Acoustic Variation in Ictalurid Catfishes

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Abstract

There are more than 35,000 ray-finned fishes (Actinopterygii) species potentially using acoustic communication. However, of the approximately 1200 known soniferous fish species, few include North American freshwater fish. To help fill this knowledge gap in fish acoustic communication, which holds great promise for conservation monitoring, I document acoustic measurements (duration 90%, bandwidth 90%, number of pulses, center frequency, and peak time) across 4 species (*Ameiurus nebulosus*, *Ameiurus natalis*, *Noturus flavus*, *Ictalurus punctatus*) from 3 genera of the North American catfish family, Ictaluridae. This was done by recording 10 trials of disturbance calls from 28 individuals and analyzing a total of 1294 sounds using Raven Pro 1.6 software. I hypothesized that: 1) more phylogenetically/morphologically related species would have more similar acoustic features, 2) acoustic features would correlate with one another, and 3) acoustic features would correlate with standard length (cm). For hypothesis 1, I instead found that *Ameriurus nebulosus* were the most acoustically dissimilar, despite having the highest level of phylogenetic/morphological similarity with *Ameirus natalis*. However, only *Ameriurus nebulosus'* number of pulses were significantly different from other species. For hypothesis 2, it was found that many acoustic measurements were correlated with one another as predicted. For hypothesis 3, only the number of pulses was found to be significantly correlated with standard length, but minimally so. These findings further support the notion that pulsation measurements may contain a high level of phylogenetic signal given it being the most important characteristic to differentiate species.

Introduction

The diversity of sound production in animals is vast, however, many people are largely unaware that fishes produce sound. This is despite the early history of philosophers such as Aristotle describing fish vocalizations (Zograf, 1890). Fish sounds take on a variety of roles, including reproductive, territorial, agonistic, aggressive, social, and feeding (Kasumyan, 2009). Despite there being more than 35,000 ray-finned fish (Actinopterygii) species potentially using acoustic communication, sounds from only approximately 1200 soniferous fish species are known (Anderson et al, 2011; Rice et al., 2020; Loobey et al., 2022). Out of these sounds, only 87 freshwater species in North America and Europe have been recorded (Rountree et al., 2018). Thus, there exists a large gap between the possible number of soniferous fish species that have been recorded, and what could be recorded.

Predominantly due to human activities, biodiversity is declining at a higher rate in freshwater than any other environment, thus making conservation efforts particularly important for freshwater habitats (Desjonquères et al., 2019). Understanding which fishes make sound, as well as having quantitative descriptions of these sounds, is essential to aquatic Passive Acoustic Monitoring (PAM); a non-invasive conservation technique that allows for autonomous audio recording over long timescales (Rountree et al., 2018; Desjonquères et al., 2019).

Although sound descriptions are lacking in the literature despite the high prevalence of soniferous fishes, phylogenetic comparisons of the evolution of fish acoustic communication are arguably even more sparse, which was recognized by a similar study focused on tetrapods (Chen & Wiens, 2020). A paper that did make phylogenetic comparisons from fish acoustic signals was done for Mediterranean gobies of the *Gobius* lineage, which compared five acoustic properties

across seven species using thirteen individuals for sampling (Horvatić et al., 2016). The degree of acoustic similarity has been found to be related to phylogenetic relationships for insects, birds, mammals, and anurans, but has not been fully explored in fish (Robillard et al., 2006; Tavares et al., 2006; Cap et al., 2008; Gingras et al., 2013b). Since fish are not known to be vocal learners, it would be assumed that their acoustic signals would have a strong genetic component. Thus, there is reason to suspect that phylogeny should be related to fish acoustic signals. I explore this phylogenetic and acoustic relationship in Ictaluridae (Figure 1).

Ictaluridae is the only family of freshwater catfish native to North America. In New York state, there are three closely related catfish genera (*Ameiurus*, *Ictalurus*, and *Noturus*) in the family Ictaluridae, which offer the opportunity to understand the evolution of sound production within this lineage (Acre-H al., 2016). Catfish are a particularly good model for studying acoustic communication, as they are already known to be widely soniferous, using either pectoral stridulation and/or swimbladder drumming as sound production mechanisms (Kaatz et al., 2010). Although capturing reproductive or agonistic sounds may be challenging outside of the natural habitat of ictalurids, disturbance sounds can be readily produced in the laboratory as they occur when a catfish is physically restrained in a way similar to a predatory attack (Kaatz & Stewart, 2012). Additionally, the varied morphology of the pectoral spine in catfish that produce sound, coupled with the diversity of environments catfish inhabit, suggest there are evolutionary links between sound production, morphology, and habitat (Kaatz et al., 2010;).

