Photopigment spectral absorbance of Lake Malaŵi cichlids

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To predict spectral sensitivity, microspectrophotometry (MSP) was used to measure absorbance of photoreceptor cells from 15 species of Lake Malaŵi cichlids. Each fish had one rod and at least three cone pigments. UV-sensitive pigments were common, but spectral sensitivity did not clearly correlate with feeding mode or habitat.

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Approximately 2000 species of cichlids, most of which are endemic, inhabit the three great lakes of Africa (Barlow, 2000). Half of this diversity is found within Lake Malaŵi (Stauffer *et al.*, 1997; Barlow, 2000). Two of the more notable theories generated to explain this astounding radiation are ecological speciation related to feeding mode and sexual selection through female choice (Danley & Kocher, 2001). Given the reliance of these theories on visually guided behaviours and that the cichlids of Lake Malaŵi exhibit both visually guided foraging and mate selection behaviour (Hert, 1991; McKaye *et al.*, 1993; Kellogg, 1997; Couldridge, 2002; Jordan *et al.*, 2003, 2004), a comparative understanding of visual physiology is necessary.

The spectral limits of visual perception are determined in part by the visual pigments within the photoreceptors. The presence of several spectral classes of cone photoreceptors in cichlids suggests that colour vision plays a role in the above-mentioned behaviours, although luminosity information mediated by the

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rods cannot be ignored as a cue (Muntz, 1976; Levine & MacNichol, 1979; Carleton *et al.*, 2000). Muntz (1976) examined only rod extracts and concluded that differences in the spectral position of maximum rod absorption ($\lambda_{\max}$) among species were not related to physical habitat differences, but might be correlated to differences in the light environment potentially driven by depth. Using microspectrophotometry (MSP), Levine & MacNichol (1979) examined cone sensitivities in *Dimidiochromis* (formerly *Haplochromis*) *compressiceps* (Boulenger) and *Metriaclima* (formerly *Pseudotropheus*) *zebra* (Boulenger), however, only data for the double cones were presented (mean ± S.D. short wavelength, SW: 536 ± 4, long wavelength, LW: 569 ± 6 and SW: 488 ± 3, LW: 533 ± 2 respectively). Carleton *et al.* (2000) reported an ultraviolet cone in a Lake Malaŵi cichlid, *M. zebra*.

While these studies were informative, a much broader analysis of the visual capabilities of Lake Malaŵi cichlids is needed in order to understand the significance of spectral sensitivity to the ecology and evolution of this astoundingly diverse group. Here, MSP was used in a preliminary study to identify the visual pigments present in rods and cones from a variety of species of Lake Malaŵi cichlids. The intention of this study was to identify possible gross differences in photoreception to serve as a baseline and impetus for deeper exploration of the correlation of spectral sensitivity and ecotypical features for these fishes. Prior to such an undertaking, a reasonable determination of presence or absence of pigments is appropriate.

The housing of animals and methods used in this study were performed under the guidelines established by University of Massachusetts' and Cornell University's Animal Care and Use Policy. The cichlid species (Table I) used were collected from the southern portion of Lake Malaŵi (14° S; 33° E), except for *Copadichromis borleyi* (Iles), *Protomelas taeniolatus* (Trewavas) and *D. compressiceps*. These three species were obtained as juveniles through the pet trade and probably bred in the U.S. Fish species were selected for their differences in habitat use and feeding mode. Phylogenetic diversity was maximized based on current interpretations of taxonomic relationships (Moran *et al.*, 1994; Parker & Kornfield, 1997; Albertson *et al.*, 1999).

In the laboratory, each species was housed separately in 110 l tanks maintained at 24–28° C, pH 7–8 and 12L : 12D photoperiod. The fishes were fed a combination of flake and stick foods three times daily. Except for feeding, occasional care and tank maintenance, the fishes were not disturbed during the 3 months prior to experimentation.

This preliminary survey of the photoreceptor types was performed by determining absorbance spectra from several photoreceptor cells from a single adult specimen of each species. Absorbance spectra were obtained for the rods and cones using a computer-controlled microspectrophotometer (Loew, 1994). The fishes were dark adapted for at least 3 h, anaesthetized using MS-222, sacrificed by decapitation, and enucleated under infra-red light using appropriate image converters. The eyecups were placed in standard phosphate buffer solution (pH 7.3) and the retina of one eye was teased away from the pigment epithelium. The retina was bisected, transferred to two cover slips, macerated using razor blades and insect pins, and overlaid with cover slips edged with silicone grease.

