SEX DIFFERENCES IN VISUAL PERCEPTION IN MELANOCROMIS AURATUS

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ABSTRACT

Moira van Staaden, Advisor

The cichlid fishes of Lake Malawi have undergone explosive speciation in the past 1.5 million years with the production of between 500-1000 species of cichlid $^1$. Sensory factors such as visual, olfactory and acoustic signals are all believed to play a role in this differentiation process. Vision is by far the most studied and best understood of the sensory modalities, but there is a distinct gap between our understanding of the molecular components of the cichlid visual system and whether, or how exactly, these impact the behavior displayed. Recent research has shown that the type of visual pigment expressed in the retina differs by sex in *Melanochromis auratus* $^{11}$. We aimed to determine whether such differences are reflected in sex-dependent variation in hue sensitivity and hue discrimination in *M. auratus*. To test this, we used a modified optomotor discrimination task to assess (i) differences in the luminance curves for each sex and (ii) sex differences in the minimum angular distance of adjacent hues required to elicit recognition as two distinct stimuli. Our results indicate significant variation in the minimum brightness required for the perception of hues across the color spectrum, but no sex-specific difference in detection thresholds. Similarly, results for the minimum angular distance required to discriminate between adjacent hues suggest there are areas of the color spectrum to which vision is tuned, but again no difference between the sexes. These results suggest that the previously reported differences in visual pigment expression levels between male and female *M. auratus* have no detectable effect on hue discrimination or sensitivity, at least as measured by behavioral means in the visual light spectrum.
I would like to dedicate my thesis to my family. Without their support and encouragement this
thesis would not have been possible. I love you all!
ACKNOWLEDGMENTS

First and foremost I wish to acknowledge with extreme gratitude my advisor Moira van Staaden for all her advice, critiques and support, without which this thesis would never have gotten off the ground. I also wish to thank my committee members, Sheryl Coombs for her support and suggestions and Robert Huber for his statistical and technical support as well as the data for the radiance spectra of the computer-generated hues. I also want to recognize all of my fellow lab members for their comments and encouragements. I especially wish to recognize my past lab mate Jeremy Didion for all his previous work and help on this thesis.

I want to express my gratitude to my mom, Jane Coniam, dad, Robert Coniam and sister, Carolyn Coniam as well as my grandpa, Richard Sidwell and aunt, Diane Jehle, for being my rock through this process and encouraging me. You all have helped me more than words can say.

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INTRODUCTION

Lake Malawi is one of three large East African Great Lakes containing more fish species than any other freshwater lake in the world and more than half of the known cichlid taxa of the African Great Lakes. Malawian cichlids have undergone extensive adaptive radiation in the past two million years. While this diversification is well documented, the processes driving it are still poorly understood. The wide variation in the use of color patterns between different species has led to the hypothesis that differences in visual signals and signal detection could be driving the diversification of the family. Consequently, the use of visual signals is by far the most studied and best understood of the diversifying cues. It is generally considered the predominant sensory modality underlying radiation of Lake Malawi cichlids with factors such as the photic environment and the cichlid visual system itself helping to shape the signals displayed.

The photic environment of Lake Malawi is believed to help shape visual signals and has lead researchers to suggest various ecological mechanisms to help explain the diversification of the cichlid species. Visibility of objects underwater is determined by spectral irradiance (the amount of light hitting a surface), spectral radiance (the amount of light emitted from a surface) and by water clarity. Since Lake Malawi is a deep lake, this reduces turbulence producing a typically clear water environment subject to only short-term seasonal perturbations. Environmental transmission is thus relatively stable throughout the year. In the rock habitat, where *M. auratus* is predominantly found, beam attenuation is lower than in the sand habitat. Consequently visual signals can be transmitted over greater distances in rocky than in the sandy habitats, creating a photic environment that should promote the use of visual cues in both inter-
and intraspecific interactions. Such cues will work most effectively, however, if the cichlid's visual system is tuned into those particular areas of the color spectrum.

