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Proc. R. Soc. B 2014 **281**, 20141197, published 30 July 2014

PROCEEDINGS OF THE ROYAL SOCIETY B

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Cite this article: Boyle KS, Colleye O, Parmentier E. 2014 Sound production to electric discharge: sonic muscle evolution in progress in *Synodontis* spp. catfishes (Mochokidae). *Proc. R. Soc. B* **281**: 20141197. http://dx.doi.org/10.1098/rspb.2014.1197

Received: 16 May 2014
Accepted: 8 July 2014

Subject Areas:
behaviour, biomechanics, evolution

Keywords:
Siluriformes, electrocyte, electric organ discharge, communication

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2014.1197 or via http://rspb.royalsocietypublishing.org.

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Sound production to electric discharge: sonic muscle evolution in progress in *Synodontis* spp. catfishes (Mochokidae)

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Elucidating the origins of complex biological structures has been one of the major challenges of evolutionary studies. Within vertebrates, the capacity to produce regular coordinated electric organ discharges (EODs) has evolved independently in different fish lineages. Intermediate stages, however, are not known. We show that, within a single catfish genus, some species are able to produce sounds, electric discharges or both signals (though not simultaneously). We highlight that both acoustic and electric communication result from actions of the same muscle. In parallel to their abilities, the studied species show different degrees of myofibril development in the sonic and electric muscle. The lowest myofibril density was observed in *Synodontis nigriventris*, which produced EODs but no swim bladder sounds, whereas the greatest myofibril density was observed in *Synodontis grandiops*, the species that produced the longest sound trains but did not emit EODs. Additionally, *S. grandiops* exhibited the lowest auditory thresholds. Swim bladder sounds were similar among species, while EODs were distinctive at the species level. We hypothesize that communication with conspecifics favoured the development of species-specific EOD signals and suggest an evolutionary explanation for the transition from a fast sonic muscle to electrocytes.

1. Introduction

Fishes have evolved diverse signalling and sensory mechanisms suited for underwater communication. This signal diversity is exemplified by some catfishes (Siluriformes), which are well known for their ability to produce sounds [1,2] and, in some cases, electrical discharges [3–8]. Members of several catfish families, including Ariidae, Doradidae and Mochokidae (which includes the diverse genus *Synodontis*), possess an elastic spring apparatus (ESA) [2,3,9,10]. Paired protractor muscles of the ESA insert on the transverse process of the fourth vertebra (Müllerian ramus), which is attached to the anterior swim bladder. Protractor muscle contractions may vibrate the swim bladder to produce low-frequency drumming sounds. However, limited descriptions of drumming sounds exist for the family [3,5,11], perhaps, because swim bladder sounds occur only in certain species [12]. In three *Synodontis* species, electric signals with species-level differences are produced by the motoneurons that innervate the protractor muscle [3], raising the possibility that the muscle fibres act as electrocytes. These observations suggest that the phylogenetic origin of electrogenesis in *Synodontis* arose for use in social communication by enhancing and regulating the electrical potentials that naturally accompany the production of muscle-generated sound signals [13].

The large diversity of the Mochokidae, which includes nine genera and approximately 200 spp. distributed across sub-Saharan Africa [14], is worthy of further study to examine the distribution and evolutionary significance of drumming sounds and electric organ discharges (EODs). Because evidence of EODs or sounds is lacking from several previously studied taxa, we hypothesize that some species are able to make sounds while others produce EODs. In this context, we further hypothesize differences should be observed at the level of the muscle ultrastructure. In addition,

we predicted that species that do not produce EODs would show a greater reliance on acoustic communication and thus possess lower hearing thresholds.

We assessed sound production, hearing thresholds, EOD formation and protractor muscle ultrastructure in several *Synodontis* species. We show that all species except *Synodontis nigriventris* produced low-frequency swim bladder sounds that are similar in spectral and temporal content, with the longest sound trains and lowest auditory thresholds observed in *Synodontis grandiops*. Distinctive, species-specific EODs were produced by all species except *S. grandiops*. We found that protractor muscle fibres had variable myofibril abundance, with the greatest density occurring in *S. grandiops*, which produced long sounds but no EODs, and the lowest myofibril abundance in the species that did not produce drumming sounds, *S. nigriventris*. We considered the possibility that EODs could result from action potentials occurring at the protractor muscle fibres (myogenic) or simply from the synchronized activity of the motoneurons that innervate the protractor muscle (neurogenic). EODs of most electric fishes are produced from electrocytes of myogenic origin, whereas adult apteronotid fishes possess specialized neurogenic electrocytes derived from the axons of spinal motoneurons [8,15,16], and simpler neurogenic EODs have not been described. We provide preliminary evidence that protractor muscle fibres are not vestigial, but rather function as myogenic electrocytes. We hypothesize that the need to signal conspecifics, especially in turbid waters, has promoted the evolution of electric communication. This study shows how remarkable variation of a single organ among closely related species provides a second pathway for communication.

2. Material and methods

(a) Sound and electric organ discharge recordings

Wild-caught fishes were obtained through the aquarium trade. Five species were examined for sound and EOD production: *Synodontis angelica* ($n = 3$), *Synodontis euptera* ($n = 4$), *S. grandiops* ($n = 6$), *Synodontis marmorata* (identified with the key of Fermon *et al*. [17], $n = 7$) and *S. nigriventris* ($n = 4$). Fishes were housed in community aquaria with an approximate 12 L : 12 D cycle and water temperature of 24–25°C, and fed a steady diet of frozen mussels and commercially prepared foods. Each species was tested separately in a series of experimental trials that took place over a period of approximately 1.5–3.5 h. All experiments occurred between 11.00 and 18.00. Sounds and EODs were recorded in a small aquarium (60 × 29 cm, water depth kept at approx. 20 cm) with an estimated minimum resonance of 4.6 kHz [18]. Sounds were recorded on a Tascam DR-05 digital recorder (44.1 kHz sampling rate) connected to an HTI-Min 96 hydrophone (−186.4 dB re: 1 V µPa^{-1}, frequency response 2 Hz–30 kHz) placed at the centre of the aquarium. Two stainless steel insulated wire electrodes (last 0.5 cm exposed) were placed 50 cm apart, near opposite ends of the aquarium. Electrode output was amplified differentially (A-M Systems model 3000), with a 50 Hz notch filter, a band pass of 10 Hz–20 kHz, gain was adjusted (50–1000×) to maximize signal-to-noise ratio without clipping, and output was digitized (44.1 kHz sampling) on a computer with an external sound card (Creative model SB0270; Creative Labs, Singapore) and Adobe Audition v. 2.1 software (Adobe, San Jose, CA, USA). EODs and sounds were recorded simultaneously with electrically isolated equipment to prevent electrical crosstalk. The Tascam line out was fed into a second channel of the USB sound card at the beginning and end of each experiment to synchronize recordings.

Three behavioural paradigms were used to test for sounds and EODs. During recording trials for each species, individual fish were recorded alone in the aquarium, while in the presence of one or two conspecifics, and when held in hand (less than or equal to 5 min at a time) in a consistent location at the centre of the aquarium, approximately 5 cm from the hydrophone.

(b) Hearing thresholds measurement: experimental set-up

Auditory thresholds were determined by using the auditory evoked potential (AEP) recording technique [19] with the experimental set-up described in detail by Colleye *et al*. [20], but at a temperature of 26°C.

The presentation of sound stimuli and the determination of thresholds followed the detailed description given by Parmentier *et al*. [21]. Three species (*S. angelica*, *S. grandiops* and *S. nigriventris*) were tested at 16 different frequencies: 50, 150, 300–3600 (in 300 Hz steps), 4000 and 4500 Hz. Sound levels at each frequency were presented at up to 158 dB re 1 µPa and attenuated in 6 dB steps until a threshold level was determined. Recording of evoked potentials and determination of threshold followed the same procedure description given by Colleye *et al*. [20].

(c) Protractor muscle histology ultrastructure

Fishes (*S. euptera* $n = 2$, and $n = 1$ for *S. angelica*, *S. nigriventris*, *S. marmorata* and *S. grandiops*) were euthanized with an overdose of MS-222 and the protractor muscle and epaxial muscle caudal to the head were quickly dissected. Small samples (2–3 mm^3) of muscle were immediately fixed in 2.5% glutaraldehyde. Muscle samples were postfixed in 1% osmium tetroxide, dehydrated through a graded ethanol–propylene oxide series and embedded in epoxy resin (SPI-PON 812, SPI-CHEM, Leuven, Belgium). Semithin sections (1 µm) and ultrathin sections (60–80 nm) were cut with a diamond knife on a Reichert Ultracut E ultramicrotome. Toluidine Blue-stained semithin sections were used for general histology and for orientation to target the area of further ultrathin sections. Ultrathin sections were classically stained with uranyl acetate and lead citrate, then viewed in a JEOL JEM 100S× transmission electron microscope (Zaventem, Belgium) at 80 kV accelerating voltage.

To determine patterns of myofibril area among species, muscle fibre cross sections were imaged with transmission electron microscopy (TEM) at 1000×. A second observer, unaware of the identity of each image, analysed the images with Adobe Photoshop CS5. With Photoshop, the number of pixels representing the total muscle fibre cross-sectional area (five to 17 fibres for each species) and the area occupied by myofibrils were determined. These data were used to estimate the proportion of muscle cell occupied by myofibrils.

(d) Protractor muscle function: preliminary experiment

We tested the hypothesis that protractor muscle fibres of electrogenic *Synodontis* species function as electrocytes and that electric discharges are not simply a product of synchronous motoneuron activity. Four *S. euptera*, which produce EODs when held, were used in this preliminary experiment. In experiments and controls, EODs were recorded before and after injection (over several hours) and amplitudes of all EOD pulses were determined. Flaxedil, a competitive acetylcholine antagonist, was dissolved in physiological saline along with 1.1 mg ml^{-1} of Trypan Blue to mark the injection site. In fish 1, 2 and 3, Flaxedil was injected into the protractor muscles in three different doses (1.8, 0.15 and 0.06 mg). In fish 4, an injection of Flaxedil was made in the axial musculature of the tail as a Flaxedil–control. One day after the Flaxedil–control experiment, a sham experiment was conducted with the same fish (fish 4) in which physiological saline and Trypan Blue were

injected into the protractor muscle. Animals were sacrificed after the experiment and injection sites were confirmed by location of the dye in dissected animals.

(e) Sound and electric organ discharge analysis

Sounds and EODs were analysed first visually and aurally with Adobe Audition v. 2.1. Low-frequency background noise (less than 60 Hz) was reduced with a high-pass filter Chebychev 1 (eighth order, 60 Hz). For analysis, swim bladder sounds were low-pass filtered and down-sampled (4 kHz with the high-quality setting). Onset and offset times of sound pulses and EODs, EOD burst cycle number, were determined visually from oscillograms. Custom Matlab v. 7.0 routines were used to analyse spectral features of sound pulses and EODs. Power spectra generated with 1024 point fast-Fourier-transforms on Hanning windowed data were used to determine spectral features: dominant frequency (the frequency with the highest peak); peak two, three and four (the second, third and fourth highest frequency peaks); and 3 dB bandwidth (the percentage of the 512 frequency bins within 3 dB from peak).

(f) Temporal patterns: sounds and electric organ discharges

In order to characterize the temporal patterns of sound and EOD events, criteria were set to distinguish clustered series of events (pulse or growl for sounds, EOD type for EODs) from relatively isolated events. Events were considered part of the same train when inter-event intervals were less than 1 s. For sounds and EODs, the following temporal variables were quantified: events per train, train duration, event rate and inter-event interval. EOD events for some species overlapped with subsequent events with no silent inter-event. In these instances, it was possible to calculate an event rate, but pulse interval calculations were only made when there was a silent separation between pulses.

(g) Waveform (dis)similarity: cross-correlation analyses

Cross-correlation analyses were used to assess similarity among individuals and species for sound and EOD waveforms. Pairwise cross-correlations were calculated from subsets of EOD and sound pulse data: 20 randomly chosen events per individual for each sound and EOD type. Custom Matlab v. 7.0 code was used to calculate the pairwise cross-correlation using the XCORR function (scaled between 0 and 1, min to max). EODs are phase specific, so the analysis included phase. Sound waveforms showed no obvious phase pattern, so the maximal pairwise correlation between sound waveforms was calculated. Classical multidimensional scaling was performed on the correlation matrix to visualize similarity relationships.

(h) Statistical analyses

Sound and EOD analyses were restricted to cases when individual identity was known and based on mean responses from individual fish. One-way ANOVAs were used to test for species-level differences of spike EOD features (duration, emission rate and dominant frequency), EOD amplitude, sound train features (event rate and duration), pulse features (duration and dominant frequency) and growl features (duration and dominant frequency). Student Newman–Keuls (SNK) post hoc tests were used when significant main effects were observed. Data were tested for normality and homogeneity of variance and when deviations were observed, a non-parametric Kruskal–Wallis test and Dunn's post hoc tests were used.

Principal components analysis (PCA) was conducted to determine whether variation in mean sound and EOD characteristics among individual fish followed a species-specific pattern. The PCA based on sound data incorporated pulse duration, growl duration, spectral variables and temporal variables. Two separate PCAs were conducted on EOD characteristics of individual fish, because some EOD types do not occur in trains. Thus, the first PCA used variables of EOD pulse duration, spectral features, and amplitude and the second PCA model incorporated EOD train temporal features. The variables included were: pulse interval, pulse number, pulse rate and train duration.

Differences between hearing thresholds for each species were tested with a Kruskal–Wallis non-parametric ANOVA at each frequency. Dunn's multiple post hoc comparison tests were used among species when auditory abilities were observed.

3. Results

(a) Sound production

All species examined produced pectoral spine stridulation sounds when handheld, whereas low-frequency (less than 500 Hz peak frequency; electronic supplementary material, table S1) swim bladder disturbance sounds (figure 1 and the electronic supplementary material, figures S1 and S2) were recorded from all individuals and species except *S. nigriventris*, for which no swim bladder sounds were observed. Swim bladder sounds were emitted in trains of pulses and growls (figure 1*a* and the electronic supplementary material, figures S1 and S2, and table S1) and were usually weak in amplitude relative to background noise levels. A total of 921 pulses, 109 growls and 112 trains were observed (see the electronic supplementary material, tables S1 and S2). *Synodontis grandiops*, however, produced robust pulse trains that are clearly visible on spectrograms and oscillograms of recordings.

Cross-correlation analyses of pulses and growls indicate that sound waveforms tend to be similar among species (figure 1*b*). In addition, little variation between species was observed for pulse duration (species means ranged from 16 to 39 ms, $F_{3,16} = 3.609$, $p = 0.037$; SNK post hoc tests $p > 0.05$), growl duration (means ranged from 183 to 242 ms, $F_{3,11} = 0.169$, $p > 0.05$), pulse dominant frequency (means ranged from 115 to 182 Hz, $F_{3,16} = 0.253$, $p > 0.05$) and growl dominant frequency (means ranged from 86 to 119 Hz, $F_{3,11} = 0.125$, $p > 0.05$). Thus, individual pulses and growls may provide limited species-specific information for communication.

Temporal patterns of swim bladder sounds varied among some species (see the electronic supplementary material, table S2). Event rates from *S. marmorata* were higher compared with *S. angelica* (median 3.8 versus 1.9 events s^{-1}, respectively; Kruskal–Wallis, d.f. = 3, $H = 12.819$, $p = 0.005$; Dunn's post hoc $p < 0.05$), but no differences existed among the other species (Dunn's post hoc tests $p > 0.05$). Inter-event intervals were greatest for *S. angelica* (*S. angelica* mean inter-event interval 0.44 s, all other species means less than or equal to 0.29 s, $F_{3,16} = 5.886$, $p = 0.007$; SNK post hoc tests $p < 0.05$), but not different among the remaining species. There was no statistical difference observed in the number of pulses and growls per sound train (medians for each species ranged from 4.0 to 16.5 events per train; Kruskal–Wallis, d.f. = 3, $H = 6.69$, $p = 0.083$). The longest mean sound train durations were produced by *S. grandiops* (mean duration 3.9 s, all other species means less than 1.1 s, $F_{3,16} = 7.691$, $p = 0.002$; SNK post hoc tests $p < 0.05$).

PCA that incorporated the spectral and duration features of individual sound events and the temporal features of sound trains, indicates broad acoustic similarity between species, except for *S. grandiops* which separates largely on

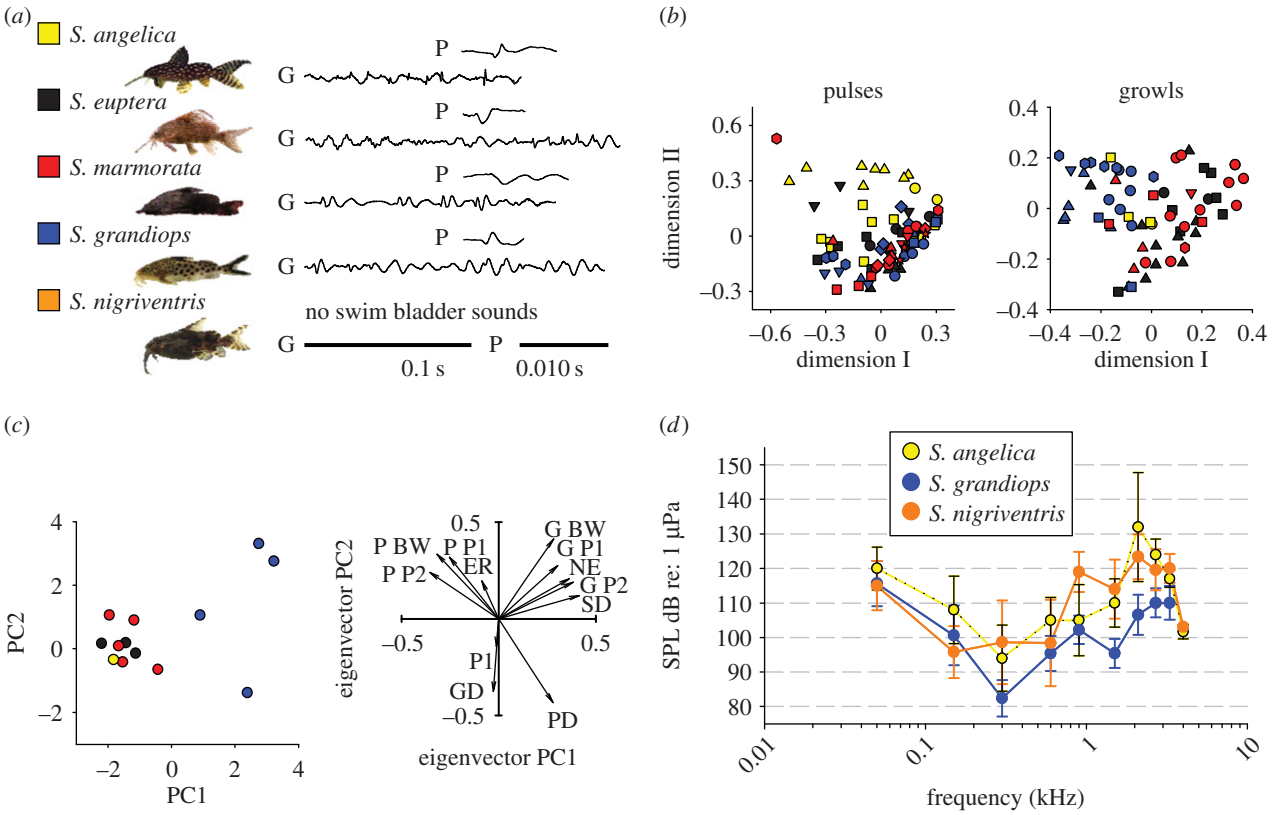

Figure 1. Similarity of *Synodontis* spp. swim bladder sounds. (*a*) Four of five species produced sounds consisting of short pulses (P) and longer growls (G). (*b*) Cross-correlation analyses show broad similarity of sounds (pulses, left and growls, right) among species. Species are indicated by different colours (indicated in *a*) and sounds from the same individuals are indicated by symbol type. (*c*) Principal component analysis based on sound characteristics (ER, event rate; GB W, growl bandwidth; GD, growl duration; G P1, growl first peak frequency; G P2, growl second peak frequency; NE, number of events; P BW, pulse bandwidth; PD, pulse duration; PI, pulse interval; P P1, pulse first frequency; P P2, pulse second frequency and SD, sound duration) indicates broad overlap among species, except *S. grandiops*, which separates mainly along principal component (PC) 1 because of TD, NE, G P1 and G P2. (*d*) Hearing thresholds of *S. angelica*, *S. grandiops* and *S. nigriventris* determined from AEPs. All three species display a broad sensitivity range to at least 3.6 kHz. Best observed thresholds were between 100 and 300 Hz and lowest for *S. grandiops*.

the basis of producing long trains with many events (figure 1*c* and the electronic supplementary material, table S3).

(b) Hearing thresholds among electric organ discharge and sound-producing species

Hearing thresholds varied among *Synodontis* species (*S. grandiops*, *S. nigriventis* and *S. angelica*) that show differences in performance of sound and EOD production. All three species have a broad range of sensitivity (figure 1*d*), but best sensitivity occurred at frequencies between 100 and 300 Hz and was lowest at 300 Hz for *S. grandiops*. This species that produces the longest sound has more sensitive hearing between 900 and 2700 Hz than the other two species (Dunn's post hoc tests $p < 0.05$).

(c) Electric organ discharges

EODs were observed in all species except *S. grandiops* (figure 2*a* and the electronic supplementary material, figure S3) and two species, *S. euptera* and *S. marmorata*, produced more than one type. A total of 1197 individual EODs and 106 EOD trains were observed (see the electronic supplementary material, tables S4 and S5). All individuals of the EOD-producing species produced EODs, except for *S. marmoratus*, for which five of seven individuals produced EODs for certain. The behavioural context of EOD formation varied among species. *Synodontis marmorata* and *S. nigriventris* readily produced EODs associated with agonistic conspecific interactions that especially occurred between approaching and contacting individuals. No swim bladder sounds were observed during such interactions. *Synodontis marmorata* was the only species observed to make solitary EODs. These spike EODs waveforms were like agonistic spikes (described below), but much weaker (*ca* less than 50× amplitude) and were not analysed further. *Synodontis angelica* and *S. euptera* only produced EODs when fish were held by an observer. When handheld, these species produced stridulations, EODs and swim bladder sounds (see the electronic supplementary material, figure S4). When observers held the pectoral spines to prevent stridulations, fish produced series of EODs and swim bladder sounds. Neither EOD trains nor sound trains preceded the other signal type in a consistent manner and EODs were more common than swim bladder sounds: 15 ± 18 (mean $\pm$ s.d.) and 12 ± 13 times more EODs than sounds for *S. euptera* and *S. angelica*, respectively. Swim bladder sounds were never completely coincident with EODs and sounds produced no measurable bioelectric signal in the aquarium.

In contrast to swim bladder sounds, cross-correlation analysis shows that *Synodontis* spp. EOD waveforms are quite distinctive among species and type (figure 2*b* and the electronic supplementary material, table S4). Spike-type EOD duration varied between all species (*S. angelica*, mean 216 ms; *S. euptera*, 92 ms; *S. marmorata*, 49 ms; one-way ANOVA, $F_{2,9} = 212.9$, $p < 0.001$; all SNK post hoc pairwise: $p < 0.001$). Dominant frequency of *S. angelica* spikes (mean 8 Hz) was lower than *S. marmorata* (49 Hz) and *S. euptera* (61 Hz)

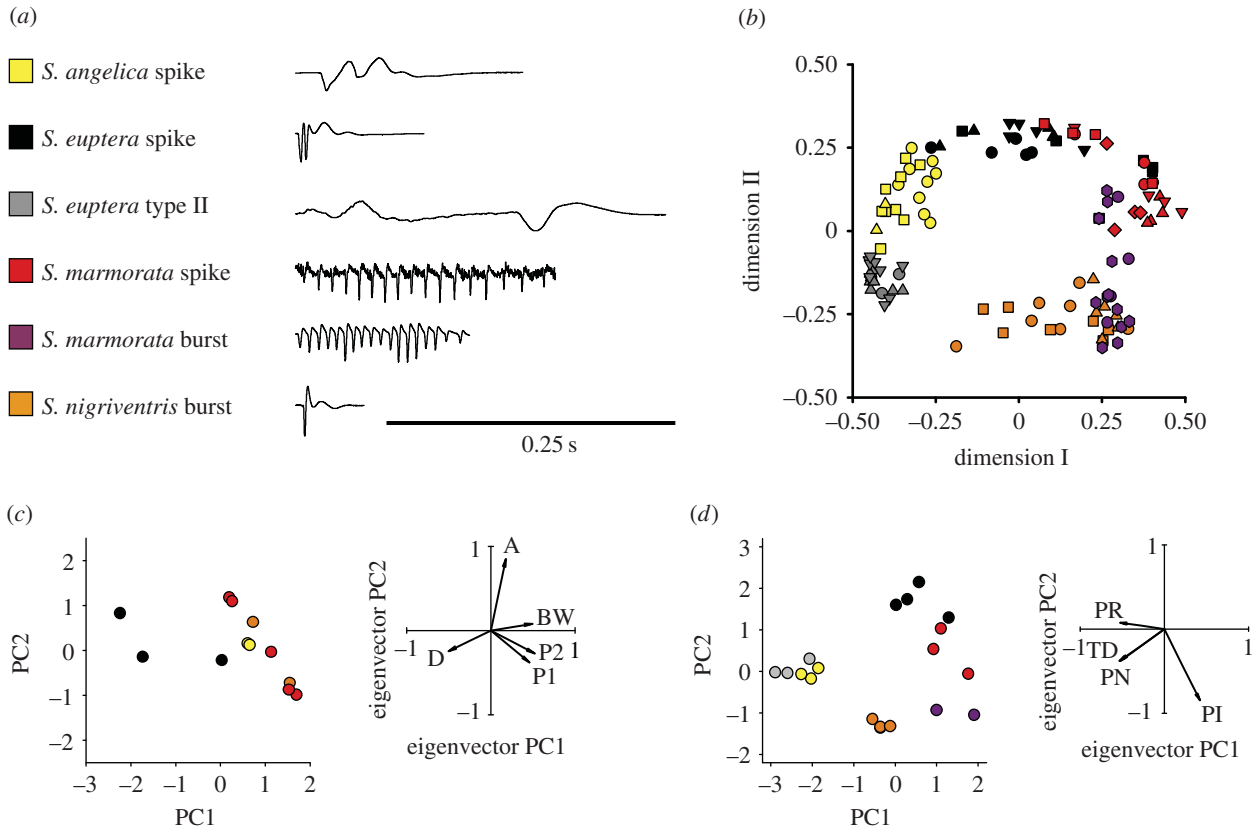

Figure 2. Distinctiveness of *Synodontis* spp. EODs. (*a*) Four of five tested species emit EODs of different types. (*b*) Cross-correlation analysis indicates a high degree of species-specificity of EOD waveforms. Species are indicated by different colours and different shades are used for species with multiple EOD types. EODs from the same individual are indicated by symbol type. (*c*) PCA of EOD pulse features (A, amplitude; BW, bandwidth; D, duration; P1, first frequency peak and P2, second frequency peak) further supports distinction between species and EOD type (colour patterns indicate species and EOD types from *a*). (*d*) PCA of EOD temporal features (PI, pulse interval; PN, pulse number; PR, pulse rate and TD, train duration), however, indicates more similarity among species in terms of EOD emission rate (colour patterns indicate species as in *a*).

and *S. marmorata* ($F_{2,9} = 6.1$, $p = 0.029$; SNK post hoc tests $p = 0.018$ and $p = 0.038$, respectively).

PCA showed that species are distinguished by EOD pulse characteristics (spectral features, pulse duration and amplitude) from different individuals (figure 2*c* and the electronic supplementary material, tables S4 and S6). Large variation in EOD amplitude existed among the species ($F_{3,12} = 80.6$, $p < 0.001$; see the electronic supplementary material, table S4). Amplitudes of *S. euptera* were greater than the other species (SNK post hoc tests all $p < 0.001$), and *S. marmorata* amplitudes were greater than the remaining species (SNK post hoc tests all $p < 0.001$).

PCA revealed that EOD train temporal patterns varied between individuals of different species (figure 2*d* and the electronic supplementary material, tables S5 and S7). Between species differences were observed for spike emission rate ($F_{2,8} = 6.4$, $p = 0.022$; *S. euptera* rates, mean 5.3 Hz, were higher than *S. angelica*, 1.6 Hz, and *S. marmorata*, 2.2 Hz; SNK post hoc tests $p = 0.046$ and $p = 0.014$, respectively).

(d) Protractor muscle ultrastructure

In all *Synodontis* spp. examined, protractor muscles displayed histological features that are unusual for vertebrate skeletal muscle fibres, including a dramatic reduction in myofibril content and small, round fibres (figure 3 and the electronic supplementary material, figure S5). The greatest myofibril area was observed in *S. grandiops*, the species with the longest sound durations, whereas the lowest myofibril density was seen in *S. nigriventris*, a species that did not produce swim bladder sounds (figure 3). Small to moderate clusters of myofibrils were present in the protractor muscle fibres of all species studied. In the longitudinal plane, myofibrils are arranged like typical vertebrate skeletal muscle, with parallel, contiguous myofibrils, triads at the Z-line, and complete Z-discs, A-bands and I-bands. Disorganized thin filament arrays were found throughout the sarcoplasm of *S. nigriventris* and *S. angelica*. Numerous canaliculi were abundant in fibres of EOD-forming species and were especially developed in *S. euptera* and *S. marmorata*. Wide canaliculi and vacuole-like vesicles were present in *S. angelica* fibres. By contrast, muscle fibres of non-EOD-forming *S. grandiops* lack canaliculi and fine filaments within the sarcoplasm but have features common to other sound-producing fishes with fast sonic muscles: ribbon-shaped myofibrils, central cores of sarcoplasm, elaborate sarcoplasmic reticulum and fingerprint-like whorl bodies.

(e) Protractor muscle electric organ discharge function

A preliminary muscle inactivation experiment with a limited sample size provided evidence that protractor muscle fibre function may be required for EOD formation in *Synodontis*. After administration of a competitive acetylcholine inhibitor, Flaxedil, EOD amplitude was reduced to 0.01 times the baseline level (figure 4). This large decrement was not present

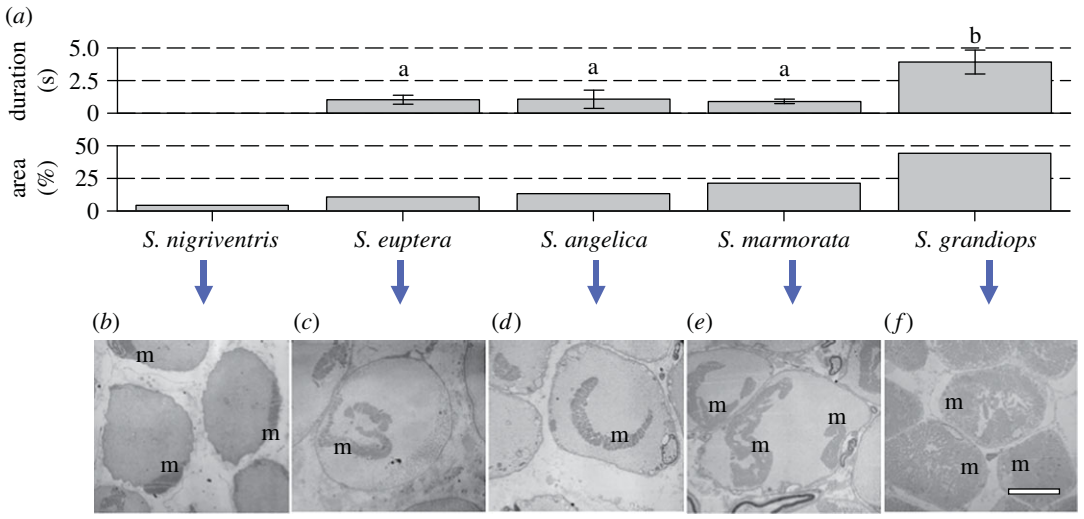

Figure 3. Protractor muscle myofibril density is associated with sound production ability. (*a*) *Synodontis grandiops* produces longer sound trains. Mean sound duration (top) measured among individual fish of each species (top). Letters indicate differences (SNK post hoc test after a one-way ANOVA). *Synodontis grandiops* does not produce EODs and has the greatest myofibril area, whereas *S. nigriventris* has the lowest myofibril area and does not produce sounds (bottom). (*b–f*) Representative protractor muscle fibre cross sections (TEM micrographs) of *Synodontis* spp. show variation in myofibril density (m). Images correspond to species in (*a*). Scale bar, 10 µm. (Online version in colour.)

when Flaxedil was injected in trunk musculature in the tail, or when saline was injected in the protractor muscle (figure 4). Thus, when sodium channel function is disrupted, EODs are nearly silenced. These observations are consistent with the hypothesis that the protractor muscle in weakly electric *Synodontis* is not merely a degenerative muscle, but rather, behaves as a population of electrocytes and that *Synodontis* EODs are thus myogenic, not neurogenic in origin.

4. Discussion

This study found remarkable differences in the proclivity of sound production and electric discharges among closely related species from the same genus. Swim bladder sounds occurred in a disturbance context similar to other catfish families [11,12,22]. These sounds were quite similar among species, both in terms of spectral features and temporal patterning. By contrast, EODs were highly divergent between species and type, regardless of phylogenetic and biogeographic affiliation [23,24].

EODs could be of potential importance for species recognition, as has been shown for the distantly related weakly electric fishes Mormyridae [25–27] and Gymnotidae [28,29]. Synchronous firing patterns from the sonic motor nucleus of the ESA protractor muscle may have facilitated the evolution of electric communication. The ESA is a synapomorphy of the Mochokidae [14] and thus swim bladder sounds may have a deep evolutionary history within the family. Swim bladder sounds and EODs of other mochokid genera, however, remain to be described. EODs appear better suited for species-specific communication than acoustic signals because their waveforms and spectral content are more distinctive at the species level. Thus, we hypothesize that the need for finding conspecifics, especially for some species that occur in turbid water, have nocturnal habits or live in environments with higher acoustic background noise, favoured the development of a second pathway of communication.

This study also found differences in hearing ability among *Synodontis* species. All species examined showed a broad range of sensitivity, as is known from other otophysan fishes [30–33].

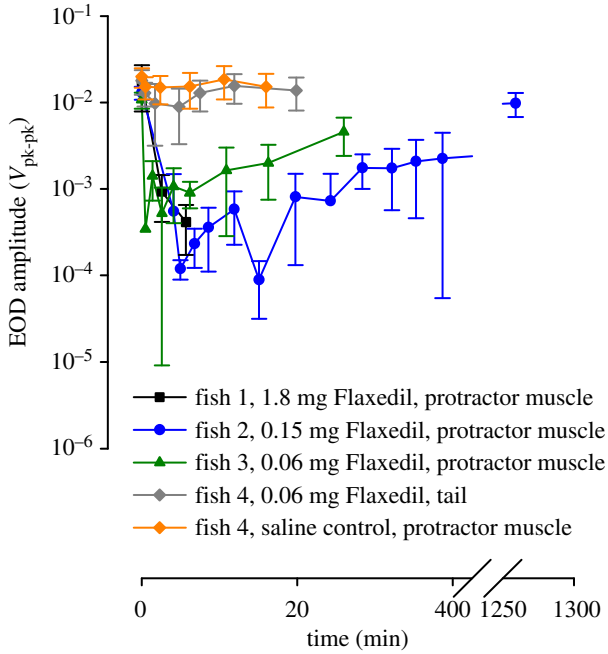

Figure 4. A preliminary experiment indicates that *S. euptera* protractor muscle fibres probably contribute to EOD amplitude. Average (+s.d.) EOD amplitude over time in treatment with a competitive acetylcholine antagonist (Flaxedil) administered in the protractor muscle versus a control with Flaxedil in the tail, and a sham with saline in the protractor muscle. Different doses in fish 1–3 all produced a dramatic reduction from baseline EOD amplitude (at time 0). Fish 1 (high dosage) died before recovery. EOD amplitudes began to recover in fish 2. In fish 3, amplitudes returned to baseline levels after recovery overnight. In fish 4, control and sham (retested the following day) resulted in little reduction of EOD amplitude. The protractor muscle probably acts as an electric organ which amplifies the signal.

However, the lowest hearing thresholds were observed in *S. grandiops*, the only species that did not produce EODs. This finding is consistent with the hypothesis that acoustic sensitivity could be more important in species that cannot produce EODs and thus have a greater reliance on acoustic

communication. Few data are available on the sensitivity of mochokid catfishes to electric stimuli [34]. Electric organ evolution occurred after the development of low-frequency ampullary receptors [35] and EODs of *Synodontis* contain frequencies that are higher than the best frequencies reported in typical ampullary receptors [36,37]. Future research should examine the response of the electrosensory system among *Synodontis* species to determine whether electroreception is tuned to the EODs of conspecifics and whether the ampullary receptors and neural circuitry permit discrimination of EODs from different *Synodontis* species. In addition, it remains to be determined whether electrogenic *Synodontis* species may be able to use EODs for active electroreception (electrolocation) like other weakly electric fishes [13,36,37].

EODs in *Synodontis* catfish did not arise from a de novo structure. Earlier work on mochokid catfishes indicated that the protractor muscle is involved with electric discharge [3,4,38]. However, it was unknown whether EODs result solely from the electrical by-product of synchronized motoneuron activity, in which case the muscle could be a degenerative vestigial sonic muscle, or whether the muscle fibres play an active role. The results of our preliminary experiment indicate that the protractor muscle fibres are likely to function as electrocytes. Synchronized activity of the motoneurons that innervate the protractor muscle is not sufficient to produce a full amplitude electric discharge. Thus, EODs are probably myogenic and require a muscle action potential to produce a typical signal.

Elucidating the origins of complex biological structures has been one of the major challenges of evolutionary studies. The protractor muscle appears to be a plastic feature among *Synodontis* spp., both in terms of morphology and function. The insertion of the muscle on the Müllerian ramus—anterior swim bladder is indicative of an evolutionary history associated with sound production. The muscle fibres with their small diameters, elaborate sarcoplasmic reticulum and reduced myofibrils, bear similarity to fast sonic muscles of distantly related fishes [2,39,40].

By contrast, myogenic electrocytes bear little resemblance to muscle fibres and have diverse morphologies associated with elaborate anterior and posterior faces [8,41]. The electrocytes of mormyrids, for example, are disc-like, thin (10–50 µm) in the rostro-caudal plane but wide (2–3 mm) in the transverse plane [41,42]. Electrocytes of other electric fishes are aligned in series along the rostro-caudal body axis and the number of rows aligned in parallel varies among taxa [8,41]. By contrast, the fibres of the *Synodontis* protractor muscle are cylindrical and similar to the small, rounded muscle fibres of fish sonic muscles [2]. Further, the protractor muscle originates on the ventral surface of the nuchal plate and along the base of the dorsal fin spine, and inserts on the anterodorsal surface of the Müllerian ramus. Thus, many fibres are oriented dorsoventrally along the axis between the Müllerian ramus and nuchal plate, whereas some fibres bend posteriorly along the dorsal contour of the swim bladder towards the base of the dorsal fin. Protractor muscle cells are elongate but possess a small cross-sectional area: diameters observed in this study ranged from 16 to 48 µm. Thus, *Synodontis* protractor muscle fibres probably possess a smaller surface area and lower surface-to-volume ratio than the electrocytes of other weakly electric fishes.

The myogenic electrocytes of other weakly electric fishes are innervated by electromotoneurons entirely on the anterior or posterior sides (but not both), depending on the taxon, and this innervation determines whether the initial EOD phase is head positive or negative, respectively [8,41]. Electrocytes of some electric fishes (e.g. Mormyridae, some Gymnoformes) are innovated on stalk-like projections, and in some mormyrids the stalks penetrate the electrocyte one or more times [41,43]. The stalks and the number of penetrations may affect the number and polarity of phases of an individual pulse EOD [41,43]. Stalks are not present in the protractor muscle fibres of *Synodontis*, and neuromuscular junctions are similar to those of other teleost fishes and bear no junctional folds. The head negative polarity of all *Synodontis* EODs in this study, except type-II EODs from *S. euptera*, is indicative of activation from the anterior side of dorsoventrally oriented protractor muscle fibres. The spatial orientation of innervation along the rostro-caudal axis in these *Syndontis* species, however, remains to be determined.

At the ultrastructural level, *Synodontis* electrocytes possess many canaliculi, proliferations from the sarcolemma that drastically increase the surface area of the sarcolemma. Canaliculi features are not present in the fibres of the non-electrogenic *S. grandiops*. Canaliculi are present in a large variety of electric fishes and their function may be to decrease resistance of the electrocyte membrane or increase its capacitance [8,41,44]. These canaliculi were present to varying extent in all EOD-forming *Synodontis*, but absent in the non-electrogenic *S. grandiops*. The degree of proliferation of canaliculi on each electrocyte face varies among taxa [44]. In this study, within EOD-forming *Synodontis* species, canaliculi density was relatively homogeneous around the protractor muscle fibre periphery. Like electrocytes of other fishes, mitochondria and nuclei of *Synodontis* were concentrated near the membrane of the cell [8,41,44]. The presence of thin filaments within the protractor muscle fibres of electrogenic *Synodontis* is similar to that of other weakly electric fishes but is indicative of a loss of myofibrils [41,44,45] rather than a functional similarity. However, unlike electrocytes of other fishes, which contain only rudiments of myofibrils [8,41,42,44,46], small to moderate clusters of myofibrils were present in the protractor muscle fibres of all species studied. Lastly, the non-electrogenic, vocal species *S. grandiops*, has the greatest myofibril area and possesses features associated with fast sonic muscles of other fishes [2,40]: ribbon-shaped myofibrils, a well-developed sarcoplasmic reticulium, cores of sarcoplasm and whorl bodies. These features of *S. grandiops* are common to sonic muscles of distantly related catfishes, though the sarcoplasmic reticulum may be less developed than in Doradidae and Pimelodidae [47].

Because the genus *Synodontis* is quite diverse (*ca* 130 spp.), it is possible that large evolutionary variation exists in protractor muscle specialization for sound production or EOD. The myogenic electrocytes of other electric fishes have evolved independently from a variety of non-sonic muscular precursors [8,13,48,49]. *Synodontis* protractor muscle, however, presents a unique example in which an electrocyte evolved from a fast-contracting sonic muscle that, in some species, retains the ability to contract in order to produce sounds. A recent study found that the independent origins of bioelectrogenesis in the Mormyridae–Gymnarchidae clade and Gymnotiformes occurred approximately 94–125 and 100–144 Myr ago, respectively [50]. The evolutionary appearance and distribution of bioelectrogenesis among mochokid catfishes remains to be determined. We have shown that within the single genus *Synodontis*, which is estimated to be approximately 21–35 Myr old [23,24], there is variation in EOD ability and electric organ morphology,

consistent with the hypothesis of a recent evolutionary event. Our study highlights that a complex system can display stepwise evolutionary transitions between functions.

The experiments were approved by the ethics committee of Liège, file no. 1226.

Acknowledgements. N. Decloux helped with microscopy. S. Riepe, G. Melotte and J. Tock assisted AEP. J. Mannard assisted with sound and EOD recordings. This is the AFFISH-RC (Applied and Fundamental Fish Research Center) publication no. 11.

Funding statement. This work was supported by the University of Liège.

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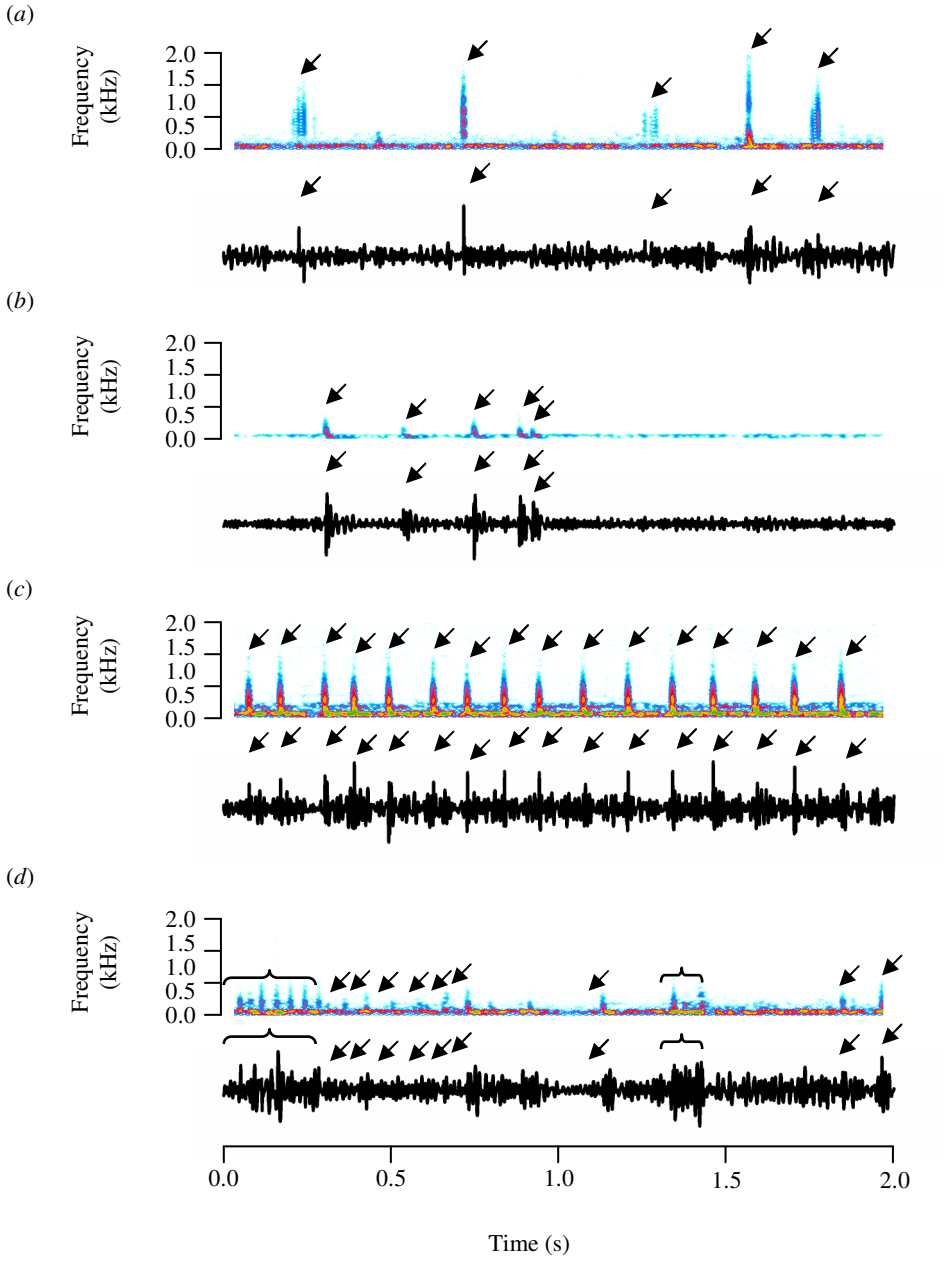
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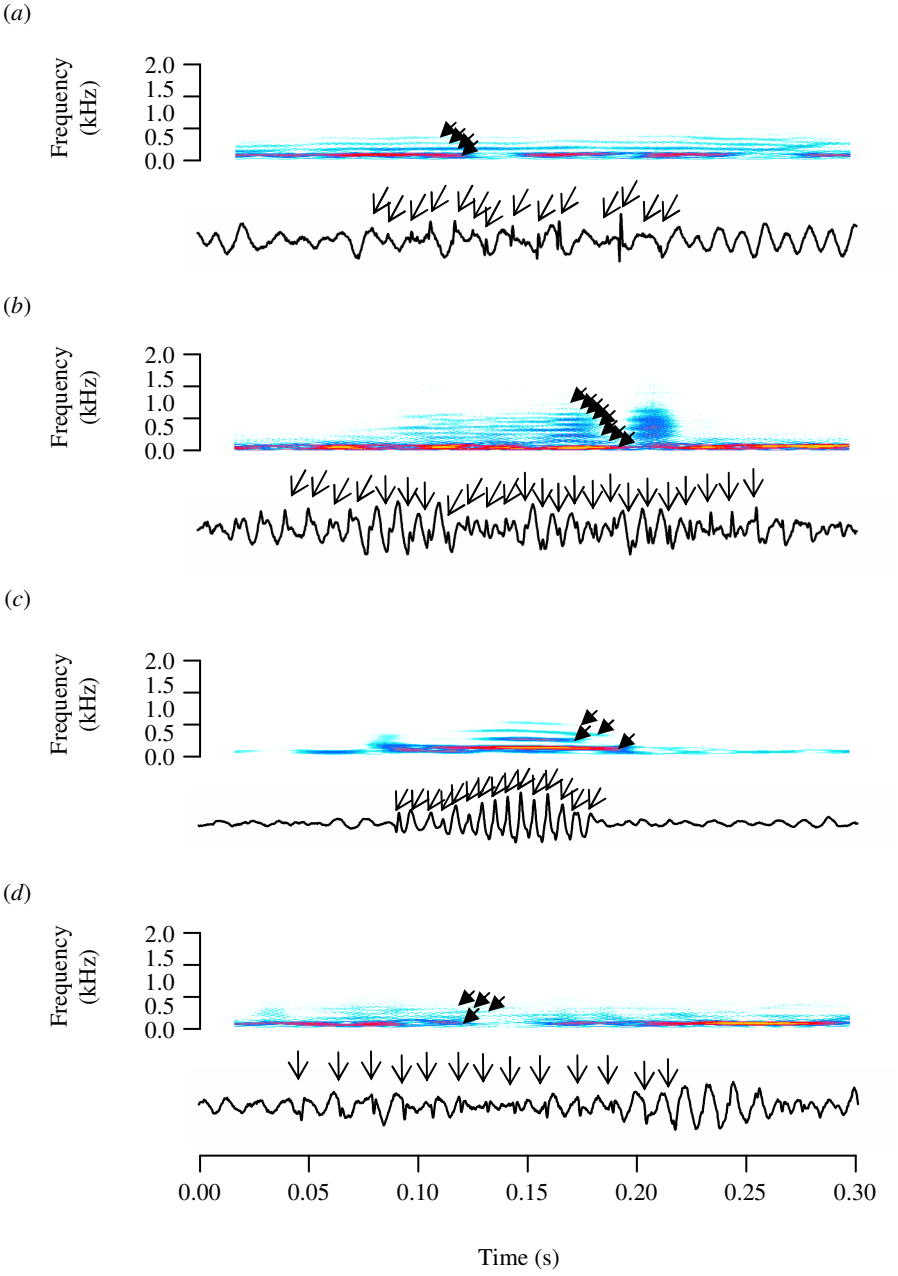
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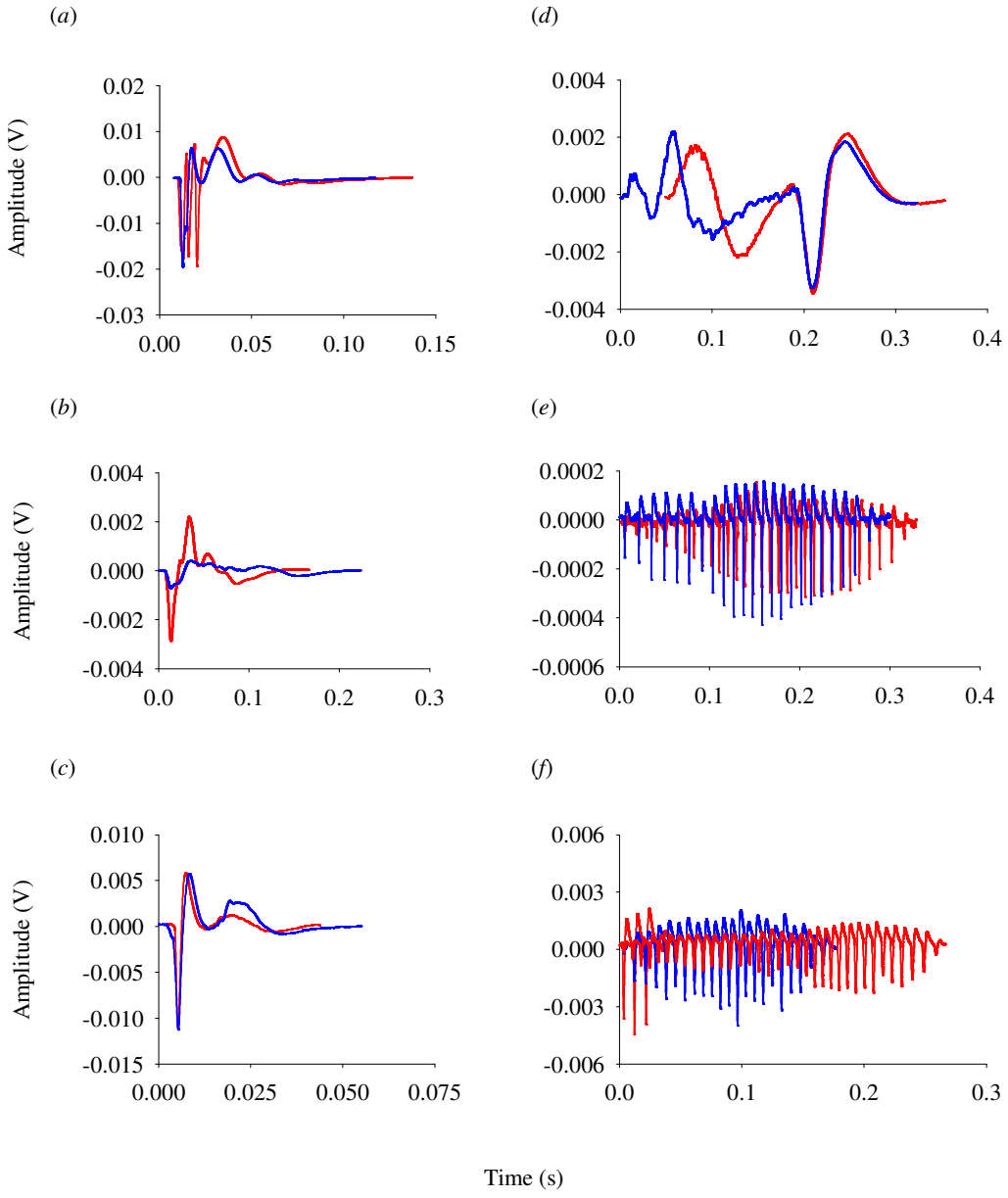
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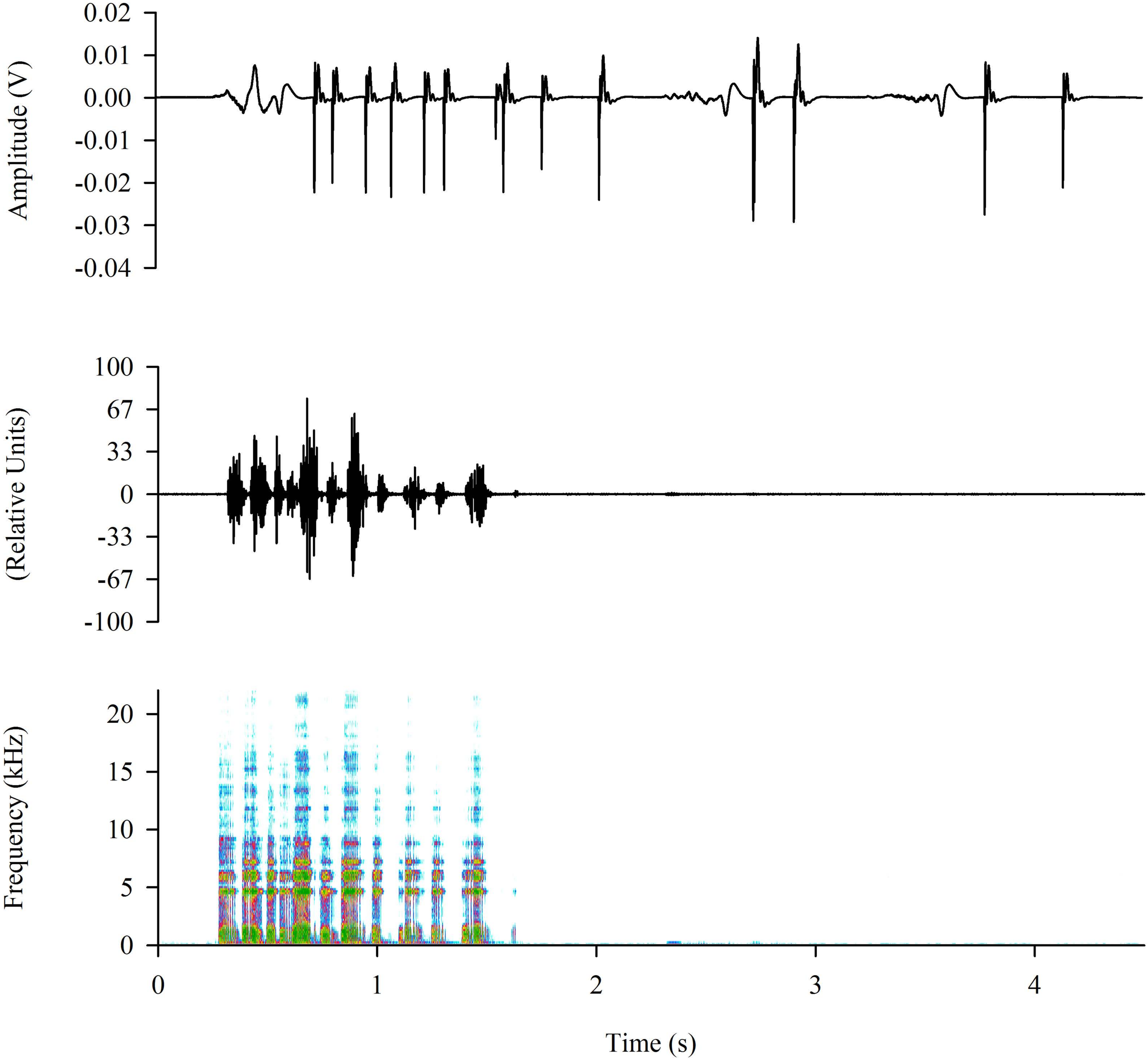
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