Zoology
December 2020, Volume 143, Pages 125831 (10p.)
https://doi.org/10.1016/j.zool.2020.125831
https://archimer.ifremer.fr/doc/00642/75385/

Acoustic signals produced by Nile tilapia *Oreochromis niloticus* and black-chinned tilapia *Sarotherodon melanotheron* during intra- and interspecific pairings

Akian Dieudonné Djétouan [1, 2, 3], Yao Kouakou [2], Parmentier Eric [4], Joassard Lucette [3], Clota Frederic [5], Baroiller Jean-François [6], Lozano Paul [6], Chatain Beatrice [7], Bégout Marie-Laure [3, 7, *]

[1] Département Eaux, Forêts et Environnement, Institut National Polytechnique Félix Houphouët Boigny, BP 1313, Yamoussoukro, Côte d'Ivoire
[2] Laboratoire de Biologie et Cytologie Animales, Unité de Formation et de Recherche Science de la Nature, Université Nangui-Abrogoua, 02 BP 801, Abidjan 02, Côte d'Ivoire
[3] Laboratoire Ressources Halieutiques, Institut Français de Recherche pour l'Exploitation de la Mer, L'Houmeau, France
[4] Laboratoire de Morphologie Fonctionnelle et Evolutive, AFFISH, Institut de chimie- B6C, Université de Liège, Sart Tilman, 4000, Liège, Belgium
[5] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France
[6] Unité Mixte de Recherche 116, Institut des Sciences de l'Evolution de Montpellier, Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Montpellier, France
[7] MARBEC, Université Montpellier, CNRS, Ifremer, IRD, Chemin de Maguelone, 34250, Palavas-les-Flots, France

* Corresponding author : Marie-Laure Bégout, email address : marie.laure.begout@ifremer.fr

Abstract :

We characterised, for the first-time, the sound production of black-chinned tilapia Sarotherodon melanotheron and show differences with that of Nile tilapia Oreochromis niloticus in a hybridization pairing context. Although both species were able to produce drum sounds, they showed different acoustic features. Drum sounds were produced in aggressive (chasing or lateral attack) and non-aggressive (courtship) contexts by O. niloticus but only in aggressive situations (fleeing or avoidance) by S. melanotheron. The second type of sounds produced by O. niloticus were grunts, produced in both aggressive (chasing and after biting) and non-aggressive contexts (nest building). The second type of sound produced by S. melanotheron was a rolling sound, produced only during courtship. Each species was able to produce common sounds (drum) and species-specific sounds (grunts and rolling). This implies that species can communicate without being able to understand each other because the sounds emitted may probably have different significance. Drumming corresponded only to aggressivity in S. melanotheron, whereas this was not true for O. niloticus. 11-ketotestosterone (11-kt) levels were significantly higher in male O. niloticus than male S. melanotheron, but there was no significant correlation between 11-kt or estradiol concentrations and the number of sounds produced in aggressive or non-aggressive behavioural contexts in either species. During interspecies interactions, O. niloticus drum sounds are likely considered to be aggressive by S. melanotheron and could potentially constitute a reproductive barrier between the two species.

Highlights

► Drum and rolling sounds have been characterized for the first time in black-chinned tilapia *Sarotherodon melanotheron*. ► Rolling sounds produced in courtship are different from drum sounds in aggressive context hampering interspecies communication. ► *Oreochromis niloticus* and *S. melanotheron* produce sounds in interspecific context both in aggressive and non-aggressive situations ► Sounds and behaviours are not correlated to sex steroid levels in these two tilapias.

Keywords : acoustic signals, behaviour, hybridization, *Oreochromis niloticus, Sarotherodon melanotheron*

1. Introduction

Numerous studies of fish behaviour have shown multimodal communication channels in mate choice, such that the choice of partner is often influenced by different kinds of signals (Candolin, 2003; Verzijden et al., 2010). In fish, the choice of sexual partner may be based on *e.g.* visual cues (Castro et al., 2009), chemical stimuli (Plenderleith et al., 2005), electric signal (Machnik and Kramer, 2008) or species-specific calls (Amorim et al., 2004; Parmentier et al., 2006). In addition to courtship, acoustic signals may also be associated with territory defence or feeding or antipredator behaviour (Amorim et al., 2003, 2004; Amorim and Neves, 2007, 2008; Patek et al., 2009; Maruska et al., 2012; Longrie et al., 2013; Pedroso et al., 2013). In reproduction context, the analysis of acoustic emissions can highlight the relevant information transmitted between the congeners, helping to understand the features used by fish in choosing sexual partners (Lobel, 1998).

As for other vertebrates, fish behaviours, including reproduction, are influenced by hormones and sex pheromones (Tacon et al., 2000; Yambe et al., 2006; Johnson and Li, 2010; Munakata and Kobayashi, 2010), implying that fish calls are also shaped by the level of circulating hormones. For example, in male *Porichthys notatus*, an increase in plasma androgen levels stimulates their vocalization by increasing sound duration (Remage-Healey, 2004; Remage-Healey and Bass, 2007).

Within teleosts, different cichlid species produce sounds either in aggressive contexts (Amorim and Almada, 2005; Amorim et al., 2003, 2004, 2008) or during courtship behaviour (Amorim et al., 2003, 2004). In Nile tilapia *Oreochromis niloticus* (Linnaeus 1758) sounds are produced during nest defence by territorial males and egg protection by females (Longrie et al., 2008, 2013). *Oreochromis mossambicus* (Peters 1852) produces sounds associated with courtship behaviour and reproduction, suggesting that sound plays a role in the synchronization of gamete emission (Amorim et al., 2003). Both species are included in the paraphyletic group of tilapias, which includes the Oreochromini and the Coptodonini tribes (Dunz and Schliewen, 2013). Various reproductive behaviours have been described in these groups (Trewavas, 1983): substrate brooder with biparental guard for *Coptodon sp*., paternal or biparental mouthbrooder for *Sarotherodon sp*., and maternal mouthbrooder for *Oreochromis sp*. Tilapia species of these last two groups belong to the Oreochromini tribe (Dunz and Schliewen, 2013).

Although reproductive behaviours can show differences between tilapia genera, hybridization between some species is possible (Toguyeni et al., 2009), implying that the reproductive barrier is not impermeable (Ptacek, 2000). This is true, for example, between black-chinned tilapia *Sarotherodon melanotheron* Rüppell 1852, a species with paternal or biparental mouthbrooding, and *O. niloticus*, a strictly maternal mouthbrooder (Trewavas, 1983) although the two species are phylogenetically close (Dunz and Schliewen, 2013) and separated some 2.3 millions years ago (Nagl et al. 2001).

If calls are an important component of behaviour, similarities in the sound features of the two species would be expected to be required to allow hybridization. In contrast to *O. niloticus*, the calling abilities of *S. melanotheron* are unknown. This information is however crucial for understanding how specimens of the two species may interact during courtship behaviour and finally mate.

We aimed to record and analyse the acoustic signals of *O. niloticus* and *S. melanotheron* in the context of intra and interspecific pairing. The objectives of this study were to characterise the sound production of *S. melanotheron* in intraspecific pairings, along with sex steroid levels, and to determine the differences in acoustic features between this species and *O. niloticus* in interspecific pairings (male: female sex ratio, 1: 1).

2. Material and methods

All procedures performed in this study involving animals were in accordance with the ethical standards of the institution and followed European Directive 2010/63 UE. This project was performed under agreement EEA# 17-190-1 with project approval under APAFIS #263.

2.1. Experimental fish

Acoustic tests and behavioural observations were carried out on mature XY males (mass 186.5 ± 73.7 g/total length 21.0 ± 2.8 cm, n = 20) and XX females (166.4 ± 73.7 g/20.4 ± 1.9 cm, n = 20) of *O. niloticus* and mature males (171.3 ± 24.1 g/21.1 ± 1.1 cm, n = 20) and females (171.8 ± 24.8 g/21.2 ± 1.0 cm, n = 20) of *S. melanotheron*. Fish of both species were raised at the station of the International Centre for Agronomic Research for Development (CIRAD) of Montpellier (France) and were maintained in four 400 l polycarbonate tanks, under a 12/12 hours light/dark photoperiod, after their transfer to the station of the French Institute for the Research and Exploitation of the Sea (Ifremer) of L'Houmeau (France). They were given a commercial diet for tilapia (EFICO YM 868, 3 mm, BIOMAR®, France) twice a day during two months before the experiment started. The water temperature was maintained at 27–28 °C. The dissolved oxygen concentration was > 4 mg l^{-1}, the pH between 7 and 8, and the NH_4^+, NO_2^-, and NO_3^- concentrations 0.3 ± 0.2, 0.6 ± 0.2, and 7.0 ± 5.8 mg l^{-1}, respectively.

2.2. Experimental protocol

The experiments were conducted in a soundproof room in the basement of Ifremer station. Four 50 l aquariums (60 × 25 × 35 cm high), each equipped with an individual biological filter, were placed on a 4-cm thick polystyrene plate on a table. This device helped to reduce the background noise from vibrations transmitted from the ground to the aquariums. Fish were weighed and measured after benzocaine anaesthesia (0.1 ml l^{-1}), dorsally marked with beads of different colours (Bégout et al., 2012), and then transferred to the aquariums for 24 h of recovery. In the experimental aquariums, four types of pairing were performed: 1 = *O. niloticus* ♂ × *O. niloticus* ♀, 2 = *O. niloticus* ♂ × *S. melanotheron* ♀, 3 = *S. melanotheron* ♂ × *O. niloticus* ♀, 4 = *S. melanotheron* ♂ × *S. melanotheron* ♀. The aquarium was divided into two compartments of the same size with a removable barrier, preventing physical and visual contact between the fish before the recordings. Ten couples were tested in each pairing mode and no individual was tested twice either in intraspecific or interspecific pairing.

