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

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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/ μ 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 ± 7.1 pulses/fish). Mean pulse duration was 1.5 ± 0.5 ms, whilst pulse peak frequency was 12008.0 ± 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 ± 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 (~ 4000 Hz) was much lower than the dominant frequency of *N.*
 235 *pulcher* sounds (~ 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

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

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

351

352 **Conclusion**

We have shown that *N. pulcher* produces high frequency (above 5 kHz, average ~12 kHz) double-pulsed calls. Sounds are most often produced jointly with aggressive or submissive visual displays, although both acoustic and visual signals can be produced 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

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370

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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 & 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

510 **Table 2** Numbers of sounds produced by *Neolamprologus pulcher* associated
511 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

512 At times multiple behaviours were displayed conjointly with a given pulse, for
513 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**