

Cutting the Gordian knot: Complex signaling in African cichlids is more than multimodal

Moira J. VAN STAADEN^{1*}, Adam R. SMITH²

¹ Department of Biological Sciences and JP Scott Center for Neuroscience, Mind & Behavior, Bowling Green State University, OH 43403, USA

² Department of Biology, University of Maryland, College Park, Maryland, 20742, USA

Abstract The active transmission of information from sender to receiver is a fundamental component of communication, and is therefore a primary facet in evolutionary models of sexual selection. Research in several systems has underlined the importance of multiple sensory modalities in courtship signals. However, we still tend to think of individuals as having a relatively static signal in consecutive communicative events. While this may be true for certain traits such as body size or coloration, behaviorally modulated signals can quickly violate this assumption. In this work, we explore how intraspecific variation may be an important component of interspecific signal divergence using cichlid fishes from Lake Malawi. Behavioral analyses were made using six species of Malawian cichlids from two divergent genera. While interspecific differences were found between congeners based on species-level analyses of both acoustic and audiovisual signals, intraspecific variation was of a similar magnitude. Specifically, individual fishes were found to possess highly plastic signal repertoires. This finding was ubiquitous across all species and resulted in a great deal of overlap between heterospecific individuals, despite statistically distinct species means. These results demonstrate that some aspects of courtship in Malawian cichlids are more plastic than previously proposed, and that studies must account for signal variability within individuals. We propose here that behavioral variability in signaling is important in determining the communication landscape on which signals are perceived. We review potential complexity deriving from multimodal signaling, discuss the sources for such lability, and suggest ways in which this issue may be approached experimentally [*Current Zoology* 57 (2): 237–252, 2011].

Keywords Cichlid, Motivation, Intraspecific variability, Mate preference, Multimodal

1 Introduction

By definition, some version of communication is a prerequisite for all proposed models of speciation via sexual selection. Given that sexual selection as a whole is a prominent mechanism for speciation (Kokko et al., 2006; Lande, 1981), the role of communication in broader evolutionary processes is readily apparent. The specific nature of the mechanisms for signal production, transmission, and perception, are key to understanding the effects of communication on speciation.

Classic models of sexual selection include a direct genetic link between male signal and female preference (Fisher, 1915; Weatherhead and Robertson, 1979; Zahavi, 1975). More recent models, however, allow for the possibility of less direct effects in communication systems. The spectrum of sensory drive and bias hypotheses involve alternate explanations in which male signals may evolve to exploit environmental transmission char-

acteristics (Coultridge and van Staaden, 2004; Seehausen et al., 2008) or even the endogenous traits of the female sensory systems (Endler, 1993).

Most present sexual selection models focus primarily on the perception of and reaction to a signal. If we assume that signaling is indeed for the ultimate purpose of communication, this still begs the question: exactly what is the information content of a sexual signal? Do male courtship displays say “I am a male of species A behaving in context X”? Are courtship signals condition dependent, as would be predicted if speciation is actively occurring via sexual selection (Boughman, 2007)? The underlying assumption that signals convey accurate, context-specific information is pervasive in most sexual selection literature (Hamilton and Zuk, 1982; Maan et al., 2006). However, several alternate options derived from general communication theory and affective neuroscience are not only possible, but also quite plausible in several systems.

Received Oct 22, 2010; accepted Jan. 01, 2011

* Corresponding author. E-mail: mvs.bgsu@gmail.com

© 2011 *Current Zoology*

2 Mate Selection and Complex Signaling

Appreciation for signal complexity and the widespread multimodality of male sexual displays is increasing (reviewed in Candolin, 2003; Hebets and Papaj, 2005). At the same time, there is a distinct paucity of studies in which the use of multiple cues in mate choice are simultaneously investigated (Coleman, 2009). Spiders and insects are notable exceptions (Elias et al., 2006; Papke et al., 2007; Uetz and Robert, 2002). In fish, analyses of complex signaling are largely limited to functions other than mating (New and Kang, 2000; Rojas and Moller, 2002), and in the context of mate choice, most claims are based on the association of signals in different modalities (Smith and van Staaden, 2009) rather than direct experimental verification (but see McLennan, 2003).

Determining the relative use of each signal is difficult because it requires a comprehensive understanding of the mating system and how this system works under natural conditions. Moreover, selective pressures may be both direct and indirect. Mating preferences may change as an immediate consequence of the signals themselves if divergent sensory adaptation affects the perception of signals (Maan and Seehausen, 2010). Additionally, such preferences can also diverge as a result of indirect effects, for instance selection within the sensory environment (Coultridge and van Staaden, 2004).

Biologists have elaborately investigated visual and acoustic modalities, but in pursuing the rich research possibilities they provide, it is possible that our view of communication and sexual selection has been too narrowly circumscribed. The application of rigid experimental or conceptual frameworks when interpreting signaling systems and their consequences may obscure relevant insight. Barlow (1998) cautioned against the use of the choice experiment as a possibly constraining tool, and the information approach (Shannon and Weaver, 1949) may be similarly restrictive when accorded a central causal role. A return to an assessment/management approach, in which signals are used to manage the behavior of others by operating on the couplings generated by others' assessment activities might be timely (Owings and Morton, 1997).

The potential roles and interactions of classical sexual selection models of signaling in speciation are manifold. Signaling may be complex in terms of the component modalities involved, but also because of

ability or individual variation. Here we consider the possible roles of signal variability, plasticity, and motivation in sexual selection. By placing these in the context of sexual signaling in the cichlid fishes of East Africa, we hope to shed light on the role that complex communication plays in the generation of rapid evolutionary radiations.

3 Cichlids as A Model for Complex Signaling

The lacustrine species flocks of cichlid fishes in Africa are the largest and most species-rich vertebrate radiation known, collectively comprising thousands of endemic species which have diverged from a common ancestor in a geologically brief time (Azuma et al., 2008). They display an astonishingly high degree of ecological and behavioral differentiation between species, although genome diversity between species is comparable to that of any two humans (Loh et al., 2008). Behavior has been frequently suggested as the ratchet driving this divergence, and sexual selection plays a central role in alternative models of cichlid speciation (Crapon de Caprona, 1986; Streelman and Danley, 2003).

Across the African continent, we have multiple dynamic natural evolutionary experiments with biologically attractive features; large numbers of species at various stages of differentiation, a broad range of divergence times, animals at high density with social systems ranging from relatively solitary individuals to communities with rigidly structured hierarchies, and utilizing many modes of communication. Laboratory investigations are generally structured around an assumption that due to the sheer number of incipient species, a single environmental or behavioral variable can be explored while keeping all others constant. But can it? In the subsequent sections we review (though not exhaustively) what is known of cichlid signaling in mate choice, suggest possible sources of variation, and argue that cognizance of internal factors is a necessary prerequisite if we are to achieve a deeper understanding of the role of behavior in cichlid signaling and divergence.

Study of cichlid communication has a venerable history dating back at least to the 1930s (Liebman, 1933; Myrberg et al., 1965; Seitz, 1940, 1949). The reality of multimodal signaling in the case of the African cichlids was well-recognized and clearly expressed in the 1970's (Silverman, 1978; Tavalga, 1976), and plastic multimo-

dal behaviors even described by Myrberg (1980)¹ as a Gordian Knot of indecipherable complexity. Similarly, in early work on cichlid courtship and signaling behavior individuality/variability was a major locus of interest (e.g., Barash, 1975; Heiligenberg, 1973, 1976; Lanzing, 1974). More recently, however, the tendency has been to downplay this variation, viewing it as 'noise' rather than 'signal'. The uncertainties of capturing a coherent picture from a fluid process (i.e., rapid diversification and consequent taxonomic instability) inevitably tempt in this direction. This is compounded by logistical constraints with respect to sampling, statistical concerns about pseudoreplication and data independence, and methodologies which do not effectively capture variation.

Given the plasticity of other aspects of cichlid biology, it would indeed be surprising if their signaling were not similarly malleable. Intraspecific plasticity through development is known for genetic or morphometric traits that can influence signal production (relative jaw morphology; Stauffer and Van Snik Gray, 2004) or perception (opsin pigment expression; Hofmann et al., 2010). Therefore, it would seem that acknowledging the extent of intraspecific or -individual variability in communication regimes will enable us to better deal with apparently inconsistent results and direct attention to alternative explanations (e.g., multiple cues along a time continuum; Jordan, 2008).

To the extent that the general cichlid pattern of coupling high species diversity with fairly small disparities in ecomorphological traits is mirrored in communication, seemingly small differences in signals could assume disproportionate importance. The best chance of identifying these will be to describe the variation in its entirety, remove genuine commonalities, and leave the telling differences whether they are qualitative or quantitative. The current resurgence of interest in the functional aspects of cichlid signaling presents an opportunity to do just that. We will describe and summarize this diffuse primary literature to focus attention on likely interactions, to highlight the variability underlying cichlid acoustic signaling, and to suggest an alternative approach which might aid in negotiating the current impasse that is uni- and multimodal signal plasticity.

4 Review of African Cichlid Communication

Visual communication is well-studied in the African

cichlids - to the extent that it is frequently (though perhaps erroneously) viewed as a 'magic trait' (Gavrilets, 2004) in speciation - but the role of other communication modalities in mate choice have received rather less attention. For convenience and clarity, we organize our presentation by this central modality, drawing attention to cross-modal interactions only where there is direct evidence to support this. (Note that genus names for the rock-dwelling mbuna of Lake Malawi follow those currently recognized by the International Commission on Zoological Nomenclature, although there is some debate cf. Oliver and Arnegard, 2010). We also exclude non-mating communication and extended phenotypes for the most part.