The cichlid visual system includes both photoreceptive rods and cones, the latter of which contains visual pigments that aid in color detection \(^6, 35\). Visual pigments are sensitive to various wavelengths of light in the color spectrum and contain opsin proteins that are coded by seven different genes \(^6\). Wild cichlids typically express three or four genes which, depending on how those genes complement each other, determine the range of sensitivities for that taxon \(^6\). This potentially grants access to a large range of spectral sensitivities from the ultraviolet to the long wavelength \(^6, 11, 34\). Color vision is crucial for cichlids signaling information in a variety of trophic and social contexts such as foraging strategies, mate selection and aggression \(^7, 8, 9, 10, 20, 23, 30, 31\).

Several studies have shown that females can use visual cues alone to discriminate between con- and heterospecifics \(^7, 8, 22, 32\). When shown images of male cichlids differing only in coloration, females of several cichlid taxa preferred males displaying species-specific color over alternatives or a similar color if their species-specific color was not present \(^7\). Female cichlids from Lake Victoria were found to select conspecific mates based on color alone, a specificity that is lost when color information is concealed under monochromatic lighting \(^8\). Male cichlids also use coloration in both male aggression and possible mate choice scenarios. *Metriaclima mbenjii* males react more aggressively to similarly colored males in Lake Malawi \(^9\), while *Pseudotropheus (Maylandia)* males preferentially associate with females displaying their mother’s color \(^10\). Furthermore, both male and female cichlids employ color vision in foraging, such as for the detection of zooplankton and algae against a light background \(^30, 31\). All of which
supports the contention that differences in signal perception and signaling preferences may be significant contributors to the process of diversification in cichlids.

Evidence for differences in signaling perception has been reported not just between species, but within species as well. One Malawian cichlid, *Melanochromis auratus*, a species known for having both sexes display bright, though different colorations, has been identified as expressing different visual pigments between males and females. This has lead to speculation of functional differences in visual processing. Sabbah et al (2010) using whole organism electrophysiology, opsin gene expression and empirical modeling, identified *M. auratus* as having the greatest intersexual difference in cone pigments of all three cichlid species investigated. While such variation in cone pigment expression levels might reasonably be expected to result in intersexual differences in color vision and visual signaling, we currently have little knowledge about how (or if) such differences in one part of the visual processing mechanism are reflected in the visual behavior of *M. auratus*. The present study focuses on sexual differences in color detection and discrimination by *M. auratus* using the optomotor response. Based on the findings of Sabbah et al (2010), we predicted that vision in female *M. auratus* would be particularly more sensitive and discriminatory at shorter wavelengths, whereas male sensitivity and discrimination would be tuned to longer wavelengths.
METHODS

Experimental Subjects

*Melanochromis auratus* is an omnivorous, rock-dwelling species of cichlid\(^{49}\). Found in the southern portion of Lake Malawi from a depth of 0 m-40 m, mature males reach a maximum length of 10 cm and are primarily a deep blue with a white horizontal stripe\(^ {49}\). Females reach a maximum length of 9 cm and both females and juveniles are yellow with a black horizontal stripe\(^ {49}\). Animals were obtained from a commercial supplier and housed individually in twenty-gallon aquaria partitioned into three sections in the University Animal Facility at Bowling Green State University. The aquaria had a layer of gravel with Terra Cotta pots for shelter. Water temperature was kept between 26-28 °C and the fish were on a 12:12 light: dark cycle. Fish were housed under standard florescent lighting because unlike other cichlid species, the lighting conditions during rearing has little effect on cone pigment expression in *M. auratus*\(^ {26, 40, 42, 44, 45}\). Fish were fed once a day with commercial cichlid flakes (Cichlid Vegi Flakes). All animals were maintained in accordance with the Bowling Green State University IACUC (protocol 11-002).

