Yellow-eyed piranhas produce louder sounds than red-eyed piranhas in an invasive population of *Serrasalmus marginatus*

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SIGNIFICANCE STATEMENT

Serrasalmus marginatus is a piranha species invasive in the upper Paraná River basin. The aim of this study was to investigate if their sounds could be used to distinguish two morphotypes: red and yellow-eyes *S. marginatus*. All the temporal and frequency features of the sounds were equivalent in both groups. However, the amplitude features were all different.

ABSTRACT

Serrasalmus marginatus is a piranha species native from the lower Paraná River basin and invasive in the upper Paraná River basin since the 1980s. In piranhas, sounds of different species have different features. The aim of this study was to investigate if the sounds produced by this species could be used to distinguish two morphotypes: red and yellow-eyes *S. marginatus* from the Araguari River (upper Paraná River basin). All the temporal and frequency features of the sounds were equivalent in both groups of eye colour; it corresponds to the species-specific signature described for *S marginatus*. However, the amplitude features were all statistically different between red and yellow eyes piranhas. Yellow-eyes specimens produced louder sounds. In different fish species, colour change in eyes can be due to the absence or the presence of a dominant allele. It can also be involved in social rank or during reproduction. Different hormones and neuropeptides can modulate vocal features. We hypothesize that a mutation or different hormonal concentrations could explain both sound amplitude and eye colour playing a role in animal communication in *S. marginatus*.

INTRODUCTION

North Brazil hydrography is dominated by the well-known Amazon River, the world's largest river on the basis of the average discharge at its mouth (Meade *et al.*, 1991). The south of the country houses the second largest river of South America, the Paraná River (Bonetto *et al.*, 1986), with a length of 4,000 km and an annual discharge of 500 million m^3 (Bonetto *et al.*, 1986). This river has allowed the largest human and industrial development of all South America (Bonetto *et al.*, 1986) and the highest concentration of large dams in the continent (Agostinho *et al.*, 2004). The construction of the Itaipu Dam in the 1980s, one of the biggest dam in the world, flooded the Sete Quedas Falls, a natural geographical barrier for fish on the waterway (Agostinho *et al.*, 1994).

The piranha *Serrasalmus marginatus* Valenciennes, 1837 (Serrasalmidae, Characiformes) was previously restricted to the Paraná River downstream from Sete Quedas Falls but now it has invaded the upper Paraná River basin (Agostinho & Júlio, 2002) resulting in a decrease of native *S. maculatus* (Agostinho & Júlio, 2002). After the first record in the invaded area in 1986, *S. marginatus* showed an increase in abundance, becoming the second most captured invasive species in the upstream floodplain (Tonella *et al.*, 2018). The success of *S. marginatus* could be explained by its aggressiveness in establishing feeding territories (Agostinho, 2003; Agostinho *et al.*, 2003) long reproductive period and offspring defence (Melo *et al.*, 2017). Recent studies showed that trophic segregation allows the co-existence of the native and the invasive species (Alves *et al.*, 2017; Rodrigues *et al.*, 2018).

Many species have evolved calls that are usually species-specific (Parmentier *et al.*, 2009; Raick *et al.*, 2018; Borie *et al.*, 2019). These specific features were already used to discriminate species within Serrasalmidae (Mélotte *et al.*, 2016, 2019; Raick *et al.*, 2020; Rountree & Juanes, 2020). And, as many other carnivorous piranhas, *S. marginatus* also produces sounds (Mélotte *et al.*, 2016). During a field work in the Araguari River, in the upper Paraná River, we

observed *S. marginatus* specimens having red or yellow eyes and living in sympatry. The aim of this study was to investigate if sounds could be used to distinguish both morphotypes, i.e. do red and yellow-eyes *S. marginatus* produce different kinds of sounds?

MATERIALS AND METHODS

Biological material

Fifty-five specimens of *Serrasalmus marginatus* have been captured with gillnets and by hook-and-line in July 2018 in the Araguari River downstream from the Amador Aguiar II Hydroelectric Dam (Paraná River basin, Brazil; 18°39′37.41″ S, 48°26′19.94″ W). For all individuals, we determined the sex and measured the standard length (SL, in mm) and the body height (BH, in mm), and the body mass (BM, in g) with a spring scale (Supporting Figure 1). The experiment was approved by the ethical commission of the University of Liège (case 1532) and the capture has been realized under the licence 10306-1 from the Brazilian ministry of the environment and with an authorisation from Consórcio Capim Branco Energia. Six fixed specimens are available at the Aquarium-Museum of the University of Liège (vouchers R.E. 15277, 15278, 15279, 15280, 15281 and 15282).

