

The fish eye view: are cichlids conspicuous?

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SUMMARY

The extent of animal colouration is determined by an interplay between natural and sexual selection. Both forces probably shape colouration in the speciose, rock-dwelling cichlids of Lake Malawi. Sexual selection is thought to drive male colouration, overcoming natural selection to create conspicuous colour patterns via female mate choice and male–male competition. However, natural selection should make female cichlids cryptic because they mouthbrood their young. We hypothesize that as a result of both sexual and natural selection, males will have colours that are more conspicuous than female colours. Cichlid spectral sensitivity, especially in the ultraviolet, probably influences how colours appear to them. Here we use simple models of the trichromatic colour space of cichlid visual systems to compare the conspicuousness of male and female nuptial colours of nine species. Conspicuousness of colours was evaluated as their Euclidian distance in colour space from environmental backgrounds and from other colours on the same fish. We find in six of the nine species that breeding males have colours that are statistically more conspicuous than female colours. These colours contrast strongly with each other or with the backgrounds, and they fall within a range of spectra best transmitted in the habitat. Female colour distances were sometimes smaller, suggesting that females of some species are more cryptic than males. Therefore, selection can differentially act to generate male colours that are more conspicuous than those in females. However, in two species, females had colours that were more conspicuous than male colours, suggesting that other selective forces and possibly sexual conflicts are acting in this system.

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INTRODUCTION

Animal colouration depends on an interplay of selective forces. Sexual selection generates conspicuous individuals, often males, that can more effectively attract mates or compete for territories. However, natural selection prevents males from becoming too conspicuous to predators (Endler, 1991; Endler, 1992; Endler, 1993) and should result in cryptic colour patterns on females that are responsible for brood care (Andersson, 1994; Wallace, 1889). Therefore, optimal colouration is predicted to be different for males and females depending on the relative strengths of natural and sexual selection.

The cichlids of Africa's Great Lakes provide a remarkable example of rapid speciation. In Lake Malawi, 500–1000 cichlid species have evolved within the last million years (Konings, 2007; Kornfield and Smith, 2000; Turner et al., 2001). Several lines of evidence suggest that sexual selection has played an important role in this diversification (Dominey, 1984). Closely related species in Lake Malawi differ primarily in male nuptial colouration (Albertson et al., 1999; Allender et al., 2003; Danley and Kocher, 2001). Visual cues are sufficient to allow female cichlids to choose conspecific mates over heterospecifics in behavioural experiments (Jordan et al., 2003; Kidd et al., 2006; Seehausen and Van Alphen, 1998). In addition, females of at least one cichlid species in Lake Malawi prefer males with colours that are more saturated and therefore often produce greater colour contrast (Pauers et al., 2004). Nuptial colouration is also important in the aggressive interactions of territorial males (Dijkstra et al., 2006; Dijkstra et al., 2005; Pauers et al., 2008; Seehausen and Schluter, 2004). Thus, both forms of sexual selection, female mate choice and male–male competition, may have driven evolution of male nuptial colouration in these fish.

In cichlids, male colour is dependent on breeding status. The majority of males in a population have colour patterns identical to females. However, when males become ready for breeding, they become territorial and develop a nuptial colour pattern that is quite different from the colouration of juveniles and females. (In this paper, we use the term *male colour* to refer to male nuptial colours and not the juvenile, non-territorial male colour patterns that are similar to those of females.) In contrast to breeding males, females are considered to be typically dull in colouration (Konings, 2007). All Malawi cichlids are maternal mouthbrooders. Females hold the eggs and developing fry in their mouths for the first 3 weeks (Keenleyside, 1991). There are a significant number of predatory cichlids which search out brooding females and ram into them, forcing them to release the eggs or fry (Barlow, 2000). Therefore, females benefit from cryptic colouration, in a manner similar to female birds sitting on a nest (Andersson, 1994; Wallace, 1889).

Sexual selection favours signals that are conspicuous to the viewer under the environmental conditions at the time of communication (Endler, 1992). To be conspicuous, a colour pattern must differ substantially from the background in colour, brightness or patch size (Endler, 1978). To the human eye, male cichlids certainly appear more brightly coloured than the females of most Lake Malawi species. However, several factors affect how colour signals and viewing backgrounds appear to a viewer such as a cichlid. These include the viewer's visual sensitivity, the spectral properties of the fish and its background, and the light which illuminates them.

The visual sensitivity of cichlids has been extensively characterized. Cichlids have seven cone opsin genes. There is little variation in opsin sequences among the rock-dwelling species of Lake Malawi (Carleton and Kocher, 2001; Spady et al., 2005), but differential expression of the seven opsin genes does result in

substantial variation in cichlid spectral sensitivity (Parry et al., 2005). Quantitative analysis of mRNA levels (Carleton, 2009; Hofmann et al., 2009) indicate that all of the species in the current study predominantly express only three opsin genes as adults. Each species expresses both *RH2B* and *RH2A α* and either *SWS1* or *SWS2B* (Table 1). The *RH2* genes are expressed in double cones and the *SWS* genes in single cones (Carleton et al., 2000; Jordan et al., 2006; Levine and MacNichol, 1979; Parry et al., 2005). The spectral properties of the visual pigments from *Metriaclima zebra* have been measured by protein expression and reconstitution *in vitro* with 11-*cis*-retinal (Parry et al., 2005). The measured absorbances (λ_{\max}) were identical or very similar to the λ_{\max} determined by microspectrophotometry (MSP) in five of the species included in this study (Table 1). In all five species, double cones were similar, usually consisting of one cone cell containing a visual pigment with λ_{\max} near 480 nm and a second cone with a visual pigment with λ_{\max} near 530 nm. In three species, single cone pigments were ultraviolet sensitive, whereas the remaining two species had violet-sensitive single cones (Carleton et al., 2000; Jordan et al., 2006; Levine and MacNichol, 1979; Parry et al., 2005). Based on these results, we conclude that rock-dwelling cichlids of Lake Malawi have one of two basic types of colour vision systems that substantially differ only in short wavelength sensitivity.

The spectrum of light reflected from a surface such as a fish or rock depends on several factors, two of the most important being the reflectance spectrum of the surface and the spectrum of light illuminating it. In aquatic environments, the illuminating spectrum is a function of the water properties and depth. As light travels through a column of relatively clear water, it generally becomes bluer because longer and shorter wavelengths are attenuated effectively. Lake Malawi is extremely clear (Muntz, 1976), but only preliminary spectral data of its waters have been published (Hofmann et al., 2009).

Male colour in numerous species of mbuna – the brightly coloured, rock-dwelling group of cichlids in Lake Malawi – was characterized by McElroy et al. (McElroy et al., 1991) and Deutsch (Deutsch, 1997). Both studies found that colour diversity seemed to occur within a limited set of hues. Blue, yellow, black and white were the most common colours. No fish were predominantly green,

red or magenta, although these colours were observed in patches or regions of a few species (McElroy et al., 1991). Although male colours seem to be drawn from a restricted palette, they appear to evolve with little phylogenetic constraint, given that they vary as much between congeners as they do between species of different genera (Deutsch, 1997; McElroy et al., 1991). There is also frequent and independent acquisition of similar male colour patterns both within and across genera (Allender et al., 2003). Because the characterizations of male mbuna colours by McElroy et al. (McElroy et al., 1991) and Deutsch (Deutsch, 1997) relied upon photographs and analytical methods based on human colour perception, they rest on the assumption that fish and human visual sensitivities are similar and that photographs accurately reproduce the appearances of fish colours.

In this study, our goal was to compare the conspicuousness of male and female mbuna colours in their environment, from the perspective of cichlid visual systems. In the evolution of colour, sexual selection and natural selection tend to be opposing forces, the former favouring conspicuous colouration and the latter, crypsis (Endler, 1992). Because sexual selection has long been thought to drive sexual dichromatism and speciation in cichlids (Dominey, 1984; Kocher, 2004; Seehausen et al., 2008), we expected males to be more conspicuous than females. Depending on viewing distance, conspicuousness may result from chromatic contrast between a fish colour and the background or between colours on an individual fish. We therefore asked whether male colours differ more than female colours from backgrounds and whether there is more colour difference within the bodies of males compared with females. In addition, we examined whether conspicuousness varies with water depth. Because brightness of colouration is easily affected by fish handling during measurements, we have ignored luminance differences and concentrated on colour, or hue, in visual signalling.

MATERIALS AND METHODS

Animals

Fish were collected at depths less than 10 m from either the southern end of Thumbi West Island or the east side of Otter Point near Cape Maclear, Malawi. The research included four species of *Metriaclima* {*Met. aurora* (Burgess 1976), *Met. callainos* (Stauffer and Hert

Table 1. Lens transmission and peak absorbances of the dominant cone types in the nine cichlid species studied

Species	Lens T50	λ_{\max}			
		SWS1	SWS2B	RH2B	RH2A
<i>Metriaclima zebra</i> (expressed)		368	423	484	528*
<i>Cynotilapia afra</i>	359	X (358)		X (472)	X (525)
<i>Labeotropheus trewavasae</i>	359	X		X	X
<i>Metriaclima aurora</i>	364	X		X	X
<i>Metriaclima callainos</i>	364.5	X		X	X
<i>Metriaclima livingstonii</i>	362	X (364)		X (473)	X (526)
<i>Metriaclima zebra</i>	360	X (368)		X (488)	X (533)
<i>Melanochromis auratus</i>	391.7		X (414)	X (482)	X (525)
<i>Melanochromis</i> 'black and white' <i>johanni</i>	357.5	X		X	X
<i>Melanochromis heterochromis</i>	402		X (418)	X (485)	X (534)

T50, wavelength at which transmission reached 50%; λ_{\max} peak absorbance.

Top row shows the peak absorbance of pigments generated by heterologously expressing *Metriaclima zebra* opsin genes [data from Parry et al. (Parry et al., 2005)].

Subsequent rows: X indicates which of the four opsin genes are predominantly expressed by each species, as determined by RT-PCR (Hofmann et al., 2009). Available MSP data are provided in parentheses.

M. heterochromis (previously *M. vermillionis*) MSP data are from Parry et al. (Parry et al., 2005), *M. zebra* SWS1 and RH2 MSP results are from Carleton et al. (Carleton et al., 2000) and Levine and MacNichol (Levine and MacNichol, 1979), respectively, and the remaining MSP data are from Jordan et al. (Jordan et al., 2006). All measurements are in nm. *The λ_{\max} was 528 nm for the expressed RH2A α pigment. Although RT-PCR methods did not distinguish between transcripts of the α and β paralogues of the RH2A gene, MSP results suggest that α is the predominantly expressed paralogue.

1976) [redescribed by Stauffer et al. (Stauffer et al., 1997)], *Met. livingstonii* (Boulenger 1899) and *Met. zebra* (Boulenger 1899)}, three species of *Melanochromis* [*Mel. auratus* (Boulenger 1897), *Mel.* “black and white” *johanni*, and *Mel. heterochromis* (Bowers and Stauffer 1993) (see Bowers and Stauffer, 1993), formerly *Mel. vermivorus*], *Labeotropheus trewavasae* (Fryer 1956), and *Cynotilapia afra* (Günther 1894). The male *L. trewavasae* had ‘red’ dorsal fins and the females were the orange blotch (OB) morph (Streelman et al., 2003). Sampled males had the nuptial colouration characteristic of territorial males rather than the juvenile, sub-dominant colouration. All procedures involving handling and measurement of live specimens were made in accordance with approved IACUC procedures.

Spectral measurements

Environmental spectra were measured using Sub-spec II, a submersible fibre-optic spectrometer based on an Ocean Optics USB2000 (Dunedin, FL, USA), fitted with a 50, 100 or 400 μm fibre, and calibrated with a tungsten halogen lamp (LS-1, Ocean Optics). Downwelling and sidewelling irradiance measurements were taken as a function of depth at three different locations: the south side of Thumbi West Island near Mitande Point (latitude $14^{\circ}1'23''\text{S}$, longitude $34^{\circ}49'27''\text{E}$), the east side of Otter Point (latitude $14^{\circ}2'17''\text{S}$, longitude $34^{\circ}49'22''\text{E}$) and Zimbabwe Rock (latitude $13^{\circ}57'53''\text{S}$, longitude $34^{\circ}48'8''\text{E}$). The first two locations had maximum depths of 15 m. Zimbabwe Rock, where the water was generally clearer, is a deeper site with maximum depth up to 40 m. At Thumbi West irradiance was measured at depths of 1, 3, 7 and 10 m. A cosine corrector (CC-3, Ocean Optics) was attached to the end of the optical fibre when measuring irradiance. Spacelight radiance was also measured at some of these depths using narrow acceptance angle probes (full angle 7 deg or 20 deg) that was attached to the collection fibre and directed horizontally into open water. For the background comparisons we used the spectra of the space light at depths of 3 m and 7 m measured at Thumbi West ($S_{3\text{m}}$ and $S_{7\text{m}}$, respectively).

The substrate backgrounds we measured were typical of the rocky habitat in which these species live. The rocks were invariably covered with a thin algal substrate often with an additional layer of fine flocculent material derived from fish excrement. Substrate reflectance was measured underwater at several different locations. Four representative substrate spectra were then selected for colour comparisons: the algae-covered brown rock (B_{rock}) and three different excrement-covered rock backgrounds ($E_{\text{rock},1}$, $E_{\text{rock},2}$ and $E_{\text{rock},3}$). Reflectance spectra were obtained by measuring the substrate reflectance compared with a white Teflon standard placed at the same location. Either downwelling light or a high intensity quartz-halogen lamp (Light and Motion, Monterey, CA, USA) were used to illuminate substrates.

Spectral reflectance was measured on live specimens, typically within 2 h of capture. We did not anesthetize fish, as cichlid colours are neurally controlled (Muske and Fernald, 1987) and therefore modified by anaesthesia. Fish were illuminated at 45 deg to their surface with either a quartz halogen bulb or a pulsed xenon lamp (PX-2, Ocean Optics). Reflected spectra were collected either at 90 deg with a 400 μm optical fibre, imaged through an ultraviolet (UV) transmitting Nikon lens (focal length 105 mm), or with a bifurcated optical fibre (Ocean Optics). Using the bifurcated fibre, light was collected on the same axis at which the illuminant exited, and thus both illumination and reflectance occurred at 45 deg to the fish. Spectra were measured with an Ocean Optics USB2000 spectrometer. Colour patch reflectance was determined by

comparison with a Spectralon diffuse reflectance standard (Labsphere, North Sutton, New Hampshire, USA). Multiple colour measurements were made for each individual with particular attention paid to measuring areas that seemed distinct, including small colour patches (e.g. blue body, black stripe, yellow egg spot). Photographs of the cichlids, taken using UV filters, did not reveal any regions of the fish with hidden UV patterns. The UV reflective areas were large, contributing uniformly to a given colour patch. Therefore, selection of colour patches using the human visual system should not have biased the colour patch selection.

Ocular media transmission measurements were made using a USB2000 spectrometer following our previous methods (Hofmann et al., 2010; Siebeck and Marshall, 2001; Siebeck and Marshall, 2007). Briefly, a window was removed from the back of the eye and the whole eye or optical elements mounted above a pinhole. Although initial measurements were also made for the individual eye elements of cornea and lens, these showed that the lens was the limiting ocular medium in all species. Therefore subsequent measurements focused solely on lens transmission. Transmission was measured using light from a quartz halogen lamp that was directed through the pinhole and up through the eye, cornea or lens. Transmitted light was collected as close as possible to the ocular media with a 100 μm fibre. Spectra were normalized to transmission at 500 nm and smoothed using a 5-point boxcar algorithm. The wavelength at which transmission reached 50% (T50) was then determined for each lens. The lens transmission curve used in the quantum catch calculations was normalized by setting the maximum reflectance to 1.

Colour space model

A trichromatic colour space model was used to characterize and compare the colours of cichlids and the backgrounds against which they might be viewed. The relative quantum catch of each cone for a fish or background spectrum was used to plot that spectrum in a chromaticity diagram derived from a Maxwell triangle (Kelber et al., 2003). Receptor quantum catch was calculated according to Eqn 1, where R_i is the sensitivity (estimated using an opsin absorbance template) of receptor i , L is the lens transmittance, S is surface reflectance, I is the illuminant, and K_i is the von Kries factor for receptor i . Downwelling irradiance was assumed to illuminate the rock substrates, whereas sidewelling irradiance was assumed to illuminate fish because their bodies are laterally compressed. Irradiance measurements from Thumbi West were used in the model because most of the fish were collected at this site.

$$Q_i \propto K_i \int R_i(\lambda)L(\lambda)S(\lambda)I(\lambda)d\lambda \quad (1)$$

We have assumed the underwater illumination and fish surface reflection are isotropic, for simplicity. The von Kries factor, given by Eqn 2, is derived from von Kries’s simple colour constancy model in which receptors adapt independently to the background (Kelber et al., 2003):

$$K_i \propto \frac{1}{\int R_i(\lambda)L(\lambda)I(\lambda)d\lambda} \quad (2)$$

We modelled situations in which photoreceptors were adapted to horizontal irradiance (I) or to light reflected from a brown rock substrate, computed as downwelling irradiance multiplied by the surface reflectance of the brown rock ($I \times S_B$). When computing Q_i of horizontal radiance, S was omitted and the horizontal radiance spectrum replaced I in Eqn 1.

To model spectral sensitivity, we used the λ_{max} values that Parry et al. (Parry et al., 2005) obtained by *in vitro* expression of the

opsin genes in *Met. zebra* (Table 1). We expect there to be only minor differences in the λ_{\max} of orthologous visual pigments from the species studied here, as there is limited opsin sequence variation amongst these rock dwelling species. Data from Spady et al. (Spady et al., 2005) and Hofmann et al. (Hofmann et al., 2009) show that there are no functionally significant sequence differences in the *SWS1*, *SWS2b*, *RH2b* or *RH2A α* genes in *Met. zebra*, *Mel. auratus*, *Mel. vermillion* (*Mel. heterochromis*) and *C. afra*. Using the λ_{\max} from *Met. zebra*, we generated absorbance templates for pigments containing the 11-*cis* retinal chromophore (Govardovskii et al., 2000). We assumed use of 11-*cis* retinal because A1 chromophore usage is consistent with previous pigment extraction (Muntz, 1976) and MSP measurements of Lake Malawi cichlids (Carleton et al., 2000; Jordan et al., 2006; Parry et al., 2005).

