

## RESEARCH ARTICLE

# Variation in swim bladder drumming sounds from three doradid catfish species with similar sonic morphologies

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## ABSTRACT

A variety of teleost fishes produce sounds for communication by vibrating the swim bladder with fast contracting muscles. Doradid catfishes have an elastic spring apparatus (ESA) for sound production. Contractions of the ESA protractor muscle pull the anterior transverse process of the 4th vertebra or Müllerian ramus (MR) to expand the swim bladder and elasticity of the MR returns the swim bladder to the resting state. In this study, we examined the sound characteristics and associated fine structure of the protractor drumming muscles of three doradid species: *Acanthodoras cataphractus*, *Platydoras hancockii* and *Agamyxis pectinifrons*. Despite large variations in size, sounds from all three species had similar mean dominant rates ranging from 91 to 131 Hz and showed frequencies related to muscle contraction speed rather than fish size. Sounds differed among species in terms of waveform shape and their rate of amplitude modulation. In addition, multiple distinguishable sound types were observed from each species: three sound types from *A. cataphractus* and *P. hancockii*, and two sound types from *A. pectinifrons*. Although sounds differed among species, no differences in muscle fiber fine structure were observed at the species level. Drumming muscles from each species bear features associated with fast contractions, including sarcoplasmic cores, thin radial myofibrils, abundant mitochondria and an elaborated sarcoplasmic reticulum. These results indicate that sound differences between doradids are not due to swimbladder size, muscle anatomy, muscle length or Müllerian ramus shape, but instead result from differences in neural activation of sonic muscles.

**KEY WORDS:** Sound production, Elastic spring apparatus, Amplitude modulation, *Acanthodoras cataphractus*, *Platydoras hancockii*, *Agamyxis pectinifrons*

## INTRODUCTION

A wide diversity of mechanisms for producing acoustic communication signals exists among teleost fishes (Ladich and Fine, 2006). Because of this functional anatomical diversity, the sounds of fishes display variation in frequency spectra, temporal patterning, frequency modulation and amplitude modulation (Amorim, 2006). Among sound production mechanisms in fish, the most well-studied examples involve muscle-driven vibrations of the swim bladder by sonic muscles that are either intrinsic to the

swim bladder (inserting and originating entirely along the swim bladder wall) or extrinsic (originating elsewhere on the body and inserting on the swim bladder or adjacent connective tissue) (Tavolga, 1971; Parmentier and Diogo, 2006).

Sounds produced by swim bladder muscles are characterized by a relatively low fundamental frequency that generally corresponds to the contraction rate of the sonic muscle (Zelick et al., 1999). Swim bladder sounds can be tonal sounds that are produced by cycles of continuous sonic muscle contraction (e.g. Fine et al., 2001; Amorim et al., 2011) or percussive pulse trains in which pulses are punctuated by silent intervals (e.g. Connaughton et al., 2000; Boyle and Tricas, 2010). Peak frequencies of the latter sound type are probably driven by the contraction and relaxation time of the sonic muscle twitch, which is much faster than the modest pulse rate of the sound (Connaughton et al., 1997).

In order to produce multiple high-speed contractions, swim bladder muscles often possess a number of apomorphic features. Sonic muscle fibers tend to be small and have modifications that increase the surface area of contact between the sarcoplasmic reticulum and myofibrils (Ladich and Fine, 2006; Parmentier and Diogo, 2006). Often, myofibrils are thin and radially arranged, the sarcoplasmic reticulum is elaborated and cores of sarcoplasm may be present (Ladich and Fine, 2006; Parmentier and Diogo, 2006).

One of the longest appreciated extrinsic swim bladder sonic mechanisms is the Springfederapparat or elastic spring apparatus (ESA) (Müller, 1842; Bridge and Haddon, 1889, 1893; Parmentier and Diogo, 2006). This mechanism is present in eight catfish (Siluriformes) families, including the South American Doradidae (Parmentier and Diogo, 2006). The ESA morphology involves the anterior transverse process of the fourth vertebra, termed the Müllerian ramus (MR), which is attached to the anterior surface of the swim bladder and forms an insertion for the ESA protractor muscle, which originates on the occiput (Ladich, 2001). Contractions of the protractor muscle pull the MR and swim bladder towards the skull and the elasticity of the MR forms a spring which returns the swim bladder to the relaxed state (Kastberger, 1977). The protractor muscle contraction rate dictates the fundamental frequency of the sound. The doradid sonic mechanism appears relatively simple compared with some fish sonic mechanisms (e.g. Parmentier et al., 2008, 2010; Kéver et al., 2012). Yet, drumming sounds from doradids can be quite complex and exhibit variation in pulse rate, temporal patterning, waveform shape, frequency modulation and amplitude modulation (Kastberger, 1977, 1978; Ladich, 1997; Papes and Ladich, 2011; Kaatz and Stewart, 2012; Knight and Ladich, 2014).

Amplitude modulation (AM) of sounds exists when sound waves have a relatively stable carrier frequency whose amplitude is varied at a lower frequency (Bradbury and Vehrencamp, 1998). Vocalizations of anuran amphibians often contain AM (Bradbury and Vehrencamp, 1998; Zelick et al., 1999) and the pattern of AM has been used to describe and compare characteristics of sounds

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among taxa (Cocroft and Ryan, 1995; Robillard et al., 2006). AM in fish sounds, however, has received comparatively less attention.

In this study, we examine features of swim bladder drumming sounds and the associated ESA morphology and sonic muscle ultrastructure for three doradid catfish species: *Acanthodoras cataphractus* (Linnaeus 1758), *Agamyxis pectinifrons* (Cope 1870) and *Platydoras hancockii* (Valenciennes 1840). We document a high degree of complexity and variability within and among individuals and species. Sounds differed mainly in the temporal and AM pattern of waveforms. The orientation and relative length of the sonic muscle and the relative swim bladder size differed among study species. Sonic muscle ultrastructure, however, was very similar among species and showed characteristics associated with fast contractions that have been observed in doradids in previous studies. These results demonstrate that differences in swim bladder shape and ESA morphology do not necessarily correspond to differences in sound frequency. Instead, patterns of activation at the level of the nervous system may allow for sounds that are distinct among species.

## RESULTS

### Swim bladder sounds

Swim bladder drumming sounds were recorded from all three species in the study. Three (all individuals tested) *Agamyxis pectinifrons* and seven (out of 8) *Platydoras hancockii* individuals produced drumming sounds when pectoral spines were held to prevent stridulation. Only three of seven *Acanthodoras cataphractus* individuals produced drumming sounds when the pectoral spines were blocked, but all individuals were observed making stridulations and sounds involving simultaneous drumming and stridulation. The latter two sound types were not investigated in further detail in this study.

Sounds of all three species displayed tonal qualities and were often accompanied by one to several harmonics (Fig. 1). Sounds displayed a great deal of variation within and among species and individuals (Fig. 1). For each species, drumming sounds could be classified qualitatively based on differences associated with their features. Within each species, these separate sound types have a range of features that are not completely distinguishable, but rather appear to occur on a continuum. Thus, there were some cases where sound waveforms resembled a combination of two sound types. In these cases, sounds were classified by the sound type they most resembled. Furthermore, each of these sound types did not clearly correspond to a particular sound type from another species. Thus, the sounds are presented with generic names for each species (e.g. type I, type II etc.).

### *Acanthodoras cataphractus*

Three sound types were observed from *A. cataphractus*. Ac type I sounds (Fig. 1B, Table 1) were of short duration and were characterized by an AM signal that had one to several modulations, sometimes without complete silence between the modulations. The first full cycle, and to a lesser extent, the second full cycle of the waveform at each AM tended to be of shorter duration (higher frequency) than the subsequent cycles. Ac type II sounds (Fig. 1B) were distinguished from Ac type I sounds by their lack of the initial fast oscillations of Ac type I sounds, and as a result, consisted of a more continuous tonal sound. Ac type III sounds (Fig. 1A,B) tended to have more AMs and a higher AM rate than Ac type II sounds. Ac type III sounds tended to lack the distinctly higher frequency oscillations present at the onset of each AM for Ac type I sounds. Ac

type III sounds had the longest duration of sound types observed for *A. cataphractus*.

### *Platydoras hancockii*

Three types of *P. hancockii* sounds were observed. Ph type I sounds (Fig. 1, Table 1) were characterized by long durations, pronounced AM and several sharp, irregularly shaped oscillations within each AM. Ph type II sounds (Fig. 1B, Table 1), were AM signals with one to three half pulses in the middle of each AM. These sounds tended to be higher frequency than the other two sound types. Ph type III sounds (Fig. 1B) were characterized by few AMs, and a fairly continuous rate of oscillations that resulted in a very tonal sound.

