ORIGINAL ARTICLE

WILEY Freshwater Biology

Potential for use of passive acoustic monitoring of piranhas in the Pacaya–Samiria National Reserve in Peru

Rodney A. Rountree[1,2] (iD) | Francis Juanes[2]

[1]The Fish Listener, East Falmouth, Massachusetts

[2]Department of Biology, University of Victoria, Victoria, British Columbia, Canada

Correspondence
Rodney A. Rountree, The Fish Listener, East Falmouth, MA.
Email: rrountree@fishecology.org

Abstract

1. Passive acoustic monitoring (PAM) can be an effective tool in the identification of fishes and mapping of their temporal and spatial distribution patterns and thereby aids in ecosystem management in remote locations. However, to date measurements of the acoustic properties of piranha have been primarily made in aquaria on captive specimens obtained through the aquarium trade. Data on the sound production of wild piranha taken under natural field conditions would enhance PAM applications.

2. Piranha captured as part of routine annual monitoring by Operation Wallacea within the Pacaya–Samiria National Reserve in Peru were auditioned for sound production prior to release. Auditioning was done by gently holding a fish underwater in the river near a suspended hydrophone, thus recordings included the piranha sound as well as natural ambient sound.

3. Seventy-nine per cent of the 129 auditioned piranha, including *Pygocentrus nattereri*, *Serrasalmus maculatus*, *Serrasalmus cf. sanchezi* and an unidentified *Serrasalmus* spp. complex, produced sounds consisting of 2 to 23 barks in a sequential series. Sound production by *S. maculatus* and *S. cf. sanchezi* are reported for the first time. Bark characteristics exhibited high variation within bark series of individual fish. Piranha could not be distinguished by single variables but did exhibit significant multivariate differences. The relationship of several variables to fish size also differed significantly among species. Measurements of within-fish variation and bark series pattern attributes were found to be useful for discrimination of sounds among piranha species.

4. We demonstrate that closely related species of piranha can be distinguished by their sounds under natural acoustic conditions based on multivariate analyses, suggesting that passive acoustic monitoring (PAM) can be an effective tool for ecosystem management in the Amazon. More broadly, our study also suggests (a) the need to report more detailed statistical descriptions of fish sounds, including measures of within-fish variation, (b) the importance of describing characteristics of sound series produced by fishes in addition to those of individual sound types and (c) the need to deposit museum voucher specimens to anchor specific sounds to specific individuals.

KEYWORDS
bioacoustics, passive acoustics, piranha, sound production, soundscape

1 | INTRODUCTION

Passive acoustic monitoring (PAM) is becoming an important tool for ecosystem and fisheries management (Rountree et al., 2006) and is especially useful in remote habitats that are difficult to sample with conventional methods (ACT, 2007). However, PAM requires a detailed knowledge of the acoustic characteristics of sounds to be effective. Further, we suggest that measurement of sound characteristics under "real-world" conditions would improve the applicability of PAM in field studies. The Pacaya–Samiria National Reserve (PSNR) is the largest in Peru, the second largest reserve in the Amazon, is located about 180 km from the city of Iquitos and more than 3,000 km from the mouth of the Amazon. Due to its remote location, its fish fauna has not been well surveyed (Ortega & Vari, 1986; Chang & Ortega, 1995). Ecological studies of fishes in the reserve, and in Peru overall, are in their infancy (Ortega & Hidalgo, 2008) and would benefit from PAM studies due to the high diversity of soniferous fishes in the Amazon (e.g., Kaatz & Stewart, 2012).

Sound production by piranha (Characidae) is well known (Meschkat, 1957; Markl, 1971; Kastberger, 1981a,b; Millot, Vandewalle, & Parmentier, 2011; Millot & Parmentier, 2014; Mélotte, Vigouroux, Michel, & Parmentier, 2016; Mélotte, Parmentier, Michel, Herrel, & Boyle, 2018); however, these studies were focused on describing sound production mechanisms and/or behaviour associated with sound production and used mainly commercially obtained specimens. In most cases, sounds were recorded under laboratory conditions, or otherwise within aquaria while in the field. However, Kastberger (1981a) apparently made some field recordings but does not distinguish field and laboratory data. Most recently, Mélotte et al. (2016) provided a detailed comparison of bark sounds among eight species of piranha (*Serrasalmus compressus*, *Serrasalmus manueli*, *Serrasalmus spilopleura*, *Serrasalmus rhombeus*, *Serrasalmus eigenmanni* and *Pygocentrus nattereri*), based on duration, fundamental frequency and cycle structure of individual sounds. Although Kastberger (1981a) reported no species-specific sounds of four piranha species, Mélotte et al. (2016) were able to differentiate sounds among piranha with a multivariate approach. Our objective was to describe the characteristics of piranha sounds under natural acoustic conditions in order to evaluate the potential of PAM monitoring for the group. We were particularly interested in determining if easily measured parameters such as duration and peak frequency (based on energy content, see Charif, Strickman, & Waack, 2010) would be sufficient for species identification under field conditions based on univariate or multivariate approaches. Although we focus on piranha, findings from our study have implications for the application of PAM for other fishes in the Amazon and elsewhere.

2 | METHODS

The study was conducted within the PSNR in Peru under the umbrella of Operation Wallacea (http://www.opwall.com/). The PSNR is contained within the confluence of the Marañón and Ucayali rivers where the main stem of the Amazon River originates. Operation Wallacea conducts regular fishing surveys of the region as part of their long-term monitoring programme (Bodmer et al., 2017). Piranha and other fishes were captured by fishing with cane poles or gill nets from small boats or canoes in shallow water (<2 m depth) while tied up to vegetation along the river bank or within the flooded forest floor. Sampling was conducted within a 2.5 km tributary of the Samiria River draining Huisto Lake (approximately at 4°53.891′S 74°20.929′W) from 6 to 25 July 2012. Operation Wallacea Peru Expedition 2012 staff and volunteers identified and measured (standard length, SL, ±0.5 cm) each specimen prior to release back into the wild. In most cases, we accompanied the survey and obtained specimens for sound auditioning prior to their release at the capture location. Occasionally, specimens captured during fishing surveys were transported to a floating dock at the base camp and held in a small inflatable pool overnight prior to auditioning and release. Because Operation Wallacea's permits did not allow for collection of specimens or removal of materials from the reserve, each fish was photo-documented and georeferenced. Species identifications were made in the field or by subsequent examination of photographic materials.

Piranha were gently hand-held underwater within 30 cm or less of the hydrophone to audition for sound production, first within a container (plastic tub, aquarium or inflatable pool as available) and then within the river. Most fish spontaneously produced sounds when grasped by hand. Only sounds recorded in the river auditions are reported herein (except where noted below), because container size and type could not be controlled and caused unquantifiable signal distortion. Sound production within the container, the river or both was recorded using an uncalibrated SQ26-H1 recorder system with a SQ26-08 Hydrophone (sensitivity = −169.00 re. 1 V/µPa rms, Cetacean Research Technology, Seattle, WA).

