

# 1 **Singing above the chorus: cooperative Princess cichlid fish (*Neolamprologus*** 2 ***pulcher*) has high pitch**

3 Rachel K. Spinks<sup>1,2</sup>, Moritz Muschick<sup>1,3</sup>, Walter Salzburger<sup>1</sup>, Hugo F. Gante<sup>1\*</sup>

4

5 <sup>1</sup> Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland

6 <sup>2</sup> Current address: ARC Centre of Excellence for Coral Reef Studies, James Cook  
7 University, Townsville 4811, Queensland, Australia

8 <sup>3</sup> Current address: Department of Fish Ecology & Evolution, EAWAG Centre for  
9 Ecology, Evolution and Biogeochemistry, 6047 Kastanienbaum, Switzerland

10 \*Correspondence: hugo.gante@unibas.ch

11

## 12 **Abstract**

13 Teleost fishes not only communicate with well-known visual cues, but also olfactory  
14 and acoustic signals. Communicating with sound has advantages, as signals propagate  
15 fast, omnidirectionally, around obstacles, and over long distances. Heterogeneous  
16 environments might favour multimodal communication, especially in socially  
17 complex species, as combination of modalities' strengths helps overcome their  
18 individual limitations. Cichlid fishes are known to be vocal, but a recent report  
19 suggests that this is not the case for the socially complex Princess cichlid  
20 *Neolamprologus pulcher* from Lake Tanganyika. Here we further investigated  
21 acoustic communication in this species. Wild and captive *N. pulcher* produced high  
22 frequency sounds (mean: 12 kHz), when stimulated by mirror images. In laboratory  
23 experiments, *N. pulcher* produced distinct two-pulsed calls mostly, but not  
24 exclusively, associated with agonistic displays. Our results suggest that male *N.*  
25 *pulcher* produce more sounds at greater durations than females. Thus, we confirm that

26 the Princess cichlid does not produce low frequency sounds, but does produce high  
 27 frequency sounds, both in combination with and independent from visual displays,  
 28 suggesting that sounds are not a by-product of displays. Further studies on the hearing  
 29 abilities of *N. pulcher* are needed to clarify if the high-frequency sounds are used in  
 30 intra- or inter-specific communication.

31

## 32 **Keywords**

33 Acoustic signals; Sound production; High frequency; Low frequency silencing;  
 34 *Neolamprologus pulcher*; Lake Tanganyika.

## 35     **Introduction**

36     In spite of the long-held view of a silent underwater world, we now know that many  
 37     teleost fishes produce sounds as part of their normal behavioural repertoire (Lobel et  
 38     al., 2010). It should come as no surprise that fish ubiquitously use sounds to  
 39     communicate, as water is a superior acoustic medium, where sound travels almost five  
 40     times faster than in air (Fine & Parmentier, 2015). Compared to other signal  
 41     modalities auditory signals can present some advantages: they propagate fast and in  
 42     all directions unlike olfactory cues, in which case the receiver must be downstream  
 43     from the sender (Fine & Parmentier, 2015); or around obstacles and to longer  
 44     distances than visual signals, which quickly become attenuated with increasing  
 45     distance, in low light or in deep water conditions (Lythgoe & Partridge, 1991). For  
 46     instance, the nocturnal New Zealand bigeye fish (*Pempheris adspersa*) produces  
 47     sounds mainly at night to promote shoal cohesion when visual cues have reduced  
 48     utility (Radford et al., 2015).

49             Nevertheless, long-range auditory signals also present some communicative  
 50     weaknesses. For instance, fish need to deal with high levels of environmental noise in  
 51     shallow water habitats (Ladich & Schulz-Mirbach, 2013; Lugli, 2015) and there is the  
 52     potential for eavesdropping by non-intended receivers, conspecifics or predators  
 53     (Verzijden et al., 2010; Bradbury & Vehrencamp, 2011; Maruska et al., 2012). The  
 54     alternate or simultaneous use of signals of different modalities combines their  
 55     strengths and reduces limitations imposed by the environment on a particular type of  
 56     signal (Stevens, 2013). Multimodal communication is thus expected to evolve under  
 57     varied and unstable environments (Munoz & Blumstein, 2012), in particular in  
 58     gregarious, territorial and socially complex species (Freeberg et al., 2012).

59 Fish commonly produce sounds in agonistic, reproductive and defensive  
60 contexts (Lobel et al., 2010), either in isolation or most often in association with  
61 visual signals (Ladich, 1990, 1997). Such sounds are usually low frequency purrs and  
62 grunts (40–1000 Hz), but higher frequency clicks and creaks (above 1 kHz) have also  
63 been reported (Ladich, 1997; Lobel et al., 2010). A group of fish that has received  
64 increasing attention regarding sound production are cichlids. In particular, those  
65 originating from the East African Great Lakes are prime models for studying  
66 diversification and adaptation due to varied life histories, morphologies and  
67 behaviours (Salzburger, 2009; Gante & Salzburger, 2012). While diversity in colour  
68 patterns and visual adaptations have long been recognised as a driving force in cichlid  
69 evolution (Santos & Salzburger, 2012; Wagner et al., 2012), the description of sound  
70 production and hearing abilities have only more recently gained momentum in spite of  
71 a long history of research (Amorim, 2006; Ladich & Fay, 2013).

