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

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23 **ABSTRACT**

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

43 **Keywords:** acoustic signals; behaviour; hybridization; *Oreochromis niloticus*; *Sarotherodon*  
44 *melanotheron*

45

## 46 **1. Introduction**

47 Numerous studies of fish behaviour have shown multimodal communication channels in mate  
48 choice, such that the choice of partner is often influenced by different kinds of signals  
49 (Candolin, 2003; Verzijden et al., 2010). In fish, the choice of sexual partner may be based on  
50 *e.g.* visual cues (Castro et al., 2009), chemical stimuli (Plenderleith et al., 2005), electric  
51 signal (Machnik and Kramer, 2008) or species-specific calls (Amorim et al., 2004; Parmentier  
52 et al., 2006). In addition to courtship, acoustic signals may also be associated with territory  
53 defence or feeding or antipredator behaviour (Amorim et al., 2003, 2004; Amorim and Neves,  
54 2007, 2008; Patek et al., 2009; Maruska et al., 2012; Longrie et al., 2013; Pedroso et al.,  
55 2013). In reproduction context, the analysis of acoustic emissions can highlight the relevant  
56 information transmitted between the congeners, helping to understand the features used by  
57 fish in choosing sexual partners (Lobel, 1998).

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

64 Within teleosts, different cichlid species produce sounds either in aggressive contexts  
65 (Amorim and Almada, 2005; Amorim et al., 2003, 2004, 2008) or during courtship behaviour  
66 (Amorim et al., 2003, 2004). In Nile tilapia *Oreochromis niloticus* (Linnaeus 1758) sounds  
67 are produced during nest defence by territorial males and egg protection by females (Longrie  
68 et al., 2008, 2013). *Oreochromis mossambicus* (Peters 1852) produces sounds associated with  
69 courtship behaviour and reproduction, suggesting that sound plays a role in the

70 synchronization of gamete emission (Amorim et al., 2003). Both species are included in the  
71 paraphyletic group of tilapias, which includes the Oreochromini and the Coptodonini tribes  
72 (Dunz and Schliewen, 2013). Various reproductive behaviours have been described in these  
73 groups (Trewavas, 1983): substrate brooder with biparental guard for *Coptodon sp.*, paternal  
74 or biparental mouthbrooder for *Sarotherodon sp.*, and maternal mouthbrooder for  
75 *Oreochromis sp.* Tilapia species of these last two groups belong to the Oreochromini tribe  
76 (Dunz and Schliewen, 2013).

77 Although reproductive behaviours can show differences between tilapia genera, hybridization  
78 between some species is possible (Toguyeni et al., 2009), implying that the reproductive  
79 barrier is not impermeable (Ptacek, 2000). This is true, for example, between black-chinned  
80 tilapia *Sarotherodon melanotheron* Rüppell 1852, a species with paternal or biparental  
81 mouthbrooding, and *O. niloticus*, a strictly maternal mouthbrooder (Trewavas, 1983) although  
82 the two species are phylogenetically close (Dunz and Schliewen, 2013) and separated some  
83 2.3 millions years ago (Nagl et al. 2001).

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

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

## 94 **2. Material and methods**

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

### 98 ***2.1. Experimental fish***

99 Acoustic tests and behavioural observations were carried out on mature XY males (mass  
100  $186.5 \pm 73.7$  g/total length  $21.0 \pm 2.8$  cm,  $n = 20$ ) and XX females ( $166.4 \pm 73.7$  g/ $20.4 \pm 1.9$   
101 cm,  $n = 20$ ) of *O. niloticus* and mature males ( $171.3 \pm 24.1$  g/ $21.1 \pm 1.1$  cm,  $n = 20$ ) and  
102 females ( $171.8 \pm 24.8$  g/ $21.2 \pm 1.0$  cm,  $n = 20$ ) of *S. melanotheron*. Fish of both species were  
103 raised at the station of the International Centre for Agronomic Research for Development  
104 (CIRAD) of Montpellier (France) and were maintained in four 400 l polycarbonate tanks,  
105 under a 12/12 hours light/dark photoperiod, after their transfer to the station of the French  
106 Institute for the Research and Exploitation of the Sea (Ifremer) of L'Houmeau (France). They  
107 were given a commercial diet for tilapia (EFICO YM 868, 3 mm, BIOMAR®, France) twice  
108 a day during two months before the experiment started. The water temperature was  
109 maintained at 27–28 °C. The dissolved oxygen concentration was  $> 4$  mg l<sup>-1</sup>, the pH between  
110 7 and 8, and the NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, and NO<sub>3</sub><sup>-</sup> concentrations  $0.3 \pm 0.2$ ,  $0.6 \pm 0.2$ , and  $7.0 \pm 5.8$  mg l<sup>-1</sup>,  
111 respectively.

