Sounds and associated morphology of *Hypostomus* species from South-East Brazil

Xavier Raick¹*, Anaïs Koussa¹, Cláudio Henrique Zawadzki², Gregório Kurchevski³, Alexandre Lima Godinho³ & Éric Parmentier¹

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

² Departamento de Biologia, Universidade Estadual de Maringá, Maringá, Paraná, Brazil

³ Fish Passage Center, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

* Corresponding author

Orcid: 0000-0002-1977-0289

Email: xavier.raick@uliege.be

**Keywords:** pectoral girdle, Loricariidae, Siluriformes, stridulation, pectoral spine, catfish, bioacoustics, sound production
Abstract

*Hypostomus* is the most diverse genus within Loricariidae. These catfish species exhibit a very conservative morphology with relatively few external characteristics that differ between different species. In consequence, there is a challenge to understand the distinction of species of this genus. This study aims to describe the sounds produced by ten species of *Hypostomus* from Araguari River and Paraopeba River (Minas Gerais, Brazil), to examine whether acoustic features could be used to distinguish between the species, and to understand the corresponding sound production mechanisms. The fish were recorded making sounds by holding them in hand underwater, in a glass-tank on the river shore next to where they had been caught. All ten species produced sounds, and no effect of body size on acoustic features was observed. Furthermore, the species could not be distinguished using acoustic features. Likewise, all the studied species exhibited similar bony ridges on the dorsal processes of the pectoral spine. Since the sounds produced by the fish do not seem to exhibit interspecific specificity, they do not appear to have a role in behaviours requiring conspecific recognition, such as reproduction. Sound production may have an alarm, distress, or acoustic aposematism function warning predators of the presence of pectoral spines, which have an anti-predatory function in this clade.

Introduction

In animal communication, signals have evolved as a consequence of their effects on receiver behaviours (Bradbury and Vehrencamp 1998). The system efficacy requires that senders and receivers have concordant coding schemes to assure the specifiable relationship that avoids confusion (Bradbury and Vehrencamp 1998). This is particularly important in sexual advertisement/mate attraction and sexual competition, because successful reproduction requires identifying a mate of the appropriate species and sex, as well as assessing indicators of mate quality (Gilliam 2011). Species-specific concordant signals between senders and receivers
are the basis of many studies aimed to describe specific acoustic signals. Moreover, this specificity of the signal could be used by scientists to distinguish between species (Raick et al. 2021). However, sounds can also be produced during other behavioural patterns such as agonistic behaviour, competitive feeding, and when disturbed (Fine and Parmentier 2015). In such cases, the signal can be addressed to conspecifics and/or heterospecifics (Ladich 1997a). In heterospecific communication, the codification of the signal could be less accurate since it should be interpreted by different species (Mann and Lobel 1998; Lagardère et al. 2005). For example, aposmatic signals are used to warn predators of the dangerous nature of the emitting species (Ruxton et al. 2004).

Catfishes are a highly diverse taxon (Howard 2019) of freshwater fishes found in a large variety of habitats (Teugels 2003). An important feature of the taxon is the modification of the first pectoral fin rays in robust lockable spines (Gosline 1977; Fink and Fink 1981) that can have an anti-predator and/or protection function (Alexander 1966; Fine and Ladich 2003) when they are abducted (Alexander 1975; Fine et al. 2011; Sismour et al. 2013). Moreover, different species from at least 18 families (Kaatz 1999; Parmentier et al. 2010) can use this sweeping mechanism to produce sounds when threatened by a predator and/or as a distress call (Bosher et al. 2006; Quan et al. 2020). Sounds are produced by the friction of the ridged proximal head of the spines within a depression of the pectoral girdle during abduction and/or adduction movements (Pfeiffer and Eisenberg 1965; Taverne and Aloulou-Triki 1974; Fine et al. 1997; Ladich 1997b; Heyd and Pfeiffer 2000; Parmentier et al. 2010). In catfishes, sound production with the pectoral spine could result from an exaptation (Parmentier et al. 2017a): some of the pectoral movements used for swimming or for defence were co-opted to make sounds in different kinds of behaviour (Alexander 1966; Fine and Ladich 2003; Parmentier et al. 2017a). Although stridulatory sounds appear mainly associated with defensive behaviours, some species use them secondarily in social communication (Fine and Ladich 2003).
Within Siluriformes, Loricariidae includes more than 1000 valid species (Fricke et al. 2021). Some of them are known to produce short clicks with a large frequency bandwidth (Smith et al. 2009; Tellechea et al. 2013) whereas other species are mute (Heyd and Pfeiffer 2000). These sounds could be used for defence against predators and/or as a warning sound (Webb and Smith 2006). Recently, the term “predator-related” sound has been introduced to avoid implying unproven functions (e.g., alarm, warning, or distress) (Ladich 2022). In Loricariidae, Hypostomus is the most diverse genus (Borba et al. 2013) with 152 species (Fricke et al. 2021). In South-East Brazil, five species (H. denticulatus, H. iheringii, H. margaritifer, H. regani, and H. strigaticeps) have been found inhabiting in sympathy in the Araguari River (Upper Paraná River Basin) and five additional species (H. alatus, H. francisci, H. aff. paulinus, H. guajupia, and H. velhochico) in the Paraopeba River (São Francisco River Basin). This study aimed to describe the sounds produced by these species and to understand the sound production mechanism in the Hypostomus genus. Sounds that are used for species-specific communication should be statistically different between species.

**Materials and methods**

**Biological materials**

In July 2018, 58 specimens of *Hypostomus* were sampled in two river basins in the state of Minas Gerais (Brazil, Fig. 1A). In the Araguari River (Upper Paraná River Basin), 53 specimens (standard length (SL): 158 – 295 mm) were sampled downstream of the Amador Aguiar I Hydropower Dam (18° 46′ 42″ S, 48° 9′ 57″ W, Fig. 1B): one *H. denticulatus* (SL: 210 mm), five *H. iheringii* (SL: 230 ± 32 mm, mean ± SD; range: 188–275 mm), 24 *H. margaritifer* (SL: 207 ± 21 mm; range: 158–263 mm), 18 *H. regani* (SL: 235 ± 28 mm; range: 199–295 mm) and five *H. strigaticeps* (SL: 233 ± 18 mm; range: 213–264 mm). In the Paraopeba River (São Francisco River Basin), one specimen of each of the following five species were sampled
downstream the Retiro Baixo Hydropower Dam (18° 52′ 35″ S, 44° 46′ 49″ W): *H. alatus* (210 mm), *H. francisci* (227 mm), *H. aff. paulinus* (173 mm), *H. guajupia* (177 mm) and *H. velhochico* (141 mm).

![Fig. 1 Sampling sites. A. Red: Paraopeba River, São Francisco River Basin. Green: Araguari River, tributary of the Paranaíba River (upper Paraná River Basin), both rivers in Minas Gerais State (Brazil). Blue: major Brazilian river basins (Amazon, Paraná, São Francisco and Tocantins). In black: state borders. B. Sampling site in Araguari River.](image)

All the specimens were collected with gillnets placed at sunset. Some of the specimens are available at the collections of the Functional and Evolutive Morphology Laboratory at the University of Liège (vouchers n°Hypostomus_001, 002, 003, 004, 005, 006 and 007). All the specimens were photographed for identification and measurements. One specimen per species was euthanized with an overdose of eugenol (CAS: 97-53-0), fixed in a 5% formaldehyde solution for two days and stored in a 70% ethanol solution. All the other specimens were released back into the river. Time between when the fish were retrieved from the net and released back into the river varied between 10 mins and 2h 30 mins.

**Recordings**

The sound recording site was the same as the specimen collection site. For logistical reasons, the sounds were recorded on the river shore in a glass-tank (108 L, water temperature:
26 ± 2°C), with a hydrophone (HTI-96-Min, High Tech Inc., Long Beach, MS, USA; sensitivity: −164.4 dB re 1V μPa⁻¹) placed in the middle of the tank and connected to a TASCAM DR5 recorder (TEAC Europe GmbH, Wiesbaden, Germany) previously calibrated with an oscilloscope. Between the net and the glass-tank, the fish were stocked in a tank on the boat during transport with a bubbler and kept in a mesocosm until recording. 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 to standardize the sound recordings as much as possible. The sound recordings of the different species were deposited in the audio-visual collections of the FNJV – Unicamp (https://www2.ib.unicamp.br/fnjv/; vouchers n°0050443 to 0050452).

**Sound-analysis**

The effects of reverberation and resonance can induce potential artifacts in the characteristics of sounds recorded in small glass tanks (Akamatsu et al. 2002). The resonant frequency of the recording tanks was 3252 Hz. Consequently, the sounds that were previously digitised on mono-channel at 44.1 kHz with a 16 bit-resolution were secondarily sub-sampled at 6 kHz to avoid sounds that could result from the tank resonance. Then, a high-pass filter at 50 Hz was applied. The amplitude of the sounds was above the noise level. The analysis was carried out with Avisoft SAS-Lab Pro 5.2 (Avisoft Bioacoustics, Glienicke/Nordbahn, Germany).

For each specimen, the first ten sounds emitted were analysed. The temporal features were measured in oscillograms while frequency features were measured in the power-spectra. The temporal features were the sound duration (d, in ms), the number of peaks in a sound (N), and the period between consecutive peaks (p, in ms) based on Fig. 2A from Raick et al. (2020a) (Fig. SP1). The frequency features were the dominant frequency ($F_{\text{AmpMax}}$, in Hz), the first, second and third frequency quartiles ($F_{Q1}$, $F_{Q2}$, $F_{Q3}$, in Hz, also named 25th, 50th and 75th percentiles obtained by dividing the power spectrum in four equal parts) and the amplitude
corresponding to the dominant frequency ($\text{AmpF}_{\text{AmpMax}}$, in dB re 1 $\mu$Pa @ 5 cm) based on Fig. 1B from Raick et al. (2020b).

**Species identification**

The identification of the specimens from the Araguari River was carried out based on the pictures with identification keys from Ota et al. 2018 and Dias and Zawadzki (2018) with additional descriptions from Zawadzki et al. (2001) for *Hypostomus margaritifer* and Zawadzki et al. (2020) for *H. albopunctatus*. These keys use a combination of features such as (1) the color of the body, (2) presence or absence of spots, (3) disposition, shape and color of these spots, (4) size difference between pectoral-fin spine and pelvic-fin spine, (5) shape of the caudal fin, (6) presence of keel in lateral series of plates, (7) orbital diameter in comparison with head length, (8) size of the spots in comparison with pupil diameter, and (9) presence or absence of bony platelets on the abdomen (Fig. 2 and 3). Species identification of the Paraopeba River specimens was carried out using previous studies devoted to this watercourse (Garavello and Garavello 2004; Zawadzki et al. 2017, 2019). In addition to the external characteristics, the number and the shape of teeth on the dentary and the premaxilla are additional identification criteria. In consequence, these were counted on the fixed specimens. To do so, pictures of the teeth were taken with a Power Shot S50 camera coupled to a LeicaM10 binocular (Leica Microsystems GmbH, Germany).
Fig. 2 A *Hypostomus iheringii* and B *Hypostomus margaritifer* from Araguari River had blotches of different color and shape: dark-brown vs. light-beige blotches respectively (Ota et al. 2018). C The size of the eye (orbital diameter 20-21.5% of head length instead of 12-17.9% for *Hypostomus ancistroides*) and D the size of the body spots (larger than the pupil diameter) are two important criteria to identify *Hypostomus iheringii* (Dias and Zawadzki 2018). E *Hypostomus velhochico* and F *Hypostomus francisci* from Paraopeba River can also be differentiated by the color of the blotches (dark-blotched vs. pale-blotched) (Zawadzki et al. 2008, 2017).
Fig. 3 A The presence and the localization of keels, the alignment of the lateral spots, and B the shape of the dorsal fin (the ventral part is longer than the dorsal one) are two criteria of identification for *Hypostomus velhochico* (Zawadzki et al. 2017). The coloration of the abdomen and the presence of plates could also help identification (Dias and Zawadzki 2018) like for example in C *Hypostomus velhochico* (blotches are visible on the abdomen), D *Hypostomus regani* (plates on the abdomen but no blotches) and E *Hypostomus margaritifer* (neither blotches nor plates).
Statistics

The description of the sounds was carried out on all the sounds analysed. Tests assumptions were tested with Shapiro-Wilk and Bartlett tests. All the statistics relative to the correlation with body size and the species comparison were conducted on means per individuals, i.e., ten sounds were averaged to obtain a single value per specimen. Only the specimens from Araguari River were used for the statistics as the number of individuals from Paraopeba River was too small. Correlation matrices were calculated using Spearman correlation coefficients ($r_s$) and their associated $P$-value matrices corrected by the Holm-Bonferroni method.

All the acoustic features of the sounds recorded from the specimens from the Araguari River were compared using Kruskal-Wallis tests. To reduce the multivariate aspect of the data, principal component analyses (PCAs) were conducted on the correlation matrices. The principal components (PCs) are presented with their associated percentage of variance and their cumulative percentage of variance. PCs for each group were compared using Kruskal-Wallis tests with the Dunn’s test as post-hoc with a Benjamini-Hochberg correction on the $P$-values. The values of the statistics $Z$ are presented with $P$-value and adjusted $P$-value ($P_{adj}$) when necessary. All the statistics were carried out using R 3.5.3. (GNU General Public License) and the significance level was $\alpha = 0.05$.

Morphology

The dorsal processes of the pectoral spines of the fixed specimens (see Fig. 5) were photographed with a Power Shot S50 camera coupled with a LeicaM10 binocular (Leica Microsystems GmbH, Germany). Micro CT scanning of one specimen from Paraopeba River was completed using an RX EasyTom (RX Solutions, Chavanod, France), with an aluminium filter. Images were generated at a voltage of 90 kV and a current of 333 $\mu$A. Reconstruction of
the pectoral girdle region was performed using X-Act software from RX. Segmentation, visualization, and analysis were performed using Dragonfly software (Object Research Systems Inc, Montreal, Canada). Direct volume renderings (iso-surface reconstruction) were used to visualize the pectoral girdle region in AMIRA 2019.2 (FEI Visualization Sciences Group and Zuse Institute Berlin, Germany). Finally, dissections were carried out to examine the musculature related to the sound-producing apparatus in several specimens. In the literature, different confusing nomenclatures are