JOURNAL OF **FISH** BIOLOGY

Disturbance calls of five migratory Characiformes species and advertisement choruses in Amazon spawning sites

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Abstract

Species-specific disturbance calls of five commercially-important characiform species are described, the Curimatidae commonly called branquinhas: *Potamorhina latior*, *Potamorhina altamazonica* and *Psectrogaster amazonica*; Prochilodontidae: jaraquí *Semaprochilodus insignis* and curimatã *Prochilodus nigricans*. All species have a two-chambered swimbladder and the sonic mechanism, present exclusively in males, utilises hypertrophied red muscles between ribs that adhere to the anterior chamber. The number of muscles is unusually plastic across species and varies from 1 to 4 pairs suggesting considerable evolution in an otherwise conservative system. Advertisement calls are produced in river confluences in the Madeira Basin during the high-water mating season (January–February). Disturbance calls and sampling allowed recognition of underwater advertisement choruses from *P. latior*, *S. insignis* and *P. nigricans*. The advertisement calls of the first two species have largely similar characteristics and they mate in partially overlapping areas in the Guaporé River. However, *P. latior* sounds have a lower dominant frequency and it prefers to call from river confluences whereas *S. insignis* shoals occur mostly in the main river channel adjacent to the confluence. These results help identify and differentiate underwater sounds and evaluate breeding areas during the courtship of commercially important characids likely to be affected by two hydroelectric dams.

KEYWORDS
advertisement call, bioacoustics, communication, Curimatidae, Prochilodontidae, sonic muscle

1 | INTRODUCTION

The Amazon |Basin is an important biodiversity hotspot for freshwater fishes, supporting more than 3500 species that live in connected water bodies (rivers, creeks and lakes) and floodplains (Junk, 1997). These wetlands are dynamic and undergo an annual hydrological cycle fluctuating between seasonal flood pulses followed by ebbing water levels that affect fish life history (Ruffino *et al*., 1995).

Some shoaling Amazon fishes undertake seasonal migrations associated with feeding and reproduction. The reproductive migration generally starts at the beginning of rising water levels and continues during the flooding season (Cella-Ribeiro *et al*., 2016) when fishes move from nutrient-poor tributaries (black and clear-water rivers) downstream toward the confluence with white-water rivers (Araujo-lima & Ruffino, 2003). The feeding migration generally occurs between resource-rich rivers (characterised by turbid white-water) and floodplains after the breeding season.

Many of the most abundant migratory species are characiforms, which comprise up to 70% of the fish biomass and 80% of total landings from South American fresh waters (Carolsfeld *et al*., 2003). Migrations of commercially important characiforms are represented by species of the families Characidae, Prochilodontidae, Curimatidae and Hemiodontidae that form large migratory schools in the Rio Madeira basin (similar to other Amazon rivers) (Goulding, 1980; Doria *et al*.,

2012). Fishes from these families are consumed in the main cities of the Amazon Basin. Characiforms form reproductive aggregations in the confluence of rivers (Goulding, 1980). Although there has been little formal work, the presence of spawning groups of migratory prochilodontid fishes of the genera *Semaprochilodus* Fowler 1941 and *Prochilodus* Agassiz 1829 have been audibly detected above water by the sounds they produce (Goulding, 1980).

Amazon fish produce sound using a variety of mechanisms including well-developed sonic intercostal muscles attached to the swimbladder as studied in piranhas (Eichelberg, 1977; Kastberger, 1978; Ladich & Bass, 2005; Millot *et al.*, 2011; Ladich, 2014; Mélotte *et al.*, 2016). An unrelated sonic muscle, which hypertrophies during the mating season, is also used by male sciaenids, such as the amazon croaker *Plagioscion squamosissimus* (Heckel 1840) (Borie *et al.*, 2014). Male *Semaprochilodus insignis* (Jardine 1841) develop intercostal sonic muscles around the swimbladder responsible for driving swimbladder motion to produce loud sounds during the spawning season (Kastberger, 1978; Junk, 1997). With the exception of captive piranhas, bioacoustics work on characiforms has been largely overlooked. Two short notes on *S. insignis* describe the sonic muscle and sounds (without quantitative analysis; Schaller, 1971; Dorn & Schaller, 1972). Recently, Smith *et al.* (2018) recorded advertisement calls of *Prochilodus argenteus* Spix & Agassiz 1829, *Prochilodus costatus* Valenciennes 1850 and *Prochilodus lineatus* (Valenciennes 1837) in cement ponds. Calls of each species consist of trains of pulses that differ in dominant frequency, pulse duration and pulse period. Additionally, the three species produce single-pulse calls.

The need for studies on ecological aspects of the Madeira River is urgent since two huge hydroelectric power dams in the region will probably affect fish migration and reproduction. Passive acoustics is an excellent non-invasive tool to determine location and timing of reproducing populations. Therefore, we characterised disturbance calls of fish held in air of three commercial curimatids species (*Potamorhina latior* (Spix & Agassiz 1829); *Potamorhina altamazonica* (Cope 1878); *Psectrogaster amazonica* Eigenmann & Eigenmann 1889)) and two prochilodontids (*Prochilodus nigricans* Spix & Agassiz 1829; and *S. insignis*). These calls allowed us to identify underwater advertisement calls in spawning aggregations for two of the species. Passive acoustic monitoring supported the hypothesis of reproductive aggregations at river confluences proposed by Goulding (1980). Additionally, sonic muscles were found to attach to ribs covering the anterior chamber of the swimbladder with different patterns of muscle investment in the two families.

2 | MATERIALS AND METHODS

This research was undertaken with the authorisation for capture, collection and transportation of biological material of fish issued by the Brazilian Ministry of the Environment, through licence number 83/2012.

2.1 | Study area

The Madeira Basin occupies the most extensive area in the Amazon region and is the largest tributary of the Amazon River in terms of sediment discharge and the second largest in flow (Goulding *et al.*, 2003). The Madeira River fish assemblage includes *c.* 1000 species, the largest recorded for any basin in the world (Ohara *et al.*, 2015; Queiroz *et al.*, 2013).

The middle Madeira Basin (Figure 1) is composed of several tributaries with different water characteristics. Rivers that originate in the Andes strongly influence biotic and abiotic ecology of the Madeira River (Guyot *et al.*, 1999, McClain & Naiman, 2008). Such rivers are considered white-water rivers with a muddy colour, an alkaline to neutral pH and a high sediment load. Black-water rivers, such as the Guaporé River (known as Iténez in Bolivia) and Machado River, come from the Brazilian shield and present different characteristics including an acidic pH, a high concentration of dissolved organic carbon and lower suspended sediments (Araujo-Lima & Ruffino, 2003).

Madeira Basin rivers begin flooding in November and may continue to rise until June; seasonal increases and decreases in river height occur 2 months earlier than in most of the Amazon Basin. The flood pulse is regulated by the Andean thaw and regional rainfall concentrated between January and March. The ebb begins in May and extends to November.

2.2 | Sound recording

Disturbance calls were recorded in air using a battery-powered digital recorder Olympus WS-400S (WMA format and sampling frequency 44.1 kHz; www.olympus-global.com) with an internal microphone placed 1–2 cm from the fish's thoracic region. Advertisement calls in eight river confluences were recorded with an H_2a hydrophone (sensitivity of −180 dB re. 1 V μPa^{-1} and useful frequency range of 10 Hz to 100 KHz: Aquarian Audio; www.aquarianaudio.com), connected to the digital recorder. Recordings were made during the fish-collecting campaign for the ichthyological collection of Rondônia Federal University (the largest and most complete collection of fishes from the Madeira |Basin) and during the fishery monitoring programme of the Jirau hydropower dam (09° 16′ 04″ S; 64° 38′ 45″ W).