Recordings and sound-analysis

For each individual, the sounds were recorded in a glass-tank of 108 L, with a hydrophone (HTI-96-Min, High Tech Inc., USA; sensitivity: −164.4 dB re 1 V μPa^{-1}) placed in the middle of the aquarium and connected to a recorder TASCAM DR5 (TEAC Corporation, USA). The water temperature of the aquarium was 26 ± 2 °C. The resonant frequency was 3252 Hz (for details on recordings in tanks, see (Akamatsu *et al.*, 2002). The fish were gently hand-held between the thumb and the index of the left hand without any pressure at approximatively five centimetres from the hydrophone. The sounds were digitalized on mono-channel at 44.1 kHz with a 16 bit-resolution and then they were sub-sampled at 4000 Hz and a high-pass filter was

applied to reduce noise below 50 Hz. The analysis was carried out with Avisoft SAS-Lab Pro 5.2 (Avisoft Bioacoustics, Germany). Ten sounds were analysed per specimen.

The following features were measured on oscillograms (Figure 1a): the sound duration (d, in ms); the number of peaks in a sound (N); the peak period (p, in ms); the maximal amplitude (Amp_{Max}, in dBre 1 µPa @ 5 cm); the duration of the energetic zone (d_{EZ}, in ms), i.e. the continuous area with an intensity of maximum 3 dB less than the maximum intensity peak (see Figure 1a); the number of peaks in the energetic zone (N_{EZ}, without unit) and the peak period inside the energetic zone (p_{EZ}, in ms). The following frequency parameters were measured on the power-spectra of the sounds (Figure 1b): the fundamental frequency (F_0, in Hz); the frequency corresponding to the maximal intensity (F_{AmpMax}, in Hz); the amplitude corresponding to the F_0 and the F_{AmpMax} frequency ($AmpF_0$ & $AmpF_{AmpMax}$, in dBre 1 µPa @ 5 cm).
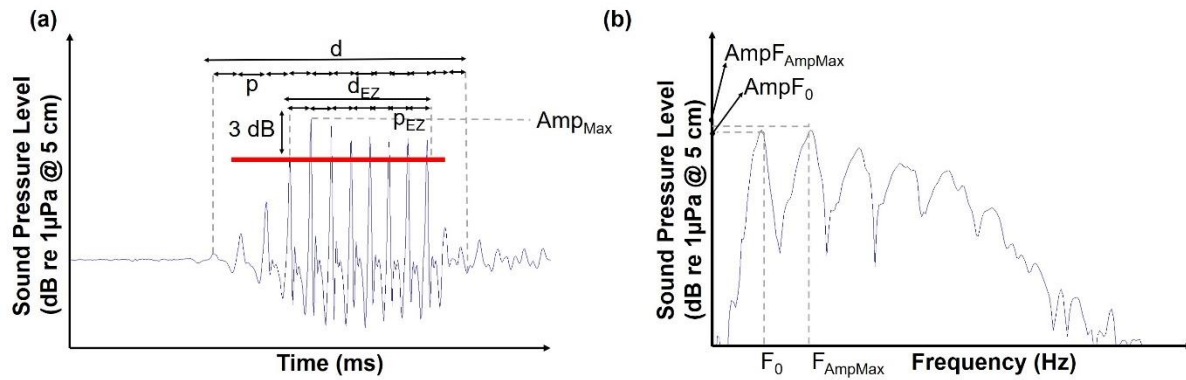

Figure 1 (a) Oscillogram and **(b)** power spectrum of a sound of *Serrasalmus marginatus* with the following measured features: d = duration, d_{EZ} = duration of the energetic zone, p = period, p_{EZ} = period of the energetic zone, N = number of peaks, N_{EZ} = number of peaks in the energetic zone, F_0 = fundamental frequency, $AmpF_0$ = amplitude of the fundamental frequency, F_{AmpMax} = frequency corresponding of the maximal intensity, $AmpF_{AmpMax}$ = amplitude of the frequency corresponding to the maximal intensity.

