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Acoustic homogeneity in the piranha *Serrasalmus maculatus*

Xavier Raick[1] | Rodney Rountree[2,3] | Gregório Kurchevski[4] | Francis Juanes[3] |
Alessia Huby[1] | Alexandre L. Godinho[4] | Éric Parmentier[1]

[1]Laboratory of Functional and Evolutionary Morphology, Freshwater and Oceanic Science Unit of Research, University of Liège, Liège, Belgium

[2]The Fish Listener, East Falmouth, Massachusetts

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

[4]Fish Passage Center, Federal University of Minas Gerais, Belo Horizonte, Brazil

Correspondence
Xavier Raick, Laboratory of Functional and Evolutionary Morphology, Freshwater and Oceanic Science Unit of Research, Bât. B6c - allée du six août, campus du Sart-Tilman, University of Liège, 4000 Liège, Belgium.
Email: xavier.raick@uliege.be

Abstract

Different studies suggest some social calls could be used in fish identification if their specificity is unambiguously assessed. Sounds of different populations of piranhas *Serrasalmus maculatus* Kner, 1858 were recorded to determine their homogeneity between rivers inside a single basin (Araguari and Grande River, upper Paraná River basin) and between separated basins (Amazon and Paraná basins). All fish from the different populations produced sounds with similar acoustic features. Consequently, the populations were not discernible based on individual sound characteristics. This high homogeneity between sounds from different populations indicates their usefulness for conservation projects using passive acoustic monitoring in piranhas. Moreover, it supports the use of acoustic features as complementary key characteristics in taxonomic studies.

KEYWORDS
bioacoustics, characiformes, Pacaya–Samiria, Serrasalmidae, upper Paraná

1 | INTRODUCTION

Many studies have stressed the importance of specific sounds in fish communication because some calls can be used for conspecific identification (Amorim *et al.*, 2011). These calls can be used by biologists in passive acoustic monitoring (PAM) projects to locate and study species in the field (Bolgan *et al.*, 2020; Di Iorio *et al.*, 2018; Rountree & Juanes, 2020). The efficiency of PAM studies relies on the acoustic homogeneity within species. Multivariate statistical procedures, such as principal components analysis (PCA), have been used to differentiate sounds among closely related species collected in the laboratory or in the field, and to assess the call specificity in different taxa (Malavasi *et al.*, 2008; Mélotte *et al.*, 2016; Parmentier *et al.*, 2017, 2019, 2020; Raick *et al.*, 2018; Rountree & Juanes, 2020). However, the extrapolation of the results from a population to the entire species requires assessing the relative homogeneity of the sounds since some acoustic features can be related to different features such as size, age, sex, *etc.*

and have been used to propose geographic dialects in a few species (*e.g.*, Fine, 1978; Parmentier *et al.*, 2009).

Piranhas are endemic to the Neotropics and are widely distributed in all major river basins of South America (Ortí *et al.*, 1996). Within the taxa, *Serrasalmus* and *Pygocentrus* species produce low-frequency harmonic sounds (Kastberger, 1981; Markl, 1971; Mélotte *et al.*, 2016, 2019; Raick *et al.*, 2020; Rountree & Juanes, 2020) that are thought to be species-specific (Mélotte *et al.*, 2016; Raick *et al.*, 2020; Rountree & Juanes, 2020). *Serrasalmus maculatus* Kner, 1858 is found in the Amazon, Paraná-Paraguay-Uruguay and Tocantins Rivers basins (Bignotto *et al.*, 2020; Jégu, 2003; Zaniboni *et al.*, 2004). The watersheds of the Amazon and Paraná Rivers are separated by the Azimuth 125° lineament (Ribeiro *et al.*, 2018). Uplift associated with intrusive magmatism in the Late Cretaceous (91–72.4 Ma) explains the origin and separation of both basins (Ribeiro *et al.*, 2018). However, sporadic contacts between both basins may occur, for example in Mato Grosso where the Aguapehy River (Paraná-Paraguay-Uruguay basin) and the Alegre River (Amazon basin) are

