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Ultraviolet reflectivity in three species of Lake Malaŵi rock-dwelling cichlids

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The body colouration extending into ultraviolet wavelengths in three species of Lake Malaŵi rock-dwelling cichlids, *Metriaclima zebra*, *Metriaclima benetos* and *Metriaclima barlowi* was quantified. All three species were ultraviolet reflective with interspecific differences in reflectivity. In addition, individuals were able to behaviourally mediate their reflectivity.

Key words: behaviour; cichlids; Lake Malaŵi; reflectivity; ultraviolet.

The cichlids of the East African Great Lakes are among the most diverse groups of fishes known (Barlow, 2000). Particularly diverse are the rock-dwelling (*mbuna*) cichlids of Lake Malaŵi, which probably account for >200 of the 1400 or more species found in these lakes (Barlow, 2000; Danley & Kocher, 2001). Lake Malaŵi *mbuna* are known for their diverse body colouration among species, especially in males (Fryer & Iles, 1972; Konings, 1990; McElroy *et al.*, 1991). Because these fishes are able to modify intensity, and in many cases pattern, within a few minutes (Nelisson, 1991), it has been widely suggested that these changes aid in the communication with other individuals. In addition, the importance of visual cues in communication for African cichlids has been well established (McElroy & Kornfield, 1990; Hert, 1991; Couldridge, 2002; Jordan *et al.*, 2003).

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In this study, male and female body reflectivity was investigated under several wavelengths and in particular under wavelengths in the ultraviolet range. Body reflectivity in several behavioural situations was also investigated with particular focus on dominance between males. For these experiments, three sympatric species, *Metriaclima zebra* (Boulenger) (males are blue with black bars), *Metriaclima benetos* (Stauffer, Bowers, Kellogg & McKaye) (males are primarily blue but exhibit dark bars under certain situations) and *Metriaclima barlowi* (McKaye & Stauffer) (males are yellow with a blue anal fin), were used. While males of all three species exhibit variation in colour patterns, females are solid brown, with the exception that *M. zebra* females have faint black vertical bars. All three species are UV sensitive (Carleton *et al.*, 1999; R.C. Jordan, K.A. Kellogg, F. Juanes, J.R. Stauffer Jr & E.R. Loew, unpubl. data).

Reflectivity was measured at the following five regions covering six to eight patches on each fish depending on the species: (1) dorsal fin, (2) operculum, (3) gular region, (4) anal fin and (5) two or four lateral flank regions (one upper region, one lower region and, for *M. zebra*, one dark and one light region of both the upper and lower regions). These regions of the body were chosen in an attempt to achieve the greatest diversity in both visible colour and morphological structure.

The reflectivity of the patches was measured with a spectroradiometer (Ocean Optics S1000) equipped with quartz optics. All measurements were collected in a dark room. Three males and three females from each of the three species were removed from the tank and lightly towelled to remove surface water. The fish were placed directly below a calibrated tungsten quartz-iodide irradiant source and reflectivity was measured with a fibre optic radiance probe set at a 45-degree angle from the measured patch. A magnesium carbonate white standard placed at the same position as the fish was used as a reflectance standard (Schneider & Jackson, 1963). The data were collected using a spectrometer programme (Cspec, Ancal Inc.) and exported into a Microsoft Excel spreadsheet. Curves were generated that were both relative to the white standard and normalized to one hundred per cent.

Next, behavioural mediation of UV reflectivity was investigated by measuring intensity from images obtained with a remote-controlled variable gain Gen II monochrome CCD video camera equipped with eight optical bandpass filters (Losey, 2003). During filming, the fishes were housed in a UV-reflective, Teflon-coated tank with Q-Panel UV-A and cool white fluorescent tubes mounted 60 cm directly overhead.

