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How viewing objects with the dorsal or ventral retina affects colour-related behaviour in guppies (*Poecilia reticulata*)

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ABSTRACT

Visual pigments can vary across the retina in many vertebrates, but the behavioural consequences of this retinal heterogeneity are unknown. Guppies (*Poecilia reticulata*) vary dorsoventrally in visual pigments and forage both on the ground and at the water surface, exposing different retinal regions to two very different visual environments. We tested guppy behaviour towards a moving stimulus presented below or above the guppy. We used 12 different narrow-band wavelength stimuli matching each of the opsin peak sensitivities presented either at the top or the bottom of our experimental apparatus. We analysed behaviours of 50 male and 50 female guppies over 4800 trials where a moving stimulus pattern was presented to each guppy. We found that wavelength, position and speed of the stimuli influenced male and female behaviour and seems to be mediated by the long wavelength sensitive photoreceptors. Males also had stronger behavioural responses than females whereas females performed more foraging-related pecking behaviour. Our results suggest that the spatial requirement of visual tasks and their ecological context are important and appear to be partly correlated with photoreceptor arrangement in the retina.

1. Introduction

For visually orientated species, it is essential that retinal function leads to efficient processing of environmental light, which is needed to maximise survival and reproduction. Species present a high level of diversity in both retinal cells (i.e. cones, rods and ganglions) and their arrangement across the retina, showing adaptation to environmental and behavioural requirements (Hart, 2001; Temple, 2011). For example, ganglion cells show differences in retinal spatial arrangement among multiple species of Australian marsupials and reed fish that relate to habitat structure, hence maximising predator detection (Collin & Pettigrew, 1988; Litherland & Collin, 2008; Navarro-Sempere, Segovia, & García, 2018). Similarly, the variation in retinal spatial arrangement of opsins of species inhabiting different microenvironments (Litherland & Collin, 2008; Temple, Hart, Marshall, & Collin, 2010), and the changes in opsins spectral sensitivity across life stages (Shand, Hart, Thomas, & Partridge, 2002), correspond to the requirements of differences in light environment, microhabitat and behaviours. The number of cone classes (photoreceptors used for colour vision and containing specific opsins) also varies greatly between species. Vertebrate species range from having one to four different photoreceptor classes (Yokoyama & Yokoyama, 1996; Osorio & Vorobyev, 2008; Sabbah, Troje, Gray, & Hawryshyn, 2013) and vary in photoreceptor relative abundance within the retina (Hart, 2001; Peichl, 2005; Temple, 2011). The rearing light environment also affects variation in retinal proportions of cone classes (Shand et al., 2008).

The relative abundance and spatial arrangement of photoreceptors should allow species to effectively detect and discriminate coloured objects in various directions in their light environments (Bowmaker & Hunt, 2006; Hart, 2001; Levine, Macnichol, Kraft, & Collins, 1979; Osorio & Vorobyev, 2008; Price, 2017; Temple, 2011; Yokoyama & Yokoyama, 1996). Although there are known differences in cone distributions between dorsal and ventral parts of the retina that appear to be tuned to different light environments (Peichl, 2005; Reckel, Melzer, & Smola, 2001; Rennison, Owens, Allison, & Taylor, 2011), we do not know how this affects visual tasks at different visual angles. Moreover, behaviour at different viewing angles may influence the evolution and development of photoreceptor cell arrangement. For example, viewing objects above the body level will influence photoreceptor cells in the ventral retina while viewing objects below the body level will influence photoreceptor cells in the dorsal retina. To our knowledge, the effects of viewing angle and cone type spatial variation on colour-based behaviours has not been investigated under experimentally controlled conditions. Here we report an experiment investigating the effects of known dorsal–ventral retinal variation in Guppies, *Poecilia reticulata* (Rennison et al., 2011) on their reactions to coloured moving stimuli.

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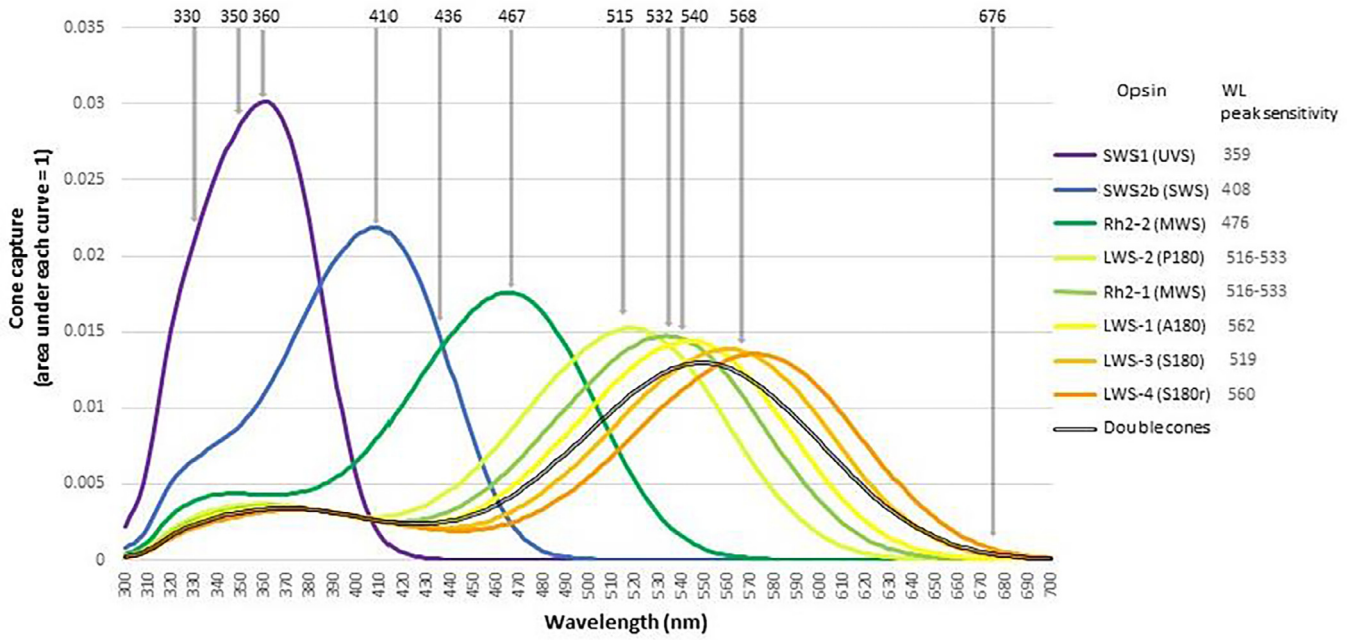

Fig. 1. Guppy opsin spectral sensitivity and narrow-band filters used to produce stimuli. Filters were selected based on Opsin peak sensitivities. Centre wavelengths of the narrow-band filters (10 nm) are indicated by the grey lines and the numbers above the lines. See Fig. S1 for the radiance spectrum of the white light stimuli. The opsin SWS2A, with a peak of sensitivity at 438 nm is not shown here because of its very low transcript abundance (Sandkam et al., 2018). Supplementary information about the opsin names are given in parentheses; these are older names in the literature.

