The Effects of Simulated Turbidity on the Mate Choice of a Female Cichlid from Lake Malawi, *Metriaclima estherae*

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Abstract

The cichlid fish (Perciformes: Teleostei) of the Rift Valley lakes of Africa are commonly viewed as non-human model evolutionary organisms that offer lessons about evolution that can be applied to many other organisms, due to the fact that new cichlid species evolve in the Rift Valley lakes at an accelerated rate when compared to other locales. In order to use them as such, however, the mechanisms by which the cichlids evolve into new species must be understood. Visual sexual selection by females has been implicated in numerous studies as a driving force in these radiations. Recently, the ability of females to discriminate in this fashion has come under threat in Lake Victoria, where water clarity has declined due to anthropogenic factors such as increased agricultural cultivation and deforestation. As a result, an increase in mating and hybridization among multiple cichlid species has been seen in the portions of the lake that are most affected by this reduction in water clarity. Comparatively little research has been done on the possible effects of reduced water clarity on the other two major lakes of the region, Lake Tanganyika and Lake Malawi. Utilizing video playback technology, it was found that reducing the amount of light transmitted (using opaque filters) between female Metriaclima estherae (a Malawian cichlid) and videos of a male of the same species and a male of a different species significantly reduces the relative amount of time the female spends with the male of the same species versus the male of a different species. The use of opaque filters is intended to simulate the effects of real-world reductions in water clarity in Lake Malawi that may occur in the future. The result found here indicates that, similar to Lake Victoria, cichlids in Lake Malawi may be more prone to choose mates of a different species when living in areas with reduced water clarity. Management plans for the lake, then, should take robust action to prevent reductions in water clarity.

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Introduction

Introduction and Current State of the Field

The present thesis seeks to examine how turbidity might affect mate choice by females in a Malawi cichlid, *Metriaclima estherae*. First, background is presented in order to provide context and justification for the experiment. The following sections will make apparent the reasons for the subject and location biases in the empirical literature on the Rift Valley cichlid system, as well as explain why cichlids are useful as model evolutionary organisms, or organisms that yield broader insights into evolution when studied. The methodology utilized for this experiment (video playback trials) will also be introduced and justified.

Cichlid research is a swiftly growing field, as suggested by the rapidly increasing number of peer-reviewed studies on the subject (with 260 articles on cichlids for the period 1910-1980, 1507 articles for 1980-2000, and 1692 for 2000 to the present, according to the online interdisciplinary database 'The Web of Science'). Some of this increase in numbers of publications may be an artifact resulting from increased recent digitization, diversification in study topics and publication foci, and greater access to study areas and organisms, but, nonetheless, cichlids clearly are important study subjects able to impart important lessons about biology.

A survey of the subjects of peer-reviewed literature on cichlids in the Rift Valley region of Africa, conducted utilizing the Web of Science database, yields some interesting results. Utilizing a subset of the number of papers described above (from 1985 onwards, excluding those that could not be efficiently categorized), there were 9 papers on conservation, 71 on

phylogentics and evolution, 17 on non-sexual behavior, 28 on non-sexual physiology and morphology, 47 on sexual selection and reproduction (broken down into 4 olfactory, 5 physiology, 16 behavior, and 23 coloration), 43 on non-phylogenetic genetics, and 11 reviews.

Breaking this literature down into a different subset of papers (ones that could be efficiently categorized) by the lake that the paper focused on, there were 219 on Lake Malawi, 259 on Lake Tanganyika, and 261 on Lake Victoria. Within each of these Lake categories yields 20 papers focused on turbidity/eutrophication in Lake Victoria, 2 papers for Lake Tanganyika, and none for Lake Malawi.

Video Playback

Using video playback technology for ethological, or behavioral, studies has gained traction in recent years, with usage in birds, fish, and other animals (Makowicz *et al.* 2010, Evans and Marler 1991). There are numerous advantages to using video playback over live animals, most notably that the former allows for efficient control of stimulus, in that the object or image inducing a behavioral response is held constant through numerous trials. This feature is particularly important in mate-choice experiments, where matching mate quality with specimens taken from a finite population can be very difficult (Rowland 1999). Other researchers have found that cichlids react appropriately to video stimulus, meaning that results are reproducible between all-live studies and video-only studies (Balshine-Earn and Lotem 1998), and other researchers have used video playback in a similar fashion in the Cruz Lab. For these reasons, use of video playback technology was deemed appropriate to test the effects of a secondary stimulus (the light-reducing filters) rather than the primary stimulus (the displaying male *C. horei* versus *M. estherae*).

Furthermore, time spent by a female near a male has been shown to indicate mate preference in cichlids (Coulridge and Alexander 2001). This means that time spent in 'preference zones' near the respective videos should be able to serve as a reasonable proxy for mate preference.

Video playback is not without its critics. D'Eath and coworkers (1996) found that hens could not discriminate on video between other hens of the same species versus those of a different species. Indeed, it seemed that the video stimulus had no effect on the hens at all in some of the experiments in the latter paper. There are numerous possible reasons for this, but most obvious is that modern screens are optimized for human viewing, not for animals, and therefore may operate in sections of the light spectrum that are only partially visible to nonhuman organisms while ignoring the portions of the spectrum those organisms do use. The refresh rate of screens, or the rate at which new data are fed into the displaying device or monitor, is also optimized for humans, and images may not be perceived in the same way by non-human observers. However, based on previous research (see above), video playback is expected to yield meaningful results in the present study.

Basic Biology

The Cichlidae are a family of freshwater paleotropical (African and Eurasian tropics) and neotropical (American tropics) fish (Leveque *et al.* 2008). They constitute one of the most diverse vertebrate families, with estimates of diversity ranging anywhere from 1500 to 4000 species, with likely many more undiscovered or driven to extinction before scientific discovery (Lowe-McConnell 1997). Certain members are valuable in the pet trade, while others are of interest for human consumption (e.g. tilapia) (Hishamunda and Ridler 2006). As a food source, cichlids have likely been exploited by local peoples for thousands of years (Canonico *et al.* 2005). The family is highly genetically, phenotypically, behaviorally, and ecologically variable (Leveque *et al.* 2008). They vary from sunfish-like flatness to torpedo-like streamlining, with a wider middle and tapered ends, and exhibit adult size differences of greater than an order of magnitude (~2.5cm to almost a meter) (Jepsen *et al.* 1999, Smith 1998).

Most cichlid species are sexually dimorphic, with the males brightly colored with assorted pigments and the females more drab and cryptic (Herler *et al.* 2010). Such features are common in fish where female sexual selection plays a prominent role in the mating system (Stelkens *et al.* 2008; Zajitschek *et al.* 2006; Pilastro *et al.* 2004).