### 112 ***2.2. Experimental protocol***

113 The experiments were conducted in a soundproof room in the basement of Ifremer station.  
114 Four 50 l aquariums (60 × 25 × 35 cm high), each equipped with an individual biological  
115 filter, were placed on a 4-cm thick polystyrene plate on a table. This device helped to reduce  
116 the background noise from vibrations transmitted from the ground to the aquariums. Fish

117 were weighed and measured after benzocaine anaesthesia (0.1 ml l<sup>-1</sup>), dorsally marked with  
118 beads of different colours (Bégout et al., 2012), and then transferred to the aquariums for 24 h  
119 of recovery. In the experimental aquariums, four types of pairing were performed: 1 = *O.*  
120 *niloticus* ♂ × *O. niloticus* ♀, 2 = *O. niloticus* ♂ × *S. melanotheron* ♀, 3 = *S. melanotheron* ♂  
121 × *O. niloticus* ♀, 4 = *S. melanotheron* ♂ × *S. melanotheron* ♀. The aquarium was divided into  
122 two compartments of the same size with a removable barrier, preventing physical and visual  
123 contact between the fish before the recordings. Ten couples were tested in each pairing mode  
124 and no individual was tested twice either in intraspecific or interspecific pairing.

### 125 **2.3. Sound recording with associated behaviours**

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

### 137 **2.4. Plasma steroid analysis**

138 At the end of each test, a blood sample (0.4–0.5 ml) was taken from each individual after  
139 anaesthesia in a bath of benzocaine at 0.1 ml l<sup>-1</sup> from a 10 % stock solution of ethyl-p-

140 aminobenzoate (benzocaine, E1501, Sigma, St Louis, MO, USA, prepared by dissolving 100  
141 g benzocaine in 1 l 100 % ethanol) and centrifuged (13,000 rpm, 6 min, 4 °C). Plasma was  
142 collected and stored at -20 °C in Eppendorf tubes for later determination of the 11-  
143 ketotestosterone (11-kt) concentration in males and females and that of estradiol (E2) in  
144 females. Plasma was diluted 1/2000 for males (n = 10 for each species) and 1/500 for females  
145 (n = 7 for *O. niloticus*, n = 12 for *S. melanotheron*) for the determination of 11-kt and 1/200  
146 for the E2 assay. Enzyme immunoassays were performed using a BioTek Synergy HT  
147 spectrofluorometer and CAYMAN kit (Cayman Chemical Ann Arbor, MI, USA) for 11-kt  
148 and an IBL kit (IBL Hamburg, Germany) for E2.

## 149 **2.5. Analysis of sounds and behaviours**

150 The software Audacity 2.0.4 was used to extract acoustic signals from the video. Sounds were  
151 digitized at 44.1 kHz (16-bit resolution) and then analysed using Avisoft software -SASLAB  
152 Pro 4.33. The characteristics of the sounds (duration (T), number of pulses, period of pulses or  
153 grunts (p), grunt duration (t), and number of grunts: see Figure 1) were measured on an  
154 oscillogram. Fundamental frequency of a pulse was obtained from logarithmic power spectra  
155 of a pulse (band pass lower cut-off frequency = 0.05 and upper cut-off frequency = 2.5 kHz,  
156 FFT size = 1024 points, time overlap = 95 %, and Hanning window, see Fig. 1B or 1D). The  
157 average of the fundamental frequencies of the pulses gave us the fundamental frequency of  
158 the sound. The behaviours associated with different sounds were annotated based on the  
159 tilapia ethogram (Longrie et al., 2013) by video observation following the fish individually.  
160 By reviewing several times the recorded videos to listen to the sounds and associate sounds  
161 and behaviour, the identity of the producer of each sound was determined. Behaviours were  
162 grouped into two categories: aggressive (biting, chasing, fleeing, avoidance, tail stroke, lateral



163 attack) and non-aggressive (courtship, quiver, circle, nest building, and no particular  
164 behaviour).

## 165 ***2.6. Statistical analysis***

166 The homogeneity of the variance of the dataset related to the mass and total length of the fish,  
167 sound characteristic variables, and E2 and 11-kt concentrations was verified using the Levene  
168 test, as well as the normality of the dataset using the Shapiro-Wilk test. Variables for data  
169 complying with these rules were compared between the species, according to the four modes  
170 of pairing, by two-factor ANOVA (pairing mode and sex as fixed factors) followed by a *post*  
171 *hoc* Tukey test. Non-parametric Kruskal Wallis and Mann-Whitney multiple comparison tests  
172 were used when the data did not fulfil the normality requirements. A repeated-measure  
173 ANOVA was used to compare the number of sounds emitted by each fish in aggressive and  
174 non-aggressive contexts for the observations between 10.00 hours and 14.00 hours. The  
175 Spearman rank correlation test ( $r_s$ ) was performed to evaluate the relationship between E2 and  
176 11-kt concentrations and the number of sounds emitted. All statistical tests were performed  
177 using Statistica 10.0 software (Statsoft, Tulsa, OK, USA) and the level of significance  
178 retained for the tests was 0.05.