72 Here we report on the production of sounds by the Princess cichlid,  
73 *Neolamprologus pulcher* (Trewavas & Poll, 1952). This cooperatively breeding  
74 species lives in rocky shores of southern Lake Tanganyika, East Africa, home to one  
75 of the most diverse freshwater fish adaptive radiations (Muschick et al., 2012;  
76 Salzburger et al., 2014), and has become a favourite in studies of animal cooperation  
77 (Wong & Balshine, 2011; Zöttl et al., 2013). In *N. pulcher* each extended family is  
78 typically formed by a dominant breeding couple and up to a few dozen subordinate  
79 helpers that collectively raise young and defend their territory from other such groups  
80 in the colony. Considering the heterogeneous nature of rocky habitats (especially  
81 when compared to sandy habitats) and the high social complexity of cooperative  
82 breeders, *N. pulcher* is expected to show increased levels of communicative  
83 complexity. Indeed it has been shown that Princess cichlids use a combination of

84 visual and olfactory signals or cues in multiple aspects of their lives, such as  
85 individual recognition, territoriality and aggression (Bachmann et al., (n.d.); Balshine-  
86 Earn & Lotem, 1998; Frostman & Sherman, 2004; Le Vin et al., 2010). It is thus  
87 puzzling that *N. pulcher* have reportedly gone completely silent (Pisanski et al., 2014).  
88 In this study we further investigate the possibility of acoustic communication in this  
89 species by examining both captive-bred and wild-caught fish, over a much wider  
90 range of sound frequencies than before.

91

## 92 **Methods**

### 93 **Acoustic recordings of wild-caught *N. pulcher* – field experiments**

94 Recordings of wild *N. pulcher* were conducted in July and August 2013.  
95 *Neolamprologus pulcher* from different social groups were carefully captured with  
96 gill nets on SCUBA in shallow waters around Kalambo Lodge, Isanga Bay, Zambia,  
97 in the south-eastern shore of Lake Tanganyika (8°37'22.1"S, 31°12'03.6"E). Around  
98 20 adult fish were placed together in a concrete pond (1 × 1 × 1 m), with lake water  
99 and without shelters, so aggression levels were reduced between individuals, and left  
100 to acclimatise for 3 days before the recordings commenced. Every second day, one-  
101 third of the water in the pond was changed. Fish were individually recorded in another  
102 concrete pond that was the same size, but only filled to 20 cm depth. An octagonal  
103 arena, with mirror panels (25 × 20 cm) on the inside, was used to elicit behaviours and  
104 sounds (Fig. 1A). Mirrors have been successfully used to induce typical agonistic  
105 behaviours in African cichlids and fish in general (Rowland, 1999; Dijkstra et al.,  
106 2012). Contrary to the use of interacting, live fish as stimuli, mirrors have the  
107 advantage that sound emitters can not be mistaken, and because only one individual is  
108 recorded at any one time, precise calculation of sound parameters is also facilitated.

109 To prevent the fish from seeing multiple mirror images, a perforated box was placed  
110 in the centre of the arena (Fig. 1A). A Teledyne Reson TC4013 hydrophone  
111 (Denmark), with a receiving sensitivity of -211 dB re: V/ $\mu$ Pa and frequency range of  
112 1 Hz to 170 kHz, was suspended inside the perforated box. Sound was intensified at  
113 500 Hz by an UltraSoundGate charge amplifier and then stored and digitalised at 48  
114 kHz (with 16 bit resolution) into Waveform Audio File Format (.wav) by the Marantz  
115 PMD670 recorder. Movements were recorded from above with a GoPro Hero 3  
116 camera that was synchronised to the sound recordings. This allowed discarding  
117 sounds that had been produced by the fish touching the setup or breaking the water  
118 surface. The pond was illuminated with indirect natural daylight and two solar-  
119 charged LED lamps. Unlike fluorescent bulbs, LEDs produce negligible levels of low  
120 frequency sound (Rumyantsev et al., 2005).

121 Individuals were introduced to the experimental arena via a box with a sliding  
122 door. After a 2-minute acclimatisation period the door of the box was opened and the  
123 box removed as soon as the fish had vacated it. If the fish did not exit right away, the  
124 box was lifted slightly to encourage departure. Each fish was recorded for eight  
125 minutes and then weighed, standard length measured, and sexed by examining the  
126 genital papilla. A total of ten (6 males and 4 females) *N. pulcher* were used in this  
127 study. Recordings of wild fish taken at Lake Tanganyika were first manually  
128 inspected for sounds and then filtered with a bandpass at 300 Hz to remove low  
129 frequency background noise. The experiments were done in accordance with the  
130 Department of Fisheries, Lake Tanganyika Research Unit, Mpulungu, Zambia.

131

132 **Acoustic recordings of captive-raised *N. pulcher* – laboratory experiments**

133 Given the recent report of silent *N. pulcher* (Pisanski et al., 2014), sound recordings  
134 were repeated under laboratory conditions, where a camera could be placed in lateral  
135 view to monitor fish behaviours with greater detail than in the field (Fig. 1B). It also  
136 allowed controlling for the effect of captive raising on sound production.

137 In order to minimise ambient background noise, acoustic recordings took  
138 place in a room with thick concrete walls, with an aquarium (40 × 30 × 25 cm) resting  
139 on 2 cm-thick acoustic absorption cotton and placed inside a large (48 × 42 × 32 cm)  
140 expanded polystyrene foam box. The inside of the expanded polystyrene foam  
141 container, except for the floor, was also covered with acoustic insulation that allowed  
142 external sounds to be reflected and internal sounds to be absorbed to reduce  
143 reverberation. Four battery-operated LED lamps were placed above the aquarium to  
144 provide adequate illumination. The aquarium contained a half terracotta flowerpot to  
145 provide shelter for the fish.

