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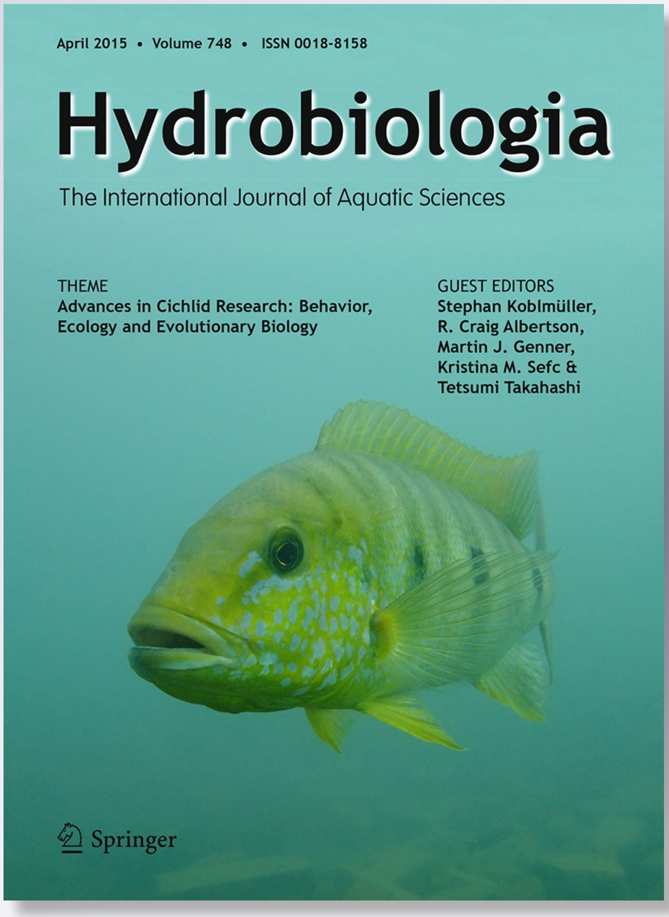

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ADVANCES IN CICHLID RESEARCH

Courting and fighting quietly: a lack of acoustic signals in a cooperative Tanganyikan cichlid fish

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Abstract Research on cichlid fish has revealed remarkable cross-species variation in social and reproductive behavior. Recently, several species of African cichlids have been shown to produce acoustic signals, typically in conjunction with the visual behavioral displays associated with mate choice and competition. However, the recent research conducted on cichlid sound production has focused largely on polygynous cichlids with female care and on species from Lake Malawi and Lake Victoria. In this study, we examined whether sound production occurs in a group-living, cooperative, biparental cichlid species, *Neolamprologus pulcher*, endemic to Lake Tanganyika, the oldest of the African Great Lakes. *Neolamprologus pulcher* is a highly social cichlid that lives in complex groups that are socially stratified. We investigated whether *N. pulcher* performed acoustic signals to each other either during competitive resource contests between size-matched males, or during courtship between male and female breeding pairs. Although the fish displayed prototypical visual displays of aggression, submission, and courtship in the appropriate context, we found no evidence of vocal sound production. Our findings suggest that cichlid sound production was not essential for the evolution of complex social behavior.

Keywords *Neolamprologus pulcher* · Acoustic communication · Sound production · Vocalization · Multimodal signaling · Social behavior · African Great Lakes · Aggression

Introduction

Many animals communicate using multiple sensory modalities that commonly include a visual and an acoustic component (Nelissen, 1991; Johnstone, 1996; Bradbury & Vehrencamp, 1998; Rowe, 1999). For example, male fowl, *Gallus gallus,* produce referential food calls in conjunction with a rhythmic motion of the head and neck collectively known as tidbitting (Smith & Evans, 2008). In wolf spiders of the *Schizocosa* genus, males produce simultaneous visual and vibratory courtship signals (McClintock & Uetz, 1996). In the Bornean ranid frog, *Staurois guttatus*, both sexes produce a foot-flagging visual display that in males is frequently interspersed with short, tonal bursts of sound (Grafe & Wanger, 2007). Similarly, some teleost fish produce complex signals that include context-dependent vocalizations often coinciding with a visual display or posture (for reviews see Ladich, 1997, 2004; Amorim, 2006).

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Due to their explosive and rapid speciation, cichlid fish, especially those from the African Great Lakes, have attracted a great deal of research interest. Cichlids are often employed as an evolutionary model for studying a diverse set of phenomena including the evolution of communication and breeding systems (Goodwin et al., 1998; Kornfield & Smith, 2000; Seehausen, 2006). Cichlid fish are well known for their visual communication (Carleton et al., 2006). However, for over forty years, researchers have known that some male and female cichlid fish from Central America and Africa also produce vocalizations toward conspecific rivals in agonistic encounters and toward their own mate throughout the breeding cycle (Myrberg et al., 1965; Schwarz, 1974, 1980). A recent influx of research on acoustic signaling has further uncovered the repeated use of vocal production in a growing number of African cichlid species. In these fish, vocalizations are typically made during agonistic or courtship interactions and appear to signal dominance or affiliation (Lobel, 1998, 2001; Amorim et al., 2004, 2008; Simões et al., 2008; Bertucci et al., 2010; Verzijden et al., 2010; Bertucci et al., 2012; Maruska et al., 2012).

