IS ACOUSTIC COMMUNICATION A CANDIDATE SIGNAL FOR SEXUAL SELECTION IN MALAWIAN CICHLIDS?

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A Thesis

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The description and classification of signals under sexual selection is a classical problem in evolutionary biology. This difficulty derives primarily from deficiencies in both the hypothetical framework describing communicative signals and in the empirical methods used to verify hypotheses of sexual selective function. Most currently accepted models of sexual selection operate only under highly specific circumstances and therefore cannot easily be applied to the complex situations surrounding selection and speciation in natural environments. In the following studies, we conducted a series of experiments in order to assess the likelihood that a sensory signal is under active sexual selection, whether by itself or in concert with other complimentary signals. For this purpose, we analyzed acoustic communication in Malawian cichlids, widely recognized as a classic model system for explosive species radiation. By describing both acoustic characteristics and the temporal concordance of visual and acoustic behaviors at multiple phylogenetic levels, we determined that the majority of signal divergence is present at levels consistent with expectations of divergence under sexual selection. Moreover, in situ data from Lake Malawi indicate that acoustic signaling by cichlids is prevalent during times when cichlids undergo courtship. We therefore conclude that acoustic signals are an important part of cichlid sexual signaling, and that contrary to the predominant view, such communication in Malawian cichlids is not entirely restricted to the visual modality.
Behavior can be as revealing as external morphology in the diagnosis of difficult species, as every naturalist knows.

-- Peter Marler, 1977
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CHAPTER I.
INTRODUCTION

Among the currently proposed mechanisms of evolution, few are more contentious than the idea that sexual selection can drive speciation in partial or even full sympatry. This is in large part due to the fact that sexual selection, by definition, can only occur in the presence of a communication system in which a badge possessed by one individual provides information to another “choosy” individual in the form of a sensory signal. The occurrence of such signals in natural populations is notoriously difficult to ascertain, as variation within signals evident to the scientific observer may simply be the non-adaptive result of genetic drift or an indirect effect of natural selection upon other traits. It is therefore important that social interactions are analyzed within a strong theoretical framework to determine the likelihood that sensory signals are candidates for sexual selection.

Few rigorous hypotheses regarding divergent signal selection have been proposed to date. Fisher originally proposed the idea that a linkage disequilibrium between female preference for a trait and male expression of that trait could drive the evolution of extravagant sexually dimorphic ornamentation (Fisher, 1915). This idea was further elaborated by Weatherhead and Robertson (1979) as the 'Sexy Son Hypothesis', which proposed that optimal mate choice for a female is determined by the likelihood that her male offspring will be attractive to other females. Because of this, Fisher’s model of runaway selection has become engrained in evolutionary thought, and is purported to be a causative agent for speciation via sexual selection, although such an application of this hypothesis has been questioned (reviewed by van Doorn et al., 2004).

Fisher’s model was, however, designed only to explain the development and maintenance of sexual ornaments, and by itself does nothing to explain how these ornaments could be used to
drive speciation via differential selection for a variant trait within parental species. In particular, it is important to note that Fisher’s model relies on two primary assumptions, such that (i) female preference for a trait is always positive, no matter the degree to which the trait has been accentuated, and (ii) the development of the trait is constrained by natural selection against extravagant ornaments. Given these assumptions, Fisher’s model can only explain divergent trait selection by females under limited circumstances viz., where there are two competing directional preferences under the control of a single genetic locus and natural selection acts as a limiting factor at both extremes of the trait distribution.

In contrast to Fisher’s hypothesis, the specific mate recognition system (SMRS) hypothesis of speciation proposed by Paterson (1978) imposes inherent stabilizing selection upon the signal through a reciprocal feedback loop in the signal recognition process, without invoking natural selection. Nonetheless, it too is still unable to explain sympatric speciation via sexual selection. Paterson’s model relies heavily on feedback between signaler and receiver creating strong stabilizing selection on a true communication system, thereby allowing signal drift and subsequent speciation only in small, allopatric founder populations. As a strictly allopatric model, the SMRS is not applicable to situations where sympatry of two incipient species continues throughout the entirety of their divergence from a common ancestor, or in cases where assortative mating is observed within a population.

A third major approach to questions concerning sexual selection is the “good genes hypothesis,” and its various forms such as the handicap principal (Zahavi, 1975; Zahavi, 1977) and the parasitism hypothesis (Hamilton & Zuk, 1982). In this model, information contained in the communication system is tied directly to offspring fitness by some heritable trait such as foraging prowess, predator avoidance, or disease resistance. Females select the male with the
best badge of fitness, thereby obtaining beneficial genes for their offspring. Unlike the previous hypotheses, the good genes hypothesis does not assume consistent directional selection over time. As the selective agent (e.g. a parasite) and the preferred phenotype co-evolve, the genotype displaying the superior badge can oscillate in response to a frequency-dependent adaptation by the selective agent. However, it is important to note that at any given point in time, female fitness is optimized by unidirectional selection on a male trait, and not by divergent selection. Therefore, the good genes hypothesis is unlikely to account for divergence via sexual selection in a sympatric population with all individuals under similar external selection pressures.

As a model system for exploring the intricacies of evolution, the cichlid fishes of Lake Malawi are both perplexing and extraordinarily important. This confirmed monophyletic group includes an estimated 500-1000 unique species (Kornfield & Smith, 2000), an unusually species-rich assemblage that has arisen in a remarkably short evolutionary time frame (1 mya, Moran et al., 1994). According to the radiation in stages model (Danley & Kocher, 2001) a substantial portion of the diversity in these cichlids is thought to have arisen via sexual selection on male nuptial coloration, although a full mechanistic explanation for this selection has not been formally proposed. The stable nature of many species, without any documented signal constraints resulting from natural selection, makes Fisher’s model unlikely. Similarly, the observed potential for broad learned tolerances in cichlid mate recognition across broadly diverged phylogenetic groupings (reviewed by Crapon de Caprona, 1986) invalidates Paterson’s strict stabilizing SMRS model, and the lack of any known adaptive fitness correlate to the mating display suggests the good genes hypothesis is unsuitable. Therefore, it seems unlikely that any
of these models affords an adequate description of the ongoing evolutionary processes occurring in Malawian cichlids.

