the dorsal-central retina. M/L double cone cells have a broader luminance detection channel, which enhances a fish's detection ability and they also act as an independent spectral channel in fish color discrimination (Marshall et al. 2018). The increased transcription levels of *RH2-1* and *LWS-3* in the predation treatment group of female guppies in the current study likely indicates that these females have more M/L double cones and/or the accessory parts of the M/L double cones express more *RH2-1*. Since predator-aware female guppies shoal near the water surface, having more numerous/sensitive M/L double cones in the dorso-central retina could help them better detect pike cichlids. By altering visual spectra instead of developing large conspicuous eyes that demand high energy consumption, guppies can enhance their visual performance while minimizing predation risk. In addition to cone cells, the density and receptive field size of retinal ganglion cells have also been linked to visual acuity (Caves et al. 2017). Therefore, further anatomical investigations are warranted to determine if predation induces any alterations in the retina and, if so, whether such changes are related to visual performance. Overall, the observed shift in predator-induced visual spectra may suggest that female guppies raised under predation risk are visually more sensitive to predators but less so to bright males. However, this hypothesis remains speculative and warrants further validation through behavioral assays, such as optomotor response and female mating preference tests.

CONCLUSIONS

The visual system is essential for sensory perception in many animals. Teleosts living in the varied lighting of aquatic environments display highly diverse visual systems, with both evolutionary adaptation and phenotypic plasticity contributing to this variation in optical structures and/or visual opsin genes among and within species. The development of visual systems is sensitive to many environmental factors, but information on what effect predators have on visual systems is limited. Predation is an important ecological factor in the inter-populational divergence of Trinidad guppies. Various characters such as behavioral, morphological, physiological, and life history traits differ between populations under high-predation and low-predation regimes, with the link between male coloration patterns and female mating preferences being one of the most obvious traits diverging between these two types of populations. Properties of the visual spectra of female guppy explain why they display different preferences for male coloration in high-predation and low-predation populations.

Predation may influence the development of the visual system of prey directly via hormonal pathways and/or indirectly through inadequate food intake and varied light conditions. By raising female guppies with or without predation, the findings of the current study demonstrate that predation does not induce phenotypic plasticity in eye size but can influence cone opsin gene expression profiles. Predation pressure suppresses transcription of the *SWS1* and *LWS-3* genes, yet enhances transcription of *RH2-1*, implying that predator-aware females have a lower preference for bright males but better detection and color discrimination ability. Alteration of visual spectra rather than enlarging eye size to improve predation awareness might represent an energy-efficient solution to avoid the drawbacks of having large eyes. Previously, the sensory drive model was proposed to explain co-evolution of male coloration and female mate preference through altered *LWS-1* allele frequencies (Sandkam et al. 2018). The present study lends further support to this model by revealing that predation itself is an important environmental factor. Predators are not only more likely to prey on bright males, but they also induce phenotypic plasticity in the visual spectra of females so that they view bright males as being less attractive, thereby promoting divergence in male coloration and female mate choice between high-predation and low-predation populations.

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Authors' contributions: C.-H. C conceived the project, was responsible for experimental animal care, data collection and analysis, and prepared the manuscript.
Competing interests: The author declares that he has no competing interests.

Availability of data and materials: The original data underpinning the findings, along with the Supplementary materials of this study, have been uploaded and are accessible at doi:10.6084/m9.figshare.29918462.

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Supplementary materials

Fig. S1. Light spectra for the full-spectrum LED tube light. (download)