TABLE I. Ecological and taxonomic information on the 15 species surveyed for the MSP analysis. Habitat, depth and food data taken from Ribbink *et al.* (1983), Konings (1990) and J.R. Stauffer (unpubl. obs.). Species group refers to two major classifications of Lake Malaŵi fishes; species which are not members of either group are left blank

Species	Habitat	Depth usually found	Food	Species group
Copadichromis borleyi	Rock	M	Open water plankton	*Utaka*
Cynotilapia afra	Rock/sand interface	M	Plankton/algae	*Mbuna*
Melanochromis auratus	Rock/sand interface	S	Algae/sediment	*Mbuna*
Metriaclima barlowi	Rock/sand interface/sediment	M	Algae/plankton/sediment	*Mbuna*
Metriaclima benetos	Rock	S	Algae/plankton	*Mbuna*
Metriaclima emmiltos	Rock	M	Algae/plankton	*Mbuna*
Metriaclima livingstonii	Sand with/shell cover	D	Algae/plankton/sediment	*Mbuna*
Metriaclima melabranchion	Rock	M	Algae/plankton	*Mbuna*
Pseudotropheus t. tropheops	Rock	S	Algae/plankton	*Mbuna*
Aulonocara hueseri	Sand	D	Sand invertebrates	
Dimidiochromis compressiceps	Vegetation	S	Fish	
Lethrinops parvidens	Rock and sand	D	Zooplankton/algae	
Mylochromis lateristriga	Sand/vegetation	S	Crustaceans	
Protomelas taeniolatus	Rock	M	Algae	
Tyrannochromis macrostoma	Rock	D	Fish	

S, 0–10 m; M, 0–30 m; D, <30 m.

Each of the two preparations from a single fish was scanned in horizontal and vertical transects to isolate morphologically unique types of photoreceptor cells for measurement. Processing time for each preparation was *c.* 2 h. The baseline spectrum for absorbance measurements was obtained from a cell-free area of the preparation and continually checked for validity. Outer segments from rods and cones were identified and aligned with the measuring beam under infra-red light, after which an absorbance spectrum was obtained from 750 to 350 nm in interleaved down and up directions. The criteria for accepting the absorbance from ultraviolet cells as being true visual pigments and not a photoproduct were similar to those used by Loew (1994) and included outer segment dichroism and bleaching characteristics. Double cones were designated as such only if found as an intact pair.

Absorbance spectra from *c.* 25% of cells measured were retained for analysis. Others were discarded because although pigment type could generally be discerned, the quality was insufficient for template fitting. Thus, sample sizes are effectively greater than reported. Similar sample sizes are common in studies of this type (Downing *et al.*, 1986; Bowmaker & Kunz, 1987; Loew, 1994; McFarland & Loew, 1994; Losey *et al.*, 2003). The Mansfield (1985)–MacNichol (1986) method was used to estimate λ_{max} of the visual pigments using templates derived from Partridge & DeGrip (1991). This is a somewhat subjective process, but adequate for discerning differences among species. Absorbance maxima were averaged to obtain a single species-specific value for each photopigment type. The cone classes were divided into UVS [single cones with average λ_{max} <400 nm (ultraviolet)], SWS-S (single cones with average λ_{max} ranging from 409 to 425 nm), SWS-LS (single cones with average λ_{max} ranging from 447 to 453 nm), SWS-LD (double cones with average λ_{max} ranging from 472 to 492 nm), MWS (double cones with average λ_{max} ranging from 514 to 539 nm) and LWS (double cones with average λ_{max} ranging from 563 to 567 nm).

All of the retinal preparations examined had at least three, and in one case, four cone spectral classes plus a single rod. Rod λ_{max} values ranged from 489 to 498 nm, with one exception at 510 nm (Table II). Template fitting identified the chromophore as vitamin A_1-based. Typical absorbance spectra are shown in Fig. 1.

Only one type of single cone, UVS, SWS-S or SWS-LS, was found in any preparation (Table II). Double cones of 12 species were SWS-LD/MWS pairs. In these cases, the λ_{max} of the medium wavelength sensitive cone was <540 nm. Three species had MWS/LWS doubles, with λ_{max} of the long wave sensitive cone >560 nm (Table II). One species had both types of double cones.

Although it cannot be said with certainty that all types of cones present in these fishes were found, the data strongly suggest that there are differences in the photoreceptive pigments of these fishes that may be phylogenetically and ecologically significant. This conclusion is supported by the finding of only one single cone type in all 15 species, and only one species with two types of double cones. All members of the monophyletic rock-dwelling group of Lake Malaŵi cichlids (*i.e.* the *mbuna*), except *Melanochromis auratus* (Boulenger), had a UVS cone. Non-*mbuna* species expressed an SWS-S pigment with the exception of *D. compressiceps* and *P. taeniolatus*, which expressed an SWS-LS.