Given the apparent difficulties of fitting sexual selection by Malawian cichlids into any strict classical framework, it is therefore necessary to construct a new hypothetical model under which potential courtship signals can be analyzed to determine the likelihood that they are indeed under sexual selection. In order to do this, three pieces of information are required about any given signal. Firstly, variation within the signal must be documented in a comparative context and at a phylogenetic level which is relevant to sexual selection, i.e. the divergence observed between closely related species that share similar niches should be equal to or greater than that observed between distant relatives. Secondly, potential sensory signals that overlap temporally with the signal in question must be described to determine whether salience is contained in the signal alone or if its meaning is derived from multi-sensory emergent effects (Partan & Marler, 2005). Thirdly, signal use in the natural environment must be documented in contexts appropriate to mate selection.

In this thesis on acoustic signaling in Malawian cichlids, I outline the first two requirements in Chapter 2, and the third in Chapter 3. Although cichlids have been known to produce sounds since Myrberg et al.’s pioneering work in 1965, little acoustic analysis has been performed in these fishes. Sounds are, however, known to be produced by males during the courtship display, and to vary interspecifically (Lobel, 1998; Amorim et al., 2004). This study represents the first attempt to study cichlid acoustic communication in an experimental paradigm designed to establish whether acoustic signaling is potentially under active sexual selection in this taxon. In doing so, it lays the foundation for both proximate analyses of acoustic signaling
in cichlids, as well as ultimate analyses of the role of acoustics in cichlid mate choice and speciation.
CHAPTER II.
CHARACTERIZATION OF CICHLID ACOUSTIC SIGNALS: HOW IS SIGNAL DIVERGENCE PHYLOGENETICALLY PARTITIONED?

Introduction

An exchange of sensory information between two courting individuals is key to sexual selection, as partners must be able to assess potential mates in order for a choice to be made. Strong selection for particular characteristics can lead to the exaggeration of these traits and extravagant phenotypes that are conspicuous both to other individuals and the scientific observer. This is particularly evident in systems with accentuated visual characteristics, as observers often utilize visually quantifiable traits to identify individuals or rate fitness. However, the presence of a conspicuous primary modality does not dictate that information from other sensory modalities is absent or unnecessary. Rather, many animals use multiple signal types in communicative displays, and the content of the message is reliant on the presence of each modality (Rovner & Barth, 1981; Narins et al., 2003).

The cichlids of the African great lakes are a classic model of rapid species divergence through explosive radiation. Although several alternatives have been proposed over the years, the prevailing model to account for this species-richness is evolution via sexual selection of a unimodal stimulus, with male nuptial coloration as a mate recognition barrier. Visual sexual selection appears to be commonplace in the rift lake cichlids, and has been hypothesized for fishes from Malawi (Arnegard et al., 1999; Kornfield & Smith, 2000; Allender et al., 2003), Victoria (Seehusen et al., 1997; Maan et al., 2004), and Tanganyika (Salzburger et al., 2006). Direct behavioral assays have demonstrated that females preferentially associate with conspecific males when given a choice between congeners, and it has been suggested that these results are
tied to differences in male coloration (Couldridge & Alexander, 2002; Jordan et al., 2003; Kidd et al., 2006). Several proximate mechanisms for the origin of female color preference have been suggested, from shifts in optical spectral sensitivity resulting in sensory drive (Carleton & Kocher, 2003; Maan et al., 2006) to parental imprinting during maternal mouthbrooding (Verzijden & ten Cate, 2007).

While existing research strongly supports the importance of visual signals in sexual selection in cichlids, there is no indication that the system is exclusively visual. A wide variety of cichlids are known to produce acoustic signals, including those from Central and South America (Myrberg et al., 1965; Schwarz, 1974a; Schwarz, 1980), African riverine species (Myrberg et al., 1965; Amorim et al., 2003), and rift lake cichlids (Lobel, 1998; Lobel, 2001; Amorim et al., 2004). Cichlid sound production has been reported almost exclusively during interactions with conspecifics, and has been shown to be both caused by, and subject to, behavioral lability (Schwarz, 1974b; Amorim & Almada, 2005). Taken together, these data suggest that cichlid vocalizations possess some communicative value, particularly as it applies to interactions with conspecifics. Furthermore, it has been noted that cichlid sound production typically accompanies characteristic visual displays that are primarily used during courtship behavior (Amorim et al., 2004; Ripley & Lobel, 2004). The temporal correlation between acoustic and visual signals during the male mating dance suggests a possible role of acoustics in sexual signaling, and thus the likelihood that female mate choice is influenced by male vocalizations as well as visual displays.

As a portion of the male courtship signal, cichlid vocalizations are a potential substrate for sexual selection. However, while species-specific differences have been documented between closely related taxa, no comparative study has yet analyzed male courtship calls at
multiple phylogenetic levels to determine whether observed differences result from sexual selection influencing active call divergence or are merely the result of drift (Lobel, 1998; Amorim et al., 2004). Since active divergence occurs only between closely-related taxa, there are testable hypotheses that can be applied to signal measurements in order to determine the likelihood that a given trait is under active sexual selection. Specifically, active sexual selection should create measurable differences at the interspecific level, but not necessarily at higher phylogenetic levels. This would cause traits under sexual selection to show interspecific divergence equal to, or greater than, that observed at the intergeneric level.

In this study, we performed comparative analyses of male acoustic behavior in six species of Malawian cichlids in two divergent genera in order to test the hypotheses that: (i) gross characteristics of male call structure and (ii) the correlation between acoustic and visual displays, deviate primarily at the intrageneric level. These hypotheses represent a critical empirical investigation into the feasibility of acoustic communication as a substrate for sexual selection in cichlids, providing the foundation for further investigations into multimodal sensory integration and selection in Malawian cichlids.