## 179 **3. Results**

### 180 ***3.1. Description of sounds produced by *O. niloticus* during intraspecific pairing***

181 Male and female *O. niloticus* emitted sounds during both aggressive and non-aggressive  
182 behaviours in intraspecific pairings. Two types of sounds were recorded (Table 1). The first  
183 type corresponded to drum sounds (Fig. 1A, B) which were associated with both aggressive  
184 (after chasing and lateral attack) and non-aggressive (courtship, circling, and quivering)  
185 behaviours. The second type corresponded to a train of grunts (Fig. 1C, D) which were also

186 emitted during both aggressive (chasing and before and after biting) and non-aggressive  
187 behaviours that corresponded to nest building, although there was no sand at the bottom of the  
188 tank. Overall, the same types of sounds were produced by *O. niloticus*, both during aggressive  
189 and non-aggressive behavioural contexts. Further, we did not observe different behaviours  
190 from the ones already described in Longrie et al. (2013) and Akian et al. (2017) even in the  
191 particular context of interspecific pairings.

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

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

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

### 209 **3.2. Description of sounds produced by *S. melanotheron* during intraspecific pairing**

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

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

### 221 **3.3. Description of sounds produced by *O. niloticus* during interspecific pairing with *S.*** 222 ***melanotheron***

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

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

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

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

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

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

252 In *O. niloticus* males and females, the grunt trains associated with non-aggressive behaviours  
253 emitted during interspecific pairings with *S. melanotheron* were not significantly different in  
254 any of the characteristics from the sounds emitted in intraspecific pairings (Tukey post-hoc

255 test,  $P > 0.05$ ), except for the fundamental frequency of the grunts, which was significantly  
256 higher (Tukey post-hoc test,  $P < 0.05$ ) during the interspecific pairings (Table 5).

257 **3.4. Description of sounds produced by *S. melanotheron* during interspecific pairing with**  
258 ***O. niloticus***

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

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

265 The duration and period of drum sounds associated with aggressive behaviour emitted by *S.*  
266 *melanotheron* females during interspecific pairings with *O. niloticus* males (Table 2) were  
267 significantly longer during interspecific than intraspecific pairings (Tukey post-hoc test,  $P <$   
268  $0.05$ ). However, there was no significant difference in the number of pulses or the frequency  
269 of the sound between the interspecific and intraspecific pairings (Tukey post-hoc test,  $P >$   
270  $0.05$ ).

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

278 were significantly less during interspecific than intraspecific pairings (Tukey post-hoc test,  $P$   
279  $< 0.05$ ). There were no significant differences in the period or fundamental frequency of the  
280 rolling sounds between the intraspecific and interspecific pairings (Tukey post-hoc test,  $P <$   
281  $0.05$ , Table 6).

### 282 **3.5. Number of sounds emitted according to the context**

283 We next considered all types of sounds produced by each fish for the analysis (for example,  
284 drum sound with grunt trains for *O. niloticus* when these two types of sounds were produced  
285 in an aggressive context and drum or rolling sounds for *S. melanotheron* when they were  
286 produced in an aggressive or non-aggressive context). There were no significant differences  
287 (Tukey post-hoc test,  $P > 0.05$  in all) in the number of sounds emitted by any tested fish  
288 during aggressive or non-aggressive behaviours whatever the time at which the recording was  
289 performed. We therefore analysed the differences in relation to the pairing mode (Figure 3).  
290 Indeed, the number of sounds emitted by *S. melanotheron* males in aggressive situations was  
291 significantly higher (Mann-Whitney U-Test,  $U = 40.00$ ,  $P < 0.01$ ) during interspecific pairing,  
292 whereas the value was significantly higher for females (Mann-Whitney U-Test,  $U = 47.00$ ,  $P$   
293  $< 0.01$ ) during intraspecific pairing. The number of sounds emitted by *O. niloticus* females  
294 during aggressive behaviours was significantly higher during intraspecific than interspecific  
295 pairing (Fig. 3A, Mann-Whitney U-Test,  $U = 46.50$ ,  $P < 0.05$ ).