The resultant position in triangular colour space was determined by normalizing the quantum catch for the short, medium and long wavelength visual pigments determined from Eqn 1 so that:

$$\begin{aligned} S &= \frac{Q_S}{Q_S + Q_M + Q_L} \\ M &= \frac{Q_M}{Q_S + Q_M + Q_L} \\ L &= \frac{Q_L}{Q_S + Q_M + Q_L} \end{aligned} \quad (3)$$

with their resulting location in cartesian space given by:

$$\begin{aligned} X &= \frac{L - M + 1}{\sqrt{3}} \\ Y &= S \end{aligned} \quad (4)$$

This colour triangle is equilateral (with altitudes equal to 1), resulting in equal weighting of the S, M, and L receptors. The degree of overlap in the absorbance spectra of the receptors determines the area inside the triangle that can be occupied by colours. This area was circumscribed by plotting a series of hypothetical, monochromatic colours, restricted to 1 nm bandwidths each. We plotted these monochromatic loci in the UV- and violet-sensitive colour spaces from 351 nm and 381 nm, the wavelengths at which the corresponding lenses begin to transmit light, to 650 nm, beyond which absorbance by the mbuna visual pigments essentially ceases.

Because spectra that are far apart in the modelled colour space are likely to appear conspicuously different to the cichlid visual system, colour distance was determined as the Euclidean distance between pairs of spectra:

$$D = \sqrt{(X_1 - X_2)^2 + (Y_1 - Y_2)^2} \quad (5)$$

where 1 and 2 correspond to a fish colour and a background, or to two fish colours on the same individual. Colour distance was computed between every fish colour and each of the six viewing backgrounds – the three excrement covered rocks, the brown rock and the two space lights at depths of 3 m and 7 m. However, at close viewing range, conspicuousness may result from contrasting colours on an individual fish. Therefore, to examine chromatic contrast within individual cichlids, we calculated the distance in conspecific colour space between all pair-wise combinations of colour spectra measured on each individual.

How cichlid visual systems process chromatic stimuli is unknown. Therefore, colour space distance, D , may not be a perfect predictor of conspicuousness. Two different pairs of points that are separated by equal distances in different directions may not be equally

distinguishable. However, it is our best estimator in that the larger D is, the more likely they can be distinguished by cichlids.

All calculations were performed for the 3 m depth. In order to test whether spectral changes in irradiance associated with depth would affect conspicuousness, we computed colour distances to the substrate backgrounds for one male and female of each species at additional depths of 1 m, 7 m and 10 m. The effect of depth on colour distances was then examined for all possible pair-wise depth comparisons.

In each species, we tested whether average male colour distances were statistically different from average female colour distances using the Wilcoxon test (implemented in R, ver 2.10 accessed 10/26/09). This nonparametric test ensured we did not have any biases from non-normality or differences in variances. Comparisons were made for male and female colour distances to each of the six backgrounds. The data for all backgrounds were then combined and tested. In addition, we compared a subset of male and female colour distances, which were selected to be greater than or equal to the median distance for that sex and background. These 'higher contrast' (HC) colours account for the possibility that some colours on a fish may function as conspicuous signals while other colours on the same individual may be more cryptic. We again used the Wilcoxon test to compare HC male *versus* HC female colour distances for each of the six backgrounds as well as a test combining all backgrounds. The significance of these tests was determined by a sequential Bonferroni correction as described in Hochberg (Hochberg, 1988). This assumed a significance threshold of $\alpha=0.05$ and a total of $m=14$ tests where significance for test j ($=1$ to 14) required that:

$$P_j \leq \alpha/(m - j + 1) \quad (6)$$

Significance values are ordered from smallest to largest and then sequentially compared with a significance level that changes, becoming less stringent with additional tests.

Additional calculations were done to test contrast within a fish. For each individual, pairwise colour distances were calculated between all possible combinations of the colours measured for that fish. Based on this diversity of colour distances, those that were greater than or equal to the median were then compared for males and females. This step removes the smaller colour distances occurring between the most similar colours on a given fish. Differences between the male within-fish and female within-fish colour distances were determined for each species by a Wilcoxon test. Results were considered significant when $P < 0.05$, since only one male–female comparison was made per species. For a few species, we did not measure many female colours and so there were not many between colour distances.

RESULTS

Water spectral properties and backgrounds

As expected for clear water, middle wavelength light (approx. 450–575 nm) is transmitted best at Thumbi West in Lake Malawi, followed by shorter wavelengths and finally the rapidly attenuated longer wavelengths (Fig. 1A). The sidewelling irradiance spectra at depths of 1 m, 3 m, 7 m and 10 m reveal a similar trend of narrowing spectra with increased depth (Fig. 1B).

Several spectra of horizontal space light occupied similar locations in colour space (data not shown). We chose the spacelight spectra measured at depths of 3 m and 7 m at Thumbi West (S_{3m} and S_{7m} , respectively) to represent spacelight backgrounds in our analysis (Fig. 1C). These spectra are similar to the downwelling and sidewelling irradiance spectra at 10 m (Fig. 1A,B).

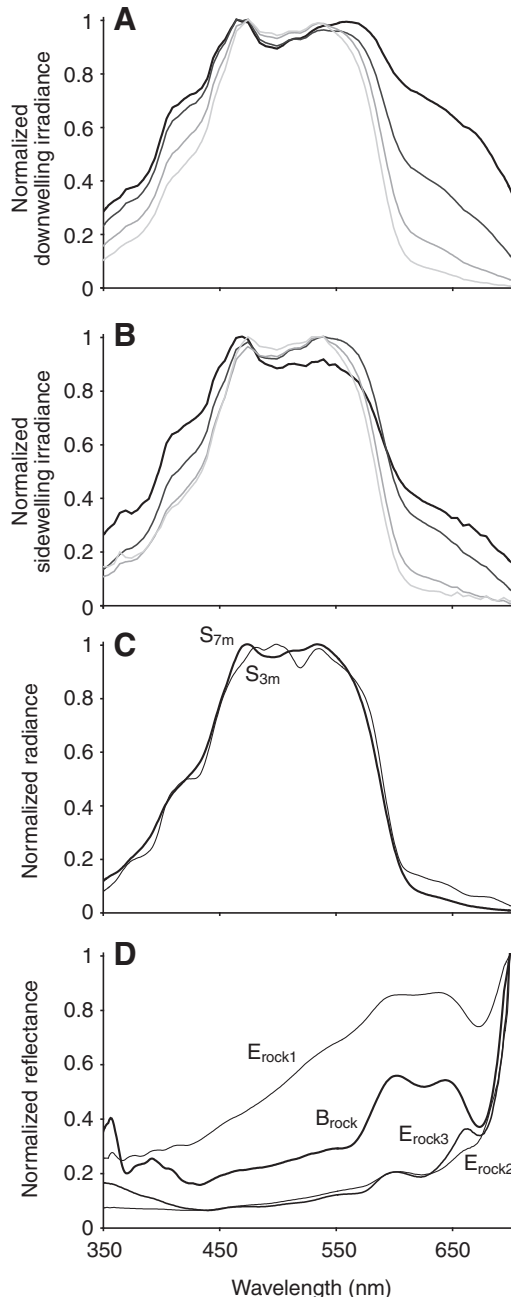


Fig. 1. Light environment of mbuna habitat in Lake Malawi. Normalized downwelling irradiance (A) and sidewelling irradiance (B) at Thumbi West Island. Irradiance was measured at depths of 1 m (darkest line), 3 m, 7 m and 10 m (lighter lines, respectively). (C) Normalized horizontal radiance (space light) at Thumbi West Island, measured at depths of 3 m and 7 m (denoted S_{3m} and S_{7m} , respectively). (D) Normalized reflectance of one brown rock (denoted B_{rock}) and three excrement-covered rock backgrounds (denoted $E_{rock1-3}$) measured at Thumbi West Island. Spectra in B and C were smoothed with a 10-point boxcar algorithm for illustrative purposes only.

A diverse set of substrate background spectra were collected and are plotted in UV colour space in supplementary material Fig. S1. Reflectance spectra from these rocks were generally broad, with highest reflectance at longer wavelengths. Reflectance in the UV–violet range varied among substrate patches. Four substrate reflectance spectra typical of Thumbi West were selected to represent this range of backgrounds: three rocks covered with fish excrement ($E_{rock1, 2}$ and 3) and one brown rock (B_{rock}) (plotted in

Fig. 1D). These four substrate spectra, together with the two space lights (S_{3m} , S_{7m}), constituted the six backgrounds included in the analyses. The reflectances of the substrates were used to compute their radiances at different depths.

Mbuna visual systems

Visual systems were characterized based on published opsin gene expression (Hofmann et al., 2009) and lens transmission spectra. Seven of the species expressed the SWS1 opsin (368 nm) and had lenses that transmitted light well into the UV, with 50% of maximum transmission ($T_{50\%}$) near 360 nm (Table 1). Only *Melanochromis auratus* and *Metriaclima heterochromis* expressed the violet-sensitive SWS2B opsin (423 nm) and had lenses that blocked most UV light, with $T_{50\%}$ near 400 nm. Previous studies indicate that all examined mbuna species have similar RH2B and RH2A α opsins (Table 1). Thus, based on lens transmission and cone visual pigments, these mbuna exhibit one of two types of colour visual system, UV sensitive or violet sensitive. In our computations we used the lens spectra of *Met. callainos* (360 nm) and *Mel. heterochromis* (400 nm) because they had relatively little noise (Fig. 2B,E). Fig. 2 shows the estimated absorbances of the various cone classes with and without lens filtering for each visual system.

Cichlid reflectance spectra and colour contrasts with backgrounds

In order to facilitate interpretation of the results, data for each species are presented in separate figures (Figs 3 and 4, supplementary material Figs S2–S8, each showing (A) photos of a representative female and male; (B) expected visual sensitivity (from Fig. 2C,F); (C) spectral reflectance curves for key male and female colours; (D) all colour spectra for that species, plus the six backgrounds, plotted in its colour space triangle; and (E) the resultant colour distances between every male and female colour and three of the backgrounds, E_{rock1} , B_{rock} and S_{7m} . The lines and symbols in C and D representing fish spectra are coloured according to the general hue perceived by humans. Noteworthy examples of reflectance spectra from breeding males and females of the nine species are shown in Fig. 3C and Fig. 4C and supplementary material Figs S2C–S8C. The reflectance spectra that were plotted (C panels) were generally those colours of a male and a female that were furthest from one or more of the backgrounds in colour space (D panels), and thus contrasted most with the backgrounds (E panels). The number of individuals and number of spectra for male and female colour distances are given in Table 2. Some species have just a few individuals, which limits our statistical power to test for differences between male and female colour distances.

Based on our hypothesis, we expected male colours to have greater distances than female colours from backgrounds. This was observed for six species. *Met. aurora* demonstrates this result: relative to each background, the male had at least one colour that was located further away in colour space than any female colour (Fig. 3D,E). Results similar to this were also found for *C. afra*, *Mel. heterochromis*, *Mel. 'B&W' johanni*, *Met. livingstonii* and *Met. zebra*. When viewed against any background, every male of these species had at least one colour that was more conspicuous than any conspecific female colour (supplementary material Figs S2–S6). Restricting comparisons to the maximum colour distances of each male and female is a sensitive method to identify differences between sexes that could be limited to single colour patches. However, sexes may differ by multiple colours that could function as signals. Therefore, we used the Wilcoxon test to compare the 'high contrast' (HC) male and female colour distances, which were greater than or equal to the median for

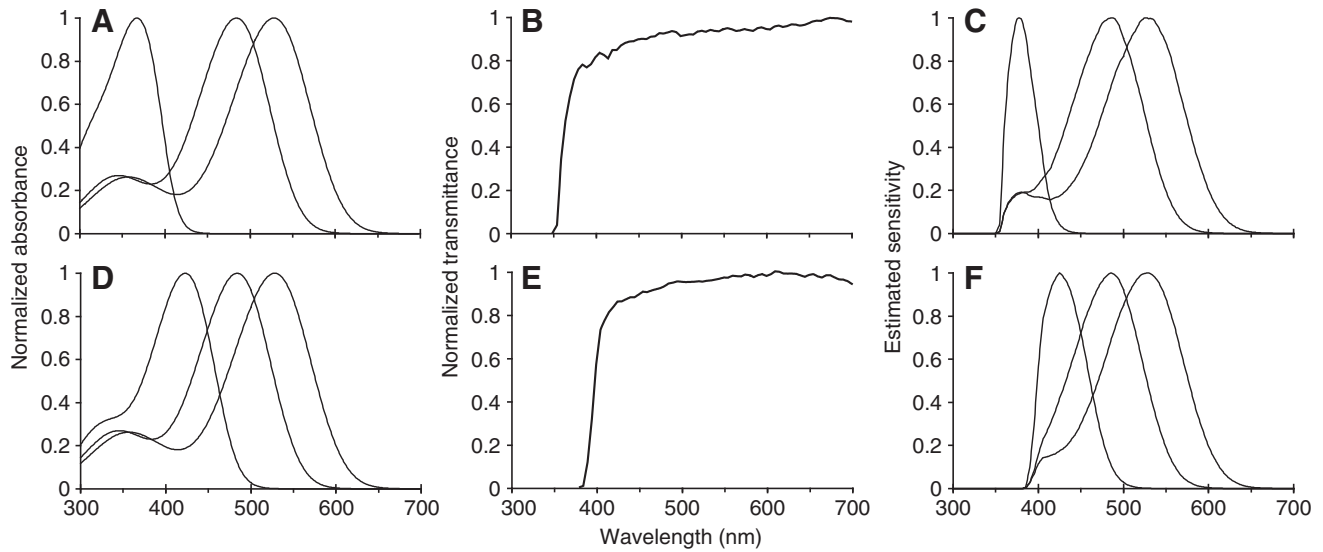


Fig. 2. Lens transmission and visual pigment absorbance of the two basic types of visual systems in mbuna. (A,D) Visual pigment templates of the UV-sensitive (A) and violet-sensitive (D) visual systems without lens filtering. (B,E) Normalized lens transmission spectra of *Metriaclima callainos* (B) and *Melanochromis heterochromis* (E). (C,F) The same sets of visual pigments filtered by the UV-transmitting lens spectrum of *Metriaclima callainos* (C) and the UV-blocking lens spectrum of *Melanochromis heterochromis* (F).

that sex and background (Table 3 and supplementary material Table S1). Four of these six species showed statistically significant differences, with HC male colour distances being larger than HC female colour distances when all backgrounds were combined. This included *C. afra*, *Met. aurora*, *Met. zebra* and *Mel. heterochromis*. Two of these species showed this statistical difference for some individual backgrounds as well. It should be noted that high contrast colours came from every individual, and therefore differences were not due to just one colourful fish. Male colour distances were significantly greater than female distances in two of these species even when all colours were compared (*C. afra*, *Mel. heterochromis*). Many of these comparisons including individual backgrounds or all colours are not significant, but some are close to significance. These likely suffer from the low numbers of spectra that were taken, especially for the females (Table 2). Similarly, *Met. livingstonii* and *Mel. 'B&W' johanni* appeared to have larger colour distances for males than females, however, these distances were not statistically different in the Wilcoxon tests. Distances to all backgrounds for HC colours are shown in supplementary material Fig. S9, and those for all colours are in supplementary material Fig. S10.

The other three species showed quite different patterns than the six we have already described. For *L. trewavasae*, females and males

both had conspicuous colours. In this species, maximum colour distances of females overlapped those of males (supplementary material Fig. S7), and colour distances to backgrounds were similar for males and females in all Wilcoxon tests (Table 3; supplementary material Tables S1, S2 and Figs S9, S10). In the last two species, females tended to have more conspicuous colours than males. *Mel. auratus* male and female colours were both highly conspicuous (Fig. 4), with HC female colour distances significantly greater than those of males for all backgrounds combined, as well as all but one individual background (supplementary material Table S1 and Fig. S9). Comparing all *Mel. auratus* colours, female distances were greater than male distances, but only significantly so with all backgrounds combined (supplementary material Table S2 and Fig. S10). Female colours were also highly conspicuous in *Met. callainos*, whereas male colours were somewhat less so (supplementary material Fig. S8). When viewed against every background, each *Met. callainos* female had at least one colour that was more conspicuous than any conspecific male colour. *Met. callainos* females had the larger colour distances for each background as well as all backgrounds combined when comparing HC colours or all colours (Table 3; supplementary material Tables S1, S2 and Figs S9, S10).