Cichlid Diversity

Within the Cichlidae family, the cichlids of the East African Rift Valley Lakes (Figure 1) are of particular interest. They represent the greatest vertebrate radiations known, with perhaps as many as 1000 species of cichlids in Lake Tanganyika alone (Day *et al.* 2008). This is even more remarkable when considering the hydrogeologic history of the lakes, which includes periods of regression and desiccation due to massive drops in lake levels (Cohen *et al.* 1997). Both Lake Malawi and Lake Tanganyika have sufficiently deep basins to accommodate great drops in water level (700m and 1500m, respectively) before total desiccation, but drops less than this can still isolate certain portions of the lake and perhaps create habitat islands (Cohen *et al.* 1997). Lake Victoria is significantly shallower than both of its more southerly companions, with a maximum depth of only some 80 meters (Johnson 1996). Evidence from sedimentation cores

and other paleoclimatological data suggest that the lake may have been nearly completely dry as little as 12.5 thousand years ago (kya), meaning that its remarkable cichlid diversity, with some several hundred species, evolved almost entirely within the period from re-hydration to the present (Cohen et al. 1997). If this were indeed correct, the lake would have the fastest rate of vertebrate speciation known for any locale. Maximum ages for the three lakes are estimated at 9-12 million years ago (mya) for Tanganyika, 4-9 mya for Lake Malawi, and ~50 kya for Lake Victoria (Johnson 1996). Both Lake Tanganyika and Lake Malawi are thought to have had at least some water continuously for most of their histories, thus suggesting an earlier date of divergence and speciation of their respective cichlid faunas in relation to that of Lake Victoria. Indeed, the age of divergence between the two major clades of Lake Malawi haplochromines (the predominant 'species flock,' or sub-subfamily, of cichlids within the Rift Valley Lakes) is estimated at 1 mya (Moran et al. 1994) and ages for Lake Tanganyika are even older, though still subject to some debate (Kocher et al. 1995). The debate about Tanganyikan divergence time hinges on whether the lake was colonized from surrounding rivers and then closed off, allowing endemic, or native and location-specific, fish to evolve, or whether the lake and surrounding waterways evolved in parallel before the lake was sealed, which several researchers have found empirical support for (Salzburger et al. 2002). This latter evidence would argue for a younger date of divergence than the former, and additional phylogenetic studies are needed to clarify which scenario is more likely. The radiations within each of the great lakes are all distinct and pairwise monophyletic, meaning that all fish within each lake are more closely genetically related to other fish within the same lake than to fish from one of the other lakes, despite apparent convergent evolution (Meyer et al. 1990).

Primary Radiation and Causes

The haplochromine cichlids of Lake Malawi, Lake Tanganyika, and Lake Victoria share several behaviors. Many haplochromines of Malawi, Tanganyika, and Victoria carry their eggs within their buccal (mouth) cavities (the fish are thus termed 'mouthbrooders') until at least some level of maturation, as a form of protection against predation or stochastic loss (Salzburger et al. 2005). Some cichlids retain their young within the buccal cavity until they are several weeks old, sucking them back into their mouth when necessary, whereas others release their young into sheltered areas upon reaching an age capable of free swimming (Wickler 1962). This mouthbrooding behavior can lead to a bizarre sort of internal fertilization: the female is occasionally too eager to pick up the eggs she lays and deprives the male of sufficient time to fertilize them; the male therefore presents the female with egg spots on his anal fin, which serve as 'dummy' eggs. While the female is attempting to pick up these 'dummy' eggs, the male releases sperm, which she in turn swallows, ensuring fertilization of the clutch (Wickler 1962). Some cichlids present in all three lakes also engage in substrate spawning, where eggs are deposited on sand or mud-bottomed areas and are externally fertilized, and may also engage in male 'lek' behavior, where territorial males defend bowers or other superior habitat in order to attract female mates (Stauffer et al. 1997; Hert 1990). This difference in parental care has been found to neatly divide each of the cichlid flocks within Tanganyika and Victoria into two separate groups, and is the first major cladogenesis, or genetically differentiating event, within the two flocks (Nagl et al. 200; Sturmbauer 1998). Results for Malawi show a similar pattern (Kocher and Danley 2001).

Another unique aspect of the Rift Valley lakes is that the mouthbrooding cichlids of Lake Tanganyika are also a target of the only known obligate brood parasite fish, the 'cuckoo catfish,'

Synodontis multipunctatus. An obligate brood parasite is entirely dependent upon its hosts for reproductive success, and is unable to raise offspring on its own. The parasitic catfish spawn at the same time as the copulating cichlids, and consume the eggs of the cichlids while depositing their own (Wisenden 1999). The female cichlid does not recognize the difference between the eggs, and picks up the catfish eggs as well as her own (Wisenden 1999). The parasitic catfish young develop significantly faster than those of the cichlids, and devour the cichlid fry while still within the female cichlid's mouth (Sato 1986). *S. mulitpunctatus* is well adapted to this form of alloparental care, which occurs when an organism cares for young to which it is unrelated. The young catfish develop at much faster rates than their closest genetic relative, *S. lucipinnis*, even at very young ages (Cruz, pers. comm.). When introduced to each other in artificial environments (such as aquaria), the catfish will readily parasitize non-sympatric species (species whose home ranges do not overlap in the wild) of mouth-brooding cichlids, such as those from Lake Victoria or Lake Malawi (Wisenden 1999).

Secondary Radiation and Causes

Ecological niche partitioning, where organisms specialize to take advantage of certain characteristics of their environment, is also evident within all three lakes. Highly specialized cichlids inhabit all manner of different trophic positions. There are snail-crushers, algae eaters, insectivores, carnivores, and even a certain Tanganyikan species (*Perissodus microlepis*) that feeds exclusively on the eyes of other fish (Hori 1993). It is thought that cichlid adaptability of the pharyngeal jaw – a second jaw set into the back of the mouth - has been critical to this trophic differentiation as well as allowing for the aforementioned mouthbrooding (Reinthal

1990; Hulsey *et al.* 2008; Liem 1974). Competition for food resources is thought to have led to this wide array of jaw morphologies, and is thought to have been the cause of the second major radiation within each of the three lakes as well as allowing for the large number of sympatric species that co-exist (Reinthal 1990; Hunter 1998).

Tertiary Radiation and Causes

The third major radiation event in Rift Valley Lake cichlids is most often linked to divergence in sexual preferences in females and attendant coloration in males (McKaye 1991; McKaye *et al.* 1984; Seehausen and van Alphen 1998). Seehausen and van Alphen (1998) showed that under natural lighting conditions, females were positively assortative in mate selection (the females preferentially selected males from their own species), whereas under monochromatic light selection became decoupled from species (females cease to select a mate from their same species), and instead activity level and size became the best predictors for selection. This is because the entirety of a male's coloration is visible under natural light, but under monochromatic light everything except a single color is washed out, such that the coloration cues normally used by a female are absent. This finding implies that coloration is important in maintaining existing diversity by sexual isolation (Seehausen *et al.* 1998). Furthermore, there is more male color variation within a species complex than between multiple species complexes (Deutsch 1997).

