

Innate sound production in the cichlid *Oreochromis niloticus*

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Abstract

The mouthbrooding cichlid *Oreochromis niloticus* is one of the world's best-studied fish and is raised extensively for aquaculture. Although it is a common behavioural model, its acoustic communication has been neglected. Because of extensive parental care, the species is a good candidate for vocal learning. In male *O. niloticus*, we investigated for the first time sound production in agonistic interactions during nest construction. Males produce short-duration (250–400 ms), often double-pulse sounds. Most energy is below 200 Hz and includes three main low-frequency peaks although energy extends beyond 1 kHz. Males (separated as eggs and raised in isolation) produce similar sounds in the same context as parental fish, indicating that the ability to produce sounds and the basic properties of the sounds are innate.

Introduction

In most animal taxa, species-specific vocal signals develop without significant environmental input, but learning has been demonstrated in a few taxa, including humans, cetaceans, elephants and some bats and birds (Beecher & Brenowitz, 2005; Jarvis, 2006). Bird species vary considerably in their song characteristics and how they acquire them. Comparative studies reveal five different song-learning strategies in the songbirds (Beecher & Brenowitz, 2005). Some have a genetically determined, structurally simple and stereotyped song, whereas in others the song is learned (Grant & Grant, 1996). Learned songs of adult songbirds consist of stereotyped units, called syllables, acquired by imitation and arranged in sequences characteristic for each species (Gardner, Naef & Nottebohm, 2005). Most fish species do not exhibit parental care after eggs are fertilized, and a single study in the cyprinid *Codoma ornata*, a species without parental care, demonstrated that the ability to produce sounds is innate (Johnston & Buchanan, 2006). Because of extended parental care, mouthbrooding cichlids are good candidates for vocal learning. In fact, some maternal mouthbrooding species of tilapias in this family have become models of social bond formation (Russock, 1999). Males form dense aggregations during the mating season where they defend territories (arenas or leks) to attract females. At the end of the mating sequence, the male quivers while circling the nest and is followed by the female,

who takes both eggs and sperm into her mouth, where the eggs are fertilized (Oliveira & Almada, 1998). The female broods the eggs until they hatch and the yolk sac is absorbed. The fry are then released and aggregate near their mother for *c.* 21 days, re-entering her mouth in times of danger (Russock, 1999). Social preference in the tilapia *Oreochromis mossambicus* is considered to be an innate behaviour, for example sexually mature individuals prefer to associate with conspecifics after being reared in social isolation (Russock & Schein, 1978). However, other behaviours including mate choice may be learned through parental care (Russock & Schein, 1977; Russock, 1986). Thus, while some studies suggest that early experience is important in behavioural development, others show little effect, if any (Ripley & Lobel, 2005).

Cichlid fish have evolved an intricate set of acoustic and visual signals including a complex series of courtship behaviours that convey information on spawning readiness and mate choice (Baerends & Baerends-van Roon, 1950; Baerends, 1984; McElroy & Kornfield, 1990; Lobel, 1998; Lobel, 2001; Amorim, Fonseca & Almada, 2003; Amorim *et al.*, 2004; Ripley & Lobel, 2005). Other sounds are emitted during agonistic interactions, often associated with territorial or brood defence (e.g. Myrberg, Kramer & Heinecke, 1965). Agonistic sounds can be produced by both sexes and are broadly classified into three groups (Amorim, 2006). The first class, a series of low-frequency pulses (growls), has been suggested, but not demonstrated, to be produced by the

pharyngeal apparatus and then amplified by the swimbladder (Lanzing, 1974; Lobel, 2001; Rice & Lobel, 2002). The second class is produced by chewing sounds, which are stridulatory with a broad frequency band. These sounds can be produced when fish are threatening conspecifics and not eating. The third class includes thump-like sounds produced during body movements such as head nodding. Each species of cichlid is capable of emitting sounds falling into one or two of these broad categories (Amorim, 2006).

