

## Potential mechanism of sound production in *Oreochromis niloticus* (Cichlidae)

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### SUMMARY

Although acoustic communication is an integral part of cichlid behaviour, its mechanism has never been identified before. In the present study, a combination of approaches was used to investigate the sound-producing mechanism of *Oreochromis niloticus*. Synchronisation of high-speed video data (500 frames s<sup>-1</sup>) and cineradiographies (250 frames s<sup>-1</sup>) with the sound recordings made it possible to locate the different body parts involved in sound production in territorial males. Sounds are made during a backward movement of the pelvic and pectoral girdles and a forward movement of the second pterygiophore of the anal fin. Various electrostimulation experiments, dissections and observation of histological cross-sections revealed a set of bundles (that we call the vesica longitudinalis) situated in the hypaxial musculature, ventro-laterally to the swimbladder. Contraction of these bundles should result in compression of the rib cage and also of the swimbladder, because of its close association with the serosa and ribs. Deflation of the swimbladder resulted in a reduced sound intensity.

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Key words: Cichlidae, sound production, swimbladder, sonic mechanism.

### INTRODUCTION

Some teleosts are well known to emit sounds during various behaviours, such as feeding competition (Amorim and Hawkins, 2005), courtship (Lobel, 1998; Amorim et al., 2003) and agonistic behaviour (Ladich, 1997). These sounds are generally low-frequency (50–500 Hz) pulses varying in duration, number and repetition rate (Winn, 1964; Amorim, 2006). Although acoustic communication appears to be an integral part of cichlid behaviour (Lobel, 2001; Amorim, 2006), cichlid speciation has usually been associated with only two competing hypotheses: (1) morphological plasticity of the pharyngeal jaw apparatus originating in trophic adaptation, and (2) sexual selection based on female recognition of conspecific male colour patterns (Seehausen and van Alphen, 1998; Turner et al., 2001; Kocher, 2004). Investigating the hypothetical link between sonic behaviour and speciation requires at least determining the sonic mechanism, which could correspond with a set of characters likely to reflect evolutionary changes (Longrie et al., 2008). In cichlids, the correlation between sound duration and sound pulse number suggests a fixed mechanism responsible for sound production (Lobel, 2001; Rice and Lobel, 2003). According to Amorim (Amorim, 2006), cichlid sounds can be grouped into three classes, probably associated with the sound-producing mechanism: (1) growls, i.e. low-frequency pulses usually associated with both agonistic and courtship contexts; (2) ‘chewing’ sounds, i.e. broad-frequency-band stridulatory sounds that can be heard when the fish are not eating and are threatening conspecifics, and (3) thump-like sounds produced apparently as a result of body movements such as head nodding. To date, these mechanisms have not been demonstrated. On the basis of their respective studies on *Oreochromis mossambicus* and *Tramitichromis intermedius*, Lanzing (Lanzing, 1974) and Rice and Lobel (Rice and Lobel, 2002) suggest that the pharyngeal jaw apparatus produces the sound, which could then be amplified by the swimbladder. In the case of *T. intermedius*, this assertion is based on the finding of a sexual

dimorphism in the physiology of two muscles (levator posterior and protactor pectoralis) of the pharyngeal jaw apparatus (Rice and Lobel, 2003), suggesting that a sex-dependent function of these muscles could be sound production.

The Nile tilapia, *Oreochromis niloticus*, is a maternal mouthbrooder cichlid. During the breeding season, males form dense nest aggregations and dig pits in the substrate. Next, they defend their territories and try to attract females. The female takes the eggs and sperm in her mouth, where fertilisation occurs. She leaves the male after the spawning act and keeps the embryos until the yolk sac is absorbed (Oliveira and Almada, 1998; Amorim et al., 2003). When they are released, the larvae stay grouped near their mother (ca. 21 days), who can take them back into her mouth in case of danger (Russock, 1999). During nest defence, *O. niloticus* males are able to produce brief (250–450 ms), often double-pulsed sounds with a main frequency below 200 Hz (Longrie et al., 2008). *Oreochromis niloticus*, as many other cichlid species (Lobel, 1998; Lobel, 2001), exhibit a belly quiver with the body during vocalisation. The aim of the present study was to investigate the mechanism of sound production by *O. niloticus* in agonistic interactions during territorial behaviour.

### MATERIALS AND METHODS

*Oreochromis niloticus* (Linnaeus 1758, Cichlidae) specimens were provided by the Aquaculture Research and Education Centre of the University of Liège (CEFRA-ULg, Tihange, Belgium). These specimens were kept in two tanks (size: 150 cm × 40 cm × 45 cm) with gravel on the bottom on a 12 h:12 h light:dark cycle and were offered food pellets once a day *ad libitum*. The environmental temperature was 28°C.

### High-speed video during sound production

#### Experimental conditions

In each trial, a male and a female were placed in the same tank (size: 150 cm×40 cm×45 cm), separated by a transparent wall. Gravel was placed on the bottom so as to see the nest built by the male. A second male was then added to the tank (Fig. 1) to induce territorial behaviour, which was usually coupled with sound production. In all, four males (standard length,  $SL\pm 15$  cm) and two females ( $SL\pm 13$  cm) were used for the high-speed recordings. Five video sequences were produced for each of the two tested territorial males. Ten landmarks were used to follow the movements performed by the fish during sound production (Fig. 2): (1) the centre of the eye, (2) the top of the neurocranium at the rostral end of the dorsal fin, (3) at the limit between the spines and the soft rays of the dorsal fin, (4) the base of the caudal end of the dorsal fin, (5) the caudal peduncle, (6) the rostral end of the base of the anal fin, (7) the proximal part of the pelvic fin, and the scapular girdle with (8) the post-cleithrum, (9) the cleithrum, and (10) the upper proximal part of the pectoral fin.