There are a number of theories that could potentially explain the latter phenomenon. Understanding how and why Rift Valley cichlids evolve is imperative if researchers do intend to use them as model evolutionary organisms, and so these theories and their pertinence to the Rift Valley cichlid systems should be explored. Three different, albeit not necessarily mutually

exclusive, hypotheses will be discussed below in the context of cichlid speciation. Firstly, Fisher's (1915) 'Runaway Hypothesis' holds that female preference for traits that originally signaled a fit mate, and therefore better potential for fit offspring (well adapted to the local environment and able to produce many offspring that survive into sexual maturity), may be taken to extremes. Over time, this may lead to exaggeration of the trait via a positive feedback loop females prefer males with the most extreme trait, which are more likely to produce male offspring with the extreme trait, and those male offspring will be preferred by the females of their own generation, and so on - until natural selection or environmental, physiological, or other constraints hold it in check (see below). An excellent and well-studied example of this type of opposing sexual selection and natural selection comes from the 'swordtails' (Xiphophorus spp.), the males of which, as the name implies, have elongated tail fins that take on a sword-like appearance (Rosenthal *et al.* 2001). The longer a male's sword, the more preferred he is by females; however, it also impairs his ability to escape predators (Basolo and Wagner 2004). Males in populations with fewer or absent predators therefore have longer, more exaggerated swords, and those in populations that suffer relatively heavier predation are constrained to shorter tails (Basolo and Wagner 2004). Theoretical objections to this model include the question of whether or not sexual selection could overcome the opposing effects of directional selection (Rowe and Houle 1996) as well as the technical difficulties of ever empirically testing such a hypothesis in a controlled experiment, as opposed to observational studies, such as those cited for the *Xiphophorus* example.

A second theory is the "Sexy Son Hypothesis" of Weatherhead and Robertson (1979). It holds that the optimal mate choice for a female is a mate that will most enhance the attractiveness of their male offspring (i.e. the male's 'sexiness' to females of the same species),

and therefore the male offspring's reproductive success, as this will produce the most grandchildren, great-grandchildren, and so on. This hypothesis has two major conditions: that the trait and preference for it be heritable and that the population or species in question has a mating system in which males compete for females, and not the other way around (since female selectiveness is the driving force; although an analogous hypothesis could be proposed for systems in which females compete for males). Cichlids do indeed fulfill both of these requirements: the aforementioned leks show male competition, and Seehausen *et al.* (1997) have shown the heritability of coloration and preference.

Lastly, the 'Handicap Hypothesis', first proposed by Zahavi (1975), suggests that males may use 'handicapping traits' to attract females by indicating overall health, bestowed by relatively more advantageous genes than fellow males possess, that allow the male to survive and thrive despite the noticeable disadvantages. In short, this means that there will be constant sexual selection in favor of the handicap, while natural selection works in opposition. The handicap also need not necessarily be a physical attribute; behavioral actions also qualify. Lek building behavior in some cichlids is an obvious example: the male must forgo foraging and self defense, which presents a handicap, in order to create the lek; therefore males with large territories and larger bowers have demonstrated their fitness despite the handicap.

The three hypotheses described above are not mutually exclusive, especially as pertaining to male nuptial coloration, but do have some distinction: the primary difference between the Handicap and Runaway hypotheses is that is that female preference for a sexually selected trait increases in frequency as a result of co-inheritance (the two traits, the preference and the trait, are passed to offspring together) of trait and preference (Runaway), rather than via direct selection owing to increased fitness of females that choose higher fitness males (Handicap). This

difference, while conventionally accepted to delineate the two, can be bypassed to synthesize the two. For instance, if allocating pigmentation to the skin is particularly physiologically draining for an individual, or an individual must go out of its way to obtain foods with which to synthesize the pigments, then the coloration could be viewed as a handicap. If the ability to sequester those pigment-creating substances were heritable, then coloration could also be viewed as falling simultaneously under the Handicap and Runaway Hypotheses. Note that this example is for illustrative purposes only; no such link or situation has been empirically established in the literature.

As the above theories and hypothetical situation illustrate, it is imperative to understand how cichlids obtain their coloration from a physiological standpoint, in order to determine which of the above theories (if any) best describes the situation, or whether it is some combination thereof, since coloration is so central to haplochromine speciation in the Rift Valley lakes. Maan et al. (2006) found that certain species' coloration was carotenoid (a type of chemical obtained from the diet) based, illustrating the potential for honest signaling of fitness, meaning that the coloration 'signal' reliably predicts the male's fitness. Maan and coworkers (2006) also found that degree of redness and size of the male cichlid's territory were independent indicators of mating success, illustrating the theory that females use multiple criteria in selecting a mate. It was found that individuals with favorable scores in both of these indicators had significantly lower parasite loads, implying that both are indeed honest indicators of fitness (Maan et al. 2008). A more parasite-ridden male would presumably have trouble defending a large territory and putting energy into coloration as compared to a male with fewer parasites, since the parasites drain energy from their host. In addition, since carotenoids are also involved in the immune response, a sickly male would have fewer to allocate towards coloration (Lin et al. 2010). This

type of parasite-mediated sexual selection may be widespread in nature (Hamilton and Zuk 1982). In addition to this, Maan and coworkers found that red coloration is energetically more costly than the other main color, yellow (which is produced by lower densities of carotenoids), of the species they examined, meaning that not only is coloration an honest indicator, but that red coloration is a superior indicator to other colors on the fish. Further work by Maan and coworkers (2008) indicates that an analogous result exists for another species of cichlid, one in which the primary male nuptial color is blue: more intense blue coloration was significantly correlated with decreased parasite loads. Should further research on other species indicate a similar trend, it would lend considerable support to the idea that the Handicap Hypothesis plays a role in these systems.

Communication and Sexual Selection in Cichlids

Understanding the basis for mate choice, and therefore speciation, is important to properly designing an experiment in which this is to be tested. For instance, if only visual selection is the object of focus (as it is in the present study), then the acoustic and olfactory elements of sexual selection must be controlled for.

Acoustic Communication

Sound production and communication via such sounds are understudied in cichlids, though this is changing. This lack of study is because most of the sounds produced by cichlids fall well outside the human range of hearing, either by being too low (< 300 Hz) or too high in frequency (> 4000 Hz) depending on what sound is being produced and what the situation is (Nelissen 1978). Nonetheless, certain species do produce sounds, typically in courtship or aggressive encounters (Verzijden *et al.* 2010, Smith and van Staaden 2009, Amorim 2008).