Table 2. Number of individuals and reflectance spectra by species and sex

Species	Number of individuals		Total number of spectra	
	Male	Female	Male	Female
<i>Cyanotilapia afra</i>	4	4	58	18
<i>Labeotropheus trewavasae</i>	4	3	61	28
<i>Melanochromis auratus</i>	4	4	76	88
<i>Melanochromis heterochromis</i>	2	1	8	4
<i>Melanochromis 'B&W' johanni</i>	1	1	6	4
<i>Metriaclima aurora</i>	2	1	22	8
<i>Metriaclima callainos</i>	4	4	59	49
<i>Metriaclima livingstonii</i>	1	1	3	4
<i>Metriaclima zebra</i>	2	1	16	3
Total	24	20	309	206

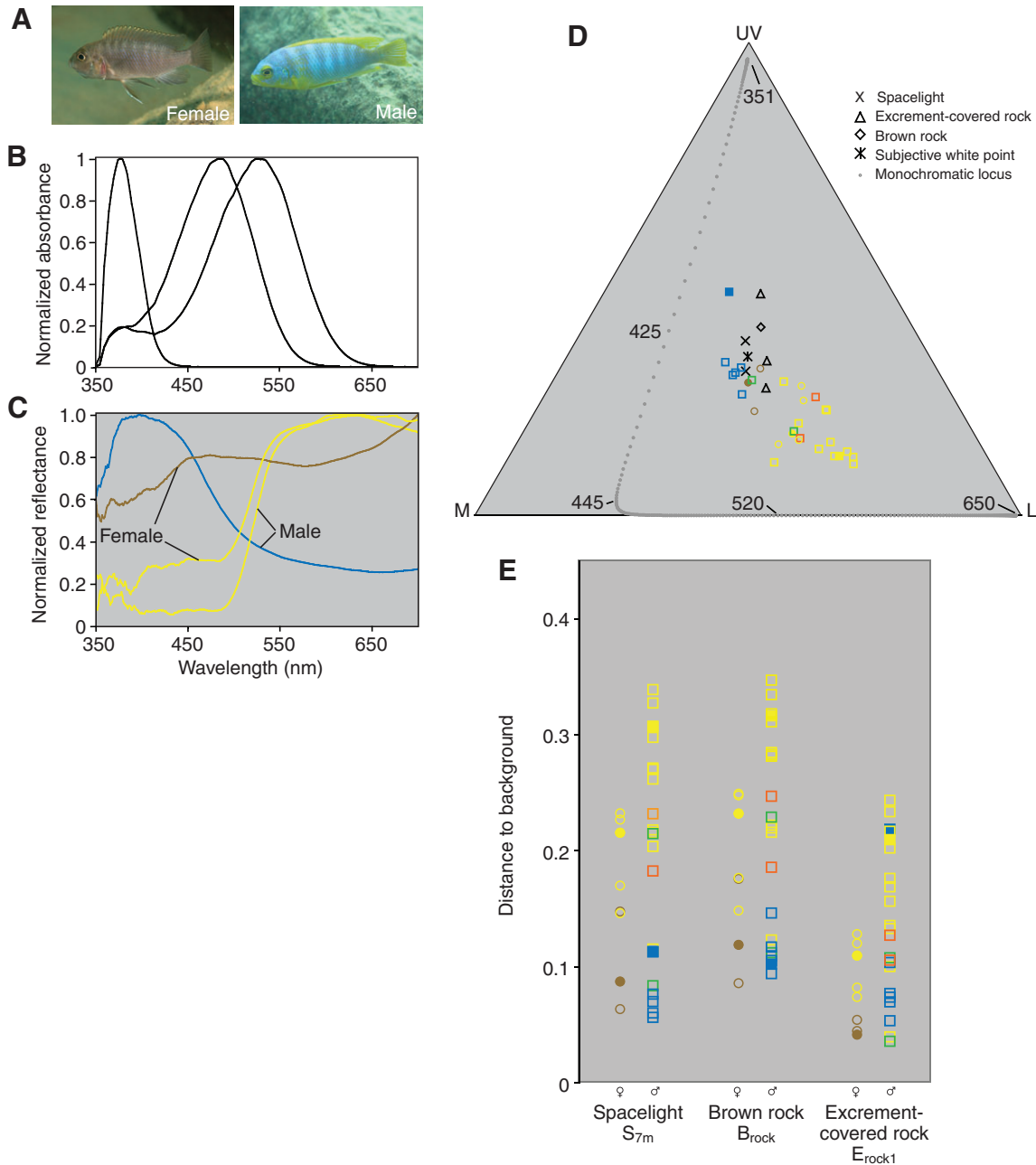


Fig. 3. *Metriaclima aurora* colours and their distances in colour space to the backgrounds at Thumbi West Island. (A) Photographs of female and male. (B) Estimated sensitivity (from Fig. 2) of the UV-sensitive visual system. (C) Sample female and male reflectance spectra. Curves were smoothed with a 10-point boxcar algorithm for illustrative purposes only. (D) All colours that were measured on females and males plotted in the colour space of the mbuna UV-sensitive visual system. Female colours (circles), male colours (squares), and rock backgrounds at 3 m deep; depths of space light were 3 m (upper X) and 7 m (lower X). Lower, middle and upper triangle symbols correspond to excrement-covered rocks 1–3 (E_{rock1} – E_{rock3}), respectively. Filled grey circles represent monochromatic loci at 1 nm intervals; wavelengths of some loci are labelled. Line and symbol colours indicate the general hues perceived by a human observer at time of measurement. Filled symbols correspond to the reflectance spectra in C. (E) Distance from each fish colour to three backgrounds.

Effect of visual system

Two of the nine species had the violet-sensitive visual system, *Mel. auratus* and *Mel. heterochromis*. We noticed that the colours of these two species and the backgrounds are compressed into a rather tight line in violet-sensitive colour space. When these colours are plotted in a UV-sensitive colour space, they spread out significantly (*Mel. auratus* data shown in supplementary material Fig. S11). Much of the dispersion in UV colour space occurs along the axis running

from the UV vertex to the M–L side of the triangle, indicating differences in relative stimulation of short-wavelength-sensitive cones. Compared with the violet visual system, the UV visual system may improve the discriminability of fish and background colours.

Effects of water depth

The spectra of downwelling and sidewelling light change with water depth. Such changes could affect cichlid conspicuousness by altering

Table 3. Summary of *P*-values from statistical colour comparisons for (A) high contrast (greater than median) colour distances to all backgrounds for males versus females, (B) colour distances to all backgrounds for all male versus all female colours, and (C) high contrast colour distances within fish: males versus females

Species	Colour distances to all backgrounds						Within fish colour distances		
	(A) High contrast male vs high contrast female colours*			(B) All male vs all female colours†			C) High contrast (colour combinations: males vs females‡		
	M>F	M=F	F>M	M>F	M=F	F>M	M>F	M=F	F>M
<i>C. afra</i>	2.2e-7			8.1e-5			2.5e-08		
<i>Met. aurora</i>	2.0e-4				0.014		1.8e-04		
<i>Met. zebra</i>	1.2e-4				0.022		4.6e-03		
<i>Mel. heterochromis</i>	1.9e-8			1.3e-9				0.35	
<i>Mel. B&W johanni</i>		0.015			0.14		0.012		
<i>Met. livingstonii</i>		0.93			0.91			0.26	
<i>L. trewavasae</i>		0.55			0.58		<2.2e-16		
<i>Mel. auratus</i>			6.8e-11			1.1e-6			2.7e-05
<i>Met. callainos</i>			<2.2e-16			<2.2e-16		0.32	

M, male; F, female.

*See supplementary material Table S1; †see supplementary material Table S2; ‡see supplementary material Table S3. If significant differences were detected between sexes, *P*-values are given in the column indicating which sex had the larger colour distances. If sexes were not significantly different, *P*-values are given under the M=F heading. Significance thresholds were determined with sequential Bonferroni correction across all tests in A and B and set at *P*<0.05 for C.

(1) the location of fish colours in colour space as a result of changes in the sidewelling irradiance; (2) the location of substrates in colour space as a result of changes in downwelling irradiance; and therefore (3) the relative distances of male and female colours to substrates. To examine these effects of water depth, we generated a single colour space plot displaying fish and substrate spectra at depths of 1 m, 3 m, 7 m, and 10 m (Fig. 5). The fish colours were those of *C. afra*, one male and one female, which represented much of the colour diversity we observed in this study. None of the fish colours moved appreciably as depth changed from 1 m to 10 m, but the substrates did move substantially with depth.

To determine the effect of depth on conspicuousness, we computed colour distances to the substrate backgrounds for one male and one female of each species at additional depths of 1 m, 7 m and 10 m. By examining all possible pair-wise depth comparisons, we observed the greatest effect of depth between 1 m and 3 m with $E_{\text{rock}3}$ as the viewing background. However, even this change in depth did not substantially affect the relative conspicuousness of male and female colours with respect to any of the substrate backgrounds. Wilcoxon tests for HC colour distances to the four rock substrates at 1 m yielded results that were qualitatively identical to those at the 3 m depth. At both depths, male HC colour distances to all backgrounds were significantly greater than those of females of the same four species (*C. afra*, *Met. aurora*, *Met. zebra* and *Mel. heterochromis*), whereas female HC distances were significantly greater than those of males in the same two species (*Mel. auratus* and *Met. callainos*), and HC distances were similar for males and females in the remaining three species (*L. trewavasae*, *Met. livingstonii* and *Mel. "B&W" johanni*). When all colours were compared to all backgrounds at 1 m, the Wilcoxon results were again similar to those at 3 m except that male colour distances were significantly greater than those of females in one additional species, *Met. auratus*. Therefore, depth does not affect the relative conspicuousness of male and female colours.

Contrast between colours within individual cichlids

Because changes in irradiance associated with water depth had little effect on the position of fish spectra in colour space, contrast within fish was analyzed using the sidewelling irradiance measured at a

single, intermediate depth (3 m). Maximum colour distances between patches on the same fish were typically higher for males than females (Table 4). Colour distances within the same fish were high for males of all species except *Met. livingstonii*, which had low colour contrast within males and females (Table 4). For these eight species, maximum colour distance within a male fish ranged from 0.23 to 0.38. Colour distances on females were quite low for six species, with maxima ranging from 0.05 to 0.15. However, maximum colour distances within females was high (from 0.19 to 0.33) in the same three species that exhibit high colour distances between females and backgrounds: *L. trewavasae*, *Met. callainos* and *Mel. auratus*.

Comparing maximum colour distances within males and females is a sensitive test for differences in conspicuousness among sexes. To further examine sexual dichromatism, we also performed a statistical analysis of HC colour distances within fish, which were at or above the median for the sex and species (supplemental material Fig. S12 and Table S3). Wilcoxon tests supported the fact that HC colour distances were greater within males for *C. afra*, *Met. aurora*, *Met. zebra*, *Mel. "B&W" johanni*, as well as *L. trewavasae*. This pattern also seems likely in *Mel. heterochromis* and *Met. livingstonii*, but statistical significance was not reached in either species probably because of the small number of spectra measured. HC colour differences within fish were similar and relatively small for both

Table 4. Maximum colour contrast between patches on individual males and females

Species	Visual system	Female	Male
<i>Cynotilapia afra</i>	UV	0.13±0.10	0.35±0.01
<i>Metriaclima aurora</i>	UV	0.16±0.02	0.38±0.05
<i>Metriaclima zebra</i>	UV	0.05	0.31±0.02
<i>Melanochromis heterochromis</i>	Violet	0.05±0.03	0.30±0.03
<i>Melanochromis "B&W" johanni</i>	UV	0.09	0.26
<i>Metriaclima livingstonii</i>	UV	0.11	0.16
<i>Labeotropheus trewavasae</i>	UV	0.21±0.06	0.33±0.08
<i>Melanochromis auratus</i>	Violet	0.33±0.07	0.29±0.08
<i>Metriaclima callainos</i>	UV	0.19±0.07	0.23±0.03

The standard deviation is provided when multiple individuals were measured.

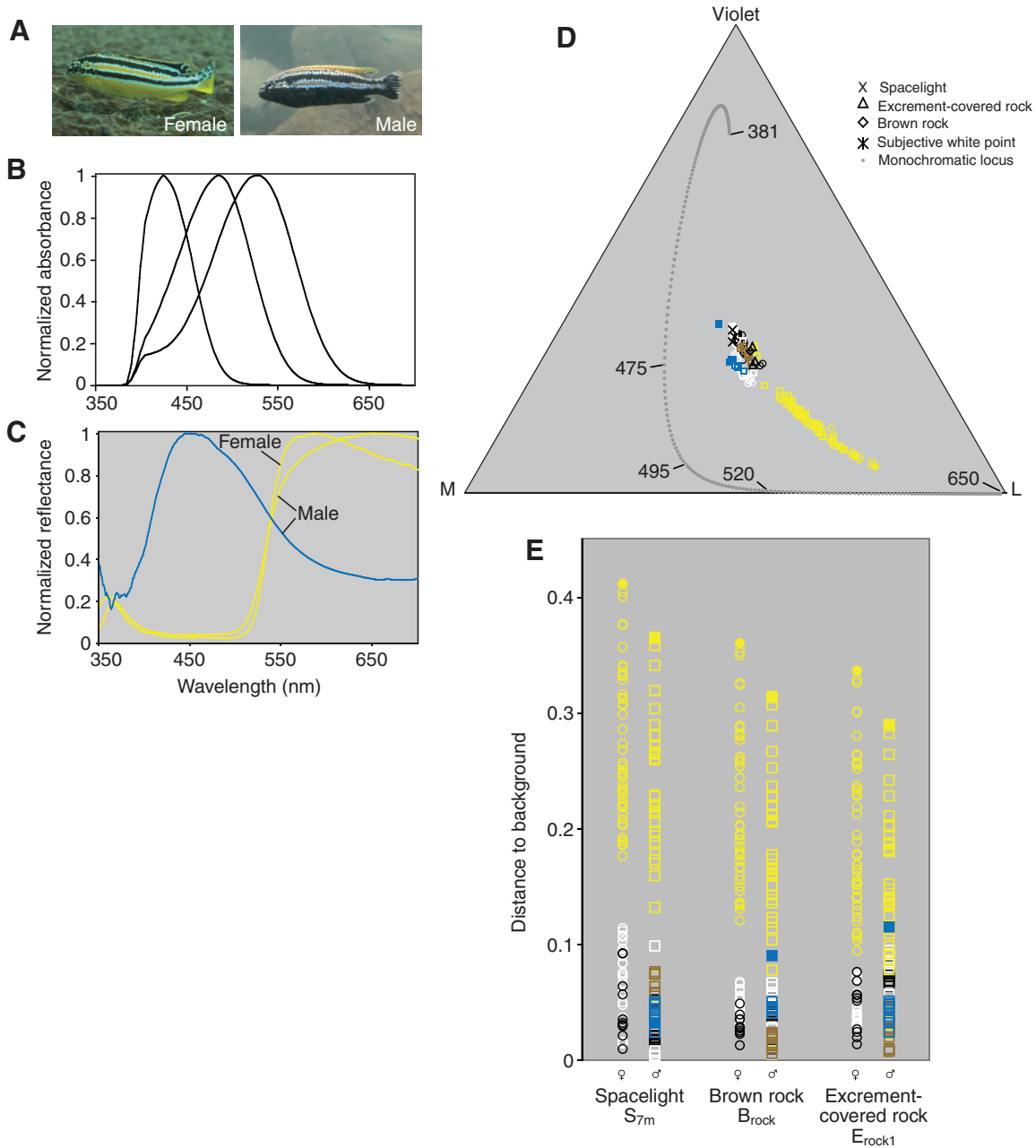


Fig. 4. *Melanochromis auratus* colours and their distances in colour space to the backgrounds at Thumbi West Island. (A) Photographs of female and male. (B) Estimated sensitivity (from Fig. 2) of the violet-sensitive visual system. (C) Sample female and male reflectance spectra. Curves were smoothed with a 10-point boxcar algorithm for illustrative purposes only. (D) All colours that were measured on females and males plotted in the colour space of the mbuna UV-sensitive visual system. Female colours (circles), male colours (squares), and rock backgrounds at 3 m deep; depths of space light were 3 m (upper X) and 7 m (lower X). Lower, middle and upper triangles represent excrement-covered rocks 1–3 (E_{rock1} – E_{rock3}), respectively. Filled grey circles represent monochromatic loci at 1 nm intervals; wavelengths of some loci are labelled. Line and symbol colours indicate the general hues perceived by a human observer at time of measurement. Filled symbols correspond to the reflectance spectra in (C). (E) Distance from each fish colour to three backgrounds.

male and female *Met. callainos*. Only in *Mel. auratus* were internal HC colour distances significantly greater in females than in males.

Maximum colour distance within each fish often occurred between the complementary colours blue and yellow. Many yellow or orange colours lacked substantial UV peaks. The spectra that contrasted most with these longer-wavelength colours tended to reflect strongly at short wavelengths, covering part or all of the UV to violet range. The greatest contrast with the backgrounds was typically produced by the same yellow and orange colours. The

short-wavelength colours of some species also contrasted strongly with backgrounds, particularly with E_{rock1} .

Cone adaptation to different backgrounds

We wanted to test whether photoreceptor adaptation to different background spectra could affect the relative conspicuousness of male and female mbuna colours. This would occur if the photoreceptors adapted to a spacelight or substrate background rather than, as we have assumed, adapting to the sidewelling irradiance. We chose to

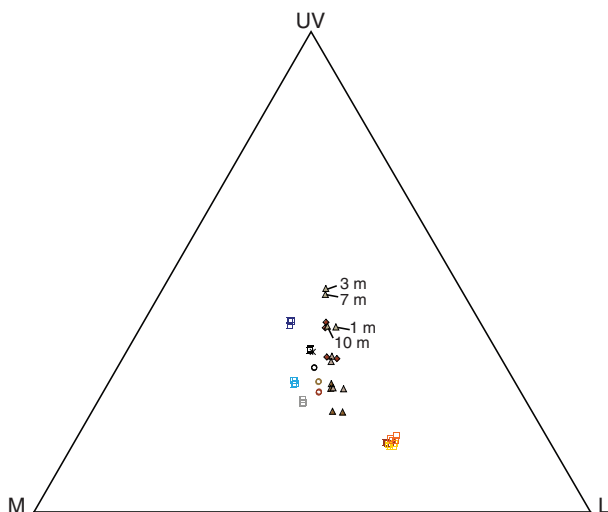


Fig. 5. Colour space plot of *Cynotilapia afra* colours and the rock backgrounds computed at four depths: 1 m, 3 m, 7 m, and 10 m. Colour constancy (von Kries) was computed from sidewelling irradiance at each depth. Symbols of the same colour are data from the same fish or background colour computed at the different depths. Depths are indicated for the E_{rock3} loci; other substrates exhibit similar depth patterns. Male colours are squares, female colours are circles, and rock backgrounds are diamonds (B_{rock}) and triangles (from highest set in colour space to lowest: E_{rock3} , E_{rock2} , E_{rock1}).

test adaptation to E_{rock1} because the spectrum of light radiating from it differs substantially from the sidewelling spectrum (Fig. 1B). E_{rock1} preferentially reflects longer wavelengths (Fig. 1D) and is a common background at Thumbi West. In calculating cone quantum catch of the fish and background spectra, we replaced I in the von Kries factor (Eqn 2) with the E_{rock1} substrate radiance calculated at a depth of 3 m. Even with this extreme change in adaptation background we detected no effect upon the relative male and female colour distances to the backgrounds for the same male and female *C. afra* whose spectra are shown in Fig. 5. Regardless of the background to which the retina was adapted, Wilcoxon tests revealed the male's colour contrast was significantly greater than that of the female relative to all backgrounds combined when we included all colours on these two fish (seven male colours, two female).

DISCUSSION

Conspicuous males result from sexual selection

Male rock-dwelling cichlids of Lake Malawi develop nuptial colours that function as signals in male-male interactions and female mate choice. To the human eye, these colours appear highly conspicuous. We tested whether these male colours are more conspicuous than female colours when viewed by the cichlids' visual systems in their habitat. We objectively measured reflectance spectra of fish and viewing backgrounds, downwelling and sidewelling irradiance, and horizontal space light. From these measurements we calculated radiance spectra reflected from fish colour patches and habitat backgrounds at depths ranging from 1 m to 10 m. We then evaluated how these fish and background radiance spectra would stimulate trichromatic visual systems based on the cone visual pigments of the mbuna species we studied. We proceeded under the assumption that two colours that are far apart in this colour space are likely to appear conspicuously different to the mbuna visual systems.