#### Kinematics

The fish were filmed at 500 frames  $s^{-1}$  with a Redlake MotionPro high-speed camera (resolution: 1280×1024 pixels, San Diego, CA, USA). This camera was connected to a computer (video chart: Asus v9280S, San Diego, CA, USA), making it possible to visualise the fish's movements in real time. This imagery system was synchronised with a hydrophone BK 8106 (Naerum, Denmark, sensitivity:  $-173$  dB re.  $1$  V/ $\mu$ Pa, flat frequency response between 7 Hz and 80 kHz) coupled to a Nexus<sup>TM</sup> conditioning amplifier (type 2690, Naerum, Denmark) by means of a data acquisition box (Midas, DA Module, BNC Breakout Box, Cambridge, MA, USA). The Midas program (Redlake, version 2.2.0.7) was used for data acquisition and to follow the movements of the landmarks in an  $x$ - $y$  referential used for analysis of the sounds and videos (Fig. 2). A buffer memory with a capacity of 2 GB was used to record the data after visualisation. For the recording sessions, a wall limiting the depth of field was placed in the tank in order to film the fish in lateral view during sound production.

#### X-ray – electrostimulation

X-ray video recordings (250 frames  $s^{-1}$ ) were made with a Redlake MotionPro high-resolution digital camera attached to the image intensifier of a Philips Optimas M200 X-ray system (Eindhoven, The Netherlands). X-rays were generated at 40 kV and the fish were filmed during application of electric stimulations. To help visualise

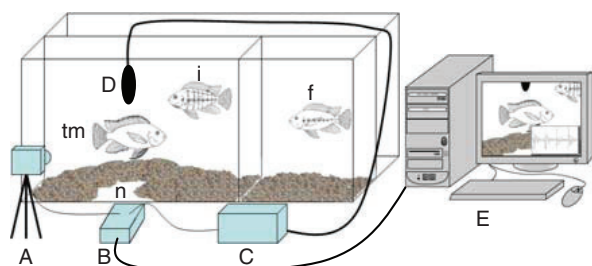


Fig. 1. Schematic view of the experimental setup during the high-speed recordings associated to the sound production in *Oreochromis niloticus*. A, high-speed camera; B, data acquisition box; C, sound amplifier nexus; D, hydrophone; E, computer with the recording/analyses program Midas; f: female; i: intruder; n: nest and tm: territorial male.

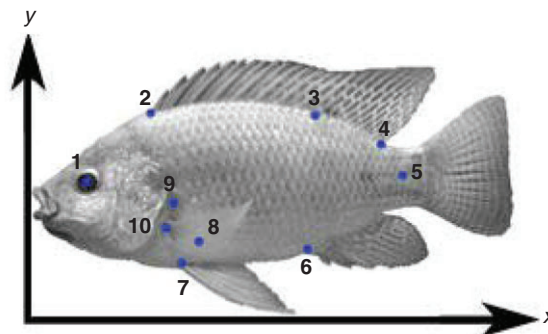


Fig. 2. Lateral view of *Oreochromis niloticus* showing the 10 landmarks followed during the high-speed movies synchronised to the sound production.

the fish movements, sets of small radio-opaque markers (Fig. 3) were inserted onto different bones under general anaesthesia in  $50$  mg  $l^{-1}$  MS 222 (Sigma Chemical Co., St Louis, MO, USA). Each marker implantation was preceded by loading a small, lead, sphere into the tip of a hypodermic needle (16-gauge on the scale of Stubs). Next, the needle was inserted subcutaneously, touching the bone at the position of interest. Then the lead marker was released from the hypodermic needle by pushing a steel wire through the back of the hypodermic needle and simultaneously retracting the needle. Finally, the positions of the markers were checked by taking X-ray pictures. Two series of recordings were produced on two males, and two sequences were analysed for each male.

#### Electrostimulation

Nineteen male *O. niloticus* ( $SL\pm 13.4$  cm) specimens were used in this experiment. A hydrophone was placed beforehand in the storage tank (150 cm×40 cm×50 cm, 28°C) to record and select one individual emitting sounds naturally during nest defence. The selected fish was then anaesthetised in phenoxy-ethanol ( $0.4$  ml  $l^{-1}$ ) and placed, belly up, in a support in the experimental tank (33 cm×17 cm×17 cm, 28°C) filled with anaesthetic. The fish was partly immersed and could breathe freely. Then a hydrophone was placed in a standardised position in the tank to record the sounds produced during electrical stimulation. The electrostimulator (HSE Stimulator Type 215/T, Hugo Sachs Elektronik, March-Hugstetten, Germany) was set to induce an electric stimulation (DC) of 5 V lasting 15 ms every 500 ms. These parameters were chosen according to the *O. niloticus* sound characteristics and to obtain analysable

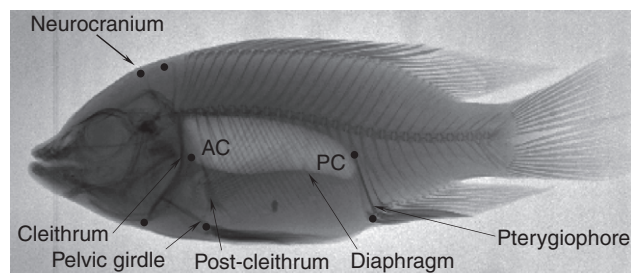


Fig. 3. Radiography in lateral view of an *Oreochromis niloticus* male. AC: anterior chamber; PC: posterior chamber; ● are the locations of lead landmarks for cineradiography.

sounds. The hydrophone (sensitivity:  $-186$  dBV/ $\mu$ Pa; flat frequency response range between 2 Hz and 37 kHz) was connected directly to a laptop computer where sounds were recorded with the help of the analysis program Avisoft-SASLab Pro version 4.38 (Berlin, Germany). Sounds were digitised at 22.05 kHz (16 bit resolution) and analysed with the Avisoft-SASLab Pro version 4.33 software (1024-point Hanning window fast Fourier transform, FFT). Temporal features (ms) were measured from oscillograms whereas frequency (Hz) and relative intensity (dB rel.) were obtained from power spectra (filter bandwidth 117 Hz, FFT size 512 points, time overlap 96.87% and a flat top window). The resonance frequency of each tank was calculated with the Akamatsu et al. equation (Akamatsu et al., 2002). The frequencies obtained were cut with a low-pass 2346 Hz filter for the storage tank and a 6352 Hz filter for the experimental tank.