Guppies exhibit differences in cone class distributions between their dorsal and ventral retinas (Rennison et al., 2011; Sandkam, Dalton, Breden, & Carleton, 2018), forage at the water surface as well as the stream bed, and perform diverse colour based behaviours (Houde & Torio, 1992; Houde, 1997; Cole & Endler, 2015a). This makes them an excellent model to study the possible association between retinal topography and behaviour. Guppies possess nine cone opsin genes coding photopigments, within cone photoreceptors with specific wavelengths of peak sensitivity. They possess one ultraviolet (SWS1), two short-wavelength (SWS2-A, SWS2-B), two medium wavelengths (RH2-1, RH2-2) and four long-wavelength (LWS1, LWS2, LWS3, LWS4) sensitive opsins, thus covering a broad range of the light spectrum (See Fig. 1 for details of the spectral sensitivity of each opsin, Kawamura et al., 2016; Kunstner et al., 2016; reviewed in Sandkam et al., 2018). Among the nine opsins, one of the medium wavelength sensitive (MWS) opsins (Rh2-1) is predominantly expressed in the ventral retina while the long wavelength sensitive (LWS) opsins are mainly expressed in the dorsal retina (Rennison et al., 2011; reviewed in Sandkam et al., 2018). In addition, sex-linked differences in guppy opsin distribution have been suggested, but evidence is mixed and may differ between wild and laboratory populations (reviewed in Sandkam et al., 2018). In two laboratory populations sex differences in MWS opsin (i.e., RH2-1) expression were found, while LWS-1 and LWS-3 show no sex difference in expression (Ehlman, Sandkam, Breden, & Sih, 2015; Sandkam et al., 2016). However, differences in LWS-1 were found in other laboratory populations originating from different wild populations (Laver & Taylor, 2011; Sakai, Kawamura, & Kawata, 2018). Evidence from natural populations are mixed. No evidence for any LWS sex difference was found in one wild population (Sandkam, Young, & Breden, 2015).

Guppies inhabit shallow clear water streams in tropical rainforest where they use colour vision for survival and reproduction (Endler, 1978; Houde, 1997). Depending upon foraging position or sexual displays, guppies view objects using different viewing angles in the water column (Krause & Godin, 1996). For example, guppies feed on different coloured nutrients such as algae, invertebrates, insect larvae, diatoms, and these foods differ in relative abundance on the stream bed and at the water surface (Houde, 1997; Zandonà et al., 2011). In addition to having very different visual backgrounds (gravel and litter versus sky and forest canopy), these two viewing angles result in different parts of the retina being stimulated by diverse objects. Additionally, male guppies display high colour polymorphism (Endler, 1978, 1983) which is used by females to recognise (Eakley & Houde, 2004) and assess mates (Cole & Endler, 2015; Houde & Endler, 1990; Houde & Torio, 1992). Males perform courtship displays in front of, and sometimes slightly lower than the female in our population, which would direct light towards the centre or dorsal part of the retina of the female. It is possible that this combination of behaviours and viewing angles has favoured specific cone distributions in the retina for both males and females; however, a link between behaviours, viewing angle and opsin distribution has never been tested experimentally.

To explore the possible relationship between guppy retinal topography and the visual angle of a coloured stimulus, we tested the behavioural response of male and female guppies to 12 moving narrow-band stimuli presented at the top or the bottom of the experimental apparatus, under controlled experimental conditions.

We suggest that, if behavioural responses are different for the same stimuli presented both dorsally and ventrally, then this may indicate the presence of intraretinal variation tuned to the stimulus, and/or potentially other differences in downstream visual processing. Given that guppy LWS opsins are more common in the dorsal retina, whereas RH2-1 (MWS opsin) is more common in the ventral retina (Rennison et al., 2011; reviewed in Sandkam et al., 2018), we predict that wavelengths that stimulate LWS opsins should lead to a stronger behavioural response when stimuli are presented at the bottom of the aquarium, which stimulates the dorsal retina, compared to when the same stimuli are presented at the top of the aquarium, which stimulates the ventral retina. Moreover, there should be no differences in wavelength-specific behaviour for light stimulating the cone types found evenly throughout the guppy retina. In addition, for all wavelength presented, the innate preference, or ecological relevance of colours viewed from the top or bottom in the guppies natural environment, could trigger different behaviours for different vertical positions.

2. Material and methods

2.1. Animal husbandry

We used guppies from a laboratory population descended from an established (90 + years) feral guppy population in Alligator Creek, Bowling Green Bay National park, Queensland (19°26.79′S 146°58.65′E). The fish were kept under laboratory conditions since 2011; approximately 28 overlapping generations. They were fed once a day with flake food (Tropical flake, Aqua One) and twice a week with brine shrimp (*Artemia cysts*, INVE Aquaculture). The laboratory was illuminated by high-frequency fluorescent lamps following a 12-hour light–dark cycle (see Kranz, Cole, Singh, & Endler, 2018 for details).

To control for fish age, we caught 200 fry from our stock tanks between 2 and 4 weeks of age, and separated them in a 196-litre glass tank. After one year, 100 adults: 50 males and 50 females were randomly selected for the experiment.

A week before the start of the experiment, we placed each fish into a single two litre tank. This allowed us to identify each individual and allowed the fish to acclimate to confinement. To avoid excess stress due to isolation, we aligned the transparent tanks next to one another so that fish could see the neighbouring fish.

2.2. Experimental apparatus and stimuli

Our trials investigated behaviour towards coloured stimuli projected through the front wall of the test tank onto one of two acrylic plates inclined at an angle of 13° from the horizontal and mounted on the top or bottom of a 200 * 250 * 185 mm glass aquarium (Fig. 2a). The surfaces of the two plates were covered with a sheet of laminated white chalk powder, with a flat reflectance at 85% for all wavelengths from 330 to 700 nm.

The coloured stimuli were generated by a broad spectrum Xe lamp source (300 to 750 nm, ABET technologies, Inc., Milford CT USA). Eleven 10 nm Bandpass Filters (Edmund Optics) were placed separately in the light beam. We also presented the unfiltered light as an additional stimulus; for simplicity, we refer to the unfiltered light as “white” for the rest of this paper. Nine of the filters were selected because they matched the peak sensitivity (λ_{max}) of one of the guppy opsins: 350, 360, 410, 436, 467, 515, 532, 540 and 568 nm. Two additional filters were outside of the range of guppy opsin peak sensitivities: 330 and 676, and the white light covered the entire wavelength range (Fig. 1, see Fig. S1 for the radiance spectrum of the white light).

A neutral density wheel in the light path was used to set the intensity of each colour stimulus to 6.86 ± 0.04 µmol photons m^{-1} sec^{-1} (mean ± SE; details for each stimulus in Table S1). Prior to the start of the experiment, we measured each stimulus spectrum inside the experimental apparatus with a calibrated sensor (Ocean Optics, USB 2000 + and a UV–VIS fibre optic cable and cosine-corrected receptor), and marked the position of the neutral density wheel needed for approximately identical total irradiance for each of the 12 stimuli. Thus, 12 equally intense stimuli were presented to the fish during each experimental session (Table S1).

We controlled the movement of the stimulus using a mirror attached to two stepper motors (Fig. 2a). The mirror reflected the stimulus onto either the top or bottom plate and the computer-controlled movements of the mirror allowed us to generate specific stimulus motion patterns and speeds on either plate. The motor control movements were generated by a MATLAB program (written by JAE) communicating through a USB cable to a Phidget21 stepper motor controller. The mirror was made out of finely polished aluminium (3 mm thick-6000 series aluminium alloy, mirror finish treatment), and had a flat 95% reflectance from 300 to 700 nm. To control the size of the light beam, two quartz lenses were placed upstream of the mirror (Fig. 2a). The beam of light was 4 mm in diameter on the plates.

The stimulus movement lasted for one minute in each trial. The stimulus moved at two different speeds during a trial: “fast” (17.7 mm.s^{-1}) and “slow” (3.47 mm.s^{-1}), both within the natural range of food speeds in the wild (Endler, unpublished data from multiple Trinidad streams). The stimulus movement pattern was as follows: fast movement during the first 18 s of the trial, slow movement during the next 38 s, and finally, fast for the remaining 4 s. The starting and finishing stimulus position was at the centre back of the tank (Fig. 2b. black circle). The fast movement traced a rectangle joining the two front corners and the middle of the plate. The slow movement traced a diamond shape within the rectangle (see Fig. 2b. for the illustration of the pattern). The speed and movement patterns were designed for the stimulus to cover the largest possible area on the plate, but also as a compromise between the area that the stimulus covers and the amount of time required to move at both speeds (fast: 390 mm in 18 s, slow: 132 mm in 38 s, Fig. 2b).