Body size has also been noted to influence sound characteristics in fishes (Ladich et al. 1992, Myberg et al., 1993). For example, it was found that sound pressure level and pulse duration increase while dominant frequency decreases in larger weakfish (*Cynoscion regalis*) (Connaughton et. al, 2000). The relationship between acoustic characteristics and body size is

not exclusive to fish and has been documented in many other groups such as anurans and mammals (Gingras et al., 2013b; Libera et al., 2015). This relationship can partially be explained by the correlation between body size and the size of sound producing organs (Fletcher, 1992). Additionally, body size has been noted as one of the most important morphological influencers on animal acoustic frequency (Marquet & Taper, 1998).

Morphological features beyond body size also play a large role in sound production. The structures involved in stridulatory catfish vocalizations are the dorsal process of the pectoral spine (Fine et al., 1997), spine locking processes, pectoral girdle (Gainer, 1967), and bony ridges on the pectoral spine (Kaatz & Stewart, 1997; Fabri et al., 2007). It is thought that the locking mechanism that makes vocalizations possible for catfish functions as a passive predator defense (Alexander, 1981). These structures also help to define the catfish order Siluriformes (Alexander, 1966). Catfish disturbance calls in particular occur when a catfish is physically restrained in such a way that is similar to interspecific attacks (Kaatz, 1999). It has been hypothesized that these sounds are a form of acoustic aposematism, in other words signaling unfavorability to predators using sound, but this hypothesis has not garnered support (Pfeiffer & Eisenberg, 1965). However, support has been shown for the idea that disturbance calls function in place of chemical signals, given the tradeoff there appears to be between chemical signaling and vocalizations in catfish (Heyd & Pfeiffer, 2000).

Therefore, this study aims to help fill in the literature gap on freshwater fish acoustic descriptions. I accomplished this by comparing the disturbance calls in air of 4 Ictaluridae species across 3 genera: Brown Bullhead (*Ameiurus nebulosus)*, Yellow Bullhead (*Ameiurus natalis)*, Stonecat (*Noturus flavus*)*,* and Channel Catfish (*Ictalurus punctatus*). Similar

methods recording fish air disturbance have been employed in past studies and have helped to inform this study (Kaatz, 2010; Knight & Ladich, 2014)

First, I ask if there are acoustic feature differences across species. I hypothesize (H1) that acoustic features between Brown Bullheads and Yellow Bullheads will be the most similar, acoustic features between Bullheads and Channel Catfish will be moderately similar, and that Stonecats will have the least similar acoustic features in comparison to other species. This is because both bullhead species are from the *Ameiurus* genus, which have knobs on the shelf of their dorsal process and have hemispheres/convolutions on their pectoral spine (Kaatz et al., 2010). *Ictalurus* morphological features are similar to *Ameriurus*, but have flat convolutions, which should result in increasingly dissimilar sounds to the bullheads (Kaatz et al., 2010). Finally, *Noturus* morphology is the most different with no knobs, hemispheres, or convolutions (Kaatz et al., 2010), which should result in the greatest acoustic differences in comparison to other Ictaluridae species tested here.

Secondly, I explore how acoustic features are correlated with one another. I hypothesize (H2) that some acoustic measurements will be correlated because acoustic features are partially a function of morphology. Thus, acoustic measurements should be correlated with one another since the morphology used to make the sound is the same, including the same constraints and affordances of that morphology.

Thirdly, I ask if body size influences acoustic characteristics. I hypothesize (H3) that acoustic measurements differ between fish of varying lengths. This is because body size has been found to be negatively correlated with fundamental frequency, setting a precedence for body size in fish impacting acoustic measurements (Myberg et al., 1993; Knight & Ladich, 2014).

Materials & Methods

<u>Fish Collection</u>

A total of 2 Brown Bullheads (*Ameiurus nebulosus*), 3 Yellow Bullheads (*Ameiurus natalis*), 9 Stonecats (*Noturus flavus*), and 14 Channel Catfish (*Ictalurus punctatus*) were recorded in this study. Brown Bullheads were collected in November 2020 from the Cornell Experimental Ponds (42.50543521664607, -76.4636035547431), Yellow Bullheads were collected in September 2021 from the Hudson River, Stonecats were collected by electrofishing in October 2021 from Fall Creek (42.45485160008576, -76.44787772736022), and Channel Catfish were purchased in October, 2021 from Fish Haven Farm located in Candor, NY. The fishes were housed in Corson-Mudd Hall on the Cornell campus.

