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DIETARY CAROTENOIDS AND THE COMPLEX ROLE OF REDNESS IN THE BEHAVIOR OF THE FIREMOUTH CICHLID *THORICHTHYS MEEKI*

By

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B.S., Viterbo University, 2010

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A Dissertation Approved on

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ABSTRACT

DIETARY CAROTENOIDS AND THE COMPLEX ROLE OF REDNESS IN THE BEHAVIOR OF THE FIREMOUTH CICHLID *THORICHTHYS MEEKI*

Sarah A. Fauque

November 18, 2015

This dissertation takes a comprehensive approach to the role of dietary carotenoids on redness and the subsequent behaviors in the firemouth cichlid, *Thorichthys meeki.*

I start with a brief introduction into signaling, the importance of carotenoids, and mate choice. The dissertation is then divided into three data chapters which are designed to stand as independent manuscripts. Chapter II documents how altering the availability of dietary carotenoids affects redness in the integument of male and female *T. meeki*. I tracked how redness changed in color and distribution in individuals over the course of 12 weeks. I confirm that a dichotomy in redness can be obtained in this time period via diet alone.

However, carotenoids are used by animals for more than red ornamentation including color vision. To account for this potential effect of carotenoids in my study animals, I examined if color vision was affected by the high- and low-carotenoid diet treatments (chapter III). Furthermore, I determined whether redness (a trait for which *T. meeki* is named) is innately attractive to this species (chapter III).

The sensory bias hypothesis suggests that males and females utilize traits that are innately appealing to the opposite sex to attract a mate. My final data chapter examines whether males and females use redness and other visual displays as a signal in mate selection (chapter IV). I conclude this dissertation by summarizing my findings and proposing future directions in which I wish to examine this system further (chapter V).

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INTRODUCTION

Animal communication uses a complex suite of signals to make decisions on various aspects that have the potential to confer fitness consequences for an individual (Endler, 1993). A successful signal is dependent upon the emiter's ability to produce a signal as well as the receiver's ability to receive and process the signal. While this seems simple enough, a signal has to pass through the environment, overcome environmental noise, and be processed physiologically by the receiver (Endler, 1993).

Signals can be of a single modality (eg.vision, olfactory, auditory) or multimodal—a combination of one or more signaling pathways. All signaling modalities have their respective advantages and disadvantages. For example, visual signals can be transmitted very quickly, but they are dependent upon the receiver being in the line of sight during a time in which ambient lighting is sufficient (Endler, 1993). Conversely, olfactory cues do not require a line of sight, but they are very slow to transmit. Furthermore, this type of signal may come at a cost to the signaler—eg. the release of cues associated with predation (Endler, 1993).

While many researchers have historically examined a single signaling modality, current research suggests that many signalers use multiple modalities (Raguso & Willis, 2002;Uetz & Roberts, 2002; Guevara-Fiore *et al.*, 2010; Estramil *et al.*, 2014;). For example, Verzijden *et al.* (2010) demonstrate that an African cichlid (*Pundamilla sp.*)

uses auditory signals for finding mates and hypothesize that these sounds may have significant influence on mate selection. However, further exploration of this subject found that auditory cues alone are not enough to attract this species. When females were presented with sound alone, they didn't respond more than to a playback of white noise. The authors hypothesize that additional cues, such as visual or olfactory signals, are necessary for finding their mate, suggesting the importance of multimodal signaling in *Pundamilla sp.* (Estramil *et al.*,2014).

Likewise, Hebets and Uetz (1999) studied wolf spiders (*Schizocosa spp.*) that are known to utilize multimodal courtship display—specifically vibratory and visual cues. By examining vibratory and visual signals independently, they were able to find that species whose courtship display was stridulation-based had females who responded more strongly to isolated vibratory cues. Similarly, species whose courtship displays were primarily visual possessed females who responded more strongly to isolated visual cues than to vibratory cues. This suggests that male courtship displays and female responses within *Schizocosa spp.* have coevolved (Hebets & Uetz, 1999). A hypothesis to explain this coevolution is that the female preferences for certain courtship displays are actually by-products of males exploiting the best sensory modality in that species.

Sensory exploitation or sensory bias is one way in which traits and preferences can coevolve (Ryan & Keddy-Hector, 1992; Fuller *et al.*,2005). Other hypotheses on mating preferences include both indirect and direct benefits to the choosing female (Kirkpatrick & Ryan, 1991; see Brooks & Griffith, 2010). The direct benefits model of sexual selection states that females will choose to mate with a male who provides her with resources that benefit her survival and fitness. For example, females can obtain nourishment via nuptial gifts such as spermatophores or body parts of the mating male that are associated with courtship displays (Vahed, 1998). Likewise, selecting a quality male in some species allows access to his territory. This access can confer benefits such as more foraging patches, protection from predation, or assistance with raising young. The indirect benefits model of sexual selection is dissimilar in that females are choosing to mate with males who will best benefit her offspring. Males who possess attractive qualities will pass on the good genes that make them attractive, thus resulting in attractive progeny. Similarly, the genes coding for preferring particular traits will be passed as well resulting in offspring who are both attractive and prefer attractive partners.

In order for males to attract females via these models, they need to convince their potential mates that they are an excellent choice. A common mode of communicating vigor is through honest signaling. An honest signal of mate quality has constraints that make it difficult to cheat. Traits that are energetically costly to produce, costly to fake, or have physiological requirements are often considered honest signals. For example, many fish species prefer larger mates. While there are adaptations that can increase the apparent size display of an animal (Neil, 1983), size is often physiologically controlled and unable to be faked.

Likewise, carotenoid-dependent coloration is considered an honest indicator of an individual’s quality. Carotenoids cannot be synthesized de novo and the color associated with it (hues of yellow, orange, and red) depend on access to carotenoid-rich foraging patches and the ability to process these pigments (Hill & Johnson, 2012). Carotenoids are essential for more than just ornamental color. These compounds are essential for immune function and vision (Hill & Johnson, 2012; Toomey & McGraw, 2012). To produce brilliant ornamental coloration there is often a trade-off between carotenoids that are used in an essential living capacity and carotenoids used for integument color. Therefore, individuals that are vibrantly colored can be assumed to be in good health as they were able to allocate this essential resource to their integument which is often not necessary for survival. The variation in carotenoid-dependent coloration and its role in mate choice has been intriguing to many scientists.

Mate choice is expected when either sex allocates significant energy into reproduction. Even if no parental care is provided, females tend to intrinsically contribute more energy into offspring than males due to anisogamy. Therefore, females are often considered the choosier sex and female mate choice has been extensively studied. However, male mate choice and mutual mate choice have been greatly overlooked until recently.

Currently, male mate choice has been examined in a variety of taxa including spiders (MacLeod & Andrade, 2014; Rundus *et al*., 2015), insects (Tigreros *et al.*,2014; Barry *et al.*,2015; Wittman & Fedorka, 2015), fish (Bahr *et al.,* 2012; Roth *et al*., 2014; Wright *et al.*,2015), and lizards (Swierk *et al.*,2013). Male mate choice has been examined specifically in organisms with sex-role reversal such as fish within the family Sygnathidae. Since the males in this system provide significant care for eggs and fry in this family, they selectively pair with females (Bahr *et al.*,2012; Roth *et al*.,2014). However, male mate choice is not confined to situations of sex-role reversal and has been investigated in systems with conventional sex roles (Jones *et al.*, 2014; MacLeod & Andrade, 2014; Ala-Honkola *et al.*,2015; Baxter *et al.*,2015; Rundus *et al.*,2015; Wittman

& Fedorka; 2015). As with females, males are expected to be choosy when reproductive or parental investment is high (Rundus *et al*, 2015).

However, male and female mate choice are not mutually exclusive. In systems where both parents invest significantly in their offspring, we would expect both sexes to selectively mate (Johnstone *et al.*,1996). Mutual mate choice studies have been increasing in frequency in the literature, but there is still a disparity when compared to the vast understanding of female mate choice and the recent studies on male mate choice. Here, I examine the complex role of carotenoid mediated redness in the firemouth cichlid, *Thorichthys meeki*.

T.meeki is a monogamous, Neotropical cichlid that displays ventral, red color in both sexes (Neil, 1984). Males and females are essentially monomorphic with the exception that the males often have longer, filamentous extensions of their medial fins; however this trait has been documented in females as well. The role of redness in *T. meeki* has been described thus far for males, where redder individuals won agonistic contests more than their less red counterparts (Evans & Norris, 1996). This suggests that redness is an honest indicator of male fighting ability in *T. meeki*. Unfortunately, the role of redness has not been examined in female *T. meeki* or whether it has a role in intersexual communication between conspecifics.

In my study, I altered the redness in *T. meeki* by varying dietary carotenoid content. I quantified how redness changed over the course of the experiment to document that this is indeed a carotenoid-dependent trait and that a dichotomy in color can be expressed. For example, redness can also be displayed by producing pteridines. This compound has been found alone in a color patch or mixed with carotenoid-dependent color patches.

As carotenoids have other biological functions, I also consider if vision is affected by diet. An inadequate ability to perceive visual signals would have a detrimental effect on the fitness of *T. meeki*. By accounting for potential variation produced by dietary carotenoids in non-target traits I can assess whether my target trait, redness, is innately attractive and plays a significant role in mate preference in this species.

CHAPTER II

CAROTENOID-MEDIATED CHANGES IN REDNESS IN THE FIREMOUTH CICHLID, *THORICHTHYS MEEKI*

Introduction

Visual signals are common throughout the animal kingdom and the conspicuousness of some signals has long been intriguing to researchers. Redness is a visual signal utilized by a wide variety of taxa for inter- and intraspecific communication. Many red signals are produced through the metabolic processing of dietary carotenoids into various red, orange, and yellow pigments. Variation in carotenoid-mediated color depends on the availability of carotenoids in the diet, the ability of the animal to absorb carotenoids, and the metabolic pathway that produces the ornamental color (Hill & Johnson, 2012; Olson & Owens, 1998). Carotenoids can also mitigate oxidative stress, stimulate immune function, and affect vision (Kodric-Bown, 1998; Olson & Owens, 1998; Hill & Johnson, 2012). According to the carotenoid trade-off hypothesis, an individual must first satisfy the demands of its immune system for carotenoids before it will invest them in brighter body coloration. Accordingly, as carotenoids cannot be synthesized de novo, colors produced by carotenoids are considered to be honest indicators of quality in carotenoid-limited environments (Kodric-Brown, 1985; Wedekind *et al.*,1998; Candolin, 2000). These colors can also demonstrate resistance to parasites;

the presence of parasites has been shown to affect the uptake and metabolism of dietary carotenoids and thus reduce red and orange coloration (Olson & Owens, 1998).

Not all reddish colors are produced solely by carotenoids, as some species possess the ability to synthesize pteridines to produce yellow, red, and orange colors (Grether *et al.*, 2001; Sefc *et al.*, 2014; Johnson & Fuller, 2015). However, at least within the fishes, individuals that can synthesize these pigments cannot use them to compensate for low carotenoid levels because although pteridines can be synthesized de novo, their presence in orange patches decreases with carotenoid scarcity instead of increasing (Grether *et al.*, 2001). Grether *et al.* (2001) suggested that pteridines are likely costly to produce, and because carotenoid availability is linked with food availability, the reduced energy available prevents increases in pteridine production.

Thorichthys meeki is a Central American cichlid commonly known as the firemouth cichlid because it has red coloration running ventrally from the mouth to the anal fin (Baerends & Baerends Van-Roon, 1950; Neil, 1984a). The degree of redness varies among individuals, and previous researchers suggested the red color is carotenoid-mediated (Evans & Norris, 1996). *T. meeki* is an opportunistic, omnivorous feeder that consumes a variety of invertebrates and algae (Neil, 1984a). Dominant males defend territories with greater food availability (Hodapp & Frey, 1980) and thus should be redder than lower-ranked conspecifics. When male redness is altered by the addition of carotenoids to their diet, the redder males won agonistic contests more frequently than their less-red opponents. (Evans & Norris, 1996).

Researchers often manipulate carotenoid-dependent coloration to study its behavioral effects (Evans & Norris, 1996; Baron *et al.*, 2008; Yasir & Qin, 2010) .

Typically, such studies are performed by dividing subjects into two groups and providing one with additional carotenoids in their food. This results in all other aspects of the animals remaining equal (such as size, health, etc.) with the exception of carotenoid-mediated color. By altering only coloration, researchers can explore how redness alone affects various behaviors. One drawback to this protocol is the lack of information on how long this manipulation takes to have an effect on color and a common failure to quantify to what extent the final coloration differs between the groups. The literature is inconclusive on how long individuals need to be on their respective diets (Evans & Norris, 1996; Wallat *et al.*,2005; Baron *et al.*, 2007; Doolan *et al.*,2009; Yasir & Qin, 2010; Adeljean *et al.*, 2013a; Adeljean *et al.*, 2013b; Yi *et al.*,2014). Researchers often note that their chosen period was sufficient to provide an obvious dichotomy in color, but few studies have quantified how the colors differ or tracked color change over time.

The aim of this study is to use digital imaging to document and quantify the color changes that occur through a manipulation in diet over 12 weeks, a time period used to alter *T. meeki* coloration in a previous study (Evans and Norris, 1996). In addition to determining if the diet provided a dichotomy in hue, I also examine how carotenoids are integrated into the integument by documenting the number of red patches as well as what proportion of the fish is red. This quantification of carotenoid-mediated integument coloration over time is the first in *T. meeki* and will be a useful parameter for those wishing to achieve this dichotomy.

Methods

Study species and diet

Thorichthys meeki were obtained as juveniles from a local pet shop which acquires their stock from various fish breeders. All fish were housed in 300L aquariua. To make conditions conducive to growth and normal behavior, all aquaria contained a gravel substrate and refugia consisting of plastic plants, pieces of slate, and clay pots. Additionally, power filters provided aeration and heaters kept water temperature at approximately 27° C. This experiment was approved by the University of Louisville's Institutional Animal Care and Use Committee (proposal #12040).

Since the sexes may differ in hue and in response to the diets, I separated them for the experiment. To determine sex, I examined the genital papilla of unsexed fish under a stereoscope every two weeks. After a fish had matured enough so that its sex could be determined, I placed it into one of four 300-liter aquaria depending on fish sex and the diet the fish would receive—high carotenoid male (HCM), low carotenoid male (LCM), high carotenoid female (HCF), and low carotenoid female (LCF).