4.1 Visual channels

The significance of vision in cichlid mate choice tends to center on the role of color-, and to a lesser extent, pattern differences. Visual sexual selection appears to be commonplace in the rift lake cichlids, and has been hypothesized for fishes from all of the great lakes (Malawi - Arnegard et al., 1999; Victoria - Seehausen et al., 1997; Tanganyika - Salzburger et al., 2006). Direct behavioral assays have demonstrated that females preferentially associate with conspecific males when given a choice between congeners as a result of differences in male coloration (Coultridge and Alexander, 2002a; Jordan et al., 2003; Kidd et al., 2006). Moreover, the degree of reproductive isolation resulting from such positive color-assortative mating is weaker amongst less distinct morphs (Salzburger et al., 2006). In multimodal tests, however, visual cues alone result in lowered male courtship and display rates compared to those with unlimited access (Silverman, 1978), and are insufficient to maintain species boundaries in certain cases (Blais et al., 2009; Plenderleith et al., 2005).

While chromatic signals appear to dominate cichlid mate choice, aesthetic patterning has inspired many colorful descriptions but few studies have examined stripe/ barring patterns directly. Seehausen et al. (1999) surveyed patterning in a phylogenetic framework and concluded that stripe patterns are constrained ecologically with no influence of sexual selection. However, significant genetic differentiation consistent with a role for male nuptial patterning in species recognition and assortative mating was identified in four sympatric putative species (genus *Diplotaxodon*) whose males differ primarily in 'monochromatic' patterning of body and

¹ Myrberg AA, 1980. Sensory mediation of social recognition processes in fishes. In: Bardach JE, Magnuson JJ, May RC, Reinhart JM ed. Fish behavior and its use in the capture and culture of fishes. ICLARM Conference Proceedings 5: 146-178.

fins (Genner et al., 2007). Similarly, the size and shape of the egg spots present on an individual's anal fin may serve as a variable recognition cue within and between species (Konings, 1990). An intraspecific sexual advertisement function in female mate choice is well established for some species. For instance, females in some species exhibit preferences for males with larger numbers of egg spots in both Lake Malawi and the satellite Lake George (Hert, 1989, 1991). In contrast, Couldridge and Alexander (2002a, b) demonstrated conclusively that the sensory basis for mate preference in *Metriaclima lombardoi* is positively associated with egg spot size and negatively associated with number. This may indicate a preference for dominant/territorial males who possess bigger and brighter egg spots than their subordinates (Naish and Ribbink, 1990). Thus in cichlids, increases in egg spot size and number are not alternative means of eliciting the same female preference, and although such preferences have been suggested to result from a sensory exploitation/bias (Tobler, 2006), there is currently no direct evidence to support this notion. Furthermore, it is important to note that egg spots are not a diagnostic male trait in all species and are present in females, suggesting that the meaning of this sexual signal is likely not consistent between species.

Direct behavioral tests of the salience of size or shape cues in cichlid mate choice are rare, and only indirect indicators allude to the possible consequence of the latter. Jordan et al. (2003) demonstrated that pattern and shape cues were sufficient to stimulate courtship and guide female choice of conspecific males, even where color was not a cue. In a system where lekking males of *Astatotilapia flavijosephi* face constraints in fertilizing multiple females and are therefore choosy (Werner and Lotem, 2003), a sequential mate preference paradigm indicates sensitivity to size information, with selective allocation of courtship effort to more attractive (i.e., heavier) females (Werner and Lotem, 2006). A landmark-based morphometric study of three sympatric *Petrotilapia* congeners found subtle morphological differentiation among species, and pronounced variation in the head region (Kassam et al., 2004). Similarly, 'barred' and 'blotched' females of *Metriaclima zebra* and *M. zebra* 'gold' are very similar in color and pattern and can often be distinguished only by small differences in head and body shape (e.g., Knight and Turner, 1999).

Unlike most other vertebrates, in which spectral deprivation has little or no effect on visual development or behavioral responses, cichlids seem to be particularly sensitive to light quality (Kröger et al., 2001). UV sensi-

tivity has been demonstrated for *Cynotilapia afra*, *Metriaclima benetos*, and *Metriaclima melabranchion* (Jordan et al., 2004). Moreover, UV-sensitive opsins are expressed in the vast majority of Malawi mbuna, having been reported for fishes from multiple genera, and the UV-sensitive pigment is expressed in all of the recently diverged *Metriaclima* species studied thus far along with many others (Carleton, 2009). Spectral sensitivities in cichlids do not clearly correlate with habitat or feeding mode (Jordan et al., 2006), although it has been suggested that planktivory is linked to UV sensitivity (Hofmann et al., 2009). Therefore, a potential role in mate choice is possible, particularly for rock-dwelling taxa inhabiting the shallower lake areas where substantial UV light penetrates (Loew and McFarland, 1990). It is reasonable to expect that cryptic structural patterning of UV-reflective pigments may be present within and between species. Similarly, polarized light is rich in shallow environments, and *Tropheops macrophthalmus* can discriminate between horizontal and vertical polarized light (Davitz and McKaye, 1978). This provides yet another visual channel to supplement the intensity and color of light, increasing the visibility of mates and enhancing contrast, object recognition, signal detection and discrimination. In sum, the contributions of the visual modality to cichlid signaling are complex. Overshadowing the lesser-explored channels are visual signals such as male nuptial coloration which, being rather stereotyped within species, are of practical application to field biologists (Markert and Arnegard, 2007), and play a rigorously demonstrated role in mate recognition (Seehausen et al., 1997; Seehausen and Van Alphen, 1998). These well established aspects of cichlid communication arguably constitute the single most important axis of rock-dwelling cichlid divergence and evolution, though several others may also be influential.

4.2 Mechanosensory signals

A variety of African cichlids are known to produce acoustic signals in the context of courtship, including both riverine (Myrberg et al., 1965), and rift lake species (Amorim et al., 2004; Lobel, 1998, 2001). Sound production is influenced by behavioral and social lability (Amorim and Almada, 2005). Although there is little experimental evidence for the influence of sound on mating decisions of females (but see Verzijden et al., 2010), a range of behavioral (Fay and Popper, 1975), psychophysical (Tavolga, 1974; Yan and Popper, 1992) and noninvasive physiological experiments (Ladich and Wysocki, 2003; Ripley et al., 2002; Smith et al., 2004) support a general correlation between hearing sensitivity

and acoustic signals in cichlids.

Signal design is characteristically broadband, covering frequencies from 20–1200 Hz, with peak energy between 180–500 Hz, and audible, upon playback to humans in the lab, as either a series of individual clicks, or more commonly, as a uniform grunt (Smith and van Staaden, 2009). Species-specific differences have been documented in lacustrine taxa (Amorim et al., 2008; Verzijden et al., 2010), but the relevance of these statistically significant variations has yet to be demonstrated in a heterospecific context. Moreover, context-dependent differences are absent between male sounds produced during aggressive displays toward males and sexual displays toward females (Verzijden et al., 2010; but see Simões et al., 2008 for a contrasting view). In the few instances where multiple populations of the same species have been investigated (Amorim et al., 2008; Smith and van Staaden, 2009), differences in signal parameters hint at the possibility of geographically differentiated dialects which could contribute to reproductive isolation of populations.

Lateral display and quivering are common features of sexual signaling in cichlids (Baerends and Baerends van Roon, 1950; McElroy and Kornfield, 1990). A signaling fish (sender) aligns itself within a few centimeters of a receiver with fins erect, and vibrates its body, so as to generate a low frequency mechanosensory stimulus. In theory, the receiver could detect and integrate this information with its lateral line system, but there have been relatively few direct tests of this hypothesis (Mongomery et al., 2009). Components of this male lateral display (e.g., amplitude, frequency) may contribute to directional components of mate attraction (Braun et al., 2002), and may be indicators of condition significant to mate choice.

4.3 Chemical cues

Olfactory cues are known to play an important role in social cognition in many fish species (Brown et al., 2006; Ladich et al., 2006) and have been suggested to play an important role in mate choice in cichlids (Cole and Stacey, 2006; Plenderleith et al., 2005; Robison et al., 1998), though direct tests are sparse. Chemical cues appear to influence female mate choice (Plenderleith et al., 2005) and assortative mating (Blais et al., 2009) in *Metriaclima emmitos*, but this is not true for all species, even close congeners. Initiation of courtship behavior by *Metriaclima lombardoi* males relies solely on female visual cues, and olfactory signals play little (if any), role in mate choice (Venesky et al., 2005). Similarly, olfactory cues neither stimulated

courtship nor guided female choice of males in four sympatric *Metriaclima* species (Jordan et al., 2003). Short-range chemical cues are significant for female mate recognition of established relationships when nest-guarding at night (*Archocentrus nigrofasciatum*; Reeb, 1994). Overall, chemical cues seem to be more important as general indicators of social or reproductive status (Almeida et al., 2005; Bender et al., 2008) and recognition (Giaquinto and Volpato, 1997) rather than for mate choice. However, individual recognition of its own olfactory cues might play a role in self-referent mate choice (Thünken et al., 2009), particularly given that MHC is extremely variable in Lake Malawi cichlids (Klein et al., 1993).

5 Case Study: Individual Malawi Cichlids Behaviorally Modify Signal

While most previous work has sought to identify interspecies differences in signal characteristics for both visual and acoustic signals in cichlids, some studies have also explored intraspecific acoustic variation related to individual size (Amorim et al., 2008; Verzijden et al., 2010). Many have alluded to the relationship between visual and acoustic signals during courtship displays (Ripley and Lobel, 2004). In quantifying this audiovisual association in a phylogenetic context, we found extensive inter- and intraspecific variation in individual usage of vocal or nonvocal courtship displays (Smith and van Staaden, 2009). This was built upon a level of individual signal variability with implications for con- and heterospecific interactions, which we detail below.