Materials and Methods

Study Organisms:

Six species were selected from two genera: *Metriaclima* (*Maylandia*) *zebra*, *Metriaclima callainos*, *Metriaclima lombardoi*, *Melanochromis auratus*, *Melanochromis johanni* (Eccles), and *Melanochromis cyaneorhabdos* (Bowers & Stauffer). Five males were used from each species except for *Melanochromis johanni* (*n* = 4). All individuals were wild-caught fishes acquired from a single locale whenever possible, with the exception of three male *Melanochromis auratus* which were captive-bred F₁ fish substituted because of mortality.
Fishes of the genus *Metriaclima* are commonly used study organisms, with *Metriaclima zebra* being a standard research model. This species exists in populations throughout the lake, with the males displaying a blue background with vertical black bars. In order to control for potential geographic polymorphism within this species, all *M. zebra* tested were from the endemic *katale* population. *Metriaclima callainos* are characterized by a loss of vertical bars, with little sexual dimorphism. *Metriaclima lombardoi* are one of the rare Malawian cichlids with yellow males and blue females, and have been the subject of research relating to female choice cued to male eggspots (Couldridge & Alexander, 2002).

In comparison to *Metriaclima*, the genus *Melanochromis* is suspected to be a second-stage trophic divergence according to the radiation in stages model (Danley & Kocher, 2001), although little work has been done with these fishes. The melanochromes do, however, display a shift to horizontal bars which represents a novel coloration pattern in Malawian cichlids that may have driven further divergence. Mature *Melanochromis auratus* males are primarily black with gold bars, while females display an inverted color pattern. *Melanochromis johanni* and *M. cyaneorhabdos* are sister species, with both possessing nearly identical blue and black males. However, whereas *Melanochromis johanni* females are yellow, *Melanochromis cyaneorhabdos* females display the same primary color pattern as do the males.

**Subject Care and Housing:**

Each animal was housed in a submerged 29cm x 19cm x 18cm plastic cage (Exo Terra, Inc.) with a vented black lid and a series of ¼" holes along the upper rim to improve water flow. Three sides of each shelter were covered with black plastic, and an 11cm section of 5cm diameter PVC pipe was added to provide structure. The enclosures were placed in two independent water tables (215cm x 79cm x 75cm reservoir with a 215cm x 79cm x 12cm flow
table) with half of the subjects housed in each system. Water temperature was maintained at 25°C and filtration was provided by Eheim 2260 canister filters (Eheim, Inc.) and Cell-Pore blocks (Metamateria, Inc.). Animals were fed a combination diet of Hikari Cichlid Staple (Kyorin Co. Ltd.) pellets, Hikari Cichlid Gold (Kyorin Co. Ltd.) pellets, and beefheart (San Francisco Bay Brand) every other day.

Experimental Set-Up:

The trial arena consisted of a 90cm x 60cm x 60cm fiberglass tank with a front viewing pane (Aquatic Eco-Systems, Inc.) mounted on a counterbalanced high-capacity vibration table (Technical Manufacturing Corporation, model # 68-561). Runners were installed in the center of the tank for a pair of acrylic barriers; one was transparent with ¼" diameter holes drilled in a grid to allow water flow and the other was a solid sheet of black acrylic. The bottom of the tank was covered with pea gravel and two small ceramic pots were placed mouth-to-mouth on each side of the runners to create caves for the fishes. A Bruel & Kjaer hydrophone (Model #8103; sensitivity -211.6 dB re 1V/µPa) was suspended above each cave, with each hydrophone running to an independent conditioning amplifier (Bruel & Kjaer Nexus Conditioning Amplifier #2692-OS1; Bruel & Kjaer Charge Amplifier Type 2635). The amplifiers connected to a digital-to-audio recorder (Marantz Solid State Recorder PMD671) to directly record stereo in-tank sounds to a memory card. Visual behaviors were recorded through the front pane with a Sony Mini-DV camcorder (Model DCR-PC1000).

Multi-Modal Probe Trials:

All trials comprised staged encounters between naïve pairs of mature conspecific males and females who had been socially isolated to ensure dominant status of the males. Before trial onset, animals were added to opposite sides of the arena with both transparent and opaque
partitions in place. After a two-hour acclimation period, the opaque barrier was removed, leaving the transparent barrier in place to prevent physical damage to the fishes. The animals were allowed to interact for one hour while their behavior was recorded and acoustic data was gathered by the hydrophones on either side of the barrier. Since females were never found to produce sound, the hydrophone audio only from the male's side of the tank was then dubbed over the video. All synchronization and audio dubbing was performed in iMovie HD (Apple Computer, Inc.). Times of behavioral onset and cessation were recorded using the program QT Movie Note Taker 0.5 (dvcreators.net). The male quiver display was chosen as a behavioral standard for visual courtship, with the duration of quivers and the association between quivers and vocalizations as specific behavioral measures.

**Sound Probe Trials:**

A second trial format was developed due to a need for improved sound quality for acoustic measurements. Following the multi-modal probe, animals were returned to their standard isolation holding for a period of three days. They were then reintroduced to the trial arena according to the procedure outlined above. In this case, however, both barriers were raised after the acclimation period and the female coaxed into the male's half of the tank. The black barrier was then replaced, restricting the fish to the male's territory. Animals were then allowed to interact for one hour while all sounds were recorded through a single hydrophone channel. The sound files were then amplified uniformly by 20 dB re 1µPa in Amadeus Pro (HairerSoft) and calls were identified by ear. Each call was saved as an individual file and transferred to Raven 1.2.2 (Cornell Lab of Ornithology Bioacoustics Research Program) for measurement of acoustic characteristics. Only vocalizations from sound probes were used for call measurements.
All sound features measured were clearly detectable above the vibrational background and no acoustic filters were applied to the vocalizations.