Four experiments were done to highlight the mechanism.

(1) Natural sounds (Nat) obtained in the storage tank were compared with sounds obtained by electrical stimulation (Stim) of the same fishes ( $N=5$ ,  $SL=14.1\pm 1.8$  cm). Also, sounds produced by the pharyngeal jaws were recorded during fish feeding ( $N=5$ ,  $SL=13.3\pm 0.7$  cm) for comparison with Nat and Stim.

(2) Sounds obtained by electrical stimulation of intact fish ( $N=5$ ,  $SL=13.3\pm 0.7$  cm) were compared with sounds obtained by electrical stimulation of the same fish in which a piece of eraser was placed between the pharyngeal jaws (PJ KO). The eraser was used to prevent the pharyngeal teeth of the upper and lower pharyngeal jaws from coming into contact.

(3) Stimulated sounds were compared with stimulated sounds of the same fish whose swimbladder had been deflated with a needle (SW KO) ( $N=4$ ,  $SL=12.1\pm 0.2$  cm).

(4) Five fish ( $SL=13.7\pm 1.6$  cm) were stimulated in different parts of the body (Fig. 4) in order to detect the area where the sound with the greatest intensity.

Electrostimulation tests were also carried out on two male specimens of the Cichlidae *Cyphotilapia frontosa* (Boulenger 1906).

Statistical analyses were carried out with Statistica 7.1. The *W* test of Shapiro–Wilk was used to test the normality of the data. Non-parametric Mann–Whitney *U*-test was then used to compare duration, frequency and relative intensity for each of the experimental conditions that we have tested.

### Morphological study

Sixteen *O. niloticus* (total length, *TL*: 6–16 cm) and two *C. frontosa* (*TL*: 12–14 cm) specimens were deeply anaesthetised with MS 222 ( $500$  mg  $l^{-1}$ ). Six were fixed in 7% formaldehyde for dissection and *in toto* staining and were dehydrated in butanol, decalcified,

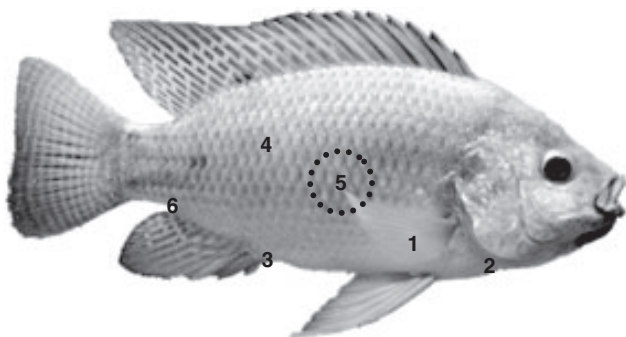


Fig. 4. Electrostimulated body areas in *Oreochromis niloticus*. (1–6). The dotted circle shows the area where sounds have the greatest intensity.

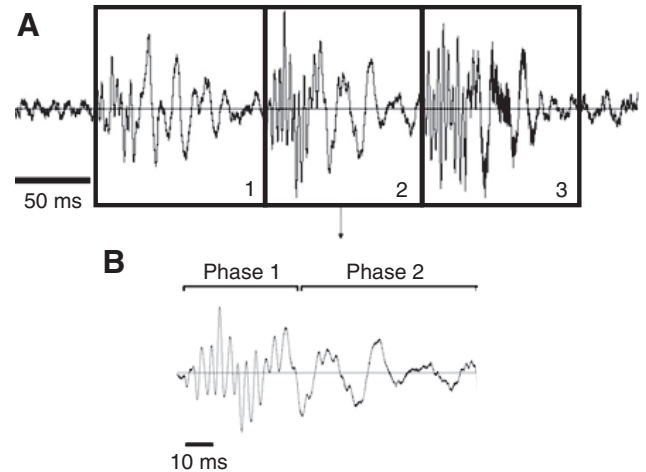


Fig. 5. (A) Oscillogram of a train of three pulses in *Oreochromis niloticus* and (B) enlargement of the oscillogram of one pulse showing the two phases characterising the pulse.

embedded in paraffin and serially sectioned with a Reichert microtome ( $15\mu$ m). Cross-sections were stained in haematoxylin-eosin (Gabe, 1976). Three specimens were coloured with Alizarin according to Taylor and Van Dyke's method (Taylor and Van Dyke, 1985) in order to visualise osseous structures. The three latter specimens and intact fishes were dissected and examined with a Wild M10 binocular microscope (Leica Camera, Leica, Wetzlar, Germany) equipped with a camera Lucida. After formaldehyde fixation, three swimbladder diaphragms were stained *in toto* in acetic carmine. Also,  $8\mu$ m cross-sections were made in the diaphragm of one *O. niloticus* specimen and stained in haematoxylin-eosin (Gabe, 1976). The different sections were observed with a Leica DM 1000 microscope. The nomenclature used to designate parts of the musculature is based on Winterbottom (Winterbottom, 1974).

## RESULTS

### Description of *O. niloticus* movements during sound production

The sounds obtained during the high-speed video recordings are presented in the form of pulse trains. These trains were generally composed of 2–4 pulses (Fig. 5A). The enlargement of the oscillogram shows that each pulse comprised two parts (Fig. 5B): a high-pitched part (phase 1) and a part consisting of broader peaks flattening over time (phase 2). The mean pulse duration was  $114\pm 7$  ms, with  $39\pm 2$  ms for phase 1 and  $75\pm 7$  ms for phase 2.