2.3. Sound recording with associated behaviours

The day after isolating the fish in the aquarium, a hydrophone (HTI-96-MIN sensitivity 164.9 dB re: 1V/µPa 1.06 mA) was placed in the centre and the movable barrier between the male and female was removed to allow contact between the fish. Five minutes after removal of the barrier, the behaviours and sounds of the fish were simultaneously recorded for each 15 min session using a camera (SONY, HDR-PJ530) positioned 1.5 m away from the front of the aquarium, with the hydrophone connected to the camera and all connected to a computer. Aeration and filter systems were removed from the aquariums during recording to minimize background noise and their influence on fish behaviour. The recordings were made three times for 15 min (starting at 10 h, 12 h, and 14 h) on the same day for each pair. The fish were not fed during the recording sessions and were placed back into a 400L tank similar to their home tank after each pairing.

2.4. Plasma steroid analysis

At the end of each test, a blood sample (0.4–0.5 ml) was taken from each individual after anaesthesia in a bath of benzocaine at 0.1 ml l^{-1} from a 10 % stock solution of ethyl-paminobenzoate (benzocaine, E1501, Sigma, St Louis, MO, USA, prepared by dissolving 100 g benzocaine in 1 l 100 % ethanol) and centrifuged (13,000 rpm, 6 min, 4 °C). Plasma was collected and stored at -20 °C in Eppendorf tubes for later determination of the 11-ketotestosterone (11-kt) concentration in males and females and that of estradiol (E2) in females. Plasma was diluted 1/2000 for males (n = 10 for each species) and 1/500 for females (n = 7 for *O. niloticus*, n = 12 for *S. melanotheron*) for the determination of 11-kt and 1/200 for the E2 assay. Enzyme immunoassays were performed using a BioTek Synergy HT spectrofluorometer and CAYMAN kit (Cayman Chemical Ann Arbor, MI, USA) for 11-kt and an IBL kit (IBL Hamburg, Germany) for E2.

2.5. Analysis of sounds and behaviours

The software Audacity 2.0.4 was used to extract acoustic signals from the video. Sounds were digitized at 44.1 kHz (16-bit resolution) and then analysed using Avisoft software -SASLAB Pro 4.33. The characteristics of the sounds (duration (T), number of pulses, period of pulses or grunts (p), grunt duration (t), and number of grunts: see Figure 1) were measured on an oscillogram. Fundamental frequency of a pulse was obtained from logarithmic power spectra of a pulse (band pass lower cut-off frequency = 0.05 and upper cut-off frequency = 2.5 kHz, FFT size = 1024 points, time overlap = 95 %, and Hanning window, see Fig. 1B or 1D). The average of the fundamental frequencies of the pulses gave us the fundamental frequency of the sound. The behaviours associated with different sounds were annotated based on the tilapia ethogram (Longrie et al., 2013) by video observation following the fish individually. By reviewing several times the recorded videos to listen to the sounds and associate sounds and behaviour, the identity of the producer of each sound was determined. Behaviours were grouped into two categories: aggressive (biting, chasing, fleeing, avoidance, tail stroke, lateral attack) and non-aggressive (courtship, quiver, circle, nest building, and no particular behaviour).

2.6. Statistical analysis

The homogeneity of the variance of the dataset related to the mass and total length of the fish, sound characteristic variables, and E2 and 11-kt concentrations was verified using the Levene test, as well as the normality of the dataset using the Shapiro-Wilk test. Variables for data complying with these rules were compared between the species, according to the four modes of pairing, by two-factor ANOVA (pairing mode and sex as fixed factors) followed by a *post hoc* Tukey test. Non-parametric Kruskal Wallis and Mann-Whitney multiple comparison tests were used when the data did not fulfil the normality requirements. A repeated-measure ANOVA was used to compare the number of sounds emitted by each fish in aggressive and non-aggressive contexts for the observations between 10.00 hours and 14.00 hours. The Spearman rank correlation test (r_s) was performed to evaluate the relationship between E2 and 11-kt concentrations and the number of sounds emitted. All statistical tests were performed using Statistica 10.0 software (Statsoft, Tulsa, OK, USA) and the level of significance retained for the tests was 0.05.

3. Results

3.1. Description of sounds produced by O. niloticus during intraspecific pairing

Male and female *O. niloticus* emitted sounds during both aggressive and non-aggressive behaviours in intraspecific pairings. Two types of sounds were recorded (Table 1). The first type corresponded to drum sounds (Fig. 1A, B) which were associated with both aggressive (after chasing and lateral attack) and non-aggressive (courtship, circling, and quivering) behaviours. The second type corresponded to a train of grunts (Fig. 1C, D) which were also emitted during both aggressive (chasing and before and after biting) and non-aggressive behaviours that corresponded to nest building, although there was no sand at the bottom of the tank. Overall, the same types of sounds were produced by *O. niloticus*, both during aggressive and non-aggressive behavioural contexts. Further, we did not observe different behaviours from the ones already described in Longrie et al. (2013) and Akian et al. (2017) even in the particular context of interspecific pairings.

During intraspecific pairing, in aggressive context, there was no significant difference between the duration of the drum sounds and the frequency between males and female in *O. niloticus* (Tukey post-hoc test, P > 0.05 for the duration and frequency, Table 2). However, males emitted a significantly higher number of pulses than females, with a significantly lower pulse period (Tukey post-hoc test, $P < 0.05$) in the aggressive context. In the characteristics of drum sounds associated with non-aggressive behaviours emitted by *O. niloticus* there were no significant differences between the two sexes during intraspecific pairings (Tukey post-hoc test, $P > 0.05$, Table 3).

The grunt trains associated with aggressive behaviours emitted by *O. niloticus* males had a duration significantly longer, with a higher number of grunt units, than those of females in the aggressive context (Tukey post-hoc test, $P < 0.05$, Table 4). However, there was no significant difference between the period or fundamental frequency between the two sexes of *O. niloticus* (Tukey post-hoc test, $P > 0.05$, Table 4).

In the grunt trains associated with non-aggressive behaviours emitted by *O. niloticus*, there was no significant difference between the duration, number of grunts, period, or frequency between the two sexes during intraspecific pairing (Tukey post-hoc test, $P > 0.05$ for all characteristics, Table 5).

3.2. Description of sounds produced by S. melanotheron during intraspecific pairing

Two types of sounds were also produced by *S. melanotheron* during intraspecific pairings (Table 1). The first corresponded to drum sounds but with a different tone than that of *O. niloticus* and was observed only in an aggressive behavioural context (avoidance, fleeing, lateral attack; Fig. 2A, B). The second type was similar to a rolling sound and was only emitted in a non-aggressive behavioural context (courtship, Fig. 2C, D).

The drum sounds associated with aggressive behaviours emitted by *S. melanotheron* were not significantly different in the characteristics between males and females (Tukey post-hoc test, $P > 0.05$, Table 2). Also, there was no significant difference between any of the characteristics of the rolling sounds associated with non-aggressive behaviours between the two sexes of *S. melanotheron* during intraspecific pairing (Tukey post-hoc test, $P > 0.05$ for all the characteristics, Table 6).

3.3. Description of sounds produced by O. niloticus during interspecific pairing with S. melanotheron

O. niloticus males and females produced the same types of sounds as during the intraspecific pairings, *i.e.* drum sounds and grunt trains (Table 1). However, there were some differences in the characteristics of the sounds associated with aggressive and non-aggressive behaviours between the intraspecific and interspecific pairings for both sexes.

The duration of the drum sounds associated with aggressive behaviours and the number of pulses were significantly higher for *O. niloticus* males during interspecific pairings than intraspecific pairings (Tukey post-hoc test, $P < 0.01$, Table 2). There was, however, no significant difference in the period or frequency for the drum sounds emitted by *O. niloticus* males between the interspecific and intraspecific contexts (Tukey post-hoc test, $P > 0.05$). In

O. niloticus females, there was no significant difference in the characteristics (duration, number of pulses, period and frequency) of the drum sounds emitted between the intraspecific and interspecific contexts in aggressive context (Tukey post-hoc test, $P > 0.05$ in all cases, Table 2).

The drum sounds associated with non-aggressive behaviours emitted by *O. niloticus* males and female during interspecific pairings with *S. melanotheron* were not significantly different in the characteristics (duration, number of pulses, period and frequency) from values in interspecific contexts (Tukey post-hoc test, $P > 0.05$, Table 3).

The second type of sound produced by *O. niloticus* males and females during the interspecific pairings was the same type of grunt train as that produced during the intraspecific pairings. This type of sound was also associated with both aggressive behaviours (chasing and after bites) and non-aggressive behaviours (nest building), as in the intraspecific context.