146 First or second generation laboratory-raised *N. pulcher* were used, originating  
147 from fish collected at Kalambo Lodge, Isanga Bay, Zambia in Lake Tanganyika. Fish  
148 were originally kept in pairs in aquaria with sandy substrate, halved terracotta  
149 flowerpots and a motorised sponge filter, and were fed once daily prior to the  
150 experiment. Ten sexually mature *N. pulcher* (5 males and 5 females) were then  
151 selected and individually recorded in April 2015. A 1.9 mm-thick glass mirror (28 ×  
152 22 cm), placed flat against a lateral wall inside the aquarium, was used to induce  
153 sound production (Fig. 1B). Fish were gently hand-netted from their home aquaria  
154 and given one hour to acclimatise in the experimental setup; however, the mirror was  
155 introduced to the aquarium only two minutes before the recording began to prevent  
156 the fish becoming accustomed to it. All nearby electrical equipment, including the

157 room lights, were shut off shortly before synchronous video and audio recordings  
158 commenced.

159 We used the same hydrophone, amplifier, recorder and settings as described in  
160 the field experiment. Although in the laboratory recordings we utilised the Raven Pro  
161 1.5 sound analysis software's adaptive broadband filter, with the default settings of a  
162 filter order of ten and a least mean squares step size of 0.01, to reduce the likelihood  
163 of filtering out potential fish sounds (Bioacoustics Research Program, 2014).  
164 Adaptive broadband filtering is useful when the preferred broadband signal is amidst  
165 narrowband background noise that could not otherwise be eliminated (Bioacoustics  
166 Research Program, 2014). This filter works just like when people talk in a noisy  
167 environment, the continuous surrounding background sounds are recognised but the  
168 focus and concentration is on the person's speech, or in this case on the sounds  
169 produced by the fish. To diminish distortion of the fish's acoustic signals in the  
170 aquarium, the hydrophone was placed within the attenuation distance of where the  
171 fish were expected to produce sound (Akamatsu et al., 2002). Behaviour was  
172 simultaneously recorded with a Nikon 1 camera with an 11-27.5 mm lens. Each  
173 recording session lasted 20 minutes. Subsequently, fish were weighed, standard length  
174 measured, sexed by examination of the external genital papilla and then returned to  
175 their home aquarium. Experiments were authorised by the Cantonal Veterinary Office,  
176 Basel, Switzerland (permit numbers 2317 & 2356).

177

# **178 Characterisation of *N. pulcher* sounds**

179 Only sounds that showed a clear structure and high signal to noise ratio were  
180 considered. All sounds were confirmed with the synchronised video footage and if,  
181 for example, the fish touched the mirror or turned around quickly, resulting in an

182 incidental sound, or an unexpected background noise occurred, then no measurements  
183 were taken at this time. For this reason, we focused on characterising sounds  
184 produced by fish only in the laboratory experiment, where behaviours could be  
185 unequivocally monitored. Based on the typical social behaviours of *N. pulcher* (Table  
186 1) we noted if a behavioural display was associated with sound. To quantify the  
187 acoustic properties of sounds produced by *N. pulcher* in the laboratory we measured  
188 pulse duration, pulse peak frequency, interpulse interval, call duration, and pulse rate  
189 (Fig. 2). In the field dataset, we focused on pulse duration and pulse peak frequency.  
190 In our study the duration of each pulse is defined as the time in milliseconds (ms)  
191 from the onset of a pulse to its end as classified by amplitude of the signal. Pulse peak  
192 frequency is the frequency with the maximum power in the pulse. The duration  
193 between each pulse, the interpulse interval, is calculated in milliseconds and is the  
194 period with only white noise levels of sound between the pulses. The duration of a  
195 call, in milliseconds, is measured from the onset of the first pulse to the end of the last  
196 pulse and may contain one pulse or many. Call duration is often subjectively  
197 measured in fish acoustics literature. We aimed to provide a non-biased, replicable  
198 classification by measuring every interpulse interval in the recordings (these periods  
199 of white noise went from milliseconds to minutes) and plotting their frequencies as a  
200 histogram. Any discontinuity would be indicative of how many pulses constitute a  
201 typical call. Lastly the pulse rate can be defined as the function of the number of  
202 pulses per call duration.

203       The aforementioned temporal parameters were measured on the oscillogram in  
204 the same preset window size and settings. Whereas peak frequency was quantified  
205 with the spectrogram (Hann, FFT size 256 samples, filter bandwidth 270Hz, with a  
206 50% overlap). All measurements were made in Raven Pro 1.5 sound analysis software,

commonly employed in animal communication research (Bioacoustics Research Program, 2014).

## Results

Of the seven (four males and three females) out of 10 *N. pulcher* that produced sound in our setup at Lake Tanganyika, there were a total of 40 pulses recorded (mean  $\pm$  SD;  $5.7 \pm 7.1$  pulses/fish). Mean pulse duration was  $1.5 \pm 0.5$  ms, whilst pulse peak frequency was  $12008.0 \pm 8312.8$  Hz. In the laboratory setting, six (four males and two females) out of 10 *N. pulcher* emitted sound. Of those six individuals, five produced sound associated with a defined social behaviour (Table 2). Sound production occurred most frequently when fish were in an aggressive posture or lateral display (Table 2). Often, these aggressive displays coupled with sound production were followed by or occurred shortly before other aggressive behaviours such as rams, bites and chases. Males only exhibited aggressive behaviours coupled with sound, whereas females in addition showed submissive displays in conjunction with sound (Table 2). One female predominantly produced sound alongside non-aggressive social and submissive behaviours (Table 2). Five doubled-pulsed calls from two fish (one male and one female) were also recorded without concurrent visual display, when both fish were motionless (Table 3). This particular female had produced sound with behavioural displays, however paused displaying for a couple of minutes and continued to call and then began displaying again. The male on the other hand did not display once, he performed a few exploratory swims of the aquarium and then stayed in the corner of the aquarium calling out the rest of the recording. These sounds did not come from background or incidental noise and were similar to the other acoustic signals produced during displays (Table 3).