**Analysis:**

Data from the multi-modal probes were analyzed via two techniques: (i) a contingency table comparing distributions of an ordinal vocal score (defined by the number of calls produced in association with a single visual quiver display) across species and genera, and (ii) a sequence of ANOVAs comparing duration of the quiver across ordinal vocal scores. Data from the sound probe trials were analyzed using discriminant function analyses (DFA) of call characteristics including primary frequency (the frequency containing the most energy across the entirety of the call), total call duration (duration from onset of the first pulse to termination of the final pulse), and the number of discrete pulses within a call. Essentially, a DFA derives a series of linear multivariate factors that maximally delineate the means of *a priori* groups while minimizing variation within groups (Seal, 1964). These analyses are generally graphically depicted via canonical centroid plots, with the centroids representing the *a priori* groups and the vector length and direction describing the relative strength and direction of each independent variable.

The utility of a discriminant function analysis is illustrated with a simple hypothetical example (Fig. 2.1). In the hypothetical example, 1000 calls are collected from two species of fish and plotted as a function of call duration and frequency (Fig. 2.1a). A large degree of overlap between the species is observed when using univariate tests for both duration (Fig. 2.1b) and frequency (Fig. 2.1c). However, if the data are analyzed along the line of a linear regression best describing the distribution of the data, a much larger degree of separation between the species is observed (Fig. 2.1d). In the DFA model, this regression line, which is a weighted composite of both duration and frequency, becomes the x-axis. A second multivariate measure is
then calculated for the y-axis, and the species are then plotted by their multivariate means and confidence intervals (Fig. 2.1e). The original independent measures (i.e. duration and frequency) are then plotted as vectors from the model origin indicating both the direction and relative strength of their effects on the multivariate model as a whole. While the example given here is a simple two-factor model, the DFA is not limited by dimensional space and can differentiate groups based upon complex multi-dimensional models when necessary.

**Results**

*Multi-modal probes:*

A simultaneous comparison of the audio-visual repertoire of all six species (as defined by vocal score) demonstrates that the correlation between acoustic and visual behaviors varies across species (Pearson $\chi^2 = 86.61$, $p < 0.0001$). An analysis comparing the overall repertoire between the genera reveals no significant difference (Pearson $\chi^2 = 11.76$, $p = 0.0674$; Fig. 2.2a). Analyses within each genus demonstrate that both *Melanochromis* (Pearson $\chi^2 = 22.43$, $p = 0.0131$; Fig 2.2b) and *Metriaclima* (Pearson $\chi^2 = 51.96$, $p < 0.0001$; Fig 2.2c) exhibit significant differences between congeners. No significant difference was found between the closely related sister taxa *Melanochromis cyaneorhabdos* and *Melanochromis johanni* (Pearson $\chi^2 = 10.15$, $p = 0.071$), nor the vocal *Metriaclima callainos* and *Metriaclima lombardoi* (Pearson $\chi^2 = 11.95$, $p = 0.0630$).

Comparisons of vocal score with total quiver duration displayed a significant correlation between higher vocal score and extended quivers in *Metriaclima callainos* ($F = 8.40$, $p = 0.0013$; Fig. 2.3a), *Metriaclima lombardoi* ($F = 5.76$, $p < 0.0001$; Fig. 2.3b), *Melanochromis johanni* ($F = 3.12$, $p < 0.0253$; Fig. 2.3c), and *Melanochromis cyaneorhabdos* ($F = 52.52$, $p < 0.0001$; Fig.
Tukey-Kramer post-hoc tests demonstrated that the relationship between vocal score and quiver duration was strongest in *Melanochromis cyaneorhabdos*, as this was the only species where quivers without a call and quivers with a single call could be distinguished (p < 0.05). Furthermore, this was the only species in which all observed vocal scores corresponded to a statistically independent range of durations (p < 0.05). No relationship was observed in *Metriaclima zebra* which displays mutually exclusive vocalizations and quivers, nor in *Melanochromis auratus* which rarely vocalizes.

**Sound Probe Trials:**

*Metriaclima zebra* and *Melanochromis auratus* were excluded from all sound probe analyses as they were found to be largely non-vocal. A DFA of the call characteristics for the remaining four species indicated that *Metriaclima callainos* was distant from both its congener and the melanochromes, which all clustered together (Wilks’ Lambda = 0.329, p < 0.0001; Fig. 2.4a). A further DFA of the cluster revealed that calls of *Metriaclima lombardoi* and *Melanochromis cyaneorhabdos* are indistinguishable, but both are diverged from the distinct grouping formed by *Melanochromis johanni* (Wilks’ Lambda = 0.877, p < 0.0001; Fig. 2.4b). A DFA analysis of call structure at the generic level reveals a strong differentiation driven by the effects of *Metriaclima callainos* (Wilks’ Lambda = 0.871, p < 0.0001; Fig. 2.4c).

**Discussion**

Acoustic signals in Malawian cichlids display a phylogenetic pattern of divergence consistent with strong selection between closely related groups, whereas variation between distantly-related taxa is likely the result of drift or indirect selection mechanisms. This phenomenon is observed both at the level of call characteristics and at the behavioral interface
between acoustic and visual displays. Amongst the data, three particular scenarios are of interest when considering the possibility of intrageneric selection: (i) the clustering of call characteristics so that no congeneric overlap is observed, (ii) the divergence of the audio-visual repertoire within, but not between, genera, and (iii) the variable strength of the audio-visual correlation between sister species *Melanochromis cyaneorhabdos* and *Melanochromis johanni*.

When considering the acoustic features of male courtship calls alone, it is evident that the genus *Metriaclima* contains intrageneric divergence equal to the degree of distance between the genera *Metriaclima* and *Melanochromis* (Fig. 2.4a). Specifically, *Metriaclima lombardoi* clusters tightly with the two *Melanochromis*, and actually overlaps with *Melanochromis cyaneorhabdos* to the extent that the two are indistinguishable. *Melanochromis johanni*, however, can be fully distinguished from its congener (Fig. 2.4b). When considering the divergence evident in the intergeneric model, the distance between the two genera can be attributed entirely to *Metriaclima callainos*, and this distance is of lesser magnitude than that between *Metriaclima callainos* and *Metriaclima lombardoi* (Fig. 2.4c). Therefore, the data for acoustic characteristics of cichlid vocalizations support our hypothesis of enhanced divergence between closely related species.