Statistics

All the measured features have been averaged per specimen to avoid pseudoreplication. A correlation matrix for all parameters has been calculated with Spearman correlation coefficients (r_s) with its associated P-value matrix corrected by the Holm method. Differences between sexes and morphotypes have been tested with Wilcoxon-Mann-Whitney tests. To test differences between sexes, the juvenile (n = 1) has been removed and only mature females (n = 21) and males (n = 32) have been kept. As the number of fish of each morphotype was quite different (11 with yellow eyes *vs* 43 with red eyes), the tests between morphotypes have been realized on five random subsamples of 22 specimens (11 of each morphotype) and the medians of Wilcoxon-Mann-Whitney statistics (W) and p-values (P) are presented. Only one aberrant value out of the 8,250 data (i.e. 15 acoustic features for 550 sounds) has been discarded. To reduce the multivariate aspect of the data, a principal component analysis (PCA) was used with all the acoustic features. In addition, a cross-validated linear discriminant analyses (LDA) with a holdout cross-validation was carried out, on previously standardised features (mean = 0, SD = 1), to discriminate the two morphotypes. The fraction of data kept for training was 66.6% and 50 random permutations have been carried out. All the statistics were carried-out with R 3.6.1. (GNU General Public License) with a significant level (α) of 0.05.

RESULTS

In the Araguari River, 550 sounds from 55 specimens of *S. marginatus* were recorded. Their standard length (SL) ranges from 134 to 225 mm (169 ± 17 mm, mean ± SD), in females from 151 to 208 mm (170 ± 12 mm) and in males from 134 to 225 mm (168 ± 19 mm). These sounds were 122 ± 21 ms (mean ± standard deviation) long with a fundamental frequency of 90 ± 7 Hz, 10 ± 2 peaks and a peak period of 12 ± 1 ms. In a sound sequence, the distance between two sounds was 1.70 ± 0.41 s. The only acoustic features that were correlated with the SL were the F_0, F_{AmpMax} ($r_s < -0.50$; P < 0.01), **p** and p_{EZ} ($r_s > 0.50$; P < 0.005).

Some fish had red eyes (n = 43) while others had yellow eyes (n = 11). The method of capture was consistent, i.e. there were red and yellow-eyes specimens captured by both net and hook and line. The difference in eye colour was not related to sex since there were both males and females for specimens of each type. Moreover, the SL, BM, BH and all the acoustic features (both examined for each feature separately and with the three first PCs of a PCA, Figure 2a) were statistically equivalent between the two sexes (Wilcoxon: $235 <$ all $W < 402$; all $P > 0.05$). Eye colour was not statistically related to the fish size as SL, BM and BH were similar in both morphotypes (Wilcoxon: $W = 46$, 48 & 42; $P = 0.36$, 0.43 & 0.22). The SL ranges from 148 to 183 mm (163 ± 12) in yellow-eyes specimens and from 134 to 225 mm (171 ± 18 mm) in red-eyes specimens.
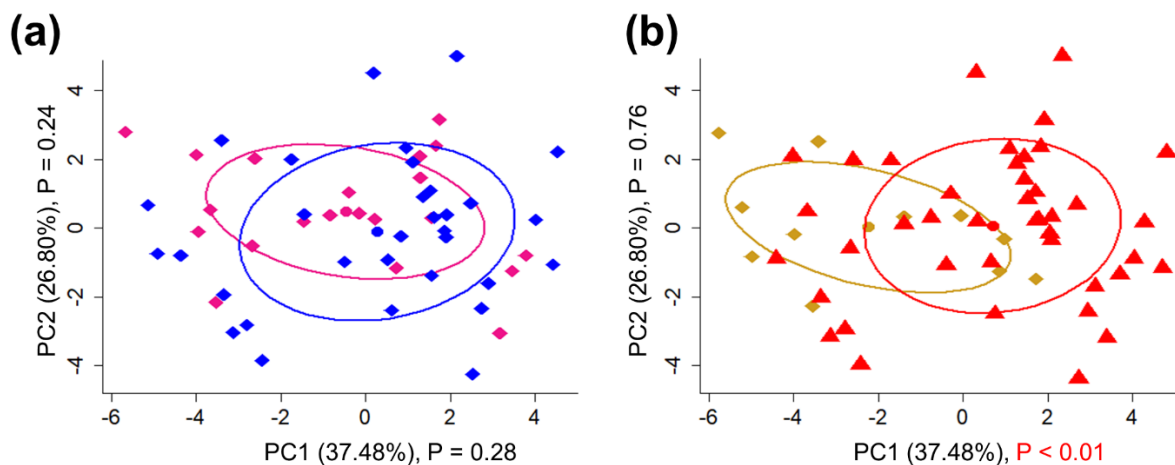