The high carotenoid (HC) diet consisted of Cobalt Aquatics Color Flakes ® while the low carotenoid (LC) diet consisted of Cobalt Aquatics Spirulina Flakes ®. These diets were chosen because they differed in the number and quantity of carotenoid-rich components they contained. Ingredients for both diets are listed in order of weight emphasizing a difference in carotenoid content. Cobalt Aquatics Color Flakes® have ingredients rich in carotenoids including: salmon fish meal, plankton, krill, and astaxanthin. Astaxanthin is an additional carotenoid that is used frequently to alter animal coloration (Wallat *et al.*,2005; Baron *et al.*,2008; Doolan *et al.*,2009; Yasir & Qin, 2010;Adeljean *et al.*,2013a; Adeljean *et al.*,2013b; Yi *et al.*,2014). The spirulina flakes are lower in carotenoids but not completely devoid of them because they are necessary for fish health (Olson & Owens, 1998; Hill & Johnson, 2012; Sefc *et al.*, 2014). This variety also contains salmon fish meal as the primary ingredient, however, spirulina flakes are more plant-based than the color flakes and include ingredients such as spirulina, algae, and kelp. Since *T. meeki* are omnivores that eat a variety of animal and plant-based organisms in the wild (Neil, 1983), it was expected that both of the chosen food types would be sufficient for their dietary needs.

When research fish reached sexual maturity, photographs were taken of each flank (see protocol below) just before the diet began (week zero) as well as every two weeks following. All fish were fed their respective diets (HC or LC) ad libitum six days per week for twelve weeks.

Data collection occurred from February to May, 2014 and March to June, 2015. Because sexual maturity did not occur simultaneously for all fish in either year, diet start date was staggered—2014 had two cohorts of fish while 2015 had three. Overall, 97 fish (HCM=29, LCM=14, HCF=28, LCF=26) completed the experiment resulting in a total of 1,358 photos.

During the 2015 trials, no photographs were taken on one planned sampling date (21 April). Because there were individuals that had been on their assigned diet for different amounts of time housed within a single aquarium, I could not simply remove one particular week from the analysis. Since not all juveniles reached maturity at the same times, they started the diet on different dates; therefore some fish missed the photograph for week 2, while others missed week 4 or week 6. Accordingly, I averaged color values for weeks 2 and 4 and for weeks 6 and 8 for all fish so that they could be combined across trials. This resulted in five time points in the experiment: week zero, weeks 2-4, weeks 6-8, week 10, and week 12.

Photography

Photographs were taken with a Cannon Elph-100 HS digital camera held parallel to focal fish on a tripod at a distance of 24.5 cm. Images were taken at high resolution (12 megapixels) with no flash under ambient, fluorescent lights. Fish were removed from water and photographed on a weigh-boat, and each picture included a DGK Digital Kolor Kard for standardization and a ruler. The flanks of each fish were dabbed with a paper towel to assist in identification as well as reduce obstruction of color patches by glare. Many studies anesthetize their fish, but this protocol is known to affect the subsequent color (Gray *et al.*, 2011). While stress and handling can also affect color to a degree, all individuals were handled in the same manner resulting in similar stress levels.

To standardize photos, I corrected images to ideal white illumination creating the same brightness intensity for all channels of RGB. Images were corrected for illumination using ImageJ freeware following methods described in Yamamoto *et al.* (2007). Original images were first split into the three RGB (red, green, and blue) channels. A rectangular region of interest (ROI) was selected within the white standard and the mean brightness (MB) was measured for each channel. The maximum value of illumination that can be displayed in an RGB photograph is 255. As most white standards are assumed to reflect 90% of the incident light, ideal white lighting should thus yield a brightness value of approximately 229 ($0.9 \times 255 \approx 229$). Accordingly, each channel was corrected to the same brightness level by multiplying each pixel by 229/MB in the appropriate channel. This process of multiplying each pixel by this calculated value standardizes the brightness across all channels. The R, G, and B channels were then merged to form a corrected, composite image which was saved as a JPEG. Fluorescent lighting generally shifts the visual spectrum towards the blue range (Yamamoto *et al.*, 2007), a tendency which was observed in my raw photos—the blue channel needed to be brightened by a lesser degree than did the other two channels. By standardizing the images in this manner the color shift was removed. The color accuracy was subsequently checked using the standards on the DGK Kolor Kard to ensure that RGB values fell where expected.

Identification

I identified individuals by their uniquely shaped opercular spots which vary in size and shape. To assign identification, an unknown photograph was visually compared to known fish of the same sex and diet from the previous session. When one flank was matched, the other flank went through the same visual comparison. When both flanks matched a known fish, the identity was assigned to the unknown photograph. When a fish was assigned a specific identity, their current photo was compared to the "before" photo to ensure that there were no misidentifications.

Photo Quantification

ImageJ was used to select specific areas of fish and examine multiple aspects of redness in *T. meeki*. All ROIs were selected using the freehand tool. Red ROIs—areas that were pink, orange, or red to an observer—were selected, then the size of the patch and the RGB composition was collected for each flank. Contiguous areas of red were considered a single patch, but if there were scales of other colors between red areas, those areas were considered distinct patches. RGB values were determined using the Measure RGB plug-in (Analyze > Measure RGB). Each red ROI was selected three times and measurements were averaged to account for possible researcher error in selection.

Hue was the color component of interest because it is dependent on the dominant wavelength of light but independent of saturation and brightness. The RGB data was used to calculate the hue of the distinct patches on each flank in Excel. Hue is measured in degrees on a color wheel, with, for example, red at 0°, orange at 30°, and yellow at 60°. Because some fish started with zero red patches and others lost their red patches over the course of the study, not all individuals had a hue value for all time points. To account for this, the hue variable was transformed into a redness rank classification that was scored from zero through fifteen. Individuals that possessed no red patches and thus no hue value were given a redness classification of 0. For the fish that did have red patches, hue values ranged from 33° for the least red fish to 5.5° for the reddest. Redness rank values one through fifteen corresponded with two-degree ranges of hue, with the least red fish (hues of 32-33°) assigned a rank value of 1 and the reddest fish (hues of 5-6°) given a rank value of 15. The distribution of redness was analyzed by counting the number of distinct red patches and by calculating the percentage of each fish's side that was occupied by the patches. The data from individual patches were combined to examine the total amount of red present on a fish.

Statistics

Statistical analysis was conducted in SPSS 21. All statistics were nonparametric because data were not normally distributed and could not be transformed. When comparing how the various aspects of redness differed among diet groups, sexes were analyzed separately. To determine whether the redness of each group (HCM, LCM, HCF, LCF) varied over the course of the experiment, I used a Friedman analysis of variance. For groups that showed significant variation in redness, I then performed post-hoc analyses with Wilcoxon signed-rank tests, using a Bonferroni corrected p-value to account for multiple tests.

Various aspects of redness were examined within and between the groups including the redness classification rank as well as the distribution of redness. Wilcoxon signed-rank tests were used to examine changes in redness distribution within the groups by comparing photos taken at weeks zero and twelve. Mann-Whitney U tests were used to determine if male and female redness varied between the diets before the diet began and at 12 weeks.

Results

Friedman analyses showed that HCM, LCM, and LCF groups all had time points that differed significantly ($X^2(4)=33.956$, $p<0.001$; $X^2(4)=22.525$), $p<0.001$; $X^2(4)=24.796$, $p<0.001$, respectively, Figures 1 and 2.). The HC females, in contrast, did not change in redness rank over the course of the experiment (Friedman analysis, $X^2(4)=6.543$, $p=0.16$, Figure 2a). For post-hoc analysis of the groups that did change in redness rank, with Wilcoxon signed-rank tests I used a Bonferroni correction, resulting in a p value of <0.005 being required for significance.

In the HCM group, Weeks 10 and 12 were significantly different from week 6-8 (Z= -2.79, p=0.005 and Z= -3.17, p=0.002, respectively), but they did not vary significantly from each other (Z= -0.7, p=0.48). Weeks 2-4 had a slightly lower rank than week zero. While weeks 6-8 did not differ significantly in rank from week 2 (Z= -0.81, p=0.42), week 10 and 12 were both significantly different from weeks 2-4 (Z= -3.67, p<0.001 and Z= -4.48, p<0.001, respectively) There were no significant differences between week zero and any other time point (Week 2-4: Z= -1.52, p=0.13; week 6-8: Z = -0.46, p=0.65; week 10: Z= -1.66, p=0.1; week 12: Z= -2.4, p=0.02). .

In the LCM group, average redness rank decreased from the beginning of the diet until the 6-8 week mark after which redness increased in weeks 10 and 12. Redness rank differed significantly only between week 6-8 and week zero(Z= -3.19, p=0.001).

The LCF group similarly showed a reduction in redness rank from the beginning of the experiment until week 6-8, when redness differed significantly from week 0 (Z= -3.89, p<0.001) but not weeks 2-4 (Z= -2.02, p= 0.04). Fish redness increased slightly in weeks 10 and 12 but remained significantly different from week zero at week 10 (Z= -3.4, p=0.001). Redness in week 12 was marginally significantly different from week 0 (Z= -2.77, p=0.006). Weeks 2-4 did not differ significantly from any other time point (week 0 : Z = -2.16, p=0.016; week 6-8: Z= -2.02, p=0.04; week 10: -1.81, p=0.07; week 12: Z=-1.02, p=0.31) . Weeks 10 and 12 did not differ significantly from weeks 6-8 . (Weeks 6-8: Z= -0.33, p=0.74 and Z= -0.42, p=0.68, respectively. Additionally, weeks 10 and 12 did not differ from each other (Z= -0.78, p=0.43).

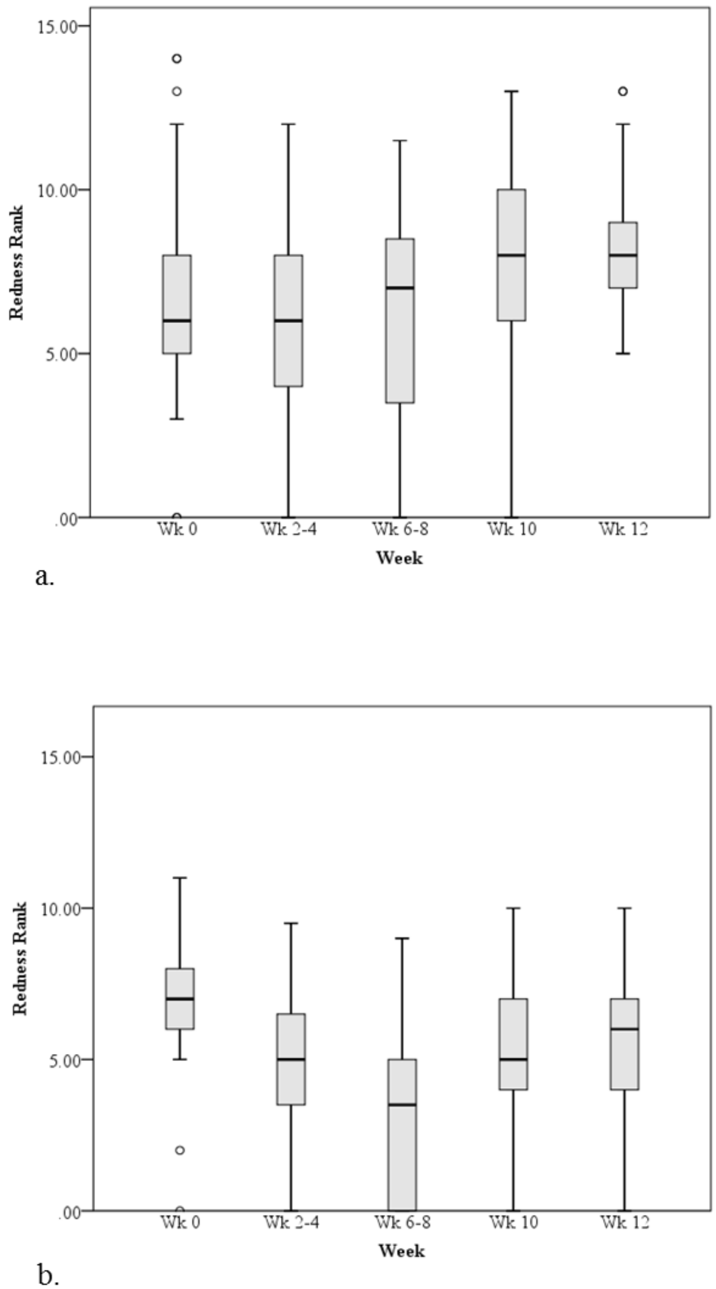
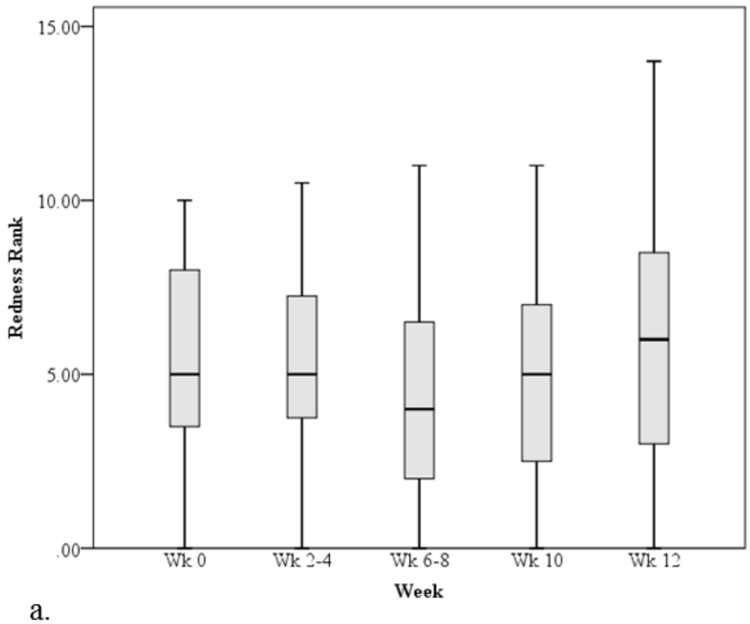
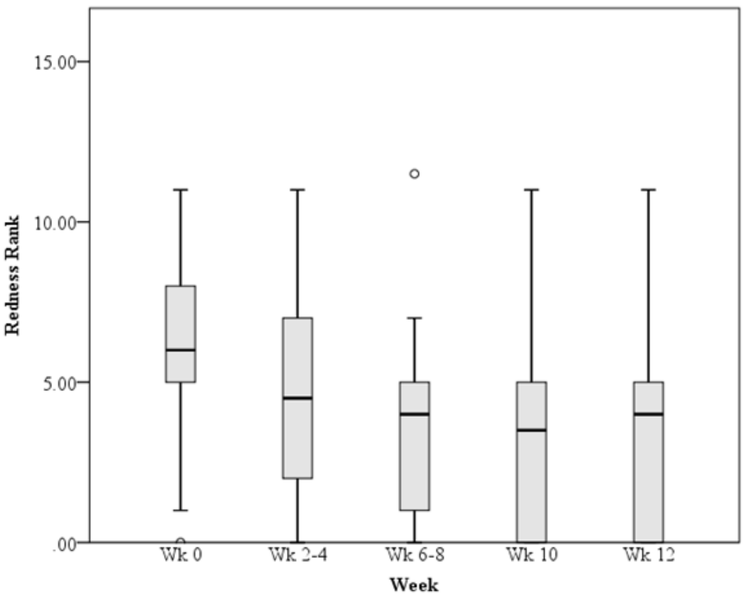


Figure 1. Male changes in median (IQR) redness rank over 12 weeks. (a) HC males (N=29) differ significantly from weeks 2-4 at the 10-week point. (b) LC males (N=14) drop in redness rank quickly and are significantly different from before the diet started at the 6-8 week mark. However, redness started to increase after this point resulting in weeks 10 and 12 showing no difference from week 0.



a.



b.