A similar trend is observed when one examines the association between the production of acoustic signals and the conspicuous visual quiver behavior. When the frequencies of overlap for these behaviors from both genera are compared, no significant difference between the *Metriaclima* and *Melanochromis* can be detected. However, comparisons of specific repertoires of these overlap frequencies within each genus reveal that a significant divergence of audio-visual repertoires is present within each genus. This intrageneric divergence is driven by the presence of a single species within each genus that is either non-vocal or which uses vocalizations in opposition to quiver behaviors (*Melanochromis auratus* and *Metriaclima zebra*)
respectively). Vocal species in both genera do not display a significant audio-visual behavioral divergence, as the vast majority of the repertoire in all vocal species is composed of quivers without a corresponding call or a single call. However, it is important to note that while there is no significant divergence between the audio-visual behavioral repertoires within each genus, there is a significant difference in the acoustic characteristics of the call itself. Thus, the inherent properties of the acoustic signal itself adds discriminatory detail to otherwise indistinguishable multi-modal systems.

Finally, it is necessary to consider not only the mutual patterns of production for both acoustic and visual behaviors, but also the predictive relationship between these two modalities in vocal species, as this may be a key component of signal fidelity. In *Metriaclima callainos*, *Metriaclima lombardoi*, and *Melanochromis johanni*, there is a weak relationship between the number of calls produced during a single quiver behavior and the duration of the quiver. This relationship is largely defined by a difference between a range of quiver durations that correlate to the production of no call or a single call, and longer quivers that correspond to an increased number of vocalizations. *Melanochromis cyaneorhabdos* displays a much stronger correlation between quiver duration and call number, such that the number of calls produced during a quiver can be specifically predicted by quiver duration or vice versa. Therefore, while the overall audio-visual repertoire of *Melanochromis cyaneorhabdos* and *Melanochromis johanni* may not be statistically different, the temporal relationships within those repertoires are species-specific. This variation in temporal patterning represents a third layer of signaling plasticity that is extant at the intrageneric level.

Amorim et al. (2004) described sound characteristics from two of the species profiled in the present study viz., *Metriaclima callainos* and *Metriaclima zebra*. While our measurements
for *M. callainos* are consistent with the values reported by Amorim, there is a stark contrast in
the primary frequency observed in *M. zebra*, with the *katale* population tested here displaying a
markedly lower mean frequency (193.8Hz vs. 460.4Hz). Moreover, Amorim does not report any
difference in the incidence of sound production between *M. callainos* and *M. zebra*, whereas our
particular population of *M. zebra* was observed to be highly non-vocal, with a total of only five
calls produced in five total hours of observation. These discrepancies suggest that there may be
extensive divergence in acoustic signaling between subpopulations of *M. zebra*, although
substantial further testing would be required to verify the consistency of this trend across the
species as a whole.

This research represents the first attempt to quantify behavioral divergence within cichlid
acoustic communication systems at multiple phylogenetic levels. As such, it allows us to
specifically determine the likelihood that male acoustic signals are under selective pressure
potentially associated with active sexual selection. Our results indicate that intrageneric
divergence within acoustic characteristics, behavioral repertoire, and temporal patterning of
behavioral correlations equaled or exceeded that at the intergeneric level. Given the hypothetical
framework previously described, the data suggest that acoustic signals are primarily under
selection at the intrageneric level. In Malawian cichlids, this is the phylogenetic level most
likely associated with sexual selection, as congener typically inhabit similar habitats, fill similar
ecological niches, and are regularly partially or fully sympatric. Taken together, these results
present a strong case for acoustic signals as candidate substrates for sexual selection by female
cichlids, and substantially raise the prospects for an integrated system of multi-modal signals.
Figure 2.1: Discriminant function analysis performed on hypothetical two-factor data. The line of best fit from a linear regression forms the first canonical axis, allowing for increased discrimination of species based on weighted multivariate factors (modified from Delago et al., 1997).
**Figure 2.2:** Contingency tables for audio-visual repertoires defined by the relationship between quiver behaviors and associated vocalizations for the (a) total intergeneric comparison (Pearson $\chi^2 = 11.76$, $p = 0.0674$), (b) interspecific comparison within *Melanochromis* (Pearson $\chi^2 = 22.43$, $p = 0.0131$), and (c) interspecific comparison within *Metriaclima* (Pearson $\chi^2 = 51.96$, $p < 0.0001$).
Figure 2.3: ANOVA comparisons of vocalizations versus quiver duration for (a) *Metriaclima callainos* (F = 8.40, p = 0.0013), (b) *Metriaclima lombardoi* (F = 5.76, p < 0.0001), (c) *Melanochromis johanni* (F = 3.12, p < 0.0253), and (d) *Melanochromis cyaneorhabdos* (F = 52.52, p < 0.0001). All species show increasing mean durations with increasing vocalization number, but *Melanochromis cyaneorhabdos* is the only one to display a unique range of quiver durations for each vocalization number.
Figure 2.4: Discriminant function analyses (DFAs) of call characteristics for (a) all four vocal species (Wilks’ Lambda = 0.329, p < 0.0001), (b) the clustered subset of vocal species (Wilks’ Lambda = 0.877, p < 0.0001), and (c) total generic vocalizations (Wilks’ Lambda = 0.871, p < 0.0001). The Melanochromis are portrayed by dashed circles while the Metriaclima are portrayed by solid circles.
Table 2.5: Call characteristics (mean ± s.d.) for the six cichlid species profiled.