5.1 Using courting pairs to investigate individual variation

We staged encounters between pairs of naive, mature conspecifics (dominant males and gravid females) in a full access restrained choice arena for 6 species from two genera (Fig. 1): *Metriaclima zebra*, *Metriaclima callainos*, *Metriaclima lombardoi*, *Melanochromis auratus*, *Melanochromis johannii*, and *Melanochromis cyaneorhabdos*. The *Metriaclima* and *Melanochromis* genera are both likely undergoing speciation by sexual selection, but are phylogenetically distant in terms of the rock-dwelling Malawian cichlids (Albertson et al., 1999). Also the presence of a striped phenotype in *Melanochromis* species and a primary barred phenotype in *Metriaclima* suggests that these genera are sexually isolated on visual cues alone. Therefore, these genera were selected as two relatively independent species radiations.

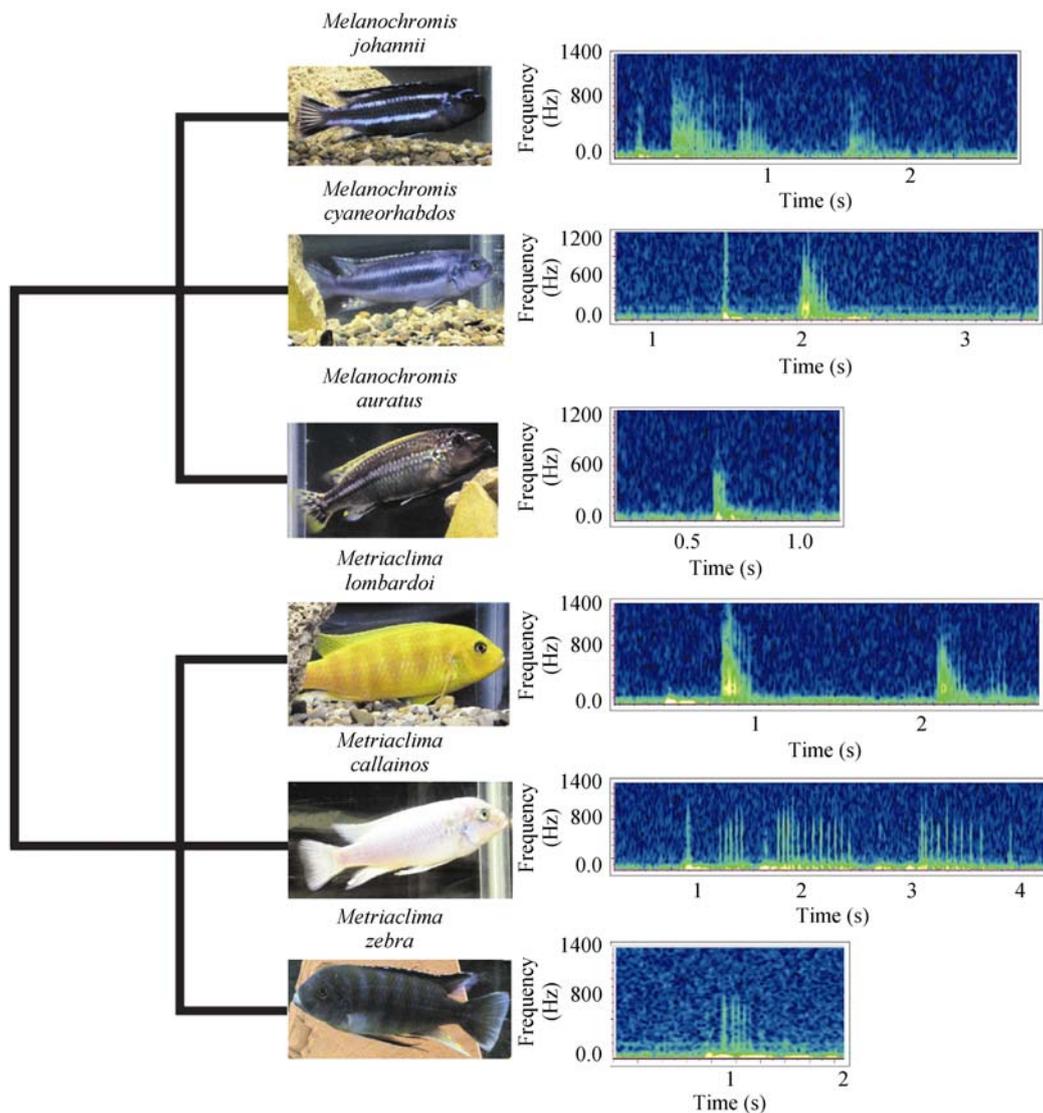


Fig. 1 Simplified phylogenetic relationships of the study species, with sonogram for a representative courtship call of each taxon

Behavior and acoustic signals were recorded over multiple courtship bouts in which we reused fish but always in unique pairings (methods detailed in Smith and van Staaden, 2009). The characteristics of courtship calls were broken down into three primary variables: (i) the primary frequency (the frequency which contained the most energy throughout the duration of a call), (ii) the total duration of the call, and (iii) the number of discrete pulses contained within each call. Multimodal cues were scored as the association between visual courtship displays and calls. In particular, the number of calls produced during a single visual display was measured. Although males rarely emitted calls outside of a visual display, all calls that coincided with visual displays occurred during the quiver phase of the male dance. Therefore, the association of the quiver displays and

courtship calls was the focal multimodal behavior.

Sound data was analyzed using multivariate discriminant function analyses (DFAs) of call characteristics identified by preliminary analyses to provide discriminatory power e.g. number of individual pulses/call was highly correlated with total call duration in the multivariate model ($r = 0.72$, data not shown) and were excluded from subsequent analyses. Preliminary analyses demonstrated pulse period was only informative in the context of comparisons between the clicking species (*M. callainos*) and the grunting species (all others). Also, since pulse period was difficult to measure in many grunting calls due to the noise threshold, this trait was excluded from analyses. An identity MANOVA with individuals nested within species was used to determine the role of individual variation in the relationship be-

tween species distributions.

5.2 Individual variation in unimodal acoustic characteristics

We found pronounced variation both between and within individuals for the characteristics of acoustic courtship signals. While multivariate analyses were able to distinguish species means based on acoustic characteristics of four highly vocal species (*Metriaclima callainos*, *Metriaclima lombardoi*, *Melanochromis cyaneorhabdos*, and *Melanochromis johannii*), intra-individual variation was of a similar magnitude to intraspecific variation within all species tested. A DFA of the call characteristics for these species indicated that *Metriaclima callainos* was distinct from its congener and both the *Melanochromis*, and that this divergence greatly exceeded the variation within *M. callainos* (Wilks' Lambda = 0.329, $P < 0.0001$). More specifically, *M. callainos* calls were composed of broadband pulses where the interpulse duration was long enough for the call to appear as a series of independent clicks to the human ear, whereas for all other species the interpulse duration was short enough that calls formed a uniform grunting noise.

A further DFA of the remaining three species revealed that calls of *Metriaclima lombardoi* and *Melanochromis cyaneorhabdos* are indistinguishable, but both are diverged from the distinct grouping formed by *Melanochromis johannii* vocalizations (Wilks' Lambda = 0.877, $P < 0.0001$). This result suggested that the courtship calls of congeners do not overlap, whereas overlap exists between heterogeneric species. A nested MANOVA, however, demonstrated that the calls of certain individuals from different species overlapped while falling outside their calculated species distribution (Wilks' Lambda = 0.624, $P < 0.0001$), i.e., call variation within individuals and species can blur the lines of what may otherwise seem to be species-specific signals. This was particularly pronounced in *M. lombardoi*, where extensive variation resulted in individuals that overlapped with both *M. cyaneorhabdos* and *M. johannii*. Fig. 2 highlights variability in the calls of individuals of two species, showing that individual lability encompasses almost the entirety of variation observed for conspecifics (from the same population). More importantly, it may also exceed the total variation observed for heterospecifics of four other taxa (data shown for one). This extensive individual variation suggests a limited utility for general analyses of individual means as accurate descriptors of individual- or species-specific communication norms in these species.

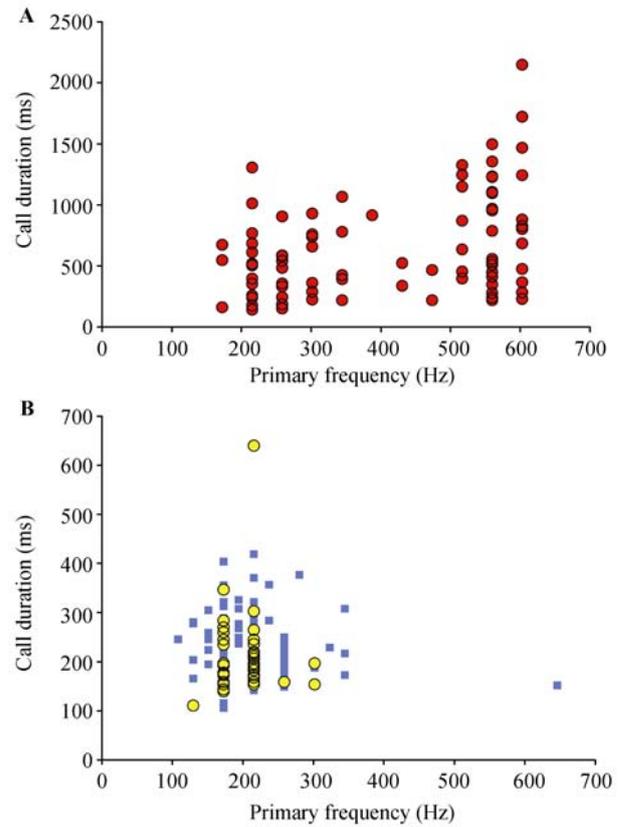


Fig. 2 Individual plasticity in acoustic signal characteristics of Malawi cichlids

A. Calls of a single *Metriaclima callainos* male span almost the entirety of variation for this species and encompasses a range of frequencies and total durations that exceeded the total variation observed for five *Melanochromis cyaneorhabdos* individuals. **B.** The calls of a focal *M. cyaneorhabdos* individual (yellow circles) covers virtually the entire range of the “species-specific” acoustic space (blue squares).

