OXFORD

Influence of ambient water coloration on habitat and conspecific choice in the female Lake Malawi cichlid, *Metriaclima zebra*

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Handling editor: Zhi-Yun Jia

Abstract

Female cichlid fish living in African great lakes are known to have sensory systems that are adapted to ambient light environments. These sensory system adaptations are hypothesized to have influenced the evolution of the diverse male nuptial coloration. In rock-dwelling Lake Malawi *mbuna* cichlids, however, the extent to which ambient light environments influence female sensory systems and potentially associated male nuptial coloration remains unknown. Yet, the ubiquitous blue flank coloration and UV reflection of male *mbuna* cichlids suggest the potential impacts of the blue-shifted ambient light environment on these cichlid's visual perception and male nuptial coloration in the shallow water depth in Lake Malawi. In the present study, we explored whether and how the sensory bias of females influences intersexual communication in the *mbuna* cichlid, *Metriaclima zebra*. A series of choice experiments in various light environments showed that *M. zebra* females 1) have a preference for the blue-shifted light environment, 2) prefer to interact with males in blue-shifted light environments, 3) do not show a preference between dominant and subordinate males in full-spectrum, long-wavelength filtered, and short-wavelength filtered light environments, and 4) show a "reversed" preference for subordinate males in the UV-filtered light environment. These results suggest that the visual perception of *M. zebra* females may be biased to the ambient light spectra in their natural habitat by local adaptation and that this sensory bias may influence the evolution of blue and UV reflective patterns in male nuptial coloration.

Key words: ambient light environment, Lake Malawi, male coloration, mbuna cichlids, *Metriaclima zebra*, sensory bias, UV coloration, UV perception, visual communication.

The variation in sensory environments such as ambient light spectrum (Endler 1992, 2005; Seehausen et al. 2008) or abiotic/biotic noise (Halfwerk and Slabbekoorn 2015) has been highlighted as an important factor that exerts selection on the sensory systems of animals living in different habitats. Such selection in non-communicative contexts (e.g. foraging, antipredation, navigation) may further promote signal evolution by the formation of preexisting sensory biases in receivers that are adapted to their local habitats (i.e. sensory drive; Endler 1992; Boughman 2002; Witte et al. 2005; Seehausen et al. 2008). In particular, given the spatial and temporal variation in ambient light environments across aquatic habitats, the diversity of fish coloration has been proposed as convincing evidence of the evolutionary relationship between sensory environments, sensory systems, and signal design in local habitats (Morrongiello et al. 2010; Fuller et al. 2022; Ricci et al. 2022).

Cichlids living in the African great lakes (Lake Tanganyika, Malawi, and Victoria) form multi-species assemblages consisting of closely related species that have diverged through multiple adaptive radiations by their diet, habitat, and behavior (Seehausen 2006, 2015; Salzburger 2009, 2018; Maan and Sefc 2013). Among the many abiotic and biotic factors influencing these radiations, many researchers suggest that variation in the ambient light environment—for example, due to water depth and turbidity—may contribute to the divergent evolution of visual systems (i.e. color vision). These divergent visual systems might influence the vast diversity of male nuptial coloration and, further, sympatric speciation by reproductive isolation (Seehausen et al. 2008; Maan and Sefc 2013; Ricci et al. 2022). In particular, Seehausen et al. (2008) revealed in Lake Victoria cichlids, *Pundamilia pundamilia* and *Pundamilia nyererei*, that variation in ambient light spectra by water depth can promote the divergence in the cichlid visual system, female preference, and male nuptial coloration; and ultimately lead to sympatric speciation without geographic isolation. Recently, Ricci et al. (2022) also showed that the interspecific variation in opsin gene expression for visual perception among 245 taxa of Tanganyikan cichlids has been promoted by the depth-related local adaptation to ambient light spectra.

In contrast to cichlids in the two other African great lakes (Lake Victoria and Lake Tanganyika), previous studies

proposed that such visual adaptation is unlikely to contribute to the evolutionary divergence of Lake Malawi cichlids. This contrasting expectation is due to the high water clarity of Lake Malawi. The underwater light spectrum of Lake Malawi mostly contains the intermediate ranges of the light spectrum (i.e. blue and green), which does not dramatically change by water depth, except for the diminished transmission of UV light (Smith et al. 2012). In addition to the homogeneous and broad spectrum range of Lake Malawi, molecular studies suggested that the interspecific variation in the molecular basis of the color vision of Lake Malawi cichlids (i.e. cone opsin gene expression) may not be driven by the habitat-specific selection. This supposition is based on the fact that most of the species studied showed general high quantum catches in the ambient light spectra (characteristic of Lake Malawi) regardless of their habitat type and water depth (Rock-dwelling vs. Sand-dwelling—Parry et al. 2005; Shallow vs. Deep—Smith et al. 2011; Reviewed in Smith et al. 2012).

Along with the weak evidence of the evolutionary divergence in visual systems by ambient light environments in Lake Malawi cichlids, Allender et al. (2003) reported that 81% of rock-dwelling cichlid species in the littoral habitats in Lake Malawi (hereafter, *mbuna* cichlids; Genner and Turner 2005) commonly have blue flank in male nuptial coloration. This blue flank nuptial coloration is shared across all of the morphologically classified morphs across *mbuna* species— "all-blue," "yellow-chest," "orange-dorsal"–except for the "all-yellow" morph. The shared visual sensitivity and shared blue nuptial coloration support the hypothesis that evolutionary divergence is not likely caused by habitat-specific sensory adaptation.

Given the 1) homogeneous ambient light spectrum across habitats (Smith et al. 2012) as compared to Lake Tanganyika (Ricci et al. 2022) and Lake Victoria (Seehausen and Schluter 2004; Smith et al. 2012), 2) limited variation in female perception such as peak sensitivity of photoreceptors (Parry et al. 2005; Dalton et al. 2010; Smith et al. 2011, 2012), and 3) limited variation in male nuptial coloration (Allender et al. 2003; Smith et al. 2012), accumulated results suggest that sensory drive may not be a major driver of the evolutionary divergence in visual systems (i.e. color vision and opsin gene expression) and male nuptial coloration in Lake Malawi cichlids. However, current evidence based on comparative analysis of opsin gene expression and male nuptial coloration is not sufficient to fully understand how the sensory environment might, or might not, influence the evolution of visual communication of Lake Malawi cichlids. Unfortunately, there is a dearth of behavioral experiments, for example,

testing sensory biases in Lake Malawi fishes; yet such experiments might be crucial for understanding behavioral traits resulting from complex perceptual mechanisms beyond the simple visual sensitivity predicted by gene expression (Smith et al. 2011). Additionally, there is the potential for observed similarities in visual systems and nuptial coloration to be the product of convergent selection due to the shared homogeneous sensory environment of sympatric Lake Malawi species. Shared general preferences for similar signal traits in a shared environment are also predicted by the sensory drive hypothesis (Fuller et al. 2005; Cummings 2007; Culbert et al. 2020).