2.3 | Disturbance calls in air

Disturbance calls of curimatid and prochilodontid species were recorded to allow identification of advertisement calls detected in river confluences. Fish were captured with a cast net from calling populations (Figure 1) and their disturbance calls were recorded immediately. Each fish was gently held on the boat until it emitted five calls or for 1–2 min. Females proved negative for sound production. Recordings were made in the late afternoon and early evening (starting *c.* 17:00 h) during the spawning season at high-water levels in January and February of 2013. Curimatid species from spawning aggregations were caught in the confluence of Guaporé and Mamoré Rivers. *P. latior* (45 calls from 9 fish; mean ± SE standard length,
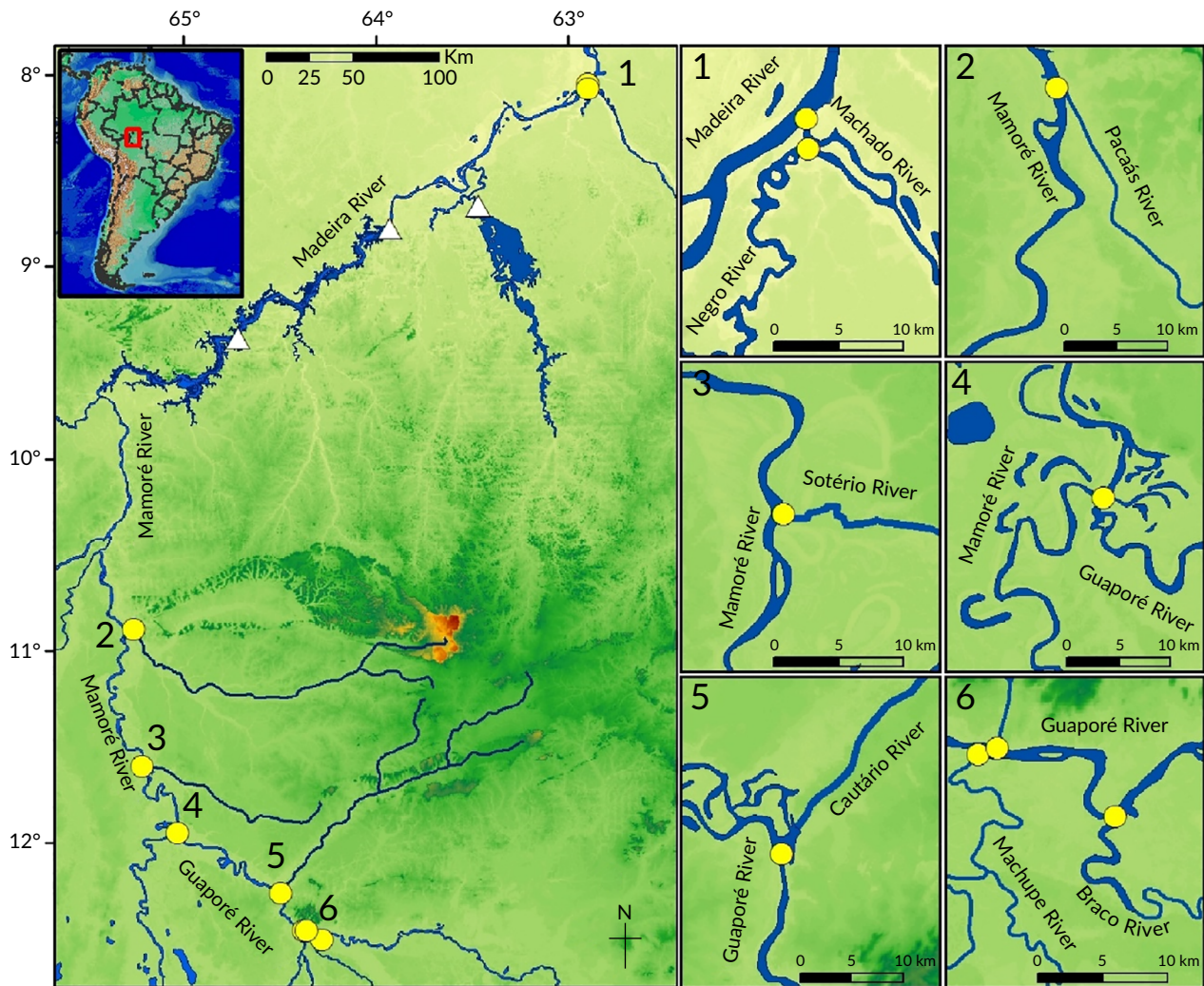
FIGURE 1   Recording areas at confluences of the Madeira–Mamoré River and black-water tributaries: 1, Machado (and their last tributary, the Negro River); 2, Pakaás; 3, Sotério; 4, Guaporé; 5, Cautário and Andes white-water rivers: 6, Machupe and Branco Rivers. Disturbance calls recorded in area 4 from the Curiamatidae and area 6 from *Semaprochilodus insignis*

L_S = 15.0 ± 0.5 cm), *P. altamazonica* (48 calls from 11 fish, 13.3 ± 0.9 cm L_S), *P. amazonica* (36 calls from 7 fish; 11.6 ± 0.4 cm L_S). The prochilodontid *S. insignis* (40 calls from 8 fish; 21.0 ± 1.6 cm L_S) were caught upstream in the Guaporé River (Figure 1) close to the river margin. Also, *P. nigricans* (42 calls from 4 adult fish) were collected and recorded from the Santo Antonio hydropower reservoir (08° 47′ 64″ S, 63° 57′ 04″ W) in the Madeira River.

After recording, fish were euthanised by cranial concussion and fixed in 10% formalin for later investigation of the sonic mechanism. Lateral muscles were removed to reveal the swimbladder and intercostal sonic muscles.

2.4 | Fish chorus distribution

The importance of river confluences to fish aggregation during the spawning season was evaluated through recordings in eight river confluences with different water characteristics in the Madeira Basin. The complex of Madeira–Mamore Rivers was considered as the main stem of the Madeira Basin. Recordings were made in both downstream and upstream directions, in the confluence of Madeira–Mamoré Rivers and black-water tributaries, such as the Machado River (and its last tributary, the Negro River), Pakaás, Sotério, Guaporé Rivers and also in the Guaporé River upstream tributaries including Cautário and Andes white-water Machupe and Branco Rivers (Figure 1). Recordings 1 km from the confluences proved negative for sound choruses.

Owing to the strong current and lower levels or non-detection of calls in the Madeira–Mamore white-water main river channel, recordings were mainly made in the mouth of its tributaries and sometimes in the river plume outside the mouth. In the Guaporé River recordings were made both at the mouths of the tributary rivers and in the main channel. The underwater recordings were made over 2–3 days for 1–2 h day^{-1} in each river confluence.

2.5 | Acoustic analysis

Wave files were downloaded to a portable computer for pre-evaluation. Segments containing acoustic recordings were saved in WAV format (16-bit amplitude resolution) and stored for later analyses.

Audacity audio software (www.audacityteam.org) was used to manually edit selections to exclude mechanical and human noise from individual calls. Selections were chosen according to signal quality and amplitude and had minimal overlapping signals whenever possible since individual sounds from dense choruses cannot be recognised. The following variables were measured: (1) number of pulses per call (*n*); (2) call duration (ms) from the start of the first pulse to the end of the last pulse; (3) pulse period (ms), time from one pulse to the next; (4) pulse rate (pulses per second, p s^{-1}), (5) pulse cycle duration (ms), period of the greatest amplitude cycle within a pulse; (6) dominant frequency (Hz); (7) pulse cycle frequency (Hz); (8) calculated frequency from pulse cycle duration (Hz). One pulse was measured in each call. These acoustic charachteristics were measured using oscillograms and spectrograms, with a fast Fourier transform (FFT) resolution of 512% and 50% overlap in Raven Pro 1.4 software (Cornell Ornithology Laboratory; ravensoundsoftware.com).

Power spectra were made using the PAMGuide tool (Merchant *et al.*, 2015) in MATLAB 2016 (www.mathworks.com). Spectra were visually evaluated for general trends in underwater and disturbance recordings. The spectrograms were plotted with a frequency band between 100 to 4000 Hz. A band between 100 and 2000 Hz was used for the power spectra analysis of underwater and disturbance recordings. The root mean square (RMS) levels of the power spectral density (PSD) values were calculated.

The parameters of disturbance and individual underwater calls were compared using non-parametric Kruskal-Wallis multiple comparison and Tukey *post hoc* tests between the different groups for each variable using STATISTICA 7 (Dell Inc.; www.statistica.com). Individual means were calculated from multiple calls from each fish. Principal component analysis and canonical modules were used for identifying important dimensions in a set of variables with similar characteristics after testing the assumption of independence between variables.

