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ACOUSTIC BEHAVIOUR IN MALAWIAN CICHLIDS (*PSEUDOTROPHEUS*, CICHLIDAE): POTENTIAL CUES FOR SPECIES RECOGNITION AND INTRASPECIFIC COMMUNICATION

AKUSTIČNO VEDENJE MALAVIJSKIH CIKLIDOV (*PSEUDOTROPHEUS*, CICHLIDAE): MOŽNA VLOGA PRI PREPOZNAVANJU VRST IN PRI INTRASPECIFIČNI KOMUNIKACIJI

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ABSTRACT

Acoustic behaviour in Malawian Cichlids (*Pseudotropheus*, Cichlidae): Potential cues for species recognition and intraspecific communication

Acoustic communication may play an important role in mate recognition systems among African cichlids and may contribute to the evolutionary processes involved in their rapid speciation. Sounds produced in early courtship (during quivering) by males of three closely related Malawi species of the *Pseudotropheus* zebra complex (*Pseudotropheus zebra*, *P. callainos* and *P.* 'zebra gold') were recorded and compared among species. In one species (*P. zebra*), sounds emitted during quivering were compared to sounds produced during later courtship behaviour, such as circle and lead-swim, and with sounds emitted during male-male and female-female interactions. The following acoustic parameters were measured: sound duration (ms), number of pulses *per* sound, initial and mean pulse periods (ms), and peak frequency of sounds (Hz). Differences in male courtship sounds were found among species. *P.* 'zebra gold' produced longer sounds with a higher number of pulses than *P. callainos*, and *P. zebra* males showed longer initial and mean pulse periods than the other two species. In *P. zebra*, male courtship, male agonistic and female agonistic sounds also differed significantly in some parameters. These results suggest that in the studied species acoustic signals could be used in species-specific recognition and potentially promote reproductive isolation, and in intraspecific communication.

Key words: Acoustic communication, speciation, sound production, *Pseudotropheus*, Cichlidae.

IZVLEČEK

Akustično vedenje malavijskih ciklidov (*Pseudotropheus*, Cichlidae): možna vloga pri prepoznavanju vrst in pri intraspecifični komunikaciji.

Zvočna komunikacija je verjetno pomembna pri prepoznavanju spolnih partnerjev afriških ciklidov in pri evolucijskih procesih, ki so udeleženi pri njihovi hitri speciaciji. Zvoki, ki jih samci rib oddajajo v zgodnji fazi dvorjenja (med drgetom) pri treh ozko sorodnih vrstah kompleksa *Pseudotropheus zebra* (*P. zebra, P. calainos* in *P.* 'zebra gold') so avtorji snemali in primerjali med seboj. Pri eni vrsti (*P. zebra*) so primerjali tudi glasove, oddane med drgetom, z glasovi ki jih ribe oddajajo med poznejšimi fazami dvorjenja in sicer med kroženjem in vodenim plavanjem kot tudi z oglašanjem med interakcijami samcev ali samic. Merili so naslednje zvočne parametre: trajanje zvoka, število pulzov v zvočnem signalu, trajanje začetnega in povprečnega cikla pulza (ms) in frekvenčni vrh zvoka. Ugotovili so medvrstne razlike pri glasovih dvorjenja. *P.* 'zebra gold' je oddajala daljše zvočne signale z višjim številom pulzov od vrste *P. callainos*, pri samcih vrste *P. zebra* pa sta trajanje začetnih pulzov in povprečno trajanje daljši kot pri drugih dveh vrstah. Pri slednji vrsti se vsaj v nekaterih parametrih signifikantno razlikujejo glasovi dvorjenja, agonistični signali samcev ter samic. Ti rezultati kažejo, da pri raziskovanih vrstah zvočni signali omogočajo vrstno prepoznavanje in morda pospešujejo reproduktivno izolacijo in omogočajo komunikacijo znotraj vrst.

Ključne besede: zvočna komunikacija, speciacija, produkcija zvoka, *Pseudotropheus*, Cichlidae.

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INTRODUCTION

The cichlids from the Great African Lakes represent one of the fastest known case of adaptive radiation among vertebrates (e.g. LANDE et al. 2001, ALBERTSON et al. 2003). It is thought that in Lake Malawi alone there are at least 295 endemic species of cichlids and at least 300 remain undescribed (GENNER & TURNER 2005). Most of these species may have arisen in the Pleistocene, 700.000 years ago (KNIGHT & TURNER 1999, COULDRIDGE 2002). Sexual selection by females, based on chromatic patterns of males seems to be the foremost mechanism responsible for the rapid speciation in these cichlids (e.g. DOMINEY 1984, MCKAYE 1991, COULDRIDGE & ALEXANDER 2002, GENNER & TURNER 2005). Visual cues are very important for inter and intraspecific recognition and can act as a sexual barrier between different species (KNIGHT & TURNER 1999, COULDRIDGE & ALEXANDER 2002). However, other channels of communication can also be important in sexual selection, such as the acoustic and chemical ones. Males of African cichlids, including *Pseudotropheus* spp., are known to produce sounds during courtship (LOBEL 1998, AMORIM et al. 2003, AMORIM et al. 2004). A preliminary study with three sisters species (AMORIM et al. 2004), showed the existence of significant differences in some of the parameters measured, suggesting that acoustic communication may have an important role in the reproductive isolation among these animals and consequently on their impressive rate of speciation.