Figure 2. Female median (IQR) changes in redness rank over 12 weeks. (a) HC females (N=28) do not differ significantly in their redness rank over the course of the experiment. (b)LC females (N=26) lose redness quickly and differ significantly at the 6-8 week point.

Mann-Whitney U tests showed that before the diets began, males in the HC (N= 29) and LC (N=14) diet groups did not differ significantly in the number of patches (mean ± SE: HC= 2.3 ± 0.18, LC= 2.3 ± 0.3, U=166, p=0.34), the percent of the fish covered by patches (mean ± SE: HC= 9.4% ± 0.01, LC= 7.3% ± 0.01, U=194, p=0.8), or the redness rank (mean ± SE: HC= 5.7 ± 0.66, LC= 6.7 ± 0.78, U=179.5, p=0.54). After 12 weeks on their respective diets, male *T. meeki* did not differ significantly in the number of patches (Mann-Whitney U test, mean ± SE: HC=2.59 ± 0.16, LC= 2.57 ± SE 0.29 U=194, p=0.8) or percent of fish covered by patches and (Mann-Whitney U tests mean ± SE: HC= 9.2% ± 0.01, LC= 6.8% ± 0.01, U=0.138, p=0.09, respectively). A Mann-Whitney U test indicated that redness rank was significantly different between HC and LC males (mean ± SE: HC= 8.2 ±0.40, LC= 5.9 ± 0.70 U=99.5, p=0.007, Figure 3) at the end of the experiment.

Female *T.* meeki followed the same pattern as males (N_{HCF}=28, N_{LCF}= 26). Before the diets began, female *T. meeki* in the two diet groups did not differ significantly in the number of patches present (Mann-Whitney U test, mean ± SE: HC=1.9 ± 0.16, LC= 2.03± 0.19, U=340.5, p=0.65), the percentage of the fish covered by patches (Mann-Whitney U test, mean ± SE: HC=7.1% ±0.01, LC= 7.5% ± 0.01, U=327.5, p=0.53), or the redness rank (Mann-Whitney U test, mean ± SE: HC=5.1 ± 0.55, LC= 5.8 ± 0.53, U=302.5, p=0.284). After 12 weeks of HC and LC diets, females did not differ significantly in the number of patches (Mann-Whitney U test, mean ± SE: HC= 2.07 ± 0.22, LC= 1.56 ± 0.25, U=275.5, p=0.1) or the percent of the fish covered by the patches (Mann-Whitney U test, mean ± SE: HC= 6.8% ± 0.01, LC= 4.6% ± 0.01,U=280, p=0.14). However, the final redness rank after 12 weeks of the experiment was significantly different between the diets (Mann-Whitney U test, mean ± SE: HC= 5.8 ± 0.73, LC= 3.6 ± 0.56, U=228.5, p=0.02, Figure 3).

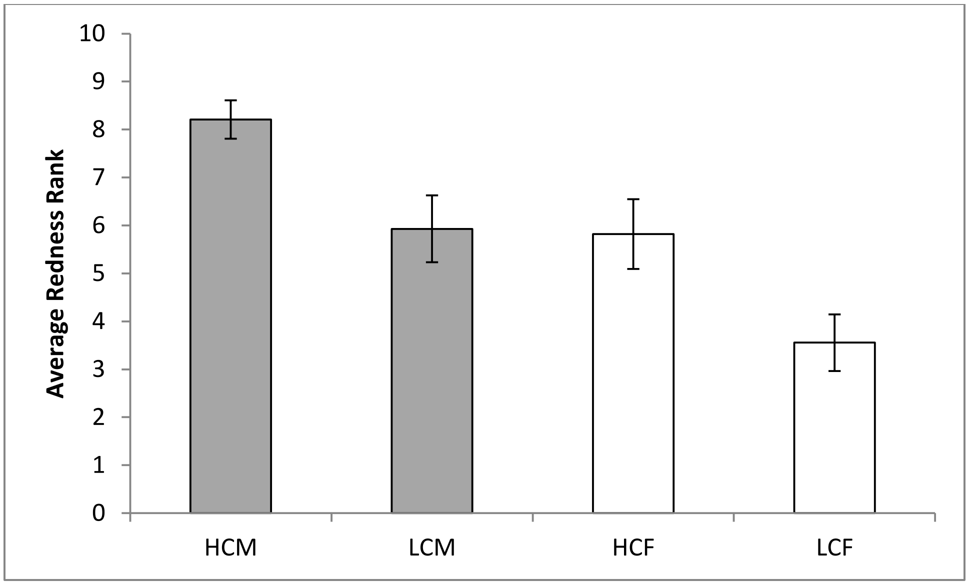


Figure 3. Both male *T. meeki* (gray bars) and female *T. meeki* (white bars) differ significantly in their redness rank at 12 weeks with those on the HC diet (N_{male} = 29, N_{female}= 28) significantly redder than the LC diet (N_{male} = 14, N_{female} = 26).

Discussion

This study confirms that integument redness is at least partially driven by dietary carotenoids in *Thorichthys meeki* and that 12 weeks on high- and low-carotenoid diets is an adequate time in which to obtain a quantitative dichotomy in color.

Remarkably, varying carotenoids seems to affect only the color of patches rather than the distribution of redness in the individual. While color seems to be controlled via current circumstance—carotenoid availability, parasite load, etc.—patch location or number may be genetically controlled. The genetic control of color has been studied in detail in various cichlid species (Magalhaes & Seehausen, 2010; Takahashi *et al.*, 2013). However, many of these studies focus on color morphs of the same species or sister species instead of variation of a trait, such as patch size, within the same species.

Interestingly, the sexes respond differently to diets that differ in carotenoids. Although males increased in redness by their tenth week on the HC diet, females on the HC diet showed no significant change in redness over 12 weeks. The mechanism behind this unexpected result is unknown. It is possible that female *T. meeki* may have started the experiment at or near their most red, which could suggest that they may have a lower need for dietary carotenoids than do males. However, it may also be the case that females need longer than males do to incorporate additional carotenoids into their integument. To my knowledge, no studies have examined whether conspecific males and females assimilate dietary pigments differently, and future studies should investigate this possibility. However, females did respond quickly to the restriction of carotenoids, decreasing red coloration by 6-8 weeks on the LC diet and then maintaining approximately the same level of redness thereafter. Since red coloration was altered so quickly in LC females, six to eight weeks of carotenoid restriction may be the better means of obtaining a dichotomy in color in female *T. meeki.* Males on the LC diet similarly became less red by weeks 6-8, but then became redder so that males' red hue after 12 weeks on the LC diet did not differ from their color before starting the experiment, although they were still significantly less red than were the HC males.

The increase in redness in males on the LC diet may be due to a use of pteridines, which can produce color alone or in conjunction with carotenoids in a patch (Grether *et al.*,2011; Sefc *et al.*,2014;Johnson & Fuller, 2015). Although the production of these compounds may be constrained by food availability (Grether *et al.*, 2011), the quantity of food I provided may have been sufficient to enable males to produce and incorporate pteridines into their skin.

Since redness changed while food availability was equal, carotenoids seem to be the primary pigment that alters redness in *T. meeki* along their ventral surface. However, carotenoids may not be responsible for all red display color in this species which can also have a red border along its dorsal fin. Johnson and Fuller (2015) demonstrated that color patches in various parts of a fish can be controlled via numerous means—carotenoids, pteridines, and structural pigments. While this red border was not examined in this experiment, it would be interesting to see if it is also mediated by carotenoids, pteridines, or a combination of the two pigments.

Conclusion

Like many other fish that display red cues, *Thorichthys meeki* are able to alter this red coloration through the ingestion and metabolism of carotenoids. A period of 12 weeks on high and low carotenoid diets is sufficient to alter redness in males. Additionally, increasing carotenoid availability in females does not alter their redness but reduction in carotenoids in the diet causes the color to change dramatically by weeks 6-8. These findings suggest that males and females may not react identically to dietary pigments, a possibility that should be further investigated. Additionally, this study adds to the literature on the appropriate amount of time to place study organisms on diets that vary in carotenoids to obtain a dichotomy in color.

CHAPTER III

SEEING RED: FIREMOUTH CICHLIDS HAVE A NATURAL AFFINITY FOR REDNESS THAT IS NOT AFFECTED BY DIETARY CAROTENOIDS

Introduction

Cichlid fishes are well known for displaying diverse patterns of vibrant color that can have large effects on individual fitness. Their colors have a variety of functions, including differentiating species and revealing reproductive and social status. The functioning of these visual signals is dependent on two fundamental factors: the ability of the sender to produce a particular color and pattern and the ability of the fish receiving those signals to perceive and process the display. Many studies of cichlids have focused on individuals' ability to produce signals and the adaptive significance and meaning of those signals. Far fewer studies have examined the ability of cichlids to perceive the signal, although in recent years researchers have explored color vision in African cichlids, a group in which color pattern diversification has sometimes been the basis of speciation. As one might expect, cichlid species in this group often have highly sensitive color vision. Although color perception varies among species, some Malawi cichlids possess an array of photoreceptors mediated by visual pigments that allow their visual sensitivity to range from ultraviolet wavelengths through the red in the visible light spectrum (Bowmaker, 2008; Carleton, 2009; Sabbah *et al.*, 2010; Weadick *et al.*, 2012). The color perception of Neotropical cichlids—a sister group to African cichlids—has not yet been well studied, although the Trinidadian pike cichlid does possess the trichromatic vision which is common among other teleosts (Weadick *et al.*, 2012).

The capability of animals to perceive color can vary even within species, and some researchers have recently begun accounting for this phenomenon when conducting behavioral analyses (Cheney *et al.*, 2013; Johnson *et al.*, 2013). In vertebrates, variation in color vision at the individual level (Toomey & McGraw, 2012) can result from differences in carotenoids deposited in intraocular filters such as the lens, cornea or oil droplets (Heinermann, 1984; Bowmaker, 2008; Toomey *et al.*, 2011). Since carotenoids must be obtained environmentally, poor diet can potentially affect how animals perceive visual signals, and an inability to perceive a color signal can result in poor decisions in agonistic interactions or reduce individuals' ability to select high-quality mates (Ronald *et al.*, 2012; Toomey & McGraw, 2012). Therefore, it is necessary to assess individual variation and take into account the dietary status of focal individuals when conducting experiments with visual cues.

Although many researchers examine the spectral sensitivity of their focal animals through microspectroscopy, dissection of the retina, or electroretinograms (ERG) of a few individuals before conducting behavioral trials, I propose that this method should be reversed. Agrillo *et. al* (2012) developed a training procedure for studying discrimination learning in fish where they focused on training guppies to associate different numerosities with food rewards. This study uses a non-invasive, behavioral assay, modeled after Agrillo *et al.* (2012), to determine whether the firemouth cichlid *Thorichthys meeki* can distinguish between colors of similar wavelength. In this Neotropical species, both sexes display the coloration for which they are named (Neil, 1984a; Neil, 1984b), a patch of color that runs ventrally from mouth to anal fin, varies across individuals in size and degree of redness, and is an honest signal of quality (Evans & Norris, 1996).

The level of carotenoids in the diet affects how red or orange the integument is in *T. meeki* (Chapter II), and the degree of redness appears to be correlated with traits that may indicate higher fitness, at least in males. Evans and Norris (1996) demonstrated that redder males were more likely to win agonistic contests than their less-red counterparts, suggesting that redness is likely to be an important signal of fighting ability in *T. meeki*. Hodapp and Frey (1982) found that dominant males possess better territories with resource-rich foraging patches, which should provide dominant males with increased access to carotenoid-rich foods.

Given that redness plays an important role in *T. meeki* interactions, individuals of this species may have an innate preference for red items. Research suggests that an underlying preference for a particular trait may be the result of a pleitropic effect (Rodd *et al.,* 2002). An attraction to red items could increase foraging efficiency, as well as enable individuals to quickly identify intruding neighbors or potential mates. Innate preferences for specific traits have been examined across various behaviors such as mate choice (Basolo, 1990; Fuller *et al.,* 2005), foraging (Cheney *et al.,* 2013), and predator avoidance (Bruce *et al.,* 2001). Recent studies have also examined the visual properties of their focal species as a parameter of exploring their sensory bias. For example, Cheney *et al.* (2013) explored response biases in the Picasso triggerfish. While they possessed a priori knowledge of the spectral sensitivity of their study organism, they were able to behaviorally confirm their ability to view color within the expected ranges. Furthermore they were able to use this information to determine that these triggerfish were innately attracted to red and green stimuli. While a natural red preference has been documented in other species (Hill, 1991; Ryan & Keddy-Hector, 1992; Rodd *et al.*, 2002; Cheney *et al.*, 2013), it has yet to be examined in *T. meeki,* even though they have been documented as an honest signal in this species (Evans & Norris, 1996).

It is ideal for researchers to possess a priori information on the spectral sensitivity of their study organism (Cheney *et al.,* 2013) before conducting behavioral trials, but the visual system of *T. meeki* has not been examined. Moreover, no studies to my knowledge examined whether carotenoids play a role in their color vision. While carotenoid-rich oil droplets affect discrimination in higher vertebrates, they have not been documented in cichlids (Hill, 1990; Toomey & McGraw, 2012); however, other intraocular filters found in the lens and cornea are common (Heinermann, 1984). Increasing carotenoid content in these areas of the eye have been shown to assist in visual acuity by reducing glare, improving detail vision, and reducing chromatic aberration (Heinermann, 1984). While it has not been shown that these intraocular filters affect color discrimination like carotenoid-rich oil droplets, there has been very little research on their properties. If carotenoids affect the vision of *T. meeki*, we may be able to detect a behavioral difference such that individuals with low-carotenoid diets are unable to differentiate between closely related hues that are distinct to individuals with carotenoid-rich diets.

The goals of this study are to determine whether *T. meeki* on low- and high-carotenoid diets differed in their ability to perceive a difference in closely related hues and to test whether members of this species are naturally attracted to red objects. I trained *T. meeki* to associate a red or orange stimulus with a food reward over a period of four days. I then tested their ability to pair that stimulus with a food reward and their ability to discriminate between their trained stimulus and a closely related hue. By using stimuli that fall within the natural coloration of *T. meeki* I can also determine if redder objects are intrinsically more attractive

Methods

Study Animal Preparation

The *Thorichthys meeki* used in this study were obtained as juveniles from a local pet shop and maintained in groups in aquaria in the laboratory. To determine their sex, I examined the genital papilla of unsexed fish under a stereoscope every two weeks. After a fish had matured enough that I could determine its sex, I placed it into one of four 300 L aquaria in which fish were segregated by sex and diet—high carotenoid male, low carotenoid male, high carotenoid female, and low carotenoid female. I used Cobalt Aquatics Color Flakes® for the high carotenoid (HC) diet and Cobalt Aquatics Spirulina Flakes® for the low carotenoid (LC) diet. All fish were fed ad libitum six days per week for a minimum of 12 weeks and additionally during approximately two weeks of acclimation and training before the experiment to provide time for diet-based color change (Chapter II; Evans and Norris, 1996).