3 | RESULTS

3.1 | Disturbance and underwater voluntary calls

Dense choruses were recorded in all river confluences. However, overlapping sounds made it impossible to isolate individual callers except near the mouth of the Guaporé and Sotério Rivers. All underwater choruses were of sufficient amplitude to be audible in air on the boat. Underwater voluntary calls were recorded in the Guaporé River (chorus 1, *n* = 59 calls) and Sotério Rivers (chorus 2, *n* = 30 calls). Cast nets employed in the middle of a chorus typically captured individuals of a single species that continued to call on the boat providing evidence of caller identity.

Recordings of disturbance calls of four characiforms were performed from fish freshly captured in the middle of choruses on the Guaporé River, mainly at the confluence with the Mamoré River (*P. latior*, *P. altamazonica* and *P. amazonica*), *S. insignis* upstream in the Guaporé River and *P. nigricans* in the Madeira River. Each species emitted distinct disturbance calls when held by hand in air. Disturbance calls consisted of a series (trains) of pulses with each pulse composed of different numbers of cycles in each species (Figure 2). Pulses varied in amplitude and introductory pulses tended to be lower in amplitude than ones that followed. Within a pulse the initial cycle was of longer duration and lower frequency than later ones and timing of later cycles exhibited various patterns of amplitude decay. The waveform for *P. nigricans* was an exception and was composed of two cycles that were not followed by a regular series of oscillations. The frequency spectra in disturbance and underwater voluntary calls maintained a high amplitude to *c.* 2 kHz in *P. latior* (Figure 3a), *S. insignis* and chorus 1 (Figure 4a), *P. altamazonica*, *P. amazonica*, *P. nigricans* (Figure 3) and chorus 2 (Figure 4b) up to 1 kHz before dropping off.

All temporal variables of disturbance and underwater voluntary calls presented significant differences between species ($P < 0.05$): number of pulses per call (Kruskal-Wallis tests, $H_{6,54} = 45.42$); call duration ($H_{6,54} = 47.64$); pulse period ($H_{6,54} = 48.06$); pulse rate ($H_{6,54} = 45.95$), pulse cycle duration ($H_{6,54} = 26.35$); dominant frequency ($H_{6,54} = 33.28$); pulse cycle frequency ($H_{6,54} = 19.90$); calculated frequency ($H_{6,54} = 33.34$; Figure 5). Calls of *P. latior* and chorus 1 were not statistically significant different for any parameter ($P > 0.05$).

The number of pulses per call was higher ($P < 0.05$) in *S. insignis* (means ± SE: 28.0 ± 3.0) than in *P. altamazonica* (5.6 ± 0.5). Pulse number was similar in both *P. amazonica* (5.4 ± 0.3) and chorus 2 (4.8 ± 0.3; Figure 5a). Although *S. insignis* calls had the greatest number of pulses, call duration was longer in *P. nigricans* (mean duration of 827 ms; Figure 5b). Pulse rate was higher in *P. amazonica* (122 ± 1.8 p s^{-1}) than in *P. nigricans* (19.7 ± 2.3 ms; Figure 5c) although pulse period was longer in *P. nigricans* (48.2 ± 5.6 ms; Figure 5d). The greatest pulse cycle duration within a pulse was similar in *P. nigricans* (2.4 ± 0.3 ms) and chorus 2 (3.1 ± 0.2 ms; Figure 5e). Also, a long pulse cycle produced a low dominant frequency in *P. nigricans* (506 ± 84.7 Hz) and chorus 2 (339 ± 14.0 Hz). Among the curimatids, dominant frequency was higher in *P. latior* (1318 ± 153.6 Hz) and similar to that of chorus 1 (1132 ± 70.2 Hz; Figure 3f). Cycle frequency (Hz) and calculated frequency (Hz) were also similar in *P. nigricans* and chorus 2 (Figure 5f and Table 1).

The number of pulses was tightly related to call duration within species ($R^2 > 0.90$, $P < 0.05$) indicating that pulses were emitted in a regular pattern (Figure 6) although correlation in chorus 2 was somewhat lower ($R^2 = 0.80$; Figure 6). The number of pulses and call duration were directly associated with pulse period. There was considerable overlap with similar regressions in *P. latior* ($y = 0.0120x - 0.0022$), chorus 1 ($y = 0.0120x - 0.0021$) and *S. insignis* ($y = 0.0120x + 0.0229$). As the pulse rate increased, the dominant frequency also tended to increase across species ($y = 8.0765x + 464.2$; Figure 7a). The pulse period overlapped in *P. latior*, *S. insignis* and chorus 1, with *c.* 13 ms between pulses. The greater pulse period in *P. nigricans* and chorus 2, 48 ms, was associated with a lower dominant frequency ($y = -23,337x + 1507$; Figure 7b).

The original set of eight acoustic parameters was transformed by PCA into two factors that accounted for 70.9% of the total variance (factor 1 = 51.1%, factor 2 = 19.8%). Factor 1 was negatively correlated (> 0.70) with pulse rate, cycle frequency (Hz), calculated
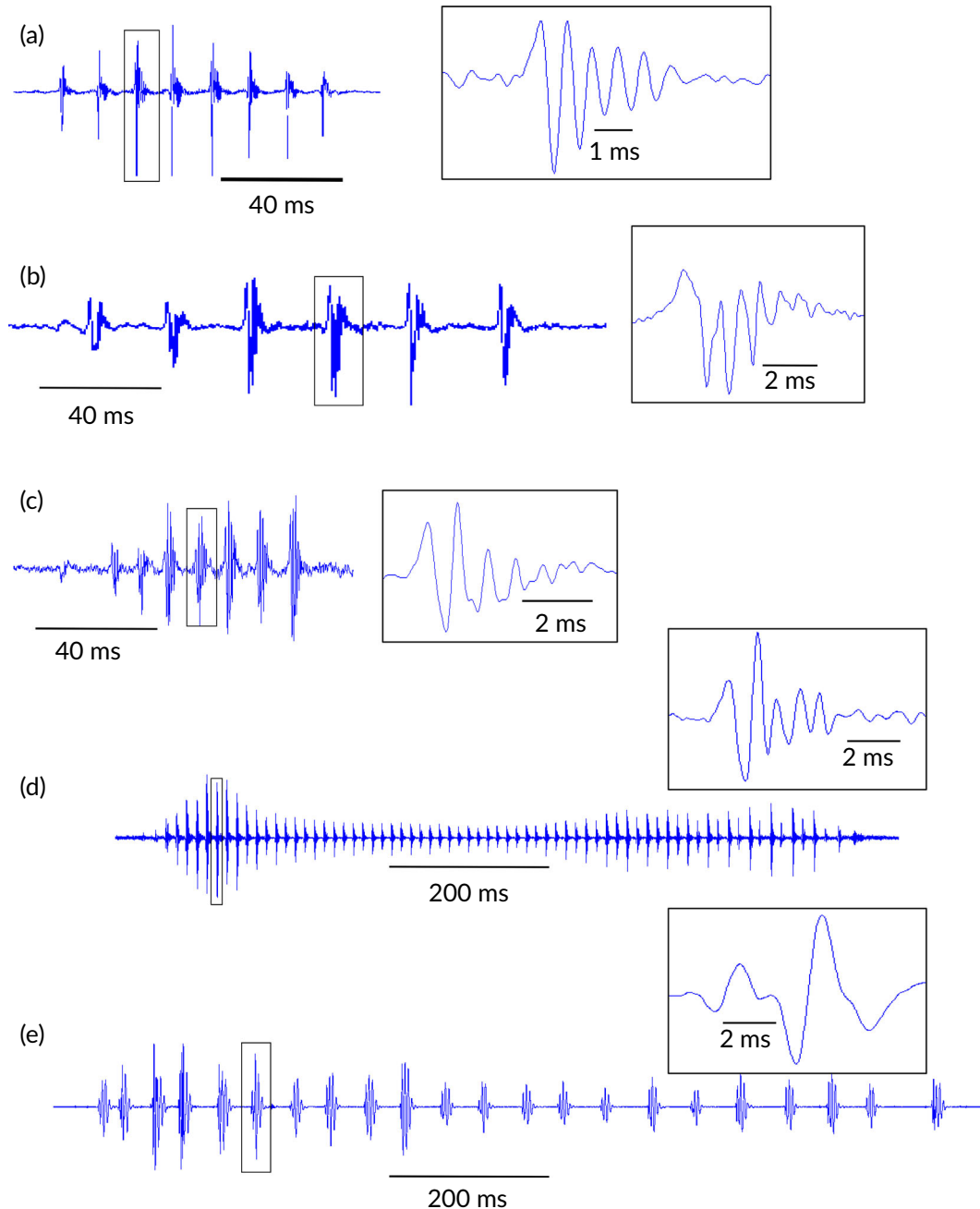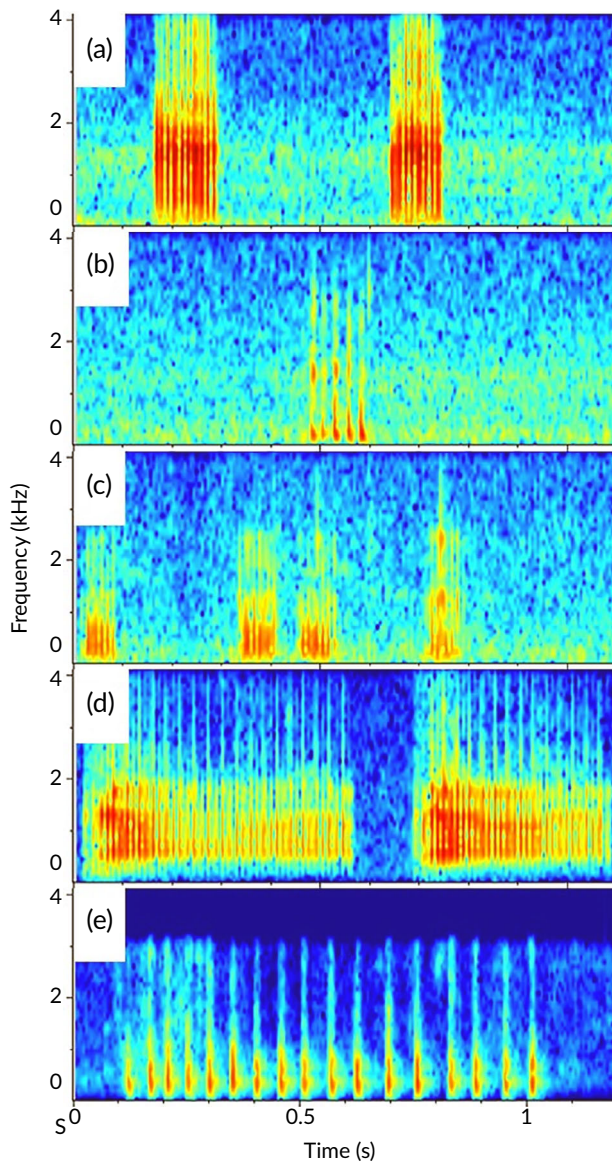
FIGURE 2 Waveform and individual pulses of characiform disturbance calls of: (a) *Potamorhina latior*, (b) *Potamorhina altamazonica*, (c) *Psectrogaster amazônica*, (d) *Semaprochilodus insignis* and (.)*Prochilodus nigricans*. Note a tendency for initial pulses to have a lower amplitude than later ones and variation in amplitude particularly in (d) and (e)

