Sounds of male Lake Victoria cichlids vary within and between species and affect female mate preferences

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Sound production in fish is widespread and occurs in several contexts, such as species recognition, mate choice, and aggression. However, there is little experimental evidence for the importance of acoustic signals in social contexts and the influence of sound on mating decisions of females. Cichlid fish are known for their bright nuptial coloration, which plays an important role in mate choice and reproductive isolation between the many species of cichlid fish in East Africa. They also produce sounds in both aggressive and courtship interactions. In this study, we show that the sounds produced by males of Lake Victoria cichlids are species specific. There is also a correlation between fish size and peak frequency of sounds across species. We did not find context-dependent differences within a species (*Pundamilia nyererei*) between male sounds produced during aggressive displays toward males or sexual displays toward females. We also show with playback experiments that courtship sounds influence the mate preferences of female cichlids. In combination with many studies in the literature on visual signaling, our results suggest that multimodal communication plays an important role in sexual selection in cichlids. *Key words:* cichlid, fish, mate choice, playback, sound, species specific. *[Behav Ecol 21:548–555 (2010)]*

M ate-choice decisions are often, if not always, influenced by multiple signals from the opposite sex. The multimodality of mating cues has been suggested to serve in conveying different aspects of mate quality (Moller and Pomiankowski 1993; Johnstone 1996) and may also operate as a signal backup for masking noise in one modality (Iwasa and Pomiankowski 1994; Johnstone 1996). Multiple signal components in general may increase detection probability and thereby expand the potential number of mates (Rowe 1999) and can enhance the receiver's motivation to respond (Partan and Marler 1999). To understand mating decisions and how they may influence processes such as sexual selection and reproductive isolation, it is important to take all sensory modalities of communication signals into account.

In cichlid fish, mate choice is an important factor in the evolution of their large numbers of species (Kornfield and Smith 2000; Kocher 2004; Seehausen et al. 2008). Especially in the East African lakes, many closely related species occur in sympatry, and several studies have addressed species-specific mate preferences based on visual (Genner and Turner 2005; Seehausen et al. 2008) and chemical (Plenderleith et al. 2005) signals. In addition to visual and chemical signals, several cichlid species are known to produce sound, which might also play a role in mating decisions and reproductive isolation (Amorim et al. 2008) but which has not been explicitly tested.

Many fish species produce sounds, and the number of species for which sounds has been described is steadily increasing (i.e., Ladich 1997; Ramcharitar et al. 2006). Sound production appears to be widespread among fish in many social contexts, such as shoaling, feeding, and territorial and courtship behavior (Brantley and Bass 1994; Ladich 1997; Myrberg and Lugli 2006; de Jong et al. 2007). The use of sound in the context of courtship indicates that sound production might influence mate choice, similar to the role of sound in well-known systems such as birds (Catchpole and Slater 2008; Byers and Kroodsma 2009), frogs (Ryan 1991), and flies (Klappert et al. 2007).

Fish sounds are often species specific (Amorim et al. 2004, 2008; Lamml and Kramer 2006; Parmentier et al. 2006) or even population specific (Lamml and Kramer 2007; Phillips and Johnston 2008). Several studies have also shown that various fish species can discriminate between own species' sounds and other species' sounds or synthetic stimuli (Myrberg and Spires 1972; Lugli 1997; Lobel 2001; Lugli et al. 2004; McKibben and Bass 1998, 2001; Rollo and Higgs 2008) and in some cases even recognize individuals by their sound (Myrberg and Riggio 1985). These aspects of sound production and perception strongly suggest that fish sounds may be used in species recognition and mate selection. However, so far, playback studies have only measured phonotactic responses, which are often a good proxy for discrimination or preferences, but validation is needed in an actual mate-choice context (e.g., Ryan 1985; Holveck and Riebel 2007). To date, no playback study in fish has shown that sound can influence mate choice among live and visibly present conspecific mates.