All aquaria in this study were maintained on a 12:12 light cycle at 27° C and had brown gravel substrates. Aquaria that held maturing fish or fish on particular diets were fitted with power filters, while aquaria used for the training and testing described below had air stones to reduce currents but maintain sufficient oxygenation. This experiment was approved by the University of Louisville’s Institutional Animal Care and Use Committee (proposal #14094).

Experimental design

For training and trials, which occurred from June through September in 2014 and June through July in 2015, *T. meeki* were housed individually in 38 L aquaria. These aquaria were considered to have three zones that each were 17 cm wide (Figure 4): a central refuge zone bounded by a choice zone on either end. The central zone contained a plant that served as refuge and was where fish typically stayed (personal observation), while the two choice zones lacked refugia. To reduce any stress caused to fish by being housed singly, each aquarium was juxtaposed with a neighboring aquarium containing a *T meeki* of the same sex, thus allowing only visual communication. Fish were acclimated for seven days prior to training. Individuals were maintained on the same HC or LC diet until two days prior to training when flake food was no longer provided in order to increase foraging motivation.

To train fish to associate a color with a food reward, squares of acrylic (LxW: 5.8x5.8 cm) were painted with a satin, latex-based paint and attached to the end of a clear, acrylic rod 33 cm in length. The training color (TC) squares were painted either red (TCR), with Valspar® Cl221 (Hue = 0.5°), or orange (TCO), with Valspar® Cl223 (Hue = 34°). These colors were selected to fall near the range of the red and orange coloration displayed by *T. meeki* in the laboratory (Hue range = 0.8°-39.5°). An identically sized blue square, painted with Valspar® 4006 (Hue = 207°), was used a control color (CC) for all fish. To test for an innate red preference, half of the fish were trained to TCR while the other half were trained to TCO. Individuals were in four groups based on diet and training color—high carotenoid red (HCR), high carotenoid orange (HCO), low carotenoid red (LCR), and low carotenoid orange (LCO). Observations were made on all training sessions for the 27 fish examined in summer of 2014. After bloodworms were placed in the aquarium, latency to enter choice zones, time spent in each choice zone, and the number of visits to each zone were recorded.

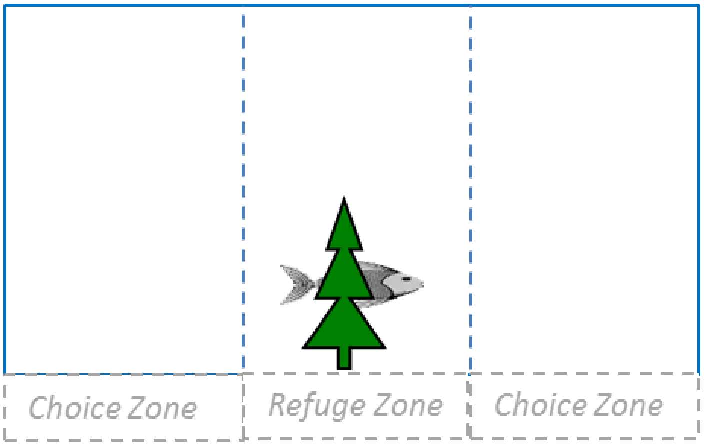


Figure 4: Schematic of aquarium viewed from the front. Choice zones (each 17 cm in width) bounded the central refuge zone (17 cm), which contained a plastic plant as shelter for the *T.meeki* focal fish .

To eliminate the possibility of social learning, fish were visually isolated from their neighbor from 30 minutes prior to the first training session until the end of the day. Training and testing was conducted between 08:00 and 13:00. I reduced the effects of researcher presence during training and testing of fish by performing slow, controlled movements and made any such effects more consistent by wearing a laboratory coat.

During training sessions, I lowered TC and CC squares into the right and left choice zones of the aquarium and simultaneously deposited a food reward (2-5 bloodworms) with the TC square via stainless steel forceps (Figure 5). Fish were then allowed to forage for ten minutes, after which any remaining bloodworms were removed. This process was repeated four times per day with approximately one hour between each training session. Color location was alternated between every training session over the course of four days for a total of 16 training periods.

Testing protocol

Testing occurred on day five to determine whether individuals associated their TC with the presence of food (test 1) as well as whether they could visually discriminate between a red and orange stimulus—one of which was novel (test 2). On the morning of testing, fish underwent a training session as a reminder of the protocol and to maintain motivation (Agrillo *et al.*, 2012). For test 1, the TC and CC were lowered into the choice zones without adding a food reward (Figure 6a). Observations were made from 4 m away for 10 minutes, and the number of seconds fish spent in each choice zone was recorded. Becuase individuals varied greatly in how active they were, the proportion of time spent in choice zones was calculated and used for analysis. Individuals were considered to have successfully associated their TC with a food reward when they spent greater than 60% of their choice time in the zone with the TC. Only individuals who successfully trained continued on to test 2. To maintain motivation, fish were given an additional training period between tests 1 and 2 (Agrillo *et al.*, 2012). The capability of *T. meeki* to discriminate between red and orange was assessed by giving individuals a choice between their TC and the novel training color (NTC), i.e the training color (red or orange) to which they had not been trained to respond (Figure 6b). In order to determine whether orientation affected choice, I noted which direction the focal fish was facing before the color squares were added. After the TC and NTC squares were placed in the aquarium, I observed the focal fish from a distance of 4 m for 10 minutes and recorded latency to entering a choice zone in seconds, the first color visited, and the number of seconds spent in each choice zone. Proportion of choice time in each choice zone was again calculated for analysis. Any individual who did not make a choice during the 10 minutes of observation was re-tested the following day.

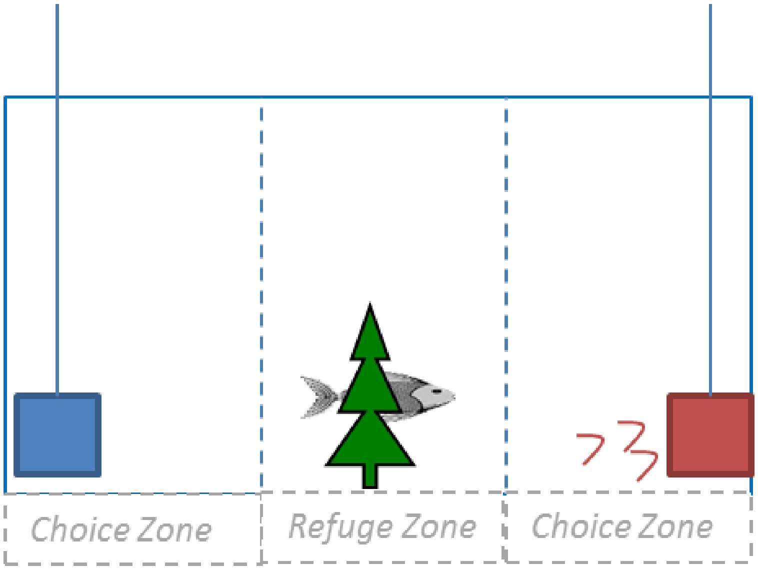


Figure 5: Training aquarium. Two colored squares were placed in the choice zones while a food reward was simultaneously provided with the TC.

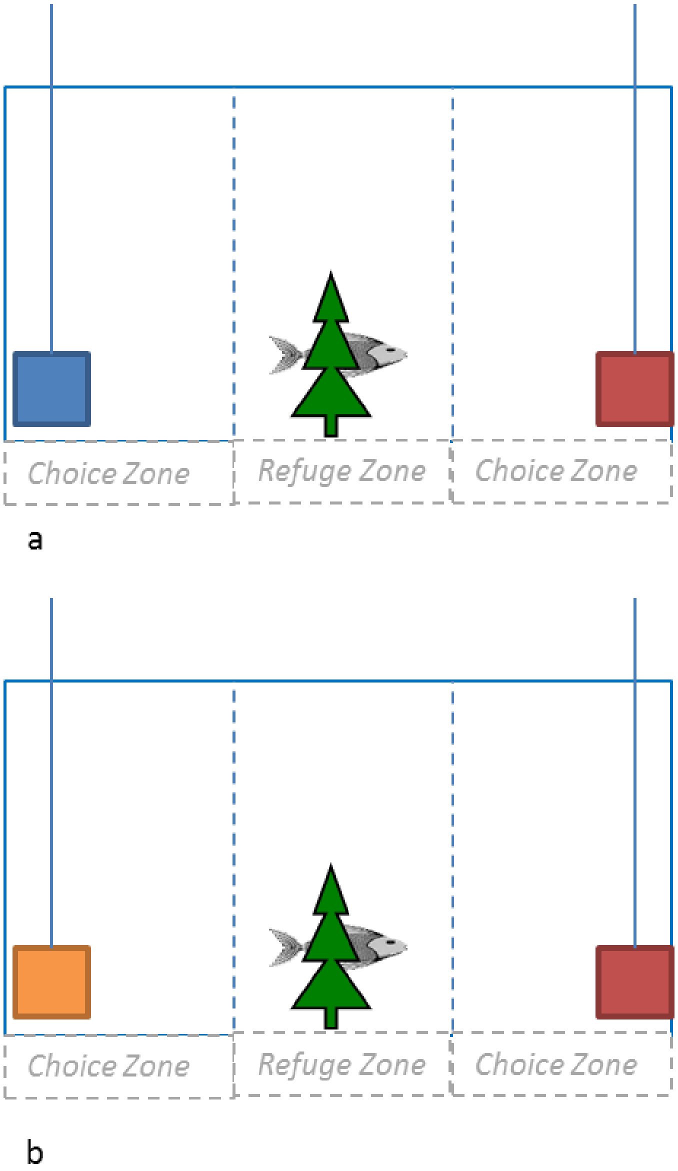


Figure 6: Schematics of testing aquaria. (a) *T. meeki* chooses between the TC and CC without the presence of a food reward. (b) The TC and NTC are placed in the choice zones without the presence of a food reward.

Statistical analysis

A total of 82 fish began the experiment, in which 33 individuals were unsuccessful in test 1 and the remaining 49 individuals successfully completed both tests 1 and 2. Data were analyzed in SPSS 21(SPSS Inc., Chicago, IL). A Kolmogorov-Smirnov test showed that the data differed significantly from normality (p=0.001), and accordingly nonparametric statistics were used.

Test 1: The effect of diet and training color on success of training. A log-linear analysis was used to determine whether success was independent of diet and TC. Training data was analyzed from the fish that were trained in summer 2014. Mann-Whitney U tests were used to examine whether red-trained and orange-trained fish differed significantly in their responses over the 16 training sessions. The responses tested included mean time spent near the TC, mean time spent near the CC, number of sessions during which fish did not visit the TC, number of sessions during which the fish did not visit the CC, and the percentage of time the fish approached their TC first.

Test 2: Do firemouths prefer red? To rule out the possibility that the initial orientation of individuals played a role in the first color approached, a Chi-square test of association was used. I used a log-linear analysis to determine whether the first color approached was dependent on TC or diet and a binomial test to examine whether one color was approached first more often than the other. Friedman tests were used to determine if diet or TC affected the latency to either red or orange choice zone. I also used Friedman tests to determine if diet or TC affected proportion of choice time spent in the red choice zone. This test was not run on the proportion of choice time spent in the orange choice zone because this value is dependent on the proportion of choice time spent in the red choice zone. To determine if there was an overall preference for red or orange, I used a Mann-Whitney U test.

Results

Test 1: Success of training. A log-linear analysis showed that training success was independent of diet (X^2=0.528, d.f.=1, p=0.467) but not TC (X^2=4.63, d.f.=1, p=0.031). Individuals trained to red were more likely to succeed than those trained to orange (Figure 7), with 80% of 38 individuals trained to red successfully associating their training color with a food reward but only 57% of the 44 fish trained to orange succeeding.

Although the probability of training success depended on training color, fish that trained to the two colors responded similarly during training. When examining a subset of individuals trained (N=27), I found that red-trained (N=14) and orange-trained (N=13) fish responded similarly to training. The mean time near the TC (red=120.8 s, orange= 109.0 s) or CC (red= 38.0 s, orange= 32.2 s) did not differ between the two training colors (U = 78, p = 0.55 and U = 90, p = 0.981, respectively). There was no significant difference in the number of times training fish failed to visit either its TC (red= 4 visits, orange= 4.3 visits) or the CC(red= 7.7 visits, orange= 8.2 visits) (Mann-Whitney U: U = 85, p = 0.79 and U = 86, p = 0.83, respectively) during training sessions. The percent of times the TC was the first zone visited was high for both training colors (mean±SE = 82% ± 2.4) and did not differ between them (Mann-Whitney U: U = 79, p = 0.58)

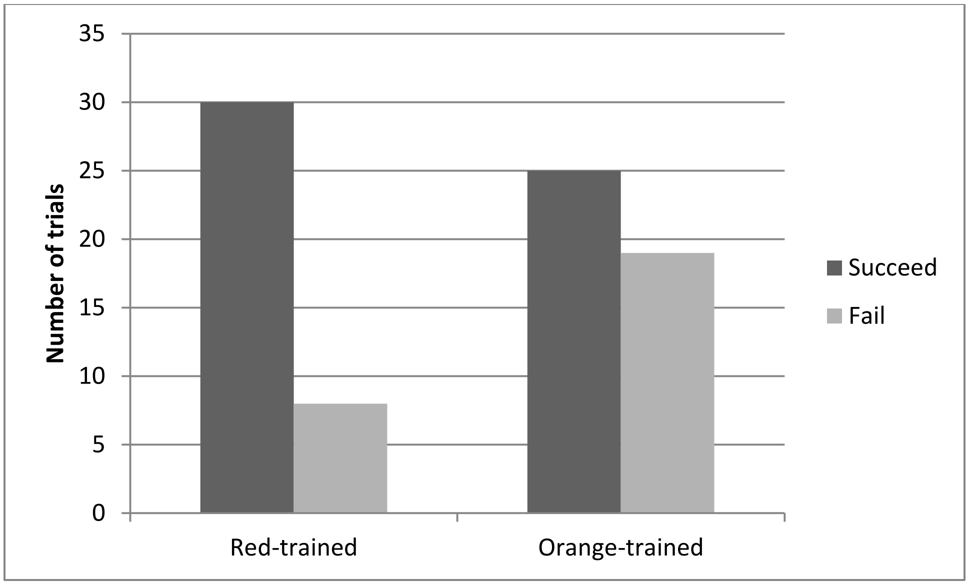


Figure 7. Those trained to red (N=38) were significantly more likely to succeed in pairing the stimulus with a food reward than those trained to orange (N=44).