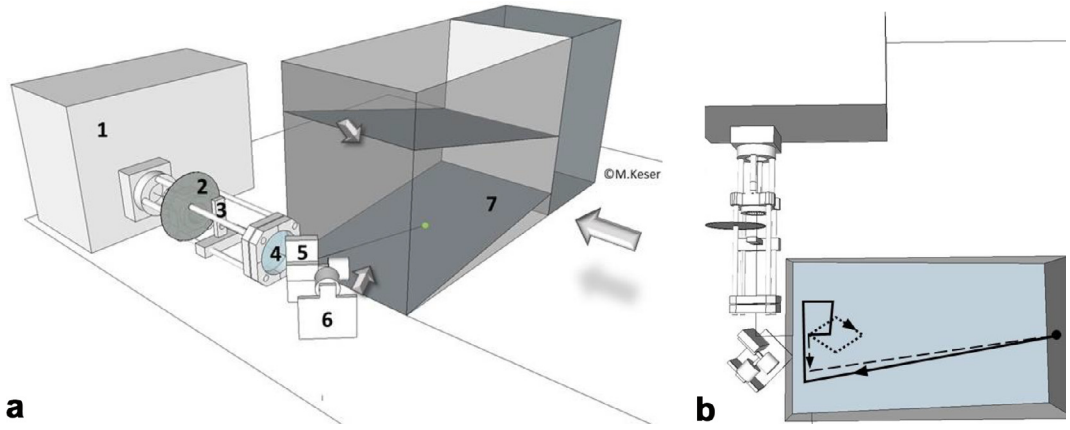

Fig. 2. Side view (2a) and overhead view (2b) of the experimental apparatus. Optical components: 1, Xe light source; 2, neutral density filter wheel; 3, narrow band filter; 4, quartz collimating lenses; 5 front-surface mirror; 6 motor and motor control electronics (connected to a computer with a USB cable); 7 lower and upper white chalk covered plates on which stimulus is projected. The stimulus in 1a is shown projected onto the lower plate. The arrows represent the cameras’ placement and orientation. The main camera faces the side of the experimental apparatus and the second camera faces the front of the apparatus, either at the top of the bottom depending on the upper or lower test position. The movement path followed by each displayed stimulus is shown in 2b: black circle: origin and arrival position for each stimulus; solid line: fast movement path at the start of the trial; dotted line: slow movement path (displayed after fast stimulus); dashed line: fast movement path at the end of the trial (displayed after the dotted line).

2.3. Experimental design

The experiment was conducted over two months. Each month 50 guppies (25 males and 25 females) were tested. An overview of the experimental design can be found in the Supplementary material, Fig. S2

Each guppy experienced four experimental sessions, one per week, over four consecutive weeks. During each session, 12 stimuli were presented sequentially to the fish, with a one-minute break between each stimulus. We used a combination of invariant and random stimuli order. In the first month, all fish experienced the same order of presentation of the stimuli in the first two weeks (invariant order: 410, 515, 330, 676, white, 540, 467, 350, 568, 436, 360 and 532 nm) and a random order in the following two weeks. In the second month, all fish experienced a random order in the first two weeks and the invariant order in the following two weeks (Fig. S2, Table S2a).

We altered the position of the stimulus between the top and bottom plates in order to stimulate differentially the lower or upper parts of the guppy's retina, respectively. The stimulus was projected on the top plate during two sessions and on the bottom plate for two sessions. We alternated the stimulus position in a Latin Square order (Fig. S2). During the first month, the stimulus was presented to the fish on the top plate on the first and third week and on the bottom plate in the second and fourth week. In the second month, the stimulus was presented to the fish in the bottom on the first and third week and the top in the second and fourth week.

We tested five males and five females each day. Each individual was tested at the same time every week because behavioural responses can vary diurnally. We defined five-time intervals of one hour during the middle of the day to limit the effects of temporal variation in opsin expression (Table S2b). Time categories were: “a” = 9:30–10:30 am, “b” = 10:30–11:30 am, “c” = 11:30 am – 12:30 pm, “d” = 2:00–3:00 pm and “e” = 3:00–4:00 pm. During the first month, at each time category, we tested a female for the first half an hour and a male for the second half an hour and reversed the sex order in the second month.

At the start of each experimental session, we placed the fish gently into the experimental apparatus for 5 min acclimation before the first colour stimulus was presented. All trials were recorded with two cameras, one on the front of the experimental apparatus (frontal) and one on the side (lateral). The trials were conducted by two investigators, A.S and M.L.K. While one investigator controlled the MATLAB program, the other recorded the behaviours of the fish. This allowed us to have a precise record of the fish movements. On the initial presentation of each stimulus, the investigator controlling the MATLAB program would say ‘start’ so that it was audible in the video replay. We fed the fish once daily with flake food after they had been in the trials.

2.4. Data collection

Because the same stimulus movement pattern and timing was used in all trials, we were able to know the exact movement of the human-invisible UV stimulus from the recorded start time. This allowed us to accurately record the behaviours directed towards the UV stimulus even though we could not see it. The fish identification label was presented to the video camera at the end of each trial, allowing us to analyse the videos blind to the fish identity.

All videos were analysed once the experiment ended and in random order. Videos from both cameras (lateral and frontal) were used to assess fish behaviours and cross-checked with the live observations. The computer monitor was set to a grey gradient when analysing the videos in order to make the observer blind to the stimulus wavelength during behaviour recording. Five wavelengths were not detectable in the videos: the UV wavelengths, 330 nm, 350 nm, 360 nm as well as 410 nm and 676 nm. For those wavelengths, a drawing of the pattern based upon a visible stimulus was made on a transparency and taped to the computer screen. We then used a timer and the auditory commentary of the video to follow the exact position of the stimulus during the entire trial and record the fish behaviour accordingly.

We used two variables to describe fish behaviour. We assessed either the propensity of the guppy to give any behavioural response or the intensity of the behavioural response. The propensity is a Boolean variable meaning that either the fish reacted to the stimulus or not. The intensity is the type of behaviour performed by the fish, estimating the degree of reaction to the stimulus. We designed and recorded six ranked categorical behaviour intensity categories based upon pilot observations. From the lowest to the highest intensity they are as follows: no behaviours (No), semi-orientation (SO), orientation (O), small movement (SM), movement (M), following (F), i.e. tracking); see Table S3 for detailed behaviour descriptions. We analysed intensity in terms of ranked categories and not as a numerical variable because it would be invalid to assume that these intensity categories fall on a linear scale. For example, we cannot affirm that movement (M) is two times more intense or costly for the guppies than orientation (O). We also recorded if the behaviours occurred during slow or fast stimulus movement, whether the fish pecked at the stimulus, and whether the fish showed any sign of stress by being still or erratic. We also recorded when the fish was looking at the mirror through the glass tank, which only happened in 0.8% of the trials and was therefore not included in further analysis.

We treated pecking behaviour differently than other behaviours because it indicates not only a strong interest in a stimulus, but also that the individual associates the stimulus with a potential food item. The other behaviours were treated separately from pecking because it is impossible to know if a given non-pecking response (e.g. orientation, movement, etc.) is due to surprise, curiosity or food-like attraction.

2.5. Data analysis

Data from 94 fish (47 males and 47 females) over 376 experimental sessions for each of the 12 wavelengths were analysed (4512 trials total). Five fish died during the final week of the experiment due to unknown causes, and one fish did not perform any behaviour during any of the trials. Consequently, we excluded these six of the original 100 fish from the analysis. For each wavelength, in half of the 376 trials stimuli were displayed at the top and half were at the bottom position. Half of the 376 trials involved females and half were males.

2.6. Statistical analysis

All statistical analyses were performed in *R* (version 4.3.3, R Core team 2013) with *R Studio* (version 0.99.8, R Studio 2016). All tests were conducted with alpha = 0.05.