Studies in Lake Malawi cichlids revealed interspecific differences in temporal aspects of male sounds, such as in the repetition rate of pulses (Lobel 1998, 2001; Amorim et al. 2004,

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2008). Species differences in frequency use have also been found in some cases (Amorim et al. 2004) but always as a covariate with body size, limiting the interpretation for species specificity. There are many similarities in the evolution of life history and secondary sexual traits between cichlids from the rift lakes of Africa (e.g., Kornfield and Smith 2000; Duponchelle et al. 2008). It is therefore likely that species differences in sound production, such as found in Lake Malawi, also exist in Lake Tanganyika (Nelissen 1978) and Lake Victoria (Myrberg et al. 1965).

Cichlid fish produce sounds in aggressive and courtship contexts during the so-called quiver display, although not with every quiver (Myrberg et al. 1965; Nelissen 1978; Amorim et al. 2004, 2008; Amorim and Almada 2005; Simoes et al. 2008). The quiver involves fast shaking movements, while extending all fins in close proximity to another individual. The sounds produced during the quiver can be described as a train of rapidly repeated pulses (Myrberg et al. 1965; Nelissen 1978; Lobel 1998; Amorim et al. 2004). Although context-dependent acoustic variation between aggression and courtship has been reported for these pulse trains, it is currently unclear whether this is a consistent phenomenon across species (Simoes et al. 2008).

Our study involved a descriptive and an experimental part. First, we tested if acoustic signals show species specificity and allometric relationships in 3 species of Lake Victoria cichlids. We found distinct temporal variation among species, while spectral variation correlated to body size within species. In one species, we tested for context-dependent variation, but we did not find differences in temporal or spectral features between sounds generated in aggressive or courtship interactions. Second, we tested the effect of male sound playback on the courtship decision of a female choosing between 2 live males and show that male sound production can positively influence female mate preferences.

MATERIALS AND METHODS

Housing

All tanks, both housing and experimental, were connected to a central recirculation water filter system and had sand substrate. Water temperature was 24 ± 1 °C, and the light regime was 12:12 h light:dark. Fish were fed once daily with fresh shrimp and peas or commercial pellets and flakes. Prior to experiments, males were housed either in groups ranging from 10 up to 15 individuals, or individually, in stock tanks (tank size 1.0 × 0.4 × 0.6 m). Females were housed in groups of 12 or more females (up to 20) in the same type of stock tanks. Prior to mate-choice experiments, individual fish were isolated for 48 h in small tanks (0.25 × 0.25 × 0.1 m).

Sound recordings

All sounds were recorded from laboratory-reared fish that were F1 or F2 descendants from wild-caught individuals from Makobe Island, in Lake Victoria, Tanzania (Seehausen 1997). All recordings were made in the same experimental tank (dimensions 2.0 × 0.4 × 0.45 m, water level was at 0.4 m), in which focal males were housed for at least 24 h before a recording session started. Males were triggered to show quiver displays and produce sounds by introducing either a male or a female conspecific individual in a Plexiglas cylinder in the tank (diameter 10 cm). Presentation of a male stimulus induces a territorial context, and presentation of a female yields a courtship context. The typical context-specific behavior of the focal male (initially described in Bearents and Baerents-van Roon 1950; as defined in Verzijden et al. 2008) was confirmed through monitoring by the experimenter. The hydrophone was suspended in the tank 3–4 cm above the sand substrate and about 10 cm away from the cylinder. A total of 11 *Pundamilia nyererei*, 11 *Pundamilia Pundamilia*, and 9 *Neochromis onmicaeruleus* males were recorded. For 9 *P. nyererei* males, we recorded sounds in both courtship and aggressive context so that we could compare these sounds pairwise within this species. We recorded at least 10 sounds of each male in each context (mean # sounds in courtship 16.7 (± 2.0 standard error of the man [SEM]), mean # sounds in aggression 15.7 [± 1.9 SEM]).