Test 2: Do firemouths prefer red? *T. meeki* are naturally attracted to red objects demonstrated by approaching the red stimulus first regardless of training color or diet. The orientation of fish at a trial began did not affect the direction the fish initially moved (Chi-square: $X^2 = 5.018$, d.f. = 4, p = 0.22) indicating that individuals did not only approach the colored square they were facing. The first color approached was independent of diet and TC (Log-linear analysis:$X^2=0.355$, d.f.= 1, p = 0.552 and $X^2 =$ 1.633, d.f. = 1, p = 0.201, respectively). When diet and TC were pooled, a binomial test showed that the red stimulus was approached first significantly more often (69% of individuals) than the orange stimulus (31% of individuals; p = 0.009).

Latency to the red choice zone was not affected by diet (Friedman test: X^2 (1)= 0.429, p = 0.51, Figure 8) or TC (Friedman test: X^2 (1) = 0.2, p = 0.66, Figure 8). I also found that latency to the orange choice zone was similarly not affected by diet (Friedman test: X^2 (1) = 2.273, p = 0.13, Figure 8) or TC (Friedman test: X^2 (1) = 1.0, p = 0.32, Figure 8). The proportion of choice time individuals spent in the red zone—and consequently the orange choice zone—was independent of diet (Friedman test: X^2 (1) = 0.8, p = 0.37, Figure 9) and TC (Friedman test: X^2 (1) = 2.333, p = 0.13, Figure 9). To determine if there was a preference for the red or orange stimulus, fish were pooled across diet and training color. Individuals spent a significantly greater proportion of choice time in the red choice zone than in the orange (Mann-Whitney U=217, p=0.03).

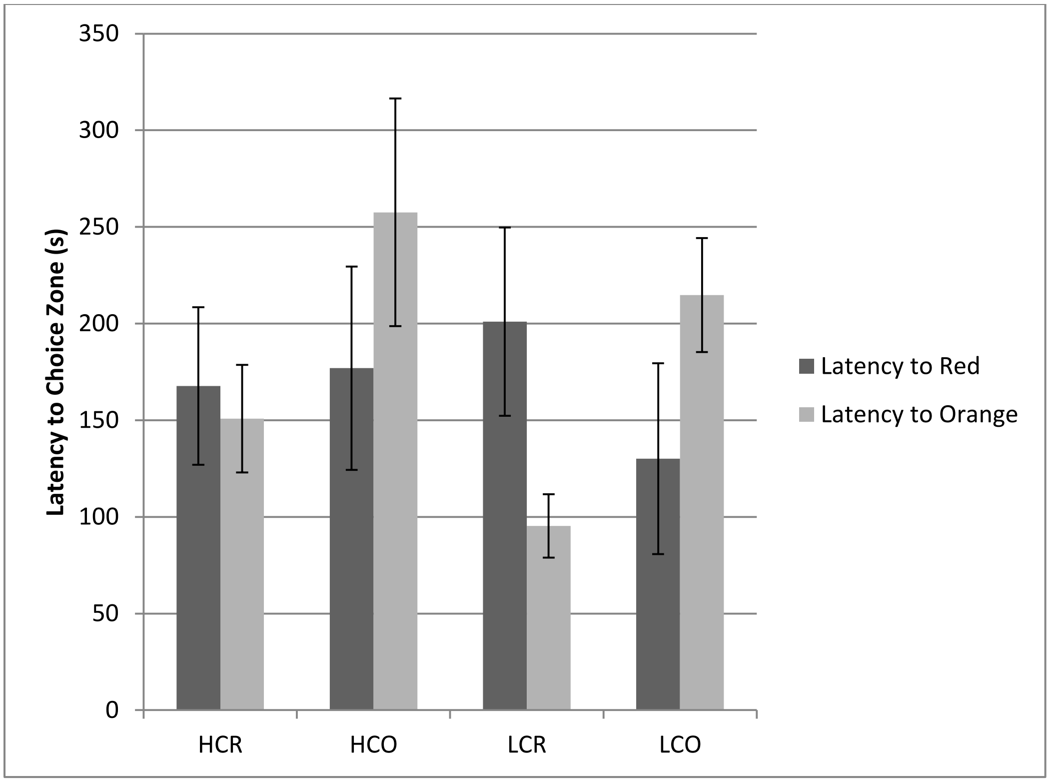


Figure 8: Average latency (±SE) to the red and orange choice zones was independent of diet and TC. (N for each group: HCR = 14, HCO = 12, LCR = 12, LCO = 11)

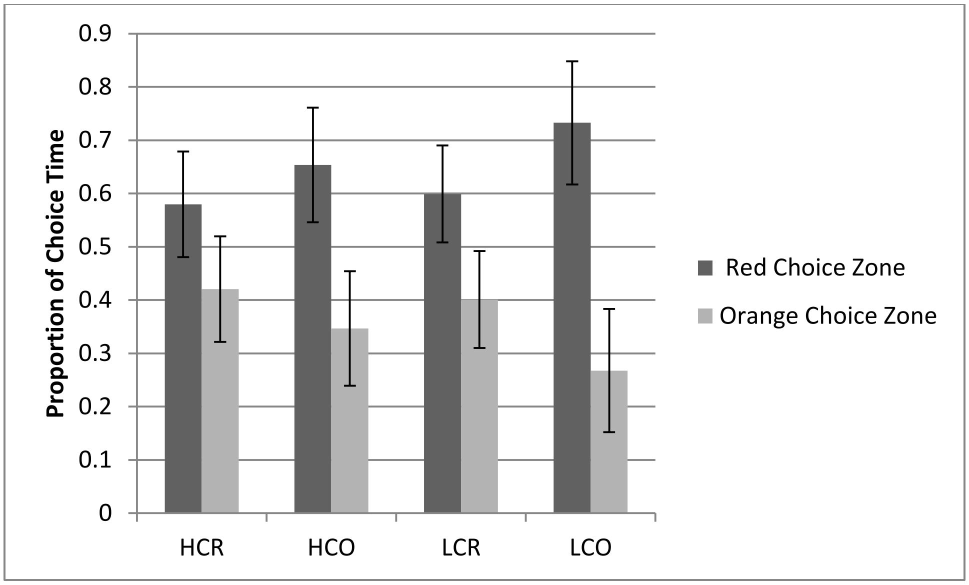


Figure 9. The average proportion of choice time in the red choice zone (±SE)(and subsequently the orange choice zone (±SE)) was independent of diet and TC (N for each group: HCR = 14, HCO = 12, LCR = 12, LCO = 11)

Discussion

Thorichthys meeki can clearly distinguish between red and orange, regardless of the level of carotenoids in their diet, and they prefer red. Individuals tended to approach the red stimulus first, regardless of TC, and overall spent a significantly greater proportion of their choice time near the red stimulus than near the orange one. Individuals thus appear to be attracted to red objects, suggesting there may be an innate attraction to red in this species. This is unsurprising since both male and female *T. meeki* display vibrant red color that is an honest signal in males (Evans & Norris, 1996). If individuals can quickly detect the presence of a red object entering their territory, they may also be able to identify quickly whether the object is a conspecific, an intruder or potential mate. Rapid identification of intruders and mates would reduce response time and thus reduce the time and energy spent fighting (Arnott & Elwood, 2009) or searching for mates. Further studies need to be done to examine the extent of this affinity and to rule out that it is not purely a result of small sample size.

Interestingly, red-trained individuals were more likely than their orange-trained counterparts to succeed in associating their TC with a food reward. It does not seem to be the training process itself because red-trained and orange-trained fish did not differ significantly in their motivation to train. While orange-trained fish did not differ in their motivation to train, they may need longer to associatively pair this color with a food resource. It has been shown that signals that are advantageous to survival and fitness are easier to pair (Wisenden & Harter, 2001). Since *T. meeki* has an affinity for red objects, it may be easier to build upon this innate preference and associate its presence with a food reward more quickly.

The use of behavioral criteria to determine non-human color vision has been used in a variety of taxa (Kelber *et al.*,2003). To my knowledge, this is the first study to use this technique as an initial step in examining visual discrimination in cichlids. While physiological techniques such as microspectrophotometry, retinal dissection, and ERG are essential to fully understand the visual spectrum and retinal composition of a study species, behavioral trials give researchers the ability to examine their focal animals' perception. Confirming that the animals can perceive the signals the experimenter is manipulating is essential for researchers. I propose that this should be tested first as it is less invasive than other common techniques. Bowmaker (2008) noted that behavioral studies to confirm spectral sensitivity are sparse and should not be overlooked. He suggests that while many organisms have been found to possess various photoreceptors, few species have been examined behaviorally to determine how the perception via these photoreceptors is biologically relevant to the organisms.

I have determined that *T. meeki* fed diets with different concentrations of carotenoids do not display a behavioral difference in the ability to discern closely related hues. The ability to associatively learn the difference between two colors has been examined before in fish in a study that focused on learning novel colors (Colwill *et al.*,2005). However, this study focused on the ability to learn rather than the ability to discriminate similar colors. My study focused on the ability of *T. meeki* to distinguish colors relevant to the biology of my study organism. The choice to use TC hues that fell within the natural variation of this species was essential to the significance of this study and to similar future studies.

Altering carotenoid availability is often used by researchers as a fast way to produce a dichotomy in carotenoid-dependent color (see Chapter II). This alteration is oftentimes the initial step to examining if this color plays a role in mate choice (Toomey & McGraw, 2012) and agonistic interactions (Evans & Norris, 1996). However, researchers take this step without considering the broad implications of using a biologically important compound such as carotenoids which are essential for more than just ornamentation (reviewed in Olson & Owens, 1998).

In some organisms the broad effects of manipulating carotenoids have been studied in detail. For example, a series of experiments in the house finch document how manipulating carotenoid availability affects traits spanning plumage coloration, circulating carotenoid concentrations, significance of the color, the effect on vision and the implications of this effect (Hill, 1991; Hill & Montgomerie, 1994; Hill *et al.*, 2009; Toomey & McGraw, 2012). Attempting to possess a full understanding of the role of carotenoids in a species is a daunting task and exceeds the scope of this dissertation. However, my goal is to add to the literature on the vast role of carotenoids and redness as a signal in *T. meeki*.

CHAPTER IV

ACTIVITY LEVEL INSTEAD OF COLOR IS MORE ATTRACTIVE IN A MATE FOR *THORICHTHYS MEEKI*

Introduction

Female mate choice has been studied exhaustively in taxa ranging from fish to primates. Because females tend to be the choosier sex in many species, their preferences and choices have been the focus of reproductive behavioral research. Until recently, males were often ignored with respect to mate choice. However, males often incur costs during mating or providing parental care. This incurred cost would suggest that males would want to selectively mate as well (Rundus *et al.,*2015). While studies have been conducted in systems with the sex-roles reversed (where males provide more parental care than females (Bahr *et al.,*2012; Roth *et al.,*2014)), male mate choice has also been documented in species that possess conventional sex roles (Jones *et al.,* 2014; MacLeod & Andrade, 2014; Baxter *et al.,*2015; Ala-Honkola *et al.,*2015; Rundus *et al.,*2015; Wittman & Fedorka; 2015). However, male and female mate choices are not mutually exclusive. Currently, mutual mate choice has been an area of increasing interest to researchers. Thus far, mutual mate choice has been examined in a variety of vertebrates including zebra finches (Holveck *et. al,* 2011), gobies (Myhre *et. al,* 2012), and sea horses (Bahr *et. al,* 2012).

There are various courtship strategies that can be used to attract and select a mate. Some of these strategies provide direct benefits to the individual making the choice such as physical resources. For example, nuptial gifts are used by numerous insect species as a form of courtship (Vahed, 1998; Rooney & Lewis, 2002; Engels & Sauer, 2006). In some species, the gift contains nutritional content upon which the female will feed such as body parts of the courting male or a spermatophore—a gift containing nutrient-rich secretions in addition to ejaculate (Vahed, 1998). Nutritional gifts provide numerous benefits including an increase of fecundity because the female can allocate spermatophore nutrients to her offspring (Rooney & Lewis, 2002).

Another way of choosing a suitable partner is by discerning the quality of a potential mate. This quality can include health (Olson & Owens, 1998; Nordeide *et al.*,2013; Wittman & Fedorka, 2015) relatedness (Ala-Honkola *et al.*,2015), or whether the potential mate contains “good genes” that can be passed on to future offspring thus increasing their survival and reproductive success. The attracting mate needs to signal to potential mates that he or she possesses these good genes. For example, the exaggerated tail of a peacock signals that he is able to avoid predation with the apparent handicap (Zahavi, 1975). The handicap as a result of producing an exaggerated tail allows this to be an honest signal of quality (Zahavi, 1977).

Honest signals result from constraints such as a physical inability to produce a signal or where the cost of cheating is greater than the reward. Carotenoid-mediated color has been examined as an honest indicator of individual quality. While carotenoids are essential for immune function and vision, it is also used in ornamental coloration. These compounds are important to vertebrates however, they are unable to produce carotenoids and must obtain them from the environment (Kodric-Bown, 1998; Olson & Owens, 1998; Hill & Johnson, 2012). Thus, in order to produce yellow, orange, or red coloration, individuals must be good foragers or hold territories rich in carotenoids and be in good health (Olson & Owens, 1998; Grether, 2000). Additionally, producing colors such as yellow, orange, and red makes individuals more conspicuous to predators (Reznick & Endler, 1982). Therefore, carotenoid-mediated signaling is an honest indicator of mate quality; they are healthy, good foragers, who are able to avoid predation with the handicap of vibrant coloration.

Thorichthys meeki is known by aquarium enthusiasts as the "firemouth cichlid". This fish is aptly named for its vibrant, red coloration that runs ventrally from mouth to anal fin in both males and females (Baerends & Baerends-Van Roon, 1950; Neil, 1984a). This color has been demonstrated to be affected by carotenoid consumption and is used by males in agonistic interactions to indicate a superior fighter—ie. dominant males (Chapter II; Evans & Norris, 1996). Hodapp and Frey (1982) demonstrated that dominant males hold better territories that contain more foraging patches. Since carotenoid-dependent redness requires access to high-quality food resources, we can predict that dominant males are also redder due to their high-quality patches.