Although there are many experimental studies about female preference for male nuptial coloration in Lake Malawi cichlids (Table 1 in Maan and Sefc 2013), most of the studies have been focused on the role of the coloration in species recognition in the context of mate choice (Knight and Turner 1998, 2004; Couldridge and Alexander 2002; Jordan et al. 2003; Blais et al. 2009; Pauers et al. 2010; Nyalungu and Couldridge 2019; reviewed in Maan and Sefc 2013). In particular, many female *mbuna* cichlids can recognize their species or populations by male coloration (Couldridge and Alexander 2002; Jordan et al. 2003; Knight and Turner 2004; Blais et al. 2009; Pauers et al. 2010). However, the degree of assortative mating is influenced by how similar male nuptial colorations are among species (Couldridge and Alexander 2002; Knight and Turner 2004), and heterospecific mating between species with similar body coloration has been reported in their natural habitat (e.g. *Metriaclima zebra* vs. *Cynotilapia afra*—Stauffer et al. 1996). Previous researchers suggested that the similarities in male nuptial coloration among species have been hypothesized as a result of constraints in genetic/physiological backgrounds or female sensory bias in color visions (Deutsch 1997; Couldridge and Alexander 2002; Allender et al 2003).

Many *mbuna* cichlids mainly feed on algae and plankton in the shallow depth of water of 5–20 m (Holzberg 1978; Ribbink et al. 1983; Genner and Turner 2005; Markert and Arnegard 2007). At these depths, the ambient light spectrum that transmits best is the blue light spectrum (approximately 450–575 nm; Deutsch 1997; Dalton et al. 2010; Smith et al. 2011). Also, these planktivorous *mbuna* cichlids often have UV sensitivity with UV-sensitive cones and UV-transparent lenses, whereas other cichlids with different diets have UV-blocking lenses to prevent potential harmful impacts of UV on their visual systems (Hofmann et al. 2010; Sabbah et al. 2010; Pauers et al. 2016; Carleton and Yourick 2020). Given the information about the *mbuna* visual system, in

Table 1 The total and average choice duration of *M. zebra* females between full-spectrum and filtered light environments (-UV, -SW, -LW) without conspecific males (*Experiment a*).

		Full versus -UV	Full versus -SW	Full versus -LW
Total choice duration	Full-spectrum (s)	200 ± 79.7	180 ± 76.1	149 ± 47.7
	Filtered (s)	138 ± 74.8	107 ± 53.4	228 ± 47.9
	Statistics	$V_{13} = 87, P = 0.030$	$V_{13} = 90, P = 0.017$	$V_{13} = 3, P < 0.001$
Average choice duration	Full-spectrum (s)	14.7 ± 6.85	11.5 ± 3.12	10.2 ± 2.69
	Filtered (s)	10.1 ± 3.13	7.67 ± 2.49	13.6 ± 3.06
	Statistics	$V_{13} = 86, P = 0.035$	$V_{13} = 91, P = 0.013$	$V_{13} = 13, P = 0.011$

The significant results of the Wilcoxon signed-rank tests are denoted in bold.

particular color visions, Dalton et al. (2010) suggested that the nuptial coloration of male *mbuna* cichlids is statistically more conspicuous than female colorations in their natural habitats (e.g. rocks).

However, whether the evolution of conspicuous male nuptial coloration has been facilitated by the sensory bias of females remains unexplored by behavioral studies. Nonetheless, previous studies do suggest selection on male nuptial coloration in *mbuna* cichlids. More dominant males of some species (i.e. *M. zebra*, *Metriaclima benetos*, *Metriaclima barlowi*) have reflectance spectra that are highly skewed toward UV wavelengths (<400 nm, Jordan et al. 2004a), and some males can engage in the plastic expression of UV reflective bands (Jordan et al. 2004a; Pauers et al. 2016). Territorial *mbuna* males vigorously fight for better territory (Genner and Turner 2005; Markert and Arnegard 2007), and dominant *mbuna* males display their dominance by changing their nuptial coloration—that is brightening their body coloration or increasing UV contrast in their coloration (Andries and Nelissen 1990; Jordan et al. 2004a; Mellor et al. 2012). Thus, the nuptial coloration of territorial males reflecting blue and UV wavelength may not only increase the conspicuousness of the males but may also provide females with information for choosing a better mate. Previous studies showed that females have a preference for dominant conspecific males (Mellor et al. 2012) and males with more food resources (Greenberg et al. 2016). However, it has not yet been tested if the blue flank or UV reflective bands in male nuptial coloration influence the female perception of *mbuna* males (Jordan et al. 2004a; Pauers et al. 2016).

We propose that like the cichlids from Lake Victoria and Lake Tanganyika, *mbuna* cichlids in Lake Malawi may have evolved preexisting sensory bias for foraging in their local environments which has led to a preference for blue male nuptial coloration. To test this hypothesis, and to increase our understanding of the evolution of male nuptial coloration in Lake Malawi *mbuna* cichlids, we use a focal cichlid species, *M. zebra* (Boulenger), to test whether females have a preexisting preference for short-wavelength light spectrum (i.e. UV, blue) in non-reproductive behavioral contexts. Our study species, *M. zebra*, has been well-studied regarding their nuptial colorations (Allender et al. 2003; Jordan et al. 2004a), visual systems (Parry et al. 2005; Dalton et al. 2010), and behavior (Pauers et al. 2008; Mellor et al. 2012; Chabrolles et al. 2017). Male *M. zebra* have nuptial coloration with blue flank and black bars whereas females are brown-colored with faint black bars (Allender et al. 2003; Dalton et al. 2010). Adult *M. zebra* males aggressively defend their territory on the rocky shore of Lake Malawi from conspecific and heterospecific intruders (Pauers et al. 2008) and dominant males often show more conspicuous bar patterns on their flank which increases UV reflection from their nuptial coloration (Jordan et al. 2004a).