Nelissen (1978) found that sounds produced vary by species, and that some species share certain sounds. Males of closely related sympatric species may use sounds to reinforce species identification and differentiation, and affect female mate preferences (Amorin *et al.* 2004, Verzijden *et al.* 2010). Males also use sound to modulate otherwise aggressive displays, in order to reduce the risk of the displays escalating into a potentially harmful physical contest (Bertucci *et al.* 2010).

However, not all sounds produced by cichlids are intentional or behaviorally significant. *Haplochromis burtoni*, for instance, produces sounds but these may be entirely accidental or simply the byproduct of other activity by the fish (Hirata and Fernald 1973). More research, particularly with updated equipment, is needed to fully establish an understanding of cichlid acoustics and their functions within a communication framework.

Chemical Communication

The importance of olfaction and pheromones in fish communication is well documented (Burnard *et al.* 2008). Olfaction may aid individuals in a wide variety of tasks, including individual recognition, mate choice, territory location, predator avoidance, and prey capture (Burnard *et al.* 2008). In cichlids, olfaction has been shown to be useful in distinguishing conspecific males from heterospecific males ('same species' and 'different species,' respectively) for spawning purposes (Plenderleith *et al.* 2005). Olfaction also allows substrate spawning cichlids to locate and fan their eggs at night, when the eggs are not visible to the parents (Reebs 1992). The parents rejected empty nests, fake eggs and nests through which deoxygenated water was flowing (which would kill the eggs, eliminating further need for parental care) but were willing to fan their own eggs hidden from view as well as the eggs of

other cichlids of the same species, indicating that tactile and visual cues alone were insufficient, while olfaction itself was necessary (Reebs 1992). Reebs (1994) also showed that chemical signaling was used for nocturnal mate recognition by the female convict cichlid, *Cichlasoma nigrofaciatum*, indicating that the females would be able to defend their broods against unfamiliar fish, even while being unable to visually identify the threat.

It has also been found that olfaction plays a part in mating behavior for the cuckoo catfish. In experimental tanks, the catfish spent significantly more time around a nozzle releasing water that had contained cichlids into the tank than a nozzle releasing purified water (Cruz, pers. comm). Further research is necessary to clarify the relative importance of chemical signals versus visual and other signs for triggering parasitism by *S. mulipunctatus*.

Visual Communication

As mentioned above, visual cues have been shown to be of utmost importance for communication between cichlids, and are the focus of the present thesis. A full description of the visual elements useful to cichlids is important to be able to draw conclusions from the results of the present study.

Body positioning, in addition to coloration, is useful in conveying information during both courtship and agonistic, or hostile, encounters (Baerends 1994). Courtship behaviors involve a 'lead swim' by the male, in which he entices a female towards his territory, 'quivering,' in which the male slightly curls his body towards the female and shakes vigorously, displaying his egg spots, and several other behaviors (Baerends and Baerends von Roon 1950). Agonistic, or aggressive, displays include directly chasing the offender, as well as a frontal

display, in which the cichlid flares its gills and holds its dorsal fin erect in an effort to make itself appear larger (Baerends and Baerends von Roon 1950).

Cichlid coloration is one of the most studied, and most interesting, aspects of the family's biology. Cichlids are able to control their own appearance by manipulating large, branched cells called chromatophores (Rican *et al.* 2005). Pigments within these cells can be aggregated or dispersed along the branches, and are particularly useful for enhancing color to attract mates or to assert dominance during intraspecific agonistic encounters (Beeching 1995). For instance, the cichlid *Astronotus ocelattus* undergoes a radical change in body color after defeat in combat: its normal olive-green coloration darkens to a near black with white barring, which makes the bearer the subject of less aggression from conspecific males (Beeching 1995). This type of physiological control is not widespread in the fish world, but it does occur in some cichlids, guppies, and sticklebacks (Kodric-Brown 1998). Morphological control of pigmentation is much more common; this is done by shifting the relative amount of pigment in each chromatophore, rather than adjusting the pigmentation's positioning within the chromatophore itself (Voss 1980).

Different chromatophore types have different coloration properties – based on the different types of carotenoids and other chemicals the cells take up - and reside in different layers of the tissue. Barlow (2000) found that there are at least three different types: melanophores, guamophores, and erythrophores. Melanophores are by far the most prevalent in most cichlid species, and are filled with melanin, a darkening agent. These cells produce much of the striping, barring, and spotting typical of many cichlids (Beeching *et al.* 2002). Guamophores typically impart a blue or green color, with an iridescent shimmer. Erythrophytes give a red color to the individual, and are typically located around the edges of the fins (Lanzing and Bower 1974). Xanthophores are a fourth type that give an orange or white-ish color to the skin (Dickman *et al.*

1988). Melanophores are typically closer to the surface of the skin, and therefore take precedence over other, more deeply set chromatophores when active (Barlow 2000). Melanophore death can lead to unintentional and perhaps permanent shifts in expressed color, highlighting the importance of this type of chromatophore (Dickman *et al.* 1988).

What is the value of all of this color-changing machinery and the energy put into elaborate coloration displays? Sexual selection is often assumed to be a driving force in evolution (see 'Tertiary Radiation and Causes,' above; Magalhaes et al. 2009) and therefore in maintaining barriers between species. Indeed, there is ample evidence that phenotypic differences override genetic differences for mate selection, since phenotypic differences are better predictors of (Stelkens and Seehausen 2009). Despite all of this, hybrids – progeny of two heterospecific individuals - do occur in the wild and in laboratory conditions (Stelkens and Seehausen 2009; Seehausen et al. 1997; Seehausen et al. 2010). These hybrids have been found to be fully viable in lab aquaria without exhibiting premature death of the individual (intrinsic breakdown) or infertility due to incompatible genetic factors for several generations (Maan et al. 2010). Extrinsic barriers, or 'external' barriers such as behavior or coloration, thus presumably maintain species barriers because intrinsic barriers are insufficient. It seems unlikely that two species with radically different trophic modalities, or modes of feeding (See 'Secondary Radiation and Causes,' above) would mate, since individuals from such species would likely be fairly differentiated in body size and shape, and female mate choice would thus prevent interbreeding. Sexual selection and mate choice with regard to coloration are directly implicated, since these two factors must be contributing to maintenance of species barriers. Females select against individuals that lack the exact proper coloration, meaning that hybrids with intermediate colors would be rejected by females from both parental species (Seehausen *et al.* 1998). This

type of pre-zygotic, or pre-fertilization, reinforcement is common in the natural world (Howard 1993; Noor 1999). Differences are thus accentuated between populations of species whose habitats overlap when compared to populations of the same species that do not have overlapping habitats (Stelkens and Seehausen 2009).