<table>
<thead>
<tr>
<th>Species</th>
<th>Calls Recorded</th>
<th>Primary Frequency</th>
<th>Total Call Duration</th>
<th>Pulse Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanochromis auratus</td>
<td>7</td>
<td>267.6 ± 157.7</td>
<td>310.6 ± 178.9</td>
<td>N/A</td>
</tr>
<tr>
<td>Melanochromis cyaneorhabdos</td>
<td>204</td>
<td>211.8 ± 48.6</td>
<td>219.6 ± 61.6</td>
<td>7.51 ± 2.97</td>
</tr>
<tr>
<td>Melanochromis johanni</td>
<td>67</td>
<td>228.4 ± 56.1</td>
<td>286.6 ± 172.2</td>
<td>9.70 ± 5.42</td>
</tr>
<tr>
<td>Metriaclima callainos</td>
<td>82</td>
<td>410.2 ± 157.0</td>
<td>659.5 ± 409.4</td>
<td>8.79 ± 4.86</td>
</tr>
<tr>
<td>Metriaclima lombardoi</td>
<td>132</td>
<td>225.6 ± 41.6</td>
<td>220.0 ± 117.0</td>
<td>8.14 ± 3.61</td>
</tr>
<tr>
<td>Metriaclima zebra</td>
<td>5</td>
<td>193.8 ± 21.5</td>
<td>914.2 ± 1005.0</td>
<td>11.00 ± 7.35</td>
</tr>
</tbody>
</table>
CHAPTER III.

THE ACOUSTIC SCENE IN LAKE MALAWI: DIEL DIFFERENCES IN SOUND PRODUCTION BY FISHES

Introduction

Whereas bird songs and frog calls have been subject to intensive quantitative analyses over the last 60 years, studies of fish calls have been sporadic and largely descriptive. In part, this is due to the technical difficulties of acoustic recording in many aquatic habitats, as shallow water environments behave as highly-bounded, acoustically complex transmission channels. This is particularly important in river and lake habitats, which are generally highly-bounded by definition. Therefore, few studies have investigated sound production in freshwater fishes in situ (Lugli & Fine, 2003), although such field studies are relatively common in marine environments (Myrberg et al., 1986; Mann & Lobel, 1995; Mann & Lobel, 1998). For this reason, we have limited knowledge of in situ sound production by many fishes, and thereby a decreased understanding of acoustic communication in aquatic environments.

Due to their extraordinary diversity in the African rift lakes, cichlid fishes have emerged as a prominent model system for the analysis of rapid and recent vertebrate radiations. Systematic and phylogenetic studies of this taxon have employed a wide array of characters ranging from classical morphological descriptors to a variety of molecular markers and behavioral phenotypes (Martin & Taborsky, 1997; Stauffer et al., 2002). In particular, most studies have focused on visually quantifiable characteristics (e.g. male nuptial coloration) for both behavioral and population studies (Fernald & Hirata, 1979; Arnegard et al., 1999). Given the spectacular color pattern variation in the family, it is no surprise that visually quantifiable
traits as well as characters subserving vision have featured prominently in these investigations (reviewed by Carleton et al., 2006).

The role of visual signals in eliciting or constraining cichlid behavior has been well documented (Seehau sen & van Alphen 1998; Maan et al., 2006b), and studies of sexual selection have focused almost exclusively on this sensory modality as the sole substrate of female choice (Seehau sen et al., 1997; Couldridge, 2002; Maan et al., 2006a). However, early studies by Myrberg et al. (1965) demonstrated sound production in phylogenetically diverse cichlids, and subsequent research has revealed that several rift lake cichlids are capable of producing acoustic signals (reviewed by Lobel, 2001). In all African cichlids profiled thus far, courting males are responsible for the vast majority of acoustic signals and their calls often display interspecific variation (Lobel, 1998; Amorim et al., 2004; Smith and van Staaden, in prep). Taken together, these laboratory findings suggest that vocalizations may play a more significant role in the social signaling of Malawian cichlids than previously suspected.

To date, no studies have described the natural auditory scene of cichlid habitats, and little is known about competition in the acoustic channel or the natural diel patterning of sound production by cichlids in situ. Therefore, the environmental context in which these calls are produced is poorly understood. Measurements of extended temporal patterns in vocalizations can provide key information regarding activity patterns of animals, as well as the contextual use of acoustic signals (Thorson & Fine, 2002; Lang et al., 2006). Similarly, characterization of ambient noise within the transmission channel is necessary to understanding signal use, as several terrestrial studies have demonstrated the importance of habitat in shaping selection on call structure (Couldridge & van Staaden, 2004; Baker, 2006). Once combined, this information
provides insight into potential purposes for the signal and allows for the development of hypotheses regarding the social value of the signal.

In this study, we deployed a remote datalogger in Lake Malawi in order to: (i) test the feasibility of long-term field recordings in the African Great Lakes, (ii) characterize the natural ambient sound environment within which fishes are signaling, and (iii) examine the diel patterning of sound production by fishes in the lake. Since Malawian cichlids primarily mate during daylight hours (McKaye, 1983) and the majority of cichlid vocalizations have been observed during male courtship dances, we hypothesized that cichlid vocalizations would display a strong diurnal bias in relation to mating behavior, and that the high density of cichlids in Lake Malawi would drive a general pattern of diurnal signaling by fishes.

**Materials and Methods**

**(a) Sound Recording**

A Long-Term Acoustic Recording System-High Frequency (LARS-HF, Loggerhead Instruments, Inc.) was outfitted with a hydrophone (High Tech, Inc. model# HTI-96-MIN, voltage sensitivity: -186.4 dB re 1V/µPa) and a Dell Axim X50 Pocket PC for data collection purposes. In order to maximize recording time from limited battery life, the sampling rate was set to 8800Hz and the Loggerhead Software was set to record a 15s sound sample to a CompactFlash card every 300s.

The logger was deployed and tethered to the substrate at 09:30 local time (05:30 GMT) on July 6, 2005 in 8m of water roughly 20m from shore at Mitande Point, Malawi (14°0'S, 34°53'E as determined by SatelliteViews.net). This site is located off Thumbi West Island, and is characterized by a rocky bottom with intermittent large boulders. Also, the site is known for
high densities of cichlids as well as an unusually rich species assemblage due to intralacustrine introductions in the 1960s and 1970s (Hert, 1990; Hert 1995). The logger was retrieved at 10:30 local time on July 13, 2005.

(b) Data Analysis

Sound visualization and analysis was carried out using Raven 1.2.2 sound analysis software (Cornell Lab of Ornithology Bioacoustics Research Program, Ithaca, NY, 2004). Ambient noise spectra were created for periods of calm and high intensity wave action by performing Fast Fourier Transform (FFT) analysis on compilations of five-second clips for each condition (n = 20). Files containing identifiable anthropogenic sources of noise were excluded from these analyses.