The high-speed video recordings showed that the fish performed a backward movement of the scapular and pelvic girdles. Movie 1 shows the girdle movement in the upper part and the corresponding sound in the lower part (see Movie 1 in supplementary material). The back-and-forth movement was performed during phase 1 of the pulse (Fig. 6), the different skeletal pieces returning to their initial positions before the beginning of phase 2. No skeletal movements were observed during phase 2 of the pulse (Fig. 6). No other landmark displayed any discernible movement during sound production.

The experiment using the high-speed video coupled with the X-ray system confirmed and complemented these results (Fig. 7). A forward movement of the second pterygiophore of the anal fin was also observed. This forward displacement took place simultaneously with the backward movement of the scapular and pelvic girdles.

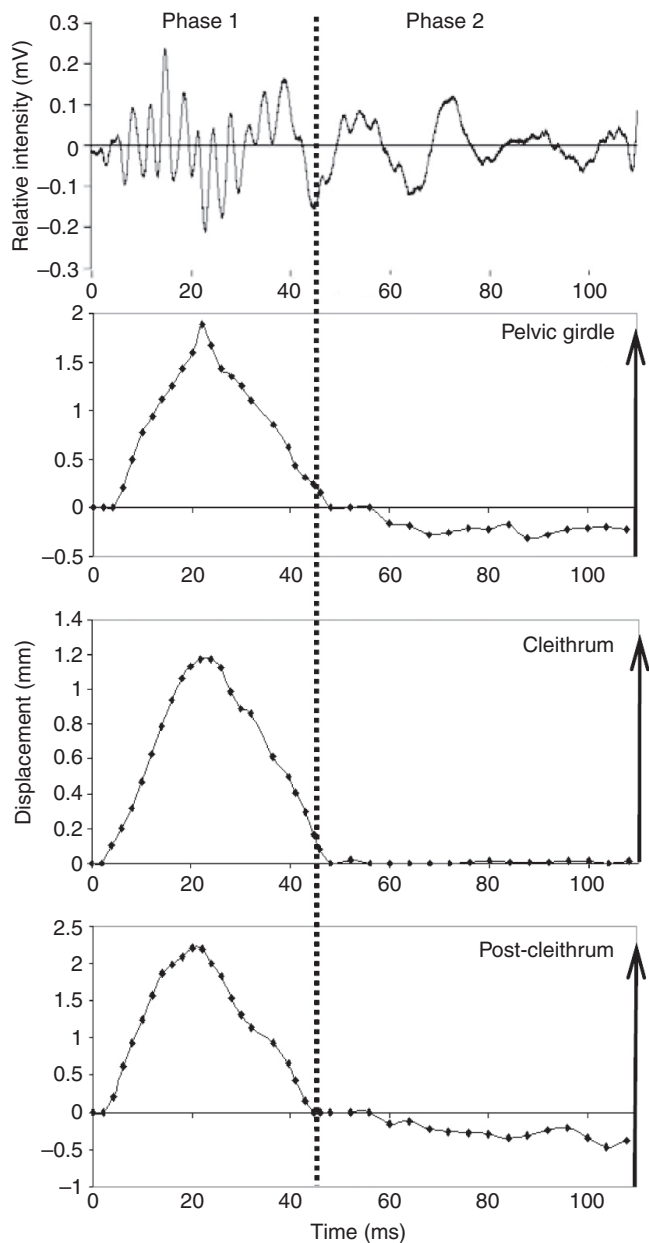


Fig. 6. Movements, according to an  $x$ - $y$  axis referential, of the pelvic girdle, post-cleithrum and cleithrum landmarks in *Oreochromis niloticus* during production of a pulse under natural conditions. The dotted line separates phases 1 and 2 of the pulse. The arrows represent the  $x$ -axis of the referential and the points at the back of the fish. The pelvic girdle, post-cleithrum and cleithrum landmarks are numbered 7, 8 and 9, respectively, in Fig. 2.

**Electrostimulation**

With the exception of sound intensity, there was no significant difference in sound duration and frequency between sounds recorded naturally in the tank and those obtained by electrostimulation (Table 1). This validates the further electrostimulation experiments. It should be stressed, however, that fewer frequencies were excited by electrostimulation than when the sounds were emitted naturally (Fig. 8A,B). Electrostimulation excited mainly the first peak ( $\pm 30$  Hz). Other peaks ( $\pm 60$  Hz, 110 Hz) usually found in the spectrum of the natural sounds of *O. niloticus* (Longrie et al., 2008) were also present but appeared to be less excited in the electrostimulation experiments

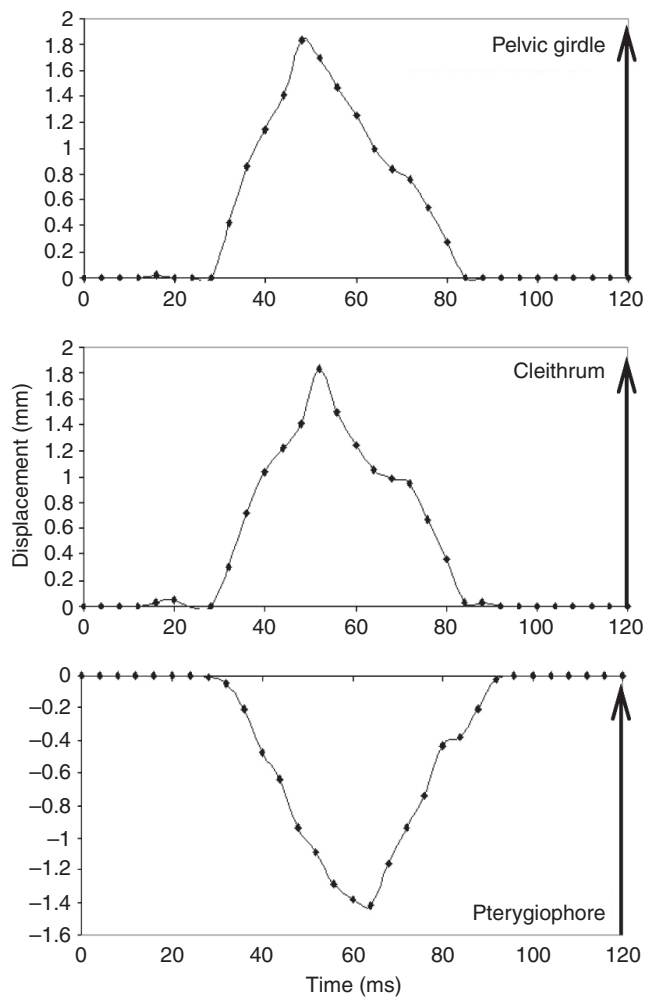


Fig. 7. Pelvic girdle, cleithrum and pterygiophore landmark movements in *Oreochromis niloticus* during production of a pulse by electrostimulation. The arrows represent the  $x$ -axis of the referential and points at the back of the fish.