Three species of the *Pseudotropheus* complex (*Pseudotropheus zebra*, *P.* 'zebra gold' and *P. callainos*) are sympatric, reproductively isolated in nature (VAN OPPEN et al. 1998) and mate assortatively in the laboratory, indicating that reproductive isolation can be maintained by direct mate choice alone (KNIGHT et al. 1998). Sounds made during the initial stages of courtship by males of these species were recorded and compared to check for differences in the acoustic signals that could potentially be used in species recognition. In addition, sounds made during late courtship stages and during agonistic interactions (male-male and female-female) were also registered for *P. zebra* to study the variability in the acoustic signals within one species.

METHODS

Trials were conducted in two aquaria (120 x 45 x 60 cm; *L* x *W* x *D*) placed on top of a concrete plate supported by two rock-wool blocks (100 x 40 x 30 cm). This setup proved to be effective to minimize external noise and building's vibration, also improving noise to signal ratio considerably. Tanks were fitted with an external power filter and maintained at 25-27°C, pH 7-9, with a 12h light/dark cycle. Each aquarium was divided into three compartments, one of 50 cm, in the middle, and two of 35 cm. Each smaller compartment had a terracotta pot and held a single male, while females were kept in the larger compartment. Males were identified by their natural marks, such as eggspots, fin length and body and fin blots.

Courtship sounds were recorded between January and September 2005. Sounds were recorded with two High Tech 94 SSQ hydrophones (sensitivity of -165 dB re $1V\mu Pa^{-1}$, flat frequency response up to 6 kHz±1 dB) and with a Pioneer DVD Recorder DVR-3100. Before the recording period, both heaters and filters were switched off. One of the opaque partitions, that separated a male from the females was removed before the start of the courtship experiments which allowed the subject male free access to the females. Sounds from females were recorded from female-female interactions that naturally occurred when they were in the middle compartment isolated from the males. Sounds from male-male interactions were recorded by placing another male the middle compartment (instead of the females), and following a similar procedure to the courtship sound recordings. Fights were stopped before escalation to avoid physical injuries in the males. Recording session durations were of 10 minutes for female-female, 15 minutes for male-male and 20 minutes for male-female interactions.

Sounds were analysed with Cool Edit Pro 2.0 and Raven 2.0. Only sounds that showed a clear structure and were recorded at a distance of 1-2 BL (body length) of the male were considered. The acoustic parameters analysed were: sound duration (ms), number of pulses in a sound, initial and mean pulse period (ms) and peak frequency 1 and 2 (Hz) (for a description of the acoustic parameters see AMORIM et al. 2004). These were the two frequency components with the highest energy in the entire sound. All parameters were analysed separately using the non-parametic Kruskal-Wallis test.

RESULTS

Sounds emitted by *Pseudotropheus* were typically pulsed short signals (500 to 800 ms), with most energy below 1 kHz. Most sounds were emitted in the initial stages of courtship, during quiver, so comparisons among species only considered 'quiver sounds'. There were significant differences between species in the following acoustic parameters: sound duration and number of pulses *per* sound were higher in *P.* 'zebra gold' and, the initial and mean pulse periods were longer in *P. zebra* than in the other two species (Kruskal-Wallis, *P. zebra* (N=12), *P.* 'zebra gold' (N=12), *P. callainos* (N=13), H=6.4-15.6, $P<0.05$). Sounds produced by *P. zebra* males during quiver differed significantly from sounds of later stages of courtship (lead swim and circle). 'Quiver sounds' (Q) were longer, with higher number of pulses, shorter mean pulse periods and higher peak frequency 1 than circle (C) and lead swim (LS) sounds (Kruskal-Wallis, Q (N=36), C (N=10), LS (N=12), H=8.5-29.1, $P<0.05$). Males and females of *P. zebra* also emitted sounds during agonistic interactions (male-male and female-female interactions). Male courtship and agonistic sounds, had longer sound duration and higher number of pulses *per* sound than female sounds and both male and female agonistic sounds had shorter initial and mean pulse periods than male courtship ones. Females produced shorter sounds and with a lower number of pulses but otherwise very similar to males' agonistic sounds (Kruskal-Wallis, M-F (N=48), M-M (N=36), F-F (N=27), H=11.7-17.5, $P<0.05$).