Neil (1984a) indicated that *T. meeki* breeding territories are established in one of two ways. Established pairs will find and defend a suitable breeding site or a male will defend a territory and actively court single females. Cichlid reproductive behavior is sometimes difficult to interpret because it appears aggressive. For example, the initial response of substrate brooding males to a potential mate in his territory is identical to the response elicited by an encroaching male (Baerends & Baerends-Van Roon, 1950). Since

T. meeki have been described as monogamous substrate breeders, they have similar, aggressive courting behaviors.

A unique feature of this species' reproductive behavior, as compared with other fishes, is that both parents provide care for the brood for approximately three months (Neil, 1984a). The drawback of providing a prolonged period of care is the loss of additional potential mating events. This cost should be particularly high for males as they don't require extra time and energy to produce viable gametes. Therefore it would be adaptive to selectively mate with the best potential partner. Since the prolonged care is provided by both parents, we would expect to see mutual mate selection to evolve.

It is easy to inadvertently interchange the terms mate choice or selection with mate preference as one is often dependent upon the other. Wagner (1998) describes mate choice as differential mating as a result of interactions between environmental conditions, mating preferences, and sampling strategies. While Wagner's definition was with respect to female mate choices, this working definition can apply to mutual mate choice situations. The aim of this study is to examine mutual mate preferences which could lead to mutual mate choice with respect to redness and behavior of the potential mates.

Redness has already been demonstrated as an important signal in *T. meeki* (Chapter III, Evans & Norris, 1996) but has yet to be examined in the realm of mating. Baerends and Baerends-Van Roon (1950) described the mating behaviors of *T. meeki* in detail but fish were not given a choice in their mate. It was noted that not all pairings were successful; some females fled the territory under highly aggressive attacks by the male. No studies on *T. meeki* have examined which aspects of a potential mate increase the likelihood of successful pairings, be it color or some behavioral aspect of the courting male or visiting female.

Methods

Study Animal Maintenance

The *Thorichthys meeki* used in this study were obtained as juveniles from a local pet shop and maintained in a 300-L aquarium in the laboratory. To determine their sex, I examined the genital papilla of unsexed fish under a stereoscope every two weeks. After a fish had matured to the point of sex determination, I placed it into one of four 300-L aquaria in which fish were segregated by sex and diet—high carotenoid male (HCM), low carotenoid male (LCM), high carotenoid female (HCF), and low carotenoid female (LCF). I used Cobalt Aquatics Color Flakes® for the high carotenoid (HC) diet and Cobalt Aquatics Spirulina Flakes® for the low carotenoid (LC) diet. All fish were fed ad libitum six days per week for a minimum of 12 weeks before the experiment to create diet-based color change (Chapter II; Evans and Norris, 1996).

All aquaria in this experiment contained a gravel substrate and various refugia. Water temperature was held at approximately 27° C via aquarium heaters and power filters were used to aerate and filter aquaria. This experiment was approved by the University of Louisville’s Institutional Animal Care and Use Committee (proposal #12040).

Experimental Design

To determine if both males and females showed a preference for individuals of the opposite sex that varied in dietary status, both males and females served as focal fish during their respective experiments and as such their protocols are identical. A HC focal fish and two choice fish (one HC and one LC) of the opposite sex were size matched (+/- 8% SL) for each trial.

The experimental set up was a 200-L aquarium divided into three compartments by clear and opaque dividers (Figure 10). The choice fish (one HC and one LC) were placed into the lateral compartments of the aquarium while the focal fish was placed in the center compartment (Figure 10). The locations of HC and LC fish were alternated every trial. The central compartment had two choice zones and a neutral zone. A choice zone began 10 cm from the clear divider which was roughly two body lengths of *T. meeki*. The remaining area of the central compartment was considered neutral or no choice. All fish were physically and visually separated via opaque dividers and allowed to acclimate for two days prior to observation.

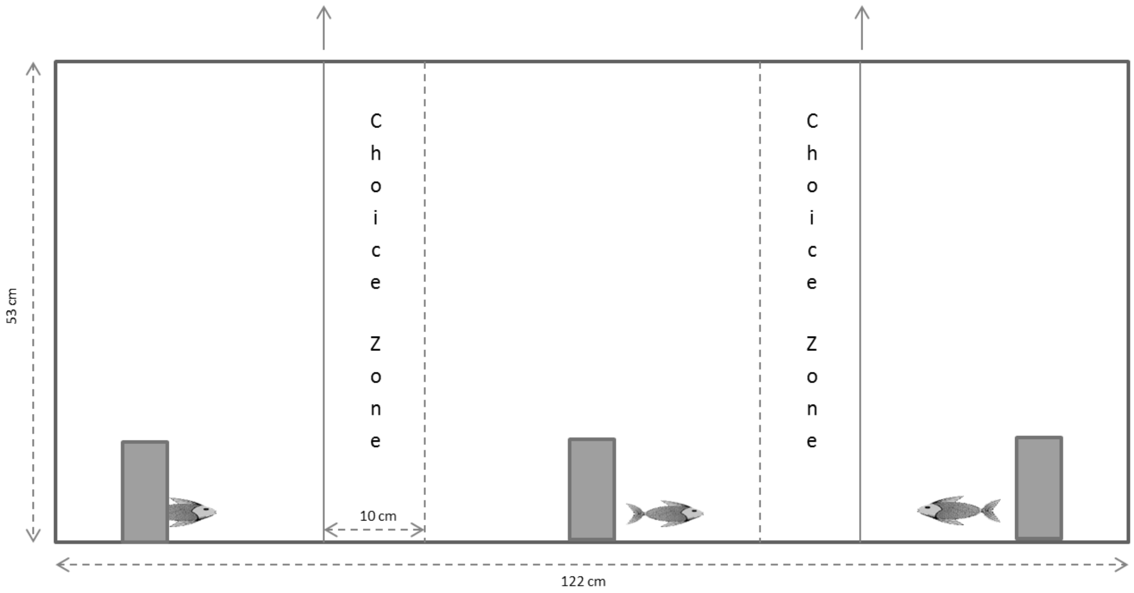


Figure 10. Schematic of experimental aquarium. A 208-L aquarium was divided into three zones via clear and opaque dividers. The center zone was additionally partitioned into choice and neutral zones. A focal fish was placed in the center compartment while HC and LC choice fish were placed in the lateral compartments. A piece of slat angled on a flower pot provided refuge in all compartments for the fish. After acclimation, the opaque dividers were removed in the direction of the arrows for observations to be made.

Observations

The opaque dividers were removed allowing the focal fish visual access to the lateral compartments. Observations were recorded directly to an external hard drive with a Canon Vivia HV30 camcorder via firewire for two hours. Video was later processed with time-budgeting data collected from the focal fish including the amount of time spent in the choice zones, the number of visits to either fish, and the average visit length with either fish. Every 30 minutes, 10 minutes of behavioral data were collected from the choice fish resulting in a total of 30 minutes of behavioral observations. The type (Table 1) and length of each behavior was recorded as well as the overall number of behaviors performed.

Table 1. Behaviors examined and their descriptions.
* Behaviors as described in Neil, 1984a.
** Behaviors as described in Baerends and Baerends-Van Roon, 1950.

Behavior	Description
Charge*	Rapid swimming towards another fish
Flare**	Raising of brachiostegal membrane
Lateral Display**	Displaying the flank towards another fish with median fins erect
Nip	Biting at the clear divider
Nosedown*	Body inclined with the head toward the substrate at an angle of at least 30 degrees, median fins erect
Tailbeat*	Exaggerated tailwags accompanied by little to no forward movement
Quiver**	Sideways trembling, often performed with the head facing the substrate

Statistics

The experiment was repeated 30 times consisting of 15 focal female trials and 15 focal male trials. All data were analyzed in SPSS 21(SPSS Inc., Chicago, IL). Normality was determined with Kolmogorov-Smirnov tests. While the time-budget data were able to be transformed to a normal distribution with a square root +1 transformation, behavioral data was unable to be transformed. Therefore, the behavioral data was analyzed using non-parametric tests.

To determine whether male and female *T. meeki* prefer to associate with redder potential mates, 2-way ANOVAs were carried out on the time spent in either choice zone and mean visit length for each zone. A Friedman's test examined whether focal fish sex and choice diet affected the number of visits to either choice zone.

There was variation in the activity level of choice fish and thus preferences were categorized according to the proportion of choice time spent near either HC or LC fish. Individuals who spent greater than 55% of their choice time near either HC or LC fish was considered to have a preference. When examining how choice fish behaved during the experiment, three trials (1 female, 2 male) were removed as these fish showed no preference for either HC or LC fish.

Considering the variation in activity level of *T. meeki* in this experiment, there were a few trials that had time-budgeting data, but no behavioral data—ie. they visited either choice zone during the experiment, but did not receive any behavioral displays during the observation times. Therefore, these trials were removed from analysis reducing my sample sizes to 12 focal female trials and 11 focal male trials. Mann-Whitney U tests were used to determine if choice males and choice females differed behaviorally. Since they did not differ significantly in any variables examined, sex was pooled to increase the power of the analysis.

To examine if preferences were driven by behavioral differences, Mann-Whitney U tests were used to determine if the number of behaviors performed and the percent of visit time spent behaving differed between preferred and non-preferred fish. To determine if preferred and non-preferred fish differed in the types of behaviors performed, the number of discrete behaviors was compared using Mann-Whitney U tests.

Results

A 2-way ANOVA examining the effect of diet and sex on choice time showed that male and female *Thorichthys meeki* do not differ significantly in the amount of time spent near either HC or LC choice fish (F(1,56) = 0.3, p = 0.59 and F(1,56) = 0.06, p = 0.80, respectively, Figure 11). Additionally, there is no interaction between sex and diet on choice time (F(1,56) = 0.039, p = 0.84) . Male and female *T. meeki* did not differ in the number of times they visited the HC and LC choice fish (Friedman Test: X^2 (1) = 0.533, p = 0.47 and X^2 (1) = 0.034, p = 0.95, respectively, Figure 12). A 2-way ANOVA examining the effect of diet and sex on average visit length found similar results (Figure 13). Focal males and females did not differ significantly in average n (F(1,56) = 0.004, p = 0.95) nor did choice diet affect average visit length (F(1,56) = 0.134, p = 0.72). There was no interaction found of sex and diet on average visit length (F(1,56) = 2.531, p = 0.12).

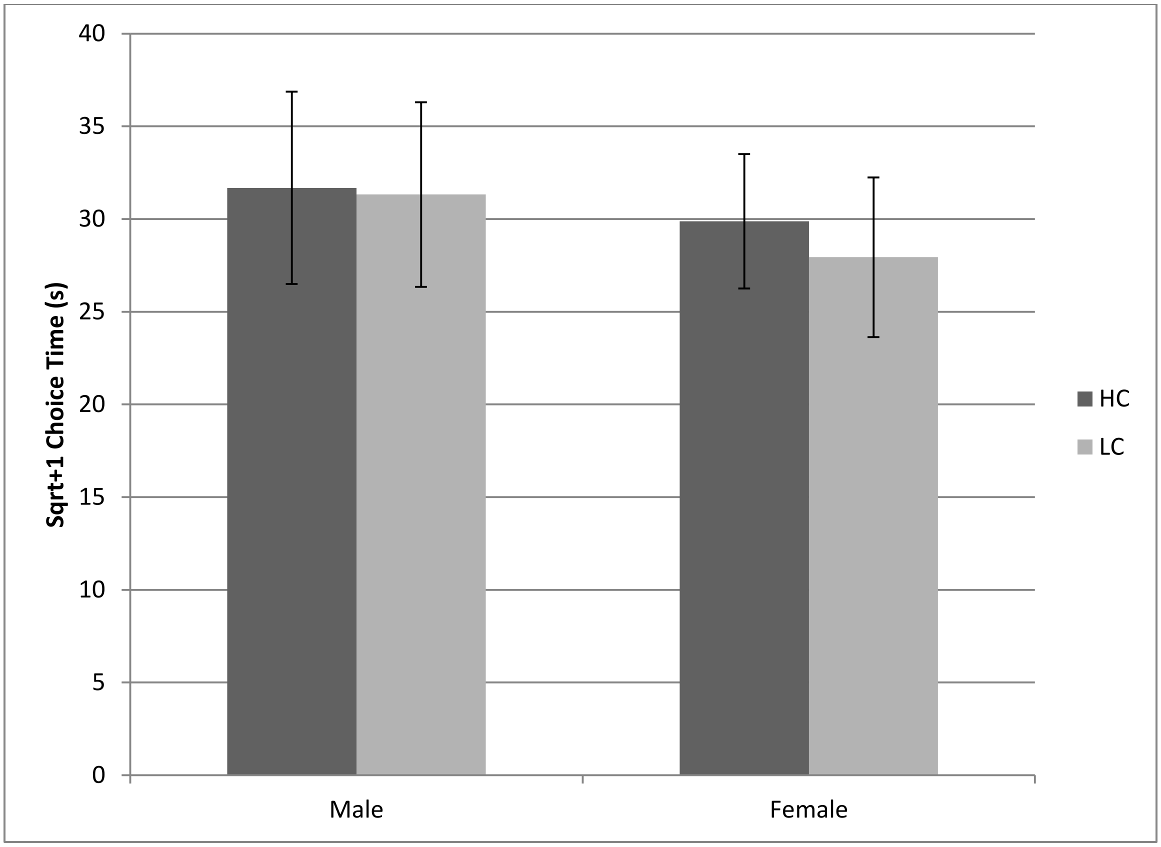


Figure 11. Male (N=15) and female (N=15) *T. meeki* do not differ significantly in the mean time (±SE) they spend near HC and LC choice fish.

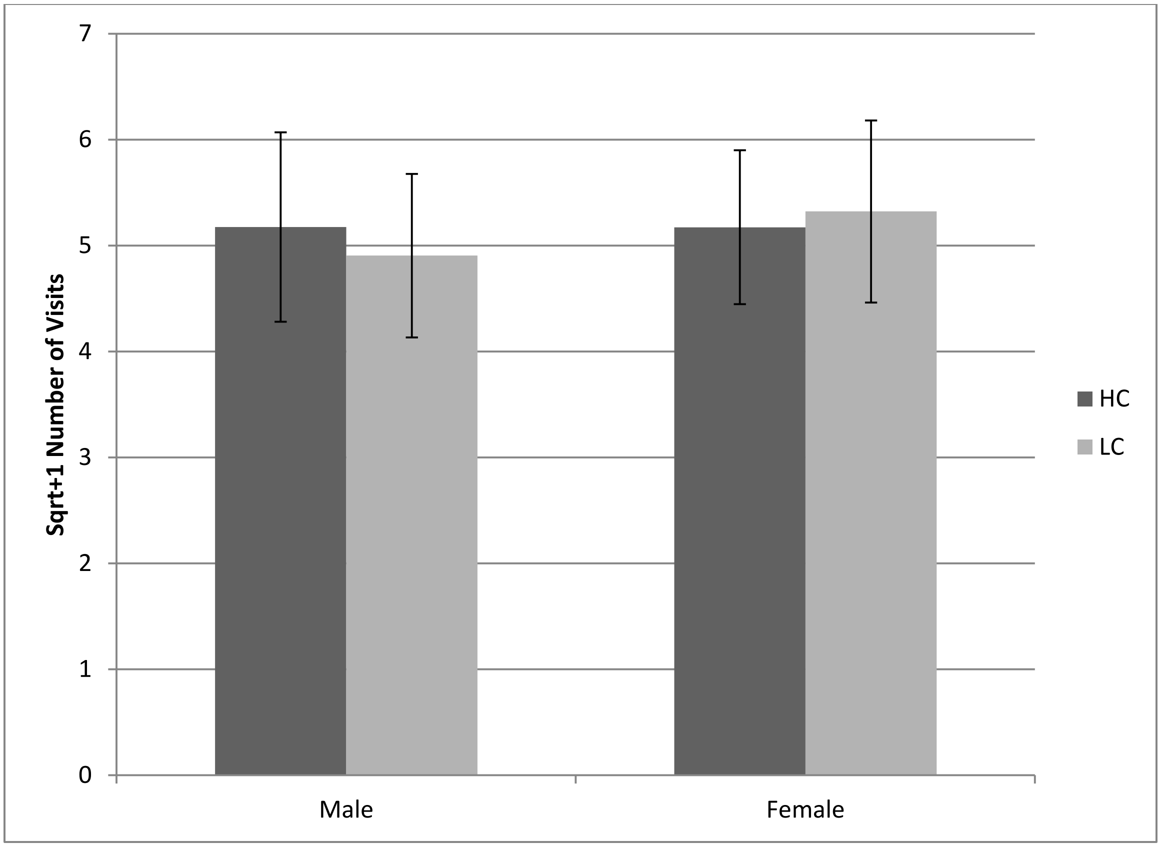


Figure 12. Male (N=15) and female (N=15) *T. meeki* visit HC and LC choice fish equally (mean±SE).