(Fig. 8B,C). This means that stimulation could trigger the sounds but not their full degree of sharpness.

When stimulations were done in air, they triggered the production of audible sounds; thus, eliminating hydrodynamic sound production as a candidate mechanism. When the pharyngeal jaws were blocked, a significant change in acoustic parameters was observed for the peak frequency (Table 2). However, this difference is not biologically important because the main peak frequencies in the different *O. niloticus* cover an interval between 30 and 75 Hz (Tables 1–3) (Longrie et al., 2008). In fish with a pierced swimbladder, the sound pressure was weaker (Fig. 9A,B; Table 3) than in intact fishes but the sounds produced did not differ in duration or frequency from those of intact fish.

When different parts of the fish body were stimulated, the maximum sound intensity was obtained by stimulation of the flanks, in zone 5 (Fig. 4; Table 4).

Electrical stimulation of the *C. frontosa* specimen gave rise to no sound emission.

**Pharyngeal sounds**

The sounds made by the pharyngeal jaws ( $N=30$  sounds from six different fishes), obtained when the fish were processing food,

displayed a duration of  $22.9 \pm 12.5$  ms and a mean dominant frequency of  $2686.1 \pm 360.7$  Hz. Both sets of data were significantly different ( $P < 0.001$ ) from those of agonistic interactions displayed during nest construction.

#### Morphology

*Oreochromis niloticus* possesses 16 precaudal vertebrae. The first three each possess a pair of epineural ribs, the third being the longest. From the fourth to the sixteenth, the vertebrae possess pairs of ventral parapophyses on which ribs articulate (Fig. 10). These ribs are dorsally closely attached to the serosa of the abdominal cavity. At the midline, between the ventral part of the swimbladder and the dorsal part of the digestive cavity, there is a groove (Fig. 11) containing different bundles of muscles. The swimbladder of *O. niloticus* is anteriorly bilobate. The two lobes surround the ventral apophysis of the third vertebra (onto which the retractor dorsalis muscle inserts) and reach the first vertebra. The posterior part of the swimbladder presses against the second pterygiophore of the anal fin (Fig. 3). The swimbladder wall is very thin and one cannot readily identify all of its layers (Fig. 12). The tunica interna of the swimbladder contains the mucosa overlying a second thin layer containing blood vessels on its ventral part. The tunica externa contains a fibrous layer, which is more developed ventrally. At this level, the tunica externa is also connected to a second fibrous layer lining the abdominal cavity (and doubled there by the coelomic epithelium) and thus the body muscles. Moreover, this fibrous layer is connected to the myosepta in relation with the ribs. As a result, the movements of the ribs have a direct influence on the whole fibrous layer. The inner swimbladder is divided into two chambers, a large anterior one representing more than two thirds of the length, and a smaller posterior one. These chambers are separated by a diaphragm, which is perforated by an orifice delimited by a sphincter, consisting of a series of circular and irradiating muscle fibres (Figs 3 and 13).

The present description of the body musculature concerns principally the region that seems responsible for sound production. Ventrally to horizontal septum, fish display a hypaxial musculature made of different muscles. The obliquus superior is the most external and consists of a set of distinct bundles separated by myosepta. It has its anterior insertion on the neurocranium. At the level of the abdominal cavity, its bundles radiate more or less obliquely towards the rear and insert on the different abdominal ribs. The closer the rib to the head, the more obliquely the bundle runs. *Oreochromis niloticus* also possesses an external latero-ventral muscle (cutaneous

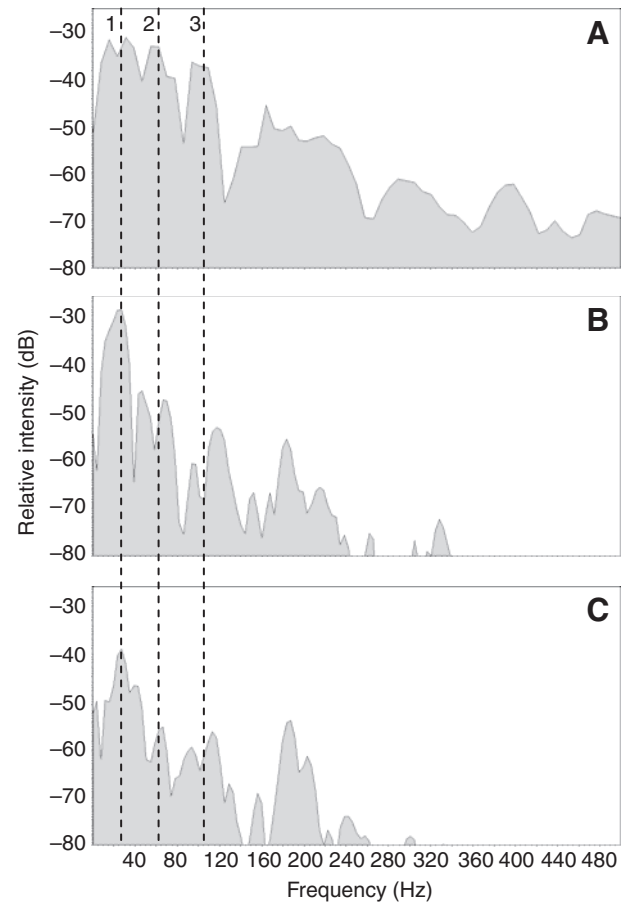


Fig. 8. Sound spectra of a single pulse in *Oreochromis niloticus* in a natural context (A), under electrostimulation (B) and under electrostimulation with a deflated swimbladder (C). Numbers 1–3 represent the main frequency peaks composing the spectrum of the Nile tilapia.