Experimental Setup

A modified optomotor response paradigm was used to assess visual responses to the projected stimuli with a similar physical setup to Smith et al (2012). Modifying this classic behavioral test for studying visual sensitivities\(^ {15, 17, 18, 39, 46}\) by using a computer-generated stimulus instead of filters allowed us to test smaller slices of the color spectrum permitting us to delimit the visual abilities of *M. auratus* with greater accuracy\(^ {26, 43}\). The test arena consisted of an acrylic tank (41.5 cm x 32.5 cm x 20.3 cm) under which a computer screen (Dell 1908FPb)
was placed so as to project the stimuli up through the translucent bottom of the tank (Figure 1A). The sides of the tank were covered with black material to eliminate all external visual cues. A clear, plastic container (36.8cm x 24.8cm x 22.2cm) was placed in the middle of the arena to keep the fish centered over the test stimulus. A high definition camera (Sony HDR-HC9) positioned 80 cm above the tank was used to videotape each trial and connected to a secondary computer screen to permit direct observation of the fish behavior. The experimenter, computer driving the stimulus presentation and secondary computer screen were separated from the experimental tank by a black curtain. Prior to each test both the test arena and internal clear container were filled to a depth of 9 cm with water from the individual’s home tank and the fish was placed in the internal clear container.

Figure 1: Setup of Arena and Generation of Test Stimuli- A. The test arena consisted of an external tank (black) (41.5 cm x 32.5 cm x 20.3 cm) with a translucent bottom through which the test stimulus was projected via computer screen (grey). An internal, clear container (light blue)
(36.8cm x 24.8cm x 22.2cm) was used to position the fish over the test stimuli. Both the external tank and internal container were filled with 9cm of water from each individual's home tank. B. A pictorial representation of the computer-generated stimuli. In the hue sensitivity test, the moving bars started at 0% brightness and slowly increased in brightness over time. In the hue differentiation test, the moving bars began to alter their hue from the background hue in either the clockwise or counter clockwise direction around the color wheel overtime. C. The color wheel divided into test sections. Each line represents twenty degrees. A total of 18 different hues were tested.

Hue Sensitivity

Ten male and ten female mature *M. auratus* were randomly chosen to compare sexual differences in the minimum brightness required for hue detection. Fish were placed individually in the test arena and allowed to acclimate for ten minutes while the computer screen displayed a black screen. The room lights were left on to allow the fish to light adapt.

After ten minutes the room lights were switched off and a customized computer program was begun which displayed colored bars, 2.97 cm wide, moving at a rate of 0.3 cm per second against a background color of 2.97 cm wide spaces. Initially the moving bars and the background hue were both black (0% brightness, saturation 100%). Against this constant background the brightness of the moving bars was then increased in a stepwise fashion (1% of the maximum brightness value every 500 ms) (Fig. 1B) until an optomotor response was noted. A positive response was defined as the fish matching the speed and direction of the moving bars. To characterize vision across the entire color spectrum a total of 18 hues spaced at 20 degree intervals around the color wheel (Fig. 1C) were selected and individually presented on the computer screen. Given that computer-generated hues are created via a combination of different wavelengths, Figure 2 demonstrates the distribution of wavelengths and radiance levels used to produce each of the hues tested. Light adaption of the fish was maintained by turning on the
overhead room lights for at least one minute between each trial (i.e. hue presentation). By maintaining light adaption, we were able to better compare our results to previous studies 26, 42, 43.

Figure 2: Radiance Spectra of the Eighteen Hue Angles- Level of radiance emitted from each wavelength constructing each hue angle. Radiance levels were measured using a colorimeter.
To control for any possible observer bias the direction of bar movement and order of hue presentation were randomized and the researcher was blind to the brightness level of the bars during the course of the trial. Each hue was tested either three or six times (for fourteen and six fish respectively). Groups tested three or six times were balanced in terms of sex composition. The minimum brightness threshold for each fish and hue was determined by averaging the lowest two or three measurements for tests of three or six repetitions respectively. Luminance curves, how bright each hue appears as weighted by the *M. auratus* eye, were then constructed for each sex using the mean of the minimum brightness levels required to detect each of the eighteen hues. Brightness levels were also converted into radiance, an absolute measure of the number of photons a light source emits, expressed as watts per steradian per square meter (W/sr/m$^2$).

**Hue Discrimination**

Ten male and ten female mature *M. auratus* were used to test for sexual differences in hue discrimination in the same physical setup employed previously. Fish were placed individually in the test tank for a ten-minute acclimation period during which a black screen (0% brightness) was presented and the room lights remained on to light adapt the visual system. The room lights were then turned off and a customized computer program began displaying moving colored bars on an identically colored background. Saturation was set at 100% for both the bars and the background hue.