We used a number of approaches to compare male and female colour distances. The most sensitive comparison examined maximum colour distances for individual fish. In addition, statistical analyses compared male and female colours for both all colours and higher contrast (HC) colours relative to six different backgrounds. Finally, we also performed a statistical analysis of within-fish contrast based on colour distances. On the whole, the different statistical analyses were concordant (Table 3). We found four species where most measures showed male colours to be more conspicuous than female colours: *C. afra*, *Met. aurora*, *Met. zebra*, and *Mel. heterochromis*. Male colours were also highly conspicuous in *L. trewavasae* and *Mel. auratus*, but the female colours were conspicuous as well. Male colour distances appear greater than those of females in *Mel. 'B&W' johanni* and *Met. livingstonii*, although this difference was statistically significant only for contrast within fish in *Mel. 'B&W' johanni*, possibly due to the small sample sizes for both species. Thus, in at least two thirds of the species male colours appear conspicuous, consistent with the hypothesis that sexual selection is driving the diversification of male colouration in Lake Malawi cichlids.

Female colours in at least four species produced significantly lower colour distance and hence less chromatic contrast with the backgrounds or other body colours, suggesting sexual selection is not a strong force in female colour evolution for these species. However, in three species female colours were conspicuous. For two species (*Mel. auratus* and *Met. callainos*), the more conspicuous colours were found on females, which had larger colour distances than males both to the backgrounds and within fish. In *L. trewavasae*, female and male colour distances relative to backgrounds were both similar and large. Interestingly, *L. trewavasae* had larger male than female within-fish colour distances, suggesting males of *L. trewavasae* may be more conspicuous than females only when viewed at close range (rather than at a distance against the background).

The patterns of male *versus* female conspicuousness described above were consistent regardless of water depth or the background to which the retina was adapted. These findings suggest that males, and in some species females, can generate conspicuous colour signals in a broad range of viewing conditions found in their habitat.

Why are some females conspicuous?

In three species, female colours were highly conspicuous. In *L. trewavasae* female colour distances were not significantly different than male distances to any background, whether HC colours or all colours were compared. For both *Mel. auratus* and *Met. callainos*, female colour distances to the backgrounds were actually larger than those of males. This held true for within fish colour distances in *Mel. auratus*, although male and female colour distances were not different in *Met. callainos*.

Photographs suggest that female colouration in these three species differs from the predominantly brown females of other species. The female colours, like those of the conspecific males, are more saturated. Two of these species have the UV visual system (*Met. callainos*, *L. trewavasae*) and one has the violet system (*Mel. auratus*), indicating that conspicuous female colours are not limited to a particular visual system or genus. Thus, evolution of female colouration may be free of strong phylogenetic constraint, as has been observed of male colouration (Deutsch, 1997; McElroy et al., 1991). Behavioural studies would help illuminate whether the conspicuous female colours function as communication signals and how they evolved. However, none of the previous cichlid behavioural work indicates that sexual selection is acting on females

in these species. Furthermore, in our field observations of these species we did not observe choosier males, which would be suggestive of male mate choice. Generally, males will court any conspecific female that enters his breeding territory (personal observation).

The presence of conspicuous female colours in these species is inconsistent with the idea that females need to remain inconspicuous. Crypsis would help mouthbrooding females avoid predators that ram the females in order to release the fry (Konings, 1990). Recent models have suggested that unique colour patterns may be linked to novel sex determining genes. New sex determiners may evolve frequently, either to modify sex ratios in small populations (Kocher, 2004) or to resolve genetic conflicts between the sexes (Roberts et al., 2009). Two of the species we identified as having conspicuous female colours, *Met. callainos* and *L. trewavasae*, have recently been shown to carry novel female sex determiners, W 'chromosomes' (Ser et al., 2009). This interesting result suggests that conspicuous female colours may mark the presence of recently evolved sex determiners in cichlid species (Kocher, 2004). Alternatively, novel sex determiners may simply interfere with the maintenance of crypsis in females.

Effects of ultraviolet sensitivity

Male colours, and in some species female colours, appeared conspicuous to both the UV- and violet-sensitive visual systems. However, differences in short-wavelength sensitivity may impact the diversity of colours that can be perceived by these two visual systems. Shifting the sensitivity of the visual system from violet to UV expanded the degree to which fish and background colours dispersed in colour space, mainly along the axis of the short-wavelength cone (supplementary material Fig.S11). To our knowledge no work has been published on cone opponency mechanisms in mbuna. However, if the mbuna visual systems are similar to those of goldfish and zebrafish, which neurally compare the signals from their shorter and longer wavelength cones (Hughes et al., 1998; Neumeyer, 1986; Neumeyer, 1992; Risner et al., 2006), the UV-sensitive visual system may have greater potential to discriminate between the colours of fishes and backgrounds found in its environment.

Effects of water depth

Our work reveals that major effects on cichlid conspicuousness from changing illumination due to water depth can be countered by simple mechanisms of colour constancy. The primary factor that would cause colours to move in colour space are the downwelling and sidewelling irradiance spectra. Both of these spectra are narrowed with depth, the sidewelling irradiance more rapidly so (Fig. 1). We assumed the substrates were illuminated by downwelling irradiance whereas the laterally compressed cichlids were illuminated by sidewelling irradiance, the same spectrum to which the retina was adapted for colour constancy. Therefore, we were not surprised that the von Kries mechanism maintained the positions of fish colours more effectively than substrate colours in colour space. Even though depth-related changes caused the substrate colours to move, the relative conspicuousness of males and females was maintained. Vision in Lake Malawi cichlids probably utilizes colour constancy, though this has not been demonstrated experimentally. Predictions of the von Kries model do agree well with the results of quantitative colour constancy behavioural tests on goldfish (Dorr and Neumeyer, 2000), the only fish in which this has been thoroughly tested.

The observation that relative conspicuousness does not vary significantly over the 1–10 m depth range explains the results of

previous work in Lake Malawi. Deutsch (Deutsch, 1997) found no correlation in Lake Malawi between water depth and the hue, saturation or brightness of mid-body colour, or with the colour distance between body regions of male mbuna. It is possible that in the relatively clear and shallow habitats of Lake Malawi, depth does not contribute to the diversification of cichlid colours. This is in contrast to studies in the more turbid environment of Lake Victoria which transmits red light better than blue light. An effect of the rapid attenuation of blue light in that lake is that fish living deeper are selected to have longer wavelength visual sensitivity and as a result prefer to mate with red fish. Shallower habitats have more blue light, and species living there are sensitive to shorter wavelengths and prefer mates which are blue (Seehausen et al., 2008). Such a steep light gradient does not exist in the clearer waters of Lake Malawi, suggesting that depth will not be as strong a factor.

Cichlid colour usage matches environmental light transmission

In examining fish from just one light environment, we cannot conclusively demonstrate that the light environment is driving evolution of fish colouration. However, it is interesting to note the relationship of light environment and fish colour. For Malawi mbuna, blue, yellow and orange were common on the conspicuous individuals of each species, whereas green and red were rare. This has long been noted of mbuna males in general (Deutsch, 1997; Levine et al., 1981; McElroy et al., 1991; Ribbink et al., 1983). Spectral properties of the lake's water and the sensitivities of mbuna visual systems both contribute to the conspicuousness of blue, yellow and orange colours among Lake Malawi cichlids and have probably favoured their evolution as signals. For the colour of a surface to be easily perceivable underwater it should have a spectral cut-off, or region where reflectance changes rapidly with wavelength, within the spectral range of light that is present at intensities sufficient for colour vision (Lythgoe, 1968). Colours with cut-offs outside this range would appear as a different colour or even colourless, depending upon the spectrum's shape. Thus, in clear oceanic water (which transmits light best between approximately 425 nm and 550 nm) blue and yellow surfaces maintain their colour over the greatest ranges in depth as well as viewing distance (Lythgoe, 1968; Lythgoe, 1979). A different set of colours is most visible in the long-wavelength transmitting habitats typical of lakes and rivers. In most fresh water, green and red colours are discernible at greater depths and viewing distances (Lythgoe, 1968; Lythgoe, 1979). Transmission in Lake Malawi near-shore rocky habitat (Fig. 1A) is similar to what is seen in clear oceanic water, though shifted slightly toward longer wavelengths. This small spectral shift probably allows orange colours to be highly visible while preserving the strong visibility of blues and yellows (Fig. 6). Red colours, however, have cut-offs beyond Lake Malawi's transmission band (Marshall, 2000b), so these colours generally would only be easily discernable from short distances and shallow depths in this clear lake water. Moreover, the UV- and violet-sensitive visual systems are both relatively insensitive to wavelengths in the red band of the spectrum (Fig. 1). Therefore, selection for red colour signals may be weak due to poor stimulation of the cichlid visual system, a result of both the light transmission spectrum in Lake Malawi and the cone spectral sensitivities of the mbuna.

From the perspectives of the mbuna visual systems, yellow or orange cichlid colours generally contrasted most with the backgrounds. Blue colours of some species also contrasted strongly with the backgrounds. Blue colours were generally absent from the inconspicuous females. In addition, the yellows on these females

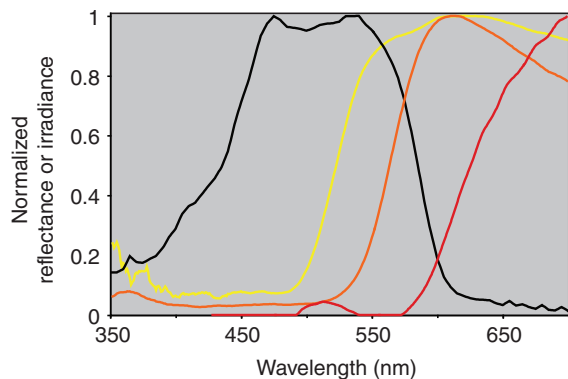


Fig. 6. As a result of the spectral properties of Lake Malawi, yellow and orange colours are visible at greater depths and viewing distances than red colours. Sidewelling irradiance spectrum 10m below the surface at Thumbi West Island (black line) illuminates fish and is similar to the lake's transmission spectrum. Reflectance of representative yellow (*Met. aurora* male) and orange (*L. trewavasae* male) colours changes rapidly at wavelengths that are prominent in the sidewelling irradiance and are transmitted well. However, reflectance of the red colour of a male cichlid from the longer-wavelength transmitting waters of Lake Victoria does not increase substantially before reaching wavelengths at the edge of Lake Malawi's main transmission envelope. Fish spectra are smoothed and then normalized to maximum reflectance between 350nm and 700nm. The Lake Victoria cichlid was a *Pundamilia neyererei* male from Makobe Island, reflectance spectrum courtesy of O. Seehausen.

were typically less conspicuous than male yellows. The female colours that were highly conspicuous tended to reflect short wavelengths relatively strongly, as in female *Met. callainos*, or they were saturated yellows. We also demonstrated that blue compared with yellow or orange produced the greatest colour distance within fish of most species. Thus a combination of yellow and blue produces a signal that transmits well in Lake Malawi and, from the mbuna's perspective, provides high colour contrast both externally with their surroundings and internally with other colours, which might be important for close-range communication.

Unlike most freshwater habitats, in Lake Malawi light transmission and spacelight backgrounds are similar to those of coral reefs (Marshall, 2000b; Marshall et al., 2003b). Lake Malawi rock substrates are also similar in colour to corals, which on average reflect light most strongly at 500 nm and beyond (Marshall, 2000a; Marshall et al., 2003b). The observation that the blue and yellow colours of coral reef fish can appear conspicuous or cryptic to humans depending on the viewing background was reported long ago (Longley, 1915). The same is probably observed by coral reef fish themselves. A model of a coral reef fish's dichromatic visual system indicates that blue patches of the angelfish *Pygoplites diacanthus* provide strong colour distance with the coral background but match the space light, whereas the reverse is true of yellow patches (Marshall, 2000a). The blue and yellow patches also contrasted highly with each other when seen with this visual system. Not surprisingly, blue and yellow colours are common among the fishes inhabiting coral reefs (Marshall, 2000b; Marshall et al., 2003a). The spectral similarities of the water and substrates of Lake Malawi and those of tropical coral reefs have probably favoured the evolution of blue and yellow colour signals in both environments.

Future work

This work is the first attempt to understand the colours of Malawi cichlids in terms of their visual system and environment. There are

numerous factors that should be studied in future work. First, examination of the brightness and pattern components of Lake Malawi cichlid colouration would add greatly to our understanding of how these fish are diversifying. For example, to our eyes the 'orange blotch' pattern on *L. trewavasae* females appears to match the mottled pattern of certain rocky substrates, but we lack sensitivity to the UV wavelengths where the female and substrate spectra could differ substantially. Viewed against other backgrounds this OB pattern may provide crypsis by means of disruptive colouration. A second research area that would yield a better understanding of the behavioural roles of the colour signals would be to study their modulation during social interactions. Conspicuous yellows often are limited to small egg spots on the retractable anal fin, and colouration on the body can be controlled neurally in cichlids (Muske and Fernald, 1987). Finally, to understand the effects of predation on the evolution of cichlid colours, we must evaluate their conspicuousness to predators. The colour patterns of cichlids living over rocks, where there are places to hide, may be more conspicuous than those of cichlids living over the open sand. Predators include birds, such as cormorants and kingfishers, and other cichlids.

Conclusions

In this work, our goal was to understand the colours of Malawi cichlids in terms of their visual system and environment. We found that male nuptial colours are conspicuous under a broad range of conditions, regardless of viewing background or depth to at least 10m. In many species, female colours are less conspicuous than those of their male counterparts. In Lake Malawi cichlids, male colour signals are known to be important in both female mate choice and male-male competition, selective forces that favour highly visible signals. Thus sexual selection has probably played a large role in the evolution of conspicuous colour signals in mbuna males. We did identify three species in which female colours were at least as conspicuous as male colours. The behavioural role and genetic basis of female colour needs to be addressed in those species. The transmission of Lake Malawi waters and the visual sensitivity of the mbuna have probably selected for preferential use of UV to blue and yellow to orange colours as conspicuous signals in these fish. The inclusion of UV reflectance in this study coupled with UV sensitivity suggests that the colours used in communication by cichlids are quite diverse, more so than can be detected by the human visual system.