232 A total of 92 pulses ( $14.8 \pm 11.5$  pulses/fish) produced by six individuals were  
 233 measured in the laboratory setup (Table 3). Since the minimum resonance frequency  
 234 of the aquarium ( $\sim 4000$  Hz) was much lower than the dominant frequency of *N.*  
 235 *pulcher* sounds ( $\sim 12000$  Hz, Table 3), according to (Akamatsu et al., 2002) resonance  
 236 distortion in the aquarium should be minimal. Inspection of interpulse duration  
 237 frequency revealed that the majority of pulses were produced less than 0.4 s apart (Fig.  
 238 3). Pulses separated by less than 0.4 s were then considered part of one call, and on  
 239 average 2 pulses were produced per call (Additional File 1). When this double-pulse  
 240 call occurred, often the first pulse had a dominant frequency between 7000 Hz and  
 241 15000 Hz and the second pulse peaked slightly higher (Fig. 2).

242 Male *N. pulcher* produced more and longer pulses than females, however the  
 243 peak frequencies of the pulses were very similar in both sexes (Table 4). The standard  
 244 two-pulsed call was found in both sexes, although males had more calls than females,  
 245 as well as a longer call duration (Table 4).

246

## 247 Discussion

### 248 Sound production by Princess cichlids

249 Multimodal communication is expected in socially complex species (Freeberg et al.,  
 250 2012) that live in unstable environments (Munoz & Blumstein, 2012). In this study  
 251 we report the production of sounds often associated with a visual display by the  
 252 cooperatively breeding Princess cichlid, *N. pulcher*. Our analyses confirm recent  
 253 findings that this species does not produce the low frequency sounds common to  
 254 many other cichlids or fish species in general (Pisanski et al., 2014), for which we  
 255 suggest the term “low frequency silencing”. However, we found strong evidence for  
 256 deliberate production of high frequency double-pulse calls by *N. pulcher*. In our field

257 and laboratory experiments we found that both males and females produce high  
258 frequency sounds (above 5 kHz, average ~12 kHz) in an agonistic context induced by  
259 mirrors.

260 High frequency sound production has long been reported in cichlids, including  
261 in species from Lake Tanganyika (e.g. (Myrberg, Jr. et al., 1965; Nelissen, 1978)).  
262 Peak frequencies are similarly high (above 5 kHz, often higher than 20 kHz) but  
263 temporal characteristics differ substantially among species. *Neolamprologus pulcher*  
264 produces a distinct double-pulse clicking call while others (*Astatotilapia burtoni*,  
265 *Simochromis diagramma*, different *Tropheus* spp.) produce a creaking or chewing  
266 multi-pulsed call (Nelissen, 1978). These short pulses of sound and high frequency in  
267 *N. pulcher* point towards a stridulatory mechanism of sound production. It has been  
268 suggested that African cichlids may produce sound by rubbing together the teeth on  
269 their pharyngeal jaws (Rice & Lobel, 2004), although this mechanism is yet to be  
270 confirmed. (Fine & Parmentier, 2015) suggest that stridulatory mechanisms should  
271 contain a wide range of frequencies, such as the broadband sound produced by *N.*  
272 *pulcher*.

273 Most of the sounds recorded in this study were produced in association with  
274 an aggressive visual display, but interestingly also in submissive displays. Importantly,  
275 since fish also produced sound with similar characteristics without an associated  
276 behaviour, we can infer that sound production is not a sole by-product of a visual  
277 display but instead can be generated independently. By examining both wild and  
278 captive fish we could also exclude any effect of captivity and captive breeding on  
279 “low frequency silencing” in *N. pulcher*. The evolutionary reasons for loss of low  
280 frequency sounds are still unclear.

281

## 282     **Acoustic differences between and within wild and captive individuals**

283     Both wild and captive individuals generate characteristic high frequency double-pulse  
 284     clicks, but pulses of *N. pulcher* in the laboratory recordings were longer in duration  
 285     compared to the field recordings (one order of magnitude on average). Interestingly,  
 286     male and female *N. pulcher* differed also in temporal parameters. Cichlid acoustic  
 287     studies have shown variation in pulse duration between closely related species,  
 288     suggesting it is evolutionarily labile: mean pulse duration in *Oreochromis*  
 289     *mossambicus* is 150 ms, compared to 10ms in *Oreochromis niloticus* (Amorim et al.,  
 290     2003; Longrie et al., 2008), and species in the genus *Maylandia* show 2–3 times  
 291     differences in mean pulse duration (Danley et al., 2012). Furthermore, context- and  
 292     sex-specific differences have been reported in *Maylandia (Pseudotropheus) zebra*  
 293     (Simões et al., 2008), and intra-individual variation in sound duration and pulse rate in  
 294     response to motivation has been demonstrated in three distantly related cichlid species  
 295     (Myrberg, Jr. et al., 1965). It is thus possible that noisier captive conditions have  
 296     induced changes on labile temporal properties of *N. pulcher* sounds (pulse  
 297     duration/period) in a similar way that environmental noise has impacted call duration  
 298     and rate in Cope’s grey treefrog, *Hyla chrysoscelis* (Love & Bee, 2010) or song  
 299     amplitude in common blackbird, *Turdus merula* and other birds (Nemeth et al., 2013).