The grunt train associated with aggressive behaviours emitted by *O. niloticus* males during interspecific pairings with *S. melanotheron* females were not significantly different in the duration, the number of grunts and the period of grunt from values in intraspecific pairings (Tukey post-hoc test, $P > 0.05$). However, the fundamental frequency was significantly higher (Tukey post-hoc test, $P < 0.05$) during the interspecific pairings. In *O. niloticus* females, the duration of the grunt train and the number of grunts were significantly higher (Tukey post-hoc test, $P < 0.05$) during the interspecific pairings than in intraspecific pairing in aggressive context (Table 4).

In *O. niloticus* males and females, the grunt trains associated with non-aggressive behaviours emitted during interspecific pairings with *S. melanotheron* were not significantly different in any of the characteristics from the sounds emitted in intraspecific pairings (Tukey post-hoc test, $P > 0.05$), except for the fundamental frequency of the grunts, which was significantly higher (Tukey post-hoc test, $P < 0.05$) during the interspecific pairings (Table 5).

3.4. Description of sounds produced by S. melanotheron during interspecific pairing with O. niloticus

S. melanotheron males and females produced drum and rolling sounds during interspecific pairing with *O. niloticus*, such as those observed during intraspecific pairing (Table 1).

The characteristics of drum sounds associated with aggressive behaviour of *S. melanotheron* males during interspecific pairings with *O. niloticus* females were not significantly different in the characteristics from the sounds emitted by *S. melanotheron* males in intraspecific pairings (Tukey post-hoc test, $P > 0.05$, Table 2).

The duration and period of drum sounds associated with aggressive behaviour emitted by *S. melanotheron* females during interspecific pairings with *O. niloticus* males (Table 2) were significantly longer during interspecific than intraspecific pairings (Tukey post-hoc test, $P < 0.05$). However, there was no significant difference in the number of pulses or the frequency of the sound between the interspecific and intraspecific pairings (Tukey post-hoc test, $P > 0.05$).

The duration of rolling sounds associated with non-aggressive behaviour emitted by *S. melanotheron* males during interspecific pairings with *O. niloticus* females and the number of pulses emitted by *S. melanotheron* males were significantly less during interspecific than intraspecific pairings (Tukey post-hoc test, $P < 0.05$, Table 6). However, there was no significant difference in the period or fundamental frequency of the sound between the intraspecific and interspecific pairings (Tukey post-hoc test, $P > 0.05$). As in males, the duration of the rolling sounds and the number of pulses emitted by *S. melanotheron* females were significantly less during interspecific than intraspecific pairings (Tukey post-hoc test, $P < 0.05$). There were no significant differences in the period or fundamental frequency of the rolling sounds between the intraspecific and interspecific pairings (Tukey post-hoc test, $P < 0.05$, Table 6).

3.5. Number of sounds emitted according to the context

We next considered all types of sounds produced by each fish for the analysis (for example, drum sound with grunt trains for *O. niloticus* when these two types of sounds were produced in an aggressive context and drum or rolling sounds for *S. melanotheron* when they were produced in an aggressive or non-aggressive context). There were no significant differences (Tukey post-hoc test, $P > 0.05$ in all) in the number of sounds emitted by any tested fish during aggressive or non-aggressive behaviours whatever the time at which the recording was performed. We therefore analysed the differences in relation to the pairing mode (Figure 3). Indeed, the number of sounds emitted by *S. melanotheron* males in aggressive situations was significantly higher (Mann-Whitney U-Test, U = 40.00, $P < 0.01$) during interspecific pairing, whereas the value was significantly higher for females (Mann-Whitney *U*-Test, U = 47.00, $P < 0.01$) during intraspecific pairing. The number of sounds emitted by *O. niloticus* females during aggressive behaviours was significantly higher during intraspecific than interspecific pairing (Fig. 3A, Mann-Whitney *U*-Test, U = 46.50, $P < 0.05$).

The number of sounds emitted by *S. melanotheron* males during non-aggressive behaviours was significantly higher (Mann-Whitney *U*-Test, U = 55.00, $P < 0.05$) during intraspecific than interspecific pairing, whereas the sounds emitted by *S. melanotheron* females and *O. niloticus* males and females were essentially the same, regardless of the pairing mode (Mann-Whitney *U*-Test, $P > 0.05$, Fig. 3B).

3.6. Sexual steroid concentrations and correlation with the number of sounds produced

Plasma 11-kt concentrations (Fig. 4A) in both species were significantly higher (Kruskal-Wallis test: $H_{(3,\ 34)} = 18.40$; $P < 0.01$) in males (*O. niloticus* = 102.7 ± 42.5 $ng.Ml^{-1}$, *S. melanotheron* = 21.8 ± 6.5 $ng.Ml^{-1}$) than females (*O. niloticus* = 3.9 ± 2.7 $ng.Ml^{-1}$, *S. melanotheron* = 8.1 ± 5.9 $ng.Ml^{-1}$). *O. niloticus* males had significantly higher 11-kt plasma concentrations than *S. melanotheron* males (Mann-Whitney *U*-test, U = 6.00, $P < 0.01$). There were no significant differences in the concentrations of 11-kt between the females of either species (Mann-Whitney *U*-test, U = 24.00, $P > 0.05$, Fig. 4A) or E2 (*O. niloticus* ♀ = 100.6 ± 58.1 $ng.Ml^{-1}$, *S. melanotheron* ♀ = 112.0 ± 56.8 $ng.Ml^{-1}$, Mann-Whitney *U*-test, U = 35.00, $P > 0.05$, Fig. 4B).

There was no significant correlation (Spearman rank correlation, $P > 0.05$) between the concentration of 11-kt and the number of sounds emitted during aggressive or non-aggressive behaviours for either species. Similarly, in females, there was no significant correlation (Spearman rank correlation, $P > 0.05$) between the concentration of E2 and the number of sounds emitted during aggressive or non-aggressive behaviours.

4. Discussion

We show that *O. niloticus* males and females produce two types of sounds (drum sounds and grunts) during intra- and interspecific pairing, as well as during aggressive and non-aggressive behaviours. During intraspecific pairings, *O. niloticus* males and females emitted drum sounds after chasing or lateral attacks and during courtship behaviours when circling around the congener and quivering. Grunt trains were also produced during chases, after biting, and after nest building when turning around to attract the attention of the opposite sex. During interspecific pairing with *S. melanotheron*, the fish produced drum sounds and grunt trains in similar behavioural contexts. Thus, *O. niloticus* produces the same sounds in aggressive and non-aggressive contexts as those observed in other cichlids by Amorim (2006). Also, similar sounds are produced in both agonistic and courtship contexts by *Hemichromis bimaculatus* and *Herotilapia multispinosa* (Amorim, 2006).

Sarotherodon melanotheron males and females were also able to produce sounds in aggressive and/or non-aggressive behavioural contexts. This species produced drum sounds in aggressive contexts (during fleeing or avoidance) and rolling sounds during non-aggressive behaviours related to courtship. The tonality of the drum sound in this species is different from that of *O. niloticus* and no grunt trains were heard. In this study, rolling sounds were only emitted by *S. melanotheron* during courtship behaviours to attract the attention of the opposite-sex partner or in response to a congener. When *S. melanotheron* males or females emitted rolling sounds during intraspecific pairings, the congener of the opposite sex responded by producing the same type of sound while approaching the congener hereby displaying an attraction sequence. In the interspecific context settings we did explore, male and female *S. melanotheron* produced such rolling sounds during courtship behaviours, possibly to attract *O. niloticus*. However, this sound does not exist in the acoustic repertoire of *O. niloticus* (Longrie et al., 2008, 2009, 2013). Generally, this was followed by aggressive behaviours from *O. niloticus*, such as chasing, after which it often emitted drum sounds or grunt trains. Aggressive behaviour by *O. niloticus* also generated fleeing or avoidance responses associated with drum sound production by *S. melanotheron*. Drum sounds were also emitted during intraspecific pairing by *S. melanotheron* males and females when fleeing during an aggressive encounter.

This study suggests that acoustic signals can induce aggressive or non-aggressive behavioural responses in both species and that *O. niloticus* and *S. melanotheron* are able to distinguish the nature of the sounds produced by congeners, as observed by Amorim et al. (2004) and Bertucci et al. (2010) in other cichlids. It also shows that these two tilapias exhibit sound-based communication for intraspecific recognition, similar to the intraspecific recognition observed in *Xenopus laevis* (Vignal and Kelley, 2007). Sound-based information may therefore play an important role in the choice of partner in *O. niloticus* and *S. melanotheron*, as shown in other cichlids (Verzijden et al., 2010). The results of this study are thus consistent with those of previous studies showing differences in the characteristics of acoustic signals involved in partner choice in cichlids (Amorim et al., 2008).

The absence of grunts and the signature of courtship sounds (rolling sound) emitted by *S. melanotheron* allows differentiation of this species from *O. niloticus* and also shows that acoustic signals may be potentially involved in the mechanisms of reproductive isolation and speciation in cichlids (Lobel, 1998; Ptacek, 2000; Amorim et al., 2008). Indeed, *O. niloticus* uses the same sounds during aggressive and non-aggressive behaviours, whereas the signals related to courtship behaviour are more distinct from those associated with aggressive behaviour in *S. melanotheron*. Moreover, the grunts recorded in this experiment have not been mentioned in previous studies on *O. niloticus* (Longrie et al., 2008, 2009, 2013). Overall, this could explain the reproductive isolation between these two species in the wild and the lack of reproduction exhibited in the interspecific context in our study. Indeed, in the artificial lake of Ayamé or in the Gô river in Grand Lahou in the south of Côte d'Ivoire where *O. niloticus* and *S. melanotheron* are found, no natural hybrid of these two species has been observed or captured by local fishermen (Koné et al., 2003 ; Adepo-Gourene and Gourene, 2008). Despite the fact that these two species are phylogenetically close and belong to the same Oreochromini tribe (Dunz and Schliewen, 2013), species-specific signals could be the basis of their reproductive isolation in the natural environment (Ptacek, 2000).