frequency (Hz) and correlated positively with pulse period (ms), pulse cycle (ms), whereas factor 2 correlated negatively with pulses per call and call duration (ms; Table 2). The analysis did not allow discrimination between the acoustic signals of *P. latior*, *S. insignis* and chorus 1. On the other hand, the scatter of *P. altamazonica* and *P. amazonica*, *P. nigricans* and chorus 2 did not overlap (Figure 8). Differences between *P. nigricans* and chorus 2 can be explained by the longer call duration in advertisement calls.

Potamorhina latior disturbance calls and chorus 1 had a series of small-amplitude oscillations visible on the power spectra.

Oscillations occurred at intervals of *c*. 100 Hz up to the dominant frequency at *c*. 1200 Hz. The exact frequency of the oscillations differed slightly between the power spectra, but the interval was similar (Figure 9a,d). Although calls of *S. insignis* were similar to those of *P. latior*, the *S. insignis* power spectra exhibited a different pattern with smaller oscillations, similar to the dense chorus in the Machupe River (Figure 9b,e). The oscillations were not observed clearly in *P. nigricans*, for which most of the energy is below 1000 Hz, with power-spectra pattern similar to chorus 2 (Figure 9c,f).

FIGURE 3   Spectrograms of characiform disturbance calls. (a) *Potamorhina latior* (two calls), (b) *Potamorhina altamazonica* (one call), (c) *Psectrogaster amazônica* (four calls), (d) *Semaprochilodus insignis* (two calls) and (e) *Prochilodus nigricans* (one call) in a 1.1 s sample. Hanning windows type, fast Fourier transform size 512 and overlap of 50%

3.2 | River signatures

The frequency band of the dense chorus in Guaporé River lost energy rapidly above 2 kHz. The frequency was similar in the Negro, Machado, Pakaás and Cautário Rivers, with a range of 250–2000 Hz. The Sotério and Branco Rivers presented a lower frequency reaching 1 kHz. The Negro and Machado Rivers presented similar patterns, possibly because the rivers are in close proximity. It was possible to differentiate between the sounds made by different species during the recordings and *P. latior* calls became more evident in the mouth of the Guaporé River and its tributary, the Cautário River, (similar spectra between 100 Hz and peak energy at 1 kHz).
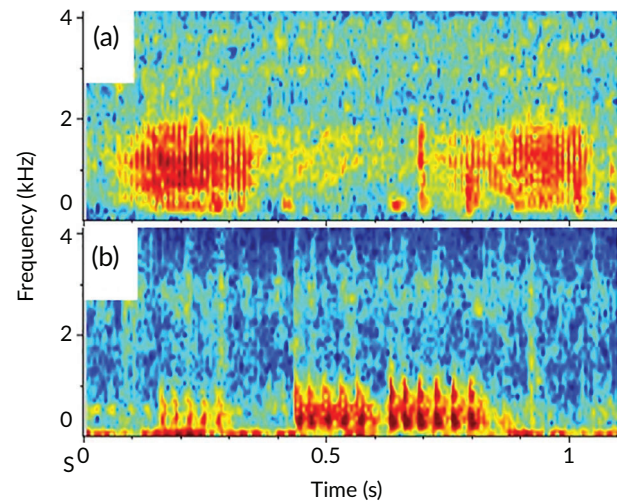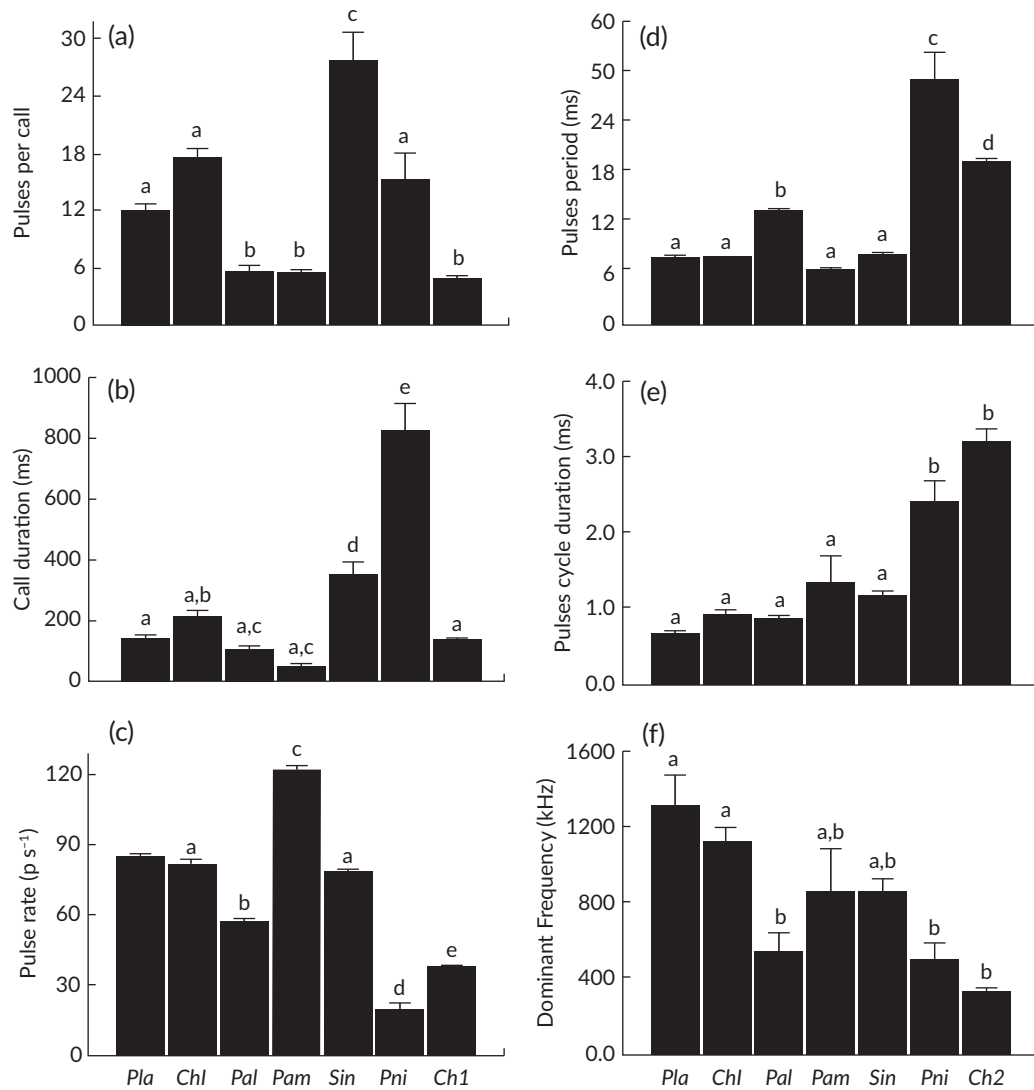