5.3 Individual variation in use of multimodal associations

Multimodal signal variation follows a pattern similar to that presented above for unimodal (acoustic) characteristics, i.e. with a large degree of both intraspecific and intra-individual variation (Smith and van Staaden, 2009). Here we use inter- and intraspecific contingency tables to analyze patterns of multimodal signal usage with visual/acoustic temporal coincidence as dependent variable. Simultaneous comparison of the audio-visual repertoire of four vocal species and two largely non-vocal species (*Metriaclima zebra* ‘katale’ and *Melanochromis auratus*) demonstrates that the correlation between acoustic and visual behaviors varies across species (Pearson $\chi^2 = 86.61$, $P < 0.0001$, Fig. 3A). However, as is the case with the general call characteristics reported above, the large degree of intraspecific variation effectively obscures the overall interspecies

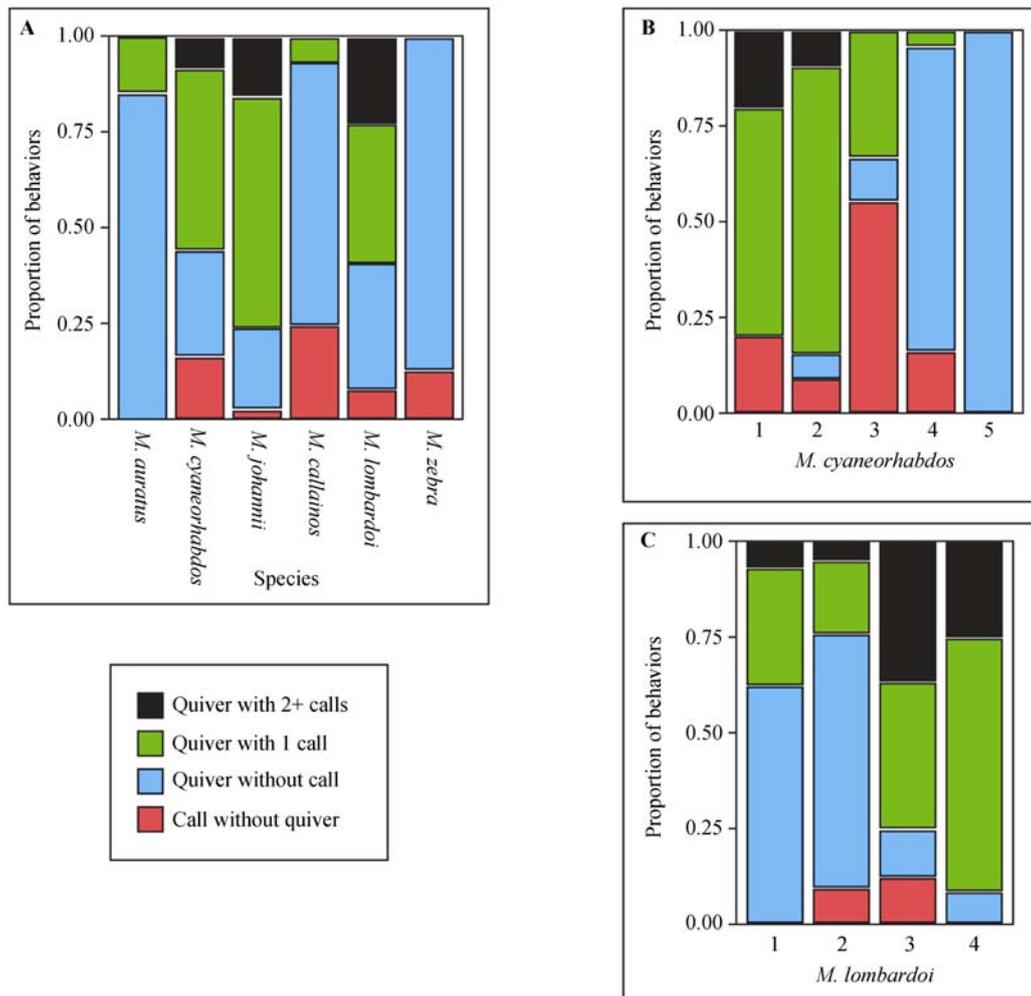


Fig. 3 Distribution of audiovisual behaviors observed (A) across all six species, (B) between individuals of *Melanochromis cyaneorhabdos*, and (C) between individuals of *Metriaclima lombardoi*

Encounters were staged between pairs of naive, mature conspecifics (dominant males and gravid females) in a full-access restrained choice arena.

analysis. This variance effect is found in both genera, as demonstrated by *Melanochromis cyaneorhabdos* (Pearson $\chi^2 = 82.00$, $P < 0.0001$, Fig. 3B) and *Metriaclima callainos* (Pearson $\chi^2 = 37.25$, $P = 0.0002$, Fig. 3C).

5.4 What does this variation mean?

Taken together, the individual preferences for vocal or nonvocal displays, combined with the large variability in acoustic characteristics, creates extraordinary potential for signal diversity from individuals. In essence, during a single courtship bout encompassing a finite number of displays, an individual male can exploit signal variation along both unimodal and multimodal axes. There are two primary points of note here. Firstly, variation in both axes is mediated by the presence and structure of acoustic signals. And secondly, individual males can produce a range of signals that encompasses almost the entire observed species range. This raises the ques-

tion of how (and why) a single fish might produce such a broad array of signals. Furthermore, how might these plastic signals be interpreted by a receiver? Perhaps this encompasses a conflation of functions with signaling components less critical for species recognition (and more critical for conveying other information, such as male quality) being the ones that are more plastic than previously proposed. Alternatively, it might reflect the relative significance of different modalities at discrete separation distances of the interactants.

6 Sources of Variability in Cichlid Signaling

The assumption that signals provided by males have endogenous information content is pervasive in cichlid communication work. This is intuitive and likely correct for many signals that remain stable on a medium tem-

poral scale, such as gross body coloration patterns, or bower shape and placement. However, for acoustic signals the lack of context specificity (Verzijden et al., 2010; see their Fig. 4), limited support for species-specificity (Verzijden et al., 2010), extensive variation in individual acoustic repertoires (in 5 above; Simões et al., 2008), and lability of multimodal use (Smith and van Staaden, 2009), calls into question what exactly these signals convey. It suggests firstly, that we ought to entertain the notion that simple information transfer may not be the ultimate function, and secondly that signals are influenced by multiple factors.

6.1 Alternative functions

Beyond a simple information transfer view, we suggest at least three plausible functions for signals in a courtship context. The overlap of display characteristics in agonistic and courtship behaviors is widely recognized in African cichlids, with several visual components, such as horizontal displays and quivers, common to both contexts (Baerends and Baerends-van Roon, 1950), and with nuptial coloration of demonstrated sig-

nificance for male-male signaling during agonistic interactions (Pauers et al., 2008; Seehausen and Schluter, 2004). Rather than having intrinsic meaning related to any particular aspect of signal structure, supplementary acoustic signals in the context of courtship may serve to mediate this generalizable display (i.e., a metacommunication function; Lewis and Gower, 1980). Alternatively, acoustic signals may manipulate receiver behavior via a startle effect to garner the attention of the female (Canfield, 2003). Or (more speculatively), the acoustic signal may serve to modulate or augment the apparent qualities of the visual signal by “hijacking” the inherent cognitive qualities of multisensory perception, e.g., via a form of the McGurk Effect (McGurk and MacDonald, 1976), or by expanding the perceived length of a quiver display (reviewed by Trout, 2001).

6.2 External sources of lability

Cichlid males face significant external challenges in acquiring mates. High densities and levels of aggression create intense competition for territory and mates as a result of their lekking behaviors. In such scenarios,

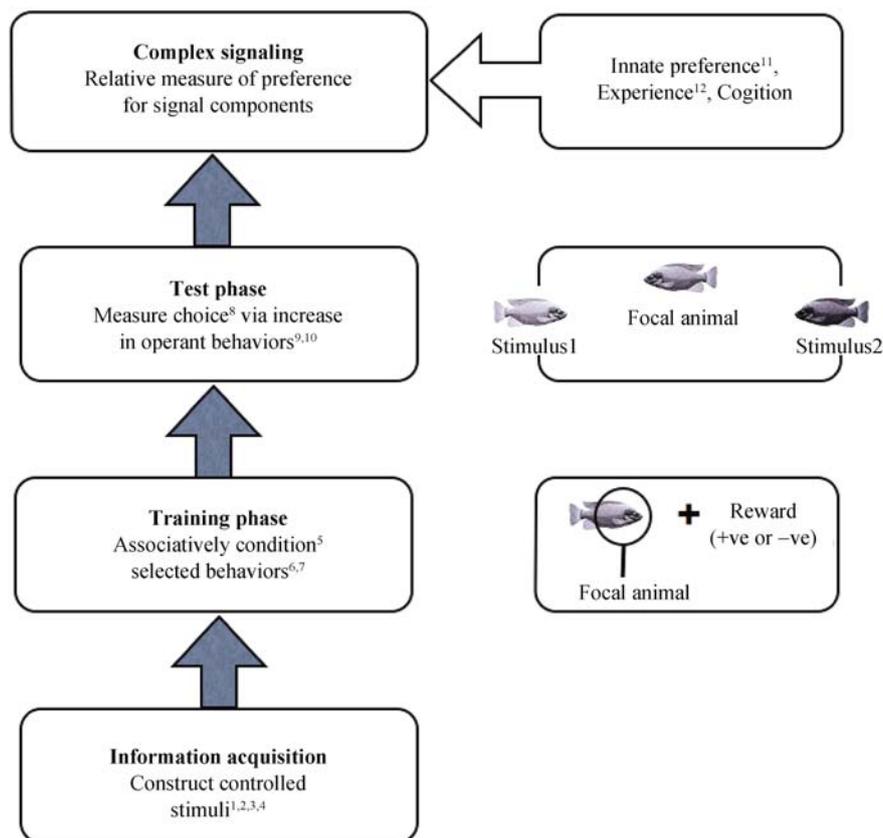


Fig. 4 Heuristic of a motivational approach for the analysis of complex signaling

¹ Balshine-Earn and Lotem, 1998; ² Rosenthal, 2000; ³ Saverino and Gerlai, 2008; ⁴ Baldauf et al., 2009; ⁵ Elliot, 2006; ⁶ Dunlop et al., 2006; ⁷ Yue et al., 2004; ⁸ Paul et al., 2005; ⁹ Braithwaite and Boulcott, 2007; ¹⁰ Braithwaite, 2006; ¹¹ Pierotti et al., 2008; ¹² Amorim and Almada, 2005.

selection for effective signaler-receiver communication is expected to be especially strong and result in the evolution of considerable diversity in signaling behaviors (e.g., Uetz and Roberts, 2002). Local population density is known to influence behavioral sequencing during courtship (Fernald and Hirata, 1977), and minor environmental disturbances limit acoustic signaling in cichlids (Lanzing, 1974).