Species Concepts

What delimits a cichlid species? Answering this question is essential to using cichlids as model evolutionary organisms because until what a species is defined as is clear, it is impossible to discuss modes or drivers of speciation. There are currently several possible definitions of 'species,' and cichlids do not fully meet any of them .Since cichlid hybrids are naturally found in the wild, and occasionally at moderately high proportions, these species fail to meet the criteria of the 'Biological Species Concept,' which maintains that a population be reproductively isolated from other such groups (Maan et al. 2010). Given the incompleteness of cichlid phylogenies, due to missing species, incomplete lineage sorting, and other confounding effects, it would be premature to declare that the cichlids of the Rift Valley meet the criteria of the 'Evolutionary' Species Concept', which is that a group is descended from a common ancestor and maintains its integrity from other such groups through space and time. Similarly, cichlids do not fulfill the requirements of the 'Ecological Species Concept,' which states that a species is a group of organisms adapted to one particular set of resources (their 'niche'); they often v utilize the same resources and differ only in coloration but are positively assortative in mate choice nonetheless (See 'Tertiary Radiation and Causes', above). It seems that a different species concept is needed to define the populations of the cichlid radiation as species as they intuitively appear to be. Hugh

Paterson (1993) provides such an additional definition with his 'Recognition Concept,' which bases species on shared reproductive behavior and mate recognition. This theory is not without its critics (Brothers and Newberry 1990), but has also received support from others (Ambrose 2003; Marin 1997; Polakow *et al.* 1995). The debate over species concepts is a lively and longlived one in the biological world, and is likely to go on. Full resolution of this issue with regards to the cichlids of the Rift Valley is not expected for some time.

Modern Threats

For all the remarkable diversity and biological importance of the Rift Valley Lake systems, they exist in a constant state of risk, loss, and degradation. Lake Victoria, in particular, has been much more affected than any of the other lakes of the region (Beeton 2002). A major source of disturbance to the ecosystem is introduced species (Vitule *et al.* 2009, Wilson *et al.* 2007, Beeton 2002). Primary amongst these are the Nile perch (*Lates niloticus*) and the water hyacinth (*Eichhornia crassipes*) (Vitule *et al.* 2009, Wilson *et al.* 2007). The perch was introduced in 1954 in order to establish Lake Victoria as a sport-fishing destination (Vitule *et al.* 2009). By the 1970s, the perch was present throughout much of the lake, though in low abundances (Pringle 2005). By the 1980s, the perch had come to dominate the lake's fisheries, and catches increased yearly since then, peaking in 2003 (van der Knaap and Ligtvoet 2010, Pringle 2005). The effect of the perch's introduction on the endemic haplochromine cichlids has been devastating (Hecky *et al.* 2010). The cichlids suffered a "catastrophic decline in abundance and biodiversity" (Hecky *et al.* 2010). A highly simplified food web, with the perch as the top predator of the food chain has replaced the diverse and complex system that previously prevailed

in the lake. Some cichlids were able to adapt to their new environment by exploiting different food resources, but most were unable to and either disappeared or became more rare within their home ranges. Subsequent to heavier exploitation of the Nile perch beginning in the 1990s and onward, a few select species of zooplanktivorous, or zooplankton eating, and small benthivorous, or bottom-dwelling, cichlids were able to recover (Kishe-Machumu *et al.* 2008; Witte *et al.* 2008). The overall downward trend continues, however (Hecky *et al.* 2010).

The second invader, the water hyacinth, has done less damage than the perch, but still poses significant problems for local inhabitants and biota (Opande et al. 2004, Wilson et al. 2007). The hyacinth was likely initially imported from South America as an ornamental plant for a domestic pond, and then accidentally introduced into the lake and its surrounding waterways. The hyacinth is free-floating and, in the herbivore-free and nutrient-rich environment of the lake, forms dense mats that can cover considerable areas, choking out native flora and blocking sunlight to the lake's waters (Kateregga and Sterner 2007). The hyacinth grows particularly well in areas that are relatively sheltered, such as bays or coves; these areas are also likely to be used by local fisherman, and the hyacinth can significantly impede this activity (Opande *et al.* 2004). The mats of hyacinth also serve as refugia for larval invertebrates, including those that become possible disease vectors, while simultaneously creating almost-anoxic zones which inhibit predation by insectivorous fish (Masifwe et al. 2001, Bienen 2005). On this front, however, there is good news. The infestation by the hyacinth may finally be contained, largely thanks to one of the few cases of successful bio-control on the record (Sikoyo and Goldman 2007). Weevils (*Neochetina* spp.) that feed on the hyacinth in South America were rigorously tested for tolerance to local flora, and, after ascertaining there was no risk of a host-plant change, were introduced to the lake (Sikoyo and Goldman 2007). These weevils, aided by physical and

chemical eradication programs, have succeeded to the point that researchers now postulate that a hyacinth resurgence is unlikely unless a major disruption occurs within the weevil populations (Wilson *et al.* 2007).

Other anthropogenic effects threaten the lake as well. Primary amongst these is the accelerating eutrophication, or excessive input of nutrients, of the lake (Stager et al. 2009). Widespread local deforestation has led to increases in sediments being carried downstream and into the lake; agricultural run-off has also significantly increased as more of the arable land in the Rift Valley comes under agricultural cultivation (Oago and Odada 2007). In addition to this land coming under cultivation, much of the intensification is done improperly and in unsustainable manners; swidden ('slash and burn') agriculture is widespread (Odada et al. 2009). This eutrophication supplies nutrients for unwanted pest species, such as the water hyacinth, and for massive algal blooms, which occur when the algal biomass and the area inhabited by it suddenly increase by a large amount (Lung'ayia et al. 2001). Such blooms are good indicators of declining water quality, and can sometimes produce toxic byproducts harmful to lake inhabitants or local humans (Lung'ayia et al. 2001). In addition to all of this, eutrophication also presents a direct threat to haplochromine cichlid species diversity (Seehausen et al. 1997). By reducing the amount of light passing through the water and constraining color vision, eutrophication limits the ability of cichlids to properly select a conspecific mate based on that mate's coloration. The incidence of hybrids in the wild is significantly greater in regions with high turbidity, even when comparing populations of the same species between these areas (Maan et al. 2010). Males in areas with high turbidity tended to be less colorful than their clear-water counterparts, and females tended to be less selective based on color (Maan et al. 2010). All of these studies were conducted on Victorian cichlids, as this is the lake in which turbidity and eutrophication are the

most advanced. As the numbers quoted at the outset of this Introduction indicate, research on the effects of turbidity and eutrophication on the other lakes, particularly Lake Malawi, is sorely lacking.