<u>Audio Recordings</u>

Using a scoop net, all the fish from a single tank were placed in a bucket filled with water from their tank. Another bucket filled with water from that tank was placed to the right of the fish-filled bucket. A Zoom H5 recorder with an attached Zoom H5 microphone and Aquarian H2A hydrophone were used to make audio recordings.

I would take a single fish and hold it in the right-side bucket which would have a hydrophone placed near the top. I would announce the species being recorded, fish number based on the order of recording, and medium (water at this stage) of the recording at this time. After one minute of recording, I would announce the end of the water recording and the start of the air recording. The fish would then be recorded in the air for another minute. The recording would exceed a minute if a call was still going by the end of the minute until the call stopped. I would

then announce the end of the air recording and proceed to take a standard length measurement of the fish, which I also announced. Thus, each sound file contained all the sounds produced from all fish from one tank. This would be repeated for all the fish in the tank, which would all be the same species. This procedure would then be repeated for the rest of the tanks. The order that each tank was recorded rotated over ten trials. At least 24 hours passed before another trial began.

Sound Analyses

Audio recordings were analyzed using Raven Pro 1.6 (https://ravensoundsoftware.com/software/raven-pro/), a bioacoustics analysis software program that allows users to visualize sounds and annotate them. First, I went through each file and boxed out my voice every time I made announcements and recorded the content from those announcements in order to know what fish recording was starting/ending. Next, I went through each air medium file and boxed out all sounds I could reasonably attribute to be fish sounds. Then, I annotated the pulses of each of these sounds to collect measurements on the number of pulses, 90% duration (s), 90% bandwidth (Hz), center frequency (Hz), and peak time (s) for each sound. A random sample of 4 out of 20 files was taken for the Channel Catfish to help bring the overall sound sample size across species closer in number, while all files were annotated for the other species to keep the number of sampled sounds more even across species. While annotating pulses, I filtered out the bottom 1500-2500 Hz depending on the level of background noise in the recording due to high levels of background noise and minimal fish sound prevalence in this frequency range (Figure 2). The waveform was on the top of the window, and the spectrogram was on the bottom of the window to more clearly identify pulses. A "sound" was classified as a discrete "sound" as opposed to a pulse in a sound if the distance between the two pulses was

greater than 0.1 seconds. I could not reasonably attribute sounds to fish in water medium files, thus those sound files were not further used or analyzed as the air medium files were.

<u>Statistical Analyses</u>

Statistical analyses were conducted in R 4.1.3(https://www.r-project.org/). To evaluate if acoustic measurement characteristics were different among species, nested ANOVAs, where individuals were nested within tanks, were conducted for all acoustic measurements (bandwidth 90% (Hz), duration 90% (s), peak time (s), center frequency (Hz), and number of pulses) versus species. For significant nested ANOVAs, Tukey HSD was then conducted to determine which species were significantly different from each other for acoustic measurement(s) found to be significantly different across species. To assess whether there were associations between signal properties, I correlated acoustic metrics with one another using the lmer package in R. I report the marginal and conditional r^2 (denoted as r^2m and r^2c, respectively). In addition, a principal component analysis was conducted to visualize the correlations between the broad suite of acoustic measurements. To evaluate if acoustic characteristics were varied with body size, I correlated standard length and acoustic features using the lmer package in R. I report the marginal and conditional r^2.

Results

<u>H1: Acoustic Feature Differences Between Species</u>

Bandwidth 90% (Hz), duration , peak time, and center frequency were not found to be significantly different across species (Table 2). The only acoustic measurement that was

significantly different across species was the number of pulses ($p = 0.003$). This difference was seen between Channel Catfish vs. Brown Bullheads ($df = 8.58$, $p = 0.026$) and Stonecats vs. Brown Bullheads ($df = 170.14$, $p = 0.011$) (Table 3 & Figure 3). All other species pairings were not statistically significant for differences in number of pulses.