FIGURE 4   Spectrogram of underwater voluntary calls and waveform recorded in (a) Guaporé River (chorus I) and (b) Sotério River (chorus II). Hanning windows type, fast Fourier transform size 512 and overlap of 50%

The similarity of power spectra of Negro, Machado, Pakaás and Cautário Rivers with disturbances calls of *P. latior* indicate that sounds recorded in these confluences were probably produced by this species. The Branco River presented a different pattern, probably caused by other unidentified species. Other sounds detected in the Pakaás River, had loud and long sounds with harmonics, represented by two peaks in the power spectra (Figure 10).

3.3 | Sonic muscles

Curimatid and prochilodontid species use paired extrinsic intercostal sonic muscles that surround the lateral walls of the anterior chamber of the two-chamber swimbladder. The anterior chamber is round and smaller than the elongated posterior chamber that tapers posteriorly. Additionally, the dorsal surface of the chamber is attached to vertebrae. Most of the swimbladder is covered by a heavy white peritoneum that lines the body cavity. One to four muscles are red in colour during the mating season in males. The muscle fibres are oriented in the horizontal plane parallel to the long axis of the fish. The first rib is thicker than posterior ones and there are major differences in sonic muscle attachment to the ribs between the two families (Figure 11). Curimatid sonic muscles primarily occur in a forward position attaching to the anterior edge of the first rib and prochilodontids have additional muscles, the first of which attaches to the posterior edge of the first rib.

In the curimatids, the simplest, perhaps most basal condition, occurs in *P. altamazonica* and *P. amazonica*, which have a single pair of muscles that connect together in front of the swimbladder *via* an aponeurosis that would compress the anterior wall of the swimbladder during muscle contraction (Figure 11). The muscle is longer in *P. amazonica* and almost covers the anterior face of the bladder with a reduced aponeurosis at the midline.

FIGURE 5 Comparison of mean (+ SE) disturbance calls from *Potamorhina latior* (*Pla*), *Potamorhina altamazonica* (*Pal*), *Psectrogaster amazonica* (*Pam*), *Semaprochilodus insignis* (*Sin*), *Prochilodus nigricans* (*Pni*) and underwater voluntary calls (chorus 1, CH1; chorus 2, CH2 and II). Different letters indicate means that are significantly different

TABLE 1 Cycle frequency and calculated frequency of the calls of five characids and two choruses

Species	Cycle frequency (mean ± SE, Hz)	Calculated frequency (mean ± SE, Hz)
P. latior	1089.4 ± 166.6[a]	1527.9 ± 73.9[a]
Chorus 1	1146.2 ± 79.8[a]	1149.3 ± 77.2[a]
P. altamazonica	906.7 ± 99.4[a]	1207.4 ± 48.4[a]
P. amazonica	956.4 ± 194.7[a]	1158.8 ± 230.6[a]
S. insignis	860.2 ± 43.9[a]	890.2 ± 39.3[b]
P. nigricans	432.2 ± 36.1[c]	446.2 ± 49.1[c]
Chorus 2	341.6 ± 11.4[c]	319.4 ± 19.2[c]

Different letters indicate significant differences (*P* < 0.05) between species based on.
Kruskal-Wallis and Tukey *post hoc* test.
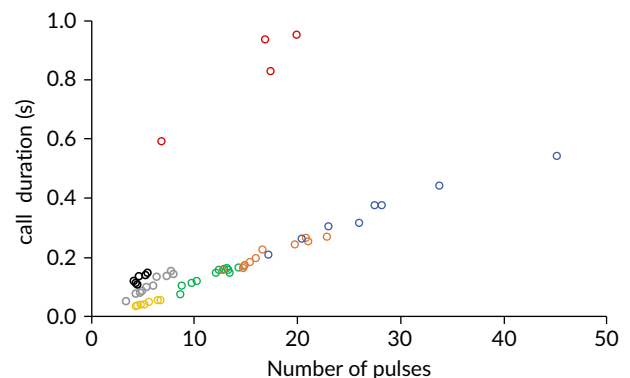
FIGURE 6 Relationship of call duration to number of pulses in disturbance calls recorded in air and underwater voluntary calls (chorus 1 and 2) detected in the Madeira Basin. Note advertisement calls in choruses are shorter than disturbance calls in *Prochilodus nigricans* and longer in *Potamorhina latior*
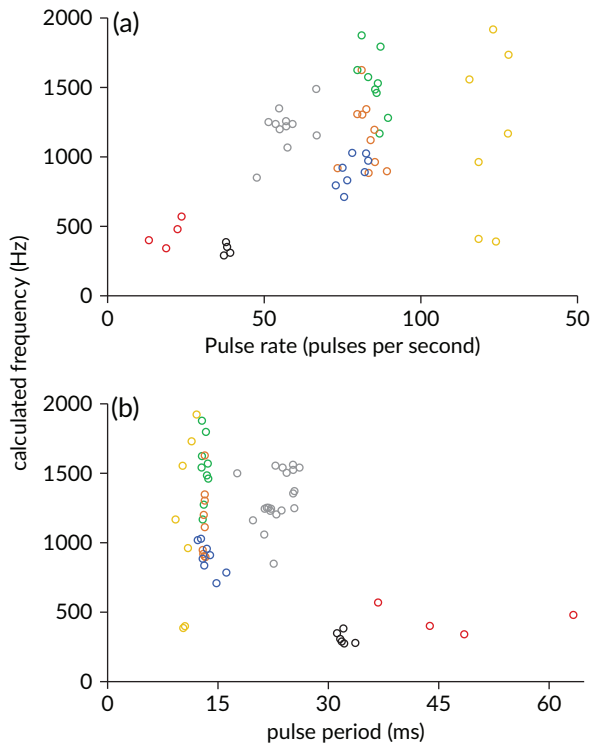
FIGURE 7 Relationship of calculated frequency to (a) pulse rate and (b) pulse period

TABLE 2 PCA factors used in separating the calls of five characid species

Variables	Factor 1	Factor 2
Pulses per call	−0.0446	−0.9223[*]
Call duration (ms)	0.5763	−0.7520[*]
Pulse rate (pulse/s)	−0.8037[*]	0.0831
Pulse period (ms)	0.8734[*]	−0.0139
Peak Frequency (Hz)	−0.5945	−0.3042
Pulse cycle (ms)	0.8508[*]	0.2066
Cycle frequency (Hz)	−0.7366[*]	−0.1675
Frequency calculated (Hz)	−0.8539[*]	0.0102
% Total variance	51.1	19.8
Cumulative %	50.1	70.9

[*], $p < 0.05$.