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

### 301 **3.6. Sexual steroid concentrations and correlation with the number of sounds produced**

302 Plasma 11-kt concentrations (Fig. 4A) in both species were significantly higher (Kruskal-  
303 Wallis test:  $H_{(3, 34)} = 18.40$  ;  $P < 0.01$ ) in males (*O. niloticus* =  $102.7 \pm 42.5$  ng.Ml<sup>-1</sup>, *S.*  
304 *melanotheron* =  $21.8 \pm 6.5$  ng.Ml<sup>-1</sup>) than females (*O. niloticus* =  $3.9 \pm 2.7$  ng.Ml<sup>-1</sup>, *S.*  
305 *melanotheron* =  $8.1 \pm 5.9$  ng.Ml<sup>-1</sup>). *O. niloticus* males had significantly higher 11-kt plasma  
306 concentrations than *S. melanotheron* males (Mann-Whitney *U*-test,  $U = 6.00$ ,  $P < 0.01$ ). There  
307 were no significant differences in the concentrations of 11-kt between the females of either  
308 species (Mann-Whitney *U*-test,  $U = 24.00$ ,  $P > 0.05$ , Fig. 4A) or E2 (*O. niloticus* ♀ =  $100.6 \pm$   
309  $58.1$  ng.Ml<sup>-1</sup>, *S. melanotheron* ♀ =  $112.0 \pm 56.8$  ng.Ml<sup>-1</sup>, Mann-Whitney *U*-test,  $U = 35.00$ ,  $P$   
310  $> 0.05$ , Fig. 4B).

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

### 316 **4. Discussion**

317 We show that *O. niloticus* males and females produce two types of sounds (drum sounds and  
318 grunts) during intra- and interspecific pairing, as well as during aggressive and non-aggressive  
319 behaviours. During intraspecific pairings, *O. niloticus* males and females emitted drum  
320 sounds after chasing or lateral attacks and during courtship behaviours when circling around  
321 the congener and quivering. Grunt trains were also produced during chases, after biting, and  
322 after nest building when turning around to attract the attention of the opposite sex. During  
323 interspecific pairing with *S. melanotheron*, the fish produced drum sounds and grunt trains in

324 similar behavioural contexts. Thus, *O. niloticus* produces the same sounds in aggressive and  
325 non-aggressive contexts as those observed in other cichlids by Amorim (2006). Also, similar  
326 sounds are produced in both agonistic and courtship contexts by *Hemichromis bimaculatus*  
327 and *Herotilapia multispinosa* (Amorim, 2006).

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

346 This study suggests that acoustic signals can induce aggressive or non-aggressive behavioural  
347 responses in both species and that *O. niloticus* and *S. melanotheron* are able to distinguish the



348 nature of the sounds produced by congeners, as observed by Amorim et al. (2004) and  
349 Bertucci et al. (2010) in other cichlids. It also shows that these two tilapias exhibit sound-  
350 based communication for intraspecific recognition, similar to the intraspecific recognition  
351 observed in *Xenopus laevis* (Vignal and Kelley, 2007). Sound-based information may  
352 therefore play an important role in the choice of partner in *O. niloticus* and *S. melanotheron*,  
353 as shown in other cichlids (Verzijden et al., 2010). The results of this study are thus consistent  
354 with those of previous studies showing differences in the characteristics of acoustic signals  
355 involved in partner choice in cichlids (Amorim et al., 2008).

356

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

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

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

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

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

418 The 11-kt concentrations in male plasma were higher than those measured in the plasma of  
419 females of both species, consistent with observations in other fish species (Oliveira, 2004;  
420 Desjardins et al., 2008; Taves et al., 2009). Differences observed between individual values  
421 may be related to stress due to confinement of the fish in tanks prior to recordings and

422 samplings. Indeed, confinement would increase serum cortisol levels, which in turn inhibit  
423 11-kt production (Consten et al., 2001; Vasconcelos et al., 2012). However, we did not  
424 measure the level of cortisol and therefore cannot confirm this hypothesis. According to  
425 Kishida and Specker (2000), male plasma androgen concentrations in *S. melanotheron* are  
426 low (less than 5 ng ml<sup>-1</sup>) during the first week of egg mouth brooding and relatively high  
427 (between 13 - 38 ng ml<sup>-1</sup>) during the second week of mouth brooding of the hatched embryos.  
428 Although we did not observe mouth brooding by the *S. melanotheron* males in our  
429 experiments, we measured 11-kt levels similar to those cited above (6 - 57 ng ml<sup>-1</sup>) in both *S.*  
430 *melanotheron* and *O. niloticus* males. The presence of dominant and subordinate males in the  
431 same breeding tank could also explain the low and high concentrations of 11-kt measured, as  
432 observed with *Astatotilapia burtoni* (Parikh et al., 2006).