300

## 301     **Significance of high pitch sounds**

302     Reports of low (i.e. below 2-3 kHz) frequency sounds in cichlid fishes have been  
 303     dominating the literature in recent years. This has likely both technical and biological  
 304     explanations. On one hand, it is possible that sounds produced by cichlids in a  
 305     reproductive context are mostly low frequency (e.g. (Nelissen, 1978)), while  
 306     recording of narrower bandwidths or applying low-pass filters to raw data could

account for masking of higher frequencies (Ripley & Lobel, 2004; Amorim et al., 2008; Longrie et al., 2008, 2009; Simões et al., 2008; Bertucci et al., 2012; Maruska et al., 2012; Pisanski et al., 2014). But perhaps the overarching reason relates to the expectation that fish are sensitive only to low frequency sounds and cannot hear above a certain threshold (e.g. (Heffner & Heffner, 1998)), which would render such high frequency sounds biologically irrelevant. It is presently unclear whether *N. pulcher* can detect such high frequencies, as hearing sensitivities have not been studied in this species and those of the close-relative *N. brichardi* (Gante et al., (n.d.)) have been investigated only in the range 100–2000 Hz (Ladich & Wysocki, 2003). Nevertheless, evidence has been mounting that some species react to high frequency sounds: for instance, behavioural studies indicate that cod *Gadus morhua* can detect ultrasonic signals up to 39 kHz and the clupeid *Alosa sapidissima* of over 180 kHz, well past human hearing (reviewed in (Popper & Lu, 2000)). Furthermore, new data indicate that species might have multiple hearing maxima, as bimodal w-shaped sensitivity curves have been described in Malawian cichlids previously thought to have only a u-shaped sensitivity curve peaking at low frequencies (van Staaden et al., 2012).

Nelissen (Nelissen, 1978) suggested that vocal complexity (measured as number of sound types) in six cichlid species from Lake Tanganyika varies inversely with number of colour patterns, such that different species would specialise along one of the two communication axes. Maruska et al. (Maruska et al., 2012) showed that acoustic signalling is an important sensory channel in multimodal courtship in the cichlid *A. burtoni*. Females responded to sounds even before seeing males (Maruska et al., 2012), which suggests that sounds could function as a long-distance attraction signal in the turbid waters of river deltas inhabited by this species. Sounds in the

332 cooperative breeding *N. pulcher* could play a role in multimodal communication in an  
 333 agonistic context and to maintain group cohesion. Since *N. pulcher* also produced  
 334 sound in the confines of the shelter, it is possible that individuals can use acoustic  
 335 signals when retreating to their shelter and other forms of communication are limited.  
 336 Importantly, high frequency signals would also transmit more efficiently above the  
 337 low frequency background noise of the underwater world, particularly in windy  
 338 conditions (van Staaden et al., 2012) or crowded fish neighbourhoods. These longer-  
 339 range high pitch sounds would allow communication among individuals belonging to  
 340 different family groups, establishing a chorus across the colony.

341         While the ability of *N. pulcher* to hear in this high frequency range is still to  
 342 be determined, several hearing ‘specialists’ inhabiting Lake Tanganyika could be  
 343 potential interspecific receivers of the acoustic signals generated by cichlids. Hearing  
 344 specialists that can detect sounds in the kHz generally have their swim bladder  
 345 acoustically coupled to the inner ear (Popper & Lu, 2000). These include several  
 346 catfish of the families Malapteruridae, Mochokidae, Claroteidae and Clariidae  
 347 (Siluriformes) that can hear higher frequency sounds and predate on cichlids. Other  
 348 potential candidates would be the many species that lurk around *Neolamprologus*  
 349 rocky habitat, such as spiny eels of the family Mastacembelidae (Synbranchiformes)  
 350 and perches of the family Latidae (Perciformes).

351

## 352 **Conclusion**

353 We have shown that *N. pulcher* produces high frequency (above 5 kHz, average ~12  
 354 kHz) double-pulsed calls. Sounds are most often produced jointly with aggressive or  
 355 submissive visual displays, although both acoustic and visual signals can be produced  
 356 in isolation. It is unclear whether the receiver of such sounds is intra- or interspecific

357 given our general lack of understanding of hearing sensitivities of fishes inhabiting  
358 Lake Tanganyika. In the event that cichlids can hear such high pitch sounds, an as of  
359 yet undescribed morphological adaptation is expected to exist. Non-visual sensory  
360 modalities in African cichlids may thus have a larger impact than originally expected  
361 and could be an important aspect in their adaptive radiation.

362

### 363 **Acknowledgements**

364 We thank Lia Albergati and Benjamin Küng for assistance in the field and Miguel  
365 Vences for use of the recording equipment. This project was funded by a travel grant  
366 from the University of Basel to RKS, by grants from the European Research Council  
367 (ERC; StG “CICHLID~X”) and the Swiss National Science Foundation (SNF) to WS,  
368 and by the “University of Basel Excellence Scholarships for Young Researchers” and  
369 “Novartis Excellence Scholarships for Life Sciences” to HFG.

370

### 371 **References**

- 372 Akamatsu, T., T. Okumura, N. Novarini, & H. Y. Yan, 2002. Empirical refinements  
373 applicable to the recording of fish sounds in small tanks. *The Journal of the*  
374 *Acoustical Society of America* 112: 3073–3082.
- 375 Amorim, M. C. P., 2006. Diversity of sound production in fish. *Diversity* 1: 71–105.
- 376 Amorim, M. C. P., P. J. Fonseca, & V. C. Almada, 2003. Sound production during  
377 courtship and spawning of *Oreochromis mossambicus*: male–female and male–male  
378 interactions. *Journal of Fish Biology* 62: 658–672.
- 379 Amorim, M. C. P., J. M. Simões, P. J. Fonseca, & G. F. Turner, 2008. Species  
380 differences in courtship acoustic signals among five Lake Malawi cichlid species  
381 (*Pseudotropheus* spp.). *Journal of Fish Biology* 72: 1355–1368.
- 382 Bachmann, J. C., F. Cortesi, M. D. Hall, N. J. Marshall, W. Salzburger, & H. F. Gante,  
383 (n.d.). Social selection maintains honesty of a plastic visual signal in a cichlid fish.  
384 Submitted .
- 385 Balshine-Earn, S., & A. Lotem, 1998. Individual recognition in a cooperatively  
386 breeding cichlid: evidence from video playback experiments. *Behaviour* 135: 369–  
387 386.
- 388 Bertucci, F., J. Attia, M. Beauchaud, & N. Mathevon, 2012. Sounds produced by the  
389 cichlid fish *Metriacroma zebra* allow reliable estimation of size and provide  
390 information on individual identity. *Journal of Fish Biology* 80: 752–766.