longitudinalis), originating on the post-cleithrum and having superficial insertions on each rib tip (Fig. 10). Below the obliquus superior musculature, a thinner layer of muscles (obliquus inferior) originates on the pectoral girdle, has a ventro-dorsal direction of its fibres and has insertions on myoseptum fibres and on the ventral part of the different ribs. In this layer of muscle, a band of bundles runs alongside the ventral part of the swimbladder and inserts on

Table 1. *Oreochromis niloticus* sound parameters (means  $\pm$  s.d.) in natural (Nat) vs stimulation (Stim) situations and results of the Mann–Whitney *U*-test

Variables	N fishes	N pulses Nat	N pulses Stim	Mean $\pm$ s.d. Nat	Mean $\pm$ s.d. Stim	M–W <i>P</i> (<0.05)
Duration (ms)	5	100	100	126.5 $\pm$ 19.6	140.8 $\pm$ 15.1	NS
Frequency (Hz)	5	100	100	57.4 $\pm$ 13.9	72.3 $\pm$ 39.1	NS

$P > 0.05 = \text{NS}$ .

Table 2. *Oreochromis niloticus* sound parameters (means  $\pm$  s.d.) under stimulation with (PJ KO) and without (Stim) the pharyngeal jaws blocked and results of the Mann–Whitney *U*-test

Variables	N fishes	N pulses Stim	N pulses PJ KO	Mean $\pm$ s.d. Stim	Mean $\pm$ s.d. PJ KO	M–W <i>P</i> (<0.05)
Duration (ms)	5	90	90	138.2 $\pm$ 12	139.3 $\pm$ 21.2	NS
Frequency (Hz)	5	90	90	60.1 $\pm$ 36	42.7 $\pm$ 25.9	***
dB rel.	5	90	90	33.3 $\pm$ 2.2	33.2 $\pm$ 2.6	NS

$P > 0.05 = \text{NS}$  and  $P < 0.001 = \text{***}$ .

Table 3. *Oreochromis niloticus* sound parameters (means ± s.d.) under stimulation, without (Stim) and with pierced swimbladder (SW KO) and results of the Mann–Whitney *U*-test

Variables	<i>N</i> fishes	<i>N</i> pulses Stim	<i>N</i> pulses SW KO	Mean ± s.d. Stim	Mean ± s.d. SW KO	M–W <i>P</i> (<0.05)
Duration (ms)	4	80	68	133.5±15,3	133.1±19.4	NS
Frequency (Hz)	4	80	68	34.6±3.1	32.1±3.3	NS
dB rel.	4	80	68	30.3±2.1	38.6±4.2	***

*P*>0.05=NS and *P*<0.001=\*\*\*.

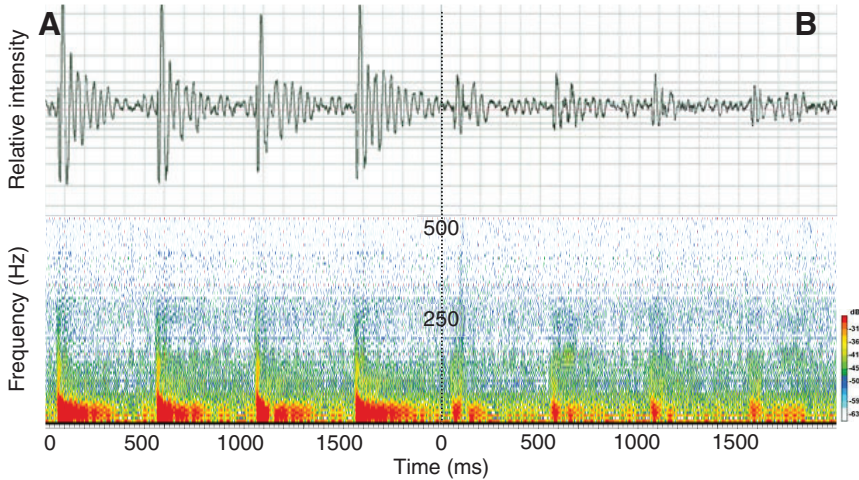


Fig. 9. Oscillogram (A) and spectrogram (B) of four artificial sounds produced by electrostimulation in *Oreochromis niloticus* in an intact fish (left) and when the swimbladder was deflated (right).

the enlarged myosepta connecting the ribs to the fibrous layer of the swimbladder. These muscle bundles, forming the vesica longitudinalis, are situated in the groove located between the swimbladder and the digestive tract (Fig. 10; Fig. 12A). From the first to the seventh vertebra, the bundles possess a ventro-dorsal orientation. From the seventh to the fourteenth vertebra, the fibre direction is dorso-ventral. Posteriorly, the vesica longitudinalis is prolonged by a tendon inserting on the pterygiophore of the second anal fin. In dissection, however, these bundles are not readily distinguishable from the obliquus inferior. On the basis of its position and of our electrostimulation results (Table 4), this muscle is the best candidate for sound production.

*Cyphotilapia frontosa* differs from *O. niloticus* in that (1) the swimbladder originates behind the third vertebra; (2) the swimbladder has no diaphragm; (3) there is no midline groove; and (4) the ventral part of the ribs is not close to the serosa (Fig. 11B). At this level, there are elongated myosepta between the ribs and the digestive cavity. As a result of this organisation, the obliquus inferior is proportionally thicker in *C. frontosa*, making it impossible to discern any sonic muscles. No cross-sections of this region were done in this species, however.