DISCUSSION

The three studied sympatric sister *Pseudotropheus* species emitted low frequency sounds during different stages of courtship. Male courtship sounds emitted during quiver showed differences among species in the sound duration, the number of pulses and in the initial and mean pulse period. In a preliminary study using males of the same *Pseudotropheus* species, AMORIM et al. (2004) also reported significant inter-specific differences for the number of pulses but only marginally non-significant differences for sound duration, probably due to the smaller sample size.

In one species, *P. zebra*, sound emission was also observed in agonistic interactions and both males and females were able to emit sounds. In this species, sounds differed between initial and late stages of courtship, and between social contexts (courtship – male-female interactions, and agonism – female-female and male-male interactions), in sound duration, number of pulses, pulse period and in peak frequency 1.

The interspecific differences in courtship sounds found in this study suggest that acoustic communication could potentially promote species isolation and play a role in the amazingly rapid speciation found in these cichlids. Temporal and pulse grouping patterns are believed to play a fundamental role in species recognition in fish (e.g. SPANIER 1979). For example, playback experiments of courtship sounds (chirps) carried out with sympatric damselfishes (*Stegastes* spp.) showed that males could distinguish conspecific from heterospecific chirps based on the number of pulses and pulse rate (MYRBERG et al. 1978, SPANIER 1979). In cichlids, LOBEL (1998) showed statistically significant differences in pulse rates and durations in the courtship sounds of two other sympatric Malawian cichlids and proposed that acoustic communication could play a role in mate choice and species recognition.

Likewise, differences found in *P. zebra* for the number of pulses, temporal and frequency parameters, for sounds emitted in different contexts (courtship *vs.* agonistic interactions) could be used in mate choice. For example, females could be able to extract information on the motivation and spawning readiness of males, as suggested for other fishes (e.g. AMORIM et al. 2003).

Evolution questions have driven attention to cichlids of the Great African Lakes leading to the hypothesis that speciation has been driven by mate choice based on male colour. The present and other recent studies (PLENDERLEITH et al. 2005) suggest that different channels of communication can be acting together in species isolation, inviting for future research on the role of different modalities in cichlid mate choice.

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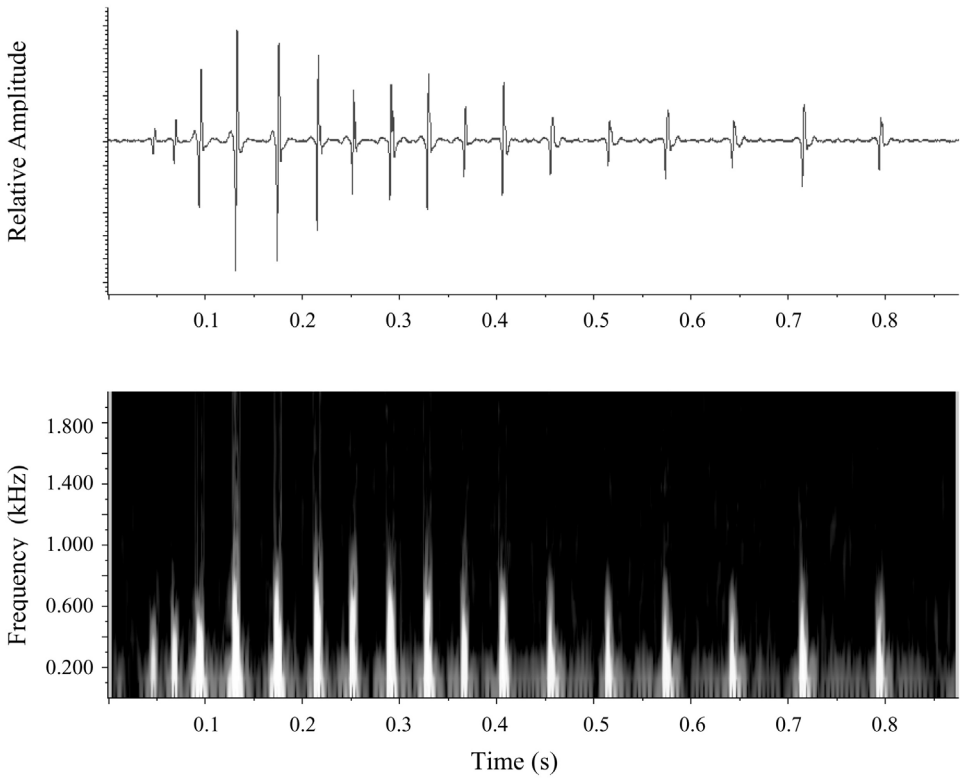

Figure 1: Oscillogram (A) and sonagram (B) of a courtship sound produced by a *Pseudotropheus zebra* male. Oscillogram was filtered with a 60 Hz high-pass filter and the sonogram used a Hamming window and a filter bandwidth of 200 Hz.