H2: Acoustic Feature Correlations With One Another

Bandwidth was found to be correlated with duration where estimated bandwidth = 4024.05 * duration + 1000 ($p = {<}0.001$, $r^2m = 0.022$, $r^2c = 0.127$, $df = 1164.11$). Bandwidth was similarly correlated with center frequency where estimated bandwidth = 0.448 * center frequency + 7410 ($p = {<}0.001$, $r^2m = 0.180$, $r^2c = 0.233$, $df = 1080$), and number of pulses where estimated bandwidth = 150.05 * number of pulses + 10108.47 ($p = {<}0.001$, $r^2m = 0.013$, $r^2c = $ df = 1278.96). Duration was also found to be correlated with peak time where estimated duration = 0.689 * peak time + 0.054 ($p = {<}0.001$, $r^2m = 0.341$, $r^2c = 0.476$, $df = 1283$), and number of pulses where estimated duration = 0.029 * number of pulses + 0.041 ($p = {<}0.001$, $r^2m = 0.392$, $r^2c = 0.630$, $df = 1247$). Finally, peak time and number of pulses were found to be correlated where estimated peak time = 0.019 * number of pulses + 0.0288 ($p = {<}0.001$, $r^2m = 0.238$, $r^2c = 0.361$, $df = 1274$). Pairings that were not found to be correlated were bandwidth and peak time, duration and center frequency, peak time and center frequency, and center frequency and number of pulses.

It was also found that 44.9% of variance could be explained by PC1, which was predominantly composed of duration, peak time, and number of pulses (Figure 4). 22.7% of variance could be explained by PC2, which was predominantly composed of center frequency and bandwidth..

<u>H3: Acoustic Feature Correlations with Standard Length</u>

Only number of pulses was found to be significantly related to standard length, where estimated number of pulses = 0.071 * standard length (cm) + 1.132 (p = 0.007, r^2m = 0.010, r^2c = 0.047, df = 66.56) (Table 5). Notably, the correlative effect is minimal. The vast majority of variation was found to be between sounds, and not between different fish or tanks.

Discussion

<u>H1: Acoustic Feature Differences Between Species</u>

Although I had hypothesized that the most morphologically/phylogenetically related species would have the most similar acoustic measurements, this was not the case as Brown Bullheads were found to be the most dissimilar compared to other Ictaluridae. This is in contrast to the pattern seen in other animal groups where phylogenies reconstructed with acoustic signals are often congruent with phylogenies based on morphological/molecular data (Cocroft & Ryan, 1995; Peters & Tonkin-Leyhausen, 1999; Laiolo & Rolando, 2003; Robillard & Desutter-Grandcolas, 2004). My findings may suggest that ecology, rather than morphology and phylogeny, plays a greater role in Brown Bullhead acoustic patterns. For example, only Brown Bullheads out of the four species studied live in thick vegetation (Fish NYS DEC Atlas, 2022). Their larger acoustic niche may be a function of them being much more prevalent across New York waterways in comparison to the other species. Thus, a wider range of acoustic measurements would allow for communication to be less hindered across the various acoustic niches they might have to compete with across habitats. It should be noted, however, that my Brown Bullhead sample size was only two individuals, so it is possible that the Brown Bullheads

I recorded had especially varied calls. However, if this were particularly a problem, I would have expected more frequent outliers in the other species which I do not see.

Another point of interest is that Brown Bullheads are significantly different from other species in the number of pulses they produce. This is similar to studies on gobies, where pulse rate was determined to be the most prevalent indicator for differentiating closely-related species with acoustics (Malavasi, 2008; Horvatić, 2016). Although pulse rate and number of pulses are not the same measurement, pulsation-related measurements consistently were reported as the strongest species-differentiating acoustic factor in closely related species. Number of pulses and pulse rate have also been found to be the predominant acoustic features damselfish used for species-species recognition (Myrberg, 1972; Spanier, 1979). Given the above, more work is needed to understand the importance of acoustic pulsation in species recognition, as well as how these measurements relate to fish phylogenies.

H2: Acoustic Feature Correlations With One Another

I did find that acoustic measurements were correlated with one another, and PCAs explained the majority of variance through PC1 and PC2. In the PCA, acoustic measurements with hertz units were grouped into PC2, while acoustic measurements measured in seconds or count were grouped into PC1. Another point of interest in the PCA is that, although Brown Bullheads were the species with the smallest sample size, they occupied the most acoustic niche space, which falls in line with the ideas discussed in H1. As for the correlations between acoustic features, this appears rarely documented in fish acoustic studies. Perhaps more data of this nature could provide further insight on sound production mechanisms and how sounds are intertwined with morphology in fishes.