Controlling the effect of time category and order of wavelength presentation on the fish behaviour

Prior to running further analyses, we tested the effects of time category and the order in which the stimuli were presented on propensity. We only tested order effects for the trials in which the stimuli were presented in random order, because when the order was invariant each order position was associated with a specific wavelength (Table S2a) and differences in responses between specific order pairs would be consistent, but not due to the order. We used a generalised linear mixed model, using glmer (R package lme4, Bates, Maechler, Bolker, & Walker, 2014), with a binomial family and individual ID as a random factor. Propensity was a binary response variable with “Y” being any behavioural response given during a trial, and “N” no behavioural response given during a trial. We used either time category or stimulus display order as the explanatory variable (fixed factors). As time category and displayed order have 5 and 12 levels respectively, we ran a post hoc analysis to control for multiple comparisons using the glht function and Holm-Bonferroni adjustment (R package multcomp, Hothorn, Bretz, & Hothorn, 2017). We found no significant effect of

time category on the propensity of guppies to display any behaviour towards any of the 12 stimuli (all $p > 0.05$, Table S4, Fig. S3a, b). We found no significant effect of the order of wavelength presentation on the propensity of behavioural response (all $p > 0.05$, except for a difference between order 4 and 5p = 0.026, Fig. S4, Table S5).

We also tested the effect of time and stimulus presentation order (for the random order) on the intensity of the behavioural response using a cumulative link mixed model clmm (R package Ordinal, Christensen, 2018) with Laplace approximation and fish ID as a random factor. CLMM models are like GLMM but allow handling of multinomial ordinal data with random effects. We used behaviour intensity as the response variable and time category or stimulus display order as the explanatory variable. We run post hoc analysis to control for multiple comparisons using the lmeans function (Tukey adjustment, R package emmeans, Lenth, Singmann, Love, Buerkner, & Herve, 2018). We did not find any significant effect of time on the intensity of guppy behavioural response to the stimulus (Table S6). We did not find any significant effect of the order of wavelength presentation on the intensity of guppy behavioural response (all $p > 0.05$, except for a difference between order 4 and 5p = 0.019, Table S7).

The absence of a significant effect of both time and order (except for one transition: less than 10% of the trials) on the fish propensity and intensity of behaviour indicates that these variables were correctly controlled in the experimental design. Consequently, we omitted those variables in the remaining analyses.

2.6.1. Effect of wavelength on the propensity of the guppy to perform any behaviour

To test for effects of wavelength on the propensity to perform any behaviour, we used a generalised linear mixed model, glmer (R package lme4, Bates et al., 2014), with a binomial family and individual ID as a random factor. We used the binary response variable with “Y” being any behavioural response given during a trial (SO, O, SM, M, F), and “N” for no behavioural response given during a trial. We used stimulus wavelengths as explanatory variables (fixed factors). We ran a post hoc analysis to control for multiple comparisons using the glht function and Holm-Bonferroni adjustment (R package multcomp, Hothorn et al., 2017).

2.6.2. Effect of sex and displayed position of the stimuli on the propensity of the guppy to perform any behaviour

To test the effect of sex (females/males) and position (top/bottom) on the propensity of the guppy to perform any behaviour towards the stimuli, we used the R function glmer with the binomial family and individual ID as a random factor (R package lme4, Bates et al., 2014). For each wavelength, the binomial behaviour was the response variable. Sex, position of the stimulus and the first order interaction between sex and position were fixed factors. We performed a backward elimination of non-significant effects of generalised linear mixed effects model and compared models using the AIC criteria. We kept the model with the smallest AIC value for each elimination step.

2.6.3. Effect of sex and displayed position of the stimuli on behavioural response intensity

To test the effect of sex and position on the intensity of the behavioural response (e.g. ranked categorical variable: orientation, movement, following, etc.) we performed cumulative link mixed models (i.e. CLMMs) using the *R* function clmm2 (package ordinal, Christensen, 2018). We used clmm2 with adaptive Guass-Hermite quadrature approximation (number of quadrature points = 3). For each wavelength, the categorical variable intensity was the response variable. The model took into consideration that the behaviour categories were ordered in terms of behavioural intensity: semi-orientation < orientation < small-movement < movement < following. Sex, position of the stimulus and the first order interaction between sex and position were the explanatory variables (fixed factors). Individual ID was added as random factor. We performed a backward elimination of non-significant effects of generalised linear mixed effects model and compared models using the AIC criteria. We kept the model with the smallest AIC value at each elimination step.

2.6.4. Effect of moving stimulus speed, sex and display position on behavioural response intensity

We tested if stimulus speed had an effect on behaviour intensity, only when fish had responded to the stimulus. Three speed categories were defined: the fish behavioural response to the stimulus was performed either during the slow stimulus speed, during the fast speed or during both slow and fast speeds. We excluded fish with no behavioural responses from this analysis because the absence of behaviour may be due to factors other than stimulus speed. We performed multinomial analyses, clmm2 (cumulative linked mixed models, R package ordinal, Christensen, 2018) for each of the 12 stimuli. For each wavelength, categorical behaviour intensity was the ordinal response variable and sex, stimulus position, stimulus speed and interaction between sex and position were explanatory variables (fixed effects). We performed a backward elimination of non-significant effects of generalised linear mixed effects model and compared models using the AIC criteria. We kept the model with the smallest AIC value in each elimination step.

2.6.5. Effect of sex, display position and speed of the moving stimuli on pecking behaviour

To assess the effect of wavelength, display position, speed and sex on pecking behaviour (food association response to the stimuli), we excluded fish with no behavioural responses from this analysis because the fish needed to show a behavioural response to the stimuli in order to peck at it. Pecking was a binary response variable with “Y” indicating that a pecking event occurred at least once during a trial, and “N” no pecking event occurred during a trial.

Wavelength effect was tested with glmer (family binomial, individual ID as random factor) and glht, Holm-Bonferroni adjustment post hoc (see previous paragraph for details). The binomial variable Pecking was the response variable and wavelength the explanatory variable.

The effect of the displayed position of the stimulus, stimulus speed and the individual sex on pecking behaviour was tested with a generalised linear mixed models, glmer (R package lme4, Bates et al., 2014), with binomial family and individual ID as random factor. For each wavelength, the binomial variable Pecking response was the response variable. Sex, position, speed of the moving stimuli and the interaction between sex and position were the explanatory variables (fixed effects). We ran a post hoc analysis to control for multiple comparisons using the glht function and Holm-Bonferroni adjustment (package multcomp, Hothorn et al., 2017). We performed a backward elimination of non-significant effects of generalised linear mixed effects model and compared models using the AIC criteria. We kept the model with the smallest AIC value.

Ethical Note

The methods adhered to the ASAB/ABS Guidelines for the Use of Animals in Research and were carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All individuals were handled with the highest care to minimise stress. During the experiment, the water quality (pH, KH and ammonia) was controlled weekly to insured the best husbandry condition for the fish. This experiment was conducted under Deakin University’s Animal Ethics Committee approval number G11-2015.

3. Results

We first describe how stimulus wavelength affected individual behaviour. Then we show tests of the effect of sex and position on (1) the propensity of the fish to perform a behavioural response (binary variable) and (2) the intensity of the behavioural response (ordered

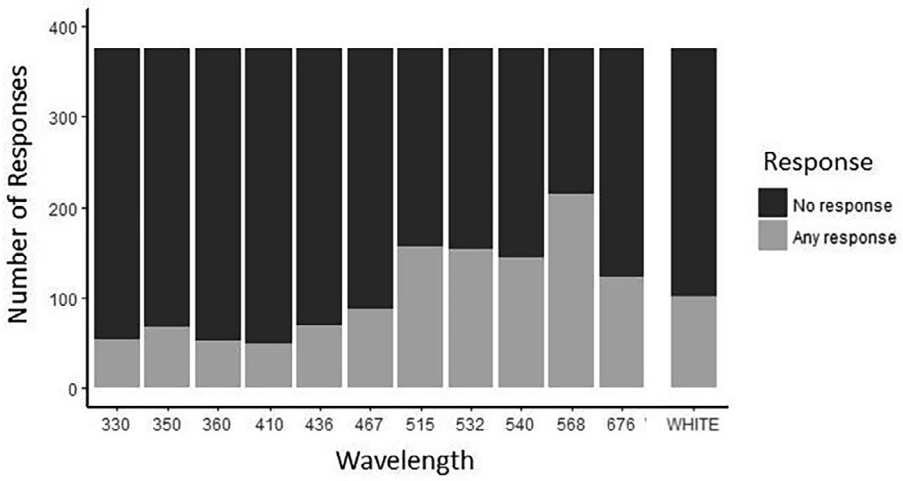

Fig. 3. Propensity of guppy to respond to the stimuli according to its wavelength. See Table S8 for results of significance tests between all pairs of colour stimuli.

categorical variable). Finally we describe the effect of sex, position and speed of the stimulus, on intensity and on a food associated response, pecking behaviour.