ACKNOWLEDGEMENTS

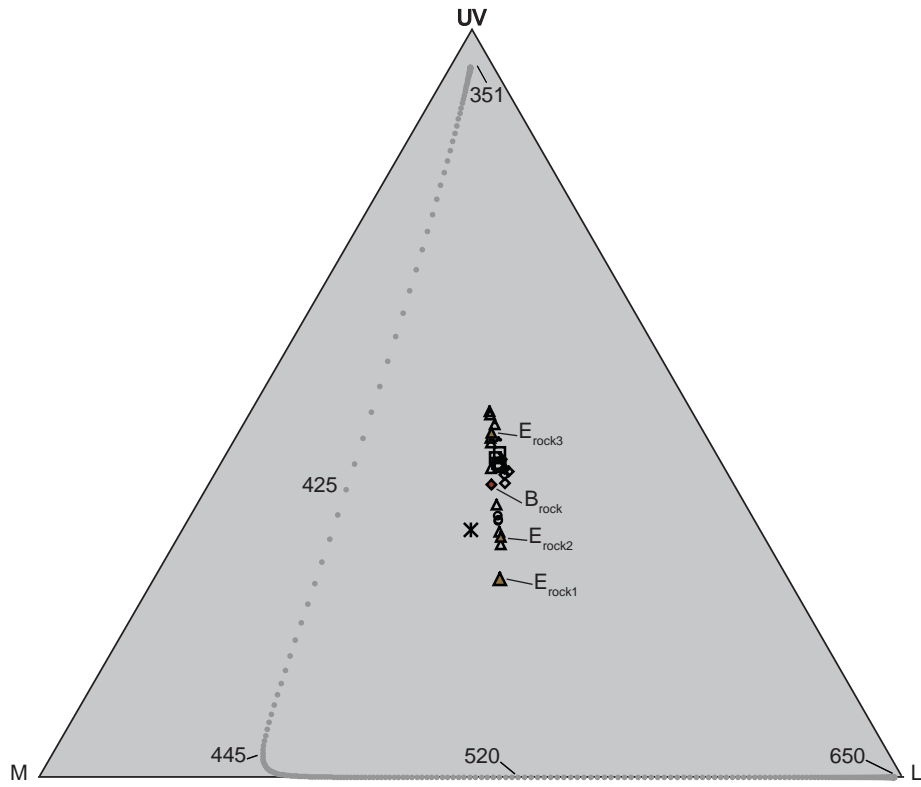
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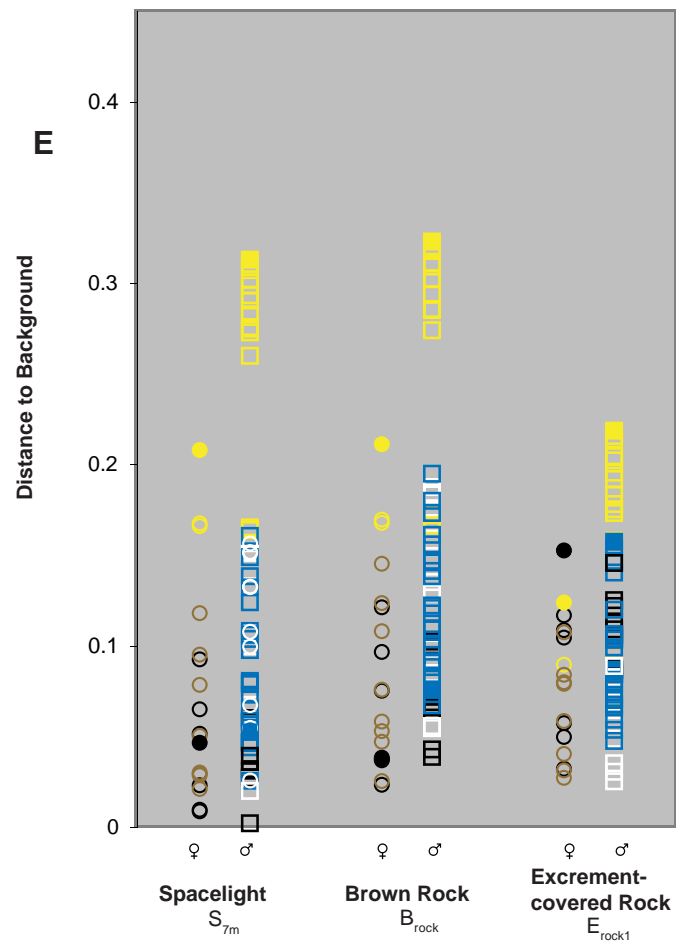
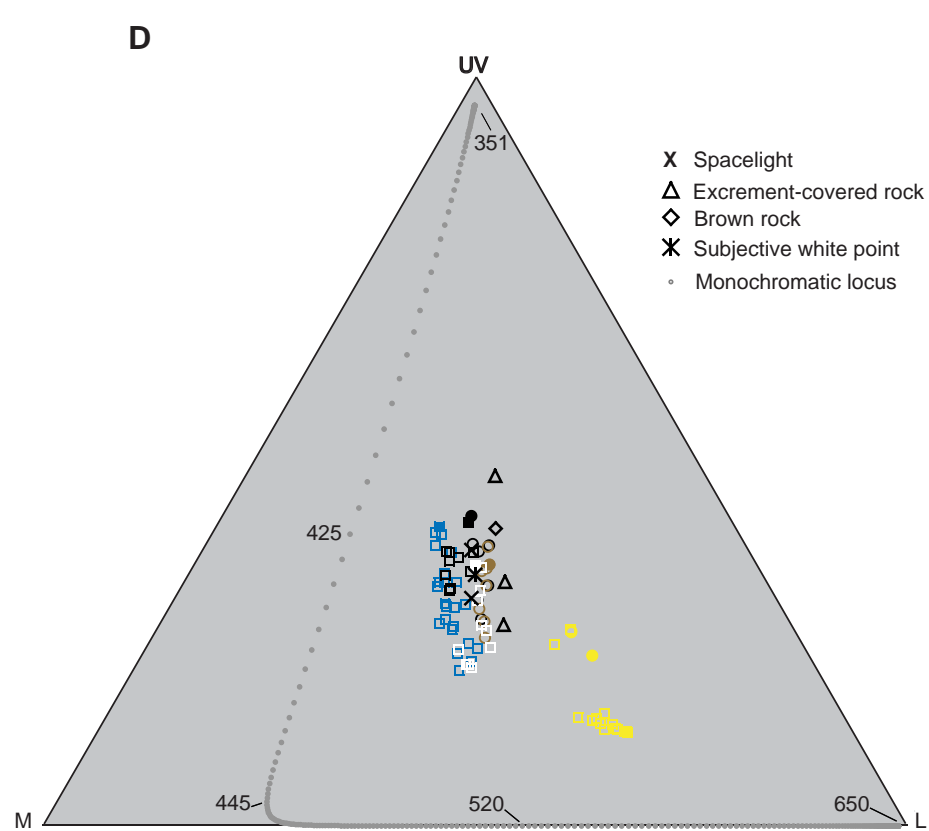
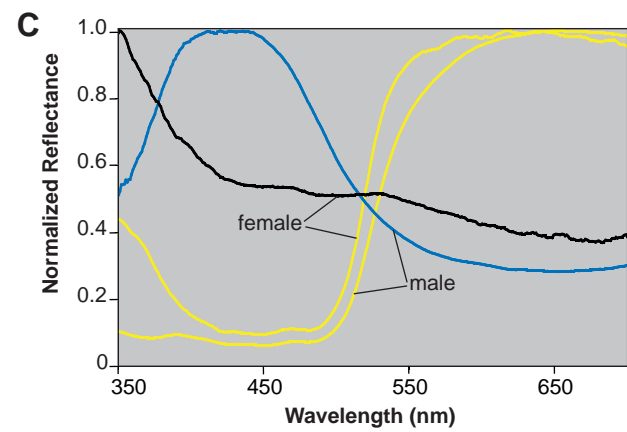
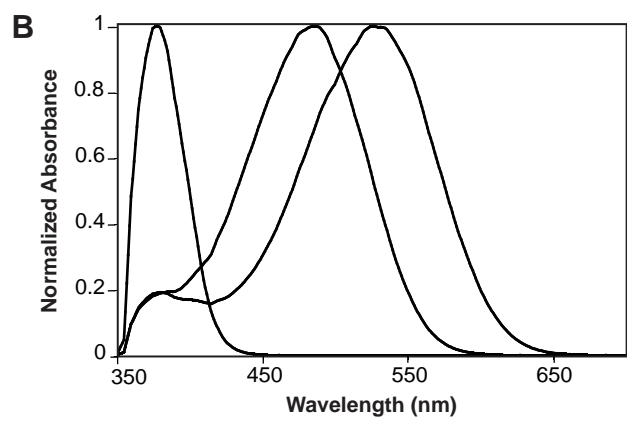
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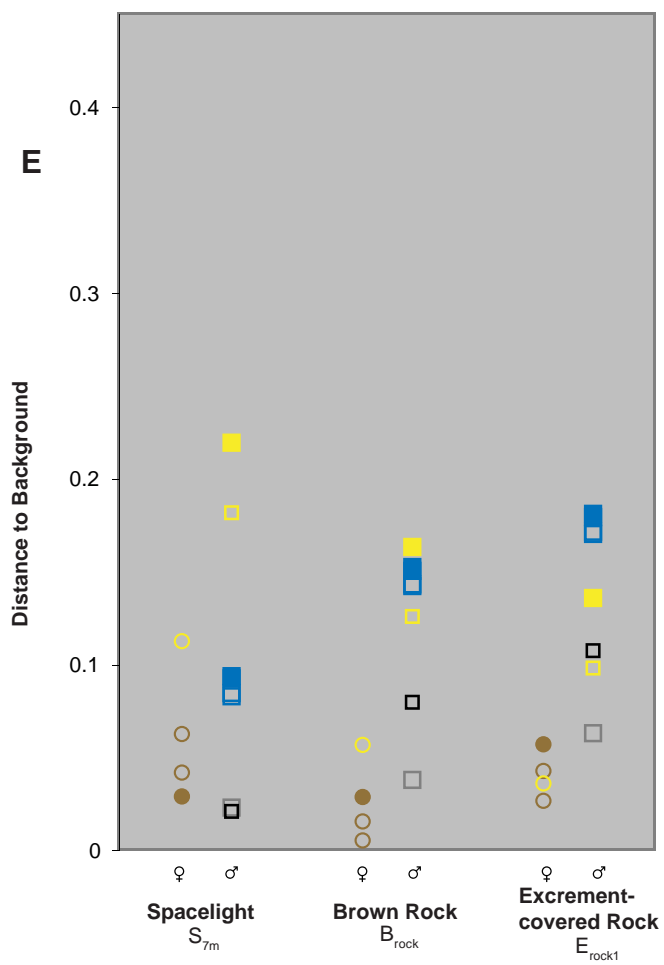
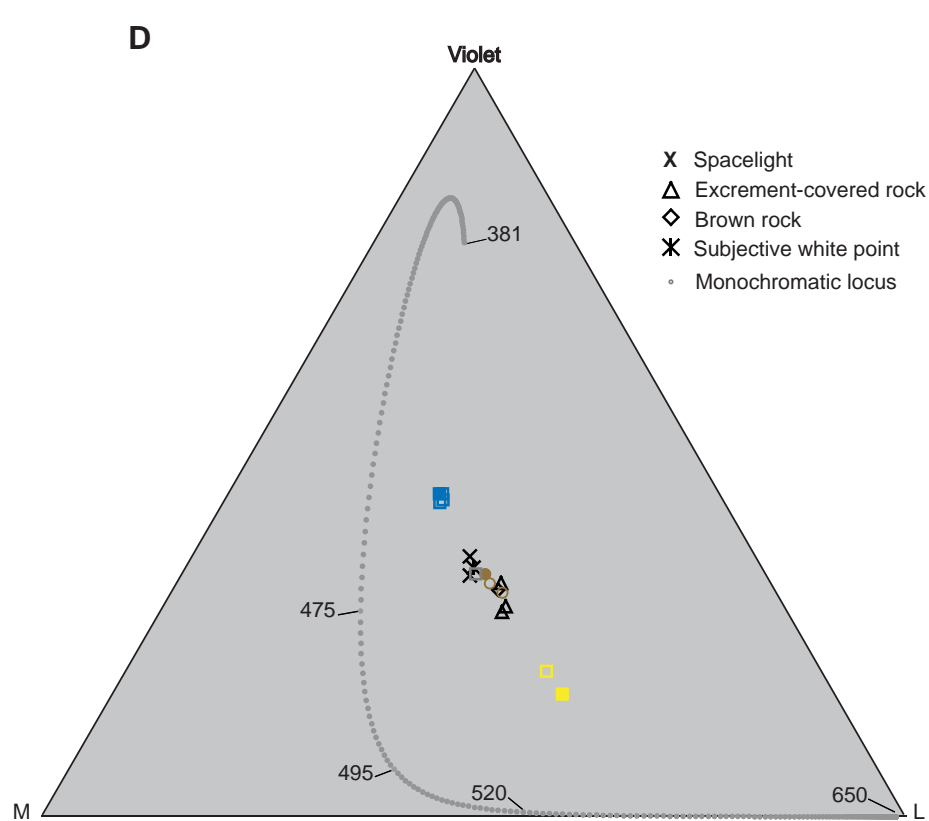
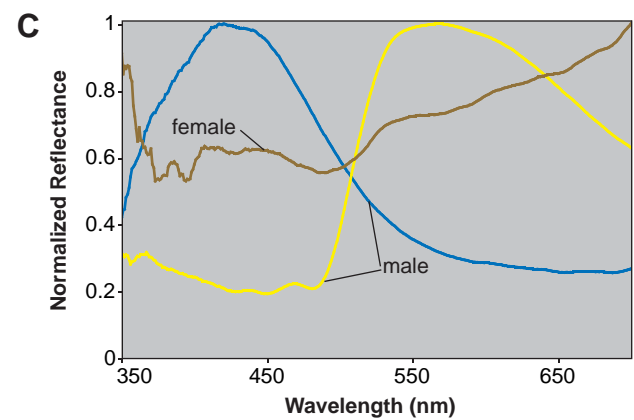
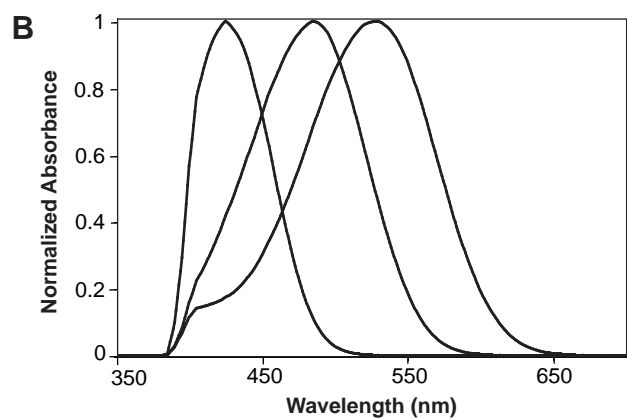
B _{rock}	brown rock background
E _{rock1-3}	excrement-covered rock backgrounds 1-3
S _{3m}	Thumbi West Island spacelight background at 3 m depth
S _{7m}	Thumbi West Island spacelight background at 7 m depth