The importance of male signal lability is apparent when considering the series of events that occur during a typical courtship bout in Lake Malawi. Generally, a male will approach a female passing his territory and perform a horizontal display involving the quivering and calling behaviors that compose the full audiovisual signal. Typically, the male gets a small number of horizontal presentations in which to “impress” the female (~3–5; personal observation) and lure her back to his territory before she leaves. During this brief time, the male can employ an expansive repertoire of possible signals in order to impress his potential mate. A male’s tenure as a territory holder has been estimated to be as short as two weeks (Barlow, 2002b), although periods of up to 18 months have been reported (Hert, 1992). Thus, the male with the highest evolutionary fitness may very well be the one who invests the most energy in mate procurement, as opposed to resource or territory defense. This is supported by the lack of association between male coloration and indicators of male dominance (e.g., male size, territory size, and -location) in a wild population (Maan et al., 2006).

6.3 Internal sources of lability

Interspecific variation would be most parsimoniously explained by fundamental differences in sound producing structures; the stridulation of pharyngeal jaws (Rice and Lobel, 2002, 2003) versus abdominal resonance via “rib-crunching” (Longrie et al., 2009). However, the pronounced similarity of the sounds presently described for African cichlids argues for a single common mechanism. Cichlid adult brain variation is appreciable (Huber et al., 1997; van Staaden et al., 1995) and ecologically relevant differences identified in the elaboration of cichlid forebrain regions (Sylvester et al., 2010) are likely associated with the behavioral control and modulation of communication as well.

Complexity may also increase ‘active time’. i.e. the period over which a signal influences a receiver’s response to that signal. Akre and Ryan (2010) showed that in túngara frogs, females do not consistently prefer greater complexity, but rather choice is due to differential memory. Female memory could thus favor the evo-

lution of increasing signal complexity through sexual selection. Other factors impacting mate-choice such as the audience effect and mate choice copying (Alonzo, 2008), and receiver learning-based biases on signal evolution (ten Cate and Rowe, 2007), all introduce further variability into the system. Comparative approaches are of limited value in such cases, since preferences emerging from the learning process are transient, and less likely to leave a phylogenetically traceable effect. Finally, variable female preferences may impact the degree of variation and complexity in male signals if (i) females are unable to evaluate some necessary features (Parker and Kornfield, 1996); (ii) sequential female assessment occurs (Werner and Lotem, 2006; Young et al., 2010); or (iii) cryptic female choice by ‘sperm shopping’ (Immler and Taborsky, 2009) drives complex sexual selection.

Taken together, the considerations outlined in sections 5 and 6 above support the notion that signaling plasticity is a fundamental trait of cichlid acoustic communication, and intraspecific variation in male signaling patterns likely results in complex patterns of mate selection and motivations.

7 Perspective: Cutting the Knot

Animals behaving in complex, three-dimensional environments receive a large amount of information from external and internal receptor arrays. Since this information feeds into motivational systems developed by evolution to generate experience, we propose addressing cichlid communication and speciation in a more complex and socially-relevant paradigm. Adopting a motivational approach to the communication landscape experienced by cichlid fishes should prove illuminating.

Mate choice tests in an artificial choice paradigm are deceptively simple, with alternatives measures (in decreasing order of confidence) ranging from spawning (Kidd et al., 2006); most courtship-, more time near-, or orientation toward- a potential mate (Barlow, 2002b). The literature is replete with variations on this theme, employing factorial design behavioral experiments, or cue-elimination and cue-conflict designs to dissect multimodal signaling and weigh the relative contributions of the various modalities in attracting females. Comparative approaches using closely related species (Verzijden et al., 2010), or geographically isolated populations (Elias et al., 2006), can be informative, as can a combination of lab and field studies in sorting out systematic problems (e.g., Holzburg, 1978 for *Metriaclima zebra*). While such strategies have proven highly

effective for invertebrates (Hebets and Uetz, 1999; Rypstra et al., 2009; Uetz and Roberts, 2002) their utility for vertebrates is less clear.

Multiple presentations of a specific stimulus rarely evoke identical responses, even under rigorously specified environmental conditions (cf. “The Harvard rule of Animal Behavior”, Grobstein, 1994). Thus, altered responses must be attributed to changes in internal state (“motivation”) of the individual. Traditionally, motivations were viewed as an array of individual, unitary properties corresponding to broad behavioral categories such as parental care or fighting (Tinbergen, 1951) collectively interacting to guide behavior, through a “great parliament of instincts” (Lorenz, 1966). While the classical ethological model of motivation has lost much of its explanatory value and been superseded by a more complex, multi-dimensional view (Huber and Kravitz, 1995; Huber et al., 2001), the term remains for want of a better alternative. Here the motivational state simply represents intrinsic forces that promote the occurrence of defined sets of behaviors within adaptive contexts, without implying a specific underlying framework (e.g., psychohydraulic model - Lorenz, 1966; neuro-evolutionary constructs - MacLean, 1990; Panksepp, 1998, 2003).

8 Affective States and Cognitive Processing

Responding to mating signals in a manner which is contingent on a multiplicity of internal, social, ecological, and/or environmental conditions, is both appropriate and adaptive. Mate choice tests of cichlids in a lab setting, indeed, provide highly variable outcomes (Barlow, 1998; Verzijden et al., 2010, Fig 3). Analysis of the processes underlying this variation is problematic since discrimination can decrease either because the task is too difficult, or because the animal is too motivated (Barlow, 2002a).

We suggest that a learning paradigm may be more illuminating of the flexibility and variability involved in complex signaling, and that an animal's motivation to work for access to various stimuli can provide an effective compromise between system complexity and practical simplicity. Moreover, it has the virtues of (i) permitting a continuous measure which is more informative than a binary one; (ii) allowing one to quantitatively separate out the specificity and sensitivity of signal components (using conditioning experiments); (iii) permitting one to assess both preference functions and choosiness; and (iv) offering the potential to distinguish affective from cognitive processes by combining these

sensitive behavioral measures with an understanding of their underlying brain substrates.

The nexus of motivation-emotion, SEEKING (Panksepp, 1998), and learning-based bias is well-recognized in humans where changes in emotional state are accompanied by concurrent changes in information processing (Clore and Ortony, 2000; Mathews and MacLeod, 1994). Such cognitive biases are now recognized also in rodents and birds (Emery, 2006; Harding et al., 2004). The neural interface between limbic and motor systems by which ‘motivation’ gets translated into ‘action’ are remarkably similar in both affective neurochemistry and neuroanatomy across all vertebrate taxa (Goodson and Bass, 2001) cf. the distribution of cannabinoid receptors in cichlids (Cottone et al., 2005). Recently researchers have begun to quantify motivational affective states in fish in the context of animal welfare (Braithwaite and Boulcott, 2007; Paul et al., 2005) and higher order cognitive processing (Braithwaite, 2006), using startle responses (Arnott and Elwood, 2009), or associative learning conditioning paradigms (Dunlop et al., 2006; Elliot, 2006; Yue et al., 2004).

We propose that an associative conditioning paradigm with positive reward will be a fruitful mechanism to assess the relative value of complex signals in driving cichlid mate preferences. In African cichlids, visual access to a conspecific has been shown to be rewarding (i.e., a positive stimulus), and modulates a conditioned response based on the intensity of the stimulus (T. Kitchin, unpubl. obs.). The technology for assessing motivation in an associative learning paradigm is readily available (Rosenthal, 2000). Video playbacks contain sufficient information to facilitate recognition in *Neolamprologus brichardi* (Balshine-Earn and Lotem, 1998), and computer-animated fishes (Baldauf et al., 2009; Saverino and Gerlai, 2008) permit precise tailoring of stimuli with respect to all aspects of visual signaling. With the exception of chemical and low frequency mechanosensory stimuli, for which temporal and spatial resolution remains a challenge, combining automated digital video tracking and computer-animated stimuli should accommodate any combination of multisensory signals and levels of lability required at both individual and higher taxonomic levels.

9 Conclusion

Models of communication have moved beyond the discrete exchange of information pioneered by Shannon and Weaver (1949) to encompass a view of communication as a creative and dynamic continuous process (e.g.,

transactional models - Barnlund, 2008; theories of co-regulation - Gallese, 2005). In the case of cichlids, we advocate a return to the motivational approach, harnessing the power of automated stimulus generating and tracking techniques, to overcome the limitations inherent in the traditional choice paradigm. Such an approach is appropriate and effective because we know cichlids have complex brains generating complex cognition (Huber et al., 1997; van Staaden et al., 1995), whereby both learning (Verzijden and ten Cate, 2007) and experiential effects (Amorim and Almada, 2005; Kotschal and Taborsky, 2010) lead to variability. In this way, the field of cichlid behavioral research might advance by returning to its classic ethological roots.

Acknowledgements We thank N. Donelson, S. Coombs, and members of the van Staaden and Huber labs for critique and discussion at earlier stages of this work. The manuscript was much improved by thoughtful comments from two anonymous reviewers. Financial support came from the J.P. Scott Center for Neuroscience Mind & Behavior (A.R.S.), and NSF grant 0757001 (M.v.S). Experiments reported here were performed under permit # 05-017 from the BGSU Institutional Animal Care and Use Committee.