P. latior is more complex. The first muscle is similar to that of the other two species with a restricted aponeurosis in front of the bladder, but there is also a second muscle between the first and second ribs (the muscles do not reach the second rib but attach to the heavy peritoneum). *Potamorhina latior* also has a slender tendon that descends from the aponeurosis, bifurcates and attaches to the left and right halves of the pectoral girdle. The action of curimatid muscles would therefore pull the anterior wall of the swimbladder posteriorly during contraction.

The prochildontids have multiple muscles. *Semaprochilodus insignis* has three pairs of muscles between the first four ribs and the distance
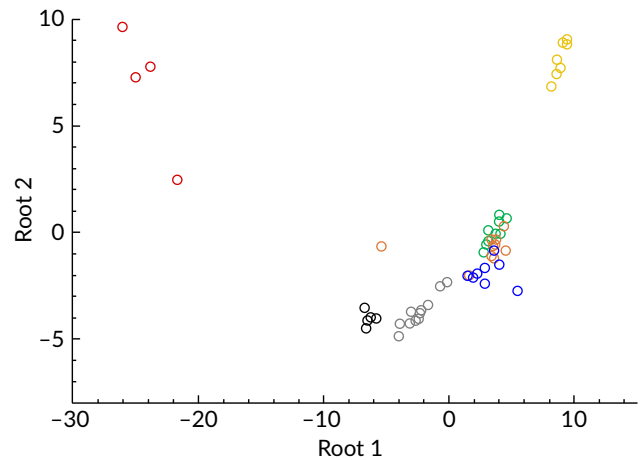
FIGURE 8 Canonical discriminant scores generated by the first two functions to illustrate the separation of disturbance calls of different species: *Potamorhina latior* (◯), *Potamorhina altamazonica* (◯), *Psectrogaster amazonica* (◯), *Semaprochilodus insignis* (◯), *Prochilodus nigricans* (◯) and the underwater calls in chorus 1 (◯) and chorus 2 (◯ black). Based on 8 discriminant functions, factor 1 and 2 accounted for 70.9% of the variance

between the first and second rib is again wider than between other ribs (Figure 11). The first muscle is darker and wider than more posterior muscles and the third muscle attaches to the peritoneum wall. *Prochilodus nigricans* has a fourth muscle that attaches to the fourth and fifth rib. Muscle contraction in the prochilodontids will pull the sides of the swimbladder backward but should not directly move the anterior wall of the chamber.

4 | DISCUSSION

Characiformes, the dominant fish taxon in the Amazon, has been largely overlooked in the bioacoustics literature. This study presents disturbance calls recorded in air for three curimatids and two prochilodontids species, acoustic recordings of dense choruses from six river systems and two choruses that sometimes were sparse enough to allow distinguishing individual calls. Based on waveform and acoustic variables of disturbance calls, it was possible to identify *P. latior* as the caller for chorus 1 and *P. nigricans* for chorus 2. These identifications were verified by cast-net captures from within the choruses. Additionally, sonic muscles of these species are briefly described.

Although imperfect because of changes to mass loading and radiation resistance, recording in air provides better free-field conditions than tank recordings (Fine *et al.*, 2004; Ghahramani *et al.*, 2014), which are affected by wall resonance and out of phase reflections (Akamatsu *et al.*, 2002; Parmentier *et al.*, 2014). Swimbladder sounds in the Atlantic croaker *Micropogonias undulatus* (L. 1766) have similar pulse repetition rates and dominant frequencies in both media although pulse duration is longer because of decreased damping and sharpness of tuning (*Q*-factor) is higher in water. Sound is generated by the same internal swimbladder motion (timing) in both media and with caution either tank or aerial recordings can be used to identify
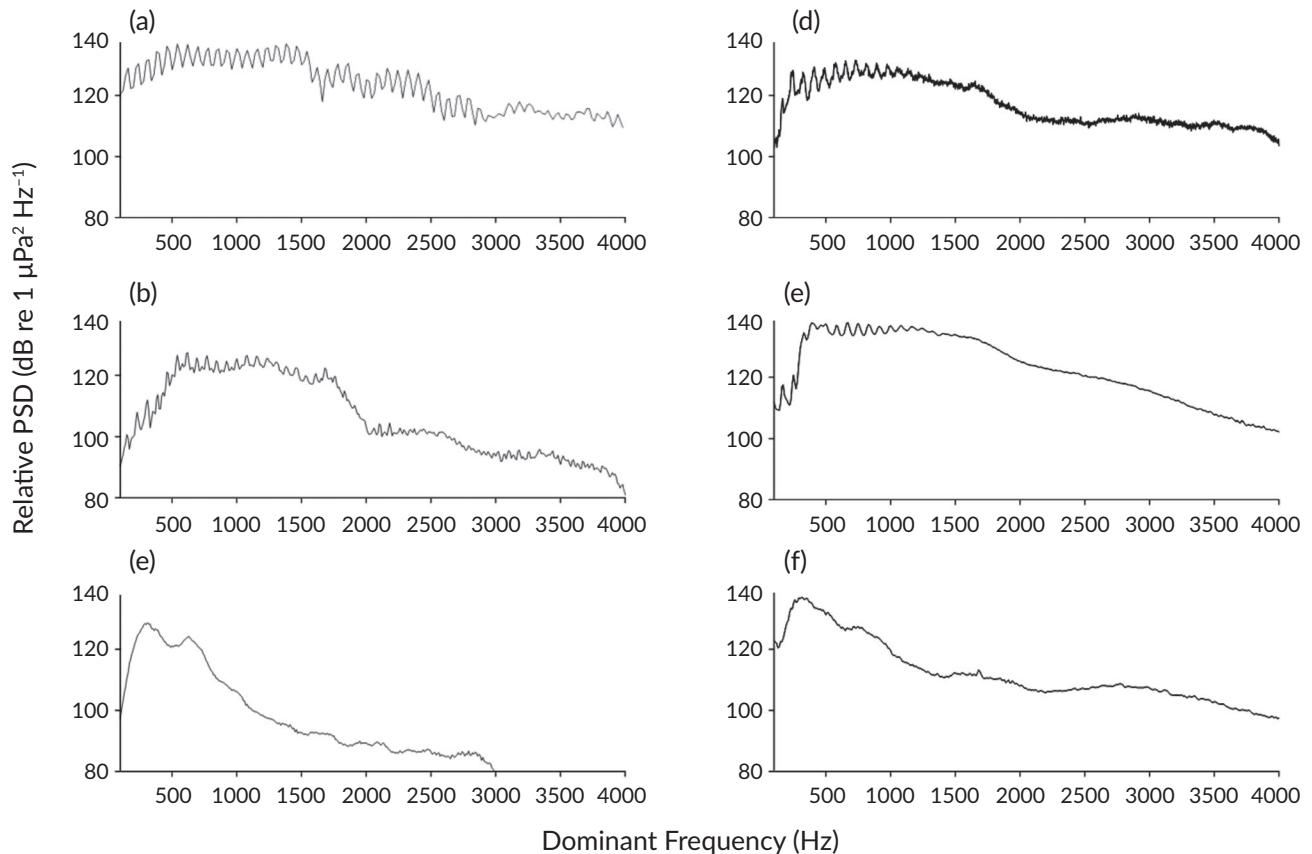
FIGURE 9 Power spectra of disturbance in air (⎯⎯) and underwater calls (⎯⎯) of (a) *Potamorhina latior*, (b) *Semaprochilodus insignis*, (c) *Prochilodus nigricans*, (d) underwater voluntary calls from the Guaporé River and (e) Sotério River. (f) Dense chorus at Machupe River

sounds produced in natural bodies of water. Similar variables of disturbance calls and advertisement calls recorded in the field allowed us to identify callers in two separate choruses with reasonable confidence.