Research on cichlid vocal behavior has mainly focused on a subset of cichlids that are all members of a recently derived clade of African cichlid fish found in Lake Malawi and Lake Victoria (the Haplochromini, a tribe in the subfamily Pseudocrenilabrinae: Lobel, 1998; Amorim et al., 2008; Smith & van Staaden, 2009; Bertucci et al., 2010; Verzijden et al., 2010; Bertucci et al., 2012). There are a few studies investigating vocal behavior in other cichlids including some cichlids from Lake Tanganyika (Nelissen, 1978; Maruska et al., 2012). These Tanganyikan cichlids, such as *Astatotilapia burtoni*, are thought to have directly seeded the speciation events in Lake Malawi and Lake Victoria (Day et al., 2007; Koblmüller et al., 2008; Sturmbauer et al., 2010, 2011). A vast majority of the cichlid species studied to date for acoustic communication are polygynous, female mouth-brooders; none are group-living cichlids (Goodwin et al., 1998; Klett & Meyer, 2002). To our knowledge, vocal communication has not been studied before in a social, group-living cichlid. Here, we explore whether sound production occurs in the cooperatively breeding cichlid, *Neolamprologus pulcher*.

Few teleosts live as rich a social life as *N. pulcher*. This small lamprologine cichlid fish from Lake Tanganyika was the first cichlid known to exhibit cooperative breeding behavior (Coeckelberghs, 1975). Some 19 other lamprologine cichlids are also cooperative (Heg & Bachar, 2006), but *N. pulcher* remains by far the best-studied cooperative cichlid fish (Wong & Balshine, 2011). *N. pulcher* live their entire lives in a social group comprised of a dominant breeding pair and subordinate helpers (Taborsky & Limberger, 1981). Together the fish co-defend a rocky territory and collectively raise the young of the breeding pair (Taborsky & Limberger, 1981; Wong & Balshine, 2011). These fish lead complex social lives with a strict dominance hierarchy, inheritance of rank, cooperation, strategic growth, and extremely frequent social interactions among group members (Balshine-Earn & Lotem, 1998; Heg & Bachar, 2006; Stiver et al., 2006; Wong & Balshine, 2011). As a consequence of their intricate sociality, there may be strong selection favoring an effective and possibly sophisticated communication system (Freeberg et al., 2012).

As a highly social species, *N. pulcher* has a particularly rich behavioral repertoire including many well-characterized visual behavioral displays and postures (see Sopinka et al., 2009; Hick et al., 2014 for recent ethograms for this species). Given the complexity of *N. pulcher's* social life and visual communication system, this species is a strong candidate for investigations of multimodal signaling. Moreover, there is evidence of visual and olfactory recognition of familiar individuals and kin in *N. pulcher* (Balshine-Earn & Lotem, 1998; Jordan et al., 2010; Le Vin et al., 2010), suggesting that acoustic signals may likewise provide information that allows discrimination among individuals, or information about social rank that would help solidify and maintain social relations (see, e.g., Bertucci et al., 2012).

Acoustic assays offer the best test of cichlid sound production because the precise biological mechanism of sound production in cichlids remains unknown (Lobel, 2001). One hypothesis is that sounds are produced by stridulation of the pharyngeal jaw apparatus and are amplified by the swim bladder (Lobel, 2001; Rice & Lobel, 2003; Ladich, 2004). More recently, however, Longrie et al. (2009) showed that in *Oreochromis niloticus*, sound was produced by a backward movement of the pelvic and pectoral girdles and a forward movement of the anal fin. In this study, we performed acoustic assays of *N. pulcher* adult males and females in both competitive and courtship contexts to determine whether sound production is used as a communication channel in this highly social Tanganyikan cichlid.

Methods and materials

Study animals and housing conditions

We used laboratory-bred sexually mature male and female *N. pulcher* ($n = 44$) derived from wild-caught stock originating from Lake Tanganyika. In the laboratory, the fish live in permanent social groups with upward of ten subordinate helpers and a single breeding pair. Each social group is housed independently in 189-l glass aquaria (92 × 41 × 50 cm) containing 3 cm of coral sand substrate, two terracotta flowerpot shelters, and two sponge filters. The temperature and chemical composition of the water are held at 25 ± 2°C and 7.6 pH, respectively, akin to the natural conditions of Lake Tanganyika. The fish are exposed to a 14:10 h light:dark cycle and maintained on a diet of prepared cichlid flakes. These housing conditions were maintained throughout the course of the study.