Using each filter, individual fish were filmed for four, 2 min periods under four social conditions: alone, with a female, with a male and while feeding in a group. From each 2 min period, six frames were randomly selected from sections of the video when the individual was swimming in a plane parallel to the camera lens. Reflectance was measured using Sigma Scan, Inc. software using the following equation: patch reflectance $= [x - b][(255w^{-1}\%wr^{-1}) - b]$, where $x =$ pixel count for the patch, $b =$ pixel count for the black standard, $w =$ pixel count for the white standard and $\%wr =$ wavelength-specific constant to adjust for the white reference standard. Within each image, monochrome pixel intensity was collected six times for each patch and methods similar to Losey (2003) were used to analyse the reflectance of the patches. In addition, when possible, intensity from the white barium sulphide and all-flat black standards was

measured. The intensity taken for each patch was then standardized to zero for black and 255 for white. Reflectance of the white standard was calibrated using a near-perfect spectralon reflectance standard. Within each social condition, reflectance of individual fish patches was averaged for each filter type. Thus, for each of the four randomly ordered social conditions, 192 frames for each individual were analysed.

The determination of a dominant *v.* subordinate male was determined by the outcome of the dominance dispute that occurred when two males were placed together in a tank. This dispute was characterized by both males increasing the intensity of their visible colour (as noted by the human observer), erecting dorsal and anal fins, and in some cases, chasing followed by nipping at the fins or posterior portion of the other male's body. Typically, within a minute, a dominant male was identified by the retention of its intense colouration, with the subordinate male maintaining a distance from the dominant male and retaining colouration similar to lone males.

Four individuals of each of two species were video-taped: three male and one female *M. zebra*, and two male and two female *M. barlowi*. *Metriaclima benetos* could not be obtained for this part of the study. Non-wavelength specific UV-only footage of this species, however, was collected.

The most notable characteristics and their analysis in this paper are mostly confined to 380 nm, which is the UV wavelength closest to the maximal sensitivity of these fishes, *i.e. c.* 372–376 nm. All data, including non-UV wavelengths and colour photographs of each species can be observed at: www.unc.edu/~rcjordan/pictures.htm.

All three species were UV reflective (Table I). Males were generally more reflective than females and reflectivity varied among body patches. When the UV only photographs were compared with colour photographs, blue and yellow patches were more reflective than the brown and dark regions. Patterning in the UV appeared to correlate with patterning in the visible spectrum.

The results from the spectroradiometer and the video were generally similar with the exception that the overall relative reflectivity values tended to be lower in the spectroradiometer data (Table I). While females tended not to change reflectivity patterns, males changed patterns under different behavioural conditions. When two males were together, even if they were foraging, one male tended to express dominance while the other was subordinate (see Table I). The dominant and subordinate UV reflectivity patterns were consistent within species. In the presence of a female, the males would often exhibit courtship behaviour (Jordan *et al.*, 2003) and express colours similar to those of dominant males in both the visible and UV spectra (R. Jordan, pers. obs.).

Metriaclima barlowi and *M. zebra* males demonstrated consistent behavioural changes in reflectivity at 380 nm. *Metriaclima benetos* males were most reflective in the flank and the dorsal fin, but video images that would determine if males altered their UV reflectivity in different behavioural situations were not obtained. In *M. barlowi*, the anal fin and the light portion of the dorsal fin were the most reflective in dominant males. In subordinate males, however, the flank was equally or more reflective than the anal fin. It appears that dominant males simultaneously increased the reflectivity of the anal fin and decreased the reflectivity of the flank, thus maximizing contrast. This phenomenon was also

TABLE I. Per cent of relative reflectance obtained from images taken under 380 nm while fishes were either still or during behavioural interactions. Increased values correspond to increased reflectivity. Values >100% are not uncommon with specular surfaces; maximal reflectivity should be assumed. The S.D. are included with the still-image data to indicate within species variance. The behavioural-image data are from single representative individuals. Dominant and subordinate describe the behavioural actions of two representative males when filmed together (see notes). △, the portions of the body that were generally most reflective in dominant males of that species