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

442 There were no differences in the E2 concentrations measured in the females of either species.  
443 Observed intraspecific individual differences may have been related to the females not all  
444 having the same reproductive status. The oestradiol values measured in *O. niloticus* females  
445 were higher than those found by Biswas et al. (2005) and Onumah et al. (2010) (mean values

446 between 3 and 22 ng.ml<sup>-1</sup> *versus* 100.59 ± 58.10 ng ml<sup>-1</sup> in this study) in females of larger size  
447 exposed to the same photoperiod. The mean values of E2 we measured in *S. melanotheron*  
448 females were also higher than those obtained by Specker and Kishida (2000) for small  
449 females (49 g) that were incubating or not incubating eggs after spawning (3 - 8 ng ml<sup>-1</sup>  
450 *versus* 112.00 ± 56.82 ng ml<sup>-1</sup> in this study). There was no link between E2 levels and the  
451 number of sounds emitted in aggressive and non-aggressive situations by females of either  
452 species. Hence, E2 levels may not be related to the production of acoustic signals. It has also  
453 been shown that oestrogens are not involved in the expression of sexual behaviours in  
454 oviparous teleosts because E2 concentrations decrease during spawning periods (Kobayashi et  
455 al., 1987; Moyle and Cech, 2000; Munakata et al., 2001; Munakata and Kobayashi, 2010).

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

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471 None.

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**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
<i>O. niloticus</i> ♂ × <i>O. niloticus</i> ♀	♂	drum (chasing, lateral attack)	drum (courtship, circling, quivering)
	♀	grunt train (chasing, biting)	grunt train (nest building)
<i>O. niloticus</i> ♂ × <i>S. melanotheron</i> ♀	♂	drum (chasing, lateral attack)	drum (courtship, circling, quivering)
	♀	grunt train (chasing, biting)	grunt train (nest building)
<i>S. melanotheron</i> ♂ × <i>O. niloticus</i> ♀	♂	drum (fleeing, avoidance, lateral attack)	rolling (courtship)
	♀	drum (chasing, lateral attack)	drum (courtship, circling, quivering)
<i>S. melanotheron</i> ♂ × <i>S. melanotheron</i> ♀	♂	grunt train (chasing, biting)	grunt train (nest building)
	♀	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 Sounds analyzed	Sound Duration (ms)	Number of pulses	Period of pulses (ms)	Fundamental frequency (Hz)
<i>O. niloticus</i> ♂ × <i>O. niloticus</i> ♀	♂	126	422.6 ± 104.9 <sup>b</sup>	3.1 ± 0.7 <sup>b</sup>	134.7 ± 32.1 <sup>b</sup>	124.6 ± 42.4 <sup>a</sup>
	♀	137	412.8 ± 118.4 <sup>b</sup>	2.5 ± 0.8 <sup>c</sup>	154.6 ± 64.4 <sup>a</sup>	107.2 ± 31.3 <sup>a</sup>
<i>O. niloticus</i> ♂ × <i>S. melanotheron</i> ♀	♂	138	510.2 ± 117.1 <sup>a</sup>	3.4 ± 0.8 <sup>a</sup>	133.4 ± 42.4 <sup>b</sup>	95.8 ± 29.6 <sup>a</sup>
	♀	168	543.9 ± 120.7 <sup>a</sup>	2.2 ± 0.5 <sup>c</sup>	196.9 ± 32.3 <sup>a</sup>	77.9 ± 6.9 <sup>b</sup>
<i>S. melanotheron</i> ♂ × <i>O. niloticus</i> ♀	♂	157	430.8 ± 127.3 <sup>b</sup>	2.3 ± 0.5 <sup>c</sup>	171.4 ± 61.7 <sup>a</sup>	91.0 ± 28.3 <sup>b</sup>
	♀	119	409.8 ± 140.9 <sup>b</sup>	2.6 ± 0.8 <sup>c</sup>	145.2 ± 54.9 <sup>ab</sup>	114.7 ± 53.5 <sup>a</sup>
<i>S. melanotheron</i> ♂ × <i>S. melanotheron</i> ♀	♂	122	456.3 ± 174.3 <sup>b</sup>	2.4 ± 0.6 <sup>c</sup>	171.9 ± 85.2 <sup>a</sup>	93.7 ± 35.2 <sup>ab</sup>
	♀	158	444.6 ± 93.4 <sup>b</sup>	2.1 ± 0.3 <sup>c</sup>	175.6 ± 69.6 <sup>a</sup>	96.9 ± 38.7 <sup>ab</sup>

**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 analyzed	Sound Duration (ms)	Number of pulses	Period of pulses (ms)	Fundamental frequency (Hz)	
<i>O. niloticus</i> ♂ × <i>O. niloticus</i> ♀	♂	159	314.8 ± 126.1 <sup>a</sup>	2.6 ± 0.9 <sup>a</sup>	142.7 ± 46.5 <sup>a</sup>	126.4 ± 42.4 <sup>a</sup>
	♀	131	272.9 ± 88.5 <sup>a</sup>	2.3 ± 0.6 <sup>a</sup>	113.2 ± 35.3 <sup>a</sup>	117.7 ± 43.7 <sup>a</sup>
<i>O. niloticus</i> ♂ × <i>S. melanotheron</i> ♀	♂	148	344.9 ± 193.4 <sup>a</sup>	1.9 ± 1.3 <sup>a</sup>	172.7 ± 95.0 <sup>a</sup>	104.5 ± 48.3 <sup>a</sup>
<i>S. melanotheron</i> ♂ × <i>O. niloticus</i> ♀	♀	126	268.9 ± 134.8 <sup>a</sup>	2.5 ± 0.7 <sup>a</sup>	143.2 ± 58.6 <sup>a</sup>	102.9 ± 47.1 <sup>a</sup>