References

- Akre KL, Ryan MJ, 2010. Complexity increases working memory for mating signals. *Current Biology* 20: 502–505.
- Albertson RC, Markert J, Danley PD, Kocher TD, 1999. Phylogeny of a rapidly evolving system: The cichlid fishes of Lake Malawi. *Proceedings of the National Academy of Sciences* 96: 5107–5110.
- Almeida OG, Miranda A, Frade P, Hubbard PC, Barata EN et al., 2005. Urine as a social signal in the Mozambique tilapia *Oreochromis mossambicus*. *Chemical Senses* 30: 1309–1310.
- Alonzo SH, 2008. Female mate choice copying affects sexual selection in wild populations of the ocellated wrasse. *Animal Behaviour* 75: 1715–1723.
- Amorim MCP, Knight ME, Stratoudakis Y, Turner GF, 2004. Differences in sounds made by courting males of three closely related Lake Malawi cichlid species. *Journal of Fish Biology* 65: 1358–1371.
- Amorim MCP, Almada VC, 2005. The outcome of male-male encounters affects subsequent sound production during courtship in the cichlid fish *Oreochromis mossambicus*. *Animal Behaviour* 69: 595–601.
- Amorim MCP, Simões JM, Fonseca PJ, Turner GF, 2008. Species differences in courtship acoustic signals among five Lake Malawi cichlid species (*Pseudotropheus* spp.). *Journal of Fish Biology* 72: 1355–1368.
- Arnegard ME, Markert JA, Danley PD, Stauffer JR Jr, Ambali AJ et al., 1999. Population structure and colour variation of the cichlid fish *Labeotropheus fuelleborni* Ahl along a recently formed archipelago of rocky habitat patches in southern Lake Malawi. *Proceedings of the Royal Society B* 266: 119–130.
- Arnott G, Elwood R, 2009. Probing aggressive motivation in a cichlid fish. *Biology Letters* 23: 762–764.
- Azuma Y, Kumazawa Y, Miya M, Mabuchi K, Nishida M, 2008. Mitogenomic evaluation of the historical biogeography of cichlids toward reliable dating of teleostean divergences. *BMC Evolutionary Biology* 8: 215.
- Baerends GP, Baerends-van Roon JM, 1950. An introduction to the study of the ethology of cichlid fishes. *Behaviour (Supplement)* 1: 1–235.
- Baldauf SA, Kullmann H, Thünken T, Winter S, Bakker TCM, 2009. Computer animation as a tool to study preferences in the cichlid *Pelvicachromis taeniatus*. *Journal of Fish Biology* 75: 738–746.
- Balshine-Earn S, Lotem A, 1998. Individual recognition in a cooperatively breeding cichlid: Evidence from video playback experiments. *Behaviour* 135: 369–386.
- Barash DP, 1975. Behavioral individuality in the cichlid fish *Tilapia mossambica*. *Behavioral Biology* 13: 197–202.
- Barlow GW, 1998. Sexual-selection models for exaggerated traits are useful but constraining. *American Zoologist* 38: 59–69.
- Barlow GW, 2002a. How behavioural studies contribute to the species problem: A piscine perspective. *Fish and Fisheries* 3: 197–212.
- Barlow GW, 2002b. *The Cichlid Fishes: Nature's Grand Experiment in Evolution*. Cambridge, MA: Perseus Publishing.
- Barnlund DC, 2008. A transactional model of communication. In: Mortensen CD ed. *Communication Theory*. New Jersey: Transaction, 47–57.
- Bender N, Heg-Bachar Z, Oliveira RF, Canario AVM, Taborsky M, 2008. Hormonal control of brood care and social status in a cichlid fish with brood care helpers. *Physiology and Behavior* 349–358.
- Blais J, Plenderleith M, Rico C, Taylor MI, Seehausen O et al., 2009. Assortative mating among Lake Malawi cichlid fish populations is not simply predictable from male nuptial color. *BMC Evolutionary Biology* 9: 1–12.
- Boughman JW, 2007. Condition-dependent expression of red colour differs between stickleback species. *Journal of Evolutionary Biology* 20: 1577–1590.
- Braithwaite VA, 2006. Cognitive ability in fish. *Fish Physiology* 24: 1–37.
- Braithwaite VA, Boulcott P, 2007. Pain perception, aversion and fear in fish. *Diseases of Aquatic Organisms* 75:131–8.
- Braun CB, Coombs S, Fay RR, 2002. What is the nature of multisensory interaction between octavolateralis sub-systems? *Brain, Behavior and Evolution* 59: 162–176.
- Brown GE, Bongiorno T, DiCapua DM, Ivan LI, Roh E, 2006. Effects of group size on the threat-sensitive response to varying concentrations of chemical alarm cues by juvenile convict cichlids. *Canadian Journal of Zoology* 84: 1–8.
- Candolin U, 2003. The use of multiple cues in mate choice. *Biological Reviews* 78: 575–595.
- Canfield JG, 2003. Temporal constraints on visually directed C-start responses: Behavioral and physiological correlates. *Brain Behavior and Evolution* 61:148–158.
- Carleton KL, 2009. Cichlid fish visual systems: Mechanisms of spectral tuning. *Integrative Zoology* 4: 75–86.
- Carleton KL, Hofmann CM, Klisz C, Patel Z, Chircus LM et al.,

2010. Genetic basis of differential opsin gene expression in cichlid fishes. *Journal of Evolutionary Biology* 23: 840–853.
- ten Cate C, Rowe C, 2007. Biases in signal evolution: Learning makes a difference. *Trends in Ecology and Evolution* 22: 380–387.
- Coleman SW, 2009. Taxonomic and sensory biases in the mate-choice literature: There are far too few studies of chemical and multimodal communication. *Acta ethologica* 12: 45–48.
- Clore GL, Ortony A, 2000. Cognition in emotion: Always, sometimes or never? In: Lane RD, Nadel L ed. *Cognitive Neuroscience of Emotion*. Oxford: Oxford University Press, 24–61.
- Cole TB, Stacey NE, 2006. Olfactory responses to steroids in an African mouth-brooding cichlid *Haplochromis burtoni* (Günther). *Journal of Fish Biology* 68: 661–680.
- Cottone E, Forno S, Campantico E, Guastalla A, Viltono L et al., 2005. Expression and distribution of CB1 cannabinoid receptors in the central nervous system of the African cichlid fish *Pelvicachromis pulcher*. *The Journal of Comparative Neurology* 485: 293–303.
- Couldridge VCK, van Staaden MJ, 2004. Habitat-dependent transmission of male advertisement calls in bladder grasshoppers (Orthoptera; Pneumoridae). *Journal of Experimental Biology* 207: 2777–2786.
- Couldridge VCK, Alexander GJ, 2002a. Color patterns and species recognition in four closely related species of Lake Malawi cichlid. *Behavioral Ecology* 13: 59–64.
- Couldridge VCK, Alexander GJ, 2002b. Experimental manipulation of male eggspots demonstrates female preference for one large spot in *Pseudotropheus lombardoi*. *Journal of Fish Biology* 60: 726–730.
- Crapon de Caprona M-D, 1986. Are 'preferences' and 'tolerances' in cichlid mate choice important for speciation? *Journal of Fish Biology* 29: 151–158.
- Davitz MA, McKaye KR, 1978. Discrimination between horizontally and vertically polarized light by the cichlid fish *Pseudotropheus macrophthalmus*. *Copeia* 1978: 333–334.
- Dunlop R, Millsopp S, Laming P, 2006. Avoidance learning in goldfish *Carassius auratus* and trout *Oncorhynchus mykiss* and implications for pain perception. *Applied Animal Behaviour Science* 97: 255–271.
- Elias DO, Hebets EA, Hoy RR, 2006. Female preference for complex/novel signals in a spider. *Behavioral Ecology* 17: 765–77.
- Elliot A, 2006. The hierarchical model of approach-avoidance motivation. *Motivation and Emotion* 30:111–116.
- Emery NJ, 2006. Cognitive ornithology: The evolution of avian intelligence. *Philosophical Transactions of the Royal Society B* 361: 23–43.
- Endler JA, 1993. Some general comments on the evolution and design of animal communication systems. *Philosophical Transactions of the Royal Society B* 340: 215–225.
- Fay RR, Popper AN, 1975. Modes of stimulation of the teleost ear. *Journal of Experimental Biology* 62: 379–387.
- Fernald RD, Hirata NR, 1977. Field study of *Haplochromis burtoni*: Quantitative behavioural observations. *Animal Behaviour* 25: 964–975.
- Fisher RA, 1915. The evolution of sexual preference. *Eugenics Review* 7: 184–192.
- Gallese V, 2005. Embodied simulation: From neurons to phenomenal experience. *Phenomenology and Cognitive Science*. 4: 23–48.
- Gavrilets S, 2004. *Fitness Landscapes and the Origin of Species*. Princeton, NJ: Princeton University Press.
- Genner MJ, Nichols P, Carvalho GR, Robinson RL, Shaw PW et al., 2007. Reproductive isolation among deep-water cichlid fishes of Lake Malawi differing in monochromatic male breeding dress. *Molecular Ecology* 16: 651–662.
- Giaquinto PC, Volpato GL, 1997. Chemical communication, aggression, and conspecific recognition in the fish Nile tilapia. *Physiology and Behavior* 62: 1333–1338.
- Goodson JL, Bass AH, 2001. Social behavior functions and related anatomical characteristics of vasotocin/vasopressin systems in vertebrates. *Brain Research Reviews* 35: 246–265.
- Grobstein P, 1994. Variability in brain function and behavior. In: Ramachandran VS ed. *Encyclopedia of Human Behavior*. Vol. 4. New York: Academic Press, 447–458.
- Hamilton WD, Zuk M, 1982. Heritable true fitness and bright birds: A role for parasites. *Science* 218: 384–487.
- Harding EJ, Paul ES, Mendl M, 2004. Animal behaviour: Cognitive bias and affective state. *Nature*. 427: 312.
- Hebets EA, Papaj DR, 2005. Complex signal function: Developing a framework for testable hypotheses. *Behavioral Ecology and Sociobiology* 57: 197–214.
- Hebets EA, Uetz GW, 1999. Female responses to isolated signals from multi-modal male courtship displays in the wolf spider genus *Schizocosa* (Araneae: Lycosidae). *Animal Behaviour* 57: 865–872.
- Heiligenberg WF, 1973. Random processes describing the occurrence of behavioural patterns in a cichlid fish. *Animal Behaviour* 21: 169–182.
- Heiligenberg WF, 1976. The interaction of stimulus patterns controlling aggressiveness in the cichlid fish *Haplochromis burtoni*. *Animal Behaviour* 24: 452–458.
- Hert E, 1989. The function of egg-spots in an African mouth-brooding cichlid fish. *Animal Behaviour* 37: 726–732.
- Hert E, 1991. Female choice based on egg-spots in *Pseudotropheus aurora* Burgess 1976, a rock-dwelling cichlid of Lake Malawi, Africa. *Journal of Fish Biology* 38: 951–953.
- Hert E, 1992. Homing and home-site fidelity in rock-dwelling cichlids (Pisces: Teleostei) of Lake Malawi, Africa. *Environmental Biology of Fishes* 33: 229–237.
- Hofmann CM, O'Quin KE, Marshall NJ, Cronin TW, Seehausen O et al., 2009. The eyes have it: Regulatory and structural changes both underlie cichlid visual pigment diversity. *PLoS Biol* 7(12): e1000266. doi:10.1371/journal.pbio.1000266
- Hofmann CM, O'Quin KE, Smith A, Carleton KL, 2010. Plasticity of opsin gene expression in cichlids from Lake Malawi. *Molecular Ecology* 19: 2064–2074.
- Holzberg S, 1978. A field and laboratory study of the behaviour and ecology of *Pseudotropheus zebra* (Boulenger), an endemic cichlid of Lake Malawi (Pisces; Cichlidae) *Journal of Zoological Systematics and Evolutionary Research*. 16: 171–187.
- Huber R, Kravitz EA, 1995. A quantitative study of agonistic behavior and dominance in juvenile American lobsters *Homarus americanus*. *Brain, Behavior and Evolution* 46: 72–83.