			Still-image		Behavioural-image		
			Male	Female	Dominant	Subordinate	Female
M. benetos							
		Opercular/gular	82 ± 17	91 ± 20			
		Anal fin	84 ± 04	50 ± 06			
	△	Dorsal fin	163 ± 32	75 ± 10			
	△	Flank	153 ± 15	79 ± 28			
M. barlowi							
		Opercular/gular	75 ± 18	81 ± 12	30	61	70
	△	Anal fin	86 ± 08	61 ± 20	152	127	119
		Flank	91 ± 15	106 ± 16	61	152	131
M. zebra							
		Opercular/gular	81 ± 16	53 ± 29	77	95	63
	△	Anal fin	73 ± 22	72 ± 13	103	80	47
	△	Dorsal fin	128 ± 48	66 ± 09	285	170	73
	△	Light flank	112 ± 16	46 ± 09	190	166	138
		Dark flank	72 ± 11	55 ± 28	104	117	0

Notes: The opercular/gular region is the averaged intensity taken at the centre of both regions. The anal fin intensity was taken at the centre of the fin. The dorsal fin measurement was taken at the centre of the rear portion of the fin. The flank intensities were averaged between the upper and mid regions. In *M. zebra*, the flank measurements are separated by light and dark barring patterns.

obvious in the *M. zebra* males. The dorsal fin, anal fin and the light part of the flank in *M. zebra* males were UV reflective while the dark bars tended to be less reflective. In dominant *M. zebra* males, the light portion of the flank was more reflective and the dark part of the flank less reflective when compared to subordinate males. Thus, the greatest contrast was achieved between the light and dark bars in the dominant males when compared to the subordinate males (Fig. 1). These behavioural changes in reflectivity were often elicited in these fish within 10 s.

Several reasons might explain why these fishes are UV reflective. First, UV reflectivity might be important to female mate choice. Male variation in UV reflectivity has been demonstrated to affect female choice in other fishes (Garcia & de Perera, 2002; Kodric-Brown & Johnson, 2002; Smith *et al.*, 2002). In guppies *Poecilia reticulata* Peters, however, specific reflective patches appear in the ultraviolet that are not as apparent in the other wavelengths (Kodric-Brown & Johnson, 2002). Such patches were absent the species in the present study. Secondly, given what is known about colour change in species closely related to