Because computer screens are optimized for the human visual system, different hues are displayed at different luminance levels, i.e. how bright a particular stimulus appears as seen by an organism, in this case the human eye \(^{21}\). In order to control for this intrinsic luminance...
variation, we standardized luminance levels at 10 cd/m\(^2\) by systematically varying the brightness levels for hues across the spectrum. These adjustments were based on cichlid radiance curves (Figure 3 and Table 1) \(^{21}\).

![Graph of Luminance versus Brightness for each of the Eighteen Hue Angles](image)

Figure 3: Graph of Luminance versus Brightness for each of the Eighteen Hue Angles- The black line represents the luminance level (10 cd/m\(^2\)) selected for which all hues were standardized. Hue angles 100° and 160° and hue angles 19° and 300° have identical curves and are therefore represented here as two single lines. Raw data obtained from Didion, unpublished \(^{21}\).

The eighteen hue angles previously described were used here as initial starting points for the discrimination task. Initially bars and background were identical hues, but the bars subsequently diverged one degree away from the background color at half-second intervals (Fig. 1B). Starting hue, direction of hue divergence (clockwise or counterclockwise around the color wheel), and direction of bar movement (to the left or right on the screen) were all randomized. In addition, the experimenter was blind to the specific values of the difference between background and bar hues. The hue divergence continued until the fish displayed an optomotor
response as previously defined. Between each trial, room lights were turned on for one minute to maintain light adaptation. Each hue was tested four times in each direction for a total of 144 separate discrimination trials. For each fish, the minimum detectable difference was calculated as the mean of the lowest three measures for each hue in each direction. A smaller difference between starting and ending hues is thus indicative of greater discrimination in that region of the color spectrum.

Table 1: Table of Radiance Levels when Scaled to a Constant Luminance- Maximum radiance level for each hue angle when each hue is scaled to a luminance level of 10cd/m².

<table>
<thead>
<tr>
<th>Hue Angle</th>
<th>Maximum Radiance (W/Sr/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.03</td>
</tr>
<tr>
<td>20</td>
<td>0.025</td>
</tr>
<tr>
<td>40</td>
<td>0.022</td>
</tr>
<tr>
<td>60</td>
<td>0.02</td>
</tr>
<tr>
<td>80</td>
<td>0.018</td>
</tr>
<tr>
<td>100</td>
<td>0.019</td>
</tr>
<tr>
<td>120</td>
<td>0.018</td>
</tr>
<tr>
<td>140</td>
<td>0.018</td>
</tr>
<tr>
<td>160</td>
<td>0.022</td>
</tr>
<tr>
<td>180</td>
<td>0.029</td>
</tr>
<tr>
<td>200</td>
<td>0.037</td>
</tr>
<tr>
<td>220</td>
<td>0.066</td>
</tr>
<tr>
<td>240</td>
<td>0.112</td>
</tr>
<tr>
<td>260</td>
<td>0.088</td>
</tr>
<tr>
<td>280</td>
<td>0.066</td>
</tr>
<tr>
<td>300</td>
<td>0.05</td>
</tr>
<tr>
<td>320</td>
<td>0.041</td>
</tr>
<tr>
<td>340</td>
<td>0.034</td>
</tr>
</tbody>
</table>
RESULTS

Hue Sensitivity

Analysis of the radiance levels showed no significant sensitivity difference between the sexes across the color spectrum. An ANOVA was performed in which the radiance levels were ranked due to the large amount of variation present for each hue (ANOVA: F= 0.9249, d.f.= 17, p > 0.5440) (Fig. 4A). However, in a pattern that was conserved across the sexes, it appeared that generally the hues primarily composed of mid to long wavelengths required less radiance to be detected, indicating greater sensitivity, than those composed primarily of shorter wavelengths, i.e. dark blue always required a higher radiance level than did light blue regardless of sex. Red to light blue (hue angles 0°-180°) required lower levels of radiance than dark blue (hue angles 240°-260°), which required higher levels of radiance to be detected (ANOVA: F= 9.9087, d.f.= 17, p < 0.0001) (Fig. 4B). The different brightness levels were also converted into luminance curves for each sex (Fig. 4C).
Figure 4: Graphs of the Results Obtained from the Hue Sensitivity Trials -

A. Mean radiance for each hue angle for females (pink line) and males (blue line). Males and females exhibited similar sensitivities, but when the radiance of the hue angle was compared across the spectrum regardless of sex it was significantly different. Letters connecting hue angles are not significantly different from each other.