Terminology used to describe the pulse structure of biological sounds is often confused and inconsistent throughout the literature due to the wide range of oscillation patterns (see Gerhardt & Huber, 2002 for a detailed discussion of the problem). For example, the individual pulsed sounds produced by fishes have variously been described as being composed of "pulses" (e.g., Millot et al., 2011), "oscillation peaks" (Kaatz & Stewart, 2012) or "cycles" (Mélotte et al., 2016). The confusion extends to parameters derived from these measurements as well, so pulse rate might refer to the cycle rate within a pulse or the rate of pulses within a sound series and it is not always clear which definition applies in a particular study. We define a piranha bark as one distinct pulsed sound made up of oscillation cycles with characteristic cycle duration, rate, period and interval. We note that in our case, the cycle rate is also a measure of the fundamental frequency of the bark (Bradbury & Vehrencamp, 1998; Gerhard, 2003). A bark series is defined as a bout of barks forming one temporally distinct sound sequence with characteristic series duration and component bark duration, interval, rate and period. Acoustic measurements of selected parameters of all sounds were made in Raven Pro 1.5 acoustic software (Bioacoustics Research

Program, 2014) following Charif et al. (2010). Variables are defined in the Supporting information Table S1. Spectrogram parameters were set at 1,024 FFT, Hanning window, 50% overlap.

To prevent bias due to pseudoreplication when comparing bark characteristics among species and within species by fish size, statistics of all acoustic parameters were summarized within each individual bark series produced by an individual fish, hereafter referred to as "within-fish" measures, including the mean, standard error of the mean (SE), minimum, maximum, range and coefficient of variation (CV) and then averaged over all individuals in a treatment group. For example, the within-fish peak frequency range is the difference between the bark with the highest peak frequency and the bark with the lowest peak frequency in an individual bark series produced by an individual fish. The within-fish peak frequency range was then used as a variate in statistical comparisons among species and to calculate the within-fish average (and SE) peak frequency range for each species. We tested for univariate among-species differences in natural-logarithm-transformed parameters with an analysis of covariance (ANCOVA) controlling for fish size using SAS/STAT software, version 12.1 (SAS Institute Inc., 2012). A significant species main effect and nonsignificant species-by-fish size interaction effect would indicate differences in the variable among species, while a significant interaction would indicate that the relationship between the variable and fish size differed among the species. Because of the low sample size (three fish), *Serrasalmus cf. sanchezi* was excluded from the ANCOVA.

Canonical discrimination analysis (CDA) and multiple analysis of variance (MANOVA) were used to test for multivariate differences in transformed within-fish sound characteristics among *P. nattereri*, *Serrasalmus maculatus*, *S. cf. sanchezi* and *Serrasalmus* spp. groups, after first conducting a stepwise discriminant analysis to select a subset of the variables for group discrimination (Pielou, 1984; Harris, 1985; Friendly, 1991; SAS Institute Inc., 2012). The CDA tests whether species can be distinguished by their sounds, and if so, which acoustic parameters contribute the most to the observed differences. Pearson correlations of the original (but transformed to normalize) variables with the derived canonical variables from the CDAs were calculated to determine which sound parameter contributed most to the group discrimination (Harris, 1985; Friendly, 1991).

Potential differences among species in sound production were also examined by qualitatively comparing their power spectra. Spectra were measured from three barks from each individual specimen (specimens with less than three barks in a series were excluded) using SpectraLab Professional Sound Analysis software (Sound Technology, Inc., Campbell, CA). Because the recording system was not calibrated, we could not calculate average spectra for each fish or species, but since our goal was to compare the shape of the spectra, rather than the amplitude, each spectra was normalized as per cent of peak amplitude within the frequency range 94–1031 Hz (which encompassed the main energy of all species). Mean and SE for normalized spectra for each species could then be calculated.

3 | RESULTS

Four types of piranha were auditioned in the field and co-occurred at sampling locations, including *P. nattereri*, *S. maculatus*, *S. cf. sanchezi* and an unidentified *Serrasalmus* spp. complex (Figure 1, Supporting information Table S2). Piranha types sampled are photo-documented herein (Figure 1) in lieu of museum specimens to help anchor sounds to specific species in the event of future taxonomic revisions of the group. Two specimens in the *Serrasalmus* complex
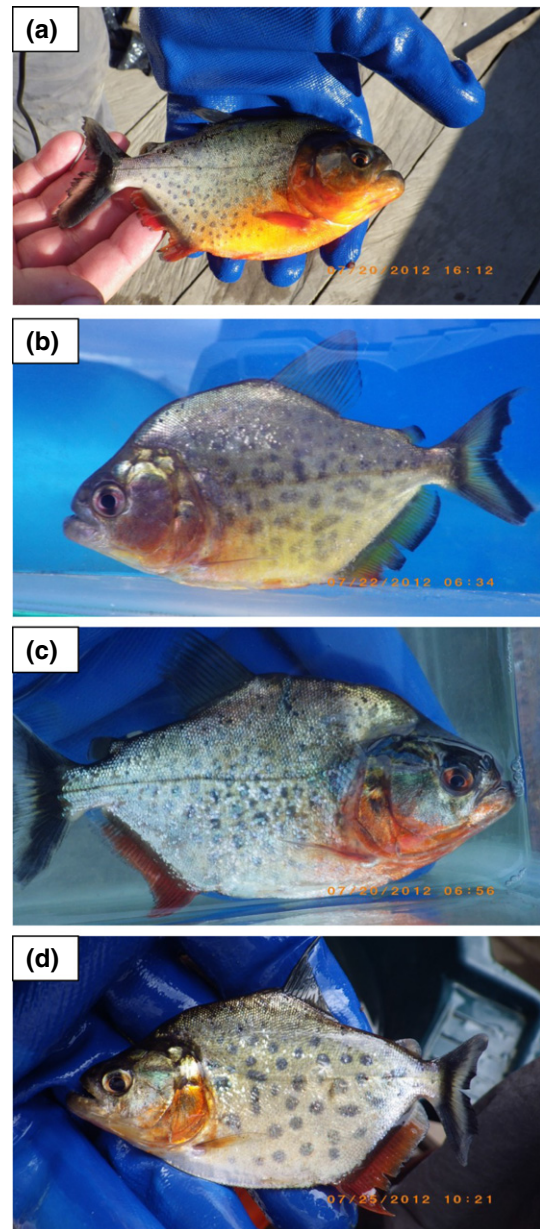
FIGURE 1 Photographs of representative specimens of each piranha species examined: (a) *Pygocentrus nattereri* (specimen 375, 11.5 cm SL, 20 July 2012 at 1612 hr); (b) *Serrasalmus maculatus* (specimen 420, 7.5 cm SL, 22 July 2012 at 634 hr); (c) *Serrasalmus cf. sanchezi* (specimen 344, 11.5 cm SL, 20 July 2012 at 657 hr); (d) *Serrasalmus* spp. complex (specimen 516, 7.5 cm SL, 25 July 2012 at 1021 hr)

were identified as possible examples of *S. compressus*. Both produced sounds, but one escaped before it could be auditioned in the river. Another 2 cm SL specimen from the *Serrasalmus* group was tentatively identified as *S. eigenmanni*, which produced vigorous sounds in the air and the inflatable pool, but also was lost before it could be auditioned in the river. We report basic bark attributes of the single river auditioned *S. cf. compressus*, and the single pool auditioned *S. cf. eigenmanni* (Tables 1 and 2), but otherwise exclude the two species from further consideration.

A total of 129 piranha were auditioned of which 102 (79%) produced sounds (Supporting information Table S2). All of the *S. maculatus* auditioned produced sounds, while only three of six *S. cf. sanchezi* did. Soniferous fish ranged from 2 to 13.5 cm SL, but *S. cf. sanchezi* individuals were significantly larger than other species

(Supporting information Table S2). Interestingly, silent individuals tended to be larger than vocal individuals. Piranha usually only produced sounds when specimens were grasped by the hand to remove them from the hook, or when held in the air or underwater for auditioning. They rarely produced sounds when hooked and landed, or while in the holding container even when pursued by a net for capture.