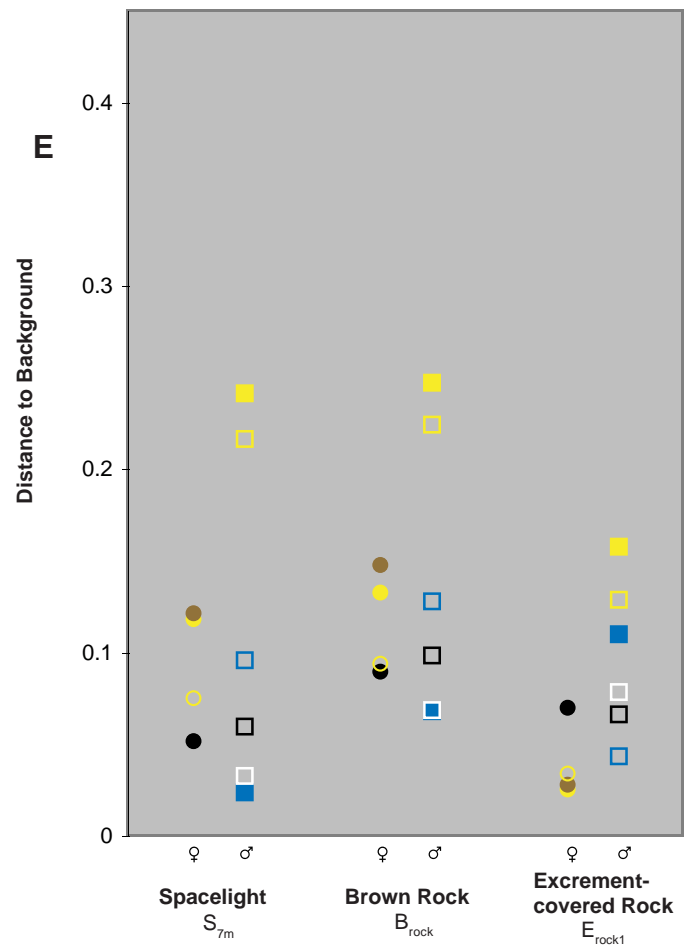
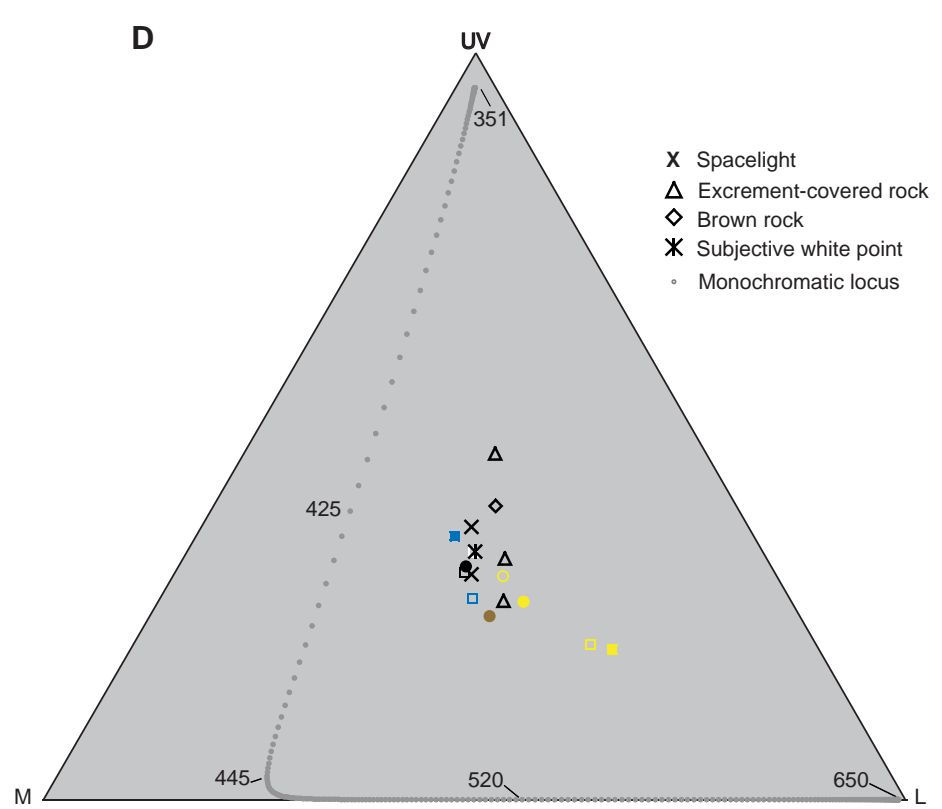
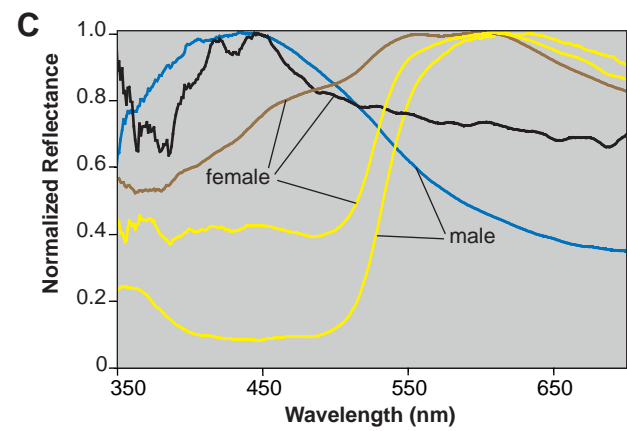
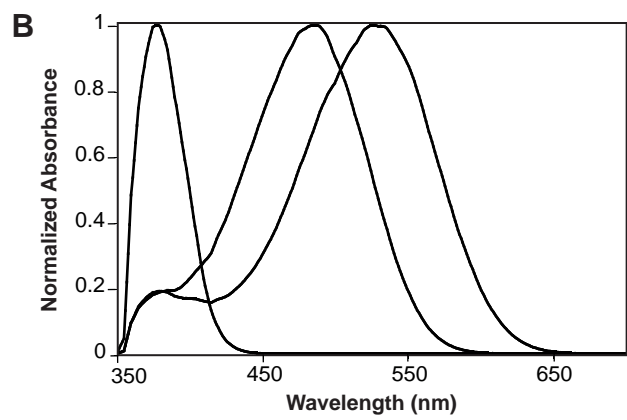
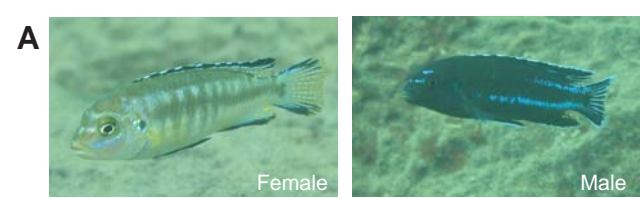
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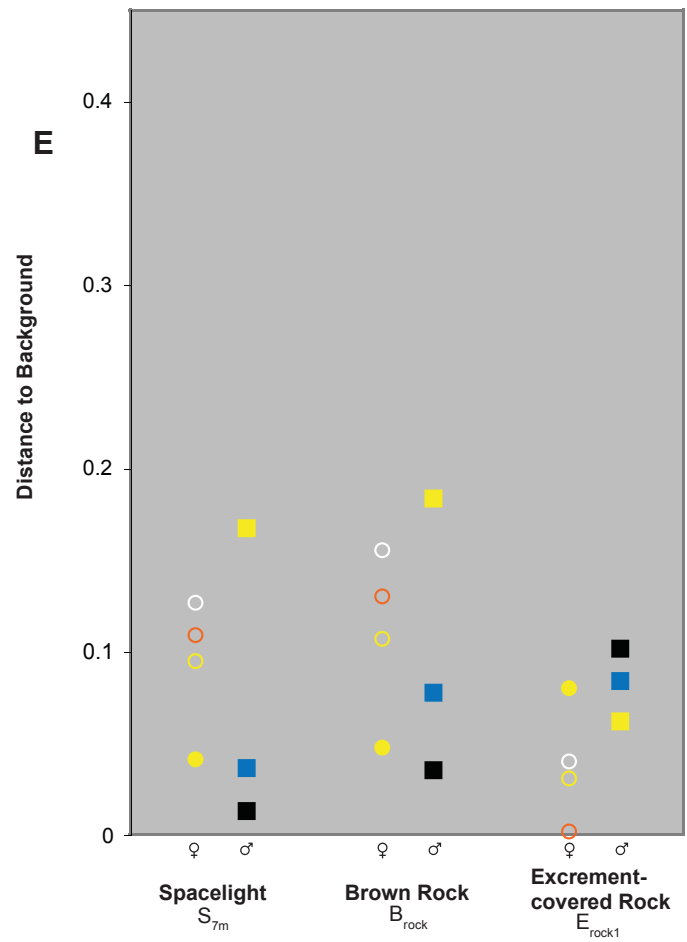
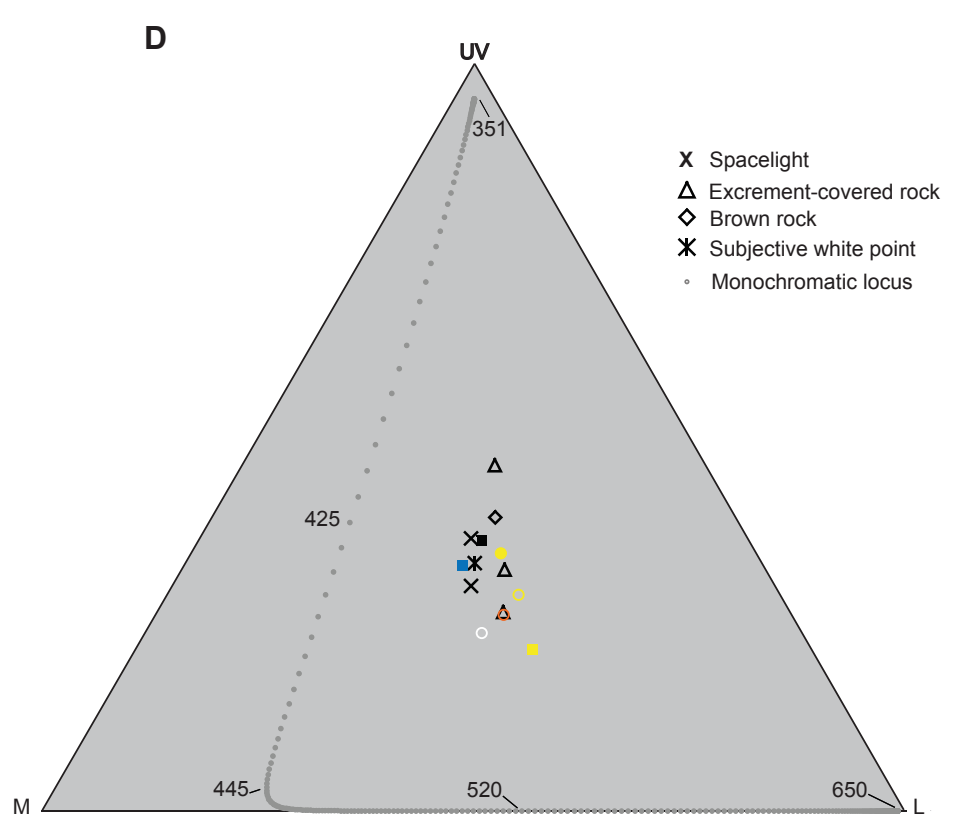
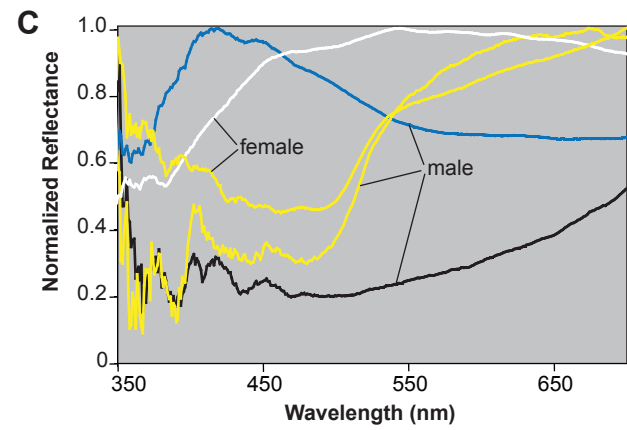
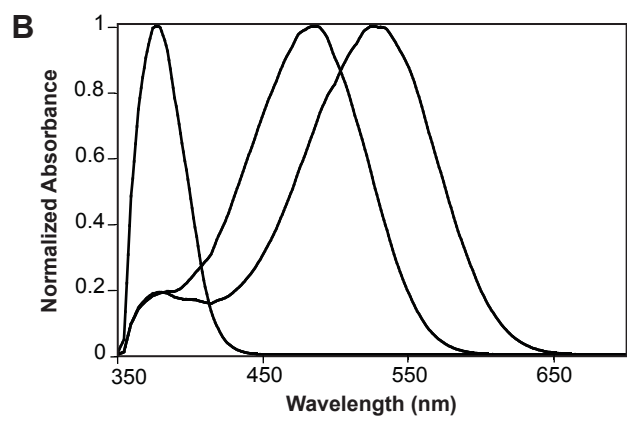
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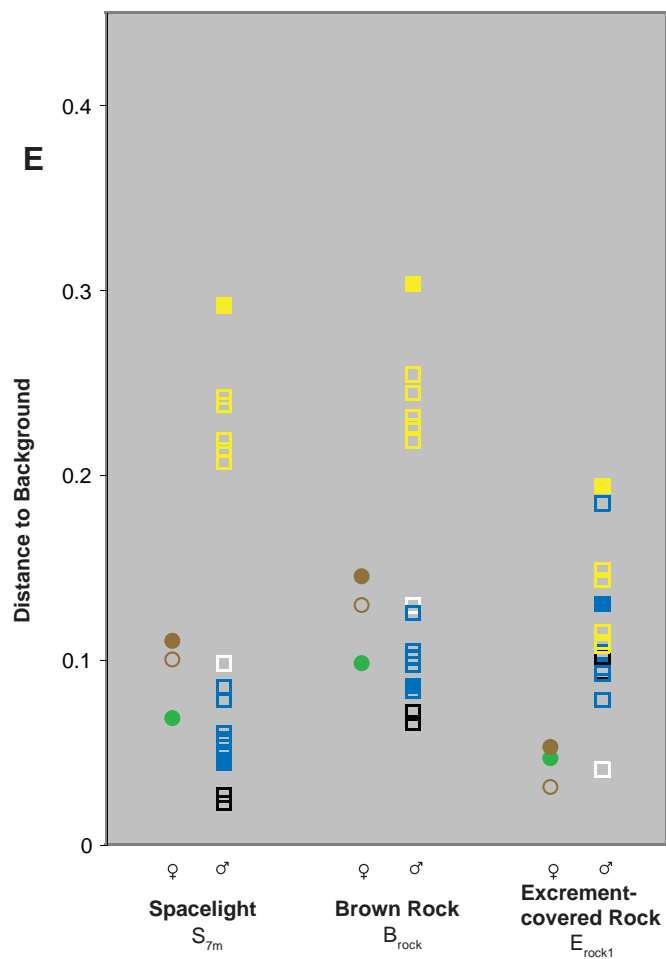
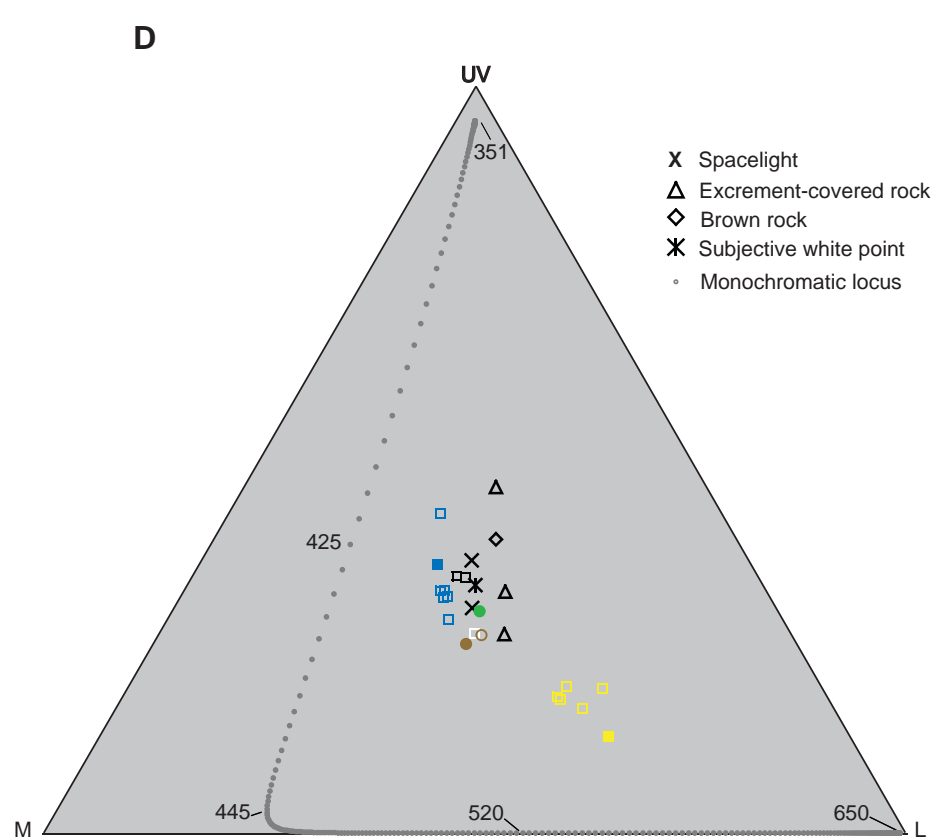
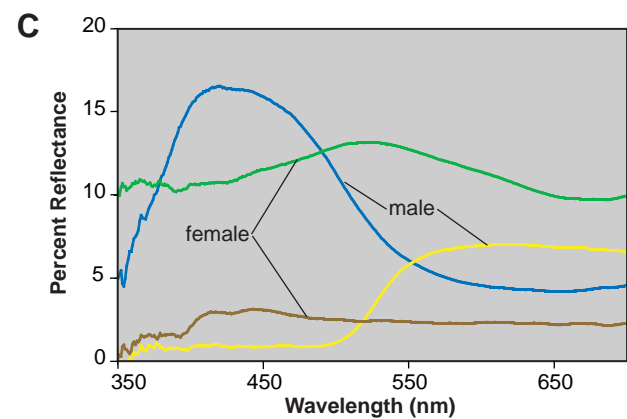
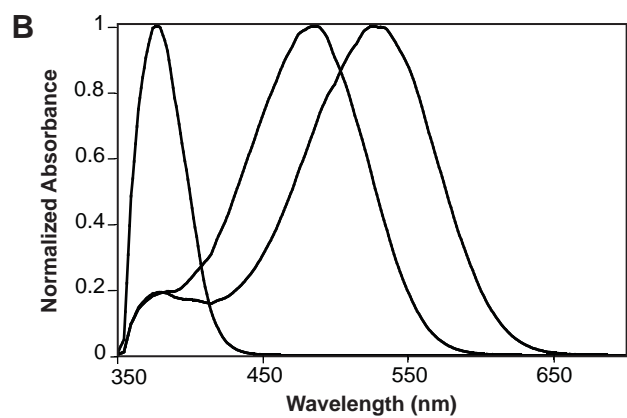
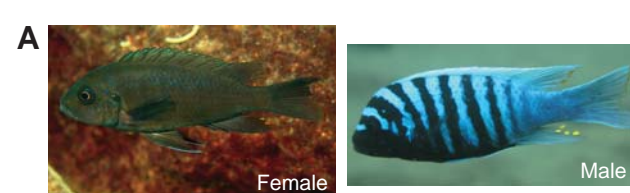


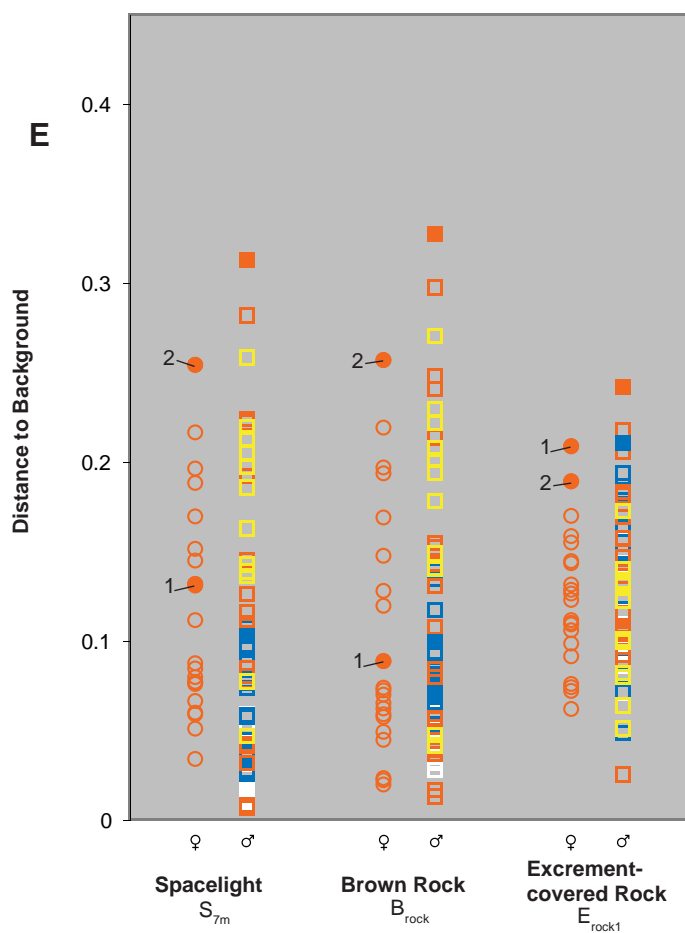
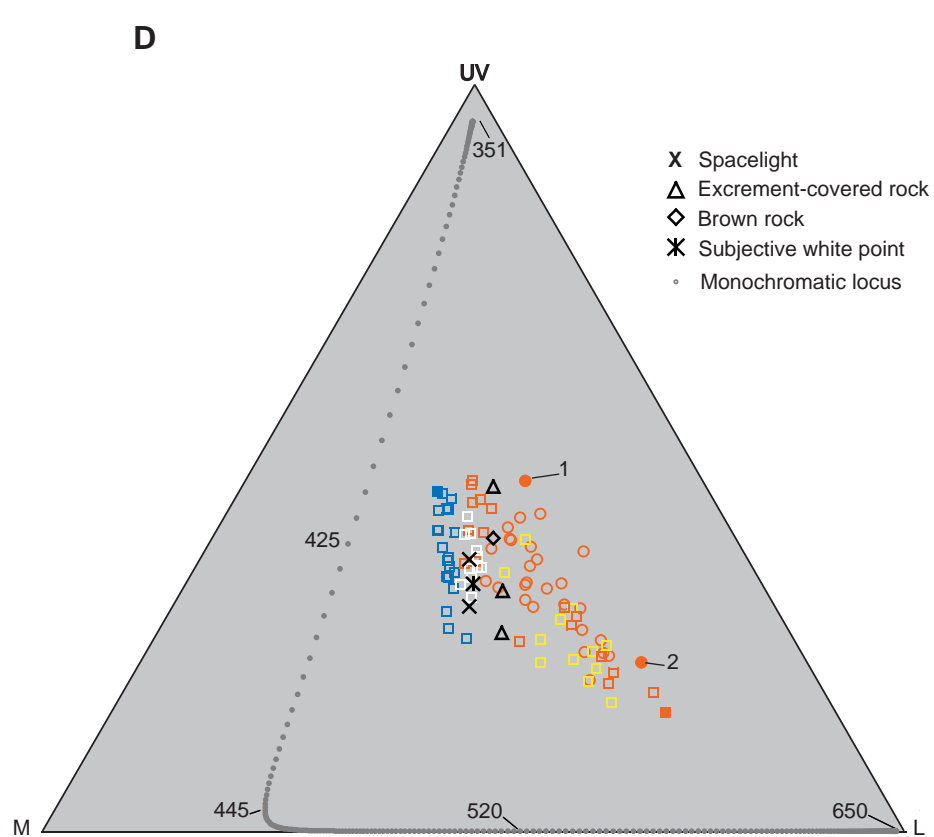
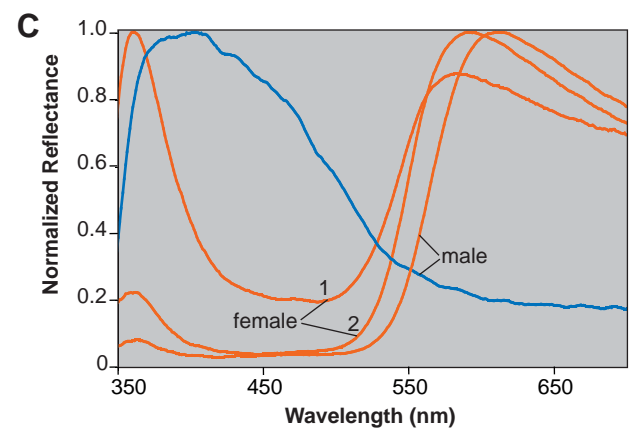
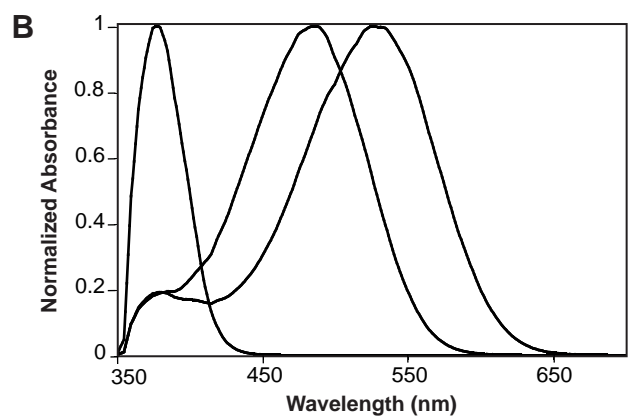