3.1. Effect of wavelength on the propensity of the guppy to perform any behaviour

The stimulus wavelength had a significant effect on the propensity of the guppy to display any behaviour. For wavelengths between 330 and 467 nm, the fish responded to the stimuli < 100 times (50–88) over 376 trials. For 515 to 676 nm responses were > 100 (123–215) over 376 trials. 102 responses were given for the white stimulus. For brevity, we refer to stimuli above 500 nm (515, 532, 540, 568, 676 nm) as "longer wavelengths" and stimuli below 500 nm (330, 350, 360, 410, 436, 467 nm) as "shorter wavelengths". Overall, guppies gave significantly less behavioural response to the shorter wavelengths than the longer wavelengths (Fig. 3; See Table S8 for significances and Fig. S5 for intensities at each wavelength).

3.2. Effect of sex and display position of the stimuli on propensity of the guppy to perform any behaviour

Guppies showed significantly more behavioural responses to the moving stimuli when presented at the bottom plate for half of the wavelengths tested: 330, 410, 436, 467, 515, 568 nm and white (Table 1, Fig. 4a). Males showed higher response propensity when 350, 436, 532 and 540 nm stimuli were presented (Table 1, Fig. 4b). For 350, 532 and 540 nm stimuli, there was a significant interaction between the effect of sex and position on the propensity to perform any behavioural response: males significantly increased their propensity to respond when the stimulus was presented at the bottom compared to the top plate, while position had no effect on the propensity of females to respond (Table 1, Fig. 4c).

3.3. Effect of sex and display position of the stimuli on behavioural response intensity

There was a significant effect of position on intensity of behaviour. When the 330, 410, 436, 467 and 515, and 568 nm and white stimuli were presented at the top plate, the fish performed significantly lower intensity behaviours compared to the bottom (Table 2, Fig. 5a, Fig. S6a, for details of the effect of position on categorical intensity see Fig. S6b, for details of the intensity ranked order see material and methods).

We also observed a significant effect of sex on the behavioural intensity. For 330, 350, 436, 532 and 540 nm stimuli, females performed significantly more low-intensity behaviours while males performed significantly more high-intensity behaviours (Table 2, Fig. 5b, Fig. S7a, for details of the effect of sex on the categorical intensity, see Fig. S7b). At 350, 532 and 540 nm this sex effect was led by the stimuli presented at the bottom of the apparatus (significant interaction Table 2). At 350 and 540 nm there was no significant interaction between the sex of the guppies and the behavioural intensity when the stimulus was presented

Table 1
Effect of sex and position on the propensity of guppies to give a behavioural response. Dash indicates variables removed during model selection with AIC. β is the coefficient which estimates the strength of the effect (including its sign), SE is the standard error and Z is a test statistic related to effect size. For each WL n = 376.

	Fixed effects																Random effects	
	Intercept				Position				Sex				Position:Sex				Fish ID, n = 94	
	β	SE	Z	P	β	SE	Z	P	β	SE	Z	P	β	SE	Z	P	variance	SD
330	−1.86	0.31	−5.94	< **0.001**	−1.06	0.33	−3.21	**0.001**	0.66	0.35	1.92	0.055	–	–	–	–	0.42	0.65
350	−1.85	0.32	−5.71	< **0.001**	0.00	0.42	0.00	1.000	1.13	0.38	2.97	**0.003**	−1.32	**0.58**	**−2.28**	**0.022**	0.06	0.24
360	−2.06	0.24	−8.68	< **0.001**	–	–	–	–	–	–	–	–	–	–	–	–	0.71	0.84
410	−1.53	0.24	−6.36	< **0.001**	−1.11	0.34	−3.26	**0.001**	–	–	–	–	–	–	–	–	0.30	0.54
436	−1.57	0.26	−5.95	< **0.001**	−0.83	0.28	−2.91	**0.004**	0.75	0.29	2.57	**0.010**	–	–	–	–	0.13	0.36
467	−0.73	0.17	−4.37	< **0.001**	−1.14	0.27	−4.25	< **0.001**	–	–	–	–	–	–	–	–	0.11	0.32
515	−0.19	0.27	−0.69	0.493	−1.11	0.26	−4.32	< **0.001**	0.55	0.35	1.55	0.120	–	–	–	–	1.41	1.19
532	−0.43	0.24	−1.77	**0.076**	−0.15	0.31	−0.47	0.636	1.01	0.35	2.90	**0.004**	−1.79	0.47	−3.78	< **0.001**	0.44	0.66
540	−0.60	0.23	−2.58	**0.010**	−0.20	0.32	−0.63	0.529	0.82	0.32	2.55	**0.011**	−0.93	0.45	−2.07	**0.038**	0.21	0.46
568	0.79	0.17	4.67	< **0.001**	−0.94	0.22	−4.23	< **0.001**	–	–	–	–	–	–	–	–	0.16	0.40
676	−1.00	0.20	−4.97	< **0.001**	–	–	–	–	0.40	0.27	1.49	0.136	–	–	–	–	0.43	0.66
White	−0.82	0.23	−3.56	< **0.001**	−0.77	0.25	−3.09	**0.002**	0.18	0.28	0.64	0.524	–	–	–	–	0.43	0.66