separated by a distance of only 1 km (Soldano, 1947). The wide geographic distribution for a single species probably explains why it is a species with a high intraspecific genetic variability (Bignotto *et al*., 2019, 2020). In addition, this species has been confused in the past with *Serrasalmus spilopleura* Kner, 1858 (Jégu & Dos Santos, 2001). The two species can be differentiated by the size of the infraorbital series bones (narrower in *S. spilopleura*), the cheek zone (broader in *S. spilopleura*) and the coloration of the caudal fine (Jégu & Dos Santos, 2001). *Serrasalmus maculatus* has a final or subterminal black bar in the caudal fin while *S. spilopleura* has a caudal fin which is two thirds dark, while the last third is hyaline (Jégu & Dos Santos, 2001).

The wide geographic distribution for a single species is an appropriate way to test the specific homogeneity of its calls. Consequently, the aim of this study was to compare the sounds of *Serrasalmus maculatus* produced by (a) specimens from two different rivers of the same drainage basin (Paraná River basin) and (b) specimens from two different drainage basins (Paraná and Amazon River basins).

2 | MATERIALS AND METHODS

2.1 | Biological material

A total of 49 specimens was sampled with hook-and-lines or gill nets. Five specimens were captured in the Araguari River (Paraná River basin, Brazil) downstream of the Amador Aguiar Dam (18°46'42''S, 48°9'57''W) with authorization from the Consórcio Capim Branco,

FIGURE 1 Map of Paraná-Paraguay and Amazon basins in South America. Rivers shown in blue. Red circles are sampling sites

and 35 in the Grande River (Paraná River basin, Brazil) in the Volta Grande Reservoir (20°10'53''S, 48°6'28''W) in June 2018 (Figure 1). Nine specimens were sampled in July 2012 at the confluence of the Marañón and Ucayali Rivers (Amazon River basin, Peru) in the Pacaya-Samiria National Reserve (4°53'53''S, 74°20'56''W) as part of the international Operation Wallacea program (Figure 1). For each specimen, the sounds were recorded and the standard length (SL) was measured. The experiment was approved by the ethical commission of the University of Liège (case 1532) and the capture of the Brazilian specimens was achieved under a licence from the Brazilian Ministry of the Environment (number 9722-1, solicitation number 10306.

2.2 | Recordings and sound analysis

Sounds of Paraná River basin specimens were recorded in a glass-tank (108 l) with a hydrophone (HTI-96-Min, High Tech Inc., Long Beach, MS, USA; sensitivity –164.4 dB re 1 V μPa^{-1}) connected to a TASCAM DR5 recorder (TEAC Corporation, Montebello, CA, USA), as described in Raick *et al*. (2020). Raw data from Amazon River basin specimens were taken from Rountree and Juanes (2020) and reanalysed to avoid bias. These specimens were recorded in the river with a hydrophone (SQ26-08, Cetacean Research Technology, Seattle, WA, USA; sensitivity: –169.0 dB re 1 V μPa^{-1}) connected to a SQ26-H1 recorder (Cetacean Research Technology), as described in Rountree and Juanes (2020). All 49 specimens made sounds when gently hand-held underwater. A total of 470 sounds, with a maximum of 10 sounds per specimen, was analysed. Sound features were averaged per specimen to avoid pseudoreplication. The following features were measured: the sound duration (d, in ms), the number of peaks in a sound (N, unitless), the period (p, in ms), the duration of the energetic zone, *i.e*., the continuous section of a sound with a maximum intensity of 3 dB less than the maximum intensity peak (Raick *et al*., 2020) (d_{EZ}, in ms), the number of peaks in the energetic zone (N_{EZ}, unitless), the percentage of peaks from the energetic zone (%N_{EZ}, unitless) and the period between peaks in the energetic zone (p_{EZ}, in ms). The frequential parameters were measured on the power spectra of the sounds: the fundamental frequency (F_0, in Hz), the frequency corresponding to the maximum intensity (F_{AmpMax}, in Hz), frequency quantiles (F_{Q1}, F_{Q2} and F_{Q3}) and the minimum frequency (F_{min}, in Hz), measured as the lower frequency value of the frequencies corresponding to a maximum intensity of 30 dB.