Males used for competitive contest trials ($n = 24$) were taken from various social groups at random and were size matched. We did not strictly control for relatedness between males in competitive trials, but the males came from separate social groups so would not have been familiar with one another. Males ($n = 10$) and females ($n = 10$) used for courtship trials were established breeding pairs taken from 10 independent social groups. We are confident that these fish were indeed established pair-bonded breeding pairs. In our lab, pairs typically breed every 2–4 weeks. All breeding pairs used in our experiment had spawned together several times prior to the experiment and more than half spawned within 2–4 days following the experiment, indicating that the females were sexually receptive at the time of the experiment. Moreover, given their high level of sociality, *N. pulcher* dominant pairs affiliate with each other on an hourly basis, every day, not only during spawning.

All fish were measured and sexed before testing. We measured the standard length (SL) of each fish from the tip of the snout to the caudal peduncle (mm) and mass (g). Fish were sexed by examination of the external genital papilla. The average body size of males used for competitive contest trials was 52.5 ± 4 mm and 4 ± 0.09 g (means ± SEMs), and the average difference in size between males within a competitive pair was 5 mm and 0.26 g. The average body size of dominant breeding pairs was 75 ± 7 mm and 12.2 ± 0.4 g for males and 69 ± 7 mm and 8.8 ± 0.25 g for females. Fish were given a unique dorsal fin clip for identification one day before testing.

Testing apparatus

Testing took place in individual 38-l glass aquaria (50 × 25 × 30 cm; see Fig. 1). Each aquarium was lined with 1.5 cm of coral sand substrate and filled with water that was chemically treated to remove chlorine and maintained at 25 ± 2°C. Aquaria were divided into two compartments using acrylic barriers; each compartment was furnished with a single terracotta flowerpot shelter. All ventilation vents in the room were covered to minimize background noise and aquaria were positioned on 10-cm-thick slabs of styrofoam to reduce low-frequency base vibrations (Simões et al., 2008; Bertucci et al., 2010). Aquarium filters and heaters were shut off prior to beginning each behavioral trial.

Size-matched males in the competitive trials were given a 1-week acclimation period to the testing apparatus in separate compartments so that each male had the opportunity to develop territorial ownership of his personal shelter (a terracotta flowerpot) and over his half of the aquarium (following Reddon et al., 2011). The breeding pairs in the courtship trials were given only a 1-h acclimation period in separate compartments prior to testing, as we did not want to separate pair-bonded males and females for too long.

Competitive and courtship trials

In total, we staged 12 competitive contest trials between size-matched males ($n = 24$ males) and 10 courtship trials between breeding pairs ($n = 10$ males; $n = 10$ females). All test trials included two phases: Phase 1 during which the two fish were separated by an acrylic barrier, followed by Phase 2 during which the fish were permitted to interact without any barrier (see Fig. 1). In courtship trials, Phase 1 was 10 min in length and the barrier was clear. Phase 2, in which no barrier was present, was also 10 min in length. In

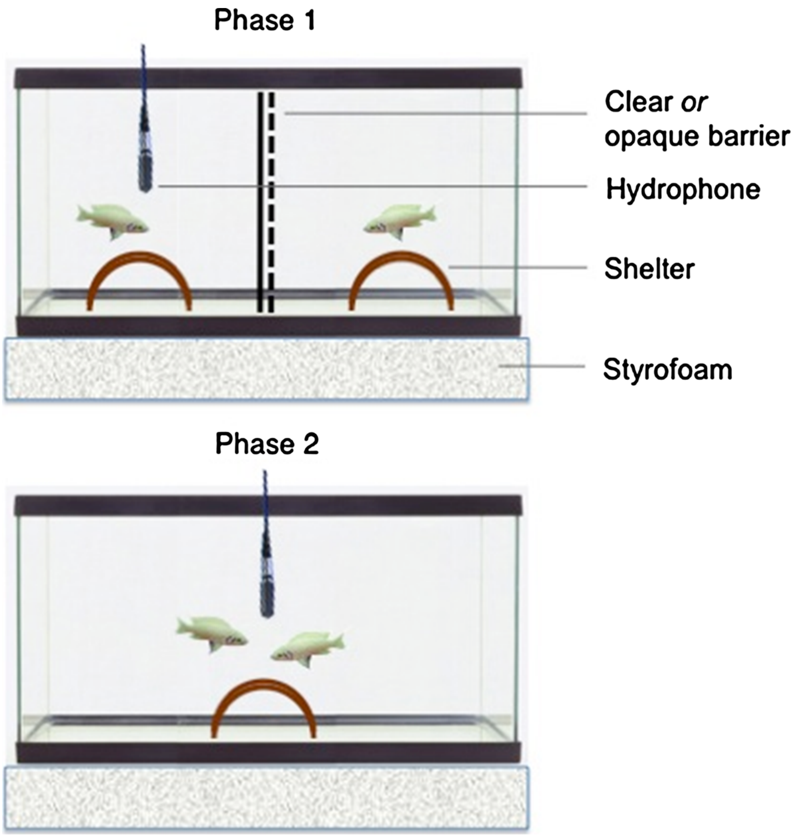

Fig. 1 Aquarium set-up for the competitive and courtship test trials. In Phase 1 of each trial, the fish were separated by a clear or opaque barrier. In Phase 2 of each trial, the fish were permitted to interact physically without any barrier. The hydrophone was centered 3–4 cm above a terracotta pot shelter. Aquaria were positioned on 10-cm-thick slabs of styrofoam to reduce low-frequency base vibrations