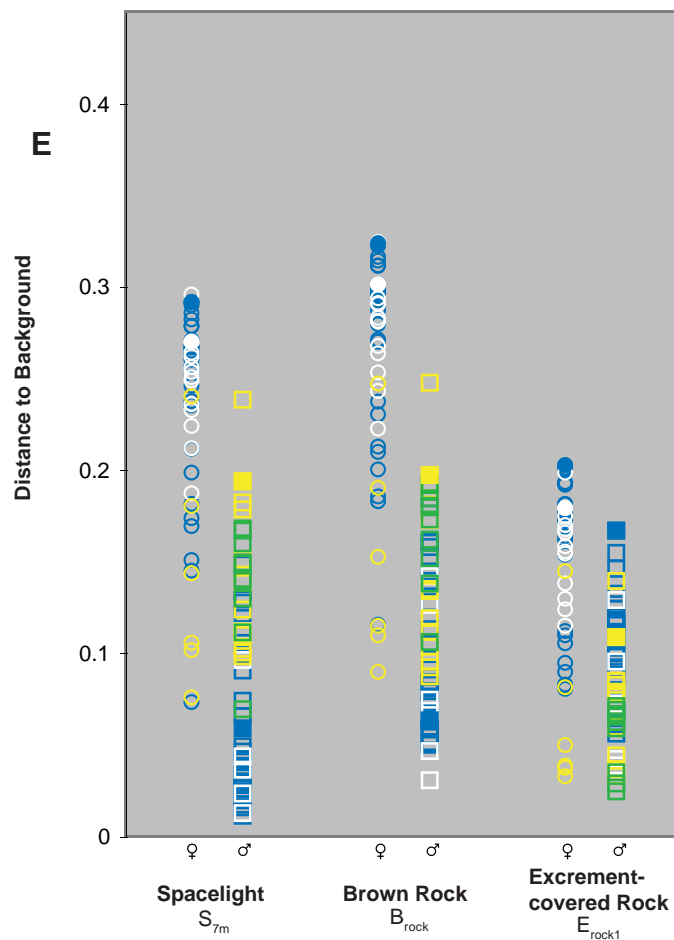
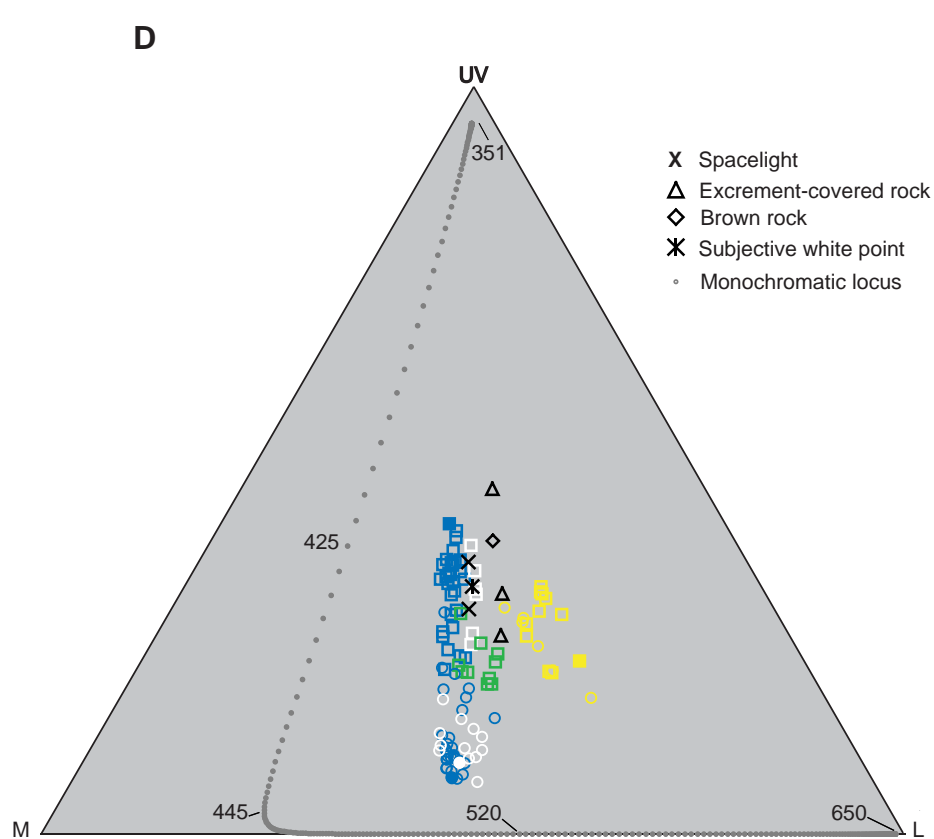
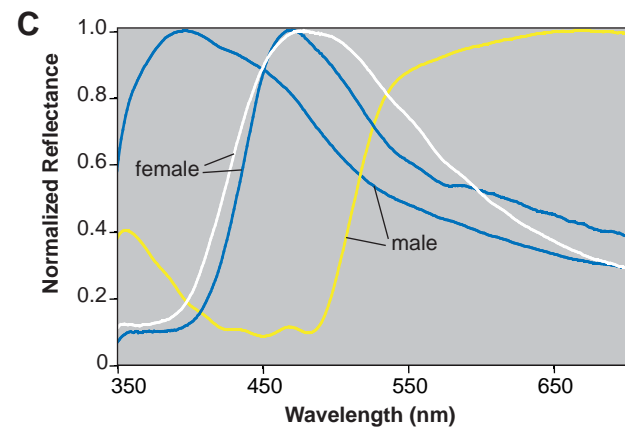
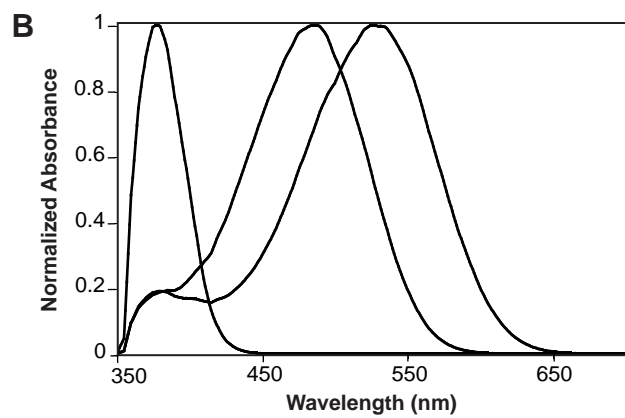


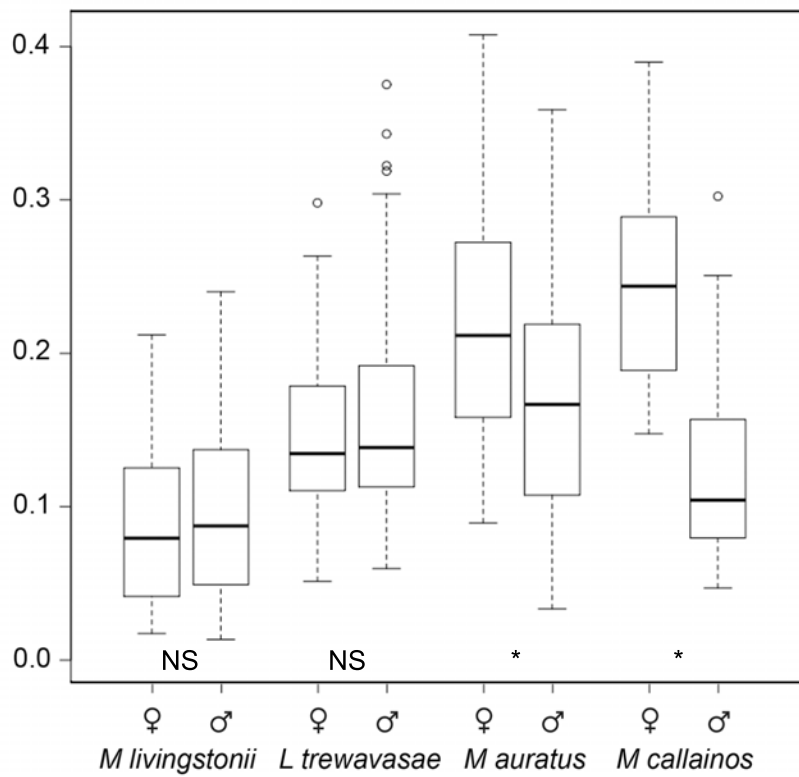
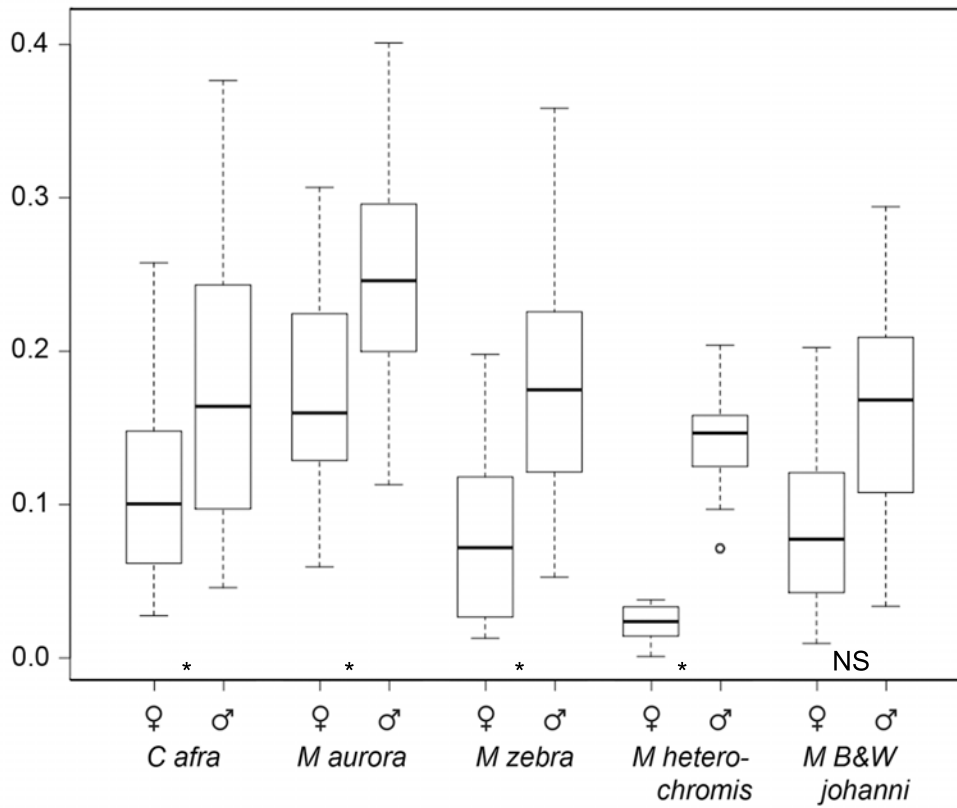


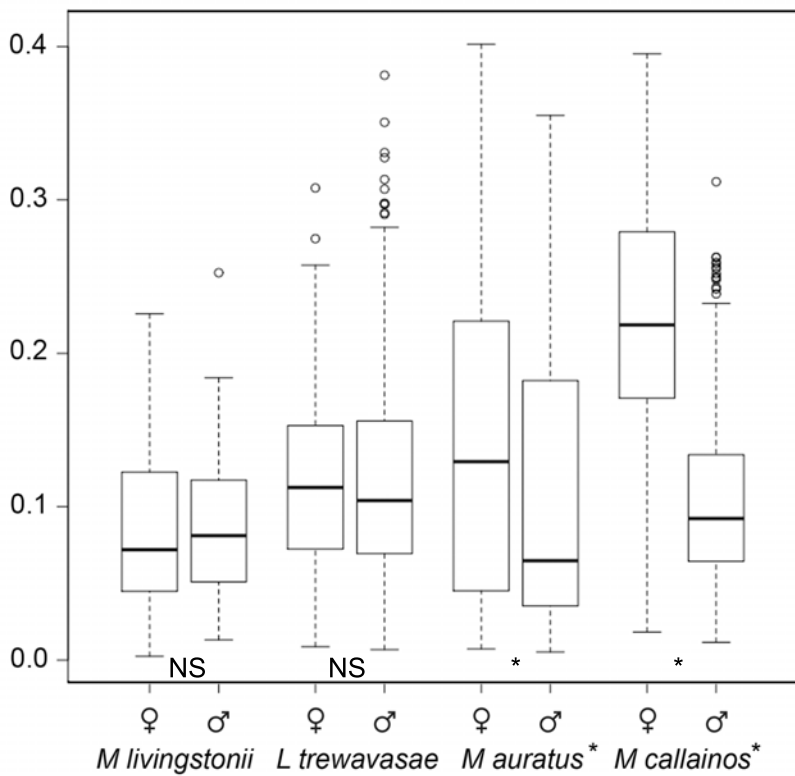
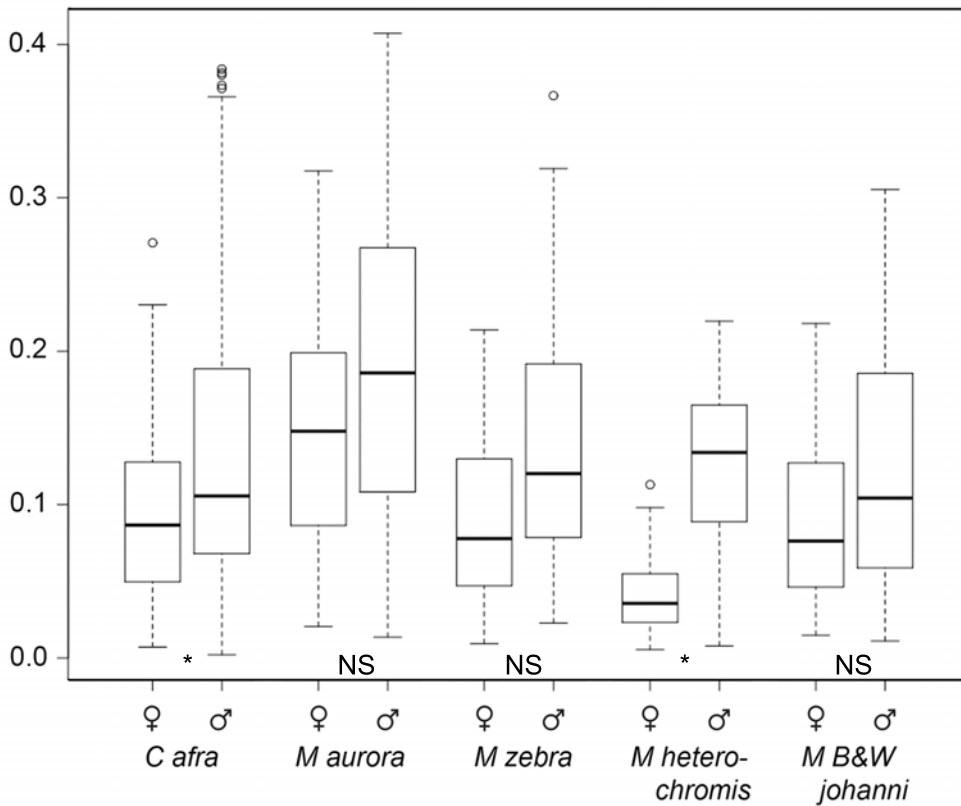


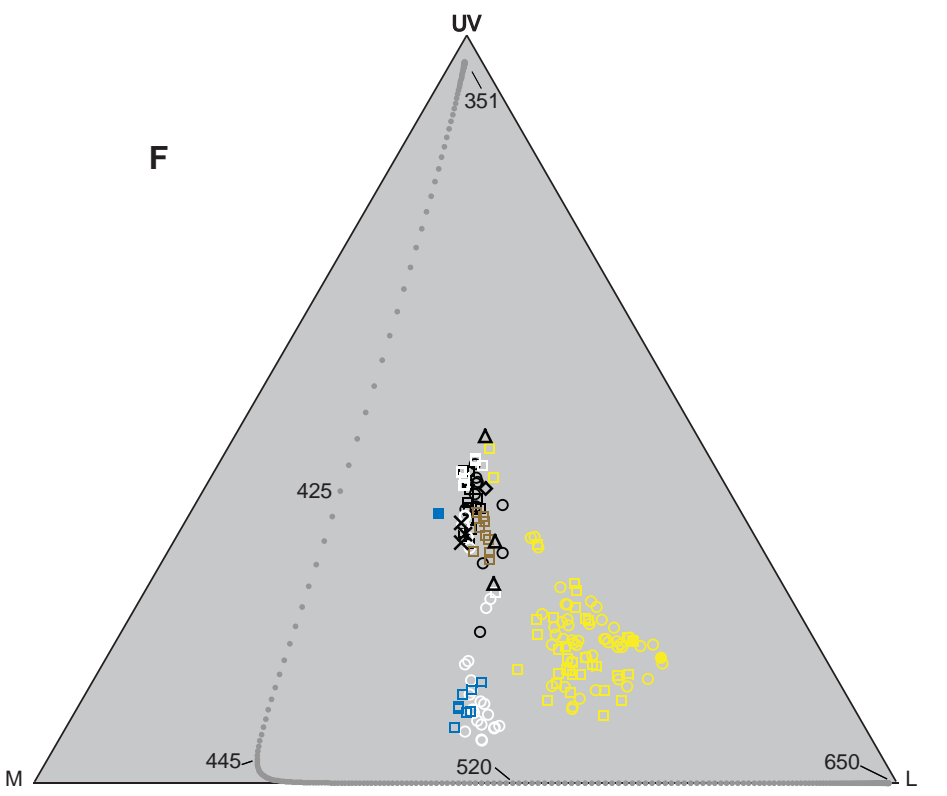
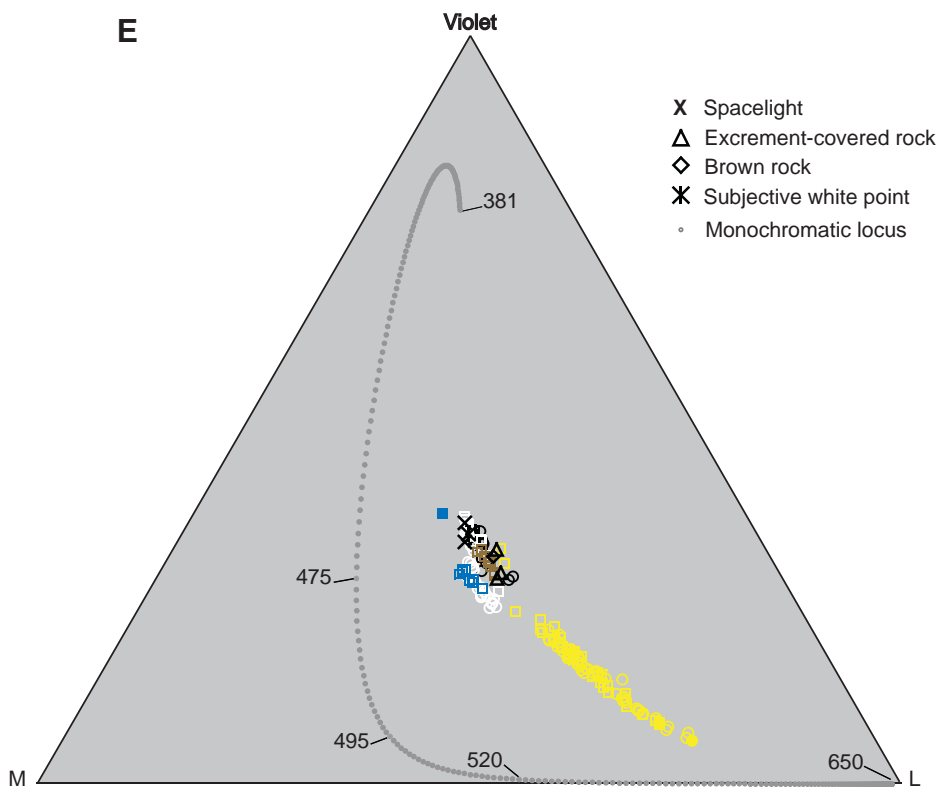
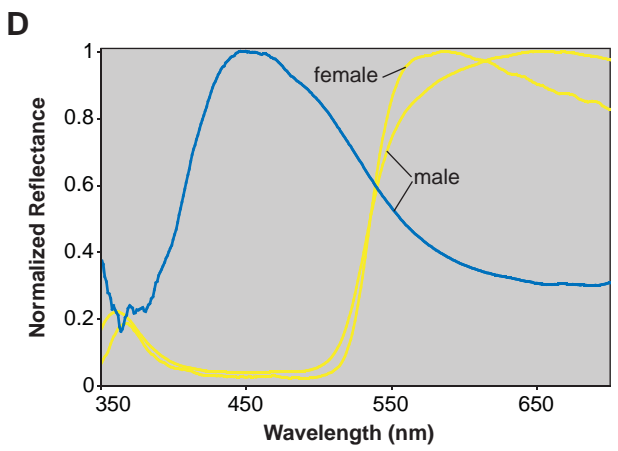
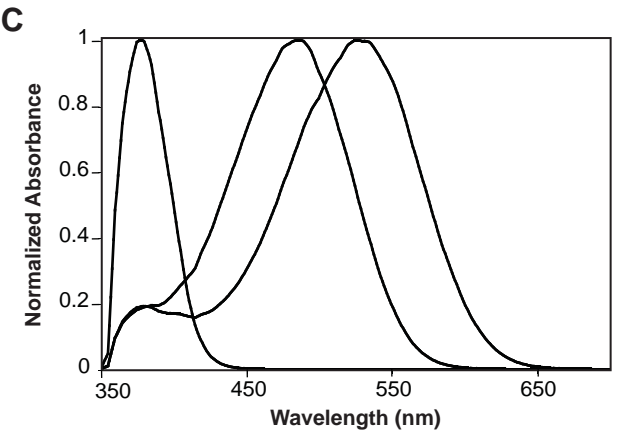
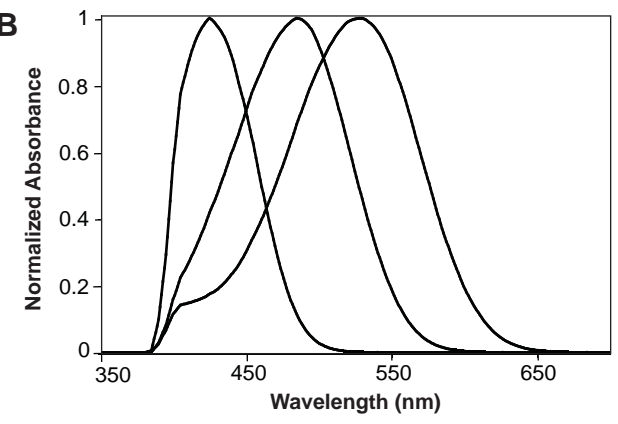