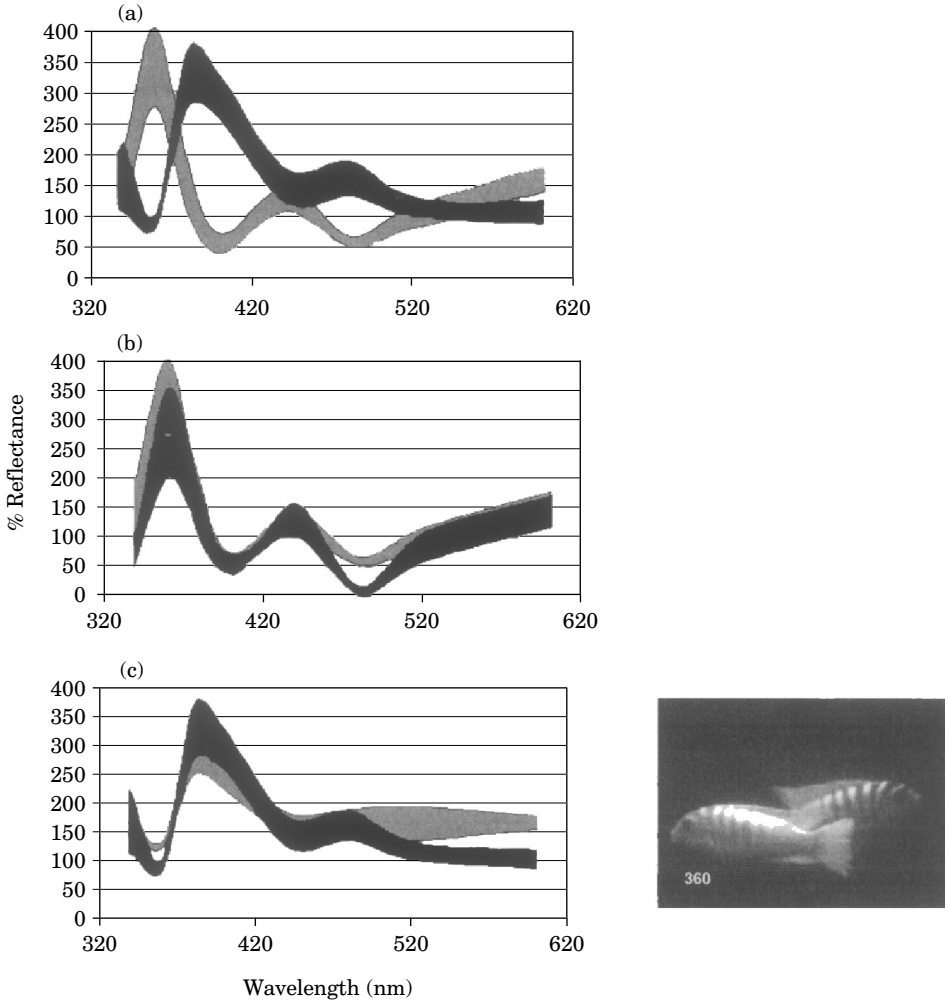

FIG. 1. Per cent reflectance of *Metriaclima zebra* during behavioural observations: (a) male (■) behaviourally dominant to the other (■) when the two fish are together, (b) both fish are behaviourally dominant because of the presence of a third male and (c) both are behaviourally subordinate because each was filmed alone. The widths of the curves represent differences in reflectance of the light portion of the flank (top of the curve) and the dark portion of the flank (bottom of the curve). Note in (a) the greater difference in reflectance at the UV wavelengths (<400 nm) for the behaviourally dominant *v.* subordinate male. Intensity was standardized and data are drawn as continuous for visual ease, but were taken at discrete wavelengths. A photograph taken from the video under one wavelength is also provided. The individual with the greater contrast in barring pattern is exhibiting dominance over the subordinate male.

those used in the present study (Nelisson, 1991), it is possible that the UV reflectivity is simply a by-product of the mechanism for colour change in the visible spectrum. Thirdly, it is possible that these fishes evolved sensitivity to UV for reasons other than communication, such as to enhance feeding success (Jordan *et al.*, in press) or to increase orientation ability (Hawryshyn, 1992). UV body reflectivity might then have evolved in response to the sensitivity.

In another species that is closely related to the ones used in the present study, *Melanochromis auratus* (Boulenger), UV sensitive cones, which are an indicator of UV sensitivity, could not be found (R.C. Jordan, K.A. Kellogg, F. Juanes, J.R. Stauffer Jr & E.R. Loew, unpubl. data). Furthermore, a crude analysis of male UV body reflectivity indicates that these individuals have fewer areas of UV reflectivity when compared to the *Metriaclima* species studied in this paper (R. Jordan, unpubl. data). More work is necessary, however, to see if a correlation between UV sensitivity and reflectivity exists for other Lake Malaŵi species. In coral reef species for example, UV colouration does not appear to be correlated with UV vision (Marshall, 2000).

Finally, UV reflectivity could be important in non-sexual communication both among and within species. Knowledge about the irradiance of the underwater environment of these fishes is insufficient to determine how animals might perceive such cues, but UV reflectivity probably enhances contrast. At Mazinzi Reef (14° S; 35° E), where these fishes were collected, both *M. zebra* and *M. barlowi* males became more obvious when dominant because of the darker patterning and sudden movement (R. Jordan, pers. obs.). It may be advantageous for individuals to demonstrate conspicuous body colouration only during a behavioural encounter with another individual or if they are guarding a territory where they can shelter while avoiding conspicuous colouration in other situations. Future investigation into the functions of UV reflectivity should include obtaining spectral information from the habitats where these fishes exist.

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