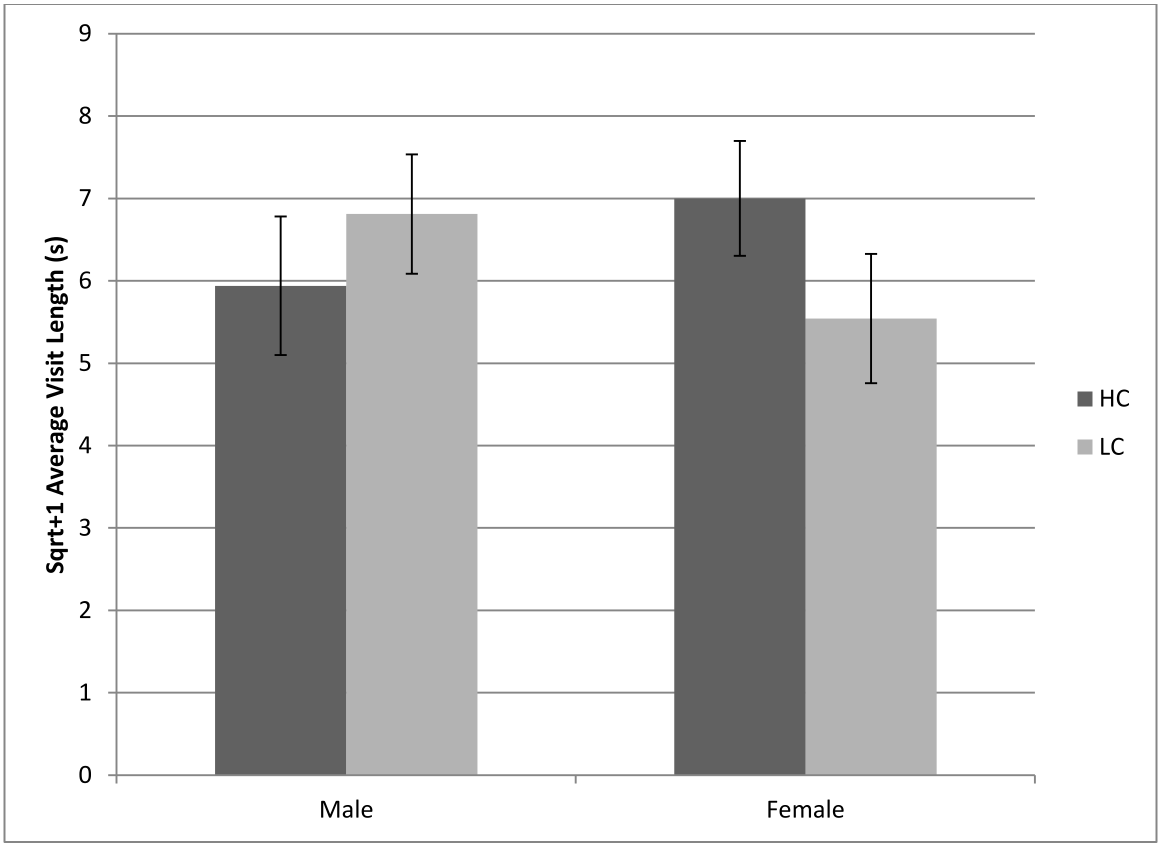


Figure 13. Average visit length (±SE) to HC and LC choice fish does not differ between male (N=15) and female (N=15) *T. meeki*.

Behavioral data was examined for only individuals who showed a preference for either HC or LC choice fish. Male and female choice fish showed no difference in the number of behaviors performed or the percent of the focal fish's visit time spent behaving (Mann-Whitney U tests: U = 222, p = 0.35and U = 218, p =0.31, respectively, Figure 14). Since sex had no role in the overall behaviors, they were pooled to examine behavioral differences between preferred and non-preferred fish.

Mann-Whitney U tests showed that preferred fish (N=23) performed more behaviors than non-preferred fish (N= 23) and spent a greater percent of the focal fish's visit time behaving (U = 165.5, p = 0.029 and U = 165, p = 0.028, respectively, Figure 15). When discrete behaviors were examined between preferred and non-preferred fish, the nosedown behavior was the only one that differed significantly between the groups (Mann-Whitney U: U = 163.5, p = 0.013, Figure 16).

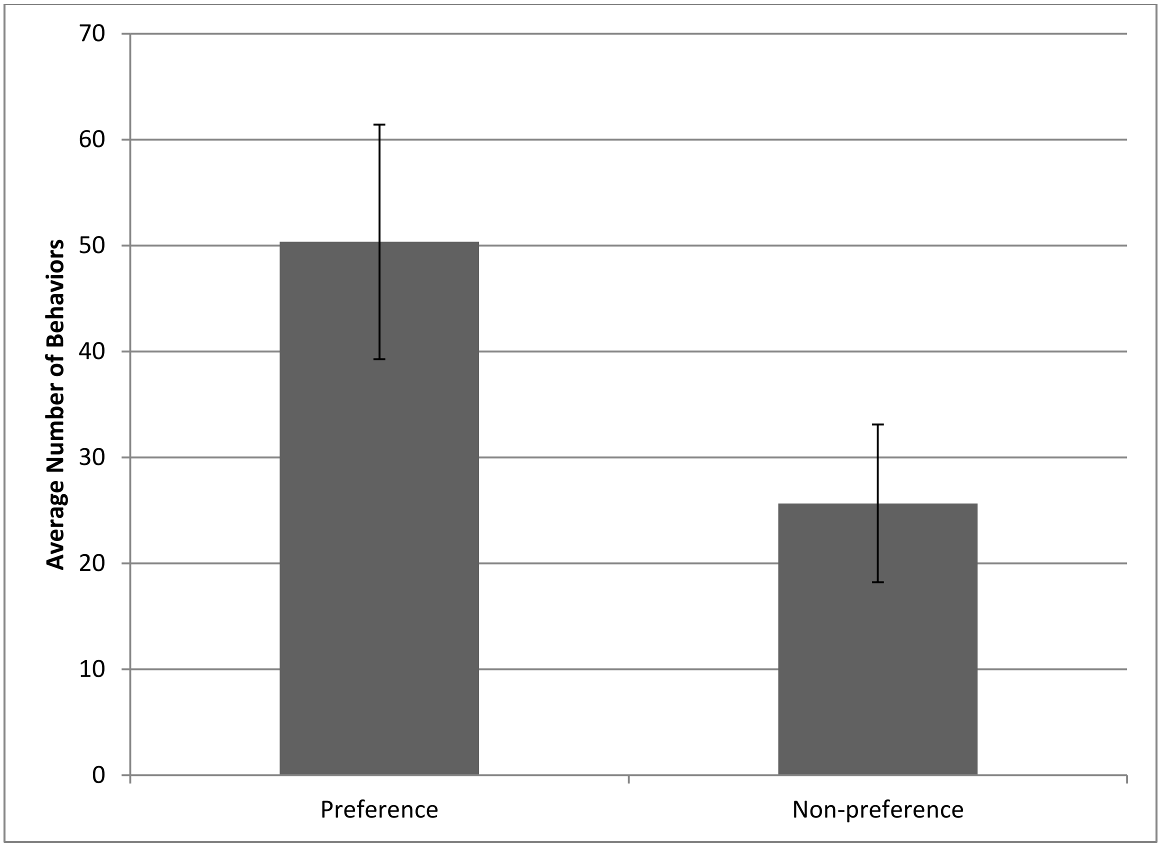


Figure 14. Preferred choice fish performed significantly more behaviors towards the focal fish than did the non-preferred fish (mean ±SE).

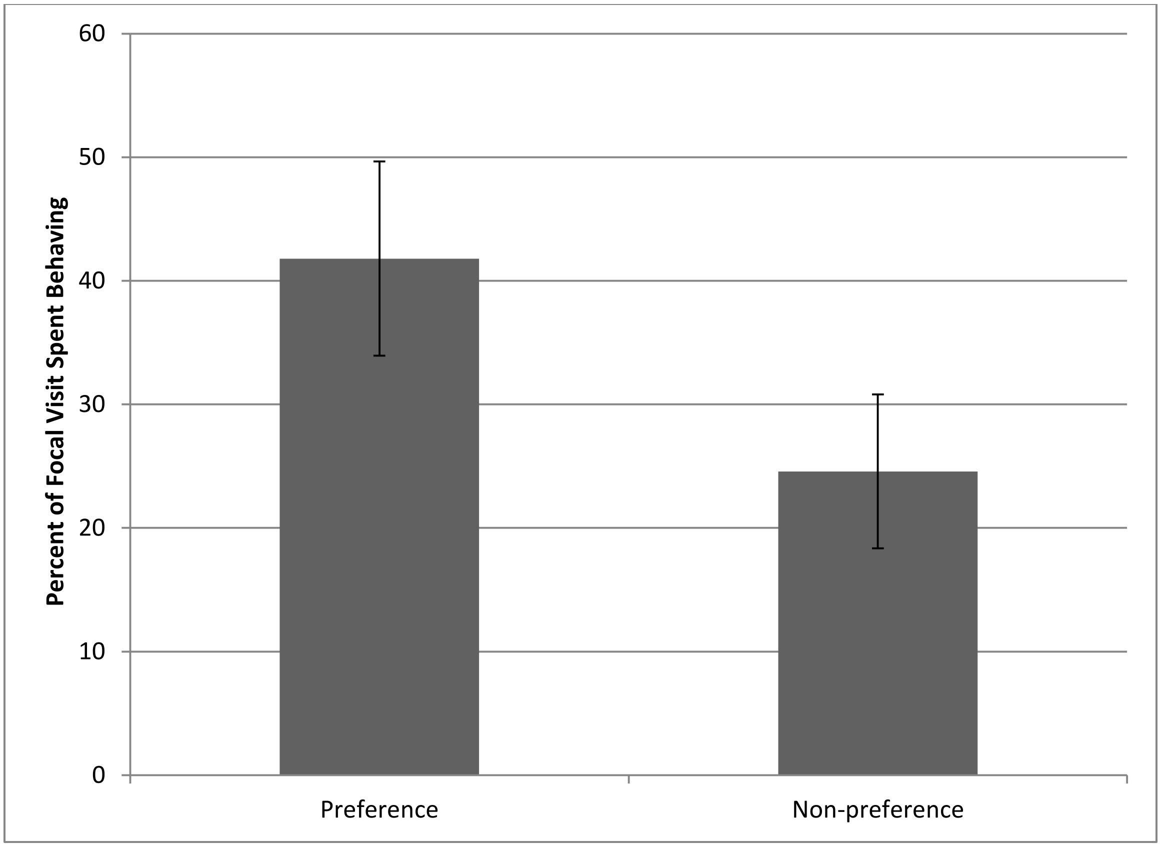


Figure 15. Preferred choice fish spent a greater percentage of the focal fish's visit performing behaviors than did the non-preferred fish (mean ±SE).

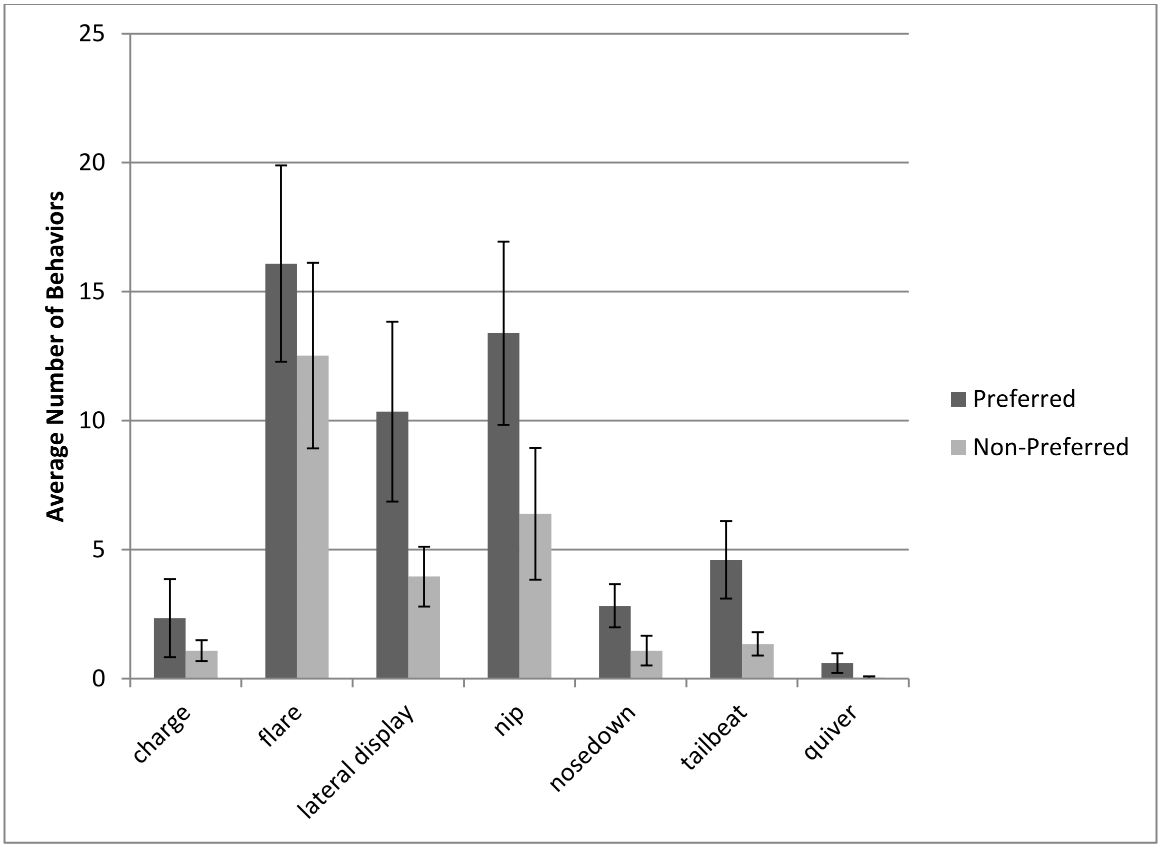


Figure 16. The behaviors displayed (mean ±SE) by preferred and non-preferred fish did not differ significantly with the exception of the “nosedown” behavior (p=0.013). While other behaviors such as “lateral display” and “tailbeat” seem to be trending in the same direction as “nosedown”, significance was not found.