We used an HTI 96-min hydrophone (High-Tech, Inc, Gulfport, MS) with a 20-dB amplifier and recorded on a digital recorder (Nomad Jukebox 3, Creative, Singapore) in WAV-format with a 44.1-kHz sampling rate. Sound recordings were individually saved and analyzed. We used Praat (Boersma and Weenink 2008) to measure the temporal parameters of the
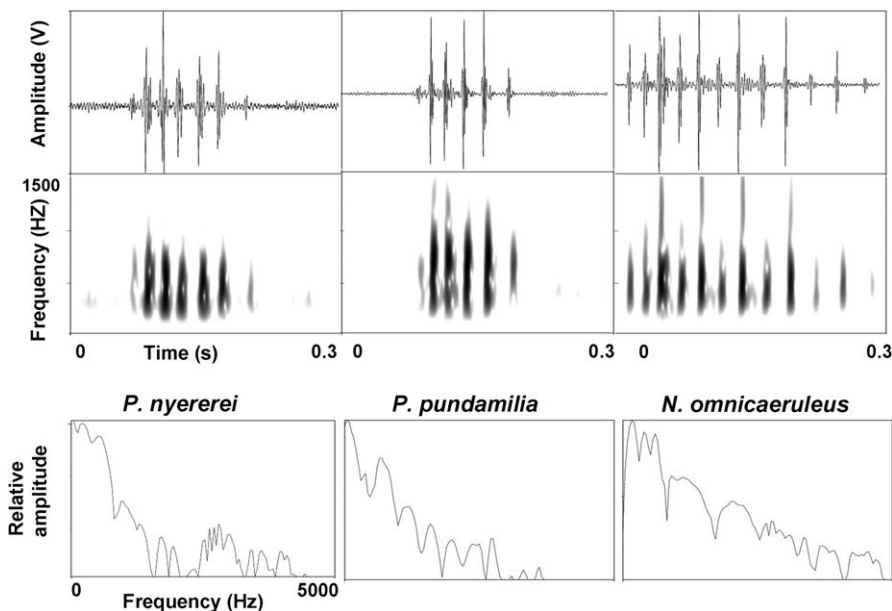

Figure 1
Oscillogram (top) and corresponding sonogram (middle) and frequency spectrum of a sound of *pundamilia nyererei*, *pundamilia pundamilia*, and *Neochromis omnicaeruleus* (from left to right). The frequency spectrum is in each case based on the third pulse of the shown sound. Peak frequencies refer to the frequency of peak amplitude from the accumulated energy over the entire period of one pulse.

typically repetitive pulses of noisy broadband sounds. We high-pass filtered the recordings at 200 Hz and counted and measured the onset of each pulse in a particular pulse train from the waveform (see Figure 1). Each pulse has a clear onset of sound (sudden increase in amplitude) after which the pulse gradually fades out. Pulse onset is therefore the most reliable measurement. We calculated the pulse period: time from the onset of one pulse to the onset of the next pulse and the sound duration: the time from the onset of the first pulse to the onset of the last pulse in a pulse train. Note that because of measurement accuracy, we decided to use a slightly different measure for sound duration and pulse period than used in other papers on cichlid sound production (e.g., Nelissen 1978; Lobel 1998; Amorim et al. 2004, 2008; Simoes et al. 2008). We used Luscinia (Lachlan 2007) to measure the peak frequency (from unfiltered recordings), which is the frequency with the highest amplitude within a pulse. We could not detect any fundamental frequency or harmonic structure in the "noisy"y' sounds produced by the males in this study.

Playback stimuli

We selected 3 calls per male from audio recordings of 10 different *P. nyererei* males for playback in the mate-choice experiment. We edited these recordings such that the stimuli were void of background noise immediately preceding and after the sound. We generated playback stimuli that consisted of 6 different calls originating from 2 males, digitally normalized to equal peak amplitude and played in random order. Each playback stimulus set was unique in being another combination of 2 males from the set of 10, reducing the impact of pseudoreplication and improving the external validity of our experiment (Slabbekoorn and Bouton 2008). We used an ING 32mV amplifier with an MR23333-000 waterproof speaker (Knowles Electronics, Itasca, IL) connected to a desktop computer. We counteracted the low-frequency cut-off speaker features by raising the amplitude up to 340 Hz, by 20 dB, using the bass boost in Audacity (www.audacity.sourceforge.net). We did not explicitly assess playback level, but playback stimuli were audible with a hydrophone at the location of the female

and of similar amplitude and quality compared with live males calling nearby.

