REGULAR PAPER

Acoustic homogeneity in the piranha *Serrasalmus maculatus*

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Keywords: Characiformes, Serrasalmidae, Bioacoustics, Pacaya–Samiria, upper Paraná

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 acoustic features could be used as complementary key characteristics in taxonomic studies.

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 explain 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 however 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 (1) by specimens from two different rivers of the same drainage basin (Paraná River basin) and (2) by specimens from two different drainage basins (Paraná and Amazon River basins).

2 MATERIALS AND METHODS

2.1 Biological material

A total of 49 specimens were sampled with hook-and-lines or gill nets. Five of them 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 authorisation from the Consórcio Capim Branco, 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 (Fig. 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 (Fig. 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.

Fig. 1. Map of Paraná-Paraguay and Amazon basins in South America. In blue: rivers. In red: sampling sites.

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., USA; sensitivity: –164.4 dB re 1V μPa^{-1}) connected to a TASCAM DR5 recorder (TEAC Corporation, USA) as described in Raick *et al.* (2020). Raw data from Amazon River basin specimens were taken from Rountree & Juanes (2020) and reanalysed to avoid bias. These specimens were recorded in the river with a hydrophone (SQ26-08, Cetacean Research Technology, USA; sensitivity: –169.0 dB re 1V μPa^{-1}) connected to a SQ26-H1 recorder (Cetacean Research Technology, USA) as described in Rountree & Juanes (2020). All 49 specimens made sounds when gently hand-held underwater. A total of 470 sounds, with a maximum of ten sounds per specimen, were 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), ($\mathbf{d_{EZ}}$, in ms); the number of peaks in the energetic zone ($\mathbf{N_{EZ}}$, unitless); the percentage of peaks from the energetic zone ($\mathbf{\%N_{EZ}}$, unitless); and the period between peaks in the energetic zone ($\mathbf{p_{EZ}}$, in ms). The frequential parameters were measured on the power-spectra of the sounds: the fundamental frequency ($\mathbf{F_0}$, in Hz); the frequency corresponding to the maximum intensity ($\mathbf{F_{AmpMax}}$, in Hz); frequency quantiles ($\mathbf{F_{Q1}}$, $\mathbf{F_{Q2}}$ & $\mathbf{F_{Q3}}$) and the minimum frequency ($\mathbf{F_{min}}$, in Hz), measured as the lower frequency value of the frequencies corresponding to an intensity of "maximum intensity − 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, were used to reduce the multivariate aspect of the data. In addition, as a measure of homogeneity, the ratio between the inter-site coefficient of variation (CVb) and the intra-site 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 (Fig. 2A and 2B). Specimens from Araguari River emitted sounds that were 121 ± 16 ms (mean ± standard deviation) long with a fundamental frequency of 82 ± 4 Hz, 9.5 ± 1.3 peaks and a peak period of 13 ± 1 ms. Specimens from 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 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.

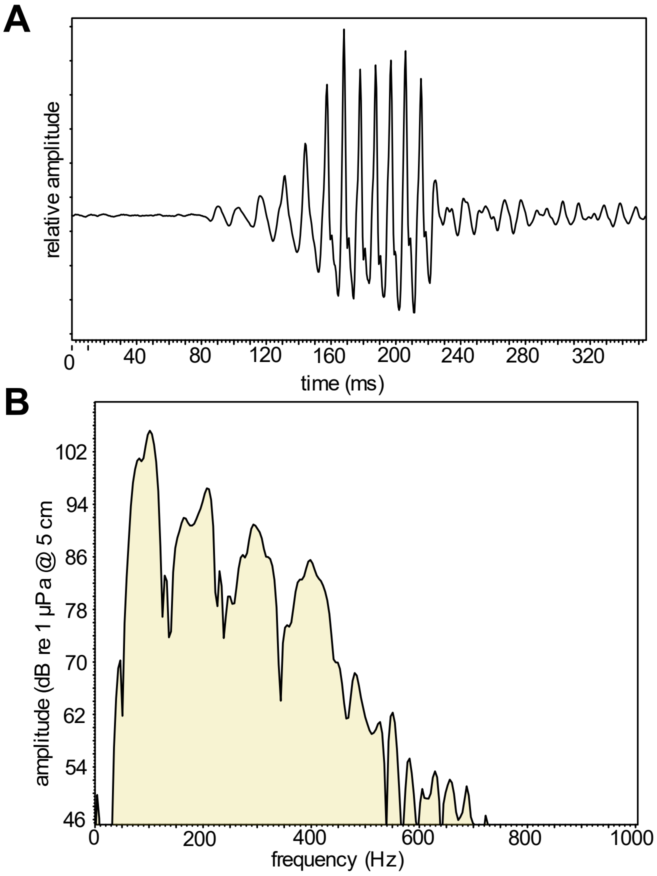

Fig. 2A. Oscillogram of a sound of *Serrasalmus maculatus* from the Grande River subsampled at 4 kHz. **B**. Logarithmic Power spectrum of the same sound (evaluation window: Hamming).