competitive trials, Phase 1 was either 2 or 10 min in length and the barrier was clear (half of trials) or opaque (half of trials). These barrier variations were implemented to test whether sound production was more or less likely to occur when visual and/or physical interaction was possible than when it was not (see, e.g., Bertucci et al., 2010). In competitive trials, Phase 2 in which no barrier was present and the males could interact was 10 min in length, analogous to the length of Phase 2 for courtship trials. In competitive trials, the male fish competed for a scarce resource, a single terracotta flowerpot placed in the middle of the aquarium (Fig. 1). Such pots are used as shelter and for breeding by *N. pulcher* in our laboratory, and the fish will readily fight over these shelters in the lab (Taves et al., 2009; Reddon et al., 2011; Hick et al., 2014).

Behavioral scoring and analysis

Synchronous audio–video recordings were made using a video camera coupled with a miniature hydrophone (TC 4013, Teledyne Reson, Denmark; sensitivity: −211 dB re 1 V/μPa; frequency response: 1 Hz to 170 kHZ) suspended 3–4 cm above the shelter following procedures tested and employed in previous studies (see, e.g., Amorim & Almada, 2005; Smith & van Staaden, 2009). Audio-visual recordings were made for both phases of each trial; however, when a barrier was present (Phase 1), audio was recorded from only one compartment of the aquarium (see Fig. 1 top panel). The compartment that we recorded from was chosen randomly for competitive trials and it was always the male's compartment in courtship trials. In total, we collected 392 min of audio-visual recording (competitive trials, Phase 1: 72 min, Phase 2: 120 min; courtship trials, Phase 1: 100 min, Phase 2: 100 min).

Audio was digitally encoded with an M-Audio Fast Track Ultra interface at a sampling rate of 48 kHz and 16-bit amplitude quantization and stored onto a computer as PCM WAV files using Praat software (Boersma & Weenink, 2013). Praat is a powerful acoustic program used widely by behavioral scientists for the recording, analysis, and manipulation of animal vocalizations (available for download at http://www.praat.org; for more information see Owren, 2008).

Table 1 This ethogram is adapted from a recent ethogram for *N. pulcher* (Sopinka et al., 2009)

Type of behavior	Description
Affiliation/courtship	
Quiver	Two fish quiver together or one will quiver to the other; the whole body trembles
Soft touch	Focal fish nips or softly makes contact with another individual
Following	Focal fish follows another in the group
Parallel swim	Two fish swim together in a parallel fashion
Aggression	
Chase	Focal fish quickly darts toward another fish
Bite	Focal fish bites another fish
Ram	Focal fish makes contact with another fish using the head or mouth region, but no obvious bite is taken (jaws remain closed)
Head shake	Fish thrashes its head from left to right repeatedly
Puffed throat	Fish flares out its operculum and lower its jaw cavity. Often this display is associated with an aggressive posture
Aggressive posture	Focal fish lowers its head toward another fish while pointing its tail upwards
Lateral display	Focal fish presents its lateral aspect to another fish while extending its unpaired fins
Mouth fight	Focal fish and another fish lock jaws and push against one another
Pseudo-mouth fight	Back-and-forth movement occurs (as if about to mouth fight), but no physical contact is established
Hook/J display	Focal fish swims toward another fish, bites or rams it, then turns away and quivers. Displayed by subordinate fish to fish higher in the dominance hierarchy
Submission	
Submissive posture	Focal fish raises its head and lowers its tail toward another individual
Submissive display	Focal fish is positioned in a submissive posture and this is accompanied by a quivering tail
Flee or chased	Focal fish quickly swims away from another fish

Raw audio files were bandpass filtered in Praat to remove any remaining low- and high-frequency noise (Hanning window, 100–3,000 Hz). These filtering techniques were suggested by Akamatsu et al. (2002) for analyzing fish sounds recorded in small aquaria and have been used by previous studies on cichlid sound production (e.g., Maruska et al., 2012). Acoustic analysis involved simultaneously listening to the audio recordings while visually inspecting the corresponding digital waveform and spectrogram of the sound recording, as well as tracking the visual behavior of the fish.