B. Concentric circle graph of male and female mean
radiance for the eighteen different hue angles. The inner circle represents a radiance of 0.025 W/Sr/m² while the outer circle represents a radiance of 0.05 W/Sr/m². Hue angles 0°-180° required lower levels of radiance while hue angles 240°-260° required higher levels of radiance to be detected. C. Luminance curves for males (blue line) and females (pink line).

Hue Discrimination

Sex appeared to have no influence on hue discrimination across the entire color spectrum. However, both the initial hue and direction of hue change around the color wheel had a significant effect on hue discrimination in a pattern which was conserved across the sexes (Fig. 5). While both directions showed higher discrimination in the same ranges of the color spectrum (i.e. short and long wavelengths), the actual hues within these ranges differed significantly depending upon direction (i.e. clockwise: hue angles 20°-40°, 180° and 300°-320° and counter-clockwise: hue angles 40°-60°, 180°-220° and 320°-340°). The difference in discrimination approached from the clockwise and counter-clockwise directions suggests that contrast plays an important role in visual discrimination.
Figure 5: Graphs of the Results Obtained from the Hue Discrimination Trials- A. Mean difference between starting and ending hues for males and females in both the clockwise and counter-clockwise directions. The inner circle denotes 50° in the clockwise direction or -50° in the counter-clockwise direction away from the starting hue. The outer circle represents 100° or -100° away from the starting hue angle. The minimum difference between hue angles was significantly different depending upon the direction around the color wheel the bars' hue was changing. In the clockwise direction, hues 60°-120° and 220° required a larger difference in hue angles to be detected as two separate hues while hues 20°-40°, 180° and 300°-320° required a smaller difference in hue angles. In the counter-clockwise direction hue angles 0°-20°, 80°-160°, and 260°-280° required a larger difference in hue angles while hue angles 40°-60°, 180°-220° and 320°-340° required a smaller difference in hue angles to be detected as two different hues. B. Mean difference required between starting and ending hue angles for males (top figure) and females (bottom figure). Average difference denotes the distance apart two hue angles must be to be perceived as two different hue angles. Both background and bar hue started at a difference of zero and over time the bars changed their hue in a clockwise or counter-clockwise manner. Lines going above the zero line denote a clockwise shift in hue angles while lines going below
the zero line denote a counter-clockwise shift in hue angle. Hue angles were determined to significantly differ in hue angle, direction and hue angle by direction. Letters connecting hue angles were determined to not be significantly different.

A complete model ANOVA was performed for sex, initial hue of the bars in each trial and direction of hue change around the color wheel (Table 2) and a Tukey test was used to determine the differences in initial hues and direction. In the clockwise direction (i.e. moving toward shorter wavelengths) hues composed primarily of short and long wavelengths were discriminated from the background much quicker than hues composed primarily of mid-wavelengths indicating possible areas of spectral tuning. *M. auratus* showed greater discriminatory ability in the clockwise direction with orange (hue angles 20°-40°), light blue (hue angle 180°) and violet to magenta (hue angles 300°-320°) ranges required a smaller difference in hue angle to be detected as two separate hues, compared to the yellow to green (hue angles 60°-120°) and dark blue (hue angle 220°) hues. In the counter-clockwise direction (i.e. moving toward longer wavelengths) generally hues composed primarily of short and long wavelengths were more quickly discriminated from the background than hue composed primarily of mid wavelengths, though in two hues composed primarily of mid wavelengths the fish did show high discrimination ability. *M. auratus* showed less discrimination ability in the counter-clockwise direction in red to orange (hue angles 0°-20°), light green to light blue (hue angles 80°-160°) and dark blue to purple (hue angles 260°-280°), while showing greater discrimination ability in orange to yellow (hue angles 40°-60°), light blue to dark blue (hue angles 180°-220°) and magenta to red (hue angles 320°-340°).
Table 2: Table of the ANOVA Results for Each Component of the Hue Discrimination Trials-
Table of the levels of significance for the different factors tested. Initial hue, direction of hue
change around the color wheel and initial hue by direction around the color wheel were found to
be significantly different from each other.