**DISCUSSION**

No study has yet identified the sound-producing structures of cichlids. The main reason is that these fish do not show any obvious structure related to this function. Given the complexity of the pharyngeal jaws, some authors have proposed that these structures

Table 4. Relative intensity (mean ± s.d.) of the sounds obtained in *Oreochromis niloticus* by electrostimulation of six different areas of the body

	1	2	3	4	5	6
mV rel.	127.6±38.8	88±13.6	/	155.3±46	277.6±65.7	/
<i>N</i> pulses	42	33	0	50	50	0

might play a role in sound production in cichlids (Marshall, 1962; Lanzing, 1974; Nelissen, 1977; Rowland, 1978; Lobel, 2001); however, nobody has actually examined tooth movements during sound production (Ladich and Fine, 2006). The sounds recorded in *O. niloticus* do not seem to support the pharyngeal teeth hypothesis. Chewing sounds related to food processing by the pharyngeal teeth last 23 ms on average and have a dominant frequency near 2700 Hz. These tooth sounds differ completely from the *O. niloticus* sounds produced during defence of the territory, whose mean duration is 250–450 ms and whose main frequency is below 200 Hz. The pharyngeal jaw hypothesis is further undermined by the observation that electrostimulation triggered sounds even when the pharyngeal jaws were prevented to come into contact. The data of the kinematics reinforce this result. The sound is realised during a backward movement of the pectoral girdle (Fig. 6). The latter possess two muscles (pharyngocleithralis externus and pharyngocleithralis

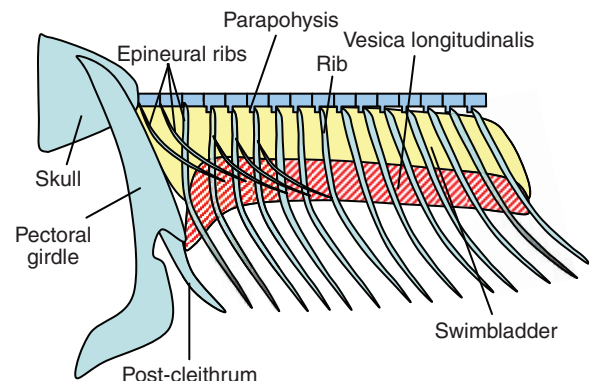


Fig. 10. Schematic lateral view of the scapular girdle and anterior part of the vertebral column in *Oreochromis niloticus*.

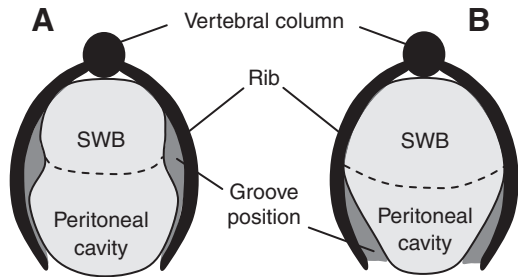


Fig. 11. Schematic transversal sections of (A) *Oreochromis niloticus* and (B) *Cyphotilapia frontosa* without the layers of hypaxial muscle. SWB, swimbladder.

internus) connecting ventrally the lower pharyngeal jaws (Vandewalle, 1972; Galis and Drucker, 1996). The backward displacement of the pectoral girdle should involve a backward and ventral displacement of the lower pharyngeal jaws, preventing the lower pharyngeal jaw from coming into contact with the upper pharyngeal jaws. Furthermore, *O. niloticus* should not be able to hear sounds resulting from tooth stridulation because they do not seem able to hear sounds above 2000 Hz (Smith et al., 2004).

A hydrodynamic sound production mechanism would seem plausible, because hydrodynamic sounds are usually produced by axial muscles (Shishkova, 1958; Moulton, 1960), but the fact that *O. niloticus* sounds are audible in air rules out this hypothesis.

Sounds produced by *O. niloticus* display many characteristics of swimbladder sounds: their main frequency is low, the pulse length is amply superior to 10 ms and a deflated swimbladder produces sounds of significantly reduced intensity (Table 3). According to Skoglund (Skoglund, 1961) and Blaxter and Tytler (Blaxter and Tytler, 1978), damage to the swimbladder (destruction, deflation, filling with water) can reduce the sound output. In the toadfish (*Opsanus tau*), partial deflation of the bladder reduces the amplitude without altering the sound spectrum (Ladich and Fine, 2006). Many fish have developed

spectacular specialisations of muscles. Generally speaking, swimbladder mechanisms involve intrinsic or extrinsic sonic muscles directly or indirectly connected to the swimbladder (Marshall, 1962; Ladich and Fine, 2006). This proximity to the swimbladder creates a resonating effect (Alexander, 1966; Demski et al., 1973). In the present case, the sonic muscles are clearly discernible from the epaxial and hypaxial musculature. Our video analyses indicate that the movements observed during sound production are due to contraction of some trunk muscles at the midline of the abdominal cavity. Electrostimulation of various parts of the body has confirmed this observation (Fig. 4). Yet it has not been possible to clearly discern any extrinsic or intrinsic muscle inserting on the swimbladder in *O. niloticus*. The muscle closest to the swimbladder is a set of bundles we have called the vesica longitudinalis. These bundles clearly belong to the axial musculature, as is the case of most of sonic muscles (Tavolga, 1971; Demski et al., 1973; Hawkins, 1993). According to Moulton (Moulton, 1958), *Pomacanthus arcuatus* (Pomacanthidae) has no intrinsic muscles on the swimbladder, and sound production could be due to contraction of the axial muscles. This author explains (but does not prove) that the intimate association between the air bladder and the surrounding peritoneum, to which many of the abundant axial muscles fibres surrounding the ribs are attached, could create a resonance within the bladder (Moulton, 1958). *Oreochromis niloticus* also possesses this close association between the ribs, the serosa and the swimbladder (Fig. 12); it is quite impossible to remove the ribs without tearing the swimbladder. In *O. niloticus*, sounds may be due to swimbladder movements related to body movements. During the first phase of sound production (Fig. 6), there is a backward movement of the pectoral girdle and a forward movement of the pterygiophore, resulting in compression of the rib cage and also of the swimbladder, because of its close association with the serosa and ribs. This compression and rubbing of the swimbladder is reinforced by the fusion existing between the ventral part of tunica externa of the swimbladder and myoseptum tissues connected to the ribs. In this respect the backward movement of the pectoral girdle corresponds with the belly quiver cichlids often exhibit during vocalisations. It