TABLE II. Average λ_{max} (nm) by species and photoreceptor cell type. Where multiple cells were tested, s.d./number of cells are given in parentheses

Species	Rods	UVS	SWS-S single	SWS-LS single	SWS-LD double	MWS double	LWS double
Copadichromis borleyi	494		424 (9/11)		478 (10/8)	539 (14/6)	
Cynotilapia afra	496 (5/2)	358 (12/6)			472 (5/6)	525 (2/7)	
Melanochromis auratus	497 (3/3)		414 (2/5)		482 (5/2)	525 (1/2)	
Metriaclima barlowi	491	366 (1/2)			483 (1/2)	523 (4/2)	
Metriaclima benetos	†	379 (13/3)			489	522	
Metriaclima emmiltos	†	383 (11/12)			482 (1/3)	521 (4/4)	
Metriaclima livingstonii	495 (2/2)	364 (2/6)			473 (3/9)	526 (2/9)	
Metriaclima melabranchion	491 (5/17)	371(1/2)			477 (5/9)	515 (5/3)	
Pseudotropheus t. tropheops	510	371 (9/7)			492 (1/2)	531 (8/6)	565
Aulonocara hueseri	496 (2/3)		415 (2/2)		484 (12/2)	526 (1/3)	
Dimidiochromis compressiceps	498 (3/4)			447 (4/4)		533 (10/10)	567 (6/13)
Lethrinops parvidens	489		426 (2/8)			514 (3/5)	563 (5/6)
Mylochromis lateristriga	496 (8/7)		419 (3/7)		482 (3/7)	522 (6/8)	
Protomelas taeniolatus	496 (1/4)			453 (4/7)		527 (9/3)	
Tyrannochromis macrostoma	494 (6/3)		410 (4/5)		482 (2/13)	529 (5/12)	566 (2/2)

UVS, single cones with $\lambda_{max} < 400$ nm; SWS-S, single cones with λ_{max} 409–425 nm; SWS-LS, single cones with λ_{max} 447–453 nm; SWS-LD, double cones with λ_{max} 472–492 nm; MWS, double cones with λ_{max} 514–539 nm; LWS, double cones with λ_{max} 563–567 nm. Data for *Dimidiochromis compressiceps* are used with permission from K. L. Carleton.

†, measurement did not meet acceptance criteria.
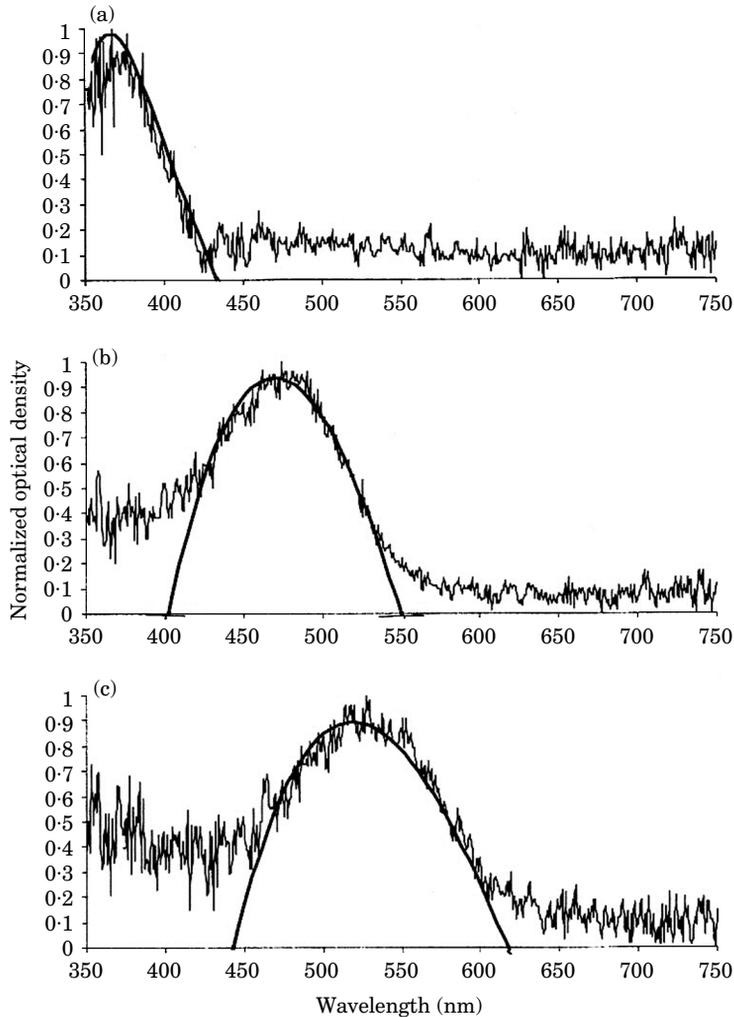
FIG. 1. Sample spectral absorbance curves from (a) a single cone receptor type UVS and from double cone receptor (b) type SWS-LD and (c) MWS type (see Table II) found in *Metriaclima livingstonii*. Each curve was normalized to the λ_{max} of the best fitting template curve indicated by the solid line.