2.3 | Statistics

Correlations between SL and acoustic features were calculated with Spearman correlation coefficients (ρ) with the family-wise error rate controlled by the Holm method. Linear regressions of the features correlated with SL were performed to remove the effect of size. Wilcoxon–Mann–Whitney tests (abbreviated Wilcoxon) were used to test differences between specimens from the Araguari and Grande Rivers, and between Peruvian and Brazilian individuals. Principal

components analysis (PCA) on the correlation matrices with all the acoustic features was used to reduce the multivariate aspect of the data. In addition, as a measure of homogeneity, the ratio between the intersite coefficient of variation (CVb) and the intrasite coefficient of variation (CVi) was calculated. A low CVb/CVi ratio is indicative of low homogeneity while a high ratio is indicative of high homogeneity (Parmentier *et al*., 2018). CVi was calculated from the sounds produced at each site while CVb was calculated on means per site. They were calculated for each acoustic feature. All the statistics were carried out with R 3.3.0. (GNU General Public License). The significance level (α) was 0.05.

3 | RESULTS

All the studied specimens produced similar sounds (Figure 2a,b). Specimens from the Araguari River emitted sounds that were 121 ± 16 ms (mean ± S.D.) long with a fundamental frequency of 82 ± 4 Hz, 9.5 ± 1.3 peaks and a peak period of 13 ± 1 ms. Specimens from the Grande River emitted sounds that were 105 ± 14 ms long with a fundamental frequency of 84 ± 10 Hz, 8.4 ± 9.5 peaks and a peak period of 13 ± 1 ms. Finally, specimens from the Amazon River emitted sounds that were 56 ± 21 ms long with a fundamental frequency of 159 ± 28 Hz, 9.0 ± 2.6 peaks and a peak period of 6 ± 1 ms.

All the specimens from the Araguari River were compared with those from the Grande River. In Brazilian specimens, *S. maculatus* populations from the Araguari River (n = 5) and the Grande River (n = 35) did not statistically differ in SL (Wilcoxon: W = 47, P = 0.11). The majority of the acoustic features (F_0, F_A, F_{min}, P, P_{EZ}, d_{EZ}, N, N_{EZ}, %N_{EZ}) were statistically equivalent (Wilcoxon: W = 74, 135, 107, 98, 106, 115, 124, 103, 76; P = 0.59, 0.054, 0.44, 0.69, 0.47, 0.27, 0.14, 0.54, 0.65; Figure 3b), while others were not (Wilcoxon: W = 144, 142, 148, 145; P = 0.02, 0.03, 0.01, 0.02 for d, F_{Q1}, F_{Q2} and F_{Q3}). The PCA did not show any differences for PC1 (49.66%) and PC2 (23.32%) between the Brazilian populations (Wilcoxon: W = 130 and 70; P = 0.09 and 0.50). The CVb/CVi ratios were very close to 1 (1.11 ± 0.14, mean ± S.D.), indicating a similar variability between and within the two rivers.