For the diel analyses, sound files were scored for the presence/absence of seven sound types. Initial categorization identified three primary types based on audible characteristics, viz., vocalizations, feeding sounds, and boat noise. Vocalizations were further divided into four subtypes on the basis of temporal characteristics (Fig. 3.1): grunts (single syllable call), drums (repeated reverberations), squeaks (two-syllable call), and click trains (series of distinct irregular clicks). Sound clips were coded into diel phase (diurnal, nocturnal, or crepuscular) based on solar cycle data obtained for the location from the U.S. Naval Observatory database (<http://aa.usno.navy.mil/data/docs/RS_OneDay.html>). Since all observations occurred during the week between the new moon and the first quarter, lunar phase data was disregarded. Data were then compared across test groups using Pearson two-tailed Chi-square analyses of ordinal means.
Results

Of the 2009 recording clips, 1150 (329 of 945 during diurnal phase, 42 of 68 during crepuscular phase, and 779 of 996 during nocturnal phase) were considered to contain relevant biological sound by our auditory criteria. However, in order to control for the effects of anthropogenic noise on the system, all files with audible boat noise were excluded (317 of 945 during diurnal phase, 8 of 68 during crepuscular phase, and 57 of 996 during nocturnal phase). This left a total of 1024 recordings with scorable sound in the remaining 1627 files.

Ambient noise in Lake Malawi is predominantly at frequencies below 1000Hz, with a peak amplitude of 62.4dB re 1µPa at 802Hz during heavy wave action and a peak amplitude of 47.5dB re 1µPa at 788Hz during calm periods (Fig. 3.2). In general, the spectra are similar to those previously reported for lacustrine habitats (Amoser & Ladich, 2005).

All four vocalization subtypes showed significant nocturnal peaks (grunt: $\chi^2 = 108.528$, p < 0.001; drum: $\chi^2 = 7.935$, p = 0.0189 click train: $\chi^2 = 107.646$, p < 0.0001; squeak $\chi^2 = 157.247$, p <0.0001; Fig. 3.3). In three of the call types (drum, click train, squeak), sound production in the twilight period is intermediate between diurnal and nocturnal production. However, this was not the case for grunts, where production during diurnal and twilight periods was of similar magnitude.

Multiple call types were significantly more likely to occur during a single sound file at night ($\chi^2 = 473.3$, p < 0.0001). In addition, nocturnal files contained more overlapping call types ($\chi^2 = 315.139$, p < 0.0001; Fig. 3.3). The temporal distribution of feeding sounds was independent of diel period ($\chi^2 = .271$, p = 0.8732).

In order to determine the effect of anthropogenic noise on call detection, analyses were performed comparing vocal scores from files with and without boat noise. Anthropogenic noise
from outboard motors significantly reduced the measured occurrence of vocalizations in both the
grun (\(\chi^2 = 11.013, p = 0.0009\)) and squeak (\(\chi^2 = 5.257, p = 0.0219\)) categories during the day,
but had no significant effect on the other two call types or during the crepuscular and nocturnal
time phases.

Discussion

Comparing the gross temporal structure of the recorded sounds with known fauna of
Lake Malawi and sound reports in the literature, it was possible to attribute each call to a likely
source family, though not to individual species. Our lab measurements (Smith and van Staaden,
in prep) and those of Amorim et al. (2004) suggest that the observed grunts are likely produced
by cichlids (Cichlidae), although grunts and moans have also been recorded from mormyrids
(Mormyridae) of the genus *Pollimyrus* (Crawford et al., 1997), of which Malawi has a single
species. Click trains are characteristic of mormyrids as well (Rigley & Marshall, 1973), of
which Malawi has five species other than *Pollimyrus*. Malawi is also home to bagrid catfishes
(Bagridae) that are known to possess a sonic swimbladder muscle (drumming; Sorenson, 1895)
and two species of "squeaking catfishes" (Mochokidae) that produce two syllable calls via
stridulation of their pectoral spines (Fine & Ladich, 2003).

Based on seven days of recording at a single locality, our data represent a snapshot of the
ambient noise over a narrow spatial and temporal range. This limitation is particularly important
when considering data acquired in shallow-water locales such as our recording site, where
seasonal changes in precipitation and wind patterns could have pronounced effects on the
magnitude and structure of environmental noise. In 8m of water, we could clearly discern
surface noise from both rain and breaking waves, and this effect is expected to be ubiquitous in
the shallow littoral zone but not necessarily in deeper pelagic waters where some fishes reside. However, we are largely unable to generalize our findings to pelagic waters or seasonal shifts due to our temporally-restricted sample. Unfortunately, we were unable to record an in situ calibration signal, so we could not back-calculate an effective transmission distance for our logger.

In natural environments the presence of background noise may affect hearing by elevation of auditory thresholds or masking. The effect of ambient noise on hearing thresholds for freshwater fishes depends crucially on the hearing mechanism employed. Amoser & Ladich (2005) found that taxa lacking specific anatomical adaptations for improving hearing thresholds are relatively unaffected by changing levels of ambient noise levels, while fishes with such adaptations (e.g. otophysans) have significantly elevated hearing thresholds with increased ambient noise. Given the lack of any known anatomical hearing specialization in Malawian cichlids and their distant phylogenetic relationship with hearing-adapted Malagasy and South Asian cichlids (Sparks & Smith, 2004), we anticipate that Malawian cichlids should be relatively unaffected by changes in noise amplitude in the littoral zone. The same is not true for potential cichlid predators, however. Catfishes and mormyrids also occur commonly in Lake Malawi, and both families are comprised of fishes with anatomical hearing adaptations for which auditory tuning and sensitivity are likely modulated by changing noise spectra (Amoser & Ladich, 2005). Based on findings by Scholik and Yan (2002) using the bluegill sunfish Lepomis macrochirus, we expect no significant changes in hearing sensitivity for fish lacking anatomical hearing adaptations if noise is less than 20 dB above hearing threshold for cichlids.