Although behaviour has been studied extensively in *Oreochromis niloticus* (Lowe, 1958; Heinrich, 1967; Bauer, 1968; El-Zarka, Shaheen & Aleem, 1970; Babiker & Ibrahim, 1979; Falter, 1987; Keenleyside, 1991; Russock, 1999; Volpato *et al.*, 2003), sound production has received no attention beyond Bauer's (1963) report that sounds occur. The aim of this paper was to describe sounds made by parental *O. niloticus* in agonistic interactions during nest construction and to investigate whether the agonistic sounds of their offspring (isolated as eggs) are learned or innate.

Methods

Adult *O. niloticus* were placed in different tanks with rough sand at the bottom to facilitate nest construction by males. The male digs a hole in the bottom by removing sand with his mouth (Falter, 1987). All fish were fed with fish commercial pellets and maintained at 28 °C with a 12L:12D photoperiod. The parental tank (2.5 × 0.7 × 0.55 m) contained two males and one female (TL: 30 cm) that reproduced in September and October 2005. The first clutch of eggs hatched in the parent tank and lived there for 177 days before individuals were transferred to three separate tanks (1.5 × 0.4 × 0.5 m) with at least three territorial males per tank. At the time of recording, the first contained 15 specimens (TL: ± 13 cm), the second contained five specimens (TL: ± 13.5 cm) and the third contained five specimens (TL: ± 16 cm). Sounds from the parents and from cichlids of the three groups formed the control group (CON). The second clutch ($n > 100$) was isolated (ISO) and placed in an identical tank in another laboratory. At the time of recording, there were 13 fish in the tank (221 days old and 13 cm TL). Isolated young began to exhibit spawning behaviour in April 2006. Distinct sounds associated with first nest construction were heard at *c.* 210 days of age and recorded initially at 220 days.

The hydrophone was placed *c.* 15 cm above the nest, and all electrical appliances were switched off, apart from the tank lights. Sounds were recorded with an Orca hydrophone (sensitivity: $-186 \text{ dB re } \{1 \text{ V mPa}^{-1}\}$) connected via an Orca-made amplifier (Orca Instrumentation, Brest, France) to a Sony TCD-D8 digital audio tape-recorder (Liège, Belgium) (recording band width: 20–22 000 Hz ± 1.0 dB). Sounds were digitized at 44.1 kHz (16-bit resolution) and analysed with the Avisoft-SASLAB Pro 4.33 software [1024-point Hanning window fast Fourier transform (FFT)]. Temporal features were measured from oscillograms and frequency variables from power spectra (filter bandwidth 300 Hz, FFT size 256 points, time overlap 96.87% overlap and a flat top window). The resonant frequencies of the parent and offspring tanks were calculated, respectively, as 1737 and 2521 Hz, using an

equation in Akamatsu *et al.* (2002). Accordingly, low-pass filters of 1.8 and 2.52 kHz were applied.

The following sound variables were measured (Fig. 1): sound (or train) duration, that is the time elapsed from the start of the first pulse to the end of the last pulse; number of pulses in a sound; and pulse duration, that is the time interval