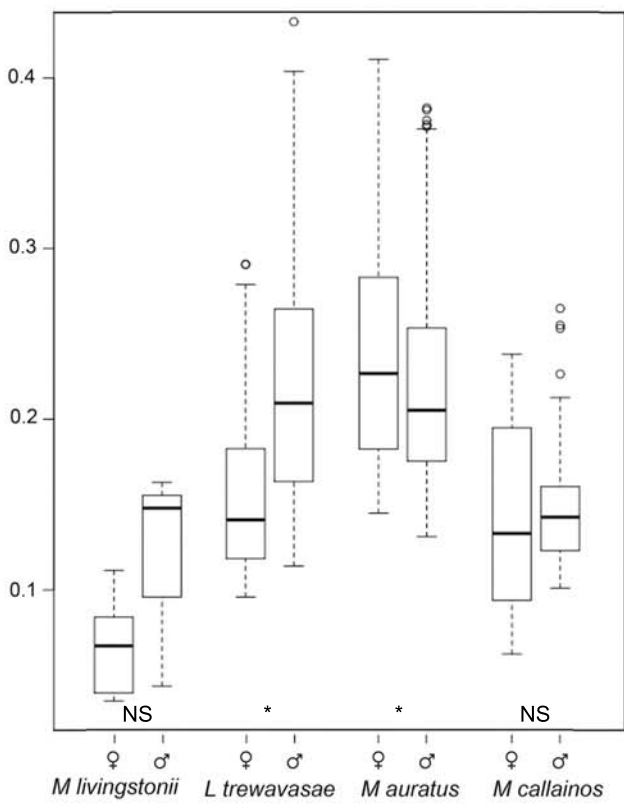
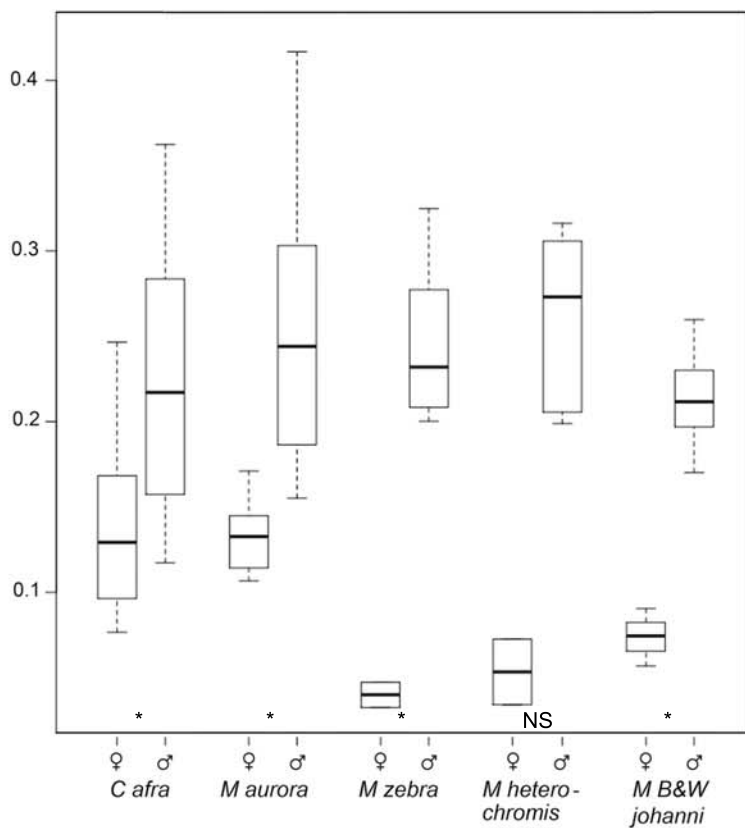


Table S1. Wilcoxon statistical comparisons between male and female colours distances that are greater than or equal to the median for each sex

Species	No. colour distances		Average colour distance (\geq median)		P-value	Bonferroni threshold
	M/F	Background	Male	Female		
<i>C. afra</i>	29/9	E _{rock1}	0.150	0.109	8.25E-03	0.0063
		E _{rock2}	0.286	0.202	8.54E-04*	0.0050
		E _{rock3}	0.162	0.088	7.30E-04*	0.0045
		B _{rock}	0.217	0.154	0.035	0.0125
		S _{3m}	0.156	0.101	0.074	0.0250
		S _{7m}	0.199	0.116	0.009	0.0071
		All	0.195	0.128	2.16E-07*	0.0036
<i>Met. aurora</i>	11/4	E _{rock1}	0.190	0.110	1.47E-03*	0.0038
		E _{rock2}	0.352	0.295	0.078	0.0071
		E _{rock3}	0.223	0.157	0.026	0.0050
		B _{rock}	0.288	0.226	0.078	0.0083
		S _{3m}	0.236	0.162	2.93E-03*	0.0042
		S _{7m}	0.277	0.211	0.026	0.0056
		All	0.261	0.193	2.01E-04*	0.0036
<i>Met. zebra</i>	8/2	E _{rock1}	0.142	0.050	0.044	0.0045
		E _{rock2}	0.282	0.207	0.400	0.0083
		E _{rock3}	0.164	0.077	0.044	0.0050
		B _{rock}	0.217	0.138	0.400	0.0100
		S _{3m}	0.170	0.043	0.044	0.0056
		S _{7m}	0.199	0.106	0.400	0.0125
		All	0.196	0.103	1.21E-04*	0.0036
<i>Mel. heterochromis</i>	4/2	E _{rock1}	0.176	0.050	0.133	0.0071
		E _{rock2}	0.150	0.044	0.133	0.0083
		E _{rock3}	0.171	0.046	0.133	0.0100
		B _{rock}	0.153	0.043	0.133	0.0125
		S _{3m}	0.150	0.073	0.133	0.0167
		S _{7m}	0.147	0.088	0.533	0.0250
		All	0.158	0.057	1.92E-08*	0.0038
<i>Mel. 'B&W' johanni</i>	3/2	E _{rock1}	0.119	0.082	0.200	0.0045
		E _{rock2}	0.295	0.185	0.800	0.0063
		E _{rock3}	0.110	0.055	0.400	0.0056
		B _{rock}	0.174	0.049	0.800	0.0071
		S _{3m}	0.198	0.048	0.800	0.0083
		S _{7m}	0.153	0.092	0.800	0.0100
		All	0.179	0.110	0.015	0.0036
<i>Met. livingstonii</i>	2/2	E _{rock1}	0.093	0.060	0.333	0.0038
		E _{rock2}	0.197	0.213	1.000	0.0125
		E _{rock3}	0.085	0.074	1.000	0.0167
		B _{rock}	0.131	0.143	1.000	0.0250
		S _{3m}	0.090	0.063	0.667	0.0050
		S _{7m}	0.102	0.118	1.000	0.0500
		All	0.116	0.112	0.932	0.0100
<i>L. trewavasae</i>	34/11	E _{rock1}	0.165	0.153	0.278	0.0042
		E _{rock2}	0.223	0.205	0.368	0.0050
		E _{rock3}	0.145	0.132	0.354	0.0045
		B _{rock}	0.165	0.152	0.540	0.0071
		S _{3m}	0.164	0.169	0.426	0.0063
		S _{7m}	0.157	0.163	0.558	0.0100
		All	0.170	0.162	0.554	0.0083
<i>Mel. auratus</i>	38/44	E _{rock1}	0.158	0.199	4.79E-03*	0.0071
		E _{rock2}	0.180	0.228	4.38E-03	0.0042
		E _{rock3}	0.160	0.201	4.65E-03*	0.0063
		B _{rock}	0.176	0.224	4.51E-03*	0.0050
		S _{3m}	0.210	0.264	4.38E-03*	0.0045
		S _{7m}	0.224	0.278	4.51E-03*	0.0056
		All	0.185	0.232	6.84E-11*	0.0036
<i>Met. callainos</i>	20/25	E _{rock1}	0.120	0.146	2.59E-15*	0.0071
		E _{rock2}	0.225	0.372	6.48E-16*	0.0042
		E _{rock3}	0.106	0.236	6.48E-16*	0.0045
		B _{rock}	0.127	0.188	2.00E-15*	0.0063
		S _{3m}	0.100	0.207	7.78E-15*	0.0125
		S _{7m}	0.102	0.229	3.11E-15*	0.0100
		All	0.139	0.262	2.20E-16*	0.0038

Calculations are done for each species relative to the six backgrounds as well as all backgrounds combined. This is a subset of the data given in Table S2, with the number of colour distances listed for each species. For each background, average male and female colour distances and the Wilcoxon P-value are given. The P-value thresholds for significance after sequential Bonferroni correction are listed. If a test is significant, the P-value is marked with an asterisk and the larger of the two average colour distances is in bold type. The sequential Bonferroni correction was applied across all tests in Tables S1–S2.

Table S2. Wilcoxon statistical comparisons between all male and all female colour distances from each species to the six backgrounds as well as all backgrounds combined

Species	No. colour distances		Back-ground	Average colour distance		P-value	Bonferroni threshold
	No. fish	M/F		Male	Female		
<i>C. afra</i>	4/4	58/18	E _{rock1}	0.129	0.125	0.070	0.0167
			E _{rock2}	0.214	0.154	0.013	0.0083
			E _{rock3}	0.118	0.063	1.35E-04*	0.0042
			B _{rock}	0.114	0.062	0.003*	0.0056
			S _{3m}	0.100	0.066	0.135	0.0500
			S _{7m}	0.124	0.072	0.032	0.0100
			All	0.134	0.093	8.13E-05*	0.0038
<i>Met. aurora</i>	2/1	22/8	E _{rock1}	0.129	0.074	0.070	0.0063
			E _{rock2}	0.273	0.247	0.475	0.0500
			E _{rock3}	0.160	0.113	0.219	0.0125
			B _{rock}	0.158	0.109	0.447	0.0250
			S _{3m}	0.159	0.113	0.202	0.0100
			S _{7m}	0.197	0.161	0.393	0.0167
			All	0.190	0.149	0.014	0.0045
<i>Met. zebra</i>	2/1	16/3	E _{rock1}	0.131	0.083	0.008	0.0038
			E _{rock2}	0.210	0.194	0.712	0.0167
			E _{rock3}	0.120	0.066	0.085	0.0063
			B _{rock}	0.117	0.058	0.958	0.0500
			S _{3m}	0.106	0.032	0.109	0.0071
			S _{7m}	0.125	0.093	0.793	0.0250
			All	0.138	0.092	0.022	0.0042
<i>Met. heterochromis</i>	2/1	8/4	E _{rock1}	0.151	0.059	4.04E-03*	0.0042
			E _{rock2}	0.123	0.029	8.08E-03	0.0050
			E _{rock3}	0.136	0.037	4.04E-03*	0.0045
			B _{rock}	0.137	0.040	8.08E-03	0.0056
			S _{3m}	0.109	0.048	0.109	0.0063
			S _{7m}	0.100	0.062	0.570	0.0500
			All	0.122	0.041	1.28E-09*	0.0036
<i>Met. 'B&W' johanni</i>	1/1	6/4	E _{rock1}	0.124	0.082	0.038	0.0038
			E _{rock2}	0.202	0.185	1.000	0.0167
			E _{rock3}	0.099	0.055	0.352	0.0050
			B _{rock}	0.096	0.049	1.000	0.0250
			S _{3m}	0.088	0.048	1.000	0.0500
			S _{7m}	0.112	0.092	0.914	0.0125
			All	0.123	0.089	0.138	0.0042
<i>Met. livingstonii</i>	1/1	3/4	E _{rock1}	0.111	0.084	0.114	0.0036
			E _{rock2}	0.165	0.180	0.857	0.0056
			E _{rock3}	0.074	0.052	0.629	0.0042
			B _{rock}	0.072	0.050	0.857	0.0063
			S _{3m}	0.071	0.061	0.857	0.0071
			S _{7m}	0.073	0.093	0.629	0.0045
			All	0.094	0.089	0.910	0.0083
<i>L. trewavasae</i>	4/3	61/28	E _{rock1}	0.168	0.156	0.904	0.0500
			E _{rock2}	0.152	0.147	0.858	0.0250
			E _{rock3}	0.106	0.093	0.387	0.0056
			B _{rock}	0.110	0.098	0.579	0.0125
			S _{3m}	0.116	0.132	0.204	0.0038
			S _{7m}	0.096	0.118	0.136	0.0036
			All	0.118	0.119	0.583	0.0167
<i>Met. auratus</i>	4/4	76/88	E _{rock1}	0.105	0.118	0.255	0.0500
			E _{rock2}	0.108	0.140	0.018	0.0100
			E _{rock3}	0.102	0.126	0.171	0.0250
			B _{rock}	0.102	0.124	0.031	0.0125
			S _{3m}	0.120	0.158	0.049	0.0167
			S _{7m}	0.116	0.151	0.013	0.0083
			All	0.110	0.141	1.11E-06*	0.0038
<i>Met. callainos</i>	4/4	59/49	E _{rock1}	0.118	0.110	1.42E-09*	0.0500
			E _{rock2}	0.175	0.328	1.55E-15*	0.0050
			E _{rock3}	0.085	0.194	1.19E-13*	0.0250
			B _{rock}	0.083	0.188	2.00E-15*	0.0056
			S _{3m}	0.074	0.170	1.04E-13*	0.0167
			S _{7m}	0.088	0.229	3.11E-15*	0.0083
			All	0.104	0.221	2.20E-16*	0.0036

Number of male and female fish and colour distances are given for each species. For each background, average male and female colour distances and the Wilcoxon P-value are given. The P-value thresholds for significance after sequential Bonferroni correction are listed. If a test is significant the P-value is marked with an asterisk and the larger of the two average colour distances is in bold type. The sequential Bonferroni correction was applied across all tests in Tables S1–S2.

Table S3. Wilcoxon statistical comparisons of distances between colours within male and female fish for each species

Species	No. available colour distances		No. high contrast colour distances compared M/F		Average male	Average female	P-value
	M/F		M/F				
<i>C. afra</i>	447/54		227/23		0.224	0.138	2.45E-08*
<i>Met. aurora</i>	110/13		55/6		0.246	0.134	1.83E-04*
<i>Met. zebra</i>	56/3		28/2		0.232	0.040	4.60E-03*
<i>Mel. heterochromis</i>	12/2		All distances		0.262	0.053	0.351
<i>Mel. 'B&W' johanni</i>	15/6		8/3		0.213	0.074	0.012*
<i>Met. livingstonii</i>	3/6		All distances		0.117	0.066	0.262
<i>L. trewavasae</i>	604/220		302/110		0.217	0.155	<2.2E-16*
<i>Mel. auratus</i>	846/1118		423/559		0.218	0.235	2.66E-05*
<i>Met. callainos</i>	470/347		235/173		0.144	0.141	0.323

Comparisons were restricted to the high contrast colour combinations, those greater than or equal to the median of each sex of each species. Two of the species did not have sufficient numbers of spectra and so included all spectra. Number of spectra included are listed for each species. For each background, average male and female colour distances and the Wilcoxon *P*-value are given. If significant at $P < 0.05$, *P*-values are marked with an asterisk and the larger of the two average colour distances is in bold type.