Examples of the spectrogram and waveform plots for individual barks of each of the four main species groups suggest some differences in the acoustic properties of individual barks (Figure 2; Table 1). Frequency parameters tended to be lowest for *S. cf. sanchezi* and *P. nattereri* (Table 1), which were also the largest fishes (Supporting information Table S2). Barks of *Serrasalmus* spp. individuals tended to have three frequency modes (Figures 2d and 3b),

TABLE 1 Summary statistics of piranha bark acoustic parameters calculated after first taking the mean of each parameter of all barks produced by an individual fish (e.g., mean of the within-fish means, minimum of within-fish means). All measurements were taken of hand-held fish in the natural environment, with the exception of the single specimen of *S. cf. eigenmanni* which was recorded in the audition pool. Values in each table cell are mean (*SEM*): minimum–maximum. Parameter definitions are provided in Supporting information Table S1

| Parameter	Pygocentrus nattereri	Serrasalmus maculatus	Serrasalmus cf. sanchezi	Serrasalmus spp.	Serrasalmus cf. compressus	Serrasalmus cf. eigenmanni
Frequency (Hz)						
Low	42 (5): 0–187	49 (7): 21–101	27 (9): 9–40	38 (3): 11–78	50	67
5th percentile	88 (6): 47–281	110 (8): 64–160	71 (11): 59–94	89 (4): 47–141	94	125
Q1	140 (10): 70–375	178 (23): 81–328	135 (38): 94–211	163 (11): 87–288	203	156
Peak	186 (16): 75–469	254 (42): 81–523	152 (38): 94–223	224 (17): 100–448	359	188
Centre	200 (13): 94–469	252 (31): 132–457	173 (44): 114–258	236 (15): 127–391	312	188
Q3	281 (16): 141–563	350 (34): 188–555	244 (78): 147–398	335 (19): 172–531	453	250
95th percentile	429 (20): 234–853	529 (37): 294–707	390 (145): 223–680	530 (24): 216–891	625	500
High	643 (32): 359–1164	911 (151): 402–2437	621 (151): 328–826	800 (36): 476–1269	1009	885
IQR bandwidth	141 (8): 47–250	172 (14): 107–277	110 (40): 54–188	171 (13): 47–281	250	94
90th percentile bandwidth	342 (17): 146–722	419 (33): 230–572	319 (134): 164–586	442 (24): 117–812	531	375
Bandwidth	601 (32): 320–1164	862 (153): 374–2415	595 (156): 287–796	761 (38): 443–1258	960	817
Duration (ms)						
IQR duration	30 (2): 11–53	22 (4): 6–48	29 (8): 19–44	19 (1): 11–34	18	14
90th percentile duration	63 (3): 34–103	46 (7): 20–98	61 (13): 43–87	42 (2): 26–62	43	36
Duration	85 (3): 47–119	64 (8): 29–125	83 (14): 63–111	62 (3): 39–85	81	49
Other measures						
Aggregate entropy	2.8 (0.1): 1.5–3.8	3.0 (0.1): 2.4–3.6	2.6 (0.4): 2.2–3.5	3.1 (0.1): 1.7–4.0	3.6	2.5
Average entropy	2.6 (0.1): 1.3–3.4	2.8 (0.1): 2.2–3.2	2.4 (0.4): 2.0–3.2	2.8 (0.1): 1.6–3.6	3.3	2.5
Number of cycles	10.1 (0.3): 7.2–13.8	9.2 (0.8): 5.1–13.6	8.3 (0.6): 7.2–9.5	8.4 (0.3): 6.1–11.5	9	8
Main cycles	7.6 (0.2): 4.0–11.2	7.3 (0.7): 4.0–11.6	5.7 (0.5): 4.8–6.4	6.0 (0.2): 4.0–8.7	5.7	8
Cycle rate (cycles/s)	128 (4): 86–265	153 (6): 107–178	105 (7): 91–116	138 (3): 114–160	129	162
Cycle period (ms/cycle)	8 (<1): 4–12	7 (<1): 6–9	10 (1): 9–11	7 (<1): 6–9	8	6
Number of fish	37–39	11–12	3	27	1	1
Number of barks	176	126	19	214	3	3

TABLE 2 Characteristics of piranha bark series (trains of repeating barks) by species. All measurements were taken of hand-held fish in the natural environment, with the exception of the single specimen of *S. cf. eigenmanni* which was recorded in the audition pool. Values in each table cell are among-fish mean (*SEM*): minimum–maximum. Parameter definitions are provided in Supporting information Table S1

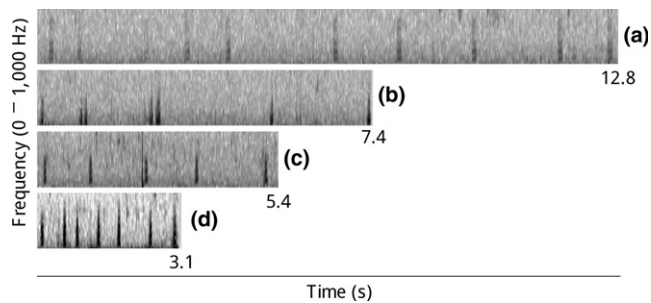| Parameter	Pygocentrus nattereri	Serrasalmus maculates	Serrasalmus cf. sanchezi	Serrasalmus spp.	Serrasalmus cf. compressus	Serrasalmus cf. eigenmanni
Barks per series	4.9 (0.6): 2–17	11.3 (1.3): 6–23	7.3 (2.0): 4–11	7.3 (0.8): 2–17	3	2
Series duration	5.1 (0.5): 0.3–10.5	10.3 (1.4): 4.4–18.9	12.6 (4.4): 7.4–21.4	6.0 (0.7): 1.6–13.8	1.3	1.8
Bark repetition	1.3 (0.2): 0.3–6.7	1.3 (0.2): 0.4–2.7	0.6 (0.2): 0.4–1.0	1.3 (0.1): 0.6–2.3	2.3	1.1
Bark period	1.1 (0.1): 0.2–3.5	1.0 (0.2): 0.4–2.4	1.8 (0.4): 1.1–2.3	0.8 (0.1): 0.4–1.6	0.4	0.9
Bark interval	2.3 (0.4): 0.3–10.2	1.1 (0.2): 0.4–2.9	2.4 (0.6): 1.3–2.9	1.9 (0.5): 0.6–14.3	0.6	1.7
Bark interval *SE*	0.7 (0.3): 0.01–7.1	0.2 (0.04): 0.05–0.6	0.5 (0.03): 0.4–0.5	1.0 (0.5): 0.04–12.6	0.4	
Bark interval CV	46 (5): 1–122	51 (5): 23–94	50 (15): 30–79	76 (8): 20–207	77	
Bark interval minimum	1.6 (0.3): 0.2–10.2	0.6 (0.2): 0.1–2.0	1.4 (0.6): 0.2–2.3	0.5 (0.1): 0.2–1.7	0.3	1.7
Number of fish	39	12	3	27	1	1
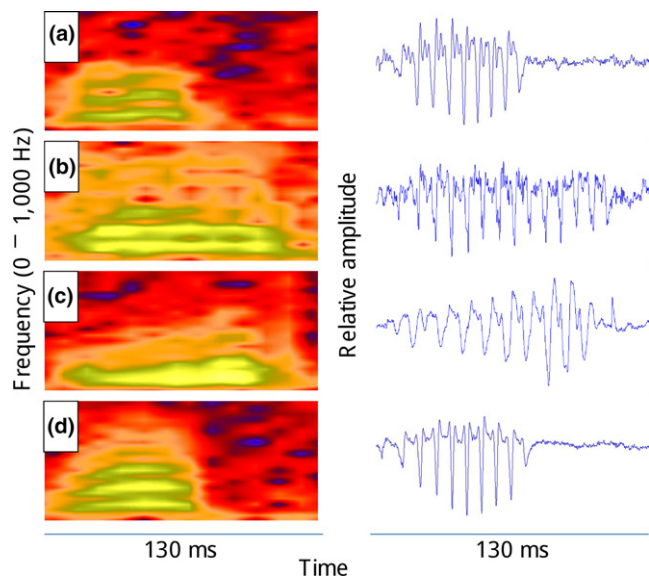
FIGURE 2 Spectrograms (left column) and waveform (right column) plots of individual piranha sounds plotted on the same 130 ms time scale (*x*-axis). Amplitudes (*y*-axis) in the waveforms are on a relative scale and not comparable among species. (a) *Pygocentrus nattereri*; (b) *Serrasalmus maculatus*; (c) *Serrasalmus cf. sanchezi*; (d) *Serrasalmus* spp. complex. Spectrogram parameters were set at 1,024 FFT, Hanning window, 50% overlap [Colour figure can be viewed at wileyonlinelibrary.com]