Furthermore, the absorbance maxima reported for double cones of *D. compressiceps* are within 3 nm of those reported in Levine & MacNichol (1979). Carleton & Kocher (2001) have investigated the molecular basis of spectral sensitivity in four species of Lake Malaŵi fishes including *D. compressiceps*. Interestingly, they report that these fishes share similar opsin sequences, and that the differences in spectral sensitivity seen among species are related to differences in gene expression. If the present methods systematically or randomly missed photopigment types, the suggestive pattern found would probably not have emerged.

The spectral sensitivities of the closely related Lake Victoria and Lake Tanganyika cichlids have been studied in great detail (Fernald & Liebman, 1980; van der Meer & Bowmaker, 1995). Though these fishes had three cones

and one rod pigment, as did many in the present study, their photopigment cells tended to be sensitive to longer wavelengths. In the Lake Victoria fishes, rod λ_{max} tended to be >500 nm, single cone λ_{max} ranged from 455 to 464 nm and double cones had a 565–569 nm pigment paired with a 522–538 nm pigment. These values are very similar to those for the Lake Tanganyika cichlid *Haplochromis burtoni* Günther (rod λ_{max} ranged from 499 to 501 nm; cone average λ_{max} was 455 nm for single, 562 and 523 nm for double cone pairs; Fernald & Liebman, 1980). The longer wavelength-sensitivity reported in these cichlids correlates with the shallow and possibly more turbid habitats where these fish live (van der Meer & Bowmaker, 1995; Hofmann *et al.*, 1999).

There is a general expectation that freshwater fishes should have substantially longer (>500 nm) wavelength sensitivity due to the 'colour' of most fresh waters. The absence of short-wavelength cones or a shift to the use of vitamin A_2-based pigments, which absorb at longer wavelengths than their A_1 counterparts, support this belief (Bowmaker, 1990). Lake Malaŵi is considered particularly clear (Muntz, 1976). Because of this clarity, ultraviolet light, along with the other short wavelengths, probably penetrates to at least 50 m in the lake (Loew & McFarland, 1990). Also important to note is that cichlids, as a family, are derived from a marine ancestor and are not strictly a freshwater group of fishes (Barlow, 2000).

Spectral sensitivity may also be responding to the demands of species or mate recognition, interspecific competition or habitat use. For example, the two species of the sympatric Lake Victoria fishes described previously that were most similar in habitat use, differed greatly in spectral sensitivity, perhaps thereby reducing interspecific competition (van der Meer & Bowmaker, 1995). A similar argument was made for the blenny *Blennius pholis* L., and the goby *Gobius paganelus* L., which can inhabit the same small tidepool, but have different visual pigments and retinal organization (Loew & Lythgoe, 1978). The pigment differences among these Lake Malaŵi species might also be driven by phylogenetic differences, because the *mbuna* are generally thought to be more closely related to each other and perhaps the deepwater species, than are the shallowwater sand dwellers. Furthermore, the *mbuna* live among rocks, and potential differences in spectral sensitivity could be attributed to the specific demands of such habitat. Davitz & McKaye (1978) found that individuals of the *mbuna* species *Pseudotropheus macrophthalmus* Ahl were able to discriminate between horizontal and vertical polarized light. This can be important for water column migration and orientation, needs that are especially strong in structural habitats.

In conclusion, evidence suggests that differences in spectral sensitivity exist in Lake Malaŵi cichlids. Despite limited sample sizes, a baseline now exists from which predictions about the visual ecology of these fishes can be drawn. Important future directions include finer-scale retinal surveys of more taxa covering a greater diversity of habitats, and a more comprehensive examination of the visual environment of Lake Malaŵi.

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