The low intensity of many fish vocalizations and high impact of background noise on recording quality limits detailed acoustic characterization of their sounds from field recordings.
Amorim *et al.* (2004) considered sounds produced by courting male cichlids within 1-2 body lengths of the hydrophone suitable for structural analysis. Our current work suggests that such analysis is compromised beyond a recording distance of 30cm, even under controlled laboratory conditions (Smith & van Staaden, in prep.). Given the extent of our field recording area, it is indeed surprising how many calls were audible above background. Although these calls cannot be reliably quantified by frequency or fine-scale temporal patterning, we are confident that human feature detection capabilities are sufficient for classification based upon gross physical call structure and audible temporal patterning (Koelsch & Siebel, 2005).

The observed biological acoustic scene in Lake Malawi presents a much different picture than initially hypothesized based on our expectations of diurnal acoustic peaks resulting from natural mating activity. The fishes in the lake, including cichlids, appear to be most acoustically active at night. Although we cannot entirely exclude the possibility, the strong visual component to cichlid courtship behavior and previous descriptions of strong diurnal preferences for mating (McKaye, 1983) suggest it is unlikely that the observed nocturnal sound is the result of nocturnal spawning. Rather, the presence of mormyrid and catfish calls in the system presents an alternative explanation. Both of these fish families possess members that are known to actively prey upon cichlids at night (Arnegard & Carlson, 2005; McKaye, 1983). The presence of calls attributable to these fishes peak at night, and their overlap with periods of cichlid calling (multi-family chorusing), suggests a form of "alarm-wave" calling (D’Spain & Berger 2001), where cichlids may use acoustic signals to alert neighbors to the presence of potential predators.

To date, no acoustic behaviors other than those performed by courting males have been described for Malawian cichlids. Our acoustic survey indicates that cichlids are highly vocal, particularly during periods when mating behavior would not be expected. Therefore, we suggest
that acoustic communication is a highly relevant aspect of cichlid behavior in multiple types of social interactions. It is therefore important that these alternate vocalizations be studied both directly in controlled lab settings and in the natural environment to tease apart their role in the cichlid communication system.
**Figure 3.1:** Representative samples of the four subtypes of vocalizations. Grunts (A) are relatively long, single-syllable calls. Click trains (B – a click pair) are series of short, temporally irregular broadband clicks. Drumming sounds (C) are regularly spaced pulses with very intense low frequency (<200Hz) components. Squeaks (D) are two-syllable calls composed of a relatively broadband first syllable followed by a less intense second syllable. All files are unfiltered and amplified 64x.
Figure 3.2: Averaged power spectra of ambient noise at high-noise time (upper line) and low-noise times (lower line). Each power spectrum was generated by averaging twenty independent 5s recordings of ambient noise. Gain was identical for all recordings.
Figure 3.3: Diel trends in fish vocalizations off Mitande Point in Lake Malawii, measured by likelihood of sound occurrence in a 15 second sample. (a) Nocturnal peaks in both cichlid calls (grunts, solid lines) and all vocalizations combined (all four subtypes, lined). (b) Increase in multi-family chorusing at night.
CHAPTER IV.

CONCLUSIONS

In sum, this combination of laboratory and field investigations of auditory signaling paints a somewhat surprising picture of acoustic communication in Malawian cichlids. The laboratory studies verified the presence of acoustic signals as key components of the male courtship display, and identified distinct intrageneric variation in both call characteristics and audio-visual behavioral repertoires. These findings are fully consistent with expectations based on our hypotheses regarding the characteristics of signals under active sexual selection. However, field data obtained from the analysis of the natural acoustic scene in Lake Malawi are less clearly interpretable. Prodigious calling was documented during the peak daylight hours when cichlids are known to actively court and spawn, thus confirming the use of acoustic signaling in a natural context related to mate choice. The occurrence of even more elevated levels of signaling at night though, was unexpected. Further complicating this issue is the incidence of nocturnal multi-species chorusing with other native fishes, a phenomenon which cannot be explained in the context of mating interactions. The most plausible explanation for this conundrum is a form of metacommunication (sensu Wilson, 1975), in which the salience of a signal derives from the broader context in which it is employed.

Context-dependent communication is by no means a novel idea, and contexts can be defined by several factors independently or in concert (e.g. environmentally, behaviorally). In particular, multi-modal signals with emergent behavioral effects have been documented in both vertebrates and invertebrates (reviewed in Partan, 2004). Given the well-established importance of coloration and body posture in the communication of social status in cichlids (Baerends & Baerends-van Roon, 1950), it seems probable that cichlid visual and acoustic displays act in
conceit to create complex multi-modal signals. Such a system would both enrich the possibilities for information transfer and enhance reliability in 'noisy' environments compared to unimodal schemes which, in the case of cichlids, are limited by the phylogenetically conserved visual display or the physical constraints that shape acoustic signals. Although the experimental regime used in this study cannot definitively identify such a system, it does highlight the potential advantages of a multisensory communication system in Malawian cichlids.

This study was designed to determine the likelihood that acoustic communication is under direct selective pressure at phylogenetic levels relevant to sexual selection. Both the laboratory and field data indicate that this is indeed the case. However, the field data suggests that acoustic signals likely serve additional functions outside the courtship context, perhaps in the service of intraspecific competition or as an alarm system. The latter prospect is particularly enigmatic, as no data exist regarding the hearing capabilities of Malawian cichlids, and it is thus unknown over what distances conspecific or heterospecific vocalizations might be detectable.

The studies presented here provide the data to unseat the old and staunchly held view that visual communication is the only relevant sensory mode in cichlid communication. Moreover, it lays the groundwork for further investigations into cichlid communication, opening several intriguing possibilities regarding both signal production and processing. Although Myrberg (1980) was of the opinion that multimodal integration in cichlids constituted an intractable problem, we suggest that audio-visual integration may be the Gordian Knot of speciation in at least one of the species-rich African cichlid radiations.
REFERENCES