P. nattereri (Figures 2a and 3a) and *S. maculatus* (Figures 2b and 3b) usually had two modes, and *S. cf. sanchezi* tended to have a single mode (Figures 2c and 3a). The fundamental frequency estimated from the first mode in the spectra plots (which is dependent on the frequency resolution of the spectral analysis) was 141 Hz for all species except for *S. cf. sanchezi* which appears to have a fundamental below 94 Hz (Figure 3). Mean fundamental frequency measured from the waveform (cycle rate) tended to be highest for *S. maculatus* and lowest for *S. cf. sanchezi* (Table 1).
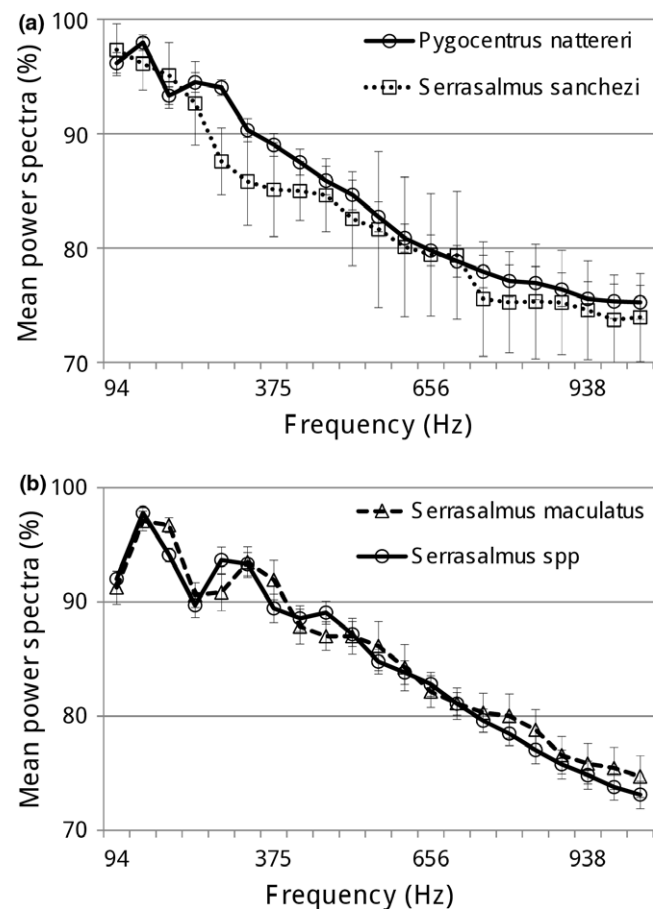
FIGURE 3 Comparison of mean normalized power spectra (normalized as per cent of peak amplitude within the frequency range 94–1031 Hz), among the four piranha types. Spectra were measured from three barks from each individual specimen (specimens with less than three barks in a series were excluded). (a) *Pygocentrus nattereri* N = 26 and *Serrasalmus cf. sanchezi* N = 3; (b) *Serrasalmus maculatus* N = 13 and *Serrasalmus* spp. complex N = 28. Vertical bars are standard error of the mean

FIGURE 4 Spectrograms of example piranha bark series plotted on the same 12.8 s time (x-axis) and 1000 Hz frequency (y-axis) scales. (a) *Serrasalmus maculatus*; (b) *Serrasalmus cf. sanchezi*; (c) *Pygocentrus nattereri*; (d) *Serrasalmus* spp. complex. Spectrogram parameters were set at 1,024 FFT, Hanning window, 50% overlap

Examples of spectrograms of bark series for each of the four main piranha types are plotted on the same scale in Figure 4 illustrating both how bark series differ among species in their temporal patterns, and how barks vary within the bark series of individual fish. Movies of the scrolling spectrogram with audio corresponding to each panel in Figure 4 are provided as Supporting information Videos S1–S4. Bark variation was high within individual fish (Supporting information Table S3). For example, within-fish peak frequency range averaged (±SE): 90 ± 17 Hz, 176 ± 30 Hz, 47 ± 27 Hz

and 137 ± 27 Hz for *P. nattereri*, *S. maculatus*, *S. cf. sanchezi and Serrasalmus* spp., respectively. The mean within-fish range in the number of cycles in barks were 3.2 ± 0.5, 5.5 ± 1.0, 3.7 ± 0.9 and 4.6 ± 0.6, respectively (Supporting information Table S3). The number of barks in a series of *S. maculatus* was twice that of *P. nattereri*, but their bark repetition rates were similar (Table 2). Bark series durations and bark periods were greatest in *S. cf. sanchezi*. The interval between barks in a series was much shorter in *S. maculatus* than the other piranha. Average bark series patterns of selected variables illustrate consistent differences among the four piranha types (Figure 5). A greater percentage of *S. maculatus* had longer bark series than *P. nattereri* (Figure 5a). Bark duration tended to decline within bark series for all species except *S. cf. sanchezi* (Figure 5c). Peak frequency declined in longer bark series for each species (Figure 5b) but the fundamental increased in *P. nattereri* (Figure 5d).