Disturbance and advertisement calls of *P. latior* and chorus 1 were similar in pulse rate, pulse period and dominant frequency although advertisement calls from chorus 1 were longer and consisted of more pulses. *S. insignis* and *P. latior* disturbance calls were similar (pulse rate, pulse period and dominant frequency), but *S. insignis* calls scattered at one end of the *P. latior* distribution but were largely separate. There was considerable overlap between parameters of *P. nigricans* and chorus 2; disturbance calls were shorter with fewer pulses than advertisement calls. We note however that species in different families such as *P. latior* (Curimatidae) and *S. insignis* (Prochilodontidae) present similar call characteristics, which can be difficult to differentiate.

There is a wide diversity of independently-derived sonic muscles in teleosts (Fine & Parmentier, 2015) and these have arisen as exaptations of structures used in other contexts such as buoyancy, swimming and feeding (Parmentier *et al.*, 2017). Sonic muscles may be intrinsic, attaching exclusively to the swimbladder, or extrinsic with a variably-distant origin and attaching directly to the swimbladder or indirectly to a bone or tendon that moves the swimbladder (Ali *et al.*, 2016; Fine *et al.*, 2016; Fine & Parmentier, 2015). Within characiforms, several intercostal muscles have been modified into extrinsic drumming muscles that move the swimbladder indirectly by attached

ribs or connecting to an aponeurosis surrounding the anterior swimbladder. This mechanism should be confirmed physiologically in future work. The families Curimatidae and Prochilodontidae are considered sister taxa (Arcila *et al.*, 2017; Oliveira *et al.*, 2011) and have distinct differences in sonic muscle investment although both involve the first rib. In curimatids the muscles attach to the anterior edge of the first rib, travel forward and connect *via* an aponeurosis in front of the swimbladder. Dorn and Schaller (1972) found similar morphology in *Potamorhina* (= *Anodus*) *laticeps* (Valenciennes 1850). *Potamorhina latior* has an additional muscle after the first rib that almost reaches the second rib but attaches to the thick peritoneum surrounding the body cavity. It also has a tendon that extends from the aponeurosis to the pectoral girdle that may serve to maintain swimbladder shape as the front of the anterior bladder is compressed during sound production.

The sister group Prochilodontidae has a distinct arrangement of intercostal muscles. They connect the first four or five ribs and the first muscle attaches to the posterior edge of the first rib. Multiple intercostals were first described briefly by Schaller (1971) in *S. insignis*. Similarly, *P. argenteus* has three pairs of sonic muscles (Godinho *et al.*, 2017; Smith *et al.*, 2018). The variation in sonic muscles in the two families should be examined in more species and the ancestral condition preceding the divergence of the muscles in the two families is unknown.
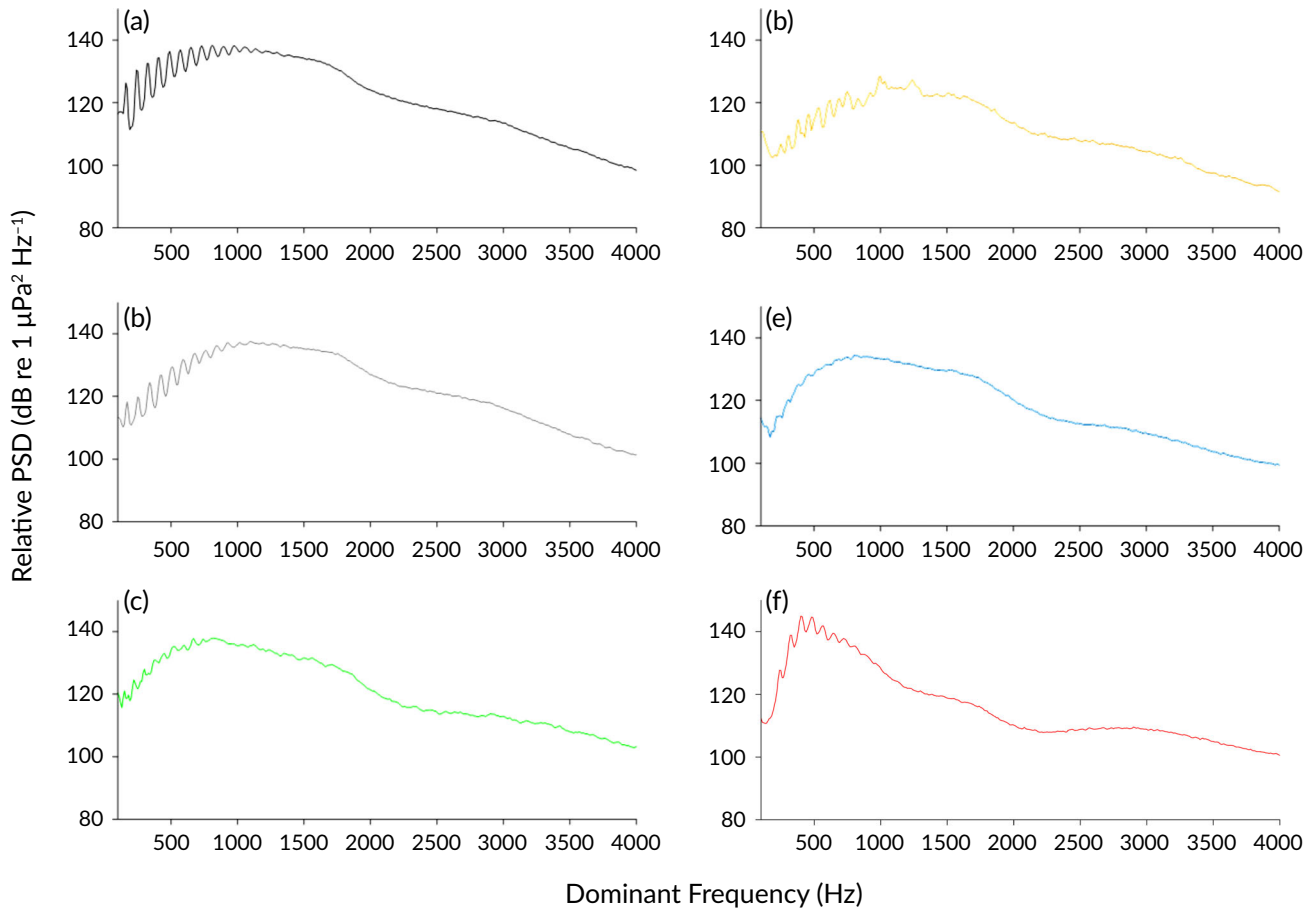
FIGURE 10 Power spectra of dense choruses at (a) Guaporé River, (b) Cautário River, (c) Machado River, (d) Pakaás River, (e) Negro River and (f) Branco River

Piranhas (Serrasalmidae) are another sonic characiform clade with sounds noted in the carnivorous genera *Serrasalmus* Lacépède 1803 and *Pygocentrus* Müller & Troschel 1844 (Markl, 1971; Millot *et al*., 2011; Mélotte *et al*., 2016; Rountree & Juanes, 2018; Mélotte *et al*., submitted). These species have extrinsic sonic muscles (Ladich & Bass, 2005; G.Mélotte, R. Vigouroux, C. Michel & E. Parmentier unpubl. data). The sonic muscles originate on vertebral processes and insert on a broad tendon that surrounds the anterior chamber of the swimbladder ventrally. However, these muscles are hypaxial rather than intercostal (G. Mélotte, R. Vigouroux, C. Michel & E. Parmentier unpubl. data) although they may have had an intercostal origin. Further they occur variably (absent to well-developed) in the three serrasalmid clades appearing to arise as exaptations of locomotory muscles (G.Mélotte, R. Vigouroux, C. Michel & E. Parmentier unpubl. data). Therefore piranha sonic muscles are distinct from those of curimatids and prochilodontids although sonic muscles from the three families have true spinal rather than occipital-spinal innervation (Markl, 1971; Ladich & Bass, 2005; G.Mélotte, R. Vigouroux, C. Michel & E. Parmentier unpubl. data). Variation in characiform sonic species will be a fruitful area for further taxonomic and evolutionary study.