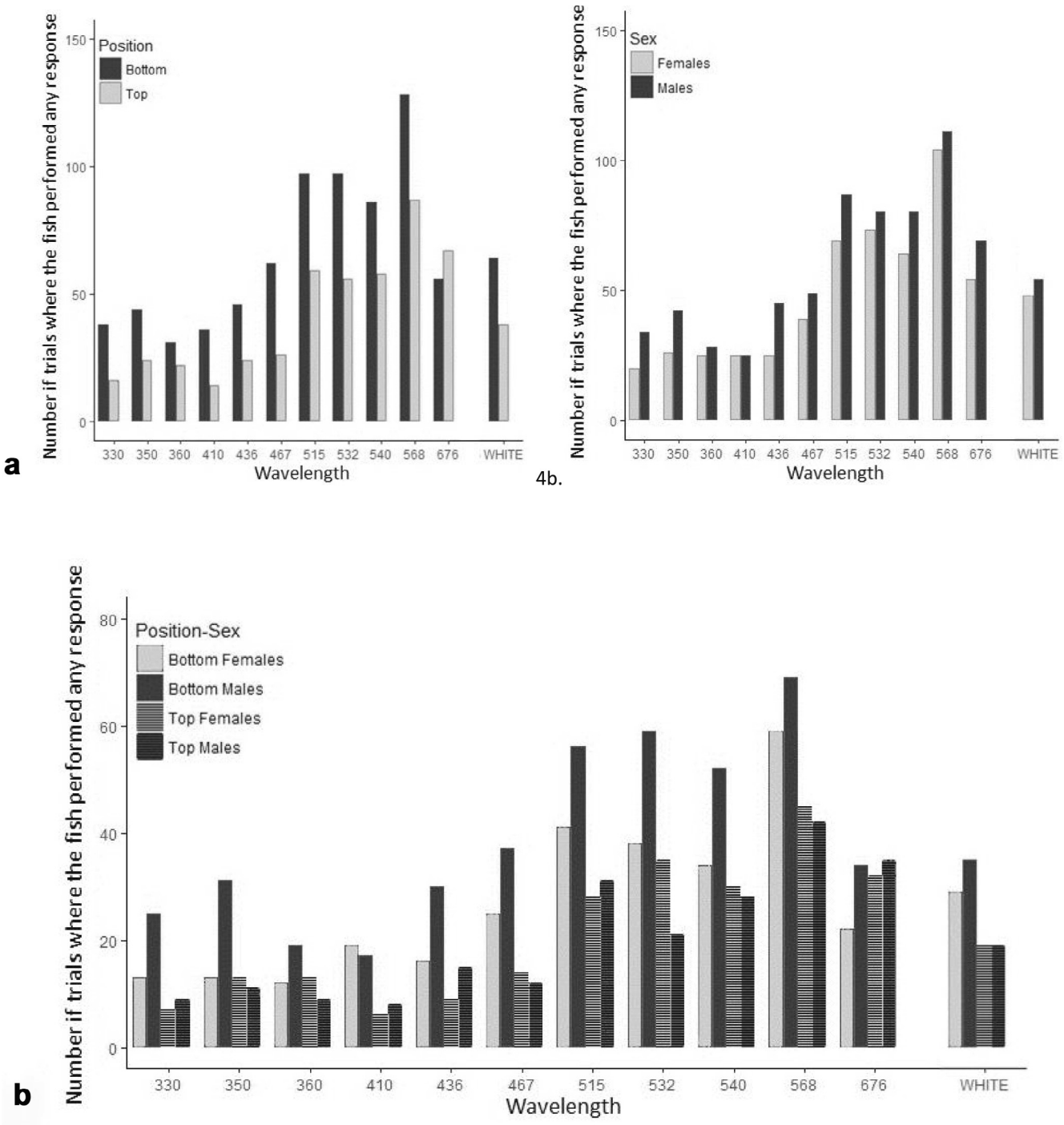

Fig. 4. Effect of position (4a), sex (4b), and their interaction (4c), on the propensity of guppies to give a behavioural response. Propensity is measured by the number of trials where guppies responded to a given stimulus.

at the top of the apparatus. At 532 nm the males tend to show a higher number of low-intensity behaviours and females higher number of high-intensity behaviours.

Fig. 5 shows an overview of the effect of position (5a) and sex (5b) on the average behaviour intensity, when intensity is considered as a numerical rather than a rank variable for illustrative purposes. No statistical analyses were performed on the numerical intensities because we cannot assume that the scale of intensity categories is linear (see material and methods). For details of the statistical effect of sex and position on the categorical intensity, see Fig. S6b and b.

3.4. Effect of moving stimulus speed, sex and display position on behavioural response intensity

Only trials where the fish performed a behavioural response were used for the analysis of the effect of speed on response intensity. The removal of “no interest” reduces the sample size and the number of behavioural response categories compared to the previous one. The speed of the stimulus had a significant effect on intensity. At 515, and 568 nm stimuli, the individuals performed higher intensity behaviour when the stimuli moved rapidly in the apparatus compared to when the movement was slow (Table 3).

Table 2
Effect of sex and position on the ranked categorical intensity of guppies' behavioural response. Dash indicates variables removed during model selection with AIC. Column symbols as in Table 1. For each WL n = 376.

	Fixed effects												*Random effects*	
	Position				Sex				Position : Sex				Fish ID	
	β	SE	Z	P	β	SE	Z	P	β	SE	Z	P	variance	SD
330	−1.03	0.33	−3.16	**0.002**	0.68	0.33	2.04	**0.041**	–	–	–	–	0.31	0.56
350	0.01	0.42	0.01	0.990	1.06	0.37	2.91	**0.004**	−1.20	0.57	−2.10	**0.035**	$1.69 * 10^{-7}$	$4.11 * 10^{-4}$
360	–	–	–	–	–	–	–	–	–	–	–	–	0.60	0.77
410	−1.08	0.34	−3.17	**0.002**	–	–	–	–	–	–	–	–	0.25	0.50
436	−0.78	0.28	−2.79	**0.005**	0.76	0.29	2.62	**0.009**	–	–	–	–	0.13	0.37
467	−1.14	0.27	−4.26	< **0.001**	–	–	–	–	–	–	–	–	0.13	0.35
515	−1.14	0.23	−5.03	< **0.001**	–	–	–	–	–	–	–	–	1.10	1.05
532	−0.29	0.30	−0.98	0.329	0.75	0.31	2.39	**0.017**	−1.44	0.44	−3.30	**0.001**	0.40	0.63
540	−0.18	0.31	−0.59	0.554	0.88	0.33	2.67	**0.008**	−1.06	0.44	−2.42	**0.015**	0.51	0.71
568	−0.78	0.20	−4.00	< **0.001**	–	–	–	–	–	–	–	–	0.31	0.55
676	0.42	0.23	1.85	0.064	–	–	–	–	–	–	–	–	0.79	0.89
White	−0.73	0.24	−2.99	**0.003**	–	–	–	–	–	–	–	–	0.47	0.68

As in Section 3.3, stimulus position and sex also significantly affected intensity. When the 360 nm stimulus was presented to the guppies, they performed significantly lower intensity behaviour when it was displayed at the bottom of the apparatus and significantly higher intensity behaviours when the stimulus was presented at the top (Table 3). When the 515 nm stimulus was presented to the guppies, they performed significantly higher intensity behaviour when it was displayed at the bottom of the apparatus and significantly lower intensity behaviours when the stimulus was presented at the top (Table 3). Also at 515 nm, the females showed higher intensity behaviour than the males (Table 3). In this analysis the significant effects of position and sex on behavioural response intensity were present for fewer wavelength stimuli, which could be due to the absence of the behavioural category "no interest".