H3: Acoustic Feature Correlations with Standard Length

Body size has often been found to be highly correlative in others clades such as anurans (Gingras et al., 2013a) and to an even further extent cetaceans, where up to 97% of their frequency variation can be attributed to body size (Matthews et al., 1999). However, this high correlation was not seen here with Ictaluridae, as only one acoustic measurement was correlated to standard length, a proxy for body size in this study. Even then, the correlative effect was almost 0. A similar finding was found in the California spiny lobster (*Panulirus interruptus*) where pulse rate was correlated with body size, but not typical measurements that are correlated with body size such as dominant frequency (Patek et al., 2009). It is somewhat counterintuitive that a stridulatory sound which is produced from the friction of body parts would not display greater correlations between acoustic measurements and body size. This might suggest that the morphological features used to make sound may not be very correlated with the rest of their body size. However, it still begs the question as to why the number of pulses in fish would be the highest correlate to body size. Given the seemingly stand alone prevalence of number of pulses in both differentiating between species and body size, it appears that acoustic pulsations would be the best acoustic means for communicating information for Ictaluridae.

Figures and Tables

	Brown Bullhead	Channel Catfish	Stonecat	Yellow Bullhead
# of Fish	2	14	9	3
# of Sounds	451	699	33	111
Length (cm)	17.06 +/- 0.50	13.85 +/- 1.22	7.73 +/- 2.05	9.14 +/- 1.17
Bandwidth (Hz)	10481.27 +/- 2255.38	10257.44 +/- 2605.21	10453.39 +/- 2745.70	10744.87 +/- 2645.74
Duration (s)	0.119 +/- 0.121	0.080 +/- 0.070	0.073 +/- 0.048	0.089 +/- 0.065
Peak Time (s)	0.082 +/- 0.090	0.054 +/- 0.065	0.054 +/- 0.038	0.056 +/- 0.052
Center Freq (Hz)	6854.72 +/- 2304.56	6256.52 +/- 2262.61	6525.21 +/- 2927.57	6753.28 +/- 2771.85
# of Pulses	2.51 +/- 2.12	1.82 +/- 1.78	1.33 +/- 0.69	2.09 +/- 1.53

Table 1. Summary Statistics of Acoustic Measurements and Sample Sizes. For the acoustic

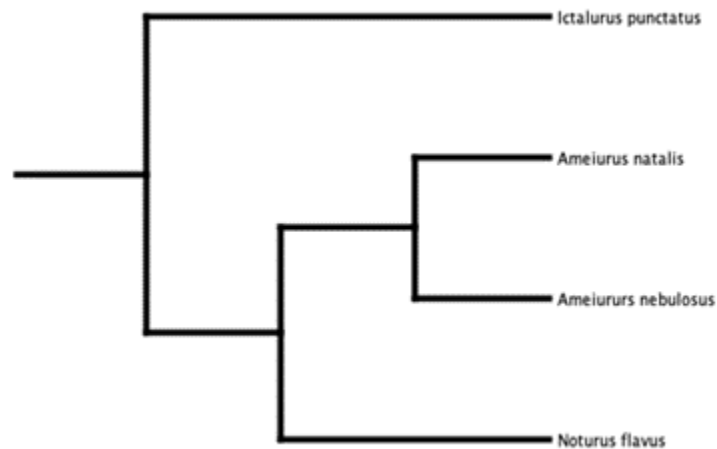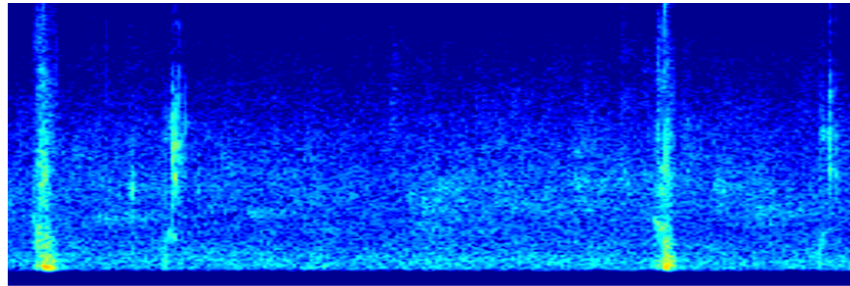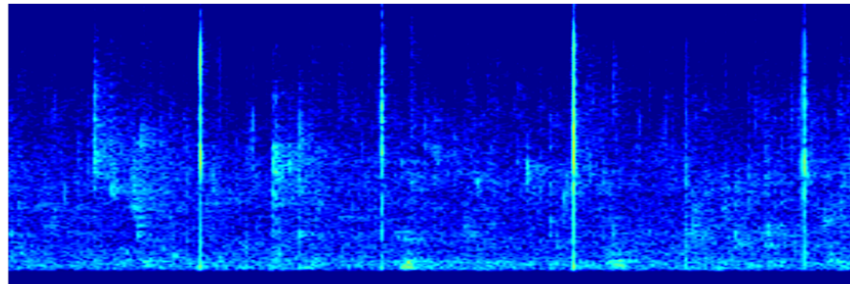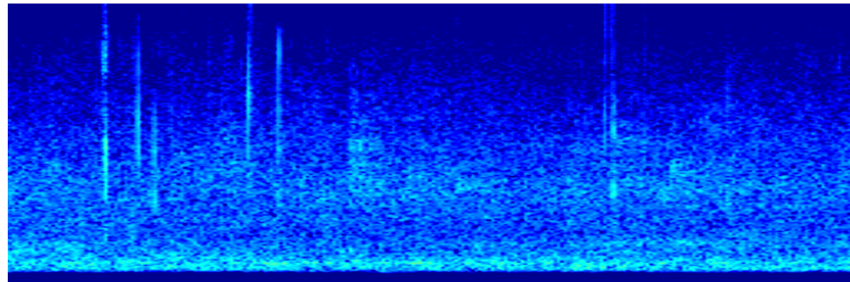measurements, the formatting is mean +/- standard deviation.