391 Bradbury, J. W., & S. L. Vehrencamp, 2011. Principles of Animal Communication,  
392 Second Edition. Sinauer Associates, Inc.; 2 edition.

393 Danley, P. D., M. Husemann, & J. Chetta, 2012. Acoustic diversity in Lake Malawi's  
394 rock-dwelling cichlids. *Environmental Biology of Fishes* 93: 23–30.

395 Dijkstra, P. D., S. M. Schaafsma, H. A. Hofmann, & T. G. G. Groothuis, 2012.  
396 “Winner effect” without winning: unresolved social conflicts increase the probability  
397 of winning a subsequent contest in a cichlid fish. *Physiology & Behavior Elsevier Inc.*  
398 105: 489–492.

399 Fine, M. L., & E. Parmentier, 2015. Mechanisms of fish sound production In Ladich,  
400 F. (ed), *Sound Communication in Fishes*. Springer Vienna, Wien: 77–126.

401 Freeberg, T. M., R. I. M. Dunbar, & T. J. Ord, 2012. Social complexity as a proximate  
402 and ultimate factor in communicative complexity. *Philosophical Transactions of the*  
403 *Royal Society B: Biological Sciences* 367: 1785–1801.

404 Frostman, P., & P. T. Sherman, 2004. Behavioral response to familiar and unfamiliar  
405 neighbors in a territorial cichlid, *Neolamprologus pulcher*. *Ichthyological Research*  
406 51: 8–10.

407 Gante, H. F., M. Matschiner, M. Malmstrøm, K. S. Jakobsen, S. Jentoft, & W.  
408 Salzburger, (n.d.). Genomics of speciation and introgression in Princess cichlid fishes  
409 from Lake Tanganyika. Submitted .

410 Gante, H. F., & W. Salzburger, 2012. Evolution: cichlid models on the runaway to  
411 speciation. *Current Biology* 22: R956–R958.

412 Heffner, H. E., & R. S. Heffner, 1998. Hearing In Greenberg, G., & M. M. Haraway  
413 (eds), *Comparative Psychology, a handbook*. Garland, New York: 290–303.

414 Ladich, F., 1990. Vocalization during agonistic behaviour in *Cottus gobio* L.  
415 (Cottidae): an acoustic threat display. *Ethology* 84: 193–201.

416 Ladich, F., 1997. Agonistic behaviour and significance of sounds in vocalizing fish.  
417 *Marine and Freshwater Behaviour and Physiology* 29: 87–108.

418 Ladich, F., & R. R. Fay, 2013. Auditory evoked potential audiometry in fish. *Reviews*  
419 *in Fish Biology and Fisheries* 23: 317–364.

420 Ladich, F., & T. Schulz-Mirbach, 2013. Hearing in cichlid fishes under noise  
421 conditions. *PloS ONE* 8: e57588.

422 Ladich, F., & L. E. Wysocki, 2003. How does tripus extirpation affect auditory  
423 sensitivity in goldfish?. *Hearing Research* 182: 119–129.

424 Le Vin, A. L., B. K. Mable, & K. E. Arnold, 2010. Kin recognition via phenotype  
425 matching in a cooperatively breeding cichlid, *Neolamprologus pulcher*. *Animal*  
426 *Behaviour Elsevier Ltd* 79: 1109–1114.

427 Lobel, P. S., I. M. Kaatz, & A. N. Rice, 2010. Acoustical behavior of coral reef fishes  
428 In Cole, K. (ed), *Reproduction and Sexuality in Marine Fishes*. University of  
429 California Press: 307–386.

430 Longrie, N., M. L. Fine, & E. Parmentier, 2008. Innate sound production in the  
431 cichlid *Oreochromis niloticus*. *Journal of Zoology* 275: 413–417.

432 Longrie, N., S. Van Wassenbergh, P. Vandewalle, Q. Manguit, & E. Parmentier, 2009.  
433 Potential mechanism of sound production in *Oreochromis niloticus* (Cichlidae). *The*  
434 *Journal of Experimental Biology* 212: 3395–3402.

435 Love, E. K., & M. A. Bee, 2010. An experimental test of noise-dependent voice  
436 amplitude regulation in Cope's grey treefrog, *Hyla chrysoscelis*. *Animal Behaviour*  
437 *Elsevier Ltd* 80: 509–515.

438 Lugli, M., 2015. Habitat acoustics and the low-frequency communication of shallow  
439 water fishes In Ladich, F. (ed), *Sound Communication in Fishes*. Springer Vienna,  
440 Wien: 175–206.

441 Lythgoe, J. N., & J. C. Partridge, 1991. The modelling of optimal visual pigments of  
442 dichromatic teleosts in green coastal waters. *Vision Research* 31: 361–371.

443 Maruska, K. P., U. S. Ung, & R. D. Fernald, 2012. The African cichlid fish  
444 *Astatotilapia burtoni* uses acoustic communication for reproduction: sound production,  
445 hearing, and behavioral significance. *PLoS ONE* 7: 1–13.

446 Munoz, N. E., & D. T. Blumstein, 2012. Multisensory perception in uncertain  
447 environments. *Behavioral Ecology* 23: 457–462.

448 Muschick, M., A. Indermaur, & W. Salzburger, 2012. Convergent evolution within an  
449 adaptive radiation of cichlid fishes. *Current Biology* 22: 2362–2368.