Using *M. zebra* females, we tested whether 1) females prefer a specific light spectrum and 2) the female preference for male nuptial coloration varies in different light environments. If a female sensory bias has played a role in the evolution of male nuptial coloration, we predicted *M. zebra* females to 1) attend more to blue-shifted light environments and 2) prefer male traits that reflect dominance, such as UV contrast on the flank (Jordan et al. 2004a), in the blue-shifted light environment that are most similar to their shallow water depth habitats.

Materials and Methods

Study animals

We purchased adult *M. zebra* (22 males and 13 females) from a commercial farm (Oxyfish, Verlinghem, France) and reared them at the ENES laboratory. We tagged *M. zebra* individuals using a transponder (PSK Transponder; Dorset Identification B.V., Dorset, the Netherlands).

In a rearing room, we stored the fish separately by sex in different holding tanks (120 × 60 cm, and 50 cm deep). We used LED lamps (Eheim Power LED+ Fresh daylight, EHEIM GmbH & Co., Deizisau, Germany) to provide a 12:12h light:dark cycle. The light spectrum in the rearing room does not contain UV light. Holding tanks were equipped with an external filter (Rena Filstar xP3; Rena France, Annecy, France), aeration, and PVC tubes as shelters. We maintained the water temperature at 25 ± 1°C with an internal heater (RenaCal 200, Rena France, Annecy, France) with a pH of 8.0. We fed fish daily with commercial cichlid food (JBL NovoRift sticks and Tetra flakes, JBL GmbH & Co., Neuhofen, Germany).

Experimental setup

To avoid any interruption from external light or sound sources, we conducted the experiments in a soundproof chamber (PRIMO Silence-Box, TipTop Wood, Saint-Etienne, France). In the chamber, an experimental tank (27 × 100 cm and 30 cm deep) was filled with tap water up to 20 cm and equipped with an external filter (Rena Filstar xP3, Rena France, Annecy, France), an aeration device, and sand substrate (~5 cm). The water temperature was maintained at 25.5 ± 1.5°C by an internal heater (RenaCal 200, Rena France, Annecy, France). During a trial, we turned off the external filter and aeration device to reduce the impact of noise from the equipment.

To test female preference, we used a two-way choice design. We partitioned the experimental tank into three sections (female, male-left, and male-right) with waterproof foamboards (Figure 1A). The foamboard between the female and male sections had windows (12 × 15 cm) that transmit full-spectrum light through a full-spectrum polyester filter (Lee filter #130, Lee filters Worldwide, Hampshire, UK); enabling visual interactions (Figure 1B).

For illumination, we deployed two different LED bulbs for full-spectrum and UV light (Highlite International, Kerkrade, the Netherlands) on the side of the tank where males were deployed and covered other sides of the experimental tank with light-absorbing material to prevent males from being able to perceive their reflection in the wall of the experimental tank. We indirectly illuminated the experimental tank using the reflected lights on the white foam board between a female and male (Figure 1).

To change the light environment of individual males, we added band-pass polyester filters to the window on the foamboard to selectively reduce the transmission of 1) UV (<400 nm, Lee filter #226), 2) short-wavelength (400 nm to 550 nm, Lee filter #512), or 3) long-wavelength light (>500 nm, Lee filter #707). We quantified the light environments with different color filters in the tank using a Red Tide USB650 spectrometer (Ocean Insights, Ostfildern, Germany) (Figure 1C). Before the measurement, the spectrometer was calibrated by the SpectraSuit software (Ocean Insights, Ostfildern, Germany) using a mercury-vapor lamp, and the intensities within a range of 300–1000 nm are measured within a range of 0–4096 arbitrary units (12 bits).
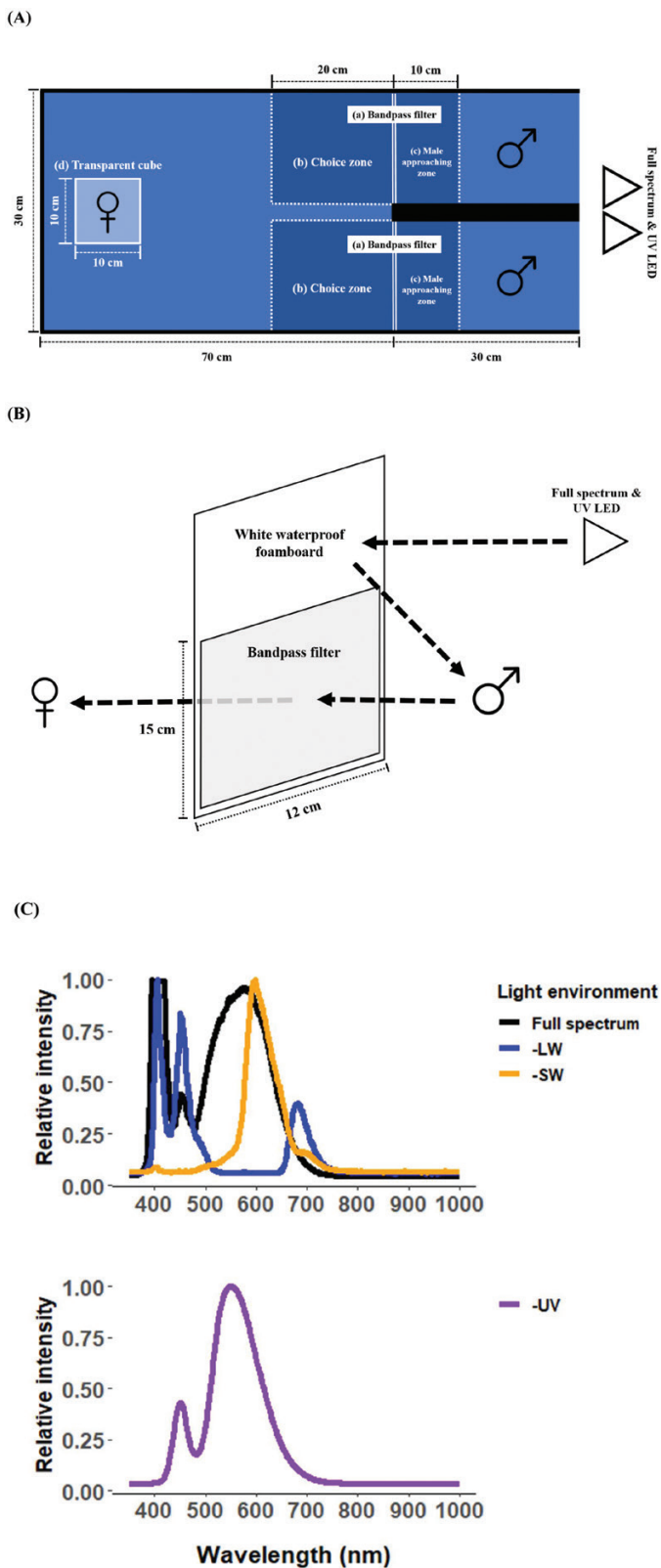
(A)