All acoustic parameters with a significant fish size-by-species interaction, based on transformed variates, in the ANCOVA are shown in Supporting information Table S4, while the strongest relationships are shown in Figure 6. No univariate within-fish, or bark series, parameters were significantly different among species groups independently from fish size. All significant parameters were negatively related to fish size except for cycle period and number of barks per series. Perhaps surprisingly, the only peak frequency variable to exhibit significantly different species-by-fish size interactions was the
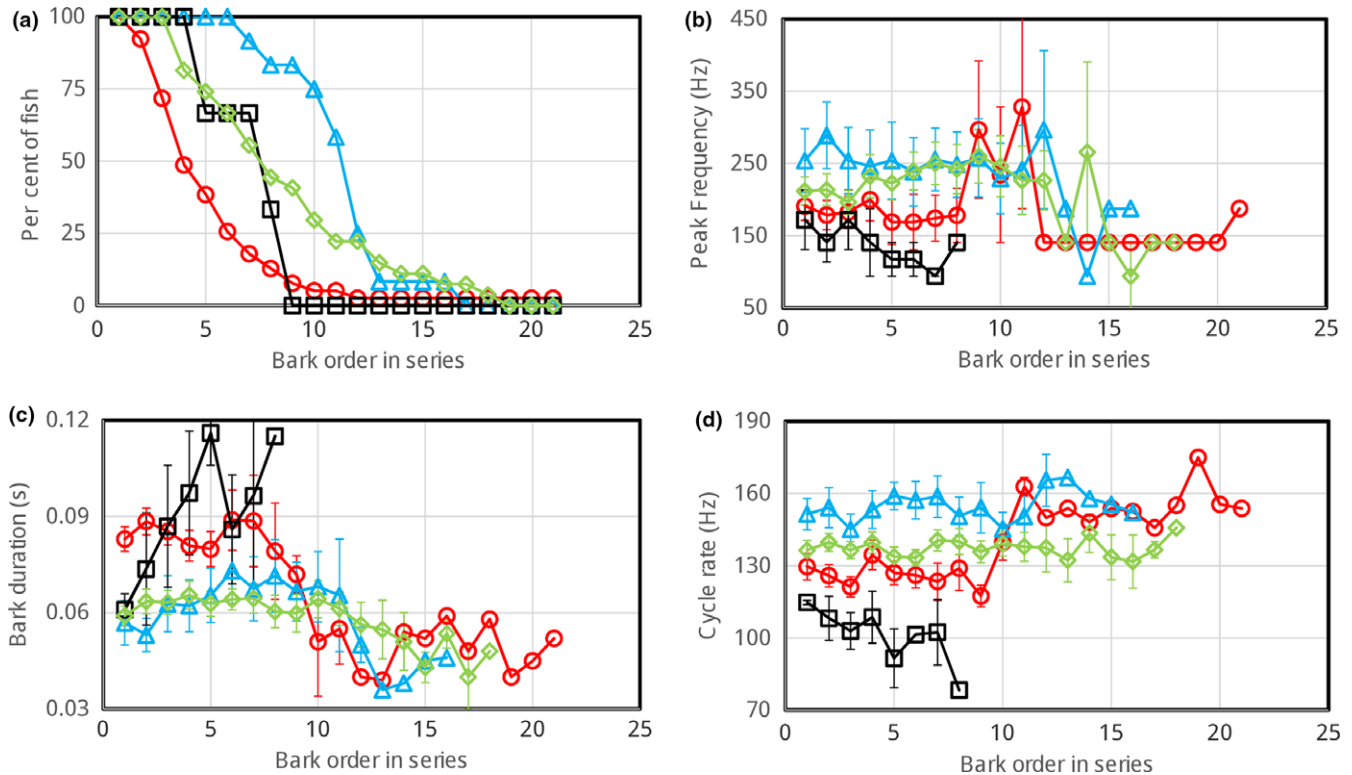
FIGURE 5 Comparison of bark series patterns among piranha species: (a) per cent of individuals exhibiting bark numbers (e.g., 49% of *Pygocentrus nattereri* have at least 4 barks in a series), (b) mean peak frequency of barks by order in the series, (c) mean duration of barks, (d) mean cycle rate (fundamental frequency) of barks. Vertical bars are one standard error of the mean. (○ = *P. nattereri*, Δ = *Serrasalmus maculatus*, □ = *Serrasalmus cf. sanchezi*, ◇ = *Serrasalmus* spp. complex) [Colour figure can be viewed at wileyonlinelibrary.com]
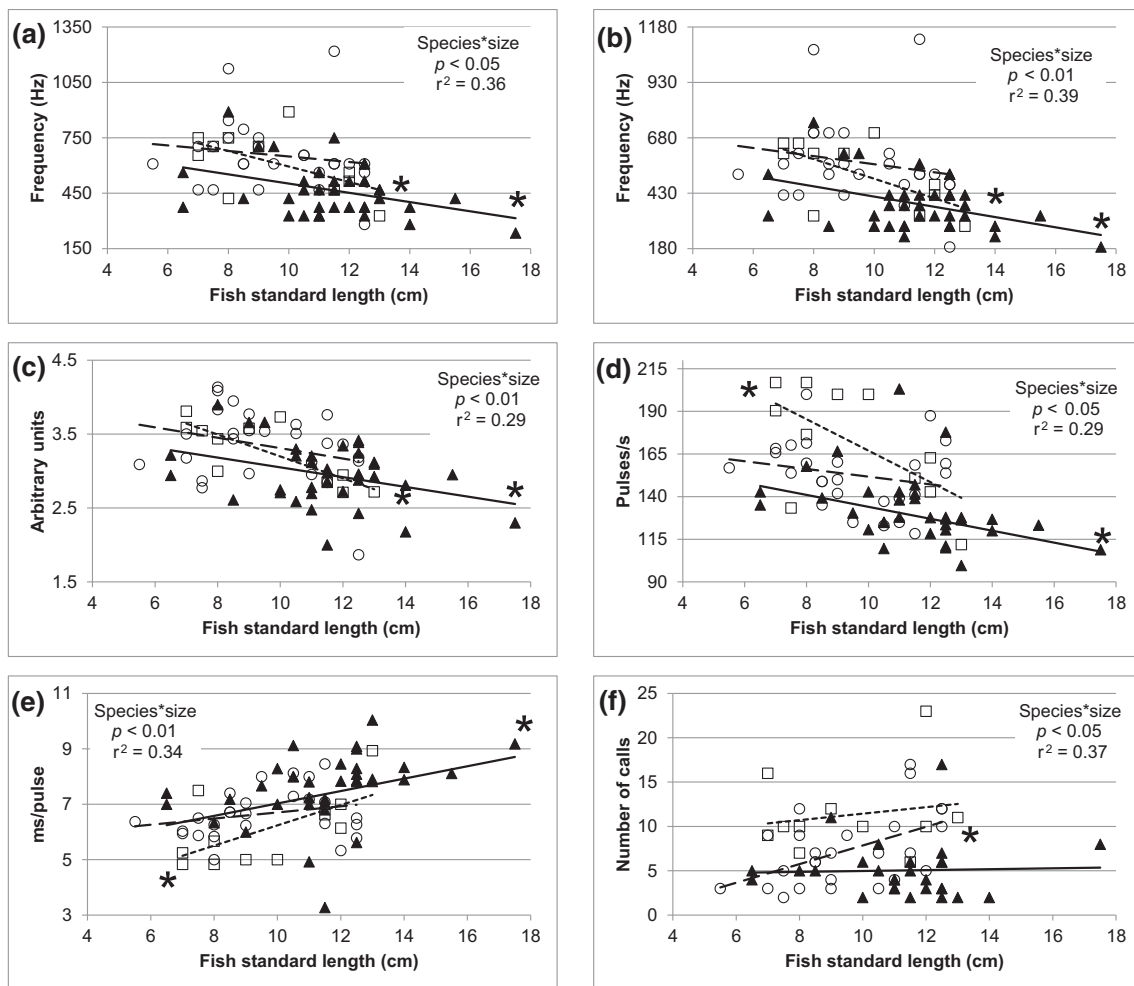
FIGURE 6 Effect of fish size on selected acoustic properties with significant differences among piranha species (see analysis of covariance results in Supporting information Table S4): (a) maximum 95th frequency percentile, (b) maximum 90th percentile of frequency bandwidth, (c) maximum aggregate entropy, (d) maximum cycle rate, (e) minimum cycle period and (f) number of barks per series. ▲ and solid line = *Pygocentrus nattereri* (N = 36 fish), □ and short-dashed line = *Serrasalmus maculatus* (N = 11 fish), ○ and long dashed line = *Serrasalmus* spp. complex (N = 26). Species with significant regressions are indicated by the asterisk "*". Plots are of untransformed variables, but statistical tests were performed on transformed variables