450 Myrberg, Jr., A. A., E. Kramer, & P. Heinecke, 1965. Sound production by cichlid  
451 fishes. *Science* 149: 555–558.

452 Nelissen, M. H. J., 1978. Sound production by some Tanganyikan cichlid fishes and a  
453 hypothesis for the evolution of their communication mechanisms. *Behaviour* 64: 137–  
454 147.

455 Nemeth, E., N. Pieretti, S. A. Zollinger, N. Geberzahn, J. Partecke, A. C. Miranda, &  
456 H. Brumm, 2013. Bird song and anthropogenic noise: vocal constraints may explain  
457 why birds sing higher-frequency songs in cities. *Proceedings of the Royal Society B:*  
458 *Biological Sciences* 280: 20122798.

459 Pisanski, K., S. E. Marsh-Rollo, & S. Balshine, 2014. Courting and fighting quietly: a  
460 lack of acoustic signals in a cooperative Tanganyikan cichlid fish. *Hydrobiologia* .

461 Popper, A. N., & Z. Lu, 2000. Structure–function relationships in fish otolith organs.  
462 *Fisheries Research* 46: 15–25.

463 Radford, C. A., S. Ghazali, A. G. Jeffs, & J. C. Montgomery, 2015. Vocalisations of  
464 the bigeye *Pempheris adspersa*: characteristics, source level and active space. *Journal*  
465 *of Experimental Biology* 218: 940–948.

466 Rice, A. N., & P. S. Lobel, 2004. The pharyngeal jaw apparatus of the Cichlidae and  
467 Pomacentridae: Function in feeding and sound production. *Reviews in Fish Biology*  
468 *and Fisheries* 13: 433–444.

469 Ripley, J. L., & P. S. Lobel, 2004. Correlation of acoustic and visual signals in the  
470 cichlid fish, *Tramitichromis intermedius*. *Environmental Biology of Fishes* 71: 389–  
471 394.

472 Rowland, W. J., 1999. Studying visual cues in fish behavior: a review of ethological  
473 techniques. *Environmental Biology of Fishes* 56: 285–305.

474 Rumyantsev, S. L., S. Sawyer, N. Pala, M. S. Shur, Y. Bilenko, J. P. Zhang, X. Hu, A.  
475 Lunev, J. Deng, & R. Gaska, 2005. Low frequency noise of light emitting diodes.  
476 *Proc SPIE Noise Dev Circ III* 5844: 75–85.

477 Salzburger, W., 2009. The interaction of sexually and naturally selected traits in the  
478 adaptive radiations of cichlid fishes. *Molecular Ecology* 18: 169–185.

479 Salzburger, W., B. Van Bocxlaer, & A. S. Cohen, 2014. Ecology and evolution of the  
480 African Great Lakes and their faunas. *Annual Review of Ecology, Evolution, and*  
481 *Systematics* 45: 519–545.

482 Santos, M. E., & W. Salzburger, 2012. Evolution. How cichlids diversify. *Science*  
483 (New York, N.Y.) 338: 619–621.

484 Simões, J. M., I. G. Duarte, P. J. Fonseca, G. F. Turner, & M. C. Amorim, 2008.  
485 Courtship and agonistic sounds by the cichlid fish *Pseudotropheus zebra*. *The Journal*  
486 *of the Acoustical Society of America* 124: 1332–1338.

487 Sopinka, N. M., J. L. Fitzpatrick, J. K. Desjardins, K. A. Stiver, S. E. Marsh-Rollo, &  
488 S. Balshine, 2009. Liver size reveals social status in the African cichlid  
489 *Neolamprologus pulcher*. *Journal of Fish Biology* 75: 1–16.

490 Stevens, M., 2013. *Sensory Ecology, Behaviour, and Evolution*. Oxford University

491 Press.  
492 van Staaden, M., L. E. Wysocki, & F. Ladich, 2012. Ecoacoustical constraints shape  
493 sound communication in Lake Malawi Cichlids [abstract]. Bioacoustics 21: 84.  
494 Verzijden, M. N., J. Van Heusden, N. Bouton, F. Witte, C. Ten Cate, & H.  
495 Slabbekoorn, 2010. Sounds of male Lake Victoria cichlids vary within and between  
496 species and affect female mate preferences. Behavioral Ecology 21: 548–555.  
497 Wagner, C. E., L. J. Harmon, & O. Seehausen, 2012. Ecological opportunity and  
498 sexual selection together predict adaptive radiation. Nature Nature Publishing Group  
499 487: 366–369.  
500 Wong, M., & S. Balshine, 2011. The evolution of cooperative breeding in the African  
501 cichlid fish, *Neolamprologus pulcher*. Biological Reviews of the Cambridge  
502 Philosophical Society 86: 511–530.  
503 Zöttl, M., D. Heg, N. Chervet, & M. Taborsky, 2013. Kinship reduces alloparental  
504 care in cooperative cichlids where helpers pay-to-stay. Nature Communications 4:  
505 1341.

## 506 **Tables**

507 **Table 1** *Neolamprologus pulcher* ethogram illustrates typical social behaviours

508 of the species (adapted from (Sopinka et al., 2009; Pisanski et al., 2014))

Behaviour	Description
<i>Non-aggressive &amp; social</i>	
Quiver	Fish quivers to mirror; the whole body trembles
Soft touch	Fish nips or softly makes contact with mirror
Parallel swim	Fish swims upwards towards the mirror
<i>Aggressive</i>	
Chase	Fish quickly darts towards mirror
Bite	Fish opens jaw and bites mirror
Ram	Fish makes forceful contact with the mouth region to the mirror, often repetitively, but jaws remain closed
Head shake	Fish thrashes head from left to right repeatedly
Puffed throat	Fish flares out its operculum and lowers its jaw
Aggressive posture	Fish lowers head towards the mirror, while it points its tail upwards
Lateral display	Fish presents its lateral aspect to the mirror while extending its unpaired fins
Pseudo-mouth fight	Back-and-forth movement occurs facing the mirror, as if fish will mouth fight, but no contact is made
Hook/J display	Fish swims towards the mirror, bites or rams it, then turns away and quivers
<i>Submissive</i>	
Submissive posture	Fish raises its head towards the mirror and lowers its tail
Submissive display	Fish in submissive posture but with a quivering tail
Flee	Fish quickly swims away from mirror