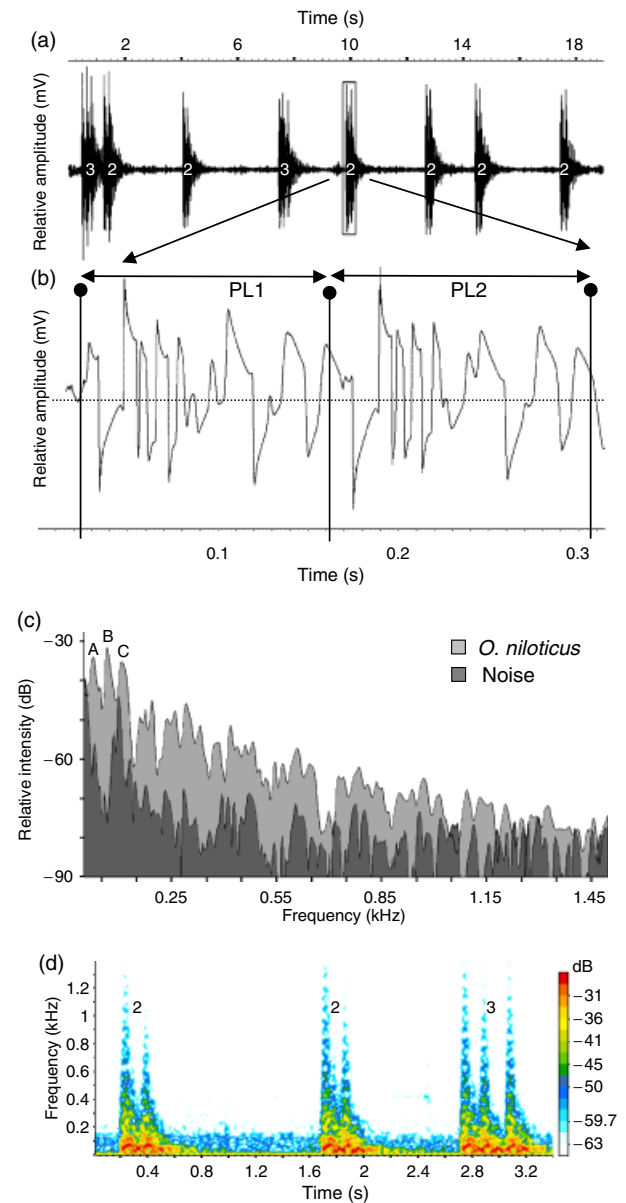


Figure 1 Male *Oreochromis niloticus* of the (CON) group. Oscillogram of different sounds (a), expanded oscillograms illustrating the waveform of two pulses (b), sound spectrum of a single pulse (c) and sonogram of different sounds. Numbers in (a) and (d) correspond to the pulse number in each sound [a total of eight different sounds are shown in (a) and three in (d)]. PL1 and PL2=pulse length (s); PL1+PL2=Sound length. A, B, C=three main peaks in a pulse. CON, control group.

Table 1 *Oreochromis niloticus*

	Sound length (ms)	# pulses	Pulse length (ms)	Main Frequency (Hz)	Peak 1 (Hz)	Peak 2 (Hz)	Peak 3 (Hz)
CON	314 ± 79	2.1 ± 0.4	143 ± 24	68 ± 33	35 ± 11	72 ± 13	121 ± 22
ISO	285 ± 80	2.4 ± 0.5	109 ± 13	46 ± 13	45 ± 7	109 ± 17	173 ± 30

Sonic characters (mean ± sd) in control (CON) and in isolate offspring (ISO).

between the onset of one pulse and its end. Three frequency peaks (A–C) were measured on each sound spectrum.

Results

A total of 607 pulses from 13 male territorial fish (eight CON, five ISO) were analysed. As males began to excavate a nest in the sand, they circled and defended the site against other males. Males were unpaired at this time. In our tanks, only males having a nest produced sounds, and the presence of a female was not required for sound production. Most sounds were made when other males approached the nest. The emitting fish stopped swimming during sound production. Sound production was accompanied by dorsal and anal fin erection, spreading of the caudal and pectoral fins, lowering of the pharyngeal floor and erection of the branchiostegal membrane. These behaviours correspond to the lateral displays of Oliveira & Almada (1998). Sound production did not occur during biting, mouth fighting and butting. After emitting sounds, males returned to the nest, circled or continued nest excavation; they also remained motionless above the nest. Aggressive sounds were usually not produced when a female approached the nest although there were several exceptions.

Sounds were mostly sequences of double or sometimes triple pulses (Fig. 1) lasting between *c.* 250 and 400 ms, and sequence duration correlated with the number of pulses. Pulse duration averaged ($X \pm sE$) 143 ms ± 24 ($n = 414$ from eight fishes). Sound amplitude maintained a steady level before exhibiting a slow decay (Fig. 1b). Because of the slow decay, pulses were continuous without an interpulse interval. Sound energy peaked between *c.* 30 and 100 Hz although energy was present to about 1000 Hz ($X = 68 \pm 33$, $n = 414$). There were generally three frequency peaks, which averaged 35 ± 11, 72 ± 13 and 121 ± 22 Hz (Fig. 1c). The frequency did not vary with fish size, and the lowest frequency was not found in the largest fish (32 cm).