Sound recordings in small tanks are affected by tank-specific resonance features (Akamatsu et al. 2002), and given the dimensions of our experimental tank, amplification phenomena are to be expected at or above 2678 Hz. Our playback stimuli consisted of rather noisily structured fish recordings with most of the sound energy distributed well below this frequency. Therefore, we assume that tank-wall reflections do not prevent the perception of directional information and that female fish are able to assess the tank side from which direction they hear a sound (cf. Rollo et al. 2007; Rollo and Higgs 2008).

Mate-choice trials

We divided the experimental tank into 3 compartments with the use of 2 grids (mesh size 160 mm × 160 mm) (see Figure 2). This allowed the females to pass through but confined the larger males to their compartments. We placed a male in each outer compartment at least 24 h prior to the experiment. Males were size matched (mean difference in standard length: 1.6 mm ± 0.5 SEM, 6 male pairs), and all males showed full nuptial coloration. At least 18 h before the introduction of the female in the setup, sound was played back to the males to habituate them to playback of sounds, in each of their compartments. We played back the same sound stimuli that they would hear later during the mate-choice trial for a total of 30 min. This playback sequence was repeated for a second time right before we introduced the female into the tank. We used 14 different females, of which 10 were tested twice, leading to a total of 24 trials. The second time a female was tested, she was presented with a different set of live males, and the speaker was placed on the other side of the tank compared with her first experiment. Each male pair was used 4 times, with both males associated with sound twice.

We placed a female cichlid in the middle compartment, after having inserted opaque dividers along the grids, so the female could not see either male. After this, we played back a series of sounds, 3 times for 20-min exposure in total, from one side,
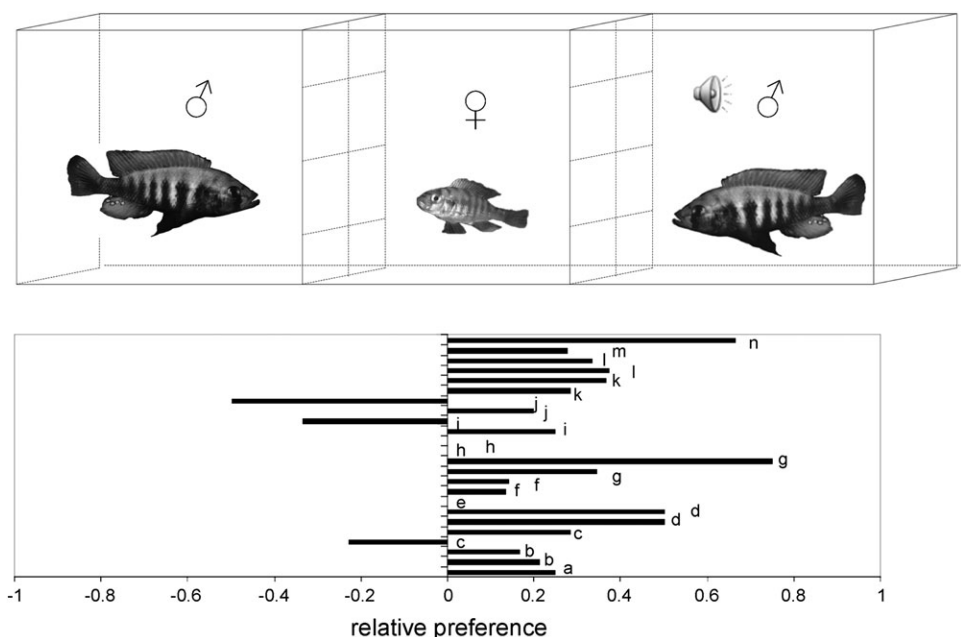
Figure 2
Setup of playback experiment (top), with size matched males on either side of the female, and a speaker in one of the male compartments. Bottom: Relative preference of females per mate-choice test after playback of male sound. Positive values indicate that females preferred the male with sound playback over the male without sound playback. In 3 trials, the "preference score" was 0. Letters arbitrarily indicate female identity.

after which the blinds were removed and the female could see both males and interact with the male on the side of her choice. The female was thus presented with a male on both sides, one courting in a silent compartment and the other courting in a compartment from which she had just heard a series of con-specific male sounds. We scored the courtship interactions between the female and the males for 30 min, as described in Seehausen and van Alphen (1998); courtship interactions are predictive of mate choice in East African cichlids (Seehausen 1997; Egger et al. 2008). We distinguished the following behaviors: male lateral display, male quiver and male lead swim, and female approach after lateral display or quiver, and female follow a male lead swim (see also Verzijden and ten Cate 2007). The presence or absence of a female approach response to male quiver turned out to be the most stereotypic interaction and was used to quantify response strength and side preferences. We detected calling activity by one of the test males during one of the trials, and we therefore, discarded this experiment from the analyses and repeated this trial with another set of individuals.