Discussion

Most focal male and female *Thorichthys meeki* preferentially associated with potential mates. However, they did not prefer to associate with a potential mate based on redness. Instead, preferences were determined according to how choice fish behaved. Preferences and behaviors did not differ between male and female *T. meeki*. Preferred fish, regardless of sex or color, were more active than the non-preferred fish. They spent a greater proportion of the focal fish's visit time behaving and had an overall greater number of behaviors than the non-preferred fish. When examining mate preference, caution needs to be extended when using time-budgeting data as a means of measurement. Time in proximity to a potential mate does not always denote a preference for that mate (Fuller, 2003). Aspects such as behavior or other biologically relevant variables should be noted as well, especially in previously unstudied taxa (Fuller, 2003). This is why activity level and behavioral observations were essential in this study. However, it is also important to note if the focal fish visits both choice fish when examining behavioral data. For example, a focal female could have been attracted to one male because he was the first to behave. The focal fish's choice could then be dependent on her continually interacting with him because he was the first to initiate contact, not necessarily that he was a better potential mate.

While the activity level of the preferred fish could be the driving force for mate preference in *T. meeki* it is interesting that the "nosedown" behavior is significantly different between preferred and non-preferred fish. This behavior is often performed as a non-contact, agonistic display (Baerends & Baerends-Van Roon, 1950; Neil, 1984a; Neil 1984b). However, the significant difference of its frequency suggests that it may play a role in courtship. This follows logically because Baerends and Baerends-Van Roon (1950) describe courtship in *T. meeki* as increasing in intensity over time leading to breeding. The final behaviors performed before breeding are nipping off a breeding surface—which has the fish in a nosedown position—and quivering in a nosedown position.

The “lateral display” behavior and “tailbeat” behavior also seem to be approaching significance when graphed (Figure 16). However, the data were unable to be transformed and significance was not detected using non-parametric statistics. With an increased sample size, or more behavioral data collected, it would not be surprising if these behaviors differed significantly as well because these behaviors are common at the beginning of courtship (Baerends & Baerends-Van Roon,1950).

What I find most interesting is that redness is innately attractive to *T. meeki* and is an honest indicator of quality yet it plays no role in attractiveness as a mate (Chapter II; Chapter III; Evans & Norris, 1996). However, signals are rarely unimodal (Sargent *et al.*, 1998; Ronald *et al.*,2012). There may have been other signals the choice fish produced that overtook the visual signal such as olfactory or auditory cues. While visual cues have been used in various mate choice experiments in fishes, experiments looking at olfactory, auditory and multimodal cues with respect to choice are becoming more common (Guevara-Fiore *et al.*,2010; Verzijeden *et al.*,2010; Ronald *et al.*,2012; Passos *et al.*, 2013; Estramil *et al.*,2014).

Olfactory cues have been shown to play a role in finding a receptive mate especially in turbid waters (Guevara-Fiore *et al.*,2010; Passos *et al.*, 2013). Guevara-Fiore *et al.* (2010) found that male guppies increase searching behavior when presented with olfactory cues of receptive females. Additionally, the olfactory cues presented by a receptive female increased her attractiveness than when the male was presented visual cues alone. Some cichlid species use acoustic signals (Amorim *et al.,* 2004; Amorim & Almada, 2005; Verzijden *et al.,* 2010; Estramil *et. al,* 2014). While it is unknown whether *T. meeki* uses auditory communication, other cichlid species have been found to use it in addition to visual and olfactory cues as a multimodal signal for mate choice (Verzijden *et al.,* 2010; Estramil *et. al,* 2014).

In this experiment, the lateral compartments were not water tight because the clear dividers were designed to be removable. This would allow for olfactory and auditory cues to be easily passed to the focal fish. Since these modalities have yet to be studied in *T. meeki* it is possible that they play a role in mate preference. *T. meeki* can be found in waters that vary in turbidity and have been shown to increase the brightness of their coloration when in more turbid waters (Neil, 1984a; Soria-Barreto & Rodiles-Hernández, 2008). It is possible that they also use other cues in these instances as well since olfactory and auditory signals are easier to transmit through turbid water than visual signals (Endler, 1992; Passos *et al.,*2013).

This study emphasizes that a signal utilized in one area of an animal's behavioral repertoire doesn't necessarily mean that it will play an important role in others. It has been demonstrated that organisms which have similar ornamentation across the sexes do not necessarily use the ornamentation in the same way (Murphy, *et al.,* 2014). This concept needs to be examined in monomorphic species such as *T. meeki* to parse apart the importance, or lack thereof, of various signals. It is evident that *T, meeki* preferentially associates with potential mates it is unclear that behaviors alone are the deciding factor.

Future studies need to be conducted with olfactory and vibratory isolation to rule out those cues.

While some studies show that association preferences predict the likelihood of reproduction, this needs to be tested in *T. meeki* (Walling *et al.,* 2010). Since courtship in cichlids is so aggressive, there are instances in which a fish will flee from a territory if aggression is too high (Baerends & Baerends-Van Roon, 1950). Focal and choice fish in this experiment were physically separated from each other and did not experience the more aggressive behaviors such jaw-locking, ramming, and chasing. It is possible that this inability may skew results towards preferring a mate that might not lead towards reproduction. Future experimentation with added interactions between the pairs need to be done to determine if the more aggressive behaviors play a significant role in reproductive success of *T. meeki*.

This is the first study of mutual mate preference in a Neotropical cichlid. Many cichlids within this group provide some length of parental care so it is surprising that this is the first time it has been documented. While the specific mode of preference has yet to be determined, this research has provided a starting point to look into multimodal signaling and more detailed behavioral interactions.

CHAPTER V

SUMMARY AND FUTURE DIRECTIONS

While dietary carotenoids have been studied extensively throughout the years, we now understand the need to examine their role from multiple perspectives. This is particularly important when examining how these pigments are used in signals for intra- and interspecific communication. Here I have demonstrated that a period of 12 weeks on a high- and low-carotenoid diet is sufficient to alter redness in male *Thorichthys meeki*. Unexpectedly, I found that females did not respond in the same manner as males. Female redness did not increase when provided more dietary carotenoids while decreasing the availability of carotenoids significantly altered their color by 6-8 weeks. This odd result warrants further investigation into how carotenoids are differentially absorbed and used by the sexes.

Furthermore, dietary carotenoids are utilized for more than color (Olson & Owens, 1998) and have been shown to affect vision in other vertebrates (Toomey & McGraw, 2012). I have demonstrated behaviorally that dietary carotenoids play no role in color discrimination in *T. meeki*. While behavioral assays are a noninvasive way to examine vision, more intensive research is necessary to fully understand the visual spectra of this species. Research on color vision in Neotropical cichlids is seriously lacking. The next step in understanding vision in *T. meeki* is to conduct specific experiments on the visual spectrum through microspectrophotometry and histological examination of the eyes themselves. By performing dissections of *T. meeki* eyes over the course of a high or low carotenoid diet we can determine if carotenoids are deposited in the eye and used as an intraocular filter. Little is known about what alters intraocular filters (Heinermann, 1989). Dissections like this will give insight into whether carotenoids are being deposited in the eye, where they are being deposited, and how long it takes for their presence to play a role in vision—if any were to occur.

My first two experiments were essential for examining how redness is used as a signal for mate preference in *T. meeki*. Here, I found that males and females have no preferential association with potential mates who are redder, but who are more actively displaying. While displays by choice fish seem to be driving the preferences of the focal fish, we do not understand what specifically makes a potential mate attractive. I intend to further examine the behaviors of the choice fish and reciprocal interactions between the choice and focal fish. This will allow me to parse apart what the preferred fish is specifically doing that the non-preferred fish is not.

Furthermore, the potential for multimodal signaling needs to be examined in this species. In addition to the visual signals produced, choice fish could be providing auditory or olfactory cues making them more attractive to the focal fish. This can be easily tested by isolating the various modes of signaling via separate, juxtaposed aquaria. Signaling pathways can then be examined individually or in combination. For example, I will have two aquaria, one holding the focal fish while the other remains empty or holds a choice fish. By placing a choice fish in the second aquarium I can examine visual cues alone. While the choice fish is displaying, a microphone in its aquarium can record any auditory cues produced and water samples can be taken from this aquarium which may hold olfactory cues. We can then observe the choice fish's behaviors when provided the auditory and olfactory cues alone (when presented an empty tank) or in conjunction with visual cues (when presented a choice fish).

In conclusion, this dissertation takes an encompassing approach to the role of carotenoids on redness and subsequent behaviors in the firemouth cichlid, *Thorichthys meeki*. This provides a starting point for further, in-depth studies of color, vision, and multimodal signaling in Neotropical cichlids.

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CURRICULUM VITAE

SARAH A. FAUQUE
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EDUCATION

Doctoral Candidate
Area of study: Behavioral Ecology
University of Louisville, Louisville, Kentucky
Anticipated Graduation Date: December 2015

Bachelor of Science in Biology, minored in Environmental Science (2010)
Viterbo University, La Crosse, Wisconsin
Recognized on the Viterbo University Dean's List

TEACHING EXPERIENCE

Graduate Teaching Assistant, Anatomy and Physiology Spring 2013 – Present

- Teach labs for nursing, physical therapy, and dental hygiene students.
- Responsible for concisely teaching students how to identify anatomical structures and understand physiological processes they will use in their careers.

Graduate Teaching Assistant Academy 2014

- A competitive, year-long program offered through the University of Louisville to obtain practical strategies, tools, and resources for teaching at the university level.

Graduate Teaching Assistant, Principles of Biology 2010 – 2012

- Teach labs for undergraduate biology major students
- Responsible for communicating clearly to students, controlling a classroom, writing and grading exams

Guest Lecturer Fall 2011

- Delivered a lecture to an Animal Behavior class on optimal foraging theory

RESEARCH AND LABORATORY EXPERIENCE

University of Louisville, Louisville Kentucky 2013-2015

- Examined the multifaceted effects of carotenoids in *Thorichthys meeki*
- Observing changes in integument, vision, and mate preference

University of Louisville, Louisville, Kentucky 2012

- Examined hierarchy formation in juvenile *Thorichthys meeki*
- Examined signaling during hierarchy formation in *T. meeki*

University of Louisville, Louisville, Kentucky 2011

- Examined antipredator behaviors in the shell-dwelling African cichlid *Neolamprologus multifasciatus*.
- Examined the effects of substrate color on the subsequent brightness of *N. multifasciatus.*

Viterbo University, La Crosse, Wisconsin Summers 2008 and 2009

- Selected as the only junior to participate in a competitive research opportunity with biology professors at Viterbo University; received and accepted invitation to participate in second year of research.
- Served as a mentor to younger students during the second year of the program

PRESENTATIONS

University of Louisville Awards Day, Louisville, Kentucky Spring 2015
“Seeing red: the potential role of carotenoids in color discrimination in fish”
Oral Presentation

Midwest Ecology and Evolution Conference, Bloomington, Indiana Spring 2015
“Making sexy fish: documenting carotenoid-mediated redness in the firemouth cichlid” Poster Presentation

CISAB Animal Behavior Conference, Bloomington, Indiana Spring 2015
“The potential role of carotenoids in color discrimination in fish” Poster Presentation

Kentucky Academy of Science Annual Meeting, Lexington, Kentucky Fall 2014
“ Bilateral mate preference in the firemouth cichlid: gender differences in a monomorphic fish” Poster Presentation

CISAB Animal Behavior Conference, Bloomington, Indiana Spring 2013
“Redness May Be Attractive to One but Not Both Sexes in Thorichthys meeki”
Poster Presentation

National Conference for Undergraduate Research, La Crosse, WI Spring 2009
“Examining the effects of different levels of experience on risk-taking behavior in the guppy *Poecilia reticulata”* Poster Presentation

Seven Rivers Research Symposium, La Crosse, Wisconsin Fall 2009
"Examining the effects of different levels of experience on risk-taking behavior in the guppy *Poecilia reticulata". *Oral Presentation

Seven Rivers Research Symposium, La Crosse, Wisconsin Fall 2008
"Effects of Past Experience on the Subsequent Bold-shy Behavior of the Guppy (*Poecilia reticulata).* Poster Presentation.

GRANTS RECEIVED

U of L BGSA Travel Grant Spring 2015
- $75 to attend conference

Guy Jordan Research Fund 2013
- $400 to increase sample size

Graduate Student Research Fund Grant Spring 2013
- $100 to attend conference

Graduate Student Research Fund Grant Fall 2012
- $100 to purchase study species

Graduate Student Research Fund Grant Spring 2011
- $100 to purchase research equipment.

Graduate Student Research Fund Grant Fall 2011
- $100 to purchase study species.

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SERVICE

Graduate Student Judge, Kentucky Academy of Science Fall 2014
- Review presentation skills of undergraduate researchers
- Collect and determine winner of graduate level ecology competitions

Biology Graduate Student Association Granting Committee Spring 2014
- Organize and determine winners for research and travel funds available to graduate students

Mentor for Undergraduate Research Assistants Spring 2013
- Examined female mate association preferences and mate choice in *Thorichthys meeki* with respect to altered redness due to dietary differences.

Summer 2012

- Examined hierarchy formation in juvenile *Thorichthys meeki* with an undergraduate assistant.

Spring – Summer 2011

- Worked with an undergraduate assistant on a collaborated research project examining changes in coloration due to substrate color.

Mentored Undergraduate Teaching Assistants Spring 2011 and Fall 2012

- Supervise and provide a teaching example for undergraduates interested in teaching at a college level or attending graduate school.

CAMPUS INVOLVEMENT

- Member, BGSA, University of Louisville Fall 2010- Present
    - Appointed Secretary Spring 2013-Fall 2014

COMMUNITY INVOLVEMENT

Docent, Louisville Zoo, Louisville, Kentucky Fall 2011

- Communicate the importance of wildlife and conservation with the local community

AWARDS

- Best Graduate Student Presentation Spring 2015