- Huber R, Panksepp JB, Yue Z, Delago A, Moore P, 2001. Dynamic interactions of behavior and amine neurochemistry during acquisition and maintenance of social rank in crayfish. *Brain, Behavior and Evolution* 57: 271–282.
- Huber R, van Staaden MJ, Kaufman L, Liem K, 1997. Microhabitat use, trophic patterns, and the evolution of brain structure in African cichlids. *Brain, Behavior and Evolution* 50: 167–182.
- Immler S, Taborsky MT, 2009. Sequential polyandry affords post-mating sexual selection in the mouths of cichlid females. *Behavioral Ecology and Sociobiology* 63:1219–1230.
- Jordan RC, 2008. Color-based association among heterospecifics in Lake Malaŵi rock-dwelling Cichlids. *Ethology* 114: 272–278.
- Jordan RC, Kellogg K, Juanes F, Stauffer Jr J, 2003. Evaluation of female mate choice cues in a group of Lake Malawi Mbuna (Cichlidae). *Copeia* 2003: 181–186.
- Jordan RC, Howe D, Juanes F, Stauffer Jr. J, Loew E, 2004. Ultraviolet radiation enhances zooplanktivory rate in ultraviolet sensitive cichlids. *African Journal of Ecology* 42: 228–231.
- Jordan RC, Kellogg K, Howe D, Juanes F, Stauffer J Jr. et al., 2006. Photopigment spectral absorbance of Lake Malawi cichlids. *Journal of Fish Biology* 68: 1291–1299.
- Kassam D, Mizoiri S, Yamaoka K, 2004. Interspecific variation of body shape and sexual dimorphism in three coexisting species of the genus *Petrotilapia* (Teleostei: Cichlidae) from Lake Malawi. *Ichthyological Research* 51: 195–201.
- Kidd MR, Danley PD, Kocher TD, 2006. A direct assay of female choice in cichlid fishes: All the eggs in one basket. *Journal of Fish Biology* 68, 373–384.
- Klein D, Ono H, O'Huigin C, Vincek V, Goldschmidt T et al., 1993. Extensive MHC variability in cichlid fishes of Lake Malawi. *Nature* 364: 330–334.
- Knight ME, Turner GF, 1999. Reproductive isolation among closely related Lake Malawi cichlids: Can males recognize conspecific females by visual cues? *Animal Behaviour* 58: 761–768.
- Kokko H, Jennions MD, Brooks R, 2006. Unifying and testing models of sexual selection. *Annual Review of Ecology and Systematics* 37: 43–66.
- Konings A, 1990. *Cichlids and All the Other Fishes of Lake Malawi*. Neptune City, NJ: T.F.H. Publications.
- Kotrschal A, Taborsky B, 2010. Environmental change enhances cognitive abilities in fish. *PloS Biology* 8: e1000351. doi:10.1371/journal.pbio.1000351
- Kröger RHH, Braun SC, Wagner H-J, 2001. Rearing in different photic and chromatic environments modifies spectral responses of cone horizontal cells in adult fish retina. *Visual Neuroscience* 18: 857–864.
- Ladich F, Collin SP, Møller P, Kapoor KP, 2006. *Communication in Fishes*. Enfield: Science Publishers.
- Ladich F, Wysocki LE, 2003. How does tripus extirpation affect auditory sensitivity in goldfish? *Hearing Research* 182: 119–129.
- Lande R, 1981. Models of speciation by sexual selection on polygenic traits. *Proceedings of the National Academy of Science* 79: 3721–3725.
- Lanzing WJR, 1974. Sound production in the cichlid *Tilapia mossambica* Peters. *Journal of Fish Biology* 6: 341–347.
- Lewis DB, Gower DM, 1980. *Biology of Communication*. New York: Wiley.
- Liebman E, 1933. Some observations on the breeding habits of Palestine Cichlidae. *Proceedings of the Zoological Society of London* 103: 885–888.
- Lobel PS, 1998. Possible species specific courtship sounds by two sympatric cichlid fishes in Lake Malawi, Africa. *Environmental Biology of Fishes* 52: 443–452.
- Lobel PS, 2001. Acoustic behavior of cichlid fishes. *Journal of Aquaculture and Aquatic Sciences* 9: 167–186.
- Loew ER, McFarland WN, 1990. The underwater visual environment. In: Douglas RH, Djamgoz MBA ed. *The Visual System of Fish*. New York: Chapman & Hall, 1–43.
- Loh YHE., Katz LS, Mims MC, Kocher TD, Yi SV et al., 2008. Comparative analysis reveals signatures of differentiation amid genomic polymorphism in Lake Malawi cichlids. *Genome Biology* 9: R113.
- Longrie N, Van Wassenbergh S, Vandewalle P, Mauguit Q, Parmentier E, 2009. Potential mechanism of sound production in *Oreochromis niloticus* (Cichlidae). *Journal of Experimental Biology* 212, 3395–3402.
- Lorenz KZ, 1966. *On Aggression*. New York: Harcourt Brace.
- Maan ME, Seehausen O, 2010. Mechanisms of species divergence through visual adaptation and sexual selection: Perspectives from a cichlid model system. *Current Zoology* 56: 285–299.
- Maan ME, van der Spoel M, Jimenez PQ, van Alphen JJM, Seehausen O, 2006. Fitness correlates of male coloration in a Lake Victoria cichlid fish. *Behavioral Ecology* 17: 691–699.
- Markert JA, Arnegard ME, 2007. Size-dependent use of territorial space by a rock-dwelling cichlid fish. *Oecologia* 154: 611–621.
- Mathews A, MacLeod C, 1994. Cognitive approaches to emotion and emotional disorders. *Annual Review of Psychology* 45: 25–50.
- MacLean PD, 1990. *The triune brain in evolution: Role in paleocerebral functions*. New York: Plenum.
- McElroy DM, Kornfield I, 1990. Sexual selection, reproductive behavior, and speciation in the mbuna species flock of Lake Malawi (Pisces: Cichlidae). *Environmental Biology of Fishes* 28: 273–284.
- McGurk H, MacDonald J, 1976. Hearing lips and seeing voices. *Nature* 264: 746–748.
- McLennan DA, 2003. The importance of olfactory signals in the gasterosteid mating system: Sticklebacks go multimodal. *Biological Journal of the Linnean Society* 80: 555–572.
- Montgomery JC, Windsor S, Bassett D, 2009. Behavior and physiology of mechanoreception: Separating signal and noise. *Integrative Zoology* 4: 3–12.
- Myrberg AA, Kramer E, Heinecke P, 1965. Sound production by cichlid fishes. *Science* 149: 555–558.
- Naish K-A, Ribbink AJ, 1990. A preliminary investigation of sex change in *Pseudotropheus lombardoi* (Pisces: Cichlidae). *Environmental Biology of Fishes* 28: 285–294.
- New JG, Kang PY, 2002. Multimodal sensory integration in the strike-feeding behaviour of predatory fishes. *Philosophical Transactions of the Royal Society B* 355: 1321–1324.
- Oliver MK, Arnegard ME, 2010. A new genus for *Melanochromis labrosus*, a problematic Lake Malawi cichlid with hypertro-