Statistical analyses

Differences in sounds between species were analyzed using Linear mixed models, with standard length and species as fixed effects, and individual identity as a random effect nested in both fixed effects. Additionally, in the case of the parameters pulse repetition time and peak frequency, we also nested sounds within individuals (in order to account for the multiple measures within one sound). We obtained the minimal adequate model by stepwise deleting nonsignificant interactions and main effects but always retaining the nested structure (Nelder and Wedderburn 1972). We checked the models for the underlying assumptions about distribution and variance. Statistical software used was Systat12 (Chicago, IL).

For the playback experiment, we calculated a relative approach ratio as follows: (number of approaches to male with sound/number of quiver displays by male with sound) − (number of approaches to male without sound/number of quiver displays male without sound). This gave us a value between −1 and 1 per experiment, with a positive value indicating a preference for the male associated with the sound, and a negative value indicating a preference for the male without sound. We then performed a nested one-sample t-test to test if females preferred the male on the side of the sound playback (ten Cate et al. 2006). We nested the experiments per male stimulus pair, to correct for any systematic differences in the attractiveness asymmetry within stimuli pairs. This is a conservative way of testing, because we test at the lowest level of replicates, while accounting for repeated measurements. Testing at the level of individual females, with repeated tests nested within females, yields very similar results. Additionally, we used a paired t-test to check if males at a side associated with sound courted more than males at a silent side and if the minor size difference between males affected female preferences.

RESULTS

Species- and size-dependent variation

We compared the number of pulses, the pulse period, sound duration, and the peak frequency of the sounds of 11 *P. nyererei* males, 12 *P. pundamilia*, and 9 *N. omnicaeruleus* "males" (mean: 22.6, ranging from 8 to 79 sounds per individual).

For the number of pulses per sound, we found significant differences between the species $F_{(2,29)} = 6.273, P = 0.005$), but we did not find a significant correlation with standard length

$F_{(1,26)} = 2.950, P = 0.1$). With a Tukey honestly significant difference (HSD) post hoc test, we determined that *N. omnicaerulaeus* differed significantly from *P. nyererei* ($P = 0.004$) but that *P. pundamilia* was not significantly different from either of the other species (mean ± SEM: *P. nyererei* = 5.8 ± 0.4; *P. pundamilia* = 6.5 ± 0.4; and *N. omnicaeruleus* = 7.8 ± 0.4).

For pulse period (figure 3A), we found significant differences between the species ($F_{(2,29)} = 51.5, P < 0.001$). With a Tukey HSD post hoc test, we determined that each species is significantly different from each other (all pairwise comparisons significant at the $P = 0.001$ level). Although small ($0.04 ± 0.01$ ms/mm), there was also a significant correlation between pulse repetition time and standard length: $R^2 = 0.317, F_{(1,31)} = 6.7, P = 0.014$.

For sound duration, we tested for a species effect and a covariance with the number of pulses. We found a significant interaction between species and the number of pulses ($F_{(2,26)} = 7.03, P < 0.01$), while the main effects "number of pulses," and species were also significant ($F_{(1,26)} = 3642.16, P < 0.001$, respectively, $F_{(2,26)} = 217.77, P < 0.001$). This indicates that although the number of pulses largely determines the length of the sound, the pulse repetition time slows progressively within a sound and does so differentially between the species. Tukey HSD showed that *N. omnicearuleus* was significantly different from both other species ($P < 0.001$) but that *P. pundamilia* and *P. nyererei* were not significantly different from each other. The regression values (±SEM) were $0.022 ± 0.0007$ for *N. omnicearuleus* and $0.016 ± 0.0005$ for *P. pundamilia* and *P. nyererei* (R^2 model = 0.94).