Oerochromis niloticus has a relatively poor hearing and does not have accessory structures connecting the swim bladder to the ear and is considered as a hearing generalist that is able to detect sound in a small bandwidth. This species can detect sounds between 100 and 1,000 Hz (Smith et al., 2004) which corresponds to the frequencies of the sounds they produce. We do not have information for *S. melanotheron*. However, once again, their anatomy, and mainly the lack of accessory structures connecting the swim bladder suggest they have the same kind of hearing ability than *O. niloticus* (Popper 1977, Schulz-Mirbach et al., 2013).

During the intraspecific pairings of *O. niloticus* and *S. melanotheron* under our experimental conditions, both males and females showed the same duration of sound and fundamental frequency associated with aggressive behaviours. In contrast, drum sounds emitted by *O. niloticus* males had a significantly higher number of pulses and significantly lower pulse period than that of the females of the same species in an aggressive context. Also, the duration of grunt trains produced by males was longer and the number of grunts higher than that of females in an aggressive context. In the interspecific context, the duration of the drum sound and the number of pulses in an aggressive context were significantly higher for *O. niloticus* males than those produced in intraspecific context. The fundamental frequency of the grunt trains emitted by males was significantly higher and the duration of the grunt trains emitted by females was significantly longer for *O. niloticus* in interspecific pairings with *S. melanotheron* than that observed in intraspecific pairings. The duration of the drum sounds associated with aggressive behaviours emitted by *S. melanotheron* females was significantly higher in interspecific than intraspecific pairings. These features could further reinforce the dominance of *O. niloticus* over *S. melanotheron* and induce a strong stress response in female *S. melanotheron* when facing *O. niloticus* males. Indeed, despite the dominance of males over females for both species, *O. niloticus* males were even more aggressive towards *S. melanotheron* females, as observed by Akian et al. (2017).

In the non-aggressive context, only the fundamental frequency of the grunts emitted by both *O. niloticus* males and females was significantly higher during interspecific than intraspecific pairings. The duration of the rolling sounds associated with non-aggressive behaviours emitted by *S. melanotheron* was significantly shorter and the number of pulses significantly lower during interspecific than intraspecific pairings. This may be due to the lack of receptivity and aggressive behaviours of *O. niloticus*, which does not stimulate *S. melanotheron* to continue to emit these types of courtship sounds.

During dyadic contests, *O. niloticus* is dominant over *S. melanotheron* (Akian et al., 2017). As a result, *S. melanotheron* males and females emitted a higher number of drumming sounds during interspecific pairing most often associated with fleeing and avoidance. During intraspecific pairing, *S. melanotheron* females produced more sounds than males in fleeing and avoidance situations because of the dominance of the males or a refusal of the females, which could be very selective in their choice of an opposite-sex partner, as reported by Balshine-Earn (1996) in *Sarotherodon galilaeus*. Indeed, *S. melanotheron* females are known to choose larger males to ensure the efficient oral incubation of offspring because it is the male that performs this task (Legendre and Trébaol, 1996), whereas all the fish in this study were size matched, which may have induced a high female refusal rate. The lack of choice and divergent reproductive behaviours such as paternal and or biparental mouth brooding in *S. melanotheron* opposite to strictly maternal mouth brooding in *O. niloticus* can hamper interspecific hybridization between the two species (Ptacek, 2000).

The 11-kt concentrations in male plasma were higher than those measured in the plasma of females of both species, consistent with observations in other fish species (Oliveira, 2004; Desjardins et al., 2008; Taves et al., 2009). Differences observed between individual values may be related to stress due to confinement of the fish in tanks prior to recordings and samplings. Indeed, confinement would increase serum cortisol levels, which in turn inhibit 11-kt production (Consten et al., 2001; Vasconcelos et al., 2012). However, we did not measure the level of cortisol and therefore cannot confirm this hypothesis. According to Kishida and Specker (2000), male plasma androgen concentrations in *S. melanotheron* are low (less than 5 ng ml^{-1}) during the first week of egg mouth brooding and relatively high (between 13 - 38 ng ml^{-1}) during the second week of mouth brooding of the hatched embryos. Although we did not observe mouth brooding by the *S. melanotheron* males in our experiments, we measured 11-kt levels similar to those cited above (6 - 57 ng ml^{-1}) in both *S. melanotheron* and *O. niloticus* males. The presence of dominant and subordinate males in the same breeding tank could also explain the low and high concentrations of 11-kt measured, as observed with *Astatotilapia burtoni* (Parikh et al., 2006).

There was no significant correlation between the 11-kt concentration and the number of sounds emitted during aggressive or non-aggressive behaviours for either species in the intraspecific and interspecific pairings. A link between the levels of several hormones (prostaglandins, GnRh) and nonapeptides (arginine-vasotocin) and sexual behaviours has been reported in fish (Bentley et al., 2006; Tsutsui and Osugi, 2009; Munakata and Kobayachi, 2010) and could also influence the production of acoustic signals. However, environmental and other signals from congeners (visual stimuli, pheromones, etc.) may be more highly related to the number of sounds produced, as observed for sexual behaviour, according to Munakata and Kobayashi (2010).

There were no differences in the E2 concentrations measured in the females of either species. Observed intraspecific individual differences may have been related to the females not all having the same reproductive status. The oestradiol values measured in *O. niloticus* females were higher than those found by Biswas et al. (2005) and Onumah et al. (2010) (mean values between 3 and 22 ng.ml^{-1} *versus* 100.59 ± 58.10 ng ml^{-1} in this study) in females of larger size exposed to the same photoperiod. The mean values of E2 we measured in *S. melanotheron* females were also higher than those obtained by Specker and Kishida (2000) for small females (49 g) that were incubating or not incubating eggs after spawning (3 - 8 ng ml^{-1} *versus* 112.00 ± 56.82 ng ml^{-1} in this study). There was no link between E2 levels and the number of sounds emitted in aggressive and non-aggressive situations by females of either species. Hence, E2 levels may not be related to the production of acoustic signals. It has also been shown that oestrogens are not involved in the expression of sexual behaviours in oviparous teleosts because E2 concentrations decrease during spawning periods (Kobayashi et al., 1987; Moyle and Cech, 2000; Munakata et al., 2001; Munakata and Kobayashi, 2010).

In conclusion, this study provides the first insights into the ability of *S. melanotheron* to produce sounds associated with aggressive and non-aggressive behaviours. Second, each species is able to produce common sounds (drum sounds) and species-specific sounds (grunts and rolling sounds). This implies that *O. niloticus* and *S. melanotheron* can communicate without being able to understand each other because the sounds emitted in the pairs may probably have different interpretation by each fish: drum sounds, for example, correspond only to aggressivity in *S. melanotheron*, whereas this is not true for *O. niloticus*. Overall, further behavioural quantification should be performed to precisely identify responses to produced sounds using for example playback sounds. More complex situation could also be studied with fish placed in different sex ratio contexts.

Funding information

This work was supported by the project of educational and institutional renovation of Agronomy School of the National Polytechnic Institute Félix Houphouet Boigny (INP-HB Côte d'Ivoire): ESA C2D - Montpellier SupAgro (France).

Declaration of Competing Interest

None.

Acknowledgements

We thank X. Cousin and S. Millot for their help in setting up the experimental device and F. Bertucci for his support in the analysis of the sound characteristics.

References

Adepo-Gourene, B., Gourene, G., 2008. Différenciation morphologique des populations naturelles d'une sous-espèce de tilapia *Sarotherodon melanotheron melanotheron* Rüppell, 1852 (Teleostei ; Cichlidae) de Côte d'Ivoire. Sciences et Nature 5, 15–27.

Akian, D.D., Yao, K., Clota, F., Lozano, P., Baroiller, J.F., Chatain, B., Bégout, M.-L., 2017. Reproductive behaviour of two tilapia species (*Oreochromis niloticus*, Linné, 1758; *Sarotherodon melanotheron*, Rüppel, 1852) in freshwater intra and interspecific pairing context. Appl. Anim. Behav. Sci. 193, 104–113.

Amorim, M.C.P., Almada, V.C., 2005. The outcome of male–male encounters affects subsequent sound production during courtship in the cichlid fish *Oreochromis mossambicus*. Anim. Behav. 69, 595–601.

Amorim, M.C.P., 2006. Diversity of sound production in fish. Communication in Fishes, 1, 71–104.

Amorim, M.C.P., Neves, A.S.M., 2007. Acoustic signalling during courtship in the painted goby, *Pomatoschistus pictus*. J. Mar. Biol. Assoc. UK. 87, 1017–1023.

Amorim, M.C.P., Neves, A.S.M., 2008. Male painted gobies (*Pomatoschistus pictus*) vocalise to defend territories. Behaviour 145, 1065–1083.

Amorim, M.C.P., Fonseca, P.J., and Almada, V.C., 2003. Sound production during courtship and spawning of *Oreochromis mossambicus:* male–female and male–male interactions. J. Fish Biol. 62, 658–672.