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>Degrees of Freedom</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>0.1522</td>
<td>1</td>
<td>&gt;0.6967</td>
</tr>
<tr>
<td><strong>Initial Hue by Sex</strong></td>
<td>0.2592</td>
<td>17</td>
<td>&gt; 0.9990</td>
</tr>
<tr>
<td><strong>Sex by Direction</strong></td>
<td>0.3801</td>
<td>1</td>
<td>&gt; 0.5376</td>
</tr>
<tr>
<td>Around the Color Wheel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sex by Initial Hue by</strong></td>
<td>0.3480</td>
<td>17</td>
<td>&gt; 0.9937</td>
</tr>
<tr>
<td>Direction Around the Color Wheel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Initial Hue</strong></td>
<td>36.6672</td>
<td>17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Direction Around the Color Wheel</strong></td>
<td>712.4885</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Initial Hue by Direction Around the Color Wheel</strong></td>
<td>48.1003</td>
<td>17</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
DISCUSSION

Our results indicate that *M. auratus* have color vision that is best tuned to the short and long wavelength ends of the spectrum, but there is no intersexual difference in either sensitivity to, or discrimination between, these various hues. Cichlids of both sexes showed similar patterns of brightness levels necessary to detect hues in different parts of the color spectrum, as well as similar patterns in the minimum angular distance necessary between two hues for them to be distinguished from each other. Vision in cichlids is thought to have many different influencing factors including physiological, environmental and social.

In our hue discrimination test, hues composed of shorter and longer wavelengths of light required a smaller hue angle difference to be detected indicating greater discriminatory ability in those areas of the color spectrum. This is concordant with evidence from other labs showing that an increased expression level of the LWS photopigment is strongly correlated with an increase in sensitivity of both long and short wavelengths \(^{26,38}\). While there is some variation at the individual level in *M. auratus*, most fish express a common suite of four cone pigment genes; SWS2b (short wavelength sensitive), RH2a and RH2b (medium wavelength sensitive) and LWS (long wavelength sensitive) \(^{40}\). Furthermore, it has been shown that in goldfish LWS cones are responsible for the detection of movement \(^{38}\). A series of experiments involving red and green moving bars set at different intensities, revealed that when the LWS cones were calculated as being in a less excited state the optomotor response was lost, even when the medium wavelength sensitive (MWS) cones were calculated as bring in an excited state. Thus increased expression of the LWS photopigment could confer an advantage in the optomotor response paradigm and may partially explain why we saw little difference in hue discrimination between males and females. Further testing using other techniques such as training individuals to recognize specific
stationary colors may provide greater insight into differences between the sexes without the confounding effects of motion detection.

The similarities in visual tuning and discrimination of male and female *M. auratus* across the color spectrum may reflect commonalities deriving from similarities in foraging strategies and social interactions. *M. auratus* are omnivorous and sensitivity to shorter or even ultraviolet wavelengths is thought to aid detection of zooplankton against the background waters of Lake Malawi while longer wavelength sensitivity facilitates detection of herbivorous food items such as algae. Similar foraging strategies would necessitate tuning to similar areas of the color spectrum. In addition, female mate choice, male-male competition and even speculation of male mate choice have been reported in Cichlidae. Unlike many other cichlid species, both male and female *M. auratus* invest in similar patterned, yet different brightly colored visual displays. Although its well-known that males invest in bright coloration in part due to the demands of female mate choice and male-male competition, this does not explain why females would similarly employ bright coloration patterns. One idea for female investment in bright colorations is male *M. auratus* do not hold permanent territories and have the potential to select females based on the area in which they choose to set up their territory. Moreover, both males and females are highly aggressive towards one another and are shown to form linear hierarchies irrespective of sex. Both sexes would thus benefit from an ability to effectively detect coloration of conspecifics in both inter- and intra-sexual selection scenarios. The necessity of tuning into similar areas of the color spectrum could explain why there is no difference in visual sensitivity or discrimination.