Visual behaviors were examined to ensure that agonistic, submissive, affiliative, and courtship behaviors occurred at rates typical for *N. pulcher* (Sopinka et al., 2009; Taves et al., 2009; Reddon et al., 2011), particularly because acoustic displays often occur in conjunction with visual displays (Ladich, 2004). The visual behaviors of fish were scored using conventional methods and classified according to an ethogram constructed for this species (see Table 1; but also see Sopinka et al., 2009; Hick et al., 2014). Each fish was observed for the entire duration of the trial during which all affiliative and aggressive behaviors were scored. For statistical analysis, visual behaviors were summed across minutes within each phase and for each individual.

In competitive trials, we classified males as winners or losers of contests following Reddon et al. (2011). A male was declared a loser when it fled from or submitted to the other male at least three consecutive times or maintained a submissive posture while avoiding the other male. Often times by the end of the trial, the operculum chevron markings of the loser had faded relative to those of the winner. Losers also typically spent a good portion of time in the upper corners of the aquarium, whereas winners did not. In the absence of an evident contest winner or loser, the contest was declared a tie.

We used generalized linear mixed models (GLMMs) with negative binomial error distributions to compare the frequency of visual aggressive behaviors performed by contest winners and losers in a competitive context, and the frequency of visual courtship behaviors performed by males and females in a courtship context. These statistical models are appropriate for analyzing overdispersed count data (Bolker, 2008). We included *trial* (i.e., pair) as a random factor in all models, because contestants or pair-bonded individuals within a single trial may not behave independently of one another. All analyses were conducted using the MASS package (Ripley et al., 2014) in R version 3.0.2 (R Development Core Team, 2013).

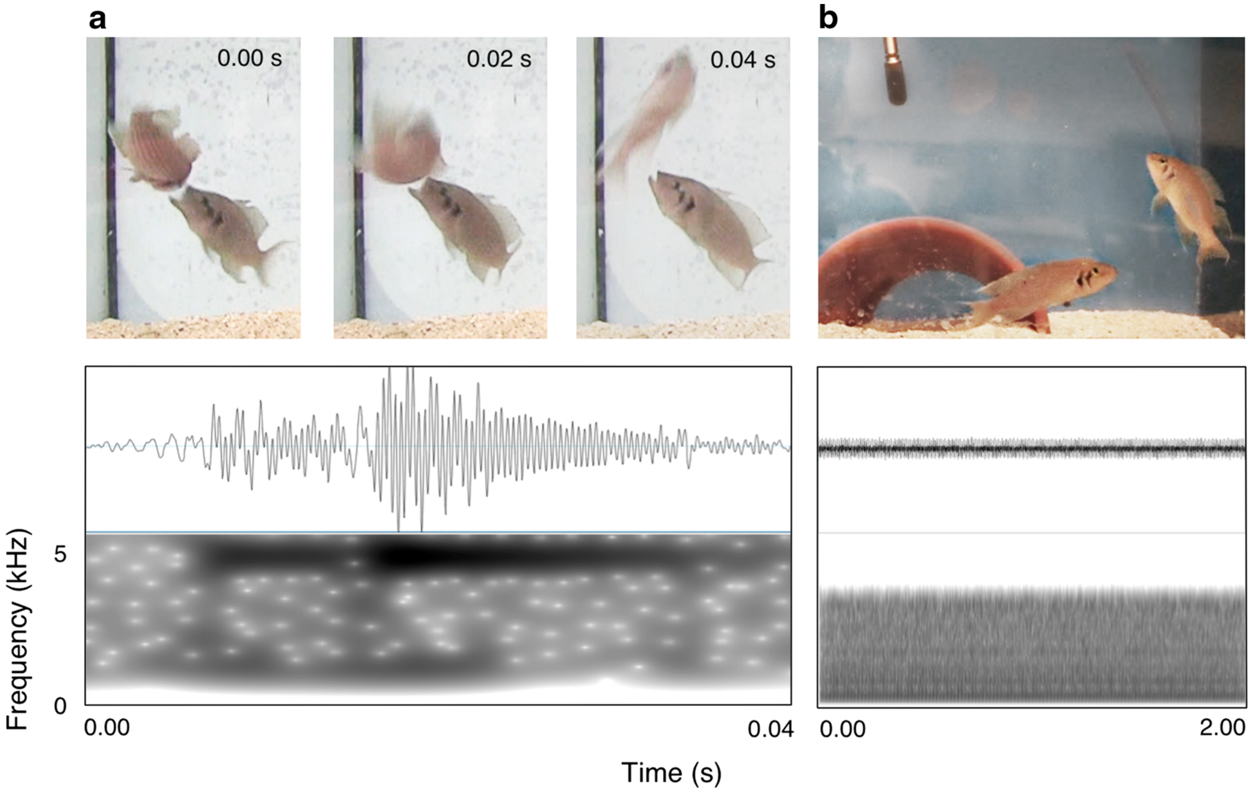

Fig. 2 Video frames illustrating an aggressive behavior and a submissive behavior with corresponding acoustic oscillograms (*center*) and spectrograms (*bottom*) illustrating the acoustic trace recorded during either interaction. **a** A very brief, high-frequency incidental sound produced as one male rapidly escaped the bite of another male (spectrogram settings: FFT, Hanning bandpass filter 1–5 kHz; window length: 0.003 s; duration: 40 ms). **b** No sound produced by a female during a submissive display to a male (spectrogram settings: FFT, Hanning bandpass filter 100 Hz to 3 kHz; window length: 0.003 s; duration: 2 s)