(B)

(C)

Figure 1 The setup of (A) the experimental tank, (B) the band-pass filter window, and (C) the intensity versus wavelength curve in different light environments. The arrows in (b) represent the direction of light. The first plot of (C) includes the wavelength curve in full-spectrum, long-wavelength filtered (-LW), and short-wavelength filtered (-SW) with two different LED bulbs for full-spectrum and UV light while the second plot shows the wavelength curve in UV filtered (-UV) without a UV light. The intensity is normalized by min–max normalization for each light environment.

Female choice experiments

We used a repeated-measure design with fourteen *M. zebra* females for three two-way choice experiments. Across the experiments, we used four different band-pass filters to manipulate the light environment such that only certain wavelengths of light could reflect off of male bodies. The filters we used were 1) **full-spectrum**—unmanipulated light environment illuminated by LED and UV lamps; 2) *UV filtered* (-**UV**)—removing UV wavelengths (<400 nm, Lee filter #226); 3) *Short-wavelength filtered* (-**SW**)—removing short wavelengths (400 nm to 550 nm, Lee filter #512); and 4) *Long-wavelength filtered* (-**LW**)—removing long wavelengths (>500 nm, Lee filter #707) (Figure 1C).

The orders of experiments and trials within an experiment were randomized to reduce the effects of orders. Also, the males were changed every experimental day but did not change the position of males within an experimental day to reduce any disturbance by catching males.

Experiment a: the presence of preference among light environments

In *Experiment a*, we deployed females in the experimental tank with full-spectrum and filtered light environments (-**UV**, -**SW**, and -**LW**). Through the experiment, we investigated whether females have a preference for a specific light environment by testing female microhabitat choice between full-spectrum and filtered light environments in the absence of conspecific males.

Experiment b: potential interaction between light environment and male presence on female microhabitat choice

In *Experiment b*, we deployed females in the same experimental setup as *Experiment a* but in the presence of two dominant conspecific males. Through the experiments, we observed whether the presence of males influences females' microhabitat choice which was observed in *Experiment a*. Also, we tested 1) if females can see the males behind band-pass filters in our experimental setup and 2) whether females perceive the variation in the manipulated male nuptial coloration by the band-pass filters (Rick et al. 2006). Additionally, we checked if females have an interest in males by comparing the results of *Experiment a* and *Experiment b* gave that we did not artificially manipulate the reproduction cycle of females by injection of hormones (details are in the below section).

We chose dominant *M. zebra* males through a 30-min observation of male–male interactions in a holding tank (Chabrolles et al. 2017). Dominant males exclusively possess shelter in a holding tank by aggression toward other subordinate males. There was no significant difference in the snout-to-tail length between males (Welch two samples *t*-test; *dominant vs. dominant M. zebra* $- t_{13.9} = -0.057$, $P = 0.955$).

Experiment c: the effects of light environment on female preference for the dominance of males

In the next experiment, we investigated female preference between *dominant versus subordinate M. zebra males* in each of four different light environments (**full-spectrum**, -**UV**, -**SW**, and -**LW**). During the experiment, we observed females' choice between dominant and subordinate males that were presented in the same light environment. Through the experiments, we investigated 1) whether females show a preference

between dominant versus subordinate males and 2) how the preference is influenced by the removal of each color component by band-pass filters.

In *Experiment c*, we chose dominant and subordinate *M. zebra* males by 30-min observation of male–male interactions in a holding tank and confirm the dominance by observing the 15-min interaction between the males in a smaller tank (16 × 30 cm and 20 cm deep) an hour before the experiment. To prevent males from getting injured, we stopped the 15-min interaction when a dominant male started to chase away a subordinate male. There was no significant difference in the snout-to-tail length between dominant and subordinate males (Welch two samples *t*-test; $t_{14.0} = -0.259$, $P = 0.800$).

General procedure

We conducted one trial per day in a randomized order for each female and had a three-day interval between each experiment. One hour before the first trial of a day, *M. zebra* females were individually secured using cuboid nets (2 × 17 × 13 cm) in their holding tank, and males were deployed in the experimental tank.

After an hour, we moved females from a holding tank to an acrylic cube (10 × 10 × 10 cm) in an experimental tank. In the acrylic cube, we gave a female a 10-min acclimation period with a waterproof foamboard (12 × 20 cm) between males and a female. During the last 1 min of the acclimation period, we removed the opaque barrier and allowed the female to observe males from within the acrylic cube. After the acclimation period, we released females into the experimental tank and observe their behavior for 10 min. All experiments were recorded by a Logitech C910 webcam (Logitech, Lausanne, Switzerland) and three Aquarian H2a-XLR hydrophones (sensitivity: –180 dB re 1 V μPa-1, flat frequency response ± 4 dB 20 Hz–4.5 kHz; Aquarian Audio & Scientific, Anacortes, WA, USA) connected to Zoom H4Next Handy Recorder (Zoom Co., Tokyo, Japan). We used hydrophones to detect potential acoustic behavior during male–female interaction (Bertucci et al. 2010) but did not detect any sounds produced in our experiment.

Data analysis

To assess the female preference in the two-way choice experiments, we compared the initial choice and time allocation of females between light environments and/or males (Couldridge and Alexander 2001). We used BORIS for Windows version 7.5.3 (Friard and Gamba 2016) to compare the behavior of females including 1) the latency to the initial choice, 2) initially chosen male/light environment, 3) total spending time in choice zones (20 × 12 cm, Figure 1A), 4) the spending time in each choice zone during a trial (**total choice duration**), and 5) the average of the spending time for each visit (**average choice duration**). Also, we measured the time that males spent in the male approaching zone (10 × 12 cm) to investigate the potential impacts of the male's position during a trial on the female choice (Figure 1A). We assessed the variation in the position of males by the log-ratio of the spending time in the male approaching zone between males (**male activity ratio**).