Fig 1. Phylogenetic Tree of Tested Species. Note that *Ameiurus natalis* and *Ameiurus nebulosus* are the most closely related. The Ameiruses are more closely related to *Noturus* than *Ictalurus*.
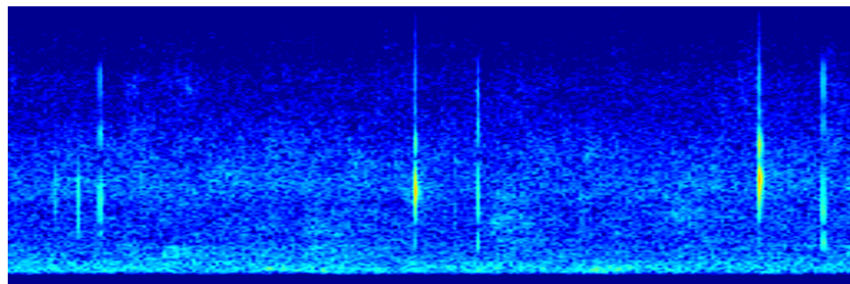
Brown Bullhead (*Ameiurus nebulosus*)

Stonecat (*Noturus flavus*)

Yellow Bullhead (*Ameiurus natalis*)

Channel Catfish (*Ictalurus punctatus*)

Fig 2. Example Spectrograms of Disturbance Calls in Ictaluridae. Y-axis = 22 kHz, X-axis = 1.5 seconds.

H1

Acoustic Measurement	p value
Bandwidth	0.709
Duration	0.413
Peak Time	0.344
Center Frequency	0.880
# of Pulses	0.003

Table 2. Nested ANOVA Results P-Value Chart for Acoustic Measurement Differences Across

All Species.

	df	p value
CC - BB	8.58	0.026
SC - BB	170.14	0.011
YB - BB	51.59	0.328
SC - CC	87.06	0.572
YB - CC	22.33	0.702
YB - SC	196.08	0.245

Table 3. P-Value & DF Chart for Number of Pulse(s) Differences Between Species. CC =