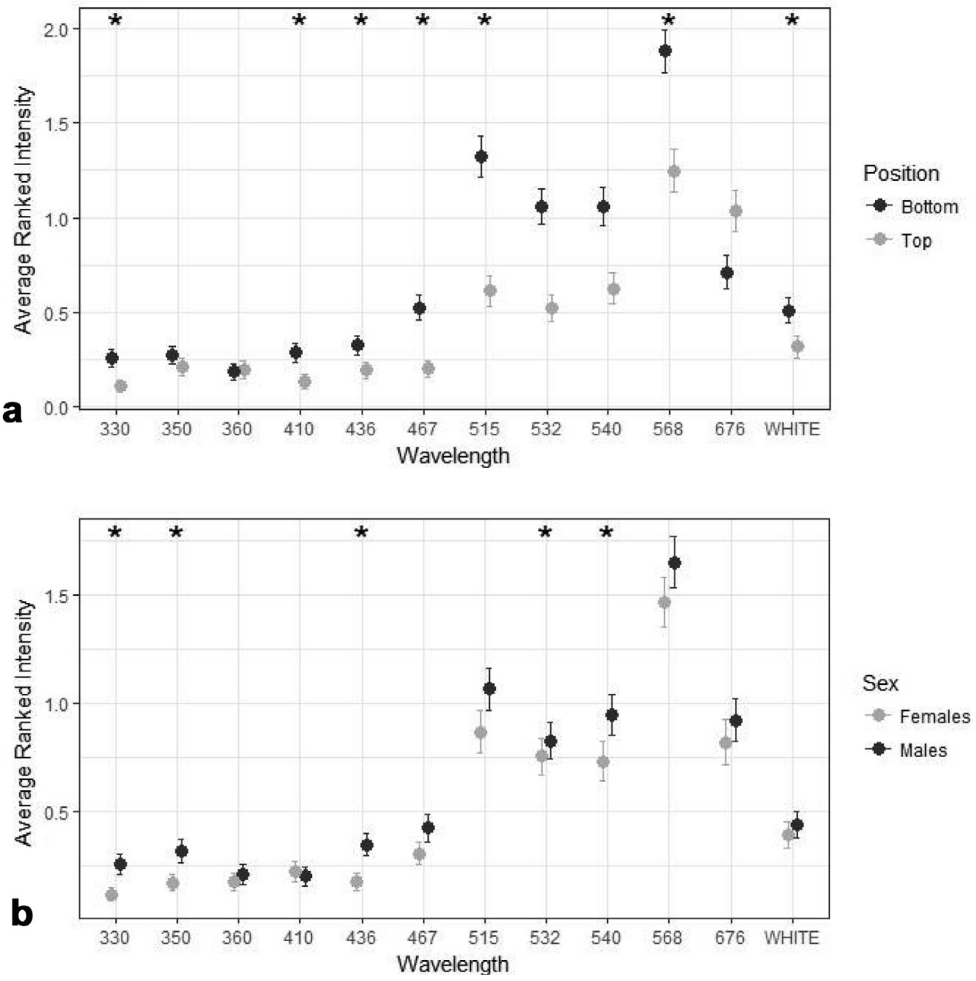

Fig. 5. Effect of the displayed stimulus position (5a) and sex (5b) on the average ranked intensity of the guppies' behavioural response when considered as a numerical variable (mean ± SE). For clarity this graph used numerical values (from Table S3) for each intensity category; 0 = no behaviour, 0.5 = semi-orientation, 1 = orientation, 2 = small movement, 3 = movement, 4 = following. We tested statistically on ranks rather than these numbers because we do not know whether the intensity categories are perceptually linear for guppies. Stars above each wavelength indicate statistical significance between bottom and top stimulus position or males and females (tests in Table 2). See Figs. S6b and S7b for categorical intensities.

Table 3
Effect of position, sex and stimulus speed on the intensity of behaviour. Specific speed comparisons are indicated on the compared column "Speed" were S = slow movement, F = fast movement, Both = both fast and slow (i.e. the fish behave during both slow and fast movement during the trial). Dash indicates variables removed during model selection with AIC. Column symbols as in Table 1.

		Fixed effects																	*Random effects*	
		Position				Sex				Position:Sex				Speed					Fish ID	
WL	n	β	SE	Z	P	β	SE	Z	P	β	SE	Z	P	β	SE	Z	P	Compared	variance	SD
330	54	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	$1.64 * 10^{-6}$	$1.28 * 10^{-3}$
350	68	0.83	0.48	1.73	0.084	–	–	–	–	–	–	–	–	–	–	–	–	–	$2.70 * 10^{-7}$	$5.20 * 10^{-4}$
360	53	1.44	0.67	2.15	**0.032**	–	–	–	–	–	–	–	–	–	–	–	–	–	0.98	0.99
410	50	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	$6.85 * 10^{-7}$	$8.27 * 10^{-4}$
436	70	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.70	0.84
467		−0.90	0.62	−1.46	0.145	−0.46	0.50	−0.93	0.353	1.90	0.91	2.08	**0.037**	−0.70	0.48	−1.47	0.142	S-F	$3.68 * 10^{-7}$	$6.07 * 10^{-4}$
	87													1.15	0.57	2.01	**0.044**	S-Both		
														−1.85	0.62	−2.97	**0.003**	Both-F		
515		−2.04	0.47	−4.35	< **0.001**	−0.90	0.39	−2.33	**0.020**	1.76	0.62	2.84	**0.005**	1.03	0.38	2.68	**0.007**	S-F	$5.32 * 10^{-10}$	$2.31 * 10^{-5}$
	154													0.59	0.46	1.28	0.199	S-Both		
														0.43	0.37	1.16	0.245	Both-F		
532		–	–	–	–	–	–	–	–	–	–	–	–	0.72	0.39	1.84	0.066	S-F	0.09	0.29
	151													1.01	0.45	2.24	**0.025**	S-Both		
														−0.29	0.37	−0.77	0.444	Both-F		
540	143	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09	0.30
568		–	–	–	–	–	–	–	–	–	–	–	–	1.15	0.42	2.73	**0.006**	S-F	0.12	0.35
	213													1.32	0.45	2.95	**0.003**	S-Both		
														−0.17	0.28	−0.61	0.541	Both-F		
676		0.59	0.39	1.50	0.133	–	–	–	–	–	–	–	–	−0.32	0.47	−0.69	0.490	S-F	1.03	1.02
	123													0.98	0.51	1.91	0.056	S-Both		
														−1.30	0.48	−2.69	**0.007**	Both-F		
White	101	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.38	0.62

3.5. Effect of sex, display position and speed of the moving stimuli on pecking behaviour

Guppies pecked significantly more at the longer (515 to 676 nm) than at the white and the shorter wavelengths (330 to 467 nm) stimuli. Guppies pecked significantly more at the 568 and 676 nm than the 515, 532, and 540 nm stimuli ($p < 0.001$, Table S9). No significant differences were found between 568 and 676 or among 515, 532 and 540 nm (Table S9).

We tested the effect of displayed position, speed of the stimulus and sex on the pecking behaviour for the longer wavelength stimuli only (515 to 676 nm). The 515 nm stimulus was pecked significantly more by females when it was displayed at the bottom of the apparatus (Table S10, Fig. S8a). The 676 nm stimulus was pecked significantly more when it was displayed at the top of the apparatus (Table S10, Fig. S8b). For the other longer wavelengths (532, 540 and 568 nm), we did not find any significant effect of the position of the stimulus or sex on pecking behaviour.

4. Discussion

We tested how the spatial display and speed of 12 narrow band wavelength moving stimuli affect male and female guppy behavioural responses. We found that (1) fish had a higher propensity to give a behavioural response toward the longer (> 500 nm) than the shorter (< 500 nm) wavelengths; (2) stimulus speed affected the behavioural response intensity for the wavelengths stimulating opsins found in the double cones; (3) behavioural spectral sensitivity was different for stimuli presented above or below the fish; and (4) there were some differences between male and female responses to the stimuli

4.1. Effect of wavelength on fish behaviour

The displayed stimulus wavelengths had a significant effect on guppy behaviour. Fish directed more behaviour towards the longer wavelength stimuli. One possible reason for this stronger response to longer wavelengths could be that guppies associate longer wavelengths with objects of interest, stimulating a positive response such as to food or potential mates. This could be innate or learned. Their food reflects mostly long wavelengths. Most algae reflect wavelengths from 500 nm to 700 nm with a trough around 670 nm and peaks of reflectance centred around 550 and 700 nm (strongly affected by the chlorophyll reflectance spectrum, Rundquist, Han, Schalles, & Peake, 1996; Gitelson, Schalles, Rundquist, Schiebe, & Yacobi, 1999). Guppies also prefer carotenoid-rich food and macroinvertebrates, which reflect wavelengths from 525 nm to infra-red. Additionally, both the flake food and brine shrimp nauplii that we fed the fish reflect more long than short wavelengths. Moreover, carotenoid-based patches on the male sexual colour pattern, strongly reflect above 500 or 600 nm (yellow or orange), are used by the females to assess male quality, and are preferred by females in some populations (Houde & Endler, 1990; Houde & Torio, 1992). However, strong response to longer wavelengths are most likely explained by foraging behaviour given that both males and females showed the same response pattern for the pecking behaviour, with significantly more pecking at longer than at shorter wavelengths. This result also matches findings from a previous foraging study in which fish responded more frequently to a long wavelength stimulus than to a short wavelength stimulus (Cole & Endler, 2015b). The stronger behavioural response for longer wavelengths supports the hypothesis that stimuli are perceived as a possible food source, which could be beneficial for the focal individual and suggests that the behaviour is ecologically relevant. Moreover, the double cones could also play a role in the behavioural responses obtained in this study because they tend to be most sensitive to longer wavelengths (Lythgoe, 1979; Campenhausen & Kirschfeld, 1998). Both foraging and double cone properties could explain the stronger response to longer wavelengths obtained in our experiment.