Statistical analysis

For *Experiment a* and *Experiment b*, we investigated whether the latency to initial choice and total spending time in both choice zones during a trial were influenced by 1) the presence of males between *Experiment a* and *Experiment b* using Wilcoxon signed-rank tests and 2) the type of filtered light environment (-UV, -SW, and -LW) paired with full-spectrum for each experiment using the Friedman tests with individual ID as a group variable. If we found a significant result in the Friedman test, we performed a post hoc Nemenyi test for pairwise comparisons. Also, we investigated the effects of the presence/absence of males, filtered light environments, and the interaction term on the initial choice of females in a mixed-effects logistic regression model with individual ID as a random variable.

Then, we compared the total/average choice durations between full-spectrum and filtered light environments using Wilcoxon signed-rank tests. If we found a significant difference in total/average choice durations, we constructed a mixed-effects linear regression model to test the relationship between the log-ratio of total/average choice durations between two choice zones as a dependent variable, and filtered light environment, male activity ratio, and the interaction term as fixed effects with individual ID as a random effect. Through the mixed-effects linear regression model, we compare the degree of female preference across the trials and confirmed whether the variation in total/average choice duration is influenced by the light environment or the male activity ratio which is not controlled by our experimental design.

For *Experiment c*, we conducted the Friedman tests to compare the latency to initial choice and total spending time among trials in four different light environments (full-spectrum, -UV, -SW, and -LW). Then, we conducted a mixed-effect logistic regression analysis to investigate the effects of the light environment on the initial choice of females between dominant and subordinate *M. zebra* males by mixed-effect logistic regression analysis with individual ID as a random effect. Then, we compared the total/average choice durations between dominant and subordinate males using Wilcoxon signed-rank tests. If we found a significant difference in total/average choice durations, we constructed a mixed-effects linear regression model to test the relationship between the log-ratio of total/average choice durations between two choice zones as a dependent variable, and filtered light environment, male activity ratio, and the interaction term as fixed effects with individual ID as a random effect.

P values of each fixed effect of regression models were obtained by likelihood ratio tests of the full model with the fixed effect in question against the model without the fixed effect. When we found a significant effect of the fixed effect, we conducted a post hoc pairwise comparison of the estimated marginal means using the "emmeans" function from the R package "emmeans." All mixed-effect regression analyses were conducted using "lmer" and "glmer" functions from the R package "lme4" (Bates et al., 2014). The *P* values of all statistical tests were adjusted by Bonferroni correction. We performed all the statistical analyses using R version 3.6.1 for Windows (R core team 2021).

Results

Experiment a: females prefer light environments with UV and blue wavelength

In the absence of males, a filtered light environment paired with the full-spectrum light environment did not influence

the latency to the initial choice of females (*Friedman* χ_2^2 = 0.462, *P* = 0.794), but did influence the total spending time in choice zones (*Friedman* χ_2^2 = 10.4, *P* = 0.005). According to post hoc tests, *M. zebra* females stayed longer in choice zones during the choice trials with full-spectrum and -LW light environments (mean ± SD (s) = 377 ± 66.4) than with full-spectrum and -SW light environments (mean ± SD (s) = 287 ± 91.8) (*P* = 0.004). There was no significant difference among other trials (-UV vs. -SW; *P* = 0.142, -UV vs. -LW; *P* = 0.382).

Females spent significantly different time between full-spectrum and filtered light environments regardless of the type of filtered light environment. During the trials with -UV and -SW light environments, females spent more time near the full-spectrum light environment in total (i.e. *total choice duration*) and also stayed longer for each visit (i.e. *average choice duration*). However, females showed a significant preference for the -LW light environment over the full-spectrum light environment in both total and average choice durations (Table 1 and Figure 2A,C).

Experiment b: females prefer males in more blue-shifted light environments

M. zebra females spent more time in choice zones in the presence of conspecific males (*Experiment b*) than in the absence of the males (*Experiment a*) across the trials (full-spectrum vs. -UV; V_{13} = 89, *P* = 0.020, vs. -SW; V_{13} = 102, *P*= 0.002, vs. -LW; V_{13} = 101, *P* = 0.001) (Figure 3).

In the presence of conspecific males, the type of filtered light environment paired with the full-spectrum light environment did not influence the latency to the initial choice (*Friedman* χ_2^2 = 1.5, *P* = 0.472) and total spending time across trials (*Friedman* χ_2^2 = 2.29, *P* = 0.318). Females spent significantly longer time near the male in the full-spectrum light environment than one in -UV or -SW but did not show a significant preference between the two males in full-spectrum and -LW light environments (Table 2 and Figure 2B,D).