maximum peak frequency exhibited among barks within an individual. Similarly, the maximum within-fish fundamental frequency (as measured from the cycle rate) was more strongly negatively related to fish size and discriminant among species than the mean fundamental (Supporting information Table S4, Figure 6a). Although the number of cycles in a bark did not show significant size or species effects, several cycle rate and cycle period variables did show significant size trend differences among species (Supporting information Table S4, Figure 6d,e). Finally, the number of barks in a series was significantly affected by fish size and different among species (Figure 6f). Among-species differences in size relationships for most parameters were driven by differences between *P. nattereri* and *S. maculatus*, whereas *Serrasalmus* spp. lacked a size effect (Supporting information Table S4, Figure 6). However, the significant positive interaction of the number of barks in a series with fish size was driven by a significant trend for *Serrasalmus* spp. (Figure 6). Aggregate entropy was negatively correlated with fish size for *P. nattereri* and *S. maculatus* indicating that the

number of harmonics declined with fish size, except for *Serrasalmus* spp. which tended to have multiple harmonics over the entire size range (Supporting information Table S4, Figure 6c).

The CDA revealed a highly significant discrimination among the four species groups tested (Figure 7, Supporting information Table S5). The first canonical best discriminates among *P. nattereri* (negative) and *S. maculatus* (positive) with *Serrasalmus* spp. falling in between. It is most strongly driven by the minimum 90th percentile bark duration and median cycle period (negative) and the number of barks in a series and mean range of the 90th percentile bandwidth (positive). The second canonical best discriminates between the three-species group (positive) and *S. cf. sanchezi* (negative) and is driven most strongly by the median cycle rate (positive) and median cycle period and mean bark period (negative). Although the CDA does not control for fish size effects, an examination of the scatter of fish sizes in the plot (i.e., the size of the dots) suggests size did not bias the discrimination.
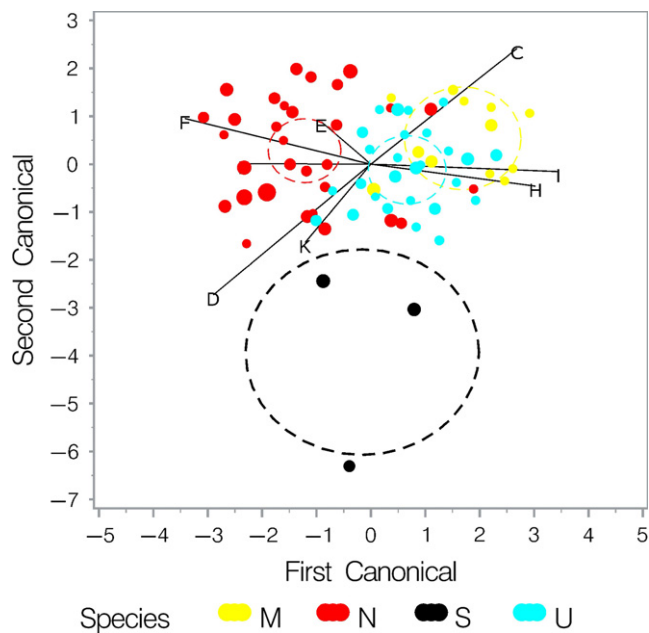
FIGURE 7 Multivariate comparison of bark characteristics among piranha types using canonical discrimination analysis of known groups: *Pygocentrus nattereri* = red circles labelled "N"; *Serrasalmus maculatus* = yellow circles labelled "M"; *Serrasalmus cf. sanchezi* = black circles labelled "S"; *Serrasalmus* spp. complex = blue circles labelled "U." Each symbol represents mean measurements from a single fish (*N* = 73). Symbol size is proportional to fish size. Dashed ellipses are the 95% confidence intervals of the group means. Vectors are proportional to the canonical loading for each attribute and indicate their relative influence on the group discrimination. Canonical statistics are provided in Supporting information Table S5

4 | DISCUSSION

The taxonomy of piranha has been problematic for decades (Géry, 1964; Jégu, Leão, & dos Santos, 1991; Machado-Allison, 2002; Jégu, 2003; Hubert et al., 2007; Ortí, Sivasundar, Dietz, & Jégu, 2008; Hubert & Renno, 2010) which has confounded our understanding of the behaviour of fishes in the group. For example, behaviour studies of *S. spilopleura* (e.g., Sazima & Zamprogno, 1985) are most likely attributable to *S. maculatus* due to taxonomic revisions (Jégu & dos Santos, 2001; Jégu, 2003). Similarly, reports of *S. spilopleura* in Peru are now known to be *S. maculatus* (Jégu, 2003). Observations by Sazima and Zamprogno (1985) of the importance of floating vegetation to *S. spilopleura* (=*S. maculatus*) may explain why we did not collect the species until later in the study when the river tributary began to be choked off by floating mats of vegetation as the water receded through the dry season.

The following species are currently known from Peru: *S. compressus*, *Serrasalmus elongatus*, *S. humeralis*, *S. maculatus*, *S. rhombeus*, *S. sanchezi* and *Serrasalmus serrulatus* (Jégu & dos Santos, 2001; Jégu, 2003; van der Sleen & Albert, 2017). Thus, our *Serrasalmus* spp. is likely a complex of these species. The occurrence of *S. cf. sanchezi* in our collections is not surprising as the species was first described from Cano Yarina, a tributary of the Pacaya River (Géry,

1964), and is known to prefer vegetated shallow shoreline habitats (Hubert & Renno, 2010). However, photographic attributes of many of the *Serrasalmus* spp. individuals we collected (e.g., Figure 1) show striking similarities with the newly described *Serrasalmus odeyssi* from Bolivia (Hubert & Renno, 2010), with many appearing intermediate between *S. cf. sanchezi* and *S. odeyssi* (Frank Magallanes, Oregon Piranha Exotic Fish Exhibit, https://www.opefe.com, and Michel Jégu, Muséum national d'histoire naturelle, Laboratoire d'ichthyologie, Pers. Comm.).

The continued revision of piranha taxonomy and expectation that additional species will be described may further confuse the attribution of specific sound characteristic to piranha species recorded in studies which lack voucher specimens. Unfortunately, in our case we were forced to rely on photo-documentation of specimens because collection of physical specimens was prohibited. Our study highlights the need for researchers to deposit georeferenced voucher specimens in museums to anchor fish sounds to specific species and specific individuals in sound production studies of fishes.

Because of this taxonomic confusion, we are uncertain of the species attribution of piranha sounds to *S. spilopleura* by Kastberger (1981a,b) who collected specimens in the Rio Negro near Manaus, Brazil, where *S. maculatus* may co-occur. Data plotted in his Figure 3 suggest similar bark duration (50–75 ms vs 64 ± 8 ms) and fundamental frequency (140–150 Hz vs 153 ± 6 Hz) between his *S. spilopleura* and our *S. maculatus*, respectively (Supporting information Table S6). Unfortunately, Kastberger (1981a,b) does not report the size of fish auditioned for sound production, and fish used for morphology were adults purchased from commercial dealers. A comparison of our data with Mélotte et al. (2016) suggests *S. maculatus* has a shorter bark duration (64 ms vs 73 ms) than *S. spilopleura* but a similar fundamental frequency (153 Hz vs 149 Hz). We therefore tentatively assume Kastberger's (1981a) *S. spilopleura* is correct and our observations for *S. maculatus* to be new. Our sound recordings of *S. cf. sanchezi* are also new.