Double cones play multiple roles in vision (e.g. luminance, polarisation and even colour vision in some species; Osorio & Vorobyev, 2005; Pignatelli, Champ, Marshall, & Vorobyev, 2010; Marshall & Cronin, 2011) and notably tend to be involved in motion detection (Boehlert, 1978; Lythgoe, 1979; Schaerer & Neumeyer, 1996; Campenhausen & Kirschfeld, 1998). This may also explain, at least partially, the effect of the stimulus speed on the fish behavioural response for some specific wavelengths. Guppies showed more intense responses to 515 and 568 nm stimuli when they were moving at fast speed compared to slow speed. In contrast, for the other 10 wavelengths there were no differences in behavioural response intensity related to speed. The 515 and 568 nm stimuli match the peak opsin spectral sensitivity of LWS2 (estimated $\lambda_{max} = 516$ nm), RH2-1 (estimated $\lambda_{max} = 516$ nm), LWS3 (estimated $\lambda_{max} = 519$ nm) and LWS1 (estimated $\lambda_{max} = 562$ nm), respectively (Kawamura et al., 2016). Both, LWS1 and LWS3 are very common across guppy retinas and are associated with the MWS opsin RH2-1 in the double cones (Sandkam et al., 2018). The important role that double cones play in motion detection in many species (Boehlert, 1978; Lythgoe, 1979; Schaerer & Neumeyer, 1996; Campenhausen & Kirschfeld, 1998) suggests that our findings are consistent with an important role that these specific opsin-containing photoreceptors may play not only in colour vision, but also in motion detection. We infer that the multi functionality of guppy double cones translates into visual behaviours that are linked to both colour and movement.

4.2. Effect of stimulus position on fish behaviour

The display position (top or bottom) of the stimulus had a significant effect on male and female propensity and intensity to respond to the stimuli.

Surprisingly, the white and shorter wavelength stimuli (except for 360 nm) led to a higher propensity and more intense responses at the bottom plate. This result was unexpected because the opsins stimulated by these wavelengths are found homogeneously throughout the retina (Rennison et al., 2011). However, there are other possible explanations. First, the dorsal retina of guppies is very likely more sensitive to light stimulation. In their natural environment guppies experience much higher light intensity coming from the water surface through Snell's window than from the stream bed. The ventral retina could therefore be adapted to permanent stimulation by higher light intensity than the dorsal retina (Kunz & Wise, 1978). This would lead to weaker responses when any wavelength stimulus was presented at the top compared to the bottom plate. Another possibility is that moving stimuli presented at the top of the apparatus is relatively less relevant to the fish and therefore generated weaker behavioural responses. Although guppies also react to small objects falling onto the water surface in the wild, this is likely to be a much less frequent and less abundant food source than algal growth on the substrate. Finally, during courtship males from our population display in front or slightly below the females in the water column and have UV, blue, and violet coloured patches in addition to orange and yellow. Thus, it could be an advantage for females to have a high sensitivity and respond to short wavelength information displayed from below. Combined with the long wavelength male patches, short wavelength patches could give additional information about male quality, given that they are used in mate choice (Cole & Endler, 2015a).

For all longer wavelength stimuli, except 676 nm, guppies had a higher propensity to give a behavioural response, and gave higher intensity responses when the stimuli were presented on the bottom plate. The 676 stimulus response was probably weak because 676 nm is relatively far from the LWS λ_{max} so few photons are captured. As for the shorter wavelengths, foraging behaviour could explain the stronger behavioural response for stimuli displayed on the bottom plate. However, those behavioural differences for position were significantly greater for the longer wavelengths than for the shorter wavelength,

probably because guppies gave more behavioural response at those wavelengths. This result matches with our expectations that the wavelengths (515, 532, 540 and 568 nm) stimulating LWS opsins (predominantly expressed in the dorsal retina, Rennison et al., 2011; Sandkam et al., 2018) would generate a higher number and more intense responses when presented at the bottom of the experimental apparatus due to cone spatial distribution in the retina. In contrast, the MWS opsin RH2-1 was found predominantly expressed on the ventral part of the retina (Rennison et al., 2011; Sandkam et al., 2018). RH2-1 opsin has a peak sensitivity estimated at 516 nm, when measured via *in vitro* expression (Kawamura et al., 2016) and is closest to the 532 nm guppy cone cell classes identified from MSP studies (reviewed in Kawamura et al., 2016; and Sandkam et al., 2018). Consequently, we would have expected a higher propensity and a higher intensity of responses when the stimuli 515 or 532 nm were presented at the top of the apparatus. However, this did not occur. On the other hand, the LWS2 opsin shows the same peak spectral sensitivity as RH2-1 and is found predominantly on the dorsal retina. Therefore, the behavioural responses to stimulation of the LWS2 opsin by the 532 nm stimulus may mask any spatial effects of RH2-1.

4.3. Differences in behaviour between males and females

For two stimuli 532 and 540 nm, we found an interaction between sex and stimulus position on the propensity and intensity of behavioural response. Our data shows that this interaction was principally driven by males; males showed higher propensity and intensity towards the 532 and 540 nm stimuli when these stimuli were displayed on the bottom. Interestingly, Laver and Taylor (2011) suggested that differences of opsin expression between males and females occurred in LWS3 (S180) expression levels. Double cones containing MWS and LWS opsins possess λ_{max} between 525.4 and 548 and LWS-3 has a λ_{max} of 519 (Kawamura et al., 2016; see Table 1 in Sandkam et al., 2018). One possibility is that the difference between males and females arose from the sex-specific presence or absence of LWS3 in the double cones.

While males showed overall higher propensity and intensity of behavioural response towards the moving stimulus, females performed significantly more pecking than males for the 515 and 676 nm stimuli. At 515 nm females significantly pecked more at the stimuli displayed at the bottom of the apparatus. At 676 nm they predominantly pecked stimuli presented at the top, although this difference was not significant. Therefore, both position and colour of the displayed stimulus affected food-related pecking behaviour. The 515 nm stimulus could be associated to a chlorophyll rich food (because it is strongly reflected by chlorophyll) that guppies might be more likely to find on the benthic layer of the stream (Reiter & Carlson, 1986) while the 676 nm stimulus could be associated to a carotenoid-rich food which might be available in higher levels of the water column, for example rainforest insects and fruit dropping onto the water surface. Finally, there was no significant effect of the fast versus the slow speed of the moving stimuli on the pecking response. However, pecking behaviour occurred during the trials reinforcing our design for speeds being within a natural range. The lack of effect of speed on pecking behaviour suggests that guppies are adaptable to different flow rates, which vary in different parts of the same stream. Stream flow rate would be faster in wet seasons than in dry and so guppies also have to be able to respond to objects in the water in all seasons.

In conclusion, our results show that (1) different coloured stimuli illicit different behavioural responses in fish, (2) the colour, position and speed of the stimuli affect the behavioural responses of guppies and (3) these three factors influence the behaviour of males and females in different ways. Our results can be partly explained by differences in the functions and spatial arrangement of the photoreceptor cells in the retina but of course could also be potentially explained by differences in downstream visual processing in the retina and brain. The more intense behaviour of the fish towards longer wavelengths (> 500 nm) possibly reflects the function of the double cones in both colour and motion detection. Differences in the behaviour of the fish towards the different positions of the longer wavelength stimuli are consistent with spatial differences in LWS opsins in the dorsal versus ventral retina. Differences between the sexes may reflect proposed differences in opsin expression in the retina of males and females. Not all of our results can be explained by retinal physiology and probably reflect interactions between the visual system and ecological factors such as mate choice and foraging. For example, sex differences in pecking response, with males having a higher general propensity and intensity of non-pecking behaviours, while pecking behaviour was mostly performed by females, may be due to differences in foraging habits between males and females. Perhaps food is more important to females who have to mature young, and male-male interactions drives their different intensity responses to stimuli. Together these results suggest that the geometry of visual tasks and their ecological context are at least partly associated with the photoreceptor layout in the retina.

5. Data availability

The raw data from this study will be available in the data repository in Brief.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.visres.2019.02.007.

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