The female preference between two males was not influenced by how the males actively moved behind the band-pass filters (i.e. *male activity ratio*; *total choice duration*—χ_1^2 = 1.597, *P* = 0.206, *average choice duration*—χ_1^2 = 1.068, *P* =
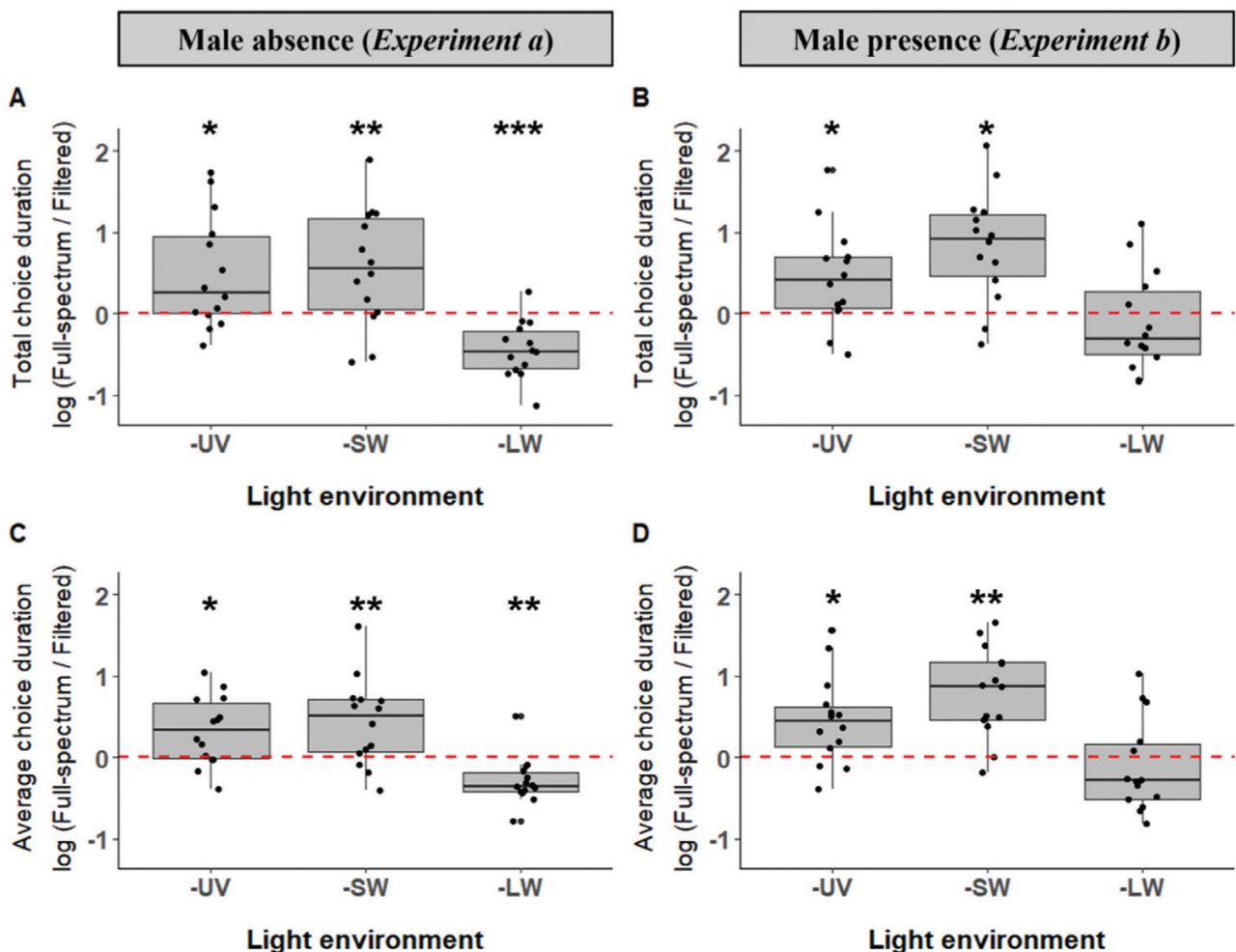

Figure 2 The log-ratio of the total/average choice duration between full-spectrum and filtered light environments in the absence (*Experiment a*; **A and C**) and presence (*Experiment b*; **B and D**) of males. The *X* axis shows the type of filtered light environment that was paired with the full-spectrum light environment. The positive *Y* values represent the preference for a full-spectrum light environment over the corresponding filtered light environments. The top and bottom of the box represent the 25th and 75th percentile, and the line in the middle represents the 50th percentile. The whiskers represent 1.5 times the 25th and 75th percentiles. The dots represent data points from each individual. The dots are scattered centering around the corresponding light environments to avoid overlap. The significant results are denoted by asterisks.

0.302), but influenced by the type of filtered light environment (*total choice duration*—χ_2^2 = 15.77, P < 0.001, *average choice duration*—χ_2^2 = 18.037, P < 0.001) and the interaction term (*total choice duration*—χ_2^2= 7.342, P= 0.025, *average choice duration*—χ_2^2 = 8.810, P = 0.012).

Experiment c: Females show a "reversed" preference for subordinate males without UV light

When *M. zebra* females had a choice between dominant and subordinate *M. zebra* males, the variation in the light environment among trials did not affect the latency to the initial choice (*Friedman* χ_3^2 = 3.699, P = 0.300), total spending time (*Friedman* χ_3^2 = 4.629, P = 0.201), and the initially chosen males (χ_3^2 = 6.115, P = 0.106).

The male dominance affected the total choice duration of *M. zebra* females between dominant and subordinate males in -UV, but not in other light environments. The average choice duration was not significantly different across all light environments. When conspecific males were presented in -UV, females spent more time near a subordinate male than a dominant male (Table 3 and Figure 4). In the mixed-effects model, the male activity ratio (χ_1^2 = 2.243, P = 0.134) and the interaction term with the light environment (χ_3^2 = 2.535, P = 0.469) did not provide significant predictions on the female preference for the dominant/subordinate males as compared to the marginal significance of the light environment(χ_3^2 = 6.719, P = 0.081).

Discussion

Through the two-way choice experiments, we found that *M. zebra* females 1) have a preference for blue-shifted light environments (-LW), 2) prefer to interact with males in the blue-shifted light environment (-LW) as compared to full-spectrum and the red-shifted light environments (-SW & -UV) when both males are dominant, 3) do not show a preference between dominant and subordinate males when the males were presented in the full-spectrum, -LW, or -SW light environments, but 4) shows a "reversed" preference to subordinate males in -UV.

Female preference for the blue-shifted light environment

In contrast to the choice between males under the same light environments, *M. zebra* females show a significant preference between full-spectrum light and filtered light environments. In the choice experiments, females preferred to spend time near the full-spectrum light environment than -UV or -SW regardless of the presence of males. However, females spent equal or more time near -LW than in the full-spectrum light environment. Also, in the absence of males, females were more likely to choose -LW first than in the full-spectrum light environment. The results suggest that *M. zebra* females may prefer a blue-shifted light environment in which short-wavelength light (blue) takes a larger proportion of ambient light spectra than long-wavelength light (red). While there is a possibility that the change in light intensity by band-pass filters may influence the female choice between full-spectrum and filtered light environments, the change in preference for different light intensities depending on the spectrum supports that female choice is exerted by the difference in the ambient spectrum rather than light intensity.

The preference for the blue-shifted light environment may suggest that *M. zebra* may have a preexisting sensory bias toward short and intermediate-wavelength (i.e. UV and blue). Hoffmann et al. (2010) provided evidence that species foraging on plankton and algae like *M. zebra* showed the highest expression of SWS1 opsin that are sensitive to short-wavelength (i.e. UV) as compared to other Lake Malawi cichlids foraging on benthic preys or other fish. Previous field researches on the natural history of *mbuna* cichlids also support that the female preference of *M. zebra* to the blue-shifted light environment may evolve as a perceptual tuning for prey and predator detection as well as mate detection (Holzberg 1978; Genner and Turner 2005; Markert and Arnegard 2007). Thus, the preexisting preference by adaptation of visual systems for non-reproductive behavioral contexts may contribute to the evolution of blue flank coloration of male *M. zebra,* which is conspicuous in the color vision (Dalton et al. 2010).