- phied lips (Teleostei: Cichlidae). *Ichthyological Exploration of Freshwaters* 21: 209–232.
- Owings DH, Morton ES, 1997. The Role of information in communication: An assessment/management approach. In: Owings DH, Beecher MD, Thompson NS ed. *Perspectives in Ethology: Communication*. New York: Plenum Press, 359–390.
- Papke RS, Kemp DJ, Rutowski RL, 2007. Multimodal signalling: Structural ultraviolet reflectance predicts male mating success better than pheromones in the butterfly *Colias eurytheme* L. (Pieridae). *Animal Behaviour* 73: 47–54.
- Panksepp J, 1998. *Affective Neuroscience: The Foundations of Human and Animal Emotions*. New York: Oxford University Press.
- Panksepp J, 2003. At the interface of the affective, behavioral and cognitive neurosciences: Decoding the emotional feelings of the brain. *Brain and Cognition* 52: 4–14.
- Parker A, Kornfield I, 1996. Polygynandry in *Pseudotropheus zebra*, a cichlid fish from Lake Malawi. *Environmental Biology of Fishes* 47: 345–352.
- Paul ES, Harding EJ, Mendl M, 2005. Measuring emotional processes in animals: The utility of a cognitive approach. *Neuroscience and Biobehavioral Reviews* 29: 469–491.
- Pauers MJ, Kapfer JM, Fendos CE, Berg CS, 2008. Aggressive biases towards similarly coloured males in Lake Malawi cichlid fishes. *Biology Letters* 4: 156–159.
- Pierotti MER, Knight ME, Immler S, Barson NJ, Turner GF et al., 2008. Individual variation in male mating preferences for female coloration in a polymorphic cichlid fish. *Behavioral Ecology* 19: 483–488.
- Plenderleith M, van Oosterhout C, Robinson RL, Turner GF, 2005. Female preference for conspecific males based on olfactory cues in a Lake Malawi cichlid fish. *Biology Letters* 1: 411–414.
- Reebs SG, 1994. Nocturnal mate recognition and nest guarding by female convict cichlids (Pisces, Cichlidae: *Cichlasoma nigrofasciatum*). *Ethology* 96: 303–312.
- Rice AN, Lobel PS, 2002. Enzyme activities of pharyngeal jaw musculature in the cichlid *Tramitichromis intermedius*: Implications for sound production in cichlid fishes. *Journal of Experimental Biology* 205: 3519–3523.
- Rice AN, Lobel PS, 2003. The pharyngeal jaw apparatus of the Cichlidae and Pomacentridae: Function in feeding and sound production. *Reviews in Fish Biology and Fisheries* 13: 433–444.
- Ripley JL, Lobel PS, Yan HY, 2002. Correlation of sound production with hearing sensitivity in the Lake Malawi cichlid *Tramitichromis intermedius*. *Bioacoustics* 12: 238–240.
- Ripley JL, Lobel PS, 2004. Correlation of acoustic and visual signals in the cichlid fish *Tramitichromis intermedius*. *Environmental Biology of Fishes* 71: 389–394.
- Robison RR, Fernald RD, Stacey NE, 1998. The olfactory system of a cichlid fish responds to steroidal compounds. *Journal of Fish Biology* 53:226–229.
- Rojas R, Moller P, 2002. Multisensory contributions to the shelter-seeking behavior of a mormyrid fish *Gnathonemus petersii* Gthr. (Mormyridae, Teleostei): The role of vision, and the passive and active electrosenses. *Brain Behavior and Evolution* 59: 211–221.
- Rosenthal GG, 2000. Design considerations and techniques for constructing video stimuli. *Acta Ethologica* 3: 49–54.
- Rypstra, AL, Schlosser AM, Sutton PL, Person MH, 2009. Multimodal signalling: The relative importance of chemical and visual cues from females to the behaviour of male wolf spiders (Lycosidae). *Animal Behaviour* 77: 937–947.
- Salzburger WH, Niederstätter H, Brandstätter A, Berger B, Parson W et al., 2006. Color-assortative mating in the cichlid species *Tropheus moorii* from Lake Tanganyika, East Africa. *Proceedings of the Royal Society B* 273: 257–266.
- Saverino C, Gerlai R, 2008. The social zebrafish: Behavioral responses to conspecific, heterospecific, and computer animated fish. *Behavioural Brain Research* 191: 77–87.
- Seehausen O, Schluter D, 2004. Male-male competition and nuptial colour displacement as a diversifying force in Lake Victoria cichlid fish. *Proceedings of the Royal Society of London Series B, Biological Sciences* 271: 1345–1353.
- Seehausen O, Van Alphen JJM, 1998. The effect of male coloration on female mate choice in closely related Lake Victoria cichlids *Haplochromis nyererei* complex. *Behavioral Ecology and Sociobiology* 42: 1–8.
- Seehausen O, Van Alphen JJM, Lande R, 1999. Color polymorphism and sex ratio distortion in a cichlid fish as an incipient stage in sympatric speciation by sexual selection. *Ecology Letters* 2: 367–378.
- Seehausen O, van Alphen JJM, Witte F, 1997. Cichlid diversity threatened by eutrophication that curbs sexual selection. *Science* 277:1808–1811.
- Seehausen O, Terai Y, Magalhaes IS, Carleton KL, Mrosso HDJ et al., 2008. Speciation through sensory drive in cichlid fish. *Nature* 455: 620–627.
- Seitz A, 1940. Die Paarbildung bei einigen Cichliden. *Zeitschrift für Tierpsychologie* 4: 40–84.
- Seitz A, 1949. Vergleichende Verhaltensstudien an Buntbarschen: (Cichlidae). *Zeitschrift für Tierpsychologie* 6: 202–235.
- Shannon CE, Weaver W, 1949. *The Mathematical Theory of Communication*. Urbana: University of Illinois Press.
- Silverman HI, 1978. Changes in male courting frequency in pairs of the cichlid fish *Sarotherodon (Tilapia) mossambicus*, with unlimited or with only visual contact. *Behavioral Biology* 23: 189–196.
- Simões JM, Duarte IG, Fonseca PJ, Turner GF, Amorim MC, 2008. Courtship and agonistic sounds by the cichlid fish *Pseudotropheus zebra*. *Journal of the Acoustical Society of America* 124:1332–1338.
- Smith ME, Kane AS, Popper AN, 2004. Acoustical stress and hearing sensitivity in fishes: Does the linear threshold shift hypothesis hold water? *Journal of Experimental Biology* 207: 3591–3602.
- Smith AR, van Staaden MJ, 2009. The association of visual and acoustic courtship behaviors in African cichlid fishes. *Marine and Freshwater Behaviour and Physiology* 2: 211–216
- van Staaden MJ, Huber R, Kaufman L, Liem K, 1995. Brain evolution in cichlids of the African Great Lakes: Brain and body size, general patterns and evolutionary trends. *Zoology - Analysis of Complex Systems* 98: 165–178.
- Stauffer JR Jr, Van Snik Gray E, 2004. Phenotypic plasticity: Its role in trophic radiation and explosive speciation in cichlids

- (Teleostei: Cichlidae). *Animal Biology* 54:137–158.
- Streelman JT, Danley PD, 2003. The stages of vertebrate evolutionary radiation. *Trends in Ecology and Evolution* 18: 126–131.
- Sylvester JB, Rich CA, Loh YHE, van Staaden MJ, Fraser GJ et al., 2010. Brain diversity evolves via differences in patterning. *Proceedings of the National Academy of Sciences* 107: 9718–9723.
- Tavolga WN, 1974. Signal/noise ratio and the critical band in fishes. *Journal of the Acoustical Society of America* 55: 1323–1333.
- Tavolga WN, 1976. Chemical stimuli in reproductive behavior in fish: Communication. *Experientia* 32:1093–1095.
- Thünken T, Waltschky N, Bakker TCM, Kullmann H, 2009. Olfactory self-recognition in a cichlid fish. *Animal Cognition* 12: 717–724.
- Tinbergen N, 1951. *The Study of Instinct*. Oxford: Oxford University Press.
- Tobler M, 2006. The eggspots of cichlids: Evolution through sensory exploitation? *Zeitschrift für Fischkunde* 8: 39–46.
- Trout JD, 2001. The biological basis of speech: What to infer from talking to the animals. *Psychological Review* 108: 523–549.
- Uetz GW, Roberts JA, 2002. Multisensory cues and multimodal communication in spiders: Insights from Video/audio playback studies. *Brain Behavior and Evolution* 59: 222–230.
- Venesky MD, Andraso GM, Ropski SJ, 2005. Behavior of male Kenyi cichlids *Pseudotropheus lombardoi* in response to visual and olfactory cues from females. *BIOS* 76: 77–83.
- Verzijden MN, ten Cate C, 2007. Early learning influences species assortative mating preferences in Lake Victoria cichlid fish. *Biology Letters* 3: 134–136
- Verzijden MN, van Heusden J, Bouton N, Witte F, ten Cate C et al., 2010. Sounds of male Lake Victoria cichlids vary within and between species and affect female mate preferences. *Behavioral Ecology* 21: 548–555.
- Weatherhead PJ, Robertson RJ, 1979. Offspring quality and the polygyny threshold: ‘The sexy son hypothesis’. *The American Naturalist* 113: 201–208.
- Werner NY, Lotem A, 2003. Choosy males in a haplochromine cichlid: First experimental evidence for male mate choice in a lekking species. *Animal Behaviour* 66: 293–298.
- Werner NY, Lotem A, 2006. Experimental evidence for male sequential mate preference in a lekking species. *Ethology* 112: 657–663.
- Yan HY, Popper AN, 1992. Auditory sensitivity of the cichlid fish *Astronotus ocellatus* (Cuvier). *Journal of Comparative Physiology A* 171: 105–109.
- Young KA, Genner MJ, Haesler MP, Joyce DA, 2010. Sequential female assessment drives complex sexual selection on bower shape in a cichlid fish. *Evolution* 64: 2246–2253.
- Yue S, Moccia RD, Duncan IJH, 2004. Investigating fear in domestic rainbow trout *Oncorhynchus mykiss*, using an avoidance learning task. *Applied Animal Behaviour Science* 87: 343–354.
- Zahavi A, 1985. Mate selection: A selection for a handicap. *Journal of Theoretical Biology* 53: 205–214.