Sound durations of *P. nattereri* barks in this study were nearly identical to those reported by Mélotte et al. (2016) but were much shorter than those reported by Millot et al. (2011), most likely due to the effects of fish size (Supporting information Table S6). Similarly, our fundamental frequency estimate of 141 Hz based on the spectra (Figure 3a) and 128 Hz based on the waveform (Table 1) falls within the range of other studies (Supporting information Table S6).

Millot and Parmentier (2014) reported that bark and cycle durations increased significantly with fish size in *P. nattereri*, but that bark dominant frequency declined, while the number of cycles increased before declining at larger sizes. Mélotte et al. (2016) reported similar findings, but also found that size effects were not exhibited by all species. Sound duration declined with fish size for *S. elongatus* but increased for *Serrasalmus marginatus* and *P. nattereri*. Cycle period of the first two cycles increased for *P. nattereri* and marginally for *S. marginatus*, while the fundamental frequency declined with fish size for *S. elongatus*, *S. rhombeus*, *S. eigenmanni* and *P. nattereri*. Our study, controlling for within-fish variation, did

not find significant relationships with fish size for bark duration or peak frequency for any species (though the trends were similar), but did find significant relationships with related variables. The fundamental frequency (cycle rate) declined with fish size, but the trend was strongest for the maximum fundamental frequency within a bark series for *S. maculatus* and *P. nattereri* (Supporting information Table S4). In other words, smaller individuals were more likely to have at least one bark in a bark series with a higher fundamental than the highest fundamental in larger fish. While the duration of individual barks was not significantly related to fish size, the number of barks within a bark series was (Figure 6f). We did not measure cycle duration directly but found cycle period to be positively related to fish size (Figure 6e), while cycle rate (fundamental frequency) was negatively related (Figure 6d). Although we did not observe the expected relationship of peak frequency to fish size, other frequency variables were significantly related to fish size, most importantly the 95th percentile and the 90th percentile bandwidth (Figure 6a,b). These observations suggest that differences in the characteristics of barks within individual bark series produced by individual fish have the potential to indicate important behavioural or physiological regulators of sound production.

Although previous studies of piranha sounds have focused on describing the characteristics of individual barks, we note that a lot of temporal information is contained within a bark series (e.g., number of barks, intervals between barks, variation in bark acoustic frequency, including the fundamental, duration, cycle structure), which can be used to identify fishes. Our observations of the influence of bark order within a bark series on bark parameters (Figure 5) suggest that studies that pool sounds from individual fish might confound effects of bark order with fish size and species. Attempts, such as ours to reduce this potential bias by using means for each fish (or other statistic), only partly work. A better approach might be to use repeated-measures analysis that take into account the ordered pattern in the bark series, but that requires large sample sizes (i.e., sounds from more individuals). It is also possible that some of this pattern information may have biological significance. For example, the length of the series (number of barks), and timing of changes in bark duration or fundamental frequency (Figure 5), may code for species, gender, individual or some aspect of physiological condition.

Although we assumed that the occurrence of silent individuals in our study resulted from fatigue after capture, the tendency for larger fish to be silent is puzzling. We note that Millot and Parmentier (2014) also reported unexpectedly finding two of the four individuals in their largest size class of *P. nattereri* to be mute. In fact, there is some suggestion that larger piranha may become mute due to changes in sonic muscle physiology with growth and/or lack of use in captive specimens (Eichelberg, 1977, 1978; Millot & Parmentier, 2014). Observations of a decline in bark amplitude in larger *P. nattereri*, after an initial increase with size (Millot & Parmentier, 2014), might also be explained by a gradual decline in function due to atrophy of sonic muscles in captive specimens.

Although the relationship among acoustic parameters and fish size was clearly different among the species (Supporting information Table S4, Figure 6), such relationships are not as useful in PAM applications. In order to use PAM to study the temporal and spatial distribution of soniferous species, we must be able to reliably identify fish by the acoustic structure of individual sounds, or of sound series when they can be identified. Like Mélotte et al. (2016), we found that even closely related piranha can be distinguished by their sounds with multivariate approaches. Unfortunately, no single parameter could be used to identify piranha species. However, our observations demonstrate that species can be distinguished by their sounds under field conditions, using easily measured characteristics, despite the presence of high levels of background ambient sound compared to controlled laboratory studies. Suggestions of bias in sound production characteristics due to muscle atrophy in captivity (see discussion above), and recent findings of unexplained differences in fish sounds recorded in the laboratory versus those recorded in the natural habitat (e.g., Holt & Johnston, 2014), suggest that *in situ* recordings may be preferable for developing PAM methods whenever possible.

Unfortunately, sound characteristics routinely reported in the literature for most fishes would not have been adequate to distinguish piranha sounds that would typically be recorded in PAM studies. That is, we need more than basic parameters like peak/fundamental frequency, bark duration, and cycle rate (although these parameters are of critical importance to behaviour and morphology studies). In fact, at least for piranha, it appears that other frequency measures, such as bandwidth, are more useful, regardless of their biological function. Publishing detailed statistical data on fish sounds, including more fully describing the frequency structure of sounds, structure of sound series and within-fish variation in these measures, is essential to rigorous comparisons of sound production among species, and they are critically needed to develop reliable PAM of habitats and ecosystems where sounds of many species occur.

In conclusion, we demonstrate that piranha species can be distinguished by relatively easily measured variables with a multivariate approach based on sounds recorded under natural conditions. However, differentiation is greatly improved with the addition of measurements of the cycle structure of the barks. Although it is generally not possible to include fish size measurements in PAM data, it is often possible to identify discrete bark series that can be attributed to individual fish due to their temporal isolation. In that case, measurements of bark series parameters and variation in bark characteristics within a bark series can greatly enhance species differentiation. Although we doubt that PAM would be effective to measure piranha abundance, it can be useful to identify locations where piranha are active and in field studies of their behaviour. For example, preliminary processing of recordings of ambient sounds in our study area suggest that piranha sounds may be useful for detecting locations where they are actively feeding (Pers. Obs.).

REFERENCES

ACT (2007). *Underwater passive acoustic monitoring for remote regions. A workshop of research scientists, technology developers, and resource managers*. Coconut Island, Hawaii: Hawaii Institute of Marine Biology, Feb. 7-9, 2007. Alliance for Coastal Technologies Ref. No. ACT-07-02. Retrieved from http://www.act-us.info

Bioacoustics Research Program (2014). *Raven pro: Interactive sound analysis software* (Version 1.5) [Computer software]. Ithaca, NY: The Cornell Lab of Ornithology. Retrieved from http://www.birds.cornell.edu/raven.

Bodmer, R., Fang, T., Antunez, M., Puertas, P., Chota, K., Pittet, M., … Mayor, P. (2017). Impact of recent climate fluctuations on biodiversity and people in flooded forests of the Peruvian Amazon. In L. Rodríguez, & I. Anderson (Eds.), *The lima declaration on biodiversity and climate change: Contributions from science to policy for sustainable development*. Technical Series No. 89 (pp. 81–90). Montreal, Canada: Secretariat of the Convention on Biological Diversity.

Bradbury, J. W., & Vehrencamp, S. L. (1998). *Principles of animal communication*. Sunderland, MA: Sinauer Associates.