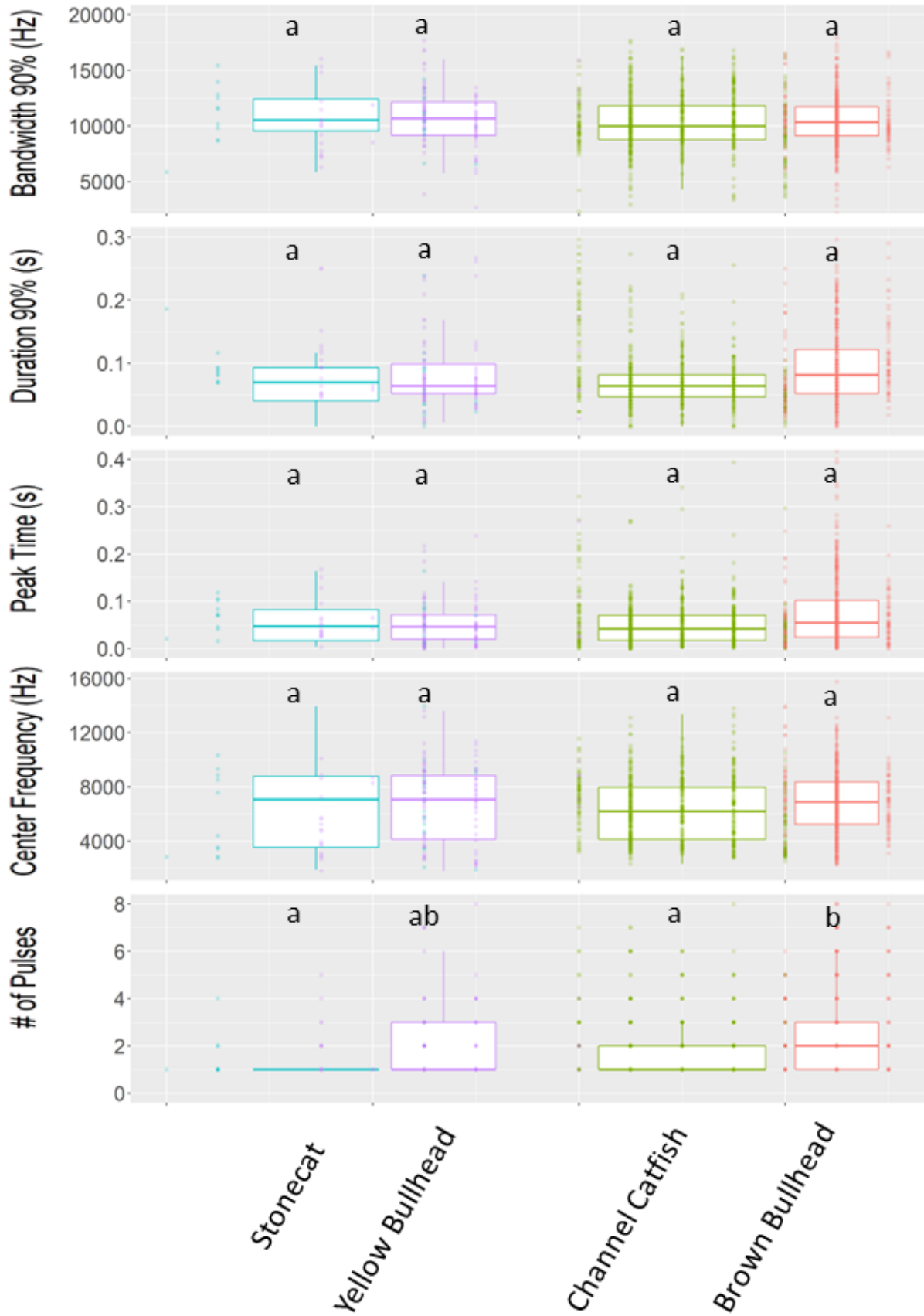Channel Catfish, SC = Stonecat, YB = Yellow Bullhead, and BB = Brown Bullhead.

Fig 3. Acoustic Measurement Differences Across Species. The letters above the boxplots represent which species belong to which group for that particular acoustic measurement, denoted on the y-axis. For example, if a boxplot is labeled "a", then it belongs to the same group as species also labeled "a" or "ab". Brown Bullheads (n sounds = 451), 14 channel catfish (n sounds = 699), 9 Stonecats (n sounds = 33), and 3 Yellow Bullheads (n sounds = 111) were used in these analyses.

H2

	Bandwidth	Duration	Peak Time	Center Frequency
Duration	$p = {<}0.001$, $r^2m = 0.022$, $r^2c = 0.127$, $y = 4024.05x + 1000$, $df = 1164.11$			
Peak Time	$p = 0.359$, $r^2m = 0.001$, $r^2c = 0.104$, $y = 879.16x + 10360.53$, $df = 1284.85$	$p = {<}0.001$, $r^2m = 0.341$, $r^2c = 0.476$, $y = 0.689x + 0.054$, $df = 1283$		
Center Frequency	$p = {<}0.001$, $r^2m = 0.180$, $r^2c = 0.233$, $y = 0.448x + 7410$, $df = 1080$	$p = 0.245$, $r^2m = {<}0.001$, $r^2c = 0.279$, $y = -1.21e{-}06 + 0.109$, $df = 1280$	$p = 0.806$, $r^2m = {<}0.001$, $r^2c = 0.140$, $y = -2.21e{-}07x + 0.069$, $df = 1257$	
# of Pulses	$p = {<}0.001$, $r^2m = 0.013$, $r^2c = 0.115$, $y = 150.05x + 10108.47$, $df = 1278.96$	$p = {<}0.001$, $r^2m = 0.392$, $r^2c = 0.630$, $y = 0.029x + 0.041$, $df = 1247$	$p = {<}0.001$, $r^2m = 0.238$, $r^2c = 0.361$, $y = 0.019x + 0.0288$, $df = 1274$	$p = 0.609$, $r^2m = {<}0.001$, $r^2c = 0.325$, $y = 16.27x + 6701.35$, $df = 1265.26$