Results

Visual observations

In competitive trials, males displayed typical visual displays of aggression and submission and engaged in frequent physical contests that included bites, rams, chases, and mouth fights (see Table 1 for a detailed ethogram and Fig. 2a). When no barrier was present (Phase 2), overt aggressive displays in the form of mouth fights were frequently initiated (mean ± SEM = 0.65 ± 0.09 per min). Mouth fights lasted for a mean duration of 28.61 ± 3.05 s but in many cases lasted well over a minute (range in durations: 2–150 s). Visual behavioral displays of aggression or submission occurred with an average frequency of 2.68 ± 0.22 per min and ranged from 0 to 18 displays per minute for any given pair (Fig. 3).

We examined the effect of contest outcome for each individual (i.e., whether an individual was a winner, loser, or had tied) on the number of aggressive behaviors produced by that individual (i.e., sum of chases, bites, rams, head shakes, puffed throat displays, aggressive postures, and lateral displays) and on the total time spent in the shelter during Phase 2. A clear winner and loser were evident in 9 of 12 contest trials. Contest winners performed significantly more aggressive acts than did losers or males who tied, with no significant difference between males who lost or tied (GLMM: $Z_{2,23} = 3.99$, $P < 0.001$). Contest winners also spent significantly more time inside the shelter than did losers (GLMM: $Z_{2,23} = 3.72$, $P < 0.001$), an indication of the winner's higher dominance rank relative to the loser's.

In the courtship trials, male and female breeding pairs displayed typical visual behavioral displays