### *Agamyxis pectinifrons*

Two types of *A. pectinifrons* sounds were observed. Ap type I sounds (Fig. 1, Table 1) tended to have few AMs and were characterized by the presence of several higher-frequency oscillations at the beginning of the AM (a feature similar to Ac type I sounds). Ap type II sounds (Fig. 1B) had a nearly continuous rate of oscillation, sometimes with some slow frequency modulation present over the entire sound wave. Ap type II sounds were often of long duration and the rate and AM number was often high and variable.

## Comparisons of sound types among species

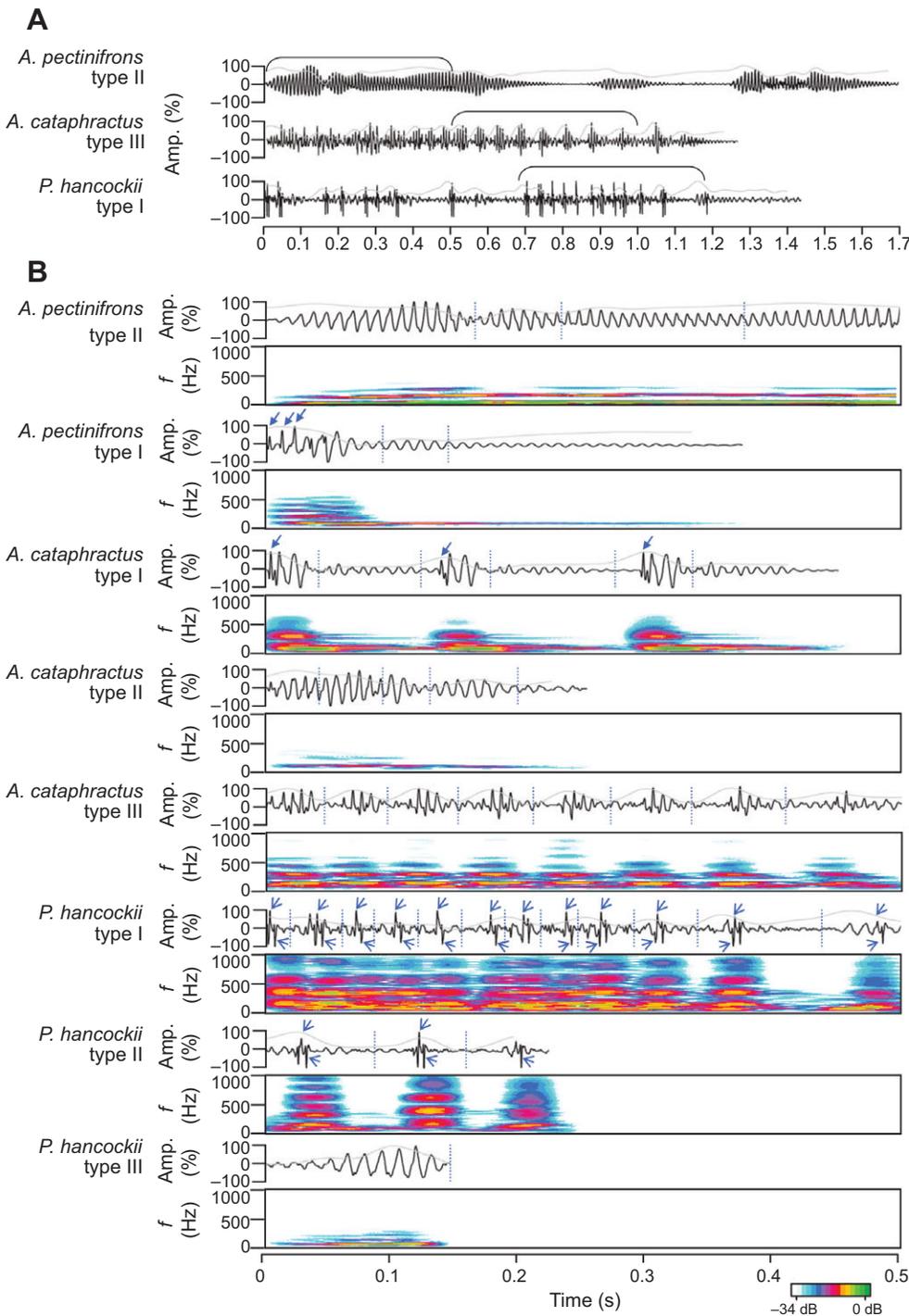
### Waveform similarity among species and sound types

In the time domain, sound types were distinctive among species and individuals (Fig. 2). Sounds clustered in multidimensional space and MANOVA results support the observed pattern of separation between species (Wilk's  $\lambda=0.313$ ,  $F_{4,466}=91.814$ ,  $P<0.001$ ), individuals (Wilk's  $\lambda=0.821$ ,  $F_{20,466}=2.410$ ,  $P=0.001$ ) and sound type (Wilk's  $\lambda=0.777$ ,  $F_{28,466}=2.242$ ,  $P<0.001$ ).

These observations support the consideration of separate sound types in the acoustic analysis below. Species and individuals produced more than one sound type, but not all individuals produced all sound types. In this analysis, *P. hancockii* type III clustered within the grouping of *A. pectinifrons* type I sounds. Type I *A. pectinifrons* sounds, however, were more variable and in some cases were closer in multidimensional space to other sounds, such as *A. cataphractus* type I sounds. Thus, there was no evidence that sound types of one species corresponded to particular sound types of another species. Thus, a single test model with a balanced and crossed statistical design in order to test all species, sound types and repeated measures was not possible. We examined the patterns of sound duration, dominant frequency and amplitude modulation with a series of statistical tests below in cases where sample sizes permitted comparison.

### Sound duration

Swim bladder sound durations were highly variable. Continuous sounds over 1 s long were observed in all three species (Table 1). Overall, duration of the entire suite of swim bladder sounds produced by each individual, regardless of type, did not differ among species (ANOVA,  $F_{2,10}=2.02$ ,  $P=0.18$ ). Among sound types representative of shorter durations (Ph type II and Ac type I), Ph II sounds were of shorter duration than Ap type II sounds – 76 versus 613 ms, respectively (ANOVA,  $F_{2,9}=11.03$ ,  $P=0.004$ , Tukey's *post hoc* test,  $P=0.0029$ ) – but not different to Ac type I sounds (*post hoc* test,  $P>0.05$ ). Sound types representative of longer durations (Ac type III, Ap type II and Ph type I) did not differ statistically (ANOVA,  $F_{2,10}=3.02$ ,  $P=0.094$ ).



**Fig. 1. Swim bladder drumming sounds of *Agamyxis pectinifrons*, *Acanthodoras cataphractus* and *Platydoras hancockii*.** (A) Long-duration sound waveforms (solid black line) from *A. pectinifrons* (Ap type II), *A. cataphractus* (Ac type III) and *P. hancockii* (Ph type I). (B) Sound waveforms (solid black line) viewed over a shorter time scale. Closer views of long duration sounds (Ap type II, Ac type III and Ph type I) correspond to the brackets in A. Waveforms of shorter sounds from *A. pectinifrons* (Ap type I), *A. cataphractus* (Ac type I and Ac type II) and *P. hancockii* (Ph type II and Ph type III) are shown in their entirety. Major regions of amplitude modulation in B are indicated by blue dotted vertical lines. The gray line is the envelope of the waveform (see Materials and methods for details), which was used to estimate the degree of modulation of sounds (number of maxima along the envelope). The beginning of each amplitude modulation of Ap type I and Ac type I sounds was often characterized by one or more short-duration positive half-cycles (solid blue arrows) followed by longer-duration, relatively symmetrical oscillations. Ph type I and type II sounds had sharp (brief duration) oscillations (blue arrowheads) in both the positive and negative direction during each amplitude modulation. Note the variation in amplitude modulation and depth among sounds and the presence of harmonics indicated by horizontal bands in the spectrograms. Spectrogram parameters: sampling rate 4 kHz, 256-point fast Fourier transforms (FFT), Hanning window, frame size 100%, overlap 75%.

### Dominant frequency

Dominant frequency of swim bladder sounds varied among species (Table 1, ANOVA,  $F_{2,10}=9.61$ ,  $P=0.005$ ). Overall, sounds of *P. hancockii* were higher frequency than *A. pectinifrons*, 130 versus 91 Hz (back-transformed mean of  $\log_{10}$  transformed data), respectively (Tukey's *post hoc* test,  $P=0.0043$ ). No other statistical differences in dominant frequency were observed among sounds overall (*post hoc* tests,  $P>0.05$ ). No differences were observed among sound types representative of lower dominant frequencies (Ac type I, Ph type I; Table 1, Kruskal–Wallis  $H=6.81$ , d.f.=2,  $P=0.033$  not significant after sequential Bonferroni correction for

multiple tests). Among sound types representative of higher frequencies (Ac type III, Ap type II, Ph type II), Ph type II sounds were higher frequency than Ap type I (152 Hz versus 89; ANOVA,  $F_{2,9}=10.75$ ,  $P=0.004$ ; Tukey's *post hoc*,  $P=0.0041$ ) but not Ac type III (Tukey's *post hoc*,  $P>0.05$ ). The dominant frequency of Ac type III was not statistically different to Ap type II.