A second major (mostly) anthropogenic threat is the establishment of a large oxygendeprived (anoxic) zone at the bottom of the lake (Nijiru et al. 2008). This zone is mostly uninhabitable, particularly to Nile perch (Nijiru et al. 2008). Occasional upwellings of this anoxic water have caused large fish kills. Interestingly, some invertebrate species, particularly shrimp, seem able to survive in the oxygen-deprived environment (Budeba and Cowx 2007). This refugium may be one of the only places in the lake that the perch is unable to exploit as a food reservoir, and, as haplochromine stocks continue to decline, these shrimp may provide an inextirpable food source for the perch (Goudswaard et al. 2006). Exactly what has created this anoxic zone is somewhat unclear. It is commonly thought that insufficient mixing of the water due to abnormally low winds has allowed oxygen-deficient water to settle to the bottom, and that these low winds are a product of a changing regional climate (Song et al. 2004; Verburg and Hecky 2003). The loss of detrivorous, or detritus consuming, cichlids also likely exacerbates the problem by increasing the amount of organic matter that makes it to the bottom. These fish consumed much of the organic matter that now settles to bottom and is aerobically decomposed by microbial communities, further reducing dissolved oxygen levels in the water (Lung'avia et al. 2001).

The human element of all of these interacting factors must also be assessed in order to fully appreciate the conservation and research issues facing the region. The fishing industry that has evolved around the Nile perch since the 1980s brings employment and export earnings to region, but at a pernicious cost. The overwhelming majority of perch caught are shipped abroad

where their filets bring a much higher market value (van der Knaap and Ligtvoet 2010). This also intensifies the deforestation problem, as large quantities of fuel, in the form of wood, are required to dry the oily flesh of the perch (Matsuishi et al. 2006). While the yearly catch is theoretically sufficient to provide sustenance to the local population, malnutrition rates are "disturbingly high" along the lake shore communities (Geheb *et al.* 2008). Cichlids previously provided a sufficient source of food for subsistence fishing, or fishing solely for one's own consumption; given the Victorian cichlids spectacular collapse this method of securing nutrition no longer works (Gehen *et al.* 2008). The wealth generated by exporting perch is also not evenly distributed within the communities with export plants: women and children are far worse off than men, and have significantly higher rates of malnutrition (Geheb et al. 2008). Furthermore, the traders that facilitate the export of the perch to Europe and the Middle East might not be simply taking a commodity away, but may be bringing something far more volatile back with them. Allegations have been made the planes that fly the fish out come back loaded with weapons for sale to local governments, militias, and warlords (Darwin's Nightmare 2005). If this is true, it could be shortening the fuse on the socio-politico-economic time bomb in what is already an unstable and dangerous region (Rwanda, site of the 1994 genocide, is in the Rift Valley).

In light of the above information, it should be clear that further research on the whole system is needed. In particular, research on the cichlids of Lake Tanganyika and Lake Malawi is needed to better anticipate, and prevent, events like those in Lake Victoria from re-occurring in order to prevent similar losses of biodiversity. Hence the present study, which aims to examine the effects of turbidity on mate choice by females from the species *Metriaclima estherae*, a Lake Malawi haplochromine cichlid.

The following questions were addressed by the present study:

1. Do the stimulus videos actually stimulate activity?

2. Do the fish used for the trials have a bias towards one side of the tank in the absence of a stimulus?

The first two questions are important in terms of assessing the rest of the experiment. If the fish show no response to the videos in terms of activity level, then any results from the experiment have no meaning, as the stimuli provided were insufficient to elicit a behavioral response. If the fish show bias in favor of one side of the tank, then appropriate statistical controls would have to be implemented.

3. Do the fish prefer the video of conspecifics over heterospecifics?

4. Do the filters affect the relative amounts of time spent with the video of conspecifics versus heterospecifics?

If the female *M. estherae* show no preference for a conspecific mate over a heterospecific mate, then assessing the effect of any other experimental treatment becomes impossible. If, however, the females do exhibit a preference, then assessing relative preferences under multiple experimental treatments is possible.

Methods

Study Animals

A population of *Metriaclima estherae*, "Red Zebras," (= *Maylandia estherae* = *Pseudotropheus estherae*) was commercially obtained from various sources (Fig 2). This species is widely available in the pet trade, but is listed as 'vulnerable' in the wild on the IUCN Red List (http://www.iucnredlist.org/apps/redlist/details/61140/0). It is endemic to five locations in Lake Malawi. The fish were reared in large glass aquaria, with daily light:dark cycles of

approximately 14:10 hours, and fed vegetarian algae flakes daily, prior to the trials. The fish were sexed into male and female populations using a combination of physiological traits (number and location of egg spots, shape of dorsal fin, and size of ovipositor vent) and behavioral traits (mouthbrooding is a guarantor of female sex). Males come in two color morphs: an entirely orange-red morph and an orange with blue stripes/iridescence morph. The video was created using an orange morph male, as most males in the purchased population were of the orange morph (there is no reliable data on prevalence of the two morphs in the wild). No females shorter than 4 cm were used for the video playback trials, as females shorter than 4 cm may not be sexually mature.

No females that had mated within the past 4 weeks were used for video trials. Females that were found to have mated (i.e. were actively mouthbrooding) were sequestered until the four weeks had expired before being used.

Stimulus Videos and Tank Setup

Two stimulus videos were created, one showing courting and spawning between two *M*. *estherae* and the other showing courting and spawning between two *Ctenochromis horei* (*C. horei* video courtesy of Marcus Cohen) (Fig 3). These two species are differently colored, and have slightly different body shapes, but have similar courtship and display patterns so should elicit similar responses from females of either species (McElroy and Kornfield 1990).

The videos were shown simultaneously by monitors flanking the central tank, where a female was housed (Fig 4). Three 'treatments' were applied to the videos: no filter, a light filter, and a dark filter. The filters were pieces of opaque Makrolon® polycarbonate (Types DP1-1853 and DP1-1873, respectively), cut to the dimensions of the tank. The light filter blocked 20% of

incoming light, and the dark filter blocked 55% of incoming light, as measured using a standard photometer.

A preference zone of 10 cm (approximately 2-2.5 cichlid female body lengths, based on the smallest female used for the video trials) was marked off at each end of the 110-liter tank. The tank's water temperature was held at 21° C, and a 10-15% water change of the tank was performed after every second fish. Water levels in the tank were held deliberately low, to coincide with the height of the monitors. Gravel was also pushed up against the sides of the tank to coincide with the bottom of the monitor. These two modifications were intended to increase the appearance of a contiguous environment for the female.

Video Playback Trials

Two trials were run per treatment per female, for a total of 6 trials. A total of 18 females were utilized for the study. The side that each video was on was swapped before each trial. The trials lasted for 25 minutes each and were each preceded by a 5-minute pre-trial, in which no stimulus was provided. Number of crosses into each preference zone was recorded, as was the length of each stay, during both the pre-trial and the trial. A 'cross' was defined as any portion of the fish passing the 10 cm line, and lasted until the last portion of the fish crossed out of the 10 cm zone.