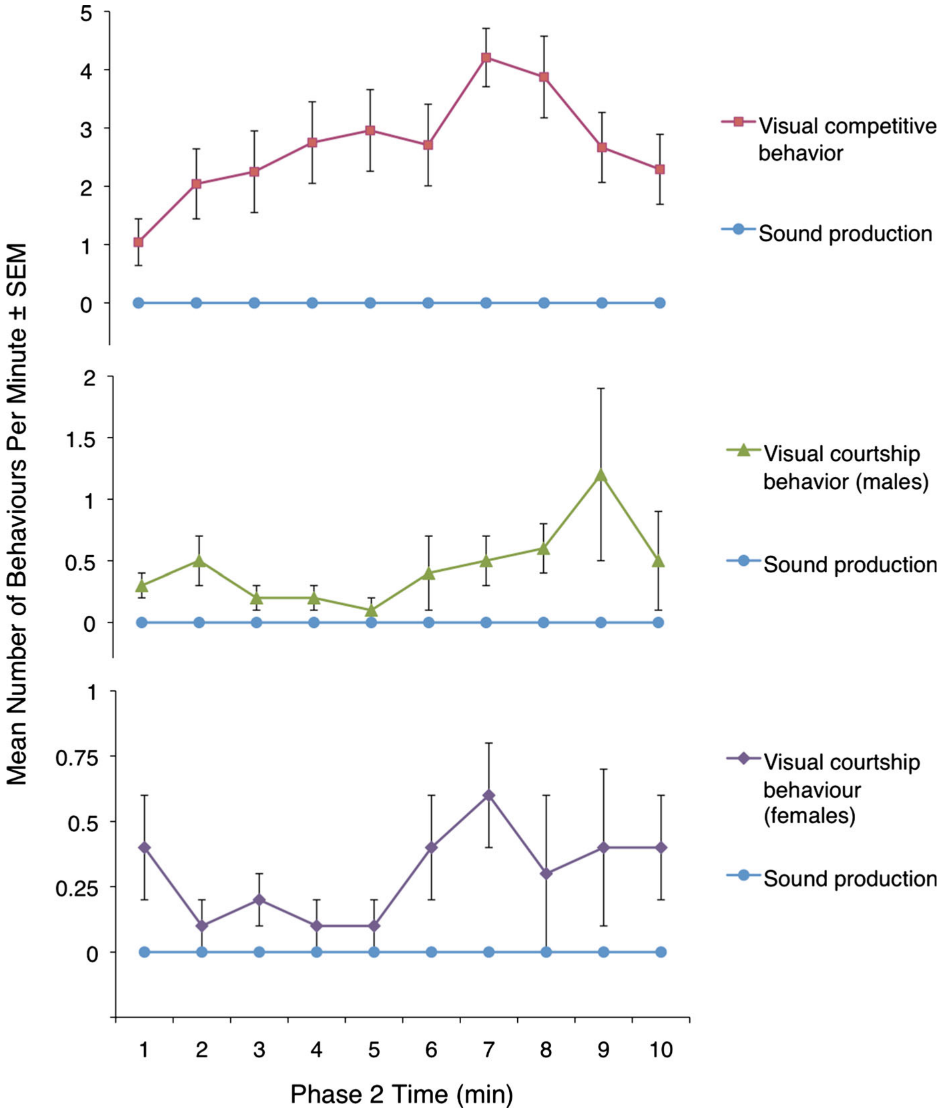

Fig. 3 Mean number of visual and acoustic behaviors per minute observed in a competitive context (*upper panel,* 12 trials, $n = 24$ males) and courtship context (*lower two panels,* 10 trials, $n = 10$ males and 10 females). *Error bars* represent the standard error of the mean. These observations were taken only during Phase 2 of the experiment when no barrier was present and the two fish could physically interact. Visual competitive behaviors shown here include mouth fights, pseudo-mouth fights, chasing, biting, ramming, puffed throat, aggressive posture, and lateral displays. Visual courtship behaviors shown here include quivering, soft touching, parallel swimming, and following (see Table 1 for details). We did not include the time spent mutually co-habiting the shelter

common in courting cichlid fish (see Table 1 for ethogram). When no barrier was present (Phase 2), courting behavior occurred with a frequency of 0.95 ± 0.09 acts per min in males and females combined. Quivering and soft touching were the most frequently exhibited courtship behaviors and were observed on average 0.24 ± 0.11 and 0.21 ± 0.04 times per min in males and 0.17 ± 0.05 and 0.13 ± 0.03 times per minute in females, respectively (see Fig. 3). Males and females mutually entered the shelter on average 0.20 ± 0.03 times per minute and spent on average 14.87 ± 2.8 s per min together in the shelter. Parallel swimming or following was observed on only a small number of occasions. Aggressive and submissive displays (0.28 ± 0.1 per min) were also observed between the breeding pairs.

We examined whether males and females differed in the number of courtship behaviors produced in Phase 2 (i.e., sum of quivers, soft touches, follows, and parallel swims). Frequency of courtship behavior did not differ significantly between males and females (GLMM: $Z_{1,19} = 0.68$, $P = 0.49$). Parallel swimming and following were observed infrequently; however, removing these two behaviors from our sum of courtship behavior did not affect the pattern or significance of our results.

Acoustic analyses

We analyzed a total of 392 min of acoustic recordings obtained from competitive and courtship trials using the methodology and criteria optimized in other studies of cichlid sound production (Amorim et al., 2004; Bertucci et al., 2010; Verzijden et al., 2010; Maruska et al., 2012). We paid particular attention to audio timeframes that coincided with visual behavioral displays (e.g., quivers) or when fish were within a 12.5 cm attenuation distance from the hydrophone (Akamatsu et al., 2002). Audio recordings were examined both before (frequency range of analysis: 1 Hz to 10 kHZ) and after (frequency range of analysis: 100–3,000 Hz) applying a bandpass filter for noise reduction. We found no evidence of vocal sound production by males or by females in either of the social contexts (Figs. 2, 3).

Although we did not detect overt vocalizations in *N. pulcher*, we picked up many incidental sounds produced by the fish's body movements and by other disturbances within the aquaria. These included, for example, sounds produced by one fish contacting another fish or an object while the fish were mouth fighting, ramming the barrier, or entering/exiting the shelter. We also recorded sounds produced by displacement of sand during rapid fin movements or mouth fighting, and those produced by the rapid turns of the fish's bodies. These incidental sounds demonstrate that our acoustic set-up was able to pick up a variety of sounds and that we would have been able to detect fish vocalizations had they been produced. To illustrate, Fig. 2 presents video frames with corresponding waveforms and spectrograms recorded (a) during an aggressive encounter between two males in a competitive trial, where an incidental sound was produced as one male rapidly maneuvered to escape the bite of the other, and (b) during a quivering bout produced by a female toward her mate in a courtship context, where no sound was produced.

Discussion

Although *N. pulcher* displayed species-typical visual displays of aggression and submission during contests and visual courtship behavior between breeding pairs, we found no evidence of social vocal sound production in either male or female *N. pulcher*. The intensity of the visual displays was comparable to what has been observed in previous studies of aggression (Buchner et al., 2004; Taves et al., 2009; Reddon et al., 2011; Hick et al., 2014) and courtship (Desjardins et al., 2008; Wong et al., 2012) in *N. pulcher*.