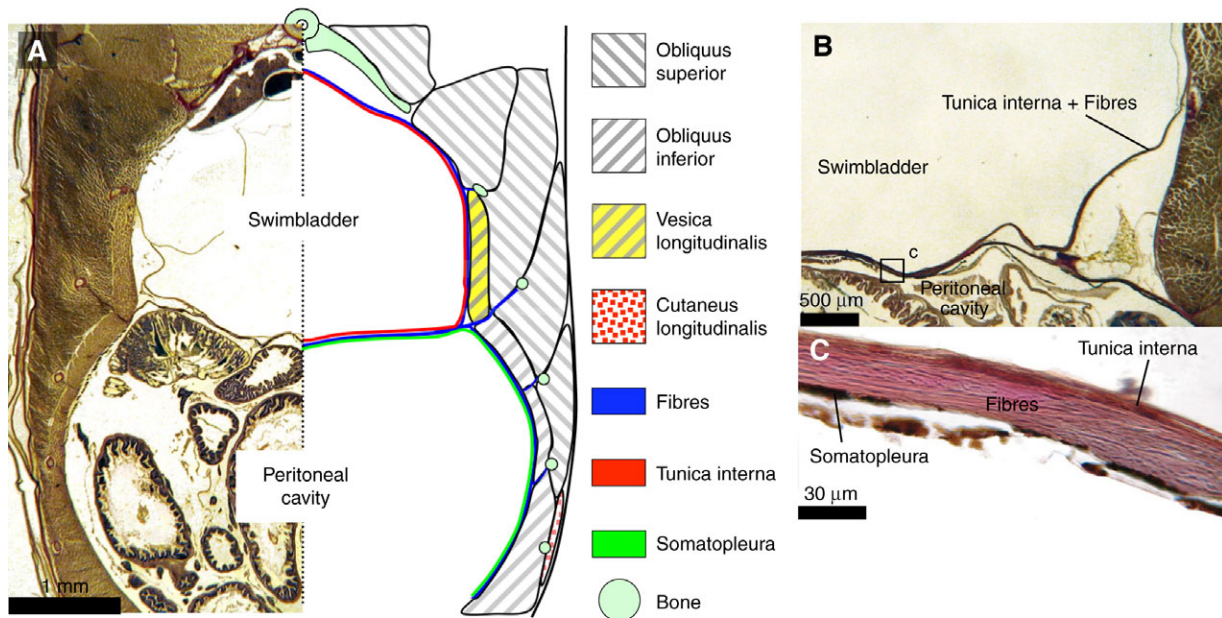


Fig. 12. Cross-section of *Oreochromis niloticus* (SL: 6.3 cm) at the level of the abdominal cavity. The schematic drawing on the right helps to distinguish the muscles, bones and swimbladder tissues. The blue line is peculiar because it results from the fusion of the tunica externa and myosepta tissues as showed in (B) and (C). C: enlargement of c. SL: standard length.

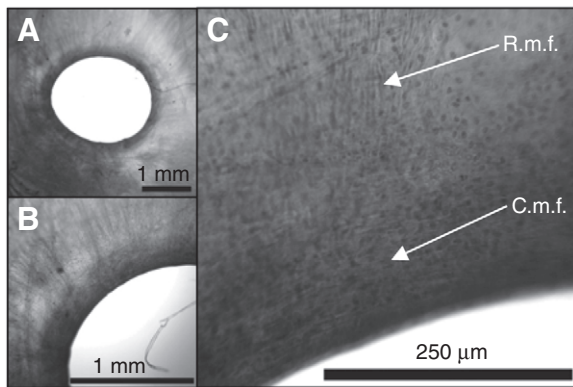


Fig. 13. (A) Picture of the whole mount of the diaphragm in *Oreochromis niloticus*. (B) Enlargement on a part of the diaphragm and (C) of the sphincter. C.m.f.: circular muscle fibres; R.m.f.: radiating muscle fibres.

does not produce the sound, which seems rather to result from contraction of the different bundles of the vesica longitudinalis. This set of bundles appears to be the best candidate for sound production in *O. niloticus*. Electrostimulation of this muscle did not enable us to recreate the sounds exactly, the spectrum of the stimulation-induced sounds being less rich than that of the natural sounds. This result indicates that sound in *O. niloticus* does not result from a single contraction. Rather, there would seem to be an antero-posterior contraction wave through the different bundles of the vesica longitudinalis (see Movie 1 in supplementary material).

The sphincter is usually implicated in the gas exchanges between the anterior chamber and the posterior one (Fange and Wittenberg, 1958; Dehadrai, 1959; Blaxter and Tytler, 1978). Contraction of the swimbladder might also create gas movement from one chamber to the other, causing vibration of the diaphragm and sound production. Such a sound production mechanism has been proposed for the Japanese Gurnard (*Chelidonichthys kumu*) by Bayoumi (Bayoumi, 1970). This role has been supposed for different fishes but it is not yet experimentally demonstrated.

The cichlid *C. frontosa* is unable to produce sound, even with electrostimulation. This fish species has no groove for a vesica longitudinalis and does not possess a diaphragm.

*Oreochromis niloticus* thus displays a particular mechanism of sound production involving the swimbladder but not the (intrinsic or extrinsic) muscles commonly associated with sound production. It could be interesting to examine whether the vesica longitudinalis is present in congeneric species, like *Oreochromis mossambicus*, which seems to produce various types of sounds (Rodman, 1966; Lanzing, 1974; Amorim et al., 2003), one of these showing features similar to those described here, such as a low-frequency peak (Longrie et al., 2008).

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