This work uses passive acoustics to support previous findings that mating aggregations of different species occur at the confluences of the main tributaries of the Madeira–Mamore and the lower Guaporé Rivers. In most river confluences, it is possible audibly to detect the occurrence of *P. latior*, commonly found in the Madeira River (Cella-Ribeiro *et al*., 2016). Curimatids are the most abundant fishes (56%) in a 1 km stretch of the black-water tributary (Belmont River) upstream from its mouth near its connection with the Madeira River (Araújo *et al*., 2009).

The mating aggregation in the Machado and Guaporé Rivers occurs inside the mouth and in the plume of those rivers (lentic area), not in the main channel of Madeira and Mamoré Rivers where the flux velocity from these Andean rivers is high and would probably decrease egg fertilisation compared with the slower Guaporé River. In black-water rivers the current velocity averages 0.4 m s^{-1} in the flood season, whereas the Andean rivers average 1.3 m s^{-1} (Barbosa *et al*., 2016). In the Madeira River the channel has a water velocity of 0.9–1.4 m s^{-1}. Therefore, the fish's capacity to maintain swimming speed higher than the water velocity necessitates substantial energy cost (Torrente-Vilara *et al*., 2011). Even so, characiforms are efficient swimmers (Makrakis *et al*. 2011).

Potamorhina latior prefers river confluences for courtship and was the dominant species during the study period based on chorus spectra detected in the river confluences. *S. insignis* shoals occur in the main river channel of the black-water Guaporé River. *Potamorhina latior*
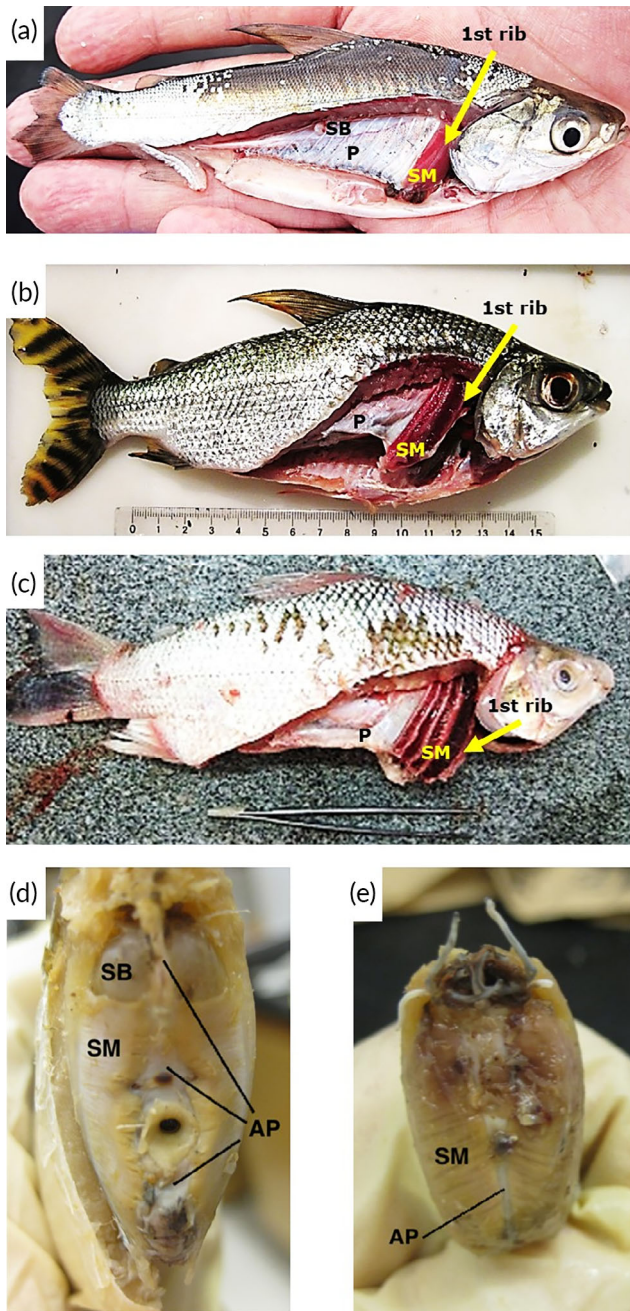
FIGURE 11 Lateral and frontal views of sonic muscles in the male characiforms (a) *Potamorhina latior* (Curimatidae), (b) *S. insignis* and (c) *P. nigricans* (Prochilodontidae) showing the reddish intercostal sonic muscles (SM) and first rib ( ). Also, note the similar structure but different sonic muscle configurations associated with the first rib and the addition of additional rib muscles in *Semaprochilodus insignis* and *Prochilodus nigricans*. A heavy white peritoneum (P) covers the swimbladder (SB). (d) Frontal view of the sonic muscle (SM) apparatus and front of the swimbladder (SB) showing the aponeurosis (AP) in *P. altamazonica* and (e) *P. amazonica*. The lateral views are of fresh material and the frontal views from fixed specimens

and *S. insignis*, produce similar acoustic patterns and these species are found breeding sympatrically in the Guaporé River although there is partial separation of the two. Both the ears of an experienced listener

and spectral similarity indicate that choruses detected in the mouths of the Machado, Negro (with dominant frequency of *c.* 800 Hz in both) and Pakaás Rivers (with a dominant frequency of 1000 Hz) include *P. latior*. It's possible that other species detected in the Negro River could be *Anodus elongatus* Agassiz 1829 (Hemiodontidae), which was the most frequently captured species during the collections in this river for the ichthyofaunal inventory of the Madeira Basin conducted by Federal University of Rondônia (UNIR). No live individuals were obtained for recording disturbance calls, due to their capture by gillnet, but it was observed in the field to have a sonic muscle system associated with the first rib similar to prochilodontids. The family Hemiodontidae is also considered as a sister group to the Prochilodontidae and Curimatidae.

The choruses detected in the present study produced by curimatid and prochilodontid species come from huge aggregations containing hundreds of thousands of individuals during the breeding season (Araujo-Lima & Ruffino, 2003), which based on the larval drift, indicates an annual reproductive period between December and April (Barthem *et al.*, 2014).

Prochilodus spp. male sounds are commonly detected during courtship–spawning behaviour (Sato *et al.*, 2005). *Prochilodus lineatus* form large spawning schools that produce a characteristic snoring sound during reproduction when huge numbers of males can be heard in the Sinos River (Pesoa & Schulz, 2010). Godoy (1975) observed males of the curimbata *P. lineatus* in a stretch of 1.5 km of the Mogi Guassu River emitting a characteristic croaking sound during spawning activity. In captive conditions, *P. argenteus* mature males emit sounds and release seminal fluid when a slight pressure was applied to their ventral region (Sato & Godinho, 2003). Similar to our results, *S. insignis* produce sounds of 300–600 Hz and 300 ms duration by contracting the drumming muscle during the breeding season (Kastberger, 1978). *Prochilodus nigricans* sounds recorded in the present work, are similar to sounds of three Prochilodontidae species (*P. argenteus*, *P. costatus*, *P. lineatus*) recorded in cement ponds, with a series of low frequency pulses, with dominant frequencies usually <500 Hz, pulse period between 29.8–38.3 ms (Smith *et al.*, 2018). There is often an initial rapid rise followed by a slow tapering of pulse amplitude. There was also a significant linear relationship between the dominant frequency of pulses and trains.

This work supports the hypothesis of reproductive aggregations in river confluences proposed by Goulding, 1980 for characiforms, such as *P. latior*, *P. altamazonica* and *P. amazonica*, *S. insignis* and *P. nigricans* since recordings 1 km from confluences were negative for sound choruses. Further quantitative work remains to demarcate the limits of these aggregations. Using passive acoustics, we show the species specificity of fish choruses, which are useful for both species identification and determination of the timing and location of reproduction. Although previous authors have proposed that spawning takes place in the main river channel in the Madeira River, current results indicate that reproductive aggregations occur in sheltered areas of the mouths of tributary rivers and could aid in developing long-term acoustics-monitoring programmes for management of these commercially important species. Since the catchment will be affected by future

hydroelectric dams, quantitative passive acoustic studies have the potential to provide a useful non-invasive tool to characterise baseline distributions of breeding populations for these important species.

ACKNOWLEDGEMENTS

CONTRIBUTIONS

All authors made an equal contribution in this work.

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