509

**Table 2** Numbers of sounds produced by *Neolamprologus pulcher* associated with behaviours in the laboratory experiment

Behaviour	#1_M	#2_F	#3_M	#9_F	#10_M	Total
Soft touch	0	0	0	1	0	1
Parallel swim	0	0	0	1	0	1
Puffed throat	0	0	2	0	8	10
Aggressive posture	0	8	5	0	20	33
Lateral display	2	6	15	1	9	33
Pseudo-mouth display	1	0	0	0	1	2
Submissive posture	0	6	0	3	0	9
Total pulses with behaviour	2	20	20	6	34	82

At times multiple behaviours were displayed conjointly with a given pulse, for example both a lateral display and a puffed throat. M = male and F = female

514 **Table 3** Parameters (mean  $\pm$  SD) of the acoustic signals associated with and  
515 without a typical *Neolamprologus pulcher* social behaviour

	No. fish	Total pulses	Pulse duration [ms]	Pulse peak frequency [Hz]	Total calls	Call duration [ms]	Pulses per call
With behaviour	5	82	11.5 $\pm$ 3.5	12280.5 $\pm$ 3740.3	43	896.0 $\pm$ 804.4	2.0 $\pm$ 0.7
Without behaviour	2	10	13.2 $\pm$ 2.8	13992.2 $\pm$ 1889.3	5	294.4 $\pm$ 324.0	2.0 $\pm$ 0.0
Pooled	6	92	12.0 $\pm$ 3.4	12938.7 $\pm$ 3494.0	48	836.0 $\pm$ 733.7	2.0 $\pm$ 0.7

516 One fish emitted sound both with and without behaviour, therefore the pulses for each  
517 were calculated separately, except when pooled

**Table 4** Sex differences in the parameters (mean  $\pm$  SD) of the acoustic signals of *Neolamprologus pulcher* in the laboratory experiments

	No. fish	Total pulses	Pulse duration [ms]	Pulse peak frequency [Hz]	Total calls	Call duration [ms]	Pulses per call
Male	4	64	14.1 $\pm$ 2.1	12710.0 $\pm$ 4303.6	36	918.0 $\pm$ 770.4	1.8 $\pm$ 0.1
Female	2	28	8.5 $\pm$ 1.5	13396.2 $\pm$ 2201.8	12	669.6 $\pm$ 910.2	2.3 $\pm$ 1.4
Pooled	6	92	12.0 $\pm$ 3.4	12938.7 $\pm$ 3494.0	48	836.0 $\pm$ 733.7	2.0 $\pm$ 0.7

All sounds produced were taken into account, both with and without a typical social behaviour

## 522    **Figures**

523    **Fig. 1** Setups used to record sounds produced by *N. pulcher*. In the field experiment  
524    (A), an octagonal mirror arena was used, while in the laboratory experiment (B), one  
525    glass mirror was placed against a wall of the aquarium

526

527    **Fig. 2** Oscillogram and spectrogram of a sound produced by *N. pulcher*. The  
528    oscillogram (A) presents the waveform of the pulses in time versus amplitude.  
529    Whereas the spectrogram (B) shows how the frequency of the pulses changes over  
530    time, and the colour indicates the relative amplitude. Here, the aforementioned  
531    temporal parameters; call duration (a), pulse duration (b) and interpulse interval (c)  
532    are illustratively defined. This double-pulsed call was made by a male in the  
533    laboratory experiments that concurrently exhibited an aggressive lateral display just  
534    after a series of rams and bites to the mirror

535

536    **Fig. 3** Histogram of interpulse duration frequency. The majority of pulses within a  
537    call are shortly separated by less than 0.4 s

538

## 539    **Additional files**

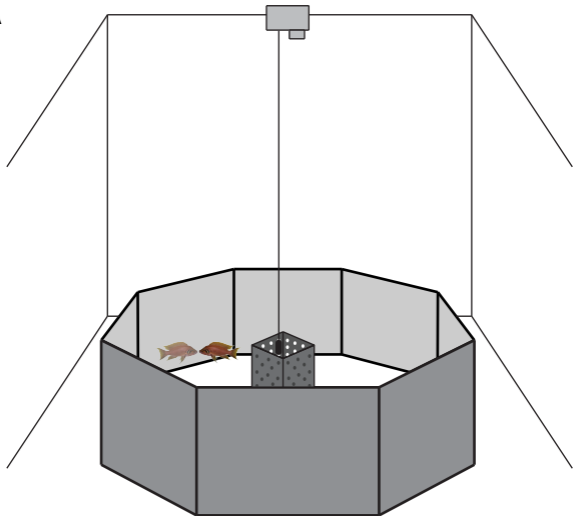
540    **Additional file 1: Audio file.** Two double-pulsed calls of a male *Neolamprologus*  
541    *pulcher*, produced during an aggressive lateral display in the laboratory experiments.  
542    The first double-pulsed call corresponds to Fig. 2

543

544    **Additional file 2: Audio file.** One double-pulsed call of a female *Neolamprologus*  
545    *pulcher*, produced during submissive posture in the lab experiments.

546

- 547     **Additional file 3: Audio file.** One double-pulsed call of a male *Neolamprologus*  
548     *pulcher*, produced without behavioural display (motionless) in the lab experiments.

**A****B**