Habitats can play a key role in the development of visual cues and can help shape areas of visual sensitivity. Lake Malawi has two main habitats, rock and sand, in which the photic
environment differs. The waters and rocks of Lake Malawi reflect mid (450-550nm) and long (600-650nm) wavelengths of light while the spectral reflectance of the color patterns of *M. auratus* show peaks at 350nm, 480nm and 500nm; short to mid wavelengths respectively. The contrast created between the shorter wavelengths of the *M. auratus* coloring and the mid to longer wavelengths of the habitat creates signals that are conspicuous and easily discernible over long distances. In our study both male and female *M. auratus* showed a greater sensitivity and discrimination of hues composed primarily of wavelengths from the short and long end of the spectrum, consistent with the photic properties of the habitat. The importance of habitat and its role in spectral tuning has been noted in several Malawian cichlid species. Dalton et al (2010) noted that none of the fish they sampled in Lake Malawi had any significant portion of their color pattern in the green or red hues and concluded this is because there would be low contrast between red or green and the background habitat. This supports the idea of the environment shaping visual cues and thus influencing spectral tuning. The impact of the photic properties of Lake Malawi on the visual system of *M. auratus* may be a driving force in the shaping of visual sensitivities and would promote males and females to tune into similar areas of the color spectrum.

There was a significant difference between hue discrimination abilities in the clockwise and counter-clockwise directions around the color wheel. These directional differences can be explained by the contrast between the background and bar hue angles. The importance of contrast has been observed in other fish species. Stickleback females prefer males with a greater contrast between eye and throat/jaw color and color contrast and luminance play a key role in object detection in Zebrafish. Using a green bar moving on a red or green background in which the irradiance level of the background was either the same, above or below the irradiance...
level of the bar, Burkhardt (2012) found that a response was elicited only when the green background was above or below (but not equal to) the irradiance, and consequently luminance level, of the green bar. Conversely, a green bar on a red background elicited a response regardless of irradiance level. This indicates that color contrast is critical in object detection, but that differing background luminance levels also influence object detection. In the present study we controlled for luminance in stimulus presentation. It is likely then that our results reflect the impact of color contrast and this may be the underlying cause for the significant directional effects noted in the hue discrimination trials.

Our study effectively suggests that there is no significant difference in hue sensitivity or hue discrimination between male and female *M. auratus* in the visible light spectrum detectable at the behavioral level. Luminance curves that we constructed for male and female *M. auratus*, equivalent to those for human vision, allow for comparison of visual abilities between species. Analysis of these luminance curves ultimately helped yield results that do not support vision differences between the sexes. Similarities in foraging strategies and social interactions, combined with strong selection by the consistent Lake Malawi photic environment may shape similar sensory biases in the sexes despite reports of significant differences in cone pigment expressions levels. Our study has important implications for visual processing in cichlids. It suggests that while male and female *M. auratus* may express different cone pigments, at least within the color spectrum, they show no difference in sensitivity levels. Further studies combining fine-scale behavioral discrimination tests with direct measures of cone pigment gene expression would enable researchers to better strengthen the link between behavioral sensitivities and cone pigment gene expression in greater detail.
REFERENCES


APPENDIX A: IACUC APPROVAL

Dr. Moira van Staaden
Biological Sciences
Bowling Green State University

Re: Annual Renewal of IACUC Protocol 11-002

Title:
Behavior and Sensory Systems of Cichlid Fishes

Dear Dr. van Staaden:

On October 17, 2012 the annual renewal for the above referenced protocol was approved after review by the IACUC. This renewal expires on October 16, 2013. Please consult with the staff of the Animal Facility about any special needs you might have to continue with this project.

Comments:

Sincerely,

[Signature]

Hillary Harms, Ph.D.
IACUC Administrator