A cooldown period of a minimum of 15 minutes was provided to the female between each trial, during which no stimulus was provided. The filming area was kept clear of people during the filming process, in order to avoid disturbing the female and altering her behavior.

Each trial was recorded using a stationary camera, and the video was later analyzed as described above. Utilizing recorded video in this way, instead of watching the trials live, allows for greater specificity in recording the duration of each cross.

Statistical Methods

Statistical significance was assessed using the program R, version 2.10.1. Normality of data sets – meaning how well the data fits a normal or 'Bell' curve - were assessed qualitatively, rather than quantitatively, using quantile-quantile plots between the data and a theoretical normal distribution. A quantile-quantile plot compares the shapes of the distributions of two data sets. When the first data set is the normal 'Bell' curve, then a linear relationship on the graph indicates normality of the second data set (see Figure 5 for an example). The Shapiro-Wilk test formally tests the normality of a data set, but was not appropriate for this experiment, since excessive use of such tests inflate the type I error rate. A type I error occurs when a null hypothesis is incorrectly rejected. Using the common significance level of 0.05 means that there is 95% confidence in the result, and so running the 14 Shapiro-Wilk tests (on each of the 8 raw data sets, plus the three untransformed ratios, plus the three transformed ratios) would mean that there is only a $.95^{14} = .4876$, or 48.76%, certainty that all of the tests returned 'true' results.

In order to assess whether or not the treatments affected relative mate preference, a ratio of the time the female spent within the *M. estherae* preference zone divided by the total time spent in all preference zones was calculated for each trial for each female. A change in the values for the ratios between one treatment and another would indicate the amount the females prefer one video over the other had changed. Data for the ratios were transformed using the arcsine transformation, p'=arcsine(sqrt(p)), as this transformation is known to improve the normality and reduce discrepancies in variance of the data (Whitlock and Schluter 2009) (Figure 5). Ratios

otherwise tend not to be normally distributed and have unequal standard deviations, which are two assumptions made when using the Student's T-test or running an ANOVA. In order to assess whether or not the videos stimulated activity, total time spent in all preference zones for each trial was compared for each of the treatment groups using paired t-tests.

Results

The male display videos significantly stimulated the activity level of the females (i.e. the females spent more time within the two preference zones with the videos playing versus when the videos were off), but differences in overall activity remained non-significant between all three of the treatment groups, with paired t-tests reporting p-values greater than 0.05 when comparing no filter versus the light filter, no filter versus the dark filter, and the light filter versus the dark filter. When comparing any of the three stimuli against the stimulus-less control, paired t-tests report p-values less than 0.002 (X \pm SEM for no filter = 196.77 \pm 20.28, for the light filter = 172.69 ± 16.40 , for the dark filter = 172.77 ± 21.26 , and for control = 63.19 ± 12.02). The female M. estherae did not prefer one side of the tank over the other, with a paired t-test reporting a p-value of 0.24 on a t-score of 1.2146 (X \pm SEM for the left side = 28.97 \pm 5.53 and for the right side = 34.222 ± 7.13). With no filter, the females significantly preferred the video of the male M. estherae with a paired t-test reporting a p-value of p<0.001 on a t-score of -4.97 (X \pm SEM for C. *horei* = 61.86 ± 8.77 and for *M. estherae* = 134.91 ± 15.37). With the light filter in place, females significantly preferred the video of the male *M. estherae*, with a paired T-test reporting a p-value of 0.003 on a t-score of -3.3961 (X \pm S.E for C. horei = 61.41 \pm 8.61 and for M. estherae = 111.27±12.96). With the dark filter in place, females significantly preferred the video of the male

M. estherae with a paired t-test reporting a p-value of 0.028 on a t-score of -2.3895 (X±SEM for *C. horei* = 73.55±9.82 and for *M. estherae* = 99.22±13.68) (Figure 6)

When comparing relative preferences on the arcsine-transformed ratios, there was no significant difference between no filter and the light filter, with a paired t-test reporting a p-value of 0.2231 on a t-score of 1.2647. There was a significant difference between no filter and the dark filter (p=0.0072, t= 3.0517) and between the light filter and the dark filter (p=0.0412, t= 2.2089) as reported by paired t-tests (X±SEM for no filter = 0.9989 ± 0.0376 , for the light filter = 0.9311 ± 0.0391 , and for the dark filter = 0.8463 ± 0.0313).

Discussion

The male display videos did stimulate the activity levels of the females which indicates that the videos hold some attractive property for the females used in the trials and that the females responses are not simply random actions. Furthermore, the females did not exhibit any bias towards one side of the tank in the absence of the stimulus videos. This latter result simplified statistical analysis, as the effects of covariance did not need to be accounted for.

In all cases the females preferred the video of the conspecific male over the heterospecific male. This result indicates that the females were able to perceive the videos and distinguish between conspecific and heterospecific organisms. This counters the objections raised by D'Eeath (see 'Video Playback' in the introduction), namely that optimizing the video for human viewing may compromise the videos ability to effectively convey its image to other species. The present results are also consistent with other studies performed using video playback technology to assess fish reactions to stimulus, particularly for species recognition (Marcowicz *et al.* 2010, Shashar *et al.* 2005, Clark and Stephenson 1999, Balshine-Earn and Lotem 1998).

The imposition of filters significantly altered the females' preference for the conspecific video versus the heterospecific video. The 80% transmission filter did not affect the cichlids relative preference when measured against having no filter in place. The 50% transmission filter significantly reduced the relative amount of time the females spent with the conspecific video when measured against both the 80% transmission filter and having no filter. The 80% transmission filter may not have reduced light transmission sufficiently enough to elicit a behavioral response in the females, because the difference was only by 20%. However, the 50% light transmission filter was dark enough to elicit a response, possibly because of the magnitude of light transmission reduction (50% less than no filter and 37.5% less than the 80% filter).

It seems that if water quality were to decline in Lake Malawi to a similar degree as what has occurred in Lake Victoria, an increase in hybridization rates for this species would be expected. Furthermore, as *M. estherae* is restricted to only a few locations around Lake Malawi, such hybridization could present a clear and present danger to the continuation of the species.

Hybridization as a result of impaired vision has been found to affect many species in Lake Victoria (Seehausen 1997). Given the results of the present study, there is a strong case for hypothesizing a similar pattern for Lake Malawi. As such, any management or conservation plan intended to preserve cichlid diversity in Malawi should include a robust component to prevent or mitigate any increase in turbidity or eutrophication in the lake.