Furthermore, our results showed that the behavior of *M. zebra* is influenced by the presence of UV in their ambient light environment. Although it has been known that some *mbuna* cichlids including *M. zebra* have UV sensitivity (Dalton et al. 2010; Maan and Sefc 2013) and UV reflective patterns in the male nuptial coloration (Jordan et al., 2004a), whether the species use the UV sensitivity in any behavioral contexts has not yet been tested (Maan and Sefc 2013). In our experiment, the preference of females for full-spectrum light over -UV in the absence of conspecific males suggests that *M. zebra* females may use the UV sensitivity in a non-reproductive context such as foraging. A comparative study on the cone pigment expression in *mbuna* cichlids also proposed that UV sensitivity may provide an advantage to find zooplankton by enhancing the contrast against the water background (Sabbah et al. 2010). However, the variation in UV sensitivity (Dalton et al. 2010) and UV reflection in male coloration (Jordan et al. 2004a) among *mbuna* cichlids suggests that the UV sensitivity may also function in inter- or intraspecific mate choice.

Female preference for male nuptial coloration

When dominant males were presented in different light environments, females did interact longer time with males in the blue-shifted light environment (-LW) than in full-spectrum and red-shifted light environments (-SW and -UV). However, when dominant and subordinate males were presented in the same light environments, in general, *M. zebra* females did not show a preference for the variation in male nuptial coloration by male dominance which is used for mate choice in other cichlids (Couldridge 2002; Pauers et al. 2004; Tobler 2006). The absence of preference for dominant males based on the nuptial coloration suggests that *M. zebra* females may not need to or cannot recognize dominant males in our experimental settings.

Although there is a significant difference in the spending time in choice zones between male-absent (*Experiment a*) and male-present (*Experiment b*) trials, we do not know if females were sexually motivated in our experiment. We did not artificially manipulate the reproductive status of females by hormone injection prior to these experiments. As such, female behavior may represent non-sexual social behavior. If this is the case, the longer spending time in the presence of males may be due to a simple aggregation behavior which is usually observed in free-living *mbuna* females (Ribbink et al 1983;
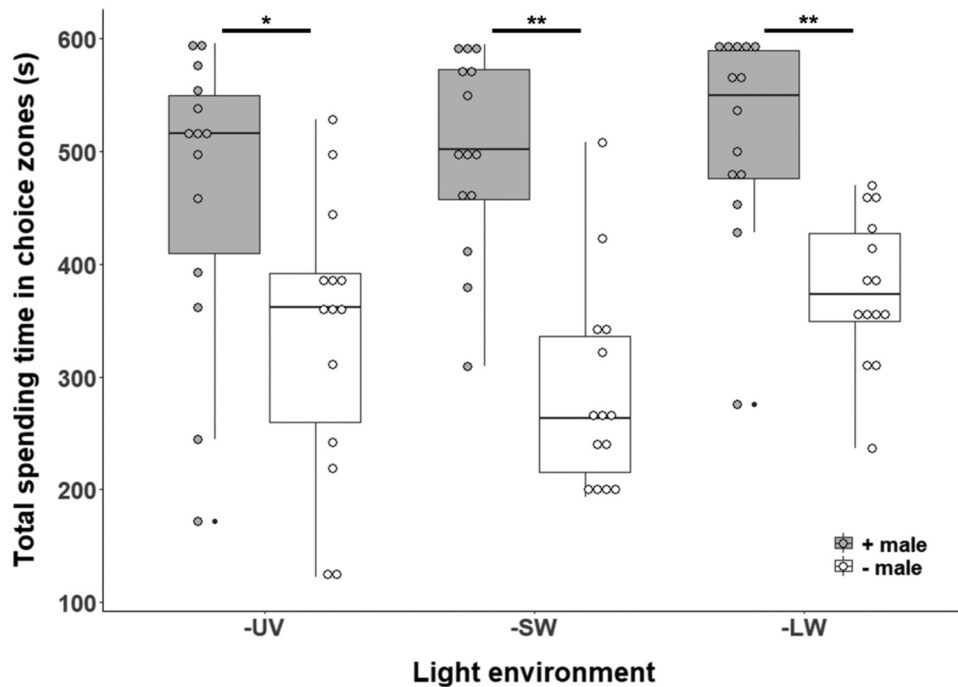
Figure 3 Total spending time in choice zones across different light environments in male-absent (*Experiment a*) and male-present trials (*Experiment b*). The top and bottom of the box represent the 25th and 75th percentile, and the line in the middle represents the 50th percentile. The whiskers represent 1.5 times the 25th and 75th percentiles. The dots represent data points from each individual. The dots are scattered centering around the corresponding light environments to avoid overlap. The significant results are denoted by asterisks.

Table 2 The total and average choice duration of *M. zebra* females between full-spectrum and filtered light environments (-UV, -SW, -LW) in the presence of two dominant conspecific males (*Experiment b*).

		Full vs. -UV	Full versus -SW	Full versus -LW
Total choice duration	Full-spectrum (s)	281 ± 102	186 ± 85.2	247 ± 94.7
	Filtered (s)	186 ± 85.2	158 ± 78.0	271 ± 89.8
	Statistics	$V_{13} = 90, P = 0.017$	$V_{13} = 100, P = 0.013$	$V_{13} = 41, P = 0.502$
Average choice duration	Full-spectrum (s)	20.6 ± 8.86	23.2 ± 17.7	14.8 ± 7.60
	Filtered (s)	13.2 ± 6.66	9.90 ± 4.73	16.8 ± 8.84
	Statistics	$V_{13} = 91, P = 0.013$	$V_{13} = 102, P = 0.001$	$V_{13} = 35, P = 0.296$

The significant results of the Wilcoxon signed-rank tests are denoted in bold.

Table 3 The total and average choice duration of *M. zebra* females between dominant and subordinate males in full-spectrum and filtered light environments (-UV, -SW, -LW) in *Experiment c*.