For peak frequency (Figure 3B), we found no species differences ($F_{(2,29)} = 2.4, P = 0.11$). There was, however, a significant negative regression with standard length: $R^2 = 0.98, F_{(1,30)} = 69.6, P < 0.001$. The regression equation (±SD) is peak frequency = $995.1(±44) − 4.9(±0.5)$*standard length.

No context-dependent differences

We detected no differences in sound parameters between the 2 contexts of aggression toward other males and courtship toward females in *P. nyererei* males (Figure 4). (Pulse repetition time: $t_8 = 1.80, P = 0.11$; Sound duration: $t_8 = 1.63, P = 0.14$; Peak frequency: $t_8 = 0.21, P = 0.84$; and number of pulses: $t_8 = 1.43, P = 0.19$).

Female preferences for males with sound

Female cichlids always explored both male compartments within the 30-min observation period, typically entering the first within a few minutes after the dividers were taken out (mean: 49.9 s ± 21.9 SEM). Females always encountered both males and also engaged in courtship interactions for a variable amount of time with one or both of them. Males typically displayed the quiver display repeatedly (mean: 8.9 per trial ± 2.5 SEM), part of which were answered by female approach (mean: 5 per trial ± 1.7 SEM). Females significantly preferred males that were associated with sound, as expressed by the relative number of approaches in response to male quiver displays, when tested at the level of male stimulus pairs: $t_5 = 4.64, P = 0.006$ (figure 2) and when tested at the level of individual females: $t_{13} = 3.16 P = 0.006$. Females did not choose to enter either males' compartment first, 14 of 24 females visited the male with sound first ($\chi^2 = 0.66, P > 0.5$). Courtship display activity of males at the side associated with sound was not different from the activity of males at the silent side: paired t-test: $t_{23} = 0.45 P = 0.65$ (mean # quivers of male on silent side 7.63 (±3.55 SEM), mean # quivers of male on playback side 9.96 (±3.39 SEM), and male standard length did not affect female preferences $t_{23} = 0.533 P = 0.59$ (mean
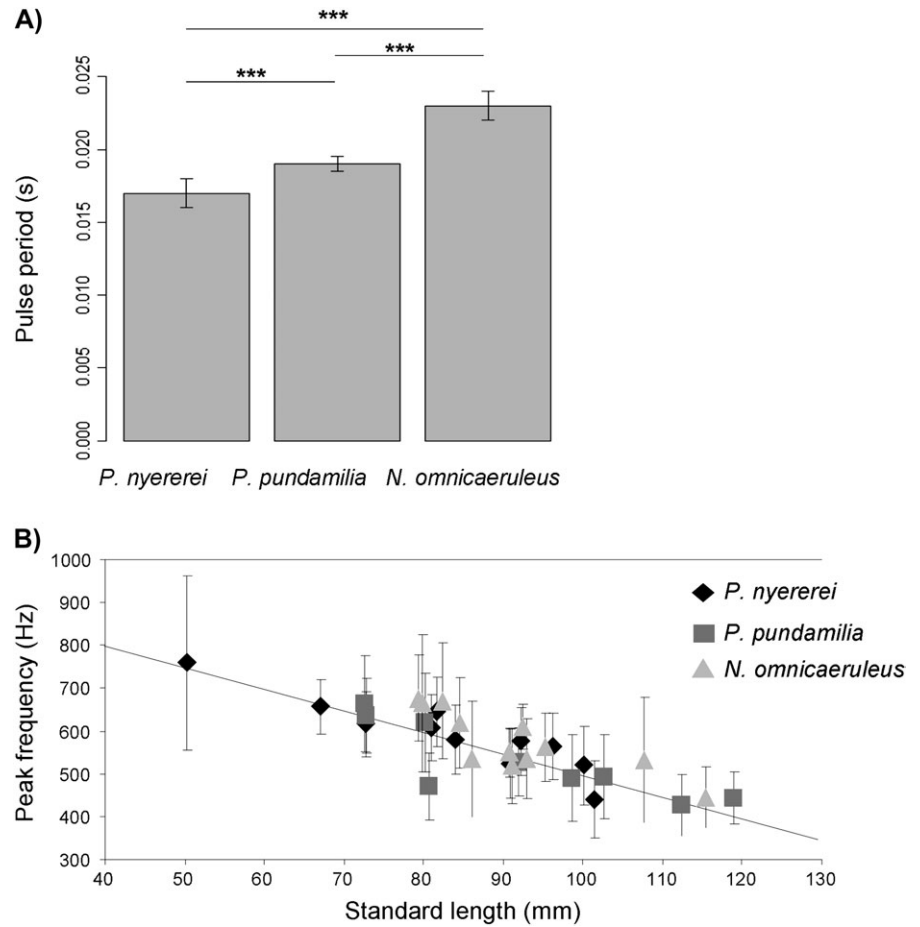
Figure 3
(A). Species differences in temporal measurements, lines with asterisk indicate significant differences ($P < 0.001$). (B) Correlation between body size and peak frequency of sound in all 3 species (means ± SD).