**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 analyzed	Sound Duration (ms)	Number of grunts	Grunt duration (ms)	Period of grunts (ms)	Fundamental frequency (Hz)
<i>O. niloticus</i> ♂ × <i>O. niloticus</i> ♀	♂	41	4612.7 ± 2108.2 <sup>a</sup>	8.1 ± 4.5 <sup>a</sup>	153.7 ± 30.6 <sup>a</sup>	581.1 ± 87.4 <sup>a</sup>	388.4 ± 158.1 <sup>b</sup>
	♀	39	2895.2 ± 1798.1 <sup>b</sup>	5.7 ± 3.1 <sup>b</sup>	170.7 ± 20.3 <sup>a</sup>	585.4 ± 77.0 <sup>a</sup>	459.3 ± 275.1 <sup>b</sup>
<i>O. niloticus</i> ♂ × <i>S. melanotheron</i> ♀	♂	50	4006.7 ± 1906.6 <sup>a</sup>	9.0 ± 4.2 <sup>a</sup>	153.1 ± 26.9 <sup>a</sup>	492.7 ± 92.7 <sup>a</sup>	571.3 ± 126.1 <sup>a</sup>
<i>S. melanotheron</i> ♂ × <i>O. niloticus</i> ♀	♀	37	4418.5 ± 2253.3 <sup>a</sup>	7.6 ± 5.3 <sup>a</sup>	202.8 ± 20.4 <sup>a</sup>	625.2 ± 71.5 <sup>a</sup>	418.6 ± 65.5 <sup>b</sup>



**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 analyzed	Sound Duration (ms)	Number of grunts	Grunt duration (ms)	Period of grunts (ms)	Fundamental frequency (Hz)
<i>O. niloticus</i> ♂ × <i>O. niloticus</i> ♀	♂	44	5434.0 ± 3125.2 <sup>a</sup>	8.9 ± 5.1 <sup>a</sup>	164.3 ± 43.2 <sup>a</sup>	612.5 ± 73.1 <sup>a</sup>	347.2 ± 120.3 <sup>b</sup>
	♀	37	5686.5 ± 2499.4 <sup>a</sup>	12.1 ± 7.3 <sup>a</sup>	158.7 ± 32.6 <sup>a</sup>	503.6 ± 93.5 <sup>a</sup>	378.2 ± 97.5 <sup>b</sup>
<i>O. niloticus</i> ♂ × <i>S. melanotheron</i> ♀	♂	31	4184.4 ± 2849.1 <sup>a</sup>	7.4 ± 3.6 <sup>a</sup>	170.1 ± 33.2 <sup>a</sup>	678.5 ± 249.7 <sup>a</sup>	506.5 ± 112.0 <sup>a</sup>
<i>S. melanotheron</i> ♂ × <i>O. niloticus</i> ♀	♀	34	4608.6 ± 2181.8 <sup>a</sup>	8.7 ± 4.6 <sup>a</sup>	186.9 ± 38.1 <sup>a</sup>	588.3 ± 110.2 <sup>a</sup>	440.8 ± 120.7 <sup>a</sup>