Amorim, M.C.P., Knight, M.E., Stratoudakis, Y., Turner, G.F., 2004. Differences in sounds made by courting males of three closely related Lake Malawi cichlid species. J. Fish Biol. 65, 1358–1371.

Amorim, M.C.P., Simões, J.M., Fonseca, P.J., Turner, G.F., 2008. Species differences in courtship acoustic signals among five Lake Malawi cichlid species (*Pseudotropheus spp*.). J. Fish Biol. 72, 1355–1368.

Balshine-Earn, S., 1996. Reproductive rates, operational sex ratios and mate choice in St. Peter’s fish. Behav. Ecol. Sociobiol. 39, 107–116.

Bégout, M.-L., Kadri, S., Huntingford, F., Damsgård, B., 2012. Tools for studying the behaviour of farmed fish. Aquacult. Behav., 65–86.

Bentley, G.E., Jensen, J.P., Kaur, G.J., Wacker, D.W., Tsutsui, K., Wingfield, J.C., 2006. Rapid inhibition of female sexual behavior by gonadotropin-inhibitory hormone (GnIH). Horm. Behav. 49, 550–555.

Bertucci, F., Beauchaud, M., Attia, J., Mathevon, N., 2010. Sounds modulate males’ aggressiveness in a cichlid fish. Ethology 116, 1179–1188.

Biswas, A.K., Morita, T., Yoshizaki, G., Maita, M., Takeuchi, T., 2005. Control of reproduction in Nile tilapia *Oreochromis niloticus* (L.) by photoperiod manipulation. Aquaculture 243, 229–239.

Candolin, U., 2003. The use of multiple cues in mate choice. Biol. Rev. 78, 575–595.

Castro, A.L.S., Gonçalves-de-Freitas, E., Volpato, G.L., Oliveira, C., 2009. Visual communication stimulates reproduction in Nile tilapia, *Oreochromis niloticus* (L.). Braz. J. Med. and Biol. Res. 42, 368–374.

Consten, D., Lambert, J.G.D., Goos, H.T., 2001. Cortisol affects testicular development in male common carp, *Cyprinus carpio* L., but not via an effect on LH Secretion. Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol. 129, 671–677.

Desjardins, J.K., Stiver, K.A., Fitzpatrick, J.L., Balshine, S., 2008. Differential responses to territory intrusions in cooperatively breeding fish. Anim. Behav. 75, 595–604.

Dunz, A.R., Schliewen, U.K., 2013. Molecular phylogeny and revised classification of the haplotilapiine cichlid fishes formerly referred to as "*Tilapia*". Mol. Phylogenetics Evol. 68, 64–80.

Johnson, N.S., Li, W., 2010. Understanding behavioral responses of fish to pheromones in natural freshwater environments. J. Comp. Physiol. 196, 701–711.

Kishida, M., Specker, J.L., 2000. Paternal mouthbrooding in the black-chinned tilapia, *Sarotherodon melanotheron* (Pisces: Cichlidae): changes in gonadal steroids and potential for vitellogenin transfer to larvae. Horm. Behav. 37, 40–48.

Kobayashi, M., Aida, K., Hanyu, I., 1987. Hormone changes during ovulation and effects of steroid hormones on plasma gonadotropin levels and ovulation in goldfish. Gen. Comp. Endocr. 67, 24–32.

Koné, T., Teugels, G.G., N'Douba, V., Gooré, B.G., Kouamélan, E.P., 2003. Premières données sur l'inventaire et la distribution de l'ichtyofaune d'un petit bassin côtier Ouest Africain : Rivière Gô (Côte d'Ivoire). Cybium 27, 101–106.

Legendre, M., Trébaol, L., 1996. Efficacité de l'incubation buccale et fréquence de ponte de *Sarotherodon melanotheron* (Rüppel, 1852) en milieu d'élevage (lagune Ebrié, Côte d'Ivoire). *In* R.S.V. Pullln, J. Lazard, M. Legendre, J.B. Amon Kothias et D. Pauly (éds.) Le Troisième Symposium International sur le Tilapia en Aquaculture. *ICLARM* Conf. Proc. 41, 375–386.

Lobel, P.S., 1998. Possible species specific courtship sounds by two sympatric cichlid fishes in Lake Malawi, Africa. Environ. Biol. Fishes 52, 443–452.

Longrie, N., Fine, M.L., Parmentier, E., 2008. Innate sound production in the cichlid *Oreochromis niloticus*. J. Zool. 275, 413–417.

Longrie, N., Van Wassenbergh, S., Vandewalle, P., Mauguit, Q., Parmentier, E., 2009. Potential mechanism of sound production in *Oreochromis niloticus* (Cichlidae). J. Exp. Biol. 212, 3395–3402.

Longrie, N., Poncin, P., Denoël, M., Gennotte, V., Delcourt, J., Parmentier, E., 2013. Behaviours associated with acoustic communication in Nile Tilapia (*Oreochromis niloticus*). PLoS ONE 8, e61467.

Machnik, P., Kramer, B., 2008. Female choice by electric pulse duration: attractiveness of the males' communication signal assessed by female bulldog fish, *Marcusenius pongolensis* (Mormyridae, Teleostei). J. Exp. Biol. 211, 1969–1977.

Maruska, K.P., Ung, U.S., Fernald, R.D., 2012. The African cichlid fish *Astatotilapia burtoni* uses acoustic communication for reproduction: sound production, hearing, and behavioral significance. PLoS ONE 7, e37612.

Moyle, P.B., Cech, Jr.J.J., 2000. Fishes: An Introduction to Ichthyology, fourth ed. Prentice Hall, Upper Saddle River, NJ.

Munakata, A., Amano, M., Ikuta, K., Kitamura, S., Aida, K., 2001. The involvement of sex steroid hormones in downstream and upstream migratory behavior of Masu salmon. Comp. Biochem. Physiol. B, 129, 661–669.

Munakata, A., Kobayashi, M., 2010. Endocrine control of sexual behavior in teleost fish. Gen. Comp. Endocr. 165, 456–468.

Nagl, S., Tichy, H., Mayer, W.E., Samonte, I.E., McAndrew, B.J., Klein, J., 2001. Classification and phylogenetic relationships of African tilapiine fishes inferred from mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 20(3), 361-374.

Oliveira, R.F., 2004. Social modulation of androgens in vertebrates: mechanisms and function. In: Slater, P.J.B., Rosenblatt, J.S., Snowdon, C.T., Roper, T.J. (Eds.), Advances in the Study of Behavior 34, 165–239. New York: Academic Press.

Onumah, E.E., Wessels, S., Wildenhayn, N., Brümmer, B., Schwark, H.G., 2010. Stocking density and photoperiod manipulation in relation to Estradiol profile to enhance spawning activity in female Nile Tilapia. Turk. J. Fish Aquat. Sc. 10, 463–470.

Parikh, V., Clement, T., Fernald, R., 2006. Androgen level and male social status in the African cichlid, *Astatotilapia burtoni*. Behav. Brain Res. 166, 291–295.

Parmentier, E., Fine, M., Vandewalle, P., Ducamp, J.-J., Lagardère, J.-P., 2006. Sound production in two carapids (*Carapus acus* and *C. mourlani*) and through the sea cucumber tegument. Acta Zool. 87, 113–119.

Patek, S.N., Shipp, L.E., Staaterman, E.R., 2009. The acoustics and acoustic behavior of the California spiny lobster (*Panulirus interruptus*). J. Acoust. Soc. Am. 125, 3434–3443.

Pedroso, S.S., Barber, I., Svensson, O., Fonseca, P.J., Amorim, M.C.P., 2013. Courtship sounds advertise species identity and male quality in sympatric *Pomatoschistus spp*. Gobies. PLoS ONE 8, e64620.

Plenderleith, M., Oosterhout, C.v., Robinson, R.L., Turner, G.F., 2005. Female preference for conspecific males based on olfactory cues in a Lake Malawi cichlid fish. Biol. Lett. 1, 411–414.

Popper, A.N., 1977. Scanning electron microscopic study of sacculus and lagena in ears of fifteen species of teleost fishes. J. Morphol. 153, 397–417.

Ptacek, M.B., 2000. The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. Behav. Process. 51 (1-3), 111–134.

Remage-Healey, L., 2004. Rapid, hierarchical modulation of vocal patterning by steroid hormones. J. Neurosci. 24, 5892–5900.

Remage-Healey, L., Bass, A.H., 2007. Plasticity in brain sexuality is revealed by the rapid actions of steroid hormones. J. Neurosci. 27, 1114–1122.

Schulz-Mirbach, T., Heß, M., Metscher, B.D., Ladich, F.,2013. A unique swim bladder-inner ear connection in a teleost fish revealed by a combined high-resolution microtomographic and three-dimensional histological study. BMC Biol. 11, 75.

Smith, M.E, Kane AS, Popper AN (2004). Acoustical stress and hearing sensitivity in fishes: does the linear threshold shift hypothesis hold water? J. Exp. Biol. 207, 3591-3602.

Specker, J.L., Kishida, M., 2000. Mouthbrooding in the black-chinned tilapia, *Sarotherodon melanotheron* (Pisces: Cichlidae): The presence of eggs reduces androgen and estradiol levels during paternal and maternal parental behavior. Horm. Behav. 38, 44–51.