### AM per sound

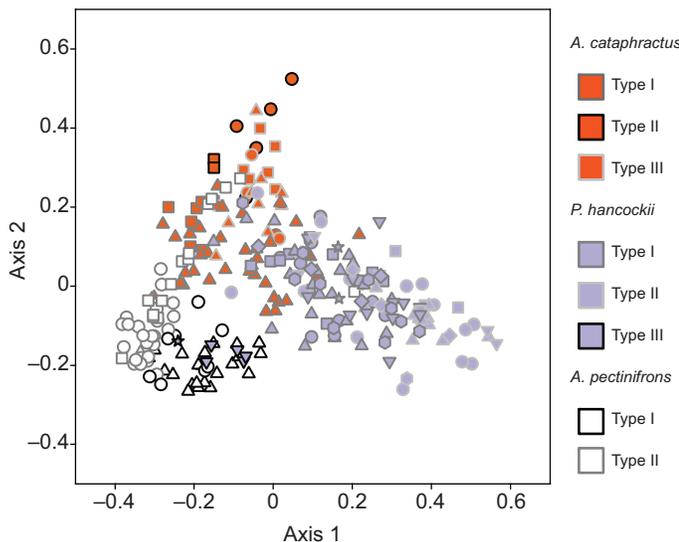
The number of amplitude modulations among species when all sound types were pooled did not vary among species (Table 1, one-way ANOVA,  $F_{2,10}=1.02$ ,  $P=0.394$ ). No differences were observed

**Table 1. Sound characteristics of three doradid catfish**

Species	Sound type	Fish (N)	Sounds (n)	Duration (ms)	Dominant frequency (Hz)	AM per sound (n)	AM rate (Hz)
<i>A. cataphractus</i> SL, 83–96 mm	All sounds	3	72	350±186	108±9	5±3	15±1
				254, 38, 1265	113, 59, 281	4, 1, 20	15, 9, 27
	Ac type I	3	46	252±176	100±9	3±2	14±2
				205, 38, 506	96, 59, 277	3, 1, 8	14, 11, 27
	Ac type II	2	7	406±146	113±0	6±3	14±1
<i>P. hancockii</i> SL, 52–62 mm	All sounds	7	117	232±154	131±20	3±2	17±1
				253, 19, 1998	131, 55, 426	4, 1, 35	17, 6, 54
	Ph type I	7	69	265±147	131±31	4±3	16±2
				266, 58, 1998	113, 66, 355	5, 1, 35	16, 6, 24
	Ph type II	6	42	76±74	152±24	1±1	22±8
<i>A. pectinifrons</i> SL, 93–105 mm	All sounds	3	72	527±354	91±2	6±4	11±1
				406, 118, 1708	91, 63, 191	4, 1, 19	11, 6, 16
	Ap type I	2	30	246±2	89±1	3±0	11±0
				236, 161, 442	89, 63, 102	3, 2, 5	11, 8, 16
	Ap type II	3	42	613±271	93±3	7±4	10±1
				477, 118, 1708	94, 82, 191	5, 1, 19	17, 6, 16

Values shown are means±s.e.m., median, min, max. Means were calculated from the average of median observations from each individual, medians are the median values of observation medians from each individual, min is the overall minimum observed value, max is the overall maximum observed value. AM rate, amplitude modulation rate; SL, standard length.

among sound types with low AM per sound (Ac type I, Ph type II) (Table 1; Kruskal–Wallis,  $H=7.52$ , d.f.=2,  $P=0.017$  not significant after seq. Bonferroni correction for multiple tests). No differences in AM per sound were observed among sound types with high AM per sound (Ac type III, Ap type II, Ph type I): one-way ANOVA,  $F_{2,10}=1.14$ ,  $P=0.357$ .



**Fig. 2. Comparison of individual drumming sound waveforms.** Two-dimensional classical multidimensional scaling biplot of cross-correlation coefficients between sounds of *Acanthodoras cataphractus*, *Agamyxis pectinifrons* and *Platydoras hancockii* (see Materials and methods for further details). Sounds from each species are indicated with different colors and sound types within each species are indicated by different symbol borders (black and gray shades). Symbol shapes within each species indicate sounds recorded from different individuals. Sound types are clustered but there is some overlap among species.

### AM rate

Rates of amplitude modulation were variable among species (Table 1, ANOVA,  $F_{2,10}=33.17$ ,  $P<0.001$ ). AM rates of *A. pectinifrons* sounds were lower than *A. cataphractus* sounds, 11 versus 15 Hz, respectively (Tukey's *post hoc* test,  $P=0.0021$ ) and *P. hancockii* sounds (16 Hz; Tukey's *post hoc* test,  $P<0.001$ ). Overall AM rates of *A. cataphractus* and *P. hancockii* did not differ (Tukey's *post hoc* test,  $P>0.05$ ). Among sound types with lower representative AM rates (Ac type I, Ap type II, Ph type I), AM rates of Ph type I sounds were higher than rates of Ap type II sounds (16 versus 11 Hz; ANOVA,  $F_{2,10}=9.50$ ,  $P=0.005$ ; Tukey's *post hoc*,  $P=0.0037$ ) but not Ac type I (Tukey's *post hoc*,  $P>0.05$ ). Ac type I AM modulation rates did not differ from Ap type II (Tukey's *post hoc*,  $P>0.05$ ). No differences were observed among sound types with higher representative AM rates (Ac type III, Ap type II, Ph type II; Table 1; ANOVA,  $F_{2,9}=4.51$ ,  $P=0.044$  not significant after seq. Bonferroni correction for multiple tests).

### Temporal features of sound trains

The tendency to produce sound trains consisting of multiple sound events was variable among taxa (Table 2). Most sounds of *P. hancockii* and nearly half of all *A. cataphractus* sounds were part of longer sound trains, whereas only 5% of the sounds of *A. pectinifrons* type II were part of a longer train. Trains of *A. cataphractus* and *P. hancockii* sometimes included more than one sound type (Table 2) and 7% of *P. hancockii* trains included three sound types.

### Sonic apparatus morphology

The sonic organ of the three species follows the same general pattern of ESA morphology that was described previously for doradids (Müller, 1842; Bridge and Haddon, 1889, 1893; Kastberger, 1977; Ladich, 2001). The sonic muscle originates on the ventral shelf formed by the supraoccipital and nuchal plate and inserts on the anterior transverse process of vertebra IV (Müllerian ramus). The

**Table 2. Temporal features of sound trains from three doradid catfish**

Species	Fish (N)	Sound trains (n)	% of sounds occurring in trains	% of sound trains with one sound type	No. of sounds per train <sup>a</sup>	Average inter-event (ms)	Sound emission rate (Hz)
<i>A. cataphractus</i>	3	9	44.8±22	52.4±50	4.8±3.0, 2, 10	503±323	2.5±0.3
<i>P. hancockii</i>	7	31	77.9±14	39.3±42	3.0±1.8, 2, 9	458±132	3.2±3.2
<i>A. pectinifrons</i>	3	2	4.8±4	100.0	2.0±0.0, 2, 2	70±2	3.2±0.5

All results are means±s.e.m.

<sup>a</sup>Results shown are means±s.e.m., min, max.

swim bladders of *A. cataphractus* and *A. pectinifrons* are cardiform shaped, whereas the swim bladder of *P. hancockii* has a more spherical morphology (Fig. 3A). Notably, the relative sizes of the swim bladders vary among the species; *P. hancockii* has a relatively smaller swim bladder than *A. cataphractus* and *A. pectinifrons*. The relative size of the caudal portion of the cranium, near the origin of the sonic muscle, is largest in *A. cataphractus* and smallest in *P. hancockii* (Fig. 3B). In *A. cataphractus*, the caudal cranium and the dorsal portion of the pectoral girdle, which are fused, are expanded laterally compared with *A. pectinifrons* and *P. hancockii* (Fig. 3B). Furthermore, the origin of the sonic muscle in *A. cataphractus* occupies a more medial position relative to the other two species (Fig. 3B). As a result, the sonic muscle from origin to insertion has a more ventrolateral trajectory, which is in contrast to *A. pectinifrons* and *P. hancockii*, where the muscles are oriented dorsoventrally.