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

## Figure captions

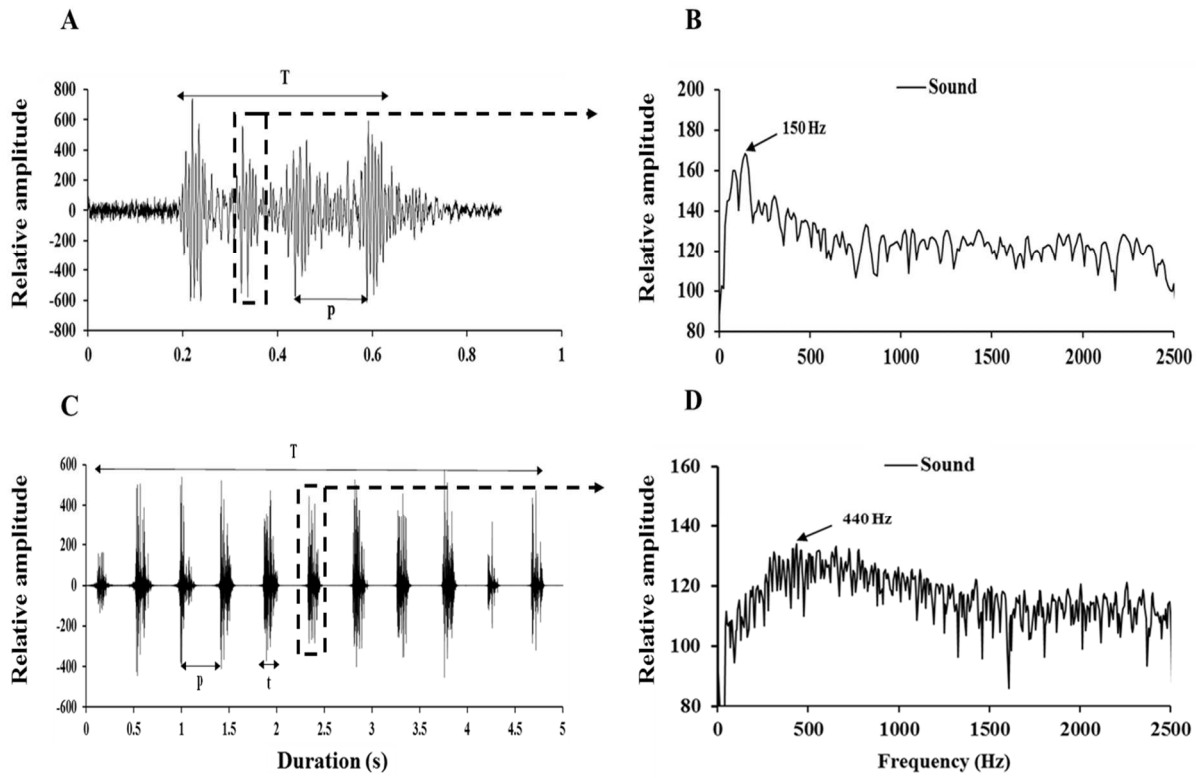
**Fig. 1.** Oscillograms and logarithmic power spectra of the drumming sound with four pulses (A and B) and a train of 11 grunts (C and D) in *Oreochromis niloticus*. (T) represents the duration of the sound, (p) the period of two pulses, and (t) the duration of a grunt. Relative amplitude means that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