Tacon, P., Baroiller, J.F., Le Bail, P., Prunet, P. & Jalabert, B., 2000. Effect of egg deprivation on sex steroids, gonadotropin, prolactin, and growth hormone profiles during the reproductive cycle of the mouthbrooding cichlid fish *Oreochromis niloticus*. Gen. Comp. Endocr. 117, 54–65.

Taves, M.D., Desjardins, J.K., Mishra, S., Balshine, S., 2009. Androgens and dominance: Sex-specific patterns in a highly social fish (*Neolamprologus pulcher*). Gen. Comp. Endocr. 161, 202–207.

Toguyeni, A., Fauconneau, B., Melard, C., Fostier, A., Lazard, J., Baras, E., Kuhn, E., van der Geyten, S., Baroiller, J.-F., 2009. Sexual dimorphism in two pure Cichlid species, *Oreochromis niloticus niloticus* (Linnaeus 1758) and *Sarotherodon melanotheron*

melanotheron Ruppel 1852, and their intergeneric hybrids. Afr. J. Aquat. Sci. 34, 69–75.

Trewavas, E.; 1983. Tilapine Fishes of the Genera Sarotherodon, Oreochromis and Danakilia. British Museum. London: Natural History, 583 p.

Tsutsui, K., Osugi, T., 2009. Evolutionary origin and divergence of GnIH and its homologous peptides. Gen. Comp. Endocr. 161, 30–33.

Vasconcelos, R.O., Carrico, R., Ramos, A., Modesto, T., Fonseca, P.J., Amorim, M.C.P., 2012. Vocal behavior predicts reproductive success in a teleost fish. Behav. Ecol. 23, 375–383.

Verzijden, M.N., van Heusden, J., Bouton, N., Witte, F., ten Cate, C., Slabbekoorn, H., 2010. Sounds of male Lake Victoria cichlids vary within and between species and affect female mate preferences. Behav. Ecol. 21, 548–555.

Vignal, C., Kelley, D., 2007. Significance of temporal and spectral acoustic cues for sexual recognition in *Xenopus laevis*. Proc. R. Soc. B: Biol. Sci. 274, 479–488.

Yambe, H., Kitamura, S., Kamio, M., Yamada, M., Matsunaga, S., Fusetani, N., Yamazaki, F., 2006. L-Kynurenine, an amino acid identified as a sex pheromone in the urine of ovulated female masu salmon. Proc. Natl. Acad. Sci. 103 (3), 15370–15374.

Figure captions

Fig. 1. Oscillograms and logarithmic power spectra of the drumming sound with four pulses (A and B) and a train of 11 grunts (C and D) in *Oreochromis niloticus*. (T) represents the duration of the sound, (p) the period of two pulses, and (t) the duration of a grunt.

Relative amplitude means that the hydrophone we used was not calibrated. The dotted rectangle means that we have selected the pulse on the oscillogram, and the arrow indicates the transformation into logarithmic power spectra to produce figures B and D. The arrows on the graphs indicate the dominant frequencies (150 Hz for a pulse in the drumming sound and 440 Hz for a grunt in these examples).

Fig. 2. Oscillograms and logarithmic power spectra of a drumming sound with three pulses (A and B) and a rolling sound with seven pulses (C and D) in *Sarotherodon melanotheron*. (T) represents the duration of the sound and (p) the period between two pulses. Relative amplitude means that the hydrophone we used was not calibrated. The dotted rectangle means that we have selected the pulse on the oscillogram, and the arrow indicates the transformation into logarithmic power spectra to produce figures B and D. The arrows on the graphs indicate the dominant frequencies (80 Hz for a pulse in the drumming sound and 97 Hz for a pulse in rolling sound in these examples).

Fig. 3. Number of sounds produced in aggressive (A) and non-aggressive (B) contexts by *Oreochromis niloticus* (male = On♂, female = On♀) and *Sarotherodon melanotheron* (male = Sm♂, female = Sm♀) according to the pairing mode. Dotted line for *O. niloticus* male and female. The letters a, b, and c in the histogram indicate significant differences (Mann-Whitney U-Test, $P < 0.05$).

Fig. 4. 11-KT (A) and E2 (B) plasma concentrations in *Oreochromis niloticus* (male = On♂, female = On♀) and *Sarotherodon melanotheron* (male = Sm♂, female = Sm♀). The letters a, b, and c in the histogram indicate significant differences (Mann-Whitney U-test, $P < 0.05$).

Fig. 1.

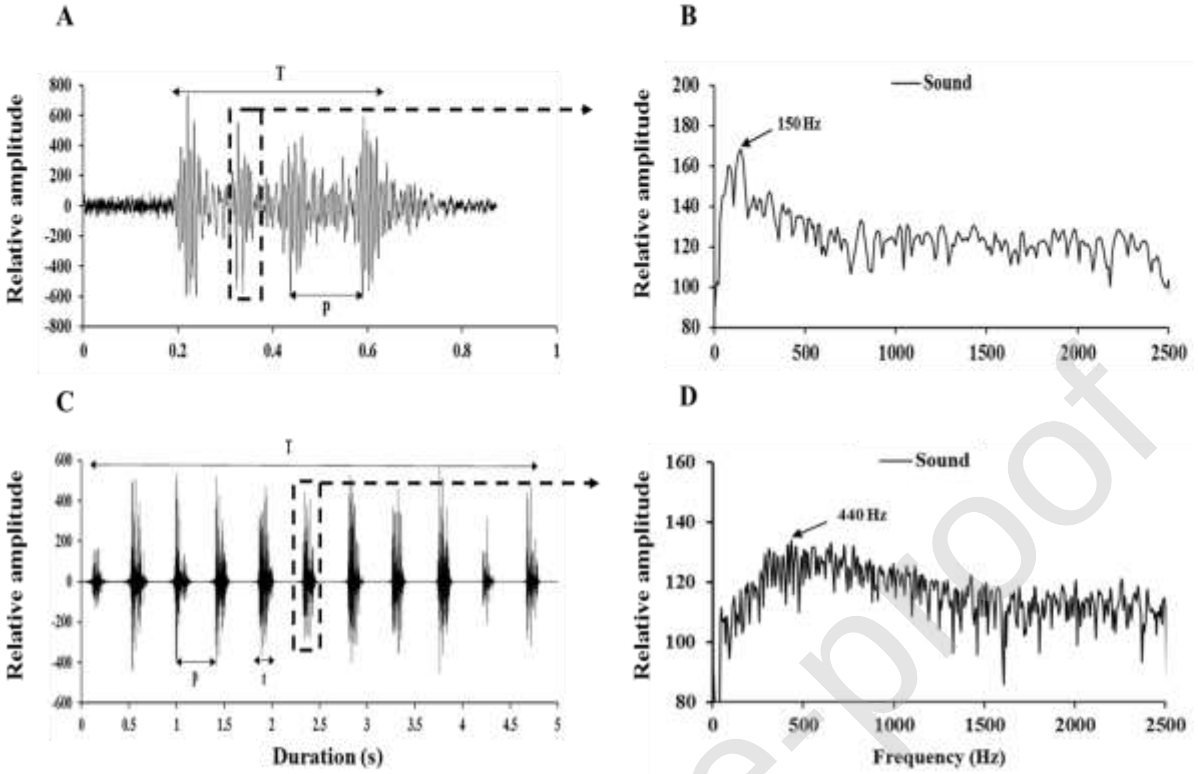
A
B
C
D
T
P
t
Relative amplitude
Duration (s)
Frequency (Hz)
Sound
150 Hz
440 Hz
800
600
400
200
0
-200
-400
-600
-800
0.2
0.4
0.6
0.8
1
0.5
1.5
2
2.5
3
3.5
4
4.5
5
200
180
160
140
120
100
80
500
1000
1500
2000
2500

Fig. 2.