Limitations of the Present Study

Unfortunately, the present study had several flaws. First and foremost, the stimulus videos were imperfect. The *M. estherae* video was zoomed in slightly more, giving the fish in it a larger appearance. As size has been found to be a factor in mate choice for many species of cichlids (Seehausen and van Alphen 1998), this presents a confounding factor in terms of

establishing reasons for preference of one video over the other. For future studies, the videos should be equal in this manner, for instance by recording the same tank set-up with each of the species while using similar sized males.

A second flaw with the videos was that the video looping used to create a constant stimulus for 25 minutes caused slight 'hiccups,' or brief moments when the video ceased to be continuous, in the video when it rolled over from finish to beginning. While the videos were created to minimize the effects of this, there was some potential for this 'unnatural' stimulus to alter female behavior. A potential solution is to create a single 25-minute stimulus video, rather than looping a several minute long video for 25 minutes. This presents its own complications, however, as capturing 25 minutes of display behavior for two males in which the males are equally active has the potential to be very difficult.

Finally, the two filters used for the experiment also have minor ultraviolet-blocking capabilities that are not necessarily commensurate to their ability to block the visible light spectrum. Since ultraviolet light is visible to cichlids (O'Quin *et al.* 2010), the filters may have affected the females' perception of the videos in ways not observable by human vision by blocking too much or too little ultraviolet light. Whether this had any effect or not is unknown at this point. Future experiments should use one set of filters that affect only ultraviolet transmission and a different set that only affects visible light transmission to determine whether blocking one spectrum and not the other affects female behavior.

Future Directions

Additional research in any field relating to the Rift Valley cichlids is important, as the above literature review showed. In particular, further study of the causes and effects of hybridization is critical to preserving cichlid diversity in the Rift Valley. As such, additional

studies similar to this one are suggested. Future researchers could potentially use other species from any of the lakes to examine how changing light conditions might affect the fishes' mate choice. Computer enhancement could also be used to directly change the coloration in a video, in lieu of using physical filters.

Another interesting avenue would be to explore how turbidity might affect hybridization between cichlid species with slightly differentiated body plans, in order to explore whether there is some critical threshold in water quality for such non-color-dependent matings. If such a threshold could be quantified, this would be useful in terms of establishing management and conservation plans.

In spite of the preceding evidence establishing the efficacy of video playback technology, its ecological value should still be further established. An experiment similar to the one presented here could be performed using all live fish. Barlow (2002) identified three general categories of behavior experiments in fish: full-access, female-only access, and restrained access. In full access experiments, fish are allowed to co-mingle freely and mate choice is evaluated. While this may most closely mimic real-world environments, mate choice behavior may be confounded by agonistic behaviors between multiple males (Barlow 2002). Such agonistic behavior may be more common in aquaria than in actual aquatic environments, as there is reduced potential for spatial separation. A second issue is that determining paternity can be difficult unless the actual spawning event is observed. In female-only access experiments, a smaller female is allowed to visit males that are confined to certain areas in a tank (potentially by using see-through walls with a hole large enough for the female but too small for the males). In this way, agonistic interference can be reduced, but the issue of paternity remains. In restricted access experiments, the fish are never allowed to come within contact with one another, perhaps

by barriers through which none of the fish may pass. These barriers may be porous (allowing transmission of e.g hormones). Video-playback trials would be considered restricted access. Any one of these types could be used, depending on the specific parameters used. For instance, a free-access or female-only access trial could be used by actually making the water turbid with some sort of water circulation system (this raises the issue of actually being able to see the fish and observe behavior, as noted in the Introduction). Such a system was used in a female-only access experiment to determine whether female sticklebacks preferred males with territory in clear or turbid waters (Engstrom-Ost and Candolin 2007).

Studies examining the affects of turbidity on other aspects of cichlid biology are also warranted. Van der Sluijs and Gray (2010) found that loss of communication viability in one category (e.g. turbidity affecting visual communication) may lead to compensation in other categories. Turbidity also directly affects these other communication categories (e.g. pollutants affect reception of hormonal signals) (van der Sluijs and Gray 2010). The plasticity of multimodal communication in cichlids should be examined further. The effects of turbidity on prey capture, prey abundance (including vegetation), and risk of predation should also investigated in the Rift Valley cichlid system.

Conclusions

The results of the present study indicate that an increase in heterospecific mate choice, and therefore hybridization, is to be expected in *M. estherae* if water clarity within the lake declines to a sufficiently low level, similar to Lake Victoria. Due to comparable results for multiple Victorian cichlid species, this hypothesis can be extrapolated to other Malawian species in addition to *M. estherae*. The decline in biodiversity that would result from an increase in

hybridization has economic and ecological impacts for the region. The fish of Lake Malawi feed a great deal of people, and a loss of fish numbers and diversity could lead to famine problems akin to those seen around Lake Victoria. The cichlids also provide numerous ecosystem services, such as controlling the larvae of disease-bearing invertebrates (Masifwa *et al.* 2001).

Further research is needed to understand how and why cichlids use visual information for mate choice and, therefore, speciation, and what mechanisms maintain those species barriers. The present study, as well as future ones, should be used to better inform and develop effective plans to conserve cichlid diversity in the region.

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Figures



Figure 1. A map showing the Great Rift Valley of Eastern Africa. The three lakes indicated with arrows are, from north to south, Lake Victoria, Lake Tanganyika, and Lake Malawi.



Figure 2. A typical male *Metriaclima estherae*. Note the solid orange color; a similar appearing male was used to create the stimulus video.



Figure 3. A typical male *Ctenochromis horei*. Note the distinctive coloration, and its differences from the male red zebra in figure 2.



Figure 4. This figure shows the experimental tank set-up. The central tank housed the female *M. estherae* while the two flanking monitors displayed the stimulus videos. The blue lines indicate preference zones, and the red arrows indicate where the filters were interposed between the tank and the monitors.



Figure 5. This shows the normal quantile-quantile plots for the untransformed ratio data for no filter (on left) and the arcsine transformed ratio data (on right). Note that the two extreme outliers in the top right of the left plot have been eliminated by the transformation and that the data qualitatively fits the line to a higher degree after transformation.



Figure 6. This displays the preferences of female *M. estherae* for each of the stimulus videos per filter type applied, and also for left side and right side of the tank in the absence of any stimulus. The error bars show standard error of the mean. The letters above each bar indicate significance. The values are as follows: Control: X±SEM for the left side = 28.97 ± 5.53 and for the right side = 34.222 ± 7.13 . No filter: X±SEM for *C. horei* = 61.86 ± 8.77 and for *M. estherae* = 134.91 ± 15.37 . The light filter: X±SE for *C. horei* = 61.41 ± 8.61 and for *M. estherae* = 111.27 ± 12.96 . The dark filter: X±SEM for *C. horei* = 73.55 ± 9.82 and for *M. estherae* = 99.22 ± 13.68

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