In other cichlids that communicate acoustically, it is courting or aggressing males that most frequently produce the sounds. Acoustic displays often occur in conjunction with visual agonistic and reproductive displays such as quivers or chasing (Myrberg et al., 1965; Amorim et al., 2004, 2008; Smith & van Staaden, 2009). However, some studies have found that cichlid vocalizations can occur independently of visual displays, and thus, vocalizations have been proposed to function in part to inhibit or avert aggressive physical behavior before it occurs (Schwarz, 1974, 1980; Bertucci et al., 2010). Hence, to maximize the likelihood of eliciting sound production in *N. pulcher*, acoustic assays in this study were taken in three different social scenarios: situation 1, in which fish could neither see nor physically interact with a conspecific (opaque barrier); situation 2, in which fish could see one another but could not physically interact (clear barrier); and situation 3, in which fish could both see and physically interact with one another (no barrier). Although the degree of possible visual and physical interaction varied across these social scenarios, acoustic communication between the fish was always possible but was not detected.

Some studies exploring cichlid sound production during courtship have housed one or two males with one or several receptive and novel females (e.g., Amorim et al., 2004). In contrast, in our study, we chose to reunite an already bonded breeding pair following a short separation (Schwarz, 1980). Such a pair-reunification paradigm is a salient and ecologically valid courtship assay for *N. pulcher*, in which a dominant breeder frequently returns to its mate after a feeding bout in the water column (Taborsky & Granter, 1998). Also, unlike most mouth-brooding cichlids where females choose a mate among courting males, *N. pulcher* achieves breeding status by inheriting or taking-over a territory that already typically contains a mate (Wong et al., 2012). This limits the scope for mate choice. Schwarz (1980) found that vocalizations in breeding pairs of biparental Central American cichlids typically accompanied or preceded aggressive interactions between the pair, such as mouth fighting and biting, but did not occur before or alongside affiliative or courting displays. In our study, we did observe both affiliative and aggressive visual behaviors between the breeding pairs but no vocal signals.

Special care was taken to acclimate the fish, minimize environmental noise, and prevent acoustic distortion during sound recording. Our analyses and set-up were based on methods used in previous studies (Akamatsu et al., 2002; Amorim et al., 2004; Amorim & Almada, 2005; Amorim et al., 2008; Simões et al., 2008; Smith & van Staaden, 2009; Bertucci et al., 2010; Verzijden et al., 2010; Maruska et al., 2012). Thus, it is unlikely that we did not detect *N. pulcher* vocal production in this study due to methodological constraints. It is also unlikely that our audio recordings were simply too brief to detect vocalizations. We recorded sounds for the same amount of time as previous studies that found evidence of sound production in other cichlid species. In those studies, fish produced sounds on average 20–25 times and as often as 50 times per 20-min trial (see, e.g., Amorim et al., 2004; Amorim & Almada, 2005; Amorim et al., 2008; Bertucci et al., 2010; Maruska et al., 2012).

We detected incidental sounds during recordings of the competitive and courtship trials. These incidental sounds were produced by physical contact during mouth fights, bites and rams, the displacement of sand in the aquarium due to rapid body movements, or physical contact with the barrier (see, e.g., Fig. 2a). This provides further evidence that our experimental set-up was appropriate for the detection of sounds at various frequencies, including those frequencies that characterize the vocalizations of other cichlid species. Although most cichlid species that vocalize produce sounds at frequencies between 100 and 1,500 Hz with peak frequencies below 500 Hz (e.g., *Astatotilapia burtoni*, Maruska et al., 2012), the full spectrum of cichlid sounds can range anywhere from 50 to 15,000 Hz (for review see Lobel, 2001 and Table 3.2 in Amorim, 2006). The incidental sounds that we detected are likely to be byproducts rather than true communication signals; however, it is worth noting that the sounds may nonetheless be ecologically or biologically relevant to the fish.

Conclusion

Our findings contribute to a growing literature on the evolution of acoustic signaling in teleost fish. To better understand the evolutionary trajectory of acoustic communication in cichlids, it is essential to determine both the origin and the social context selecting for sound production in this diverse family of fish. The complex social lifestyle of *N. pulcher* makes this species an obvious candidate for additional modes of communication. Indeed, the *social complexity hypothesis* predicts that highly social species will exhibit analogously complex communication systems (Freeberg et al., 2012). However, in our study, we provide the first evidence that males and females of the cooperatively breeding cichlid *N. pulcher* do not produce sounds in two socially relevant contexts.

Divergent selection on vocal signals may have contributed to the reproductive isolation and speciation of African cichlids (Amorim, 2006). Most of the African cichlids in which we do observe vocal communication vocalize in a mating context where males multiply mate, interacting with females only briefly, and in which females provide parental care alone. There may be multiple origins of sound production in cichlids, as some recently derived African species as well as New World cichlids produce sounds (Myrberg et al., 1965; see also Schwarzer et al., 2009), and of course, we cannot rule out the possibility that *N. pulcher* have gone silent secondarily. However, our findings suggest that sound did not evolve to facilitate more accurate or efficient communication among group-living cichlids. Future empirical work should investigate whether any close relatives of *N. pulcher* communicate acoustically. Ideally, sound production will be investigated in group-living cooperative species as well as more solitary cichlid species.

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