approach ratio to larger male 0.19 (±0.04 SEM), mean approach ratio to smaller male 0.23 [±0.05 SEM]).

DISCUSSION

Our results show that the sounds of males from 3 sympatric species of cichlids from Lake Victoria differ significantly in the repetition rate of the pulses within a sound, the number of pulses in a sound, and in covariance between the number of pulses and the duration of the sound but not in peak frequency. The peak frequency of the sound is negatively correlated with male size in all 3 species. Although we cannot exclude that sounds may vary with context when 2 individuals would be swimming around freely, we found no differences for sounds that male *P. nyererei* produced during aggression and courtship

toward individuals in a Plexiglas container (the other 2 species were not tested for this). Finally, we also show that the presence of conspecific sounds influences female choice when selecting one male over another to court. Males that were associated with playback of sounds were preferred over males that were not associated with sound. These playback results provide, to our knowledge, the first experimental evidence for sounds to affect mate preferences in any fish species.

Fish sounds vary with species and size

Our descriptive findings on sound structure and interspecific variation show a strong parallel with findings in Lake Malawi cichlids. The sound that is produced in conjunction with the lateral visual display in courtship and aggression appears
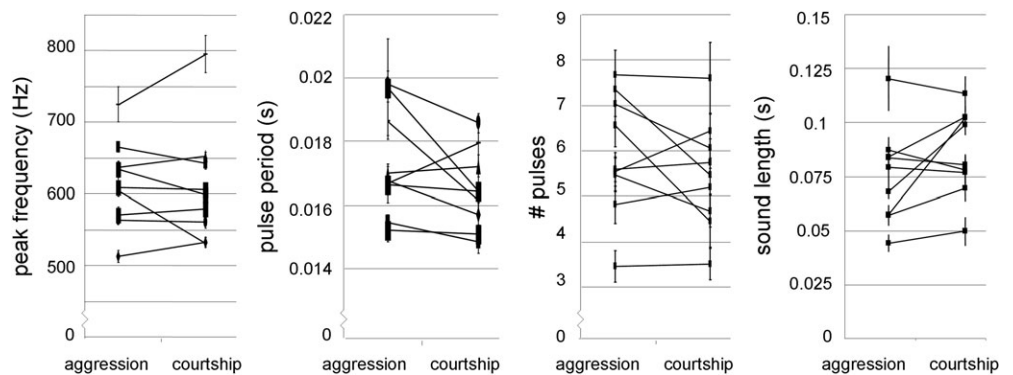
Figure 4
The lack of context-dependent acoustic variation illustrated by the means (± standard error) per male in both recording contexts for the 4 measurements. Lines connecting means indicate which were of one male.

to be similar in all East African species: rapidly repeated pulses (11–50 pulses/s) with a relatively low peak frequency (±500 Hz) (Lobel 1998, 2001; Amorim et al. 2004, 2008). Closely related, co-occurring species of cichlids from Lake Malawi also show differences in the same 2 temporal measurements as the species in our study, pulse repetition time, and the number of pulses in a sound (Lobel 1998; Amorim et al. 2004, 2008). The species differences in sound duration we found in our study on Lake Victoria cichlids were not found in other studies on cichlids (Lobel 1998; Amorim et al. 2004, 2008). Species differences in sound production of Lake Tanganyika cichlids have not been studied quantitatively but may also show similar differentiation in temporal aspects (Nelissen 1978). The cichlid species flocks of East Africa thus not only show many parallels in their morphological diversity, life history, nuptial color diversity, and behavior (Kocher et al. 1993; Duponchelle et al. 2008; Salzburger 2009) but also in acoustic signals.