**Fig. 2.**

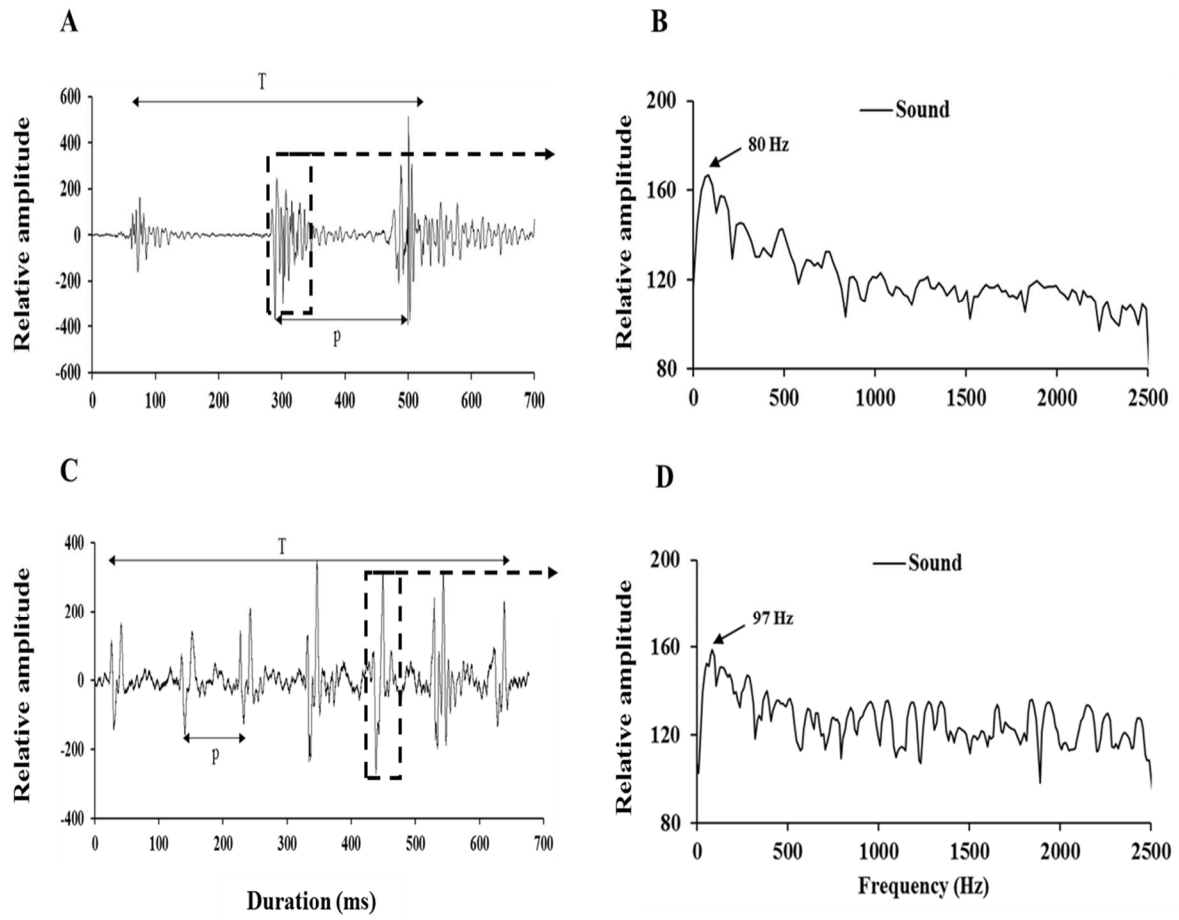


Fig. 3.

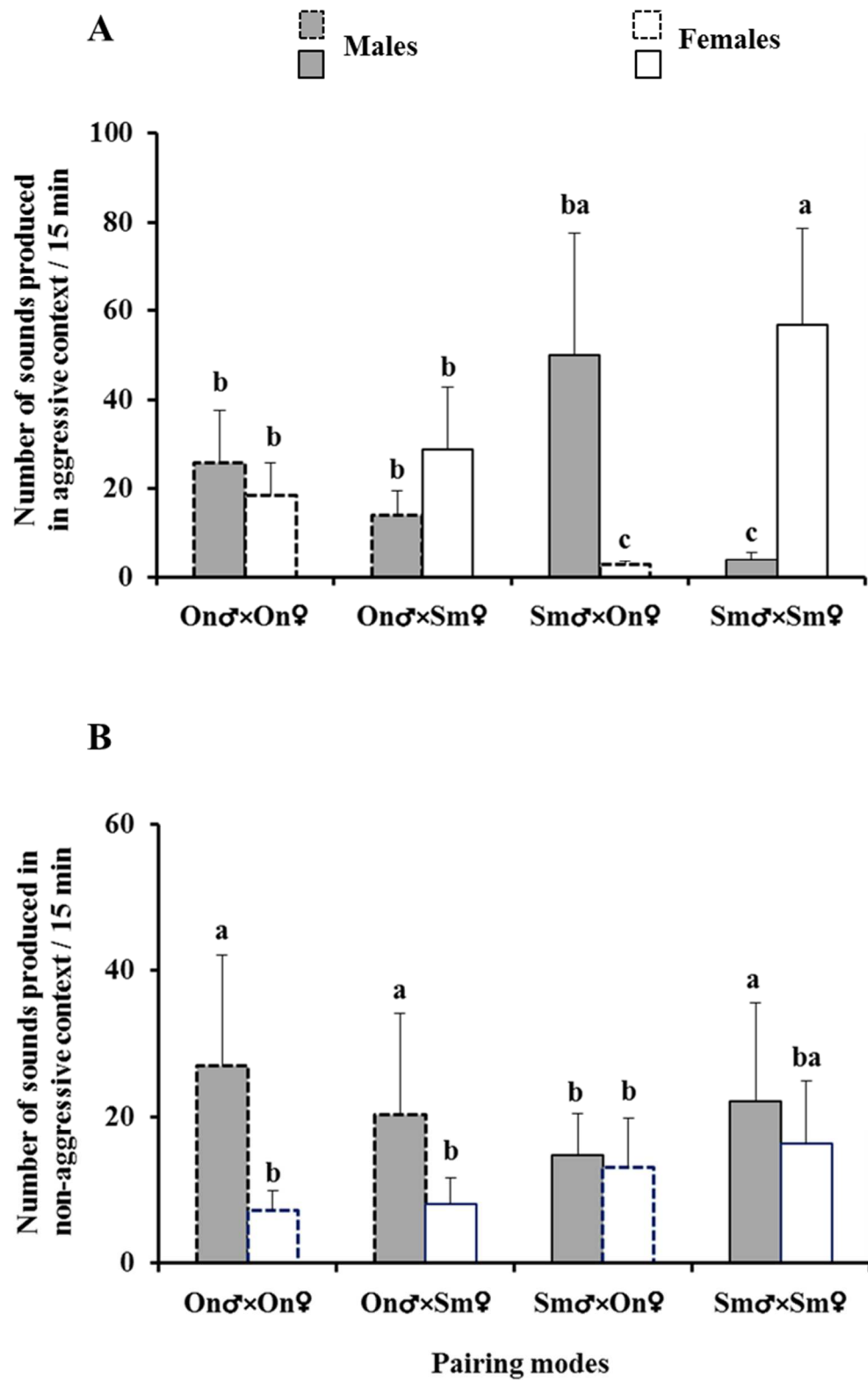


Fig. 4.