Figure 2 Scores of the principal component analysis on all the adults *Serrasalmus marginatus* with **(a)** males (in blue) and females (in pink) and **(b)** red-eyes (in red) and yellow-eyes (in golden) specimens with concentration ellipses of 50% for each group. P-values from Wilcoxon tests are indicated for each principal component (PC). Significative P-values are in red.

We observed that the temporal (**d**, **d$_{EZ}$**, **N**, **N$_{EZ}$**, **p** and **p$_{EZ}$**) and frequency features (**F$_0$**, **F$_{AmpMax}$**) were equivalent in both groups of eye colour (Wilcoxon: $37 < W < 82$; all $P > 0.05$; Figure 3). However, the amplitude features (**AmpF$_0$**, **AmpF$_{AmpMax}$**, **Amp$_{Max}$**) were all statistically

different between red and yellow eyes piranhas (Wilcoxon: W = 93, 95 & 101; P = 0.03, 0.02 & 0.007; Figure 3). Yellow-eyes specimens produced louder sounds.
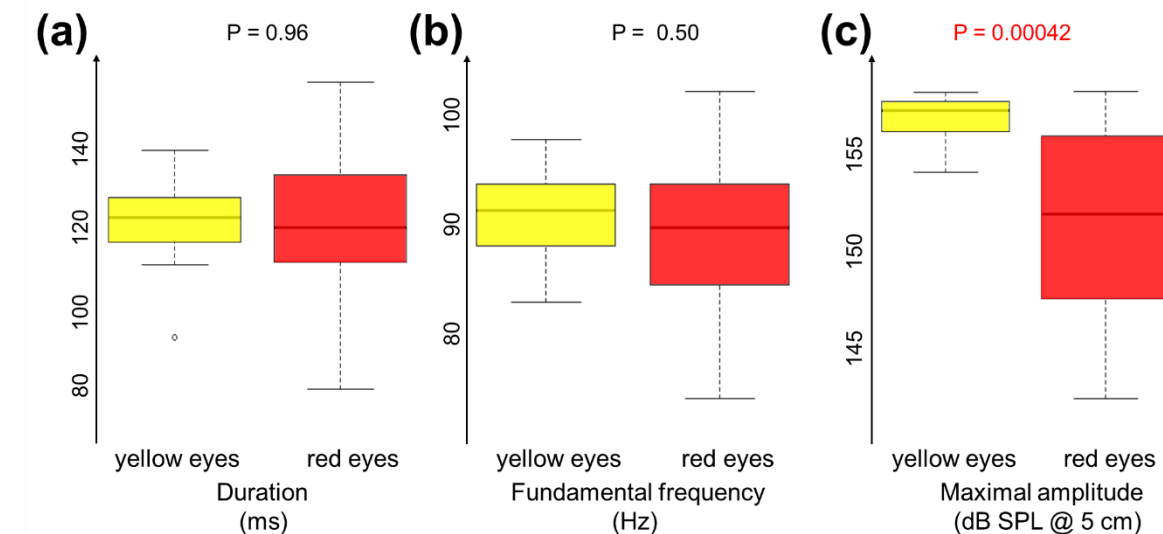

Figure 3 Comparison (median, interquartile range (IQR) and 1.5*IQR) of three acoustic features between all red and yellow-eyes specimens of *Serrasalmus marginatus*. Significative P-value is in red.

According to the PCA on all the features, PC1 (39.9 ± 3.7 %) was correlated to all the amplitude features (all $P < 0.005$). At the contrary, PC2 ($29.3 \pm 1.4\%$, $69.2 \pm 2.4\%$ cumulative) was only correlated to all other sonic features (all $P < 0.05$). PC1 separated both morphotypes (Wilcoxon: W = 92; P = 0.04) while it was not the case for PC2 (Wilcoxon: W = 70; P = 0.33; Figure 2b). When performing an LDA on all the sounds, sounds were associated with the correct group, with an overall correct classification rate of 82.15% with a better classification rate for the red-eyes specimens (93.94%).