Species differences in sound production could be correlated with trophic specializations and may result in a linkage of ecological specialization and mate choice (as suggested for birds: Slabbekoorn and Smith 2000; Podos 2001). However, it is as yet unclear what the sound production mechanism in cichlids is. Some have suggested that stridulation of the pharyngeal jaws produces the sounds (Rice and Lobel 2002, 2003), perhaps analogous to the stridulation of the oral jaws in clown fish *Amphiprion clarkii* (Parmentier et al. 2007). If this is the sound-producing mechanism, species-specific trophic specializations of bones and muscles are likely to affect acoustic features. However, we need more knowledge on the sound-producing mechanism in cichlids to draw any conclusions. Such knowledge may also reveal how the observed relation between body size and peak frequency is brought about. A relation between size and sound frequency is known for other animals groups, such as birds (cf. ten Cate et al. 2002) and frogs (cf. Ryan 1991) and might be used by signal receivers to assess the quality of potential competitors or mates.

Sounds affect mate preferences in fish

Our experimental data clearly demonstrate that sounds can affect female mate preferences in fish. Female cichlids preferred to engage in courtship with males that were associated with sound playback over males that were not. The playback of the sound in one of the compartments did not appear to have changed the males' behavior or appearance. The males associated with the extra playback sequence audible to the female, in addition to the 30-min playback that both males were exposed to beforehand, did not show increased courtship behavior compared with the males on the side of the fish tank without the extra playback sequence. Nuptial coloration is mediated by testosterone, which also influences territoriality and courtship behavior (Fernald 1976; Oliveira and Almada 1998; Dijkstra et al. 2007), but both males showed full coloration at the start of the trial, as assessed by human observers. Therefore, we conclude that it was the presence of sound that increased the attractiveness of the male, either by drawing attention, or by signaling the presence of an occupied territory with a conspecific male motivated to court.

We tested females for their preference between conspecific sound and no sound. The specificity of the sound perception of haplochromine females is at present not investigated, and it could be that female preference for males is enhanced by any sound. Also, we cannot be certain that the females perceived the playback as a sound from a live male, because the speakers and the manipulation of the recordings may inadvertently have distorted the sound. However, Estramil N, Bouton M, Verzijden MN, Hofker K, Riebel K, and Slabbekoorn H (unpublished data) found that *P. nyererei* females discriminate

between white noise and conspecific sounds played back through the same setup as used in this study. Future studies could also address whether females prefer males associated with conspecific sound over heterospecific sound or sounds of big males over sounds of small males.

A sound-producing male is indeed either defending his territory or courting another female (Simoes et al. 2008), and sound could therefore be an important indication of his motivation to mate. The females in our experiment heard the sound before they could see the males. The sound thus influenced the female's mate preference before she assessed the live males visually. This raises interesting hypotheses about female mate-choice behavior. Males produce the sound only while making a quiver display at short distance from another individual, either male or a female. If female preference is affected by the sound she has heard from an interaction between 2 other individuals, this means that sound communication in cichlids could involve more than short distance private signals, and females may be eavesdropping, on fighting or courting males (McGregor 1993; Doutrelant and McGregor 2000).

In combination with many studies in the literature on visual signaling (Van Oppen et al. 1998; Couldridge and Alexander 2002; Egger et al. 2008), our results, on intra and interspecific acoustic variation and sound-dependent mate preferences suggest that multimodal communication plays an important role in sexual selection in cichlids. It is currently unclear how signal components in each modality covary with each other and whether relationships among modalities may constrain or facilitate for example signal evolution or the process of speciation. Therefore, measuring the combined effects of all signal components across sensory modalities promises to be a fruitful and necessary avenue of future research. Importantly, the playback data provide experimental evidence for the potential of sounds in general to be important in fish reproductive decisions.

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