The size of the 40 specimens sampled in Brazil was higher (range 113–283 mm, mean: 209 mm, Wilcoxon: W = 6, P < 0.0001) than the nine specimens from Peru (range 70–130 mm, mean
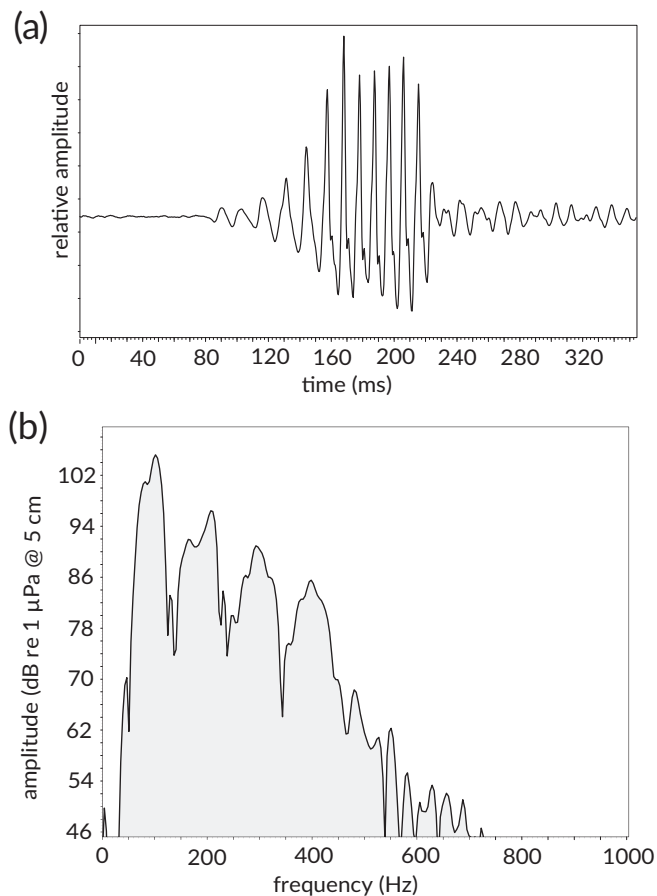
FIGURE 2   (a) Oscillogram of a sound of *Serrasalmus maculatus* from the Grande River subsampled at 4 kHz. (b) Logarithmic power spectrum of the same sound (evaluation window: Hamming)
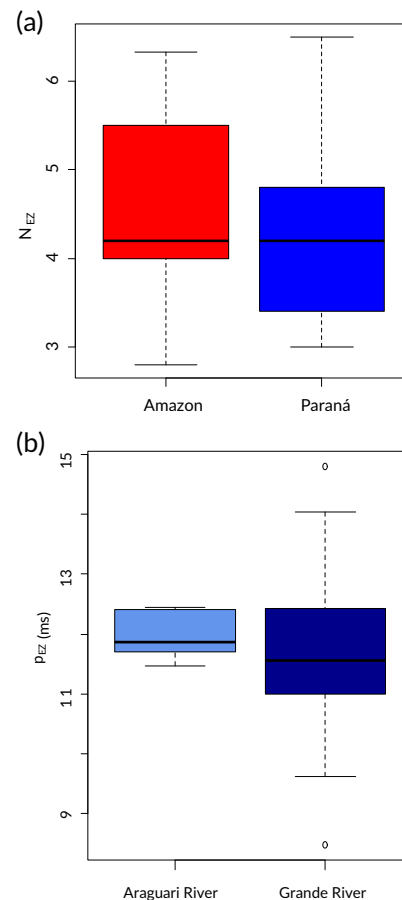
FIGURE 3   (a) Boxplot of the number of peaks in the energetic zone (N_{EZ}) for specimens from the Amazon River basin and the smallest specimens from the Paraná River basin. (b) Boxplot of the peak period in the energetic zone (p_{EZ}) for specimens from the Araguari River and the Grande River (both in the Paraná River basin)
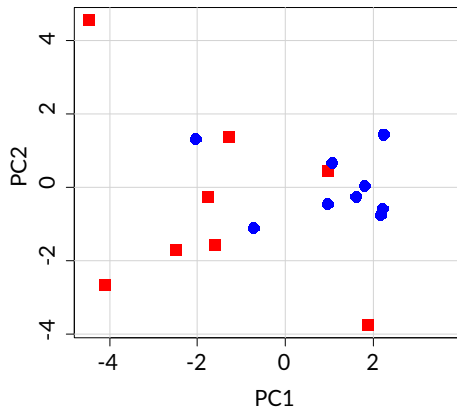
FIGURE 4 Scatterplot of the principal components (PCs) for specimens from the Amazon River basin and the smallest specimens from the Paraná River basin (■) Amazon, (●) Paraná