DISCUSSION

All the temporal and frequency features of the sounds were similar between red and yellow-eyed morphotypes of *S. marginatus* and were similar to those previously reported for this species (Mélotte *et al.*, 2016). However, the comparison of the sounds recorded in this study with those described in smaller specimens of *S. marginatus* in the literature (Mélotte *et al.*,

2016) showed that the number of peaks in the sound is the only comparable acoustic feature that is not influenced by the fish size (Wilcoxon: $W = 194$; $P = 0.49$).

In *S. marginatus,* red and yellow-eyes fish show differences in sound intensity. Both the eye colour and the acoustic differences could be the link to one or several mutation(s) leading to the formation of new populations. Natural populations of the January tetra *Hemigrammus hyanuary* (Characidae, Characiformes) are known to exhibit two distinct iris coloration phenotypes: silver and green (Frankel, 2000). Crosses experiments indicate that the green iris phenotype is inherited as a monogenic autosomal recessive at the gene locus encoding the silver phenotype (Frankel, 2000). In the rainbow trout *Oncorhynchus mykiss* (Salmonidae, Salmoniformes), the red eye colour is caused by the absence of a dominant allele controlling colour development (Dobosz *et al.*, 1999).

In fish, colour change in eyes can also be involved in social rank or during reproduction. In the stickleback, *Gasterosteus aculeatus* Linnaeus, 1758 (Gasterosteidae, Gasterosteiformes), for example, the blue iridescent eye of the male during the mating period is a primary courtship signal in female mate choice (Flamarique *et al.*, 2013). These eye pigments have been shown to be mediated by hormones in different species (Fujii, 2000; Franco-Belussi *et al.*, 2018). In *Oreochromis niloticus* (Linnaeus, 1758) (Cichlidae, Perciformes), the relative position in the social hierarchy influences the levels of hepatic Insulin-like Growth Factor (IGF-I) mRNA expression and the eye colour pattern (Volpato *et al.*, 2003; Vera Cruz & Brown, 2007). The level of IGF-I mRNA is related to the level of aggressivity (Vera Cruz & Brown, 2007) and most fish have been shown to display a decrease in eye darkening during aggressive interactions (Vera Cruz & Brown, 2007). Subordinate fish showed eye-darkening patterns which acted as a social signal of submission while dominant fish had paler eye colour patterns than subordinates (Volpato *et al.*, 2003; Vera Cruz & Brown, 2007). In the sound producer *Terapon jarbua*

(Forsskål, 1775) (Terapontidae, Perciformes), the coloration of the eyes can also change with the level of aggressivity (Schneider, 1964; Parmentier *et al.*, 2016).

In fish, as in many vertebrates, different hormones and neuropeptides can also modulate vocal features because acting on vocal mechanisms (Remage-Healey, 2004; Remage-Healey & Bass, 2005; Bass *et al.*, 2015). Bringing together the two known phenomena, we hypothesize that different hormonal concentrations could explain both sound amplitude and eye colour playing a role in animal communication in *S. marginatus*. In our study, the few specimens that produce louder sounds have yellow eyes whereas the numerous specimens that produce weaker sounds possess red eyes. Louder sounds should be related to more powerful muscles and testify better health condition (Amorim *et al.*, 2013). In this case, yellow eyes could testify a dominating status and it seems logical to have more submissive specimens than dominant ones as suggested by our sample. Additional studies are required to determine the hormonal status in both morphotypes.

Competing interests

The authors declare no competing interests.

Author contributions

X.R. design the study. E.P., X.R., A.H., A.G., G.K. planned and organised the field mission. X.R., G.K. & A.H. performed the field mission. X.R. recorded the sounds. X.R. analysed the data. X.R. interpreted the data. X.R. wrote the manuscript with inputs from E.P. The manuscript was corrected and implemented by A.H., A.G. & G.K. All authors gave final approval for publication.

Data availability

Pictures of the specimens are available in the Supporting Information. Six fixed specimens are available at the Aquarium-Museum of the University of Liège (vouchers R.E. 15277, 15278, 15279, 15280, 15281 and 15282). Derived data supporting the findings of this study are available from the corresponding author X.R. on request.

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