Table 4. Correlations Between Acoustic Measurements. The first numeric value in the cell represents the p-value, the second is their y = mx + b equation, and the third numeric value represents df.
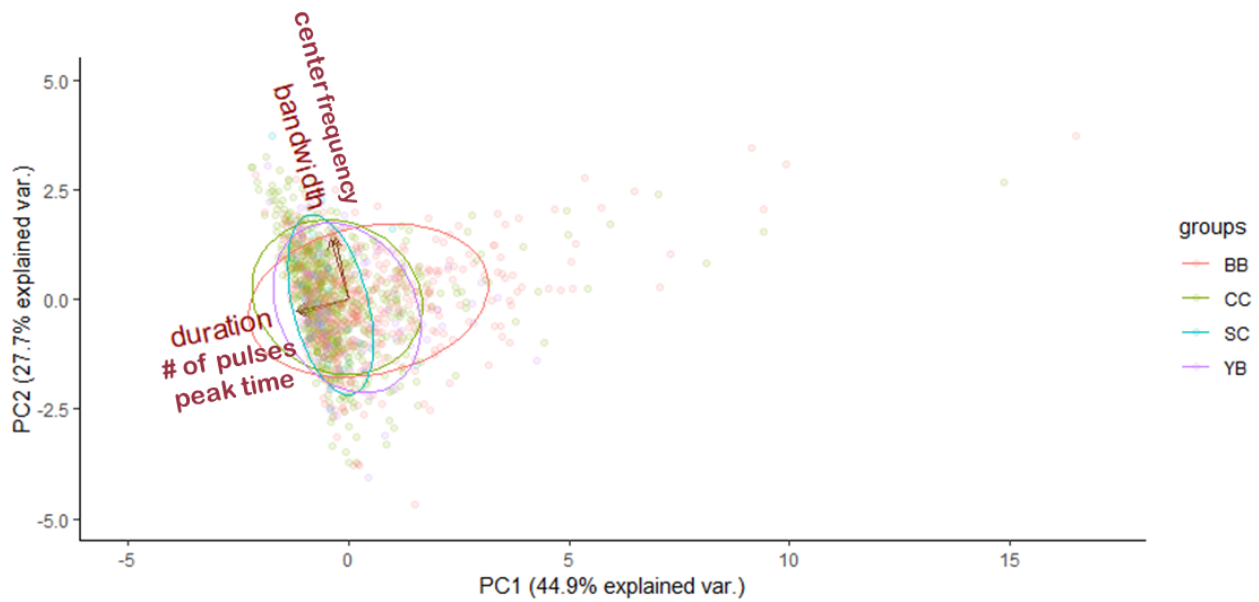

Fig 4. Principal Component Analysis Biplot. 44.9% of variance is explained by peak time, duration, and number of pulses (PC1). 22.7% of variance is explained by center frequency and bandwidth.

H3

	Bandwidth	Duration	Peak Time	Center Freq	# of Pulses
p value	0.917	0.375	0.124	0.849	0.007
r^2 marginal	< 0.001	0.003	0.006	< 0.001	0.010
r^2 conditional	0.108	0.274	0.134	0.330	0.047
df	45.53	52.37	54.84	47.26	66.56
y = mx + b	y = -4.508x + 10480.25	y = 0.002x + 0.077	y = 0.002x + 0.403	y = 11.37x + 6691.62	y = 0.071x + 1.132
% var by fish	6%	17%	5%	3%	2 %
% var by tank	5%	10%	8%	30%	2 %
% var by sound	89%	73%	87%	67%	96 %

Table 5. Acoustic Measurement Differences Between Standard Lengths Results Chart.

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