94 mm). Therefore, the nine Peruvian specimens were compared with the nine smallest Brazilian specimens (from both rivers). In both Peruvian and Brazilian individuals, some features were correlated with SL (d, F_0, F_{AmpMax}, F_{Q1}, F_{Q2}, F_{Q3}, F_{min}, p, p_{EZ}) while others were not (d_{EZ}, N, N_{EZ}, %N_{EZ}). All the features that were not correlated with the SL were not significantly different between the basins (Wilcoxon: W = 27, 36, 45, 59, P = 0.26, 0.72, 0.72, 0.11; Figure 3a). The effect of size was removed from the remaining acoustic features using regression to compare residuals between specimens from both basins. All residuals of d, F_0, F_{AmpMax}, F_{Q1}, F_{Q2}, F_{Q3}, F_{min}, p and p_{EZ} were not significantly different between Peruvian and Brazilian fish (Wilcoxon: W = 21, 59, 50, 50, 60, 57, 48, 21, 28; P = 0.09, 0.11, 0.44, 0.44, 0.09, 0.16, 0.55, 0.09, 0.30). A PCA was conducted with d_{EZ}, N, N_{EZ}, %N_{EZ} and the residuals of the remaining acoustic features (Figure 4). PC1 and PC2 were equivalent between Peruvian and Brazilian individuals (Wilcoxon: W = 21 and 36; P = 0.09 and 0.73). The CVb/CVi ratios very close to 1 (1.15 ± 0.16, mean ± S.D.) indicated a high similarity between CVb and CVi, *i.e*., a similar variability between and within basins.

4 | DISCUSSION

The acoustic features of *S. maculatus* did not vary among populations distributed across a large geographic area in South America. This high homogeneity of acoustic features within the species shows species-specific acoustic features can be a useful tool for species identification in PAM studies focusing on piranha species. We suggest using a combination of acoustic features as a complementary tool to describe vocal species. In the case of *S. maculatus*, fish having a size of 70–283 mm produce sounds that were made of 8.7 ± 1.5 peaks (mean ± S.D.) with a peak period of 11 ± 3 ms, giving a total duration of 97 ± 24 ms with a fundamental frequency of 99 ± 32 Hz. This dataset could also be useful to distinguish this species from other piranha species. For example, *S. spilopleura*, which might be phenotypically confused with *S. maculatus* in the field (Jégu & Dos Santos, 2001;

Rountree & Juanes, 2020), produces sounds that are made of 10 ± 2 peaks with a peak period of 6 ± 0.4 ms, giving a total duration of 73 ± 18 ms long with a fundamental frequency of 149 ± 12 Hz (based on seven specimens, SL 79 ± 4 mm) (Mélotte *et al*., 2016). When comparing specimens of similar size, *S. maculatus* seems to produce sounds with a shorter duration and fewer peaks compared to *S. spilopleura* sounds, as described by Mélotte *et al*. (2016). Although this size effect is quite low in comparison to other teleost species such as clown fishes, for example (Colleye *et al*., 2009), the sounds produced by the different species of piranha are still affected by fish length (Mélotte *et al*., 2016; Raick *et al*., 2020; Rountree & Juanes, 2020). PAM studies need to take into account the size of the target specimens or to examine in more detail the acoustic features that are not correlated to size. This homogeneity is essential to formalize their use in PAM and to be able to distinguish the target species. Several studies in marine environments have shown long-term consistency (10–17 years) in the acoustic features of fish from the same geographic areas, including the brown meagre *Sciaena umbra* Linnaeus, 1758 (Parmentier *et al*., 2018) and Atlantic cod *Gadus morhua* Linnaeus, 1758 (Caiger *et al*., 2020), further supporting the usefulness of PAM for fishes.

CONFLICT OF INTEREST

The authors declare no competing interest.

AUTHOR CONTRIBUTIONS

X.R. designed the study. E.P., X.R., A.H., A.G. and G.K. planned and organized the field mission in Brazil. X.R., G.K. and A.H. performed the field mission. X.R. recorded the sounds in Brazil. R.R. and F.J. provided the data from Peru. X.R. analysed and interpreted the data. X.R. wrote the manuscript with inputs from E.P. and R.R. The manuscript was corrected and implemented by A.H., A.G., F.J. and G.K. All authors gave final approval for publication.

ORCID

Xavier Raick https://orcid.org/0000-0002-1977-0289

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