		Full-spectrum	-UV	-SW	-LW
Total choice duration	Dominant (s)	273 ± 102	217 ± 55.4	246 ± 79.4	237 ± 86.3
	Subordinate (s)	204 ± 78.1	296 ± 95.5	221 ± 96.3	237 ± 99.9
	Statistics	$V_{13} = 77, P = 0.135$	$V_{13} = 20, P = 0.042$	$V_{13} = 65, P = 0.463$	$V_{13} = 59, P = 0.715$
Average choice duration	Dominant (s)	12.9 ± 6.17	16.7 ± 15.7	13.7 ± 7.10	15.8 ± 6.04
	Subordinate (s)	12.2 ± 8.50	20.5 ± 15.5	13.1 ± 6.33	15.9 ± 6.89
	Statistics	$V_{13} = 58, P = 0.761$	$V_{13} = 40, P = 0.463$	$V_{13} = 64, P = 0.502$	$V_{13} = 57, P = 0.442$

The significant results of the Wilcoxon signed-rank tests are denoted in bold.

Marsh and Ribbink 1986). In *Experiment b*, females then may have been more likely to interact with males in blue-shifted light environments simply due to the high conspicuousness of male nuptial coloration in such light environments. This would suggest that the blue flank coloration of *mbuna* males may evolve to increase conspicuousness in their habitat which has a blue-shifted ambient light spectrum (Seehausen et al. 2008; Dalton et al. 2010; Smith et al. 2012). Moreover, in our
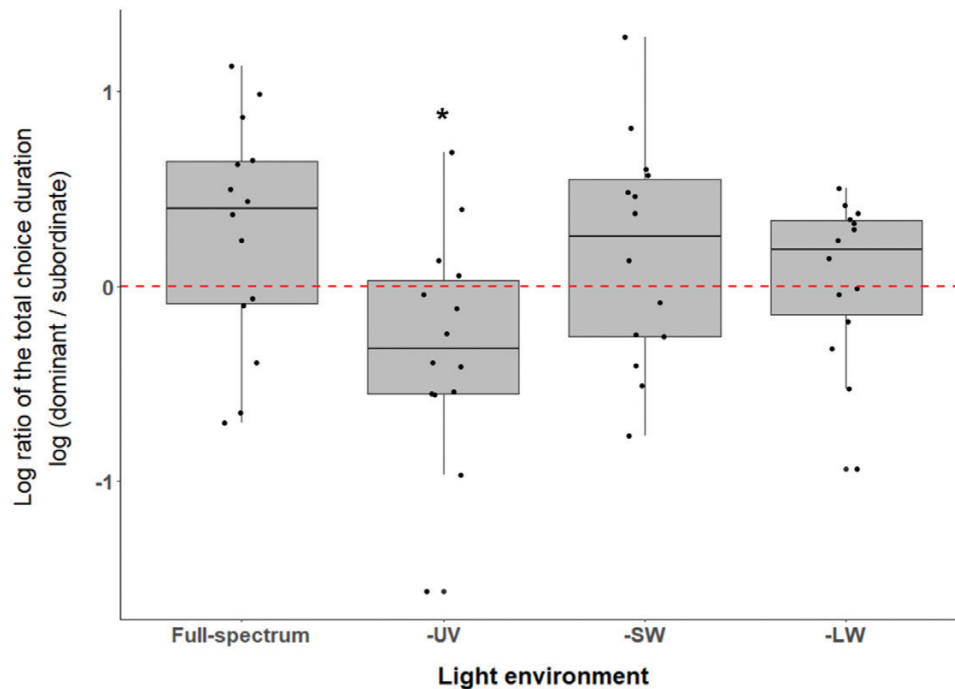
Figure 4 The log-ratio of the total choice duration between dominant and subordinate males. The *X* axis shows the type of light environment in that males were presented. The positive *Y* values represent the preference for dominant males over subordinate males in the corresponding filtered light environments. The top and bottom of the box represent the 25th and 75th percentile, and the line in the middle represents the 50th percentile. The whiskers represent 1.5 times the 25th and 75th percentiles. The dots represent data points from each individual. The dots are scattered centering around the corresponding number of neighbors to avoid overlap. The significant results are denoted by asterisks.

experimental settings, males did not hold a resource or territory that influence female mate choice in *M. zebra* (Greenberg et al. 2016), so the dominance perceived by nuptial coloration may be insufficient to elicit a sexual or reproductive context such as the female choice between dominant and subordinate males.

Interestingly, females showed a weak preference for subordinate males in the absence of UV wavelength. Given that the reflectance spectra of dominant *M. zebra* males are highly skewed toward UV wavelengths (<400 nm; Jordan et al. 2004a), in the -UV, subordinate males may be more conspicuous than dominant males and the reduced conspicuousness of the nuptial coloration of dominant males may result in the "reversed" preference for male dominancy in our experiment. If so, the UV-reflected bar patterns of dominant *M. zebra* males may not be adaptive to attract females without the pre-evolved UV sensitivity of females that has been proposed to evolve in non-communicative contexts such as foraging zooplanktons in the natural signaling environment of *mbuna* cichlids that includes UV wavelengths (i.e. shallow water in the Lake Malawi) (Jordan et al. 2004b; Sabbah et al. 2010). Although further research is required to test whether UV-reflected patterns of male nuptial coloration enhance the reproductive success of *M. zebra* males, our results suggest evidence of the potential role of sensory drive in the evolution of UV reflections in *mbuna* males (Jordan et al. 2004a; Smith et al. 2012; Carleton et al. 2016).

Metriaclima zebra females used in our experiments had been raised in an artificial light environment that is different from their natural ambient light environment. Previous research has reported the potential for rapid change in the cichlid visual system, such as opsin gene expression patterns, based on the rearing conditions and artificial light

environments (Hoffmann et al. 2010). It is unclear if our animals were similarly influenced by their rearing environment. Thus, the replication of our experimental designs with fish from their natural habitat will provide a better insight into signal evolution in *mbuna* cichlids.

In summary, the choice experiments in various light environments with *M. zebra* females showed that 1) females have a preference for the blue-shifted light environment, 2) male *M. zebra* coloration may maximize the conspicuousness in the blue-shifted light environment, 3) UV sensitivity may contribute to intraspecific mate choice or foraging of *M. zebra* females, and 4) the evolution of the UV-reflected patterns of dominant *M. zebra* males may be promoted by female sensory bias for non-communicative contexts such as foraging (i.e. sensory drive; Endler 1992; Cummings 2007).

Acknowledgment

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