Chang, F., & Ortega, H. (1995). *Additions and corrections to the list of freshwater fishes of Peru*. Publicaciones del Museo Historia Natural. UNMSM, pp. 1–11.

Charif, R. A., Strickman, L. M., & Waack, A. M. (2010). *Raven Pro 1.4 user's manual*. Ithaca, NY: The Cornell Lab of Ornithology.

Eichelberg, H. (1977). Fine structure of the drum muscles of the piranha (Serrasalminae, Characidae). *Cell and Tissue Research*, 185, 547–555.

Eichelberg, H. (1978). Local degenerative changes in the drum muscles of piranhas (Serrasalminae, Characidae). *Cell and Tissue Research*, 188, 75–82.

Friendly, M. (1991). *SAS system for statistical graphics*. NC: SAS Institute Inc.

Gerhard, D. (2003). *Pitch extraction and fundamental frequency: History and current techniques*. Regina, Canada: Department of Computer Science, University of Regina. pp. 0–22.

Gerhardt, H. C., & Huber, F. (2002). *Acoustic communication in insects and anurans: Common problems and diverse solutions*. Chicago, IL: The University of Chicago Press.

Géry, J. (1964). Poissons characoïdes de l'Amazonie péruvienne: Avec 27 figures dans le texte. *Studies on Neotropical Fauna and Environment*, 4(1), 1–44.

Harris, R. J. (1985). *A primer of multivariate statistics*. New York, NY: Academic Press Inc.

Holt, D. E., & Johnston, C. E. (2014). Sound production and associated behaviours in blacktail shiner *Cyprinella venusta*: A comparison between field and lab. *Environmental Biology of Fishes*, 97, 1207–1219. https://doi.org/10.1007/s10641-013-0208-5

Hubert, N., Duponchelle, F., Nuñez, J., Garcia-Davila, C., Paugy, D., & Renno, J. F. (2007). Phylogeography of the piranha genera *Serrasalmus* and *Pygocentrus*: Implications for the diversification of the Neotropical ichthyofauna. *Molecular Ecology*, 16(10), 2115–2136. https://doi.org/10.1111/j.1365-294X.2007.03267.x

Hubert, N., & Renno, J. F. (2010). *Evolution of the neotropical ichthyofauna - molecular and evolutionary perspectives about the origin of the fish communities in the Amazon*. Saarbrücken, Germany: VDM Publishing House.

Jégu, M. (2003). Serrasalminae (Pacus and piranhas). In R. E. Reis, S. O. Kullander, & C. J. Ferraris Jr (Eds.), *Checklist of the freshwater fishes of South and Central America* (pp. 182–196). Porto Alegre, Brasil: EDIPUCRS.

Jégu, M., & dos Santos, G. M. (2001). Mise au point à propos de *Serrasalmus spilopleura* Kner, 1858 et réhabilitation de *S. maculatus* Kner, 1858 (Characidae: Serrasalminae). *Cybium*, 25(2), 119–143.

Jégu, M., Leão, E. L. M., & dos Santos, G. M. (1991). *Serrasalmus compressus*, une espece nouvelle du Rio Madeira, Amazonie (Pisces: Serrasalmidae). *Ichthyological Exploration of Freshwaters*, 2(2), 97–108.

Kaatz, I. M., & Stewart, D. J. (2012). Bioacoustic variation of swimbladder disturbance sounds in Neotropical doradoid catfishes (Siluriformes: Doradidae, Auchenipteridae): Potential morphological correlates. *Current Zoology*, 58(1), 171–188. https://doi.org/10.1093/czoolo/58.1.171

Kastberger, G. (1981a). Economy of sound production in piranhas (Serrasalminae, Characidae): I. Functional properties of sonic muscles. *Zoologische Jahrbücher. Abteilung für allgemeine Zoologie und Physiologie der Tiere*, 85, 113–125.

Kastberger, G. (1981b). Economy of sound production in piranhas (Serrasalminae, Characidae): II. Functional properties of sound emitter. *Zoologische Jahrbücher. Abteilung für allgemeine Zoologie und Physiologie der Tiere*, 85, 393–411.

Machado-Allison, A. (2002). *Los peces caribes de Venezuela: una aproximación a su estudio taxonómico*. Bol. Acad. C. Fís., Mat. y Nat. Vol. LXII No. 1 Marzo:35–88.

Markl, H. (1971). Schallerzeugung bei piranhas (Serrasalminae, Characidae). Sound production in piranhas (Serrasalminae, Characidae). *Zeitschrift für Vergleichende Physiologie*, 74(1), 39–56. https://doi.org/10.1007/BF00297789

Mélotte, G., Parmentier, E., Michel, C., Herrel, A., & Boyle, K. (2018). Hearing capacities and morphology of the auditory system in Serrasalmidae (Teleostei: Otophysi). *Scientific Reports*, 8(1), 1281. https://doi.org/10.1038/s41598-018-19812-1

Mélotte, G., Vigouroux, R., Michel, C., & Parmentier, E. (2016). Interspecific variation of warning calls in piranhas: A comparative analysis. *Scientific Reports*, 6, 36127. https://doi.org/10.1038/srep36127

Meschkat, A. (1957). Von den stimmen der fische im Amazonas. *Fischwirt*, 7, 67–68.

Millot, S., & Parmentier, E. (2014). Development of the ultrastructure of sonic muscles: A kind of neoteny? *BMC Evolutionary Biology*, 14, 24. Retrieved from http://www.biomedcentral.com/1471-2148/14/24.

Millot, S., Vandewalle, P., & Parmentier, E. (2011). Sound production in red-bellied piranhas (*Pygocentrus nattereri*, Kner): An acoustical, behavioural and morphofunctional study. *Journal of Experimental Biology*, 214, 3613–3618. https://doi.org/10.1242/jeb.061218

Ortega, H., & Hidalgo, M. (2008). Freshwater fishes and aquatic habitats in Peru: Current knowledge and conservation. *Aquatic Ecosystem Health & Management*, 11(3), 257–271. https://doi.org/10.1080/14634980802319135

Ortega, H., & Vari, R. P. (1986). *Annotated checklist of the freshwater fishes of Peru*. Smithsonian Contributions to Zoology, number 437, 25 pages, 1 figure, 1 table.

Ortí, G., Sivasundar, A., Dietz, K., & Jégu, M. (2008). Phylogeny of the Serrasalmidae (Characiformes) based on mitochondrial DNA sequences. *Genetics and Molecular Biology*, 31(1 suppl), 343–351. https://doi.org/10.1590/S1415-47572008000200030

Pielou, E. C. (1984). *The interpretation of ecological data*. New York, NY: John Wiley & Sons.

Rountree, R. A., Gilmore, R. G., Goudey, C. A., Hawkins, A. D., Luczko-vich, J., & Mann, D. A. (2006). Listening to fish: Applications of passive acoustics to fisheries science. *Fisheries*, *31*(9), 433–446. https://doi.org/10.1577/1548-8446(2006)31[433:LTF]2.0.CO;2

SAS Institute Inc (2012). *SAS/STAT® 12.1 user's guide*. Cary, NC: SAS Institute Inc.

Sazima, I., & Zamprogno, C. (1985). Use of water hyacinths as shelter, foraging place, and transport by young piranhas, *Serrasalmus spilopleura*. *Environmental Biology of Fishes*, *12*(3), 237–240. https://doi.org/10.1007/BF00005154

van der Sleen, P., & Albert, J. S., eds. (2017). *Field guide to the fishes of the amazon*. Orinoco and Guianas: Princeton University Press.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.