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 ($\mathbf{F_0}$, $\mathbf{F_A}$, $\mathbf{F_{min}}$, **P**, $\mathbf{P_{EZ}}$, $\mathbf{d_{EZ}}$, **N**, $\mathbf{N_{EZ}}$, $\mathbf{\%N_{EZ}}$) 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; Fig. 3B), while others were not (Wilcoxon: W = 144, 142, 148 & 145; *P* = 0.02, 0.03, 0.01 & 0.02 for **d**, $\mathbf{F_{Q1}}$, $\mathbf{F_{Q2}}$ & $\mathbf{F_{Q3}}$). The PCA did not show any differences for PC1 (49.66%) and PC2 (23.32%) between the Brazilian populations (Wilcoxon: W = 130 & 70; *P* = 0.09 & 0.50). The CVb/CVi ratios were very close to 1 (1.11 ± 0.14, mean ± SD) 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 9 specimens from Peru (range: 70 – 130 mm, mean: 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**, $\mathbf{F_0}$, $\mathbf{F_{AmpMax}}$, $\mathbf{F_{Q1}}$, $\mathbf{F_{Q2}}$, $\mathbf{F_{Q3}}$, $\mathbf{F_{min}}$, **p** & $\mathbf{p_{EZ}}$) while others were not ($\mathbf{d_{EZ}}$, **N**, $\mathbf{N_{EZ}}$, $\mathbf{\%N_{EZ}}$). 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; Fig.3A). Then, the effect of size from the remaining acoustic features was removed using regression to compare residuals between specimens from both basins. All residuals of **d**, $\mathbf{F_0}$, $\mathbf{F_{AmpMax}}$, $\mathbf{F_{Q1}}$, $\mathbf{F_{Q2}}$, $\mathbf{F_{Q3}}$, $\mathbf{F_{min}}$, **p** & $\mathbf{p_{EZ}}$ 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 $\mathbf{d_{EZ}}$, **N**, $\mathbf{N_{EZ}}$, $\mathbf{\%N_{EZ}}$ and with the residuals of the remaining acoustic features (Fig. 4). PC1 and PC2 were equivalent between Peruvian and Brazilian individuals (Wilcoxon: W = 21 & 36; *P* = 0.09 & 0.73). The CVb/CVi ratios very close to 1 (1.15 ± 0.16, mean ± SD) indicated a high similarity between CVb and CVi, i.e. a similar variability between and within basins.

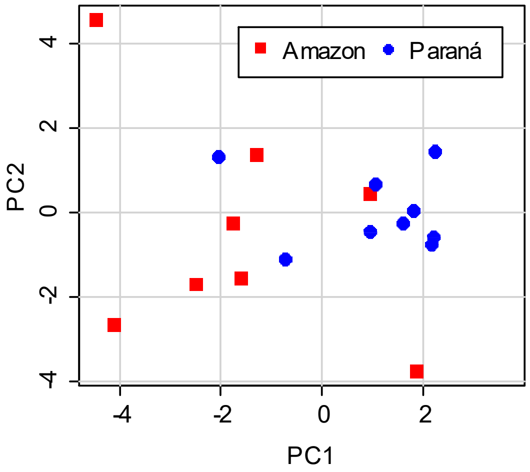

Fig. 4. Scatterplot of the Principal Components (PCs) for specimens from the Amazon River basin and the smallest specimens from the Paraná River basin.

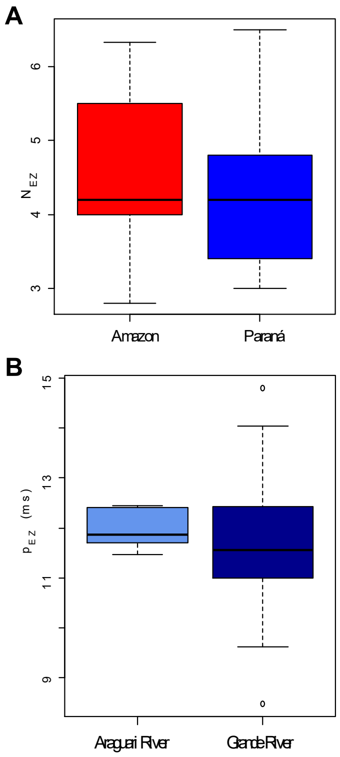

Fig. 3A. Boxplot of the number of peaks in the energetic zone (**N_{EZ}**) for specimens from the Amazon River basin and the smallest specimens from the Paraná River basin. **B**. Boxplot of the peak period in the energetic zone (**p_{EZ}**) for specimens from the Araguari River and the Grande River (both in the Paraná River basin).

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 ± standard deviation) with a peak period of 11 ± 3 ms, giving a total duration of 97 ± 24 ms long with a fundamental frequency of 99 ± 32 Hz. This dataset could also be useful to distinguish this species from others 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 smaller duration and less 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 formalise 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.

Acknowledgements

The authors thank the FRS-FNRS and the King Leopold III Fund for Nature Exploration and Conservation for their financial support and Antônio Bezerra da Silva for his help in Grande River.

Conflict of interest

The authors declare no competing interest.

Funding

This work was supported by the Fonds de la Recherche Scientifique FRS-FNRS (2018/V 3/5/199 - JG/MS – 2718, 2018/V 3/5/200 - JG/MS – 2717 and PDR no. 23625340) and the King Leopold III Fund for Nature Exploration and Conservation (2018/1.12.).

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Author contributions

X.R. designed the study. E.P., X.R., A.H., A.G., G.K. planned and organised the field mission in Brazil. X.R., G.K. & A.H. performed the field mission. X.R. recorded the sounds in Brazil. R.R. & 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. & G.K. All authors gave final approval for publication.