The basic amplitude, frequency and time patterns of the calls were similar in CON and isolate offspring (ISO), indicating an innate basis for sound production and sound characteristics in this species (Table 1). Sound characters of isolates were based on 193 pulses from 10 fish (ranging from 13 to 16 cm TL). Pulse duration averaged 109 ± 13 ms, peak frequency was 46 ± 13 Hz and the three frequency peaks were 45 ± 7, 109 ± 17 and 173 ± 30 Hz. Call parameters from the CON and ISO groups were relatively similar. We could not evaluate whether differences were significant because it was not possible to always identify the sounds of individual callers.

Discussion

Sound production in *O. niloticus* occurred during agonistic interactions in nest defence, and only territorial males produced sounds. These sounds can be described by several variables: three frequency peaks, peak energy below 100 Hz and a duration between 250 and 400 ms. These characters differ from other cichlids (Schwarz, 1974; Nelissen, 1975, 1977; Brown & Marshall, 1978; Amorim *et al.*, 2004; Ripley & Lobel, 2004) and even from *O. mossambicus*, a congeneric mouthbrooding species (Amorim *et al.*, 2003). Compared with *O. mossambicus*, *O. niloticus* has a lower number of pulses (2 vs. at least 10), a longer pulse duration (150 vs. 10 ms), continuous pulses and a lower peak frequency. However, *O. mossambicus* sounds also include a strong frequency component peaking at *c.* 40 Hz. Such a low-frequency peak is not common in fish sounds (Amorim *et al.*, 2003). However, the possibility of it being an artefact of the recording environment and equipment in two closely related species recorded in different laboratories is unlikely: note these peaks are not present in recordings of background noise (Fig. 1). Further, the maximum auditory sensitivity is 100 Hz in the cichlid *Sarotherodon melanotheron* (Fay & Popper, 1975) and in *O. niloticus* (Smith, Kane & Popper, 2004). The latter authors did not test lower frequencies.

The sonic mechanism is incompletely understood in cichlids. Lobel and colleagues have suggested that sounds are made by stridulation of the pharyngeal teeth (Lobel, 2001; Rice & Lobel, 2004) and amplified by the swim bladder (Brown & Marshall, 1978; Lobel, 2001). However, the long sound duration and the low frequency do not support the teeth hypothesis in *O. niloticus*. Stridulatory sounds are usually short (<10 ms) with high (>1000 Hz) main frequencies (Tavolga, 1971).

The juvenile fish raised in isolation produced species-specific acoustic signals and courtship displays. However, sounds exhibited minor differences from those from the conspecifics that were not isolated. Further studies on a larger group will be necessary to evaluate and understand these differences. Because the juveniles of the ISO groups had no opportunity to hear acoustic signals, the ability to produce sounds is innate as in many bird species that produce simple and stereotyped song (Grant & Grant, 1996). In Cichlidae, the radiation in Lake Malawi has been categorized into three historical stages (Kocher, 2004; Gerner & Turner, 2005). The first stage, habitat divergence, is represented by the evolution of distinct rock- and sand-dwelling clades. The second stage is the elaboration of morphologically distinct genera, which is characterized by the differentiation of the feeding apparatus. In the third stage, species differ primarily in colour pattern, which

indicates the action of sexual selection (Kocher, 2004). Because many cichlid species lack larval or juvenile dispersal, significant differences in allele frequencies have been found among populations that are separated by only a few metres (Van Oppen *et al.*, 1997; Arnegard *et al.*, 1999; Genner & Turner, 2005; Kidd, Kidd & Kocher, 2006). Colour pattern is thought to be the basis of sexual selection and may be a factor leading to sympatric speciation in cichlids (Kocher, 2004; Schliewen *et al.*, 2006). However, different studies indicate that sounds could also play an important function. Lobel (1998) found differences in pulse rates and durations for two sympatric but not congeneric Malawian cichlids and proposed that courtship sounds could play a role in mate choice and species recognition. In three sympatric cichlid species (*Pseudotropheus*), courtship sounds differed significantly and might indicate different regimes of intraspecific sexual selection (Amorim *et al.*, 2004). Unlike courtship sounds, agonistic sounds would not be directly related to reproductive isolation. Low-frequency peaks in *O. niloticus* and *O. mossambicus* are related to a similar mechanism of sound production. However, differences between temporal properties of their calls suggest modifications in central nervous pattern generators underlying sound production and are likely reflective of evolutionary changes.

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