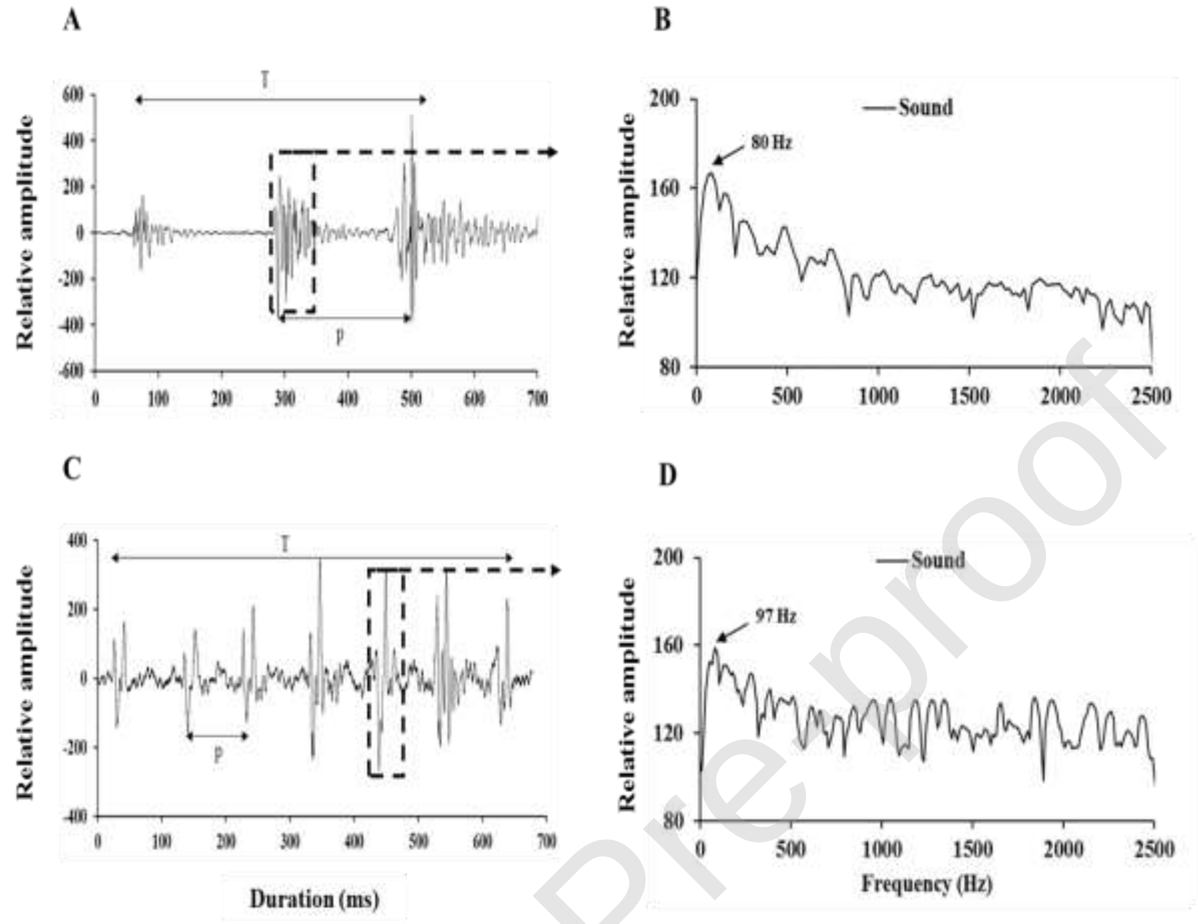
A
B
C
D
Relative amplitude
T
p
Duration (ms)
Sound
80 Hz
97 Hz
Frequency (Hz)
600
400
200
0
-200
-400
-600
100
300
500
700
160
120
80
1000
1500
2000
2500

Fig. 3.

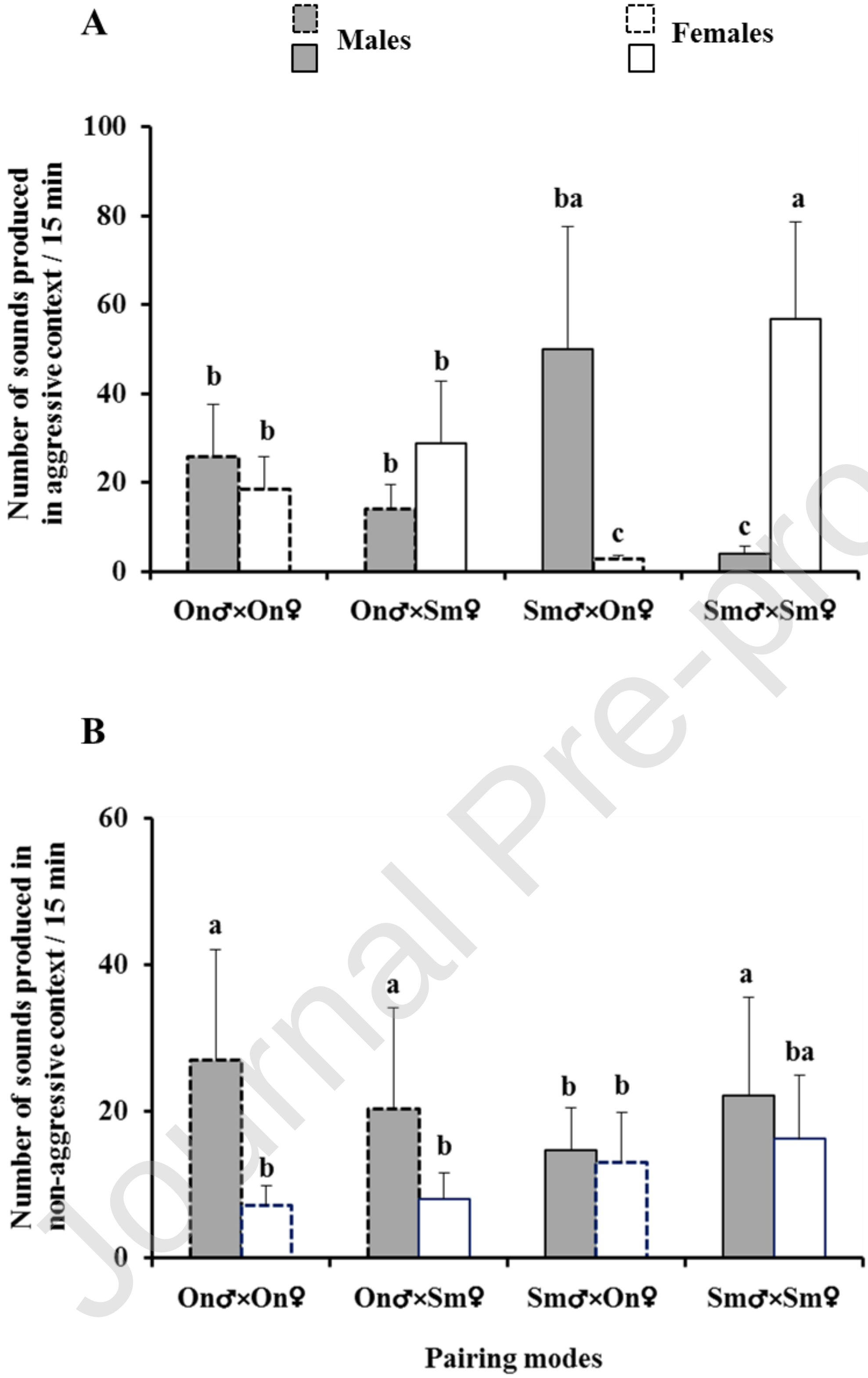
A
Males
Females
Number of sounds produced in aggressive context / 15 min
100
80
60
40
20
0
b
b
b
b
ba
c
c
a
On♂×On♀
On♂×Sm♀
Sm♂×On♀
Sm♂×Sm♀
B
Number of sounds produced in non-aggressive context / 15 min
60
40
20
0
a
b
a
b
b
b
a
ba
On♂×On♀
On♂×Sm♀
Sm♂×On♀
Sm♂×Sm♀
Pairing modes

Fig. 4.

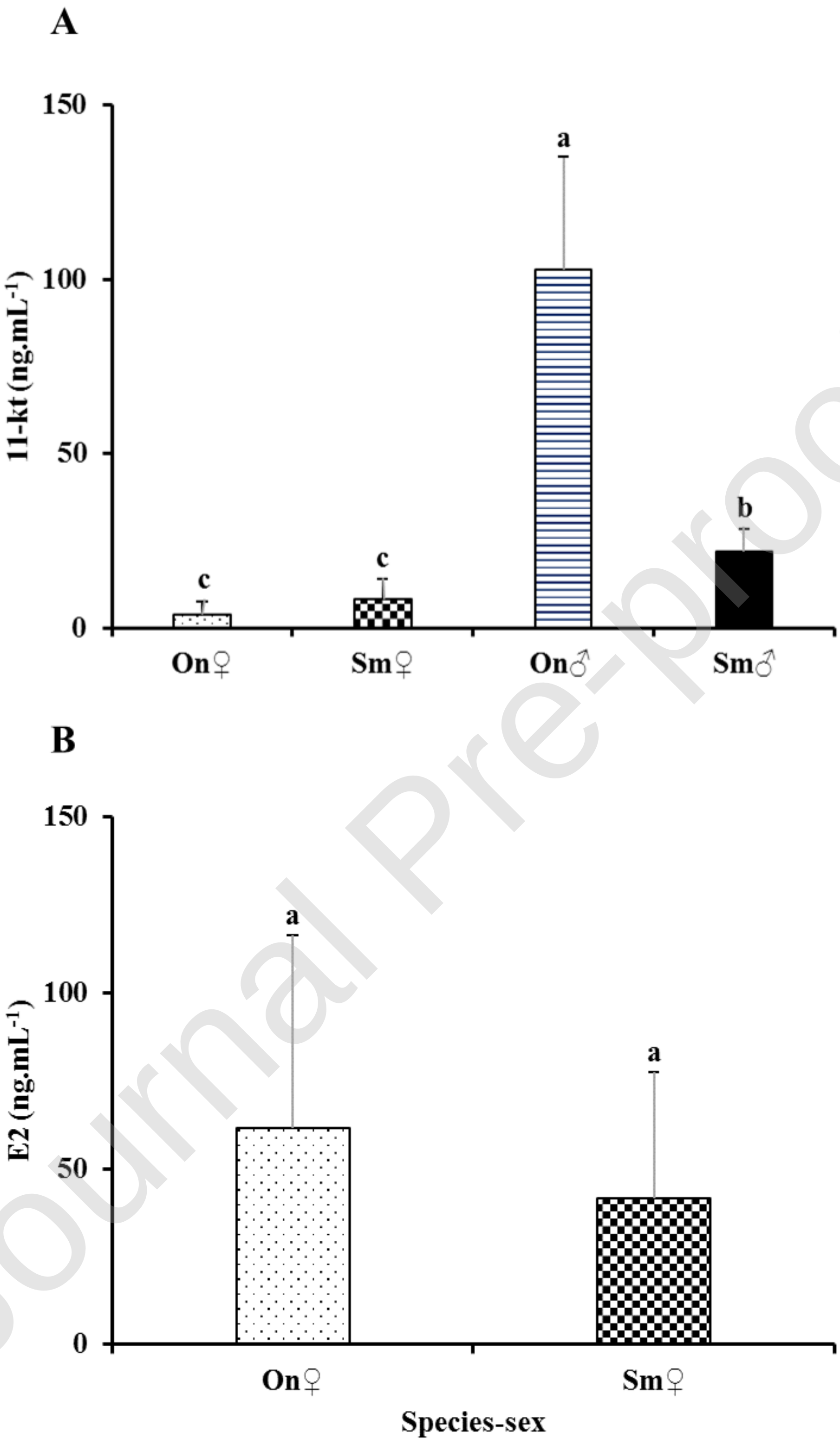
A
150
100
50
0
11-kt (ng.mL^{-1})
a
b
c
c
On♀
Sm♀
On♂
Sm♂
B
150
100
50
0
E2 (ng.mL^{-1})
a
a
On♀
Sm♀
Species-sex

TABLE 1: Sounds produced with associated aggressive and non-aggressive behaviours by *Oreochromis niloticus* and *Sarotherodon melanotheron* according to the pairing modes.

Pairing modes		**Sounds with aggressive behaviours**	**Sounds with non-aggressive behaviours**
O. niloticus ♂ × *O. niloticus* ♀	♂	drum (chasing, lateral attack)	drum (courtship, circling, quivering)
	♀	grunt train (chasing, biting)	grunt train (nest building)
O. niloticus ♂ × *S. melanotheron* ♀	♂	drum (chasing, lateral attack) grunt train (chasing, biting)	drum (courtship, circling, quivering) grunt train (nest building)
	♀	drum (fleeing, avoidance, lateral attack)	rolling (courtship)
S. melanotheron ♂ × *O. niloticus* ♀	♂	drum (fleeing, avoidance, lateral attack)	rolling (courtship)
	♀	drum (chasing, lateral attack) grunt train (chasing, biting)	drum (courtship, circling, quivering) grunt train (nest building)
S. melanotheron ♂ × *S. melanotheron* ♀	♂ ♀	drum (fleeing, avoidance, lateral attack)	rolling (courtship)

TABLE 2. Characteristics of the drum sounds emitted by *Oreochromis niloticus* and *Sarotherodon melanotheron* during aggressive behaviours during intraspecific and interspecific pairing. The letters a, b, and c in the columns indicate significant differences according to Tukey's post-hoc test (*P* < 0.05).

Pairing modes	**Sound characteristics**				
	Number of	**Sound Duration**	**Number of**	**Period of**	**Fundamental frequency**

O. niloticus ♂ × *O. niloticus* ♀	♂	126	422.6 ±	3.1 ± 0.7^{b}	134.7 ±	124.6 ± 42.4^{a}
	♀	137	412.8 ±	2.5 ± 0.8^{c}	154.6 ±	107.2 ± 31.3^{a}
O. niloticus ♂ × *S. melanotheron* ♀	♂	138	510.2 ± 117.1^{a}	3.4 ± 0.8^{a}	133.4 ± 42.4^{b}	95.8 ± 29.6^{a}
	♀	168	543.9 ±	2.2 ± 0.5^{c}	196.9 ±	77.9 ± 6.9^{b}
S. melanotheron ♂ × *O. niloticus* ♀	♂	157	430.8 ± 127.3^{b}	2.3 ± 0.5^{c}	171.4 ± 61.7^{a}	91.0 ± 28.3^{b}
	♀	119	409.8 ±	2.6 ± 0.8^{c}	145.2 ±	114.7 ± 53.5^{a}
S. melanotheron ♂ × *S. melanotheron* ♀	♂	122	456.3 ± 174.3^{b}	2.4 ± 0.6^{c}	171.9 ± 85.2^{a}	93.7 ±35.2^{ab}
	♀	158	444.6 ± 93.4^{b}	2.1 ± 0.3^{c}	175.6 ± 69.6^{a}	96.9 ± 38.7^{ab}

TABLE 3. Characteristics of the drum sounds emitted by *Oreochromis niloticus* during non-aggressive behaviours during intraspecific and interspecific pairing with *Sarotherodon melanotheron*. The letter a in the columns indicate no significant differences according to Tukey's post-hoc test (*P* > 0.05).

Pairing modes		**Sound characteristics**				
		Number of sounds	**Sound Duration**	**Number of pulses**	**Period of pulses**	**Fundamental frequency**
O. niloticus ♂ × *O. niloticus* ♀	♂	159	314.8 ± 126.1^{a}	2.6 ± 0.9^{a}	142.7 ± 46.5^{a}	126.4 ± 42.4^{a}
	♀	131	272.9 ± 88.5^{a}	2.3 ± 0.6^{a}	113.2 ±	117.7 ± 43.7^{a}
O. niloticus ♂ × *S. melanotheron* ♀	♂	148	344.9 ± 193.4^{a}	1.9 ± 1.3^{a}	172.7 ± 95.0^{a}	104.5 ± 48.3^{a}
S. melanotheron ♂ × *O. niloticus* ♀	♀	126	268.9 ± 134.8^{a}	2.5 ± 0.7^{a}	143.2 ± 58.6^{a}	102.9 ± 47.1^{a}

TABLE 4. Characteristics of the grunt trains emitted by *Oreochromis niloticus* during aggressive behaviours during intraspecific and interspecific pairing with *Sarotherodon melanotheron*. The letters a and b in the columns indicate significant differences according to Tukey’s post-hoc test ($P < 0.05$).

Pairing modes		**Sound characteristics**					
		Number of sounds	**Sound Duration**	**Number of grunts**	**Grunt duration**	**Period of grunts**	**Fundamental frequency**
O. niloticus ♂ × *O. niloticus* ♀	♂	41	4612.7 ± 2108.2^{a}	8.1 ± 4.5^{a}	153.7 ± 30.6^{a}	581.1 ± 87.4^{a}	388.4 ± 158.1^{b}
	♀	39	2895.2 ±	5.7 ± 3.1^{b}	170.7 ±	585.4 ±	459.3 ± 275.1^{b}
O. niloticus ♂ × *S. melanotheron* ♀	♂	50	4006.7 ± 1906.6^{a}	9.0 ± 4.2^{a}	153.1 ± 26.9^{a}	492.7 ± 92.7^{a}	571.3 ± 126.1^{a}
S. melanotheron ♂ × *O. niloticus* ♀	♀	37	4418.5 ± 2253.3^{a}	7.6 ± 5.3^{a}	202.8 ± 20.4^{a}	625.2 ± 71.5^{a}	418.6 ± 65.5^{b}

TABLE 5. Characteristics of the grunt trains emitted by *Oreochromis niloticus* during non-aggressive behaviours during intraspecific and interspecific pairing with *Sarotherodon melanotheron*. The letters a and b in the columns indicate significant differences according to Tukey's post-hoc test ($P < 0.05$).

Pairing modes		**Sound characteristics**					
		Number of sounds	**Sound Duration**	**Number of**	**Grunt duration**	**Period of grunts**	**Fundamental frequency**
O. niloticus ♂ × *O. niloticus* ♀	♂	44	5434.0 ± 3125.2^{a}	8.9 ± 5.1^{a}	164.3 ± 43.2^{a}	612.5 ± 73.1^{a}	347.2 ± 120.3^{b}
	♀	37	5686.5 ±	12.1 ±	158.7 ±	503.6 ±	378.2 ± 97.5^{b}
O. niloticus ♂ × *S. melanotheron* ♀	♂	31	4184.4 ± 2849.1^{a}	7.4 ± 3.6^{a}	170.1 ± 33.2^{a}	678.5 ± 249.7^{a}	506.5 ± 112.0^{a}
S. melanotheron ♂ × *O. niloticus* ♀	♀	34	4608.6 ± 2181.8^{a}	8.7 ± 4.6^{a}	186.9 ± 38.1^{a}	588.3 ± 110.2^{a}	440.8 ± 120.7^{a}

TABLE 6. Characteristics of the rolling sounds emitted by *Sarotherodon melanotheron* during non-aggressive behaviours during intraspecific and interspecific pairing with *Oreochromis niloticus*. The letters a and b in the columns indicate significant differences according to Tukey's post-hoc test ($P < 0.05$).

Pairing modes		**Sound characteristics**				
		Number of sounds	**Sound Duration (ms)**	**Number of pulses**	**Period of pulses (ms)**	**Fundamental frequency (Hz)**
S. melanotheron ♂ × *S. melanotheron* ♀	♂	167	535.8 ± 288.7^{a}	3.8 ± 2.6^{a}	196.3 ± 103.0^{a}	107.6 ± 69.2^{a}
	♀	152	682.1 ± 283.8^{a}	3.7 ± 2.2^{a}	181.6 ± 84.3^{a}	87.7 ± 28.5^{a}
O. niloticus ♂ × *S. melanotheron* ♀	♀	108	325.1 ± 179.7^{b}	2.6 ± 1.2^{b}	206.3 ± 116.4^{a}	92.4 ± 45.1^{a}
S. melanotheron ♂ × *O. niloticus* ♀	♂	117	302.5 ± 167.9^{b}	2.5 ± 0.7^{b}	200.3 ± 55.6^{a}	92.1 ± 34.8^{a}