#### Sonic muscle ultrastructure

Sonic muscle ultrastructure was similar for all three species (Fig. 4). Muscle fibers for each species were rounded in cross section. Myofibrils were ribbon-like and radially arranged in a spoke-like pattern. Muscle fibers possessed a central core of sarcoplasm, and in larger muscle fibers, smaller additional cores were often present (Fig. 4). Mitochondria were abundant near myofibrils in the peripheral sarcoplasm and central core. The sarcoplasmic reticulum was well developed in each species with a dense network of sarcotubules present between myofibrils. The relative cross sectional area of myofibrils did not differ among species (Kruskal–Wallis,  $H=0.9389$ , d.f.=2,  $P=0.625$ ) with the observed median relative myofibril areas (% of fiber area) of 47.6% for

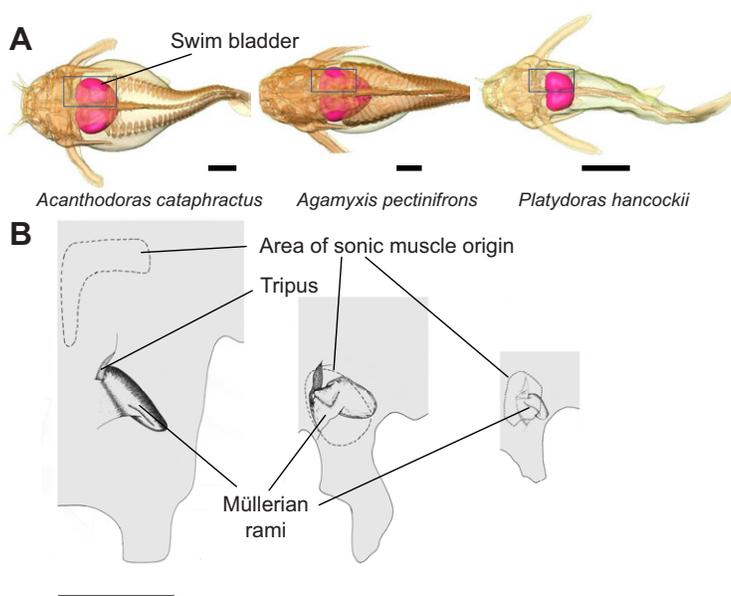
*A. cataphractus* ( $n=7$  fibers), 50.1% for *A. pectinifrons* ( $n=5$ ) and 47.2% for *P. hancockii* ( $n=11$  fibers).

#### DISCUSSION

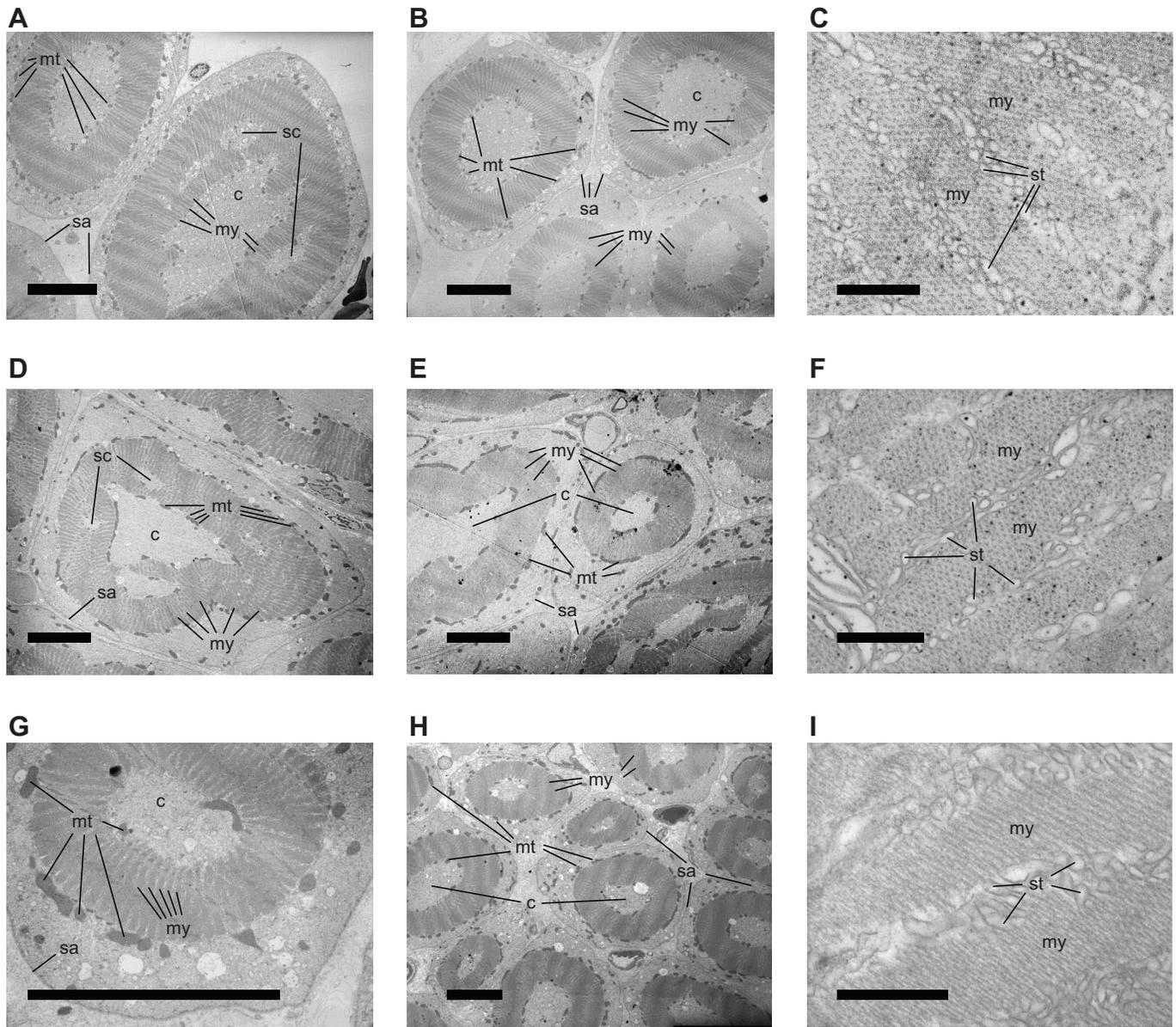
Several types of drumming sounds were observed from all three species in this study. Furthermore, individuals of each species were able to modulate their activation patterns, which allows them to produce multiple sound types.

The fundamental frequency of swim bladder drumming sounds is determined by the sonic muscle contraction rate (Tavolga, 1971; Ladich and Fine, 2006). This relationship has been shown experimentally in a variety of fish families that evolved sonic muscles independently and are capable of producing long, continuous sounds. Examples include batrachoidid toadfishes (Skoglund, 1961; Cohen and Winn, 1967; Fine et al., 2001), triglid sea robins (Bass and Baker, 1991; Connaughton, 2004), ariid catfishes (Tavolga, 1962) and doradid catfishes (Kastberger, 1977). Because sound frequency in these species is physiologically determined, it increases with temperature (Fine, 1978; Bass and Baker, 1991). Higher drumming sound frequencies were observed at warmer temperatures in a study on the doradid catfish *Platydoras armatulus* (Papes and Ladich, 2011).

Swim bladders have been modeled as highly resonant air bubbles (Bergeijk, 1964; Harris, 1964). Evidence from experimental studies, however, indicates that unlike air bubbles, swim bladders are not highly resonant. Experiments measuring the vibration of exposed swim bladders in toadfish (Fine et al., 2009) and piranha (Millot et al., 2011) indicate that the swim bladder is a highly damped structure. Because of this damping, the swim



**Fig. 3. 3D volume rendered images obtained from CT scans.** Reconstructed dorsal views of (A) *Acanthodoras cataphractus*, *Agamyxis pectinifrons* and *Platydoras hancockii*. (B) Schematic dorsal views of the left elastic spring apparatus in *A. cataphractus*, *A. pectinifrons* and *P. hancockii* (corresponding to the rectangles in A). The grayed zone represents the dorsal part of the fused neurocranium, pectoral girdle and nuchal plate. The dotted line outlines the origin of the sonic muscle on the ventral surface of the neurocranium. The three diagrams in B are shown at the same scale. Scale bars: 1 cm.



**Fig. 4. Transmission electron micrographs of cross sections of sonic muscle, the protractor muscle of the elastic spring apparatus in three doradid catfish species.** Muscles of *A. cataphractus* (A–C), *A. pectinifrons* (D–F) and *P. hancockii* (G–I) share similar characteristics: radially arranged ribbon-like myofibrils, cores of sarcoplasm, abundant mitochondria around the periphery of myofibrils in the core and surrounding sarcoplasm and an elaborate sarcoplasmic reticulum. Larger fibers have smaller cores of sarcoplasm in addition to the large central core. c, core of sarcoplasm; mt, mitochondria; my, myofibril; sa, sarcolemma; sc, smaller core of sarcoplasm; st, sarcotubule. Scale bars: 10  $\mu\text{m}$  (A,B,D,E,G,H), 0.5  $\mu\text{m}$  (C,F,I).

bladder is able to respond to a wider range of frequencies that are driven by the contraction rate of the sonic muscles and the resulting sounds are not constrained by the natural frequency of the swim bladder. These studies show that the swim bladder soft tissue, and not just the surrounding muscles, is responsible for damping. Because of these properties, the spectral frequencies of swim bladder sounds are likely to be relatively insensitive to changes in body size of signaling fish. Observations of male toadfish choruses in the wild show narrow frequency ranges, even though the sounds are likely to be produced by individuals of varying sizes (Fine, 1978). Differences in the relative and absolute swim bladder volumes among fish in this study (Fig. 3) are unlikely to contribute to spectral variation because of the expected damped properties of the swim bladder. The relatively narrow

range of observed sound frequencies in our study is consistent with this prediction.

Though muscle contraction speed is the principal driver of sound frequency for doradids, effects of body size and the relative size of individual sonic apparatus elements may be expected to affect the spectra of sounds. As sonic muscle lengths increase, contraction cycle duration is expected to increase (Wainwright and Richard, 1995), and the resulting sound frequency should decrease (Connaughton et al., 2000). Consistent with this prediction, Knight and Ladich (2014) observed a very modest inverse relationship between dominant frequency and body size among individuals of five doradid catfishes. In our study, muscle lengths probably varied somewhat among species because of the overall size differences among fish and as a result of species differences in

relative sonic muscle length. The smallest individuals in our study, *P. hancockii*, have a relatively shorter sonic muscle, which runs in a predominately dorsal–ventral course from origin to insertion (Fig. 3). Variation in dominant frequency among the sounds of the doradid species examined in this study, however, was relatively modest. The average dominant frequency ranged from 91 to 131 Hz among species and the ranges of individual sounds were largely overlapping (Table 1). In general, sounds of *P. hancockii* were slightly higher frequency than *A. pectinifrons*. It is possible that longer muscle fibers would impart a physiological limitation in contraction frequency that would be reflected in the highest observed sound frequencies. Consistent with this prediction, the highest observed dominant frequencies of *P. hancockii* sounds were over 400 Hz, whereas *A. pectinifrons* and *A. cataphractus* sound frequencies were all less than 200 and 300 Hz, respectively. Body size variation among species, however, does not appear to explain variation between the sounds of species.

The sonic muscles of the three species in this study possessed features that are indicative of fast contractions. Muscle fibers had central cores of sarcoplasm, an elaborated sarcoplasmic reticulum and radially arranged myofibrils. These features were observed previously in a related species, *P. armatulus* (formerly *P. costatus*; Ladich, 2001). Larger muscle fibers observed during this study sometimes had smaller additional cores, a feature observed in the fast sonic muscles of oyster toadfish *Opsanus tau* (Fine et al., 1993; Loesser et al., 1997) and the doradid *P. armatulus* (Ladich, 2001). The sonic muscle fiber morphology with alternating radiations of myofibrils and sarcoplasmic reticula is most efficient for calcium transport to the site of contraction but results in a trade-off that restricts mitochondria to the periphery and center of the contractile cylinder (Fine et al., 1993). Fine et al. (1993) hypothesized that sarcoplasmic cores mitigate the effect of this trade-off and the addition of cores in larger fibers prevents an increase in the distance of ATP transport to the myofibrils.

Many of the unusual ultrastructural features of doradid sonic muscles are shared by taxonomically disparate groups of sound producing teleosts (Ladich and Fine, 2006; Parmentier and Diogo, 2006). All three species in this study were capable of producing long continuous sounds (>1 s) and produced sounds with fundamental frequencies >100 Hz. Sounds of such characteristics have physiological demands that may require the apomorphic features observed in these drumming muscles. Notably, however, no obvious differences in fine structure of drumming muscles were observed between these species, even though sounds are distinctive at the species level.

The principal variation in sounds within and among species observed in this study occurred not from spectral variation, but rather from complex temporal patterning. These differences appear most likely to result from differences in the pattern of neural activation of the sonic muscles, rather than from differences in the gross morphology of the sonic apparatus and swim bladder or the fine structure of the sonic muscles. Much of the observed variation appears to involve the envelope of amplitude modulation among sounds.

Amplitude modulation has been noted in the sounds of other fishes that produce long, continuous sounds. Examples include a mormyrid (Crawford et al., 1997), batrachoidid fishes (Dos Santos et al., 2000; Rice and Bass, 2009), and gobiids (Lugli et al., 1996; Parmentier et al., 2013). In this study, the number of amplitude modulations per sound was variable and not consistently different among sound types. The rate of AM in sounds, however, did differ. AM rate of *A. pectinifrons* sounds overall was lower than the other

two species, but *A. cataphractus* type I and *A. pectinifrons* type II sounds did not differ in terms of AM rate. In addition, the overall waveform shape of sounds from different species and sound types differed irrespective of sound duration.

High variation in swim bladder sounds has been observed in other studies on drumming sounds in doradid catfishes and the closely related auchenipterids. Kaatz and Stewart (2012) described ‘continuous growl’ sounds, which lack a silent pulse interval, but display a repeated pattern of peaks of alternating amplitudes; sounds with ‘fixed interval pulse groups’, which are short duration clusters of repeated pulses that somewhat resemble *P. hancockii* type I and II sounds; and sounds with irregular intervals between sounds. Their results parallel the high observed variation in sound duration and inter-event interval in sounds in this study. A continuous growl-like sound with alternating pulse amplitudes was observed also in *Orinocodoras eigenmanni* (Kaatz and Lobel, 2001). Kaatz and Stewart (2012) observed two sound types (mentioned but not shown) from *A. pectinifrons* and one type from *A. cataphractus*, which is in partial agreement with the diverse acoustic repertoire observed for these species in this study. Papes and Ladich (2011) report two sound types for *Platydoras armatulus*, a short and long sound type, the latter which resembles the harmonic moan-like *A. pectinifrons* type II sounds of this study. Similar sounds were reported from *P. armatulus* (formerly *P. costatus*) in an earlier study (Ladich, 1997). In a study of sounds from five doradid species including *A. pectinifrons*, Knight and Ladich (2014) observed continuous ‘single drumming sounds’ in all five species and ‘series of short drumming pulses’ that were separated by silent interpulse intervals in two of the five species, *Megalodoras uranoscopus* and *Oxydoras niger*. Sharply peaked pulses, similar to *P. hancockii* type I sounds were observed in *Doras* sp. (Kastberger, 1978). A continuous oscillation of pulses, similar to *A. pectinifrons* type II and *A. cataphractus* type II was observed in *Acanthodoras* sp. (Kastberger, 1978). These studies show that both continuous tonal sounds and trains of separated pulses are widespread among doradids and many species appear capable of producing sounds of both sound types.

Morphological features of the doradid ESA and swim bladder and drumming sound features do not appear to be strongly correlated (Kaatz and Stewart, 2012). Some doradid catfish species possess numerous diverticula of the swim bladder, which in some species are quite elaborate (Birinidelli et al., 2009; Kaatz and Stewart, 2012). No clear relationship between sound properties or complexity and swim bladder diverticula has been observed in doradid sounds (Kaatz and Stewart, 2012). Species examined in this study lack numerous diverticula and possess relatively simple, cardiform swim bladders; yet, complex and diverse sounds were observed among species and even individual fish. Higher-frequency sounds of some doradoid fishes are associated with a conical MR morphology (Kaatz and Stewart, 2012). The doradids of the present study, however, all possess a discoidal plesiomorphic MR and, although there were differences in dominant frequency among species, the observed range of sound frequencies was relatively narrow. Furthermore, no clear relationship between body size and dominant frequency of drumming sounds in doradoid catfish exists (Kaatz and Stewart, 2012).

Patterns of sonic muscle activation may explain differences in sound features among species that are less associated with ESA morphology and muscle fine structure. A previous study indicates that contractions of the bilateral protractor muscles of some doradid species are synchronous with a one-to-one correspondence with the fundamental frequency (Kastberger, 1977). The sonic motor neurons of doradids are located along the midline of the medulla

and rostral spinal cord on the ipsilateral side of the protractor muscle (Ladich and Bass, 1998). The moderate differences in fundamental frequency among sound types and species could potentially be explained by differences in firing rate. The sharp pulses at the beginning of waveforms that have a knock-like quality (*A. cataphractus* type I, *A. pectinifrons* type I and *P. hancockii* type II) could be produced by rapid, more synchronous activation. Amplitude modulation could be driven by the number of firing motor units of the protractor muscle, in which an increase in recruited motor units would result in larger-amplitude movements of the MR. Some sounds displayed slow decreases in amplitude and an increase in cycle duration (decreasing fundamental frequency). The reduction of amplitude in these sounds could be indicative of fatigue, which may be a limiting factor for fish sonic muscles (Mitchell et al., 2008). The decreasing fundamental frequency is indicative of a lowering of motoneuron firing rate, perhaps from spike frequency adaptation (Granit et al., 1963; Miles et al., 2005). A recent study with the plainfin midshipman *Porichthys notatus* demonstrated that differential motoneuron recruitment associated with motoneuron size (smaller soma with lower recruitment thresholds after activation from premotor nucleus neurons) can cause amplitude modulation of sounds (Chagnaud et al., 2012). Perhaps a similar mechanism exists for doradid catfish.

Intrinsic sonic muscles, which have short fiber lengths and an origin and insertion on the swim bladder are predicted to have more efficient relaxations during sound production (Fine and Ladich, 2003), are not known from any catfishes. Instead of this system, most families of catfishes with swimbladder muscles possess an ESA morphology. Notable exceptions are the Pimelodidae and Pseudopimelodidae, in which sonic muscles insert directly on the ventral side of the swim bladder (Ladich and Fine, 1994; Heyd and Pfeiffer, 2000; Ladich, 2001; Birinidelli and Shibatta, 2011). It is hypothesized that the ESA may provide an evolutionary alternative to intrinsic sonic muscles because the bony structure allows for fast relaxation of sonic muscle contractions (Fine and Ladich, 2003).

Further study is needed to determine what advantage the ESA confers for sound production. The Müllerian ramus at least permits the application of a powerful force on a focused point of the anterior chamber of the swimbladder that is the major radiator. It allows for sound production in small species and in species that have proportionally small swim bladders, which is usually the case for catfish. Moreover, the entire swimbladder does not seem to be involved in sound production in these species.

Sound production mechanisms could also be linked to the mode of life and the evolutionary function conveyed in a particular behavioral context. In species with swim bladder muscles, the fundamental frequency may not vary much with fish size since muscle contraction rate determines the fundamental frequency (Skoglund, 1961; Fine et al., 2001; Millot et al., 2011). In these fish, reproductive success would be determined by calling effort (ability to make calls for long periods) and not by dominant frequency (Parmentier and Fine, 2016). Despite substantial variation in size (from 56 to 99 mm), sounds from all three doradid species had similar mean dominant frequencies, and if the same relationship applies to their reproductive sounds, then mate quality would be reflected in the ability to sustain sounds rather than frequency.

Moreover, the catfish ESA is likely to have evolved multiple times within the Siluriformes (Parmentier and Diogo, 2006). In addition, the ESA morphology exists in some catfish species that may rarely (if at all) produce drumming sounds. Some species of *Synodontis* (Mochokidae) possess an ESA and produce electric organ discharges with the ESA protractor muscle in addition to

sounds, whereas others may not produce sounds at all (Hagedorn et al., 1990; Baron et al., 2001; Boyle et al., 2014).

Malapterurid catfishes possess an ESA morphology and a protractor muscle with ultrastructural features similar to fast sonic muscles, but at least some of the sounds observed from this family are not consistent with an ESA-driven sound (Boyle et al., 2015). Because detailed life-history information is lacking for many catfishes with swim bladder sonic mechanisms, it is not yet known if changes occur in the condition of sonic muscle with reproductive season and sex. The intra- and interspecific diversity of disturbance sounds observed in the current and previous studies (Kaatz and Stewart, 2012; Knight and Ladich, 2014) indicates the potential for these sound types to signal different kinds of information in other behavioral contexts. Different context-dependent swim bladder sounds were observed in doradoid fishes by Kaatz (1999) in which agonistic sounds were continuous and reproductive sounds were pulsed. In addition, by producing a diversity of sounds in distress situations, catfish may increase the efficacy of startling a wider range of predatory species.

## MATERIALS AND METHODS

### Fish

Fish were obtained from commercial tropical fish wholesalers and kept in a communal aquarium maintained with tap water kept at 24–25°C and on a 12 h light:12 h dark cycle. Fish were fed a diet of commercially prepared food (JBL Novotab, Neuhofen, Rhineland-Palatinate, Germany) every other day.

### Sound recordings

Sound recordings were attempted with *A. cataphractus* ( $N=5$ , mean standard length $\pm$ s.d.,  $88\pm 7.2$  mm), *A. pectinifrons* ( $N=3$ ,  $99\pm 6.0$  mm) and *P. hancockii* ( $N=8$ ,  $56\pm 3.1$  mm). Recording experiments were conducted in a small aquarium (60 $\times$ 29 cm) with an approximate water depth of 20 cm, and an estimated minimum resonance frequency of 4.6 kHz (Akamatsu et al., 2002). A hydrophone (HTI Min-96,  $-164.4$  dB re.  $1 \text{ V } \mu\text{Pa}^{-1}$ ; flat frequency response range between 2 Hz and 30 kHz) (Long Beach, MS, USA) was placed in the center of the aquarium and sounds were recorded with a Tascam DR-05 recorder (TEAC, Wiesbaden, Germany) at a 44.1 kHz sampling rate. For recordings of distress sounds, fish were held in the hand by an observer at approximately the same position, 5 cm from the hydrophone. All species readily produced stridulatory sounds with the pectoral spine and spinal fossa. These sounds were not included in the analysis. All three species are capable of producing sounds with the ESA and pectoral spines (stridulation) simultaneously; thus an effort was made to hold the fish in a manner to block abduction of the pectoral spines. Pectoral spines were held adjacent to the humeral process of the pectoral girdle by the tips of the observer's fingers in order to avoid contact with the 'exomembrane' (Kastberger, 1977) area of the fish. Swim bladder sounds obtained in this manner (see Results) were similar to recordings in which the fish's pectoral spines were free to stridulate (supplementary material Fig. S1), but facilitated measurements of swim bladder sound features.

### Sound analysis

#### Sound duration and dominant frequency

Sounds were initially screened using Adobe Audition 2.1 (San Jose, CA, USA) and Avisoft-SAS Lab Pro version 4.33 software (Avisoft Bioacoustics, Glienicke, Germany). Spectrograms for figures were produced with Avisoft. Sound duration was estimated from visual inspections of sound waveforms. Dominant frequency was estimated from power spectra produced from 1024-point FFTs on zero-padded data with a Hanning window in Matlab 7.0 (MathWorks, Natick, MA, USA).

#### Amplitude modulation of sound waveforms

A procedure was conducted in order to estimate the extent and rate of AM in individual swim bladder sounds. A custom routine in Matlab 7.0 was used to first rectify sounds. Because the rates of AM were low, sounds were low-

pass filtered at 75 Hz with a fifth-order Butterworth filter. Low-frequency noise in the rectified waveform was filtered with a 5 Hz high-pass, fifth order Butterworth filter. The resulting waveform presented an acoustic envelope that closely matched the visible AM in the original sound wave (Fig. 1). The number of peaks (extrema) of the envelope was then calculated. The rate of amplitude modulation (Hz) was approximated based on the number of extrema/duration of the sound.

### Sound waveform similarity among species and sound types

Similarity of sound waveforms was assessed by calculating pairwise cross-correlation coefficients of all sounds in the time domain. A custom routine was run in Matlab 7.0 software using the XCORR function. For each sound-pair, alignment with the maximal absolute cross-correlation value (ignoring phase) was used and scaled between 0 and 1. To visualize and analyze the relationship among sounds, two-dimensional classical metric multidimensional scaling (MDS) was performed on the matrix of sound correlation values in Matlab 7.0. This procedure produced was used to produce a biplot in order to represent patterns of similarity among sounds as distances in two-dimensional space.

### Temporal features of sound trains

The temporal patterns of sound emission were examined for each species. Individual sounds were sometimes emitted after relatively brief, silent inter-event interval. To characterize and compare the temporal patterning of sounds, we considered sounds with an inter-event interval less than 1 s to be part of a sound train. Mean sound train characteristics were calculated for each species based on an average of the mean sound train characteristics for each individual fish.

### Morphology of the swim bladder and elastic spring apparatus

In order to visualize the swim bladder shape, size and position relative to the body and skeleton, one live fish from each species was scanned with computerized tomography (CT) at the Veterinary Clinic of the University of Liège. Each fish was anesthetized with 200 mg l<sup>-1</sup> MS-222 (Sigma-Aldrich, St Louis, MO, USA). Scans were performed with a Siemens Somatom Sensation (Siemens AG, Munich, Germany) with a slice thickness of 0.6 mm: *A. pectinifrons*, 369 slices; *A. cataphractus*, 364 slices; *P. hancockii*, 237 slices. Amira 5.4.0 (VSG, FEI Company, Eindhoven, The Netherlands) was used for segmentation and 3D reconstruction of the swim bladder, body and skeleton from CT scan data. ESA morphology was examined in preserved specimens (fixed in 7% formalin and stored in 70% ethanol). Fish were dissected and examined with a stereo dissection microscope (Leica, Wild M10, Wetzlar, Germany) coupled to a camera lucida.

### Protractor muscle ultrastructure

For ultrastructural examinations, one fish from each species (*A. cataphractus*, 89 mm SL; *A. pectinifrons* 93 mm SL that was used in sound recordings, see above; and *P. hancockii*, 67 mm SL) was killed with an overdose of MS-222. Fish were quickly dissected to sample ESA protractor muscle (Bridge and Haddon, 1893). Small muscle samples (2–3 mm<sup>3</sup>) were immediately fixed in 2.5% glutaraldehyde. All muscle samples were post-fixed in 1% osmium tetroxide, dehydrated through a graded ethanol-propylene oxide series and embedded in epoxy resin (SPI-PON 812, SPI-CHEM, JEOL Europe, Zaventem, Belgium). Semithin (1 µm) and ultrathin sections (60–80 nm) were cut using 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 100SX transmission electron microscope (Zaventem, Belgium) at 80 kV accelerating voltage.

### Muscle fiber size and myofibril density

Muscle fiber cross-sectional area and myofibril density were estimated from TEM images (1000× magnification). Adobe Photoshop CS5 software (San Jose, CA, USA) was used to calculate myofibril area from 18 fibers. Myofibril area was estimated from the percentage of pixels occupied by myofibrils relative to the total muscle fiber cross section.

### Statistical analyses

All data for parametric statistics were tested for assumptions of normality and homogeneity of variance. When tests indicated a deviation from these assumptions, data were transformed. When suitable transformations were not possible, non-parametric tests were used. A sequential Bonferroni (Rice, 1989) procedure was used to adjust the family-wide type I error rate ( $\alpha=0.05$ ). Statistical tests were conducted using Minitab version 13 (State College, PA, USA) and GraphPad Prism 5.0 software (La Jolla, CA, USA).

### Sound waveform similarity

To test for patterns among species and sound types based on waveform similarity, i.e. clustering in multidimensional space among species and sound type, a type III GLM multivariate analysis of variance (MANOVA) was conducted using the scores from the two MDS axes as response variables. The MANOVA model tested species, individual fish (nested within species) and sound type (nested within species and individual). Data were scaled as a proportion and arc-sine square root transformed.

### Sound features between species and sound types

We tested for differences in duration, dominant frequency, AM number and AM rate between species with all sound subtypes included. Individual fish were used as replicates in these analyses. Because sound data for each individual tended to be non-normal, the median observation for each fish was used. Differences were tested with a series of univariate tests, one-way ANOVA or Kruskal–Wallis, followed by Tukey's or Dunn's *post hoc* tests, respectively. We first examined whether differences existed among drumming sounds from each species overall.

Variable drumming sound types were observed (see Results), so we considered that pooling all drumming sounds for each species may conceal differences that exist when sound types were analyzed separately. Because sound types were unique to each species and not all individuals within a species made each sound type, a balanced statistical comparison was not possible. Thus we performed a subset of univariate comparisons (one-way ANOVA or Kruskal–Wallis) with the most common sound type of *A. pectinifrons* (the second sound type was observed in only two individuals) and the two most common sound types of *A. cataphractus* and *P. hancockii*. Separate tests were conducted on sound duration among shorter and longer duration sound types, dominant frequency among lower and higher frequency sound types, AM number among sound types with few or many AM per sound and AM rate among sounds with low and high AM rates. Because a single *A. pectinifrons* sound type was most common, it was used in both series of tests.

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### Competing interests

The authors declare no competing or financial interests.

### Author contributions

K.S.B., S.R. and E.P. conceived and designed the experiments. K.S.B. and S.R. recorded and analyzed fish sounds. K.S.B., S.R. and E.P. performed conducted electron microscopy. E.P. analyzed the myofibril density in muscle fibers and described the sonic apparatus anatomy. G.B. performed the CT scan. K.S.B. and E.P. wrote the manuscript and K.S.B., S.R., G.B. and E.P. revised the manuscript.

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### Supplementary material

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### References

- Akamatsu, T., Okumura, T., Novarini, N. and Yan, H. Y. (2002). Empirical refinements applicable to the recording of fish sounds in small tanks. *J. Acoust. Soc. Am.* **112**, 3073–3082.
- Amorim, M. C. P. (2006). Diversity of sound production in fish. In *Communication in Fishes*, Vol. I (ed. F. Ladich, S. P. Collin, P. Moller and B. G. Kapoor), pp. 71–104. Enfield: Science Publishers.

- Amorim, M. C. P., Simões, J. M., Almada, V. C. and Fonseca, P. J.** (2011). Stereotypy and variation of the mating call in the Lusitanian toadfish, *Halobatrachus didactylus*. *Behav. Ecol. Sociobiol.* **65**, 707-716.
- Baron, V. D., Morshnev, K. S., Olshansky, V. M., Orlov, A. A., Pavlov, D. S. and Teferi, I.** (2001). Observations of the electric activity of silurid catfishes (Siluriformes) in Lake Chamo (Ethiopia). *J. Ichthyol.* **41**, 542-549.
- Bass, A. H. and Baker, R.** (1991). Evolution of homologous vocal control traits. *Brain Behav. Evol.* **38**, 240-254.
- Bergeijk, W. A. V.** (1964). Directional and nondirectional hearing in fish. In *Marine Bioacoustics* (ed. W. N. Tavolga), pp. 291-299. New York: Pergamon Press.
- Birnidelli, J. L. O. and Shibatta, O. A.** (2011). Morphology of the gas bladder in bumblebee catfishes (Siluriformes, Pseudopimelodidae). *J. Morphol.* **272**, 890-896.
- Birnidelli, J. L. O., Sousa, L. M. and Sabaj Pérez, M. H.** (2009). Morphology of the gas bladder in thorny catfishes (Siluriformes: Doradidae). *Proc. Acad. Nat. Sci. Phila.* **158**, 261-296.
- Boyle, K. S. and Tricas, T. C.** (2010). Pulse sound generation, anterior swim bladder buckling and associated muscle activity in the pyramid butterflyfish, *Hemitaenichthys polylepis*. *J. Exp. Biol.* **213**, 3881-3893.
- Boyle, K. S., Colley, O. and Parmentier, E.** (2014). Sound production to electric discharge: sonic muscle evolution in progress in *Synodontis* spp. catfishes (Mochokidae). *Proc. R. Soc. Lond. B Biol. Sci.* **281**, 20141197.
- Boyle, K. S., Bolen, G. and Parmentier, E.** (2015). Agonistic sounds and swim bladder morphology in a malapterurid electric catfish. *J. Zool.* **296**, 249-260.
- Bradbury, J. W. and Vehrencamp, S. L.** (1998). *Principles of Animal Communication*. Sunderland, MA: Sinauer.
- Bridge, T. W. and Haddon, A. C.** (1889). Contributions to the anatomy of fishes. I. The air-bladder and Weberian ossicles in the Siluridae. *Proc. R. Soc. Lond. B Biol. Sci.* **46**, 309-328.
- Bridge, T. W. and Haddon, A. C.** (1893). Contributions to the anatomy of fishes. II. The air-bladder and Weberian ossicles in the siluroid fishes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **184**, 65-333.
- Chagnaud, B. P., Zee, M. C., Baker, R. and Bass, A. H.** (2012). Innovations in motoneuron synchrony drive rapid temporal modulations in vertebrate acoustic signaling. *J. Neurophysiol.* **107**, 3528-3542.
- Cocroft, R. B. and Ryan, M. J.** (1995). Patterns of advertisement call evolution in toads and chorus frogs. *Anim. Behav.* **49**, 283-303.
- Cohen, M. J. and Winn, H. E.** (1967). Electrophysiological observations on hearing and sound production in the fish, *Porichthys notatus*. *J. Exp. Zool.* **165**, 355-370.
- Connaughton, M. A.** (2004). Sound generation in the searobin (*Prionotus carolinus*), a fish with alternate sonic muscle contraction. *J. Exp. Biol.* **207**, 1643-1654.
- Connaughton, M. A., Fine, M. L. and Taylor, M. H.** (1997). The effects of seasonal hypertrophy and atrophy on fiber morphology, metabolic substrate concentration and sound characteristics of the weakfish sonic muscle. *J. Exp. Biol.* **200**, 2449-2457.
- Connaughton, M. A., Taylor, M. H. and Fine, M. L.** (2000). Effects of fish size and temperature on weakfish disturbance calls: implications for the mechanism of sound generation. *J. Exp. Biol.* **203**, 1503-1512.
- Crawford, J. D., Cook, A. P. and Heberlein, A. S.** (1997). Bioacoustic behavior of African fishes (Mormyridae): potential cues for species and individual recognition in *Pollimyrus*. *J. Acoust. Soc. Am.* **102**, 1200-1212.
- Dos Santos, M. E., Modesto, T., Matos, R. J., Grober, M. S., Oliveira, R. F. and Canário, A. V. M.** (2000). Sound production by the Lusitanian toad fish, *Halobatrachus didactylus*. *Bioacoustics* **11**, 309-321.
- Fine, M. L.** (1978). Seasonal and geographical variation of the mating call of the oyster toadfish *Opsanus tau* L. *Oecologia* **36**, 45-57.
- Fine, M. L. and Ladich, F.** (2003). Sound production, spine locking, and related adaptations. In *Catfishes* (ed. G. Arratia, B. G. Kapoor, M. Chardon and R. Diogo), pp. 249-290. Enfield, NH: Science Publisher Co.
- Fine, M. L., Bernard, B. and Harris, T. M.** (1993). Functional morphology of toadfish sonic muscle fibers: relationship to possible fiber division. *Can. J. Zool.* **71**, 2262-2273.
- Fine, M. L., Malloy, K. L., King, C. B., Mitchell, S. L. and Cameron, T. M.** (2001). Movement and sound generation by the toadfish swimbladder. *J. Comp. Physiol. A* **187**, 371-379.
- Fine, M. L., King, C. B. and Cameron, T. M.** (2009). Acoustical properties of the swimbladder in the oyster toadfish *Opsanus tau*. *J. Exp. Biol.* **212**, 3542-3552.
- Granit, R., Kernell, D. and Shortness, G. K.** (1963). Quantitative aspects of repetitive firing of mammalian motoneurons, caused by injected currents. *J. Physiol.* **168**, 911-931.
- Hagedorn, M., Womble, M. and Finger, T. E.** (1990). Synodontid catfish: a new group of weakly electric fish. *Brain Behav. Evol.* **35**, 268-277.
- Harris, G. G.** (1964). Considerations on the physics of sound production by fishes. In *Marine Bioacoustics* (ed. W. N. Tavolga), pp. 233-247. New York: Pergamon Press.
- Heyd, A. and Pfeiffer, W.** (2000). Über die Lauterzeugung der Welse (Siluroidei, Ostariophysi, Teleostei) und ihren Zusammenhang mit der Phylogenie und der Schreckreaktion \*. *Rev. Suisse Zool.* **107**, 165-211.
- Kaatz, I. M.** (1999). *The Behavioral and Morphological Diversity of Acoustic Communication Systems in a Clade of Tropical Catfishes (Pisces: Siluriformes)*, p. 367. Syracuse, New York: State University of New York.
- Kaatz, I. M. and Lobel, P. S.** (2001). A comparison of sounds recorded from a catfish (*Orinocodoras eigenmanni*, Doradidae) in an aquarium and in the field. *Biol. Bull.* **201**, 278-280.
- Kaatz, I. M. and Stewart, D. J.** (2012). Bioacoustic variation of swimbladder disturbance sounds in Neotropical doradoid catfishes (Siluriformes: Doradidae, Auchenipteridae): potential morphological correlates. *Curr. Zool.* **58**, 171-188.
- Kastberger, G.** (1977). Der Trommelapparat der Doradiden (Siluriformes, Pisces). *Zool. Jb. Physiol.* **81**, 281-309.
- Kastberger, G.** (1978). Produção de sons em doradideos e auchenipterideos (Siluriformes, Pisces). *Acta Amaz.* **8**, 455-468.
- Kéver, L., Boyle, K. S., Dragičević, B., Dulčić, J., Casadevall, M. and Parmentier, E.** (2012). Sexual dimorphism of sonic apparatus and extreme intersexual variation of sounds in *Ophidion rochei* (Ophidiidae): first evidence of a tight relationship between morphology and sound characteristics in Ophidiidae. *Front. Zool.* **9**, 34.
- Knight, L. and Ladich, F.** (2014). Distress sounds of thorny catfishes emitted underwater and in air: characteristics and potential significance. *J. Exp. Biol.* **217**, 4068-4078.
- Ladich, F.** (1997). Comparative analysis of swimbladder (drumming) and pectoral (stridulation) sounds in three families of catfishes. *Bioacoustics* **8**, 185-208.
- Ladich, F.** (2001). Sound-generating and -detecting motor system in catfish: design of swimbladder muscles in doradids and pimelodids. *Anat. Rec.* **263**, 297-306.
- Ladich, F. and Bass, A. H.** (1998). Sonic/vocal motor pathways in catfishes: comparisons with other teleosts. *Brain Behav. Evol.* **51**, 315-330.
- Ladich, F. and Fine, M. L.** (1994). Localization of swimbladder and pectoral motoneurons involved in sound production in pimelodid catfish. *Brain Behav. Evol.* **44**, 86-100.
- Ladich, F. and Fine, M. L.** (2006). Sound-generating mechanisms in fishes: a unique diversity in vertebrates. In *Communication in Fishes*, Vol. I (ed. F. Ladich, S. P. Collin, P. Moller and B. G. Kapoor), pp. 3-43. Enfield, NH: Science Publishers Inc.
- Loesser, K. E., Rafi, J. and Fine, M. L.** (1997). Embryonic, juvenile, and adult development of the toadfish sonic muscle. *Anat. Rec.* **249**, 469-477.
- Lugli, M., Torricelli, P., Pavan, G. and Miller, P. J.** (1996). Breeding sounds of male *Padogobius nigricans* with suggestions for further evolutionary study of vocal behaviour in gobioid fishes. *J. Fish Biol.* **49**, 648-657.
- Miles, G. B., Dai, Y. and Brownstone, R. M.** (2005). Mechanisms underlying the early phase of spike frequency adaptation in mouse spinal motoneurons. *J. Physiol.* **566**, 519-532.
- Millot, S., Vandewalle, P. and Parmentier, E.** (2011). Sound production in red-bellied piranhas (*Pygocentrus nattereri*, Kner): an acoustical, behavioural and morphofunctional study. *J. Exp. Biol.* **214**, 3613-3618.
- Mitchell, S. L., Poland, J. and Fine, M. L.** (2008). Does muscle fatigue limit advertisement calling in the oyster toadfish *Opsanus tau*? *Anim. Behav.* **76**, 1011-1016.
- Müller, J.** (1842). Beobachtungen über die Schwimmblase der Fische, mit Bezug auf einige neue Fischgattungen. *Arch. Anat. Physiol. Wiss. Med.* **1842**, 307-329.
- Papes, S. and Ladich, F.** (2011). Effects of temperature on sound production and auditory abilities in the striped Raphael catfish *Platydoras armatulus* (Family Doradidae). *PLoS ONE* **6**, e26479.
- Parmentier, E. and Diogo, R.** (2006). Evolutionary trends of swimbladder sound mechanisms in some teleost fishes. In *Communication in Fishes*, Vol. I (ed. F. Ladich, S. P. Collin, P. Moller and B. G. Kapoor), pp. 43-68. Enfield, NH: Science Publishers Inc.
- Parmentier, E. and Fine, M. L.** (2016). Fish sound production. An Exaptation? In *Vertebrate Sound Production and Acoustic Communication* (ed. R. A. Suthers, W. T. Fitch, R. R. Fay and A. N. Popper), New York: Springer Science and Business Media.
- Parmentier, E., Lagardère, J.-P., Chancerelle, Y., Dufrane, D. and Eeckhaut, I.** (2008). Variations in sound-producing mechanism in the pearlfish Carapini (Carapidae). *J. Zool.* **276**, 266-275.
- Parmentier, E., Bouillac, G., Dragičević, B., Dulčić, J. and Fine, M.** (2010). Call properties and morphology of the sound-producing organ in *Ophidion rochei* (Ophidiidae). *J. Exp. Biol.* **213**, 3230-3236.
- Parmentier, E., Kever, L., Boyle, K., Corbisier, Y.-E., Sawelew, L. and Malavasi, S.** (2013). Sound production mechanism in *Gobius paganellus* (Gobiidae). *J. Exp. Biol.* **216**, 3189-3199.
- Rice, W. R.** (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223-225.
- Rice, A. N. and Bass, A. H.** (2009). Novel vocal repertoire and paired swimbladders of the three-spined toadfish, *Batrachomoeus trispinosus*: insights into the diversity of the Batrachoididae. *J. Exp. Biol.* **212**, 1377-1391.
- Robillard, T., Höbel, G. and Gerhardt, H. C.** (2006). Evolution of advertisement signals in North American hylid frogs: vocalizations as end-products of calling behavior. *Cladistics* **22**, 1-13.

- Skoglund, C. R.** (1961). Functional analysis of swim-bladder muscles engaged in sound production of the toadfish. *J. Biophys. Biochem. Cytol.* **10**, 187-200.
- Tavolga, W. N.** (1962). Mechanisms of sound production in the ariid catfishes *Galeichthys* and *Bagre*. *Bull. Am. Mus. Nat. Hist.* **124**, 1-30.
- Tavolga, W. N.** (1971). Sound production and detection. In *Fish Physiology*, Vol. 5 (ed. W. S. Hoar and D. J. Randall), pp. 135-205. New York: Academic Press.
- Wainwright, P. C. and Richard, B. A.** (1995). Scaling the feeding mechanism of the largemouth bass (*Micropterus salmoides*): motor pattern. *J. Exp. Biol.* **198**, 1161-1171.
- Zelick, R., Mann, D. A. and Popper, A. N.** (1999). Acoustic communication in fishes and frogs. In *Comparative Hearing: Fish and Amphibians* (ed. R. R. Fay and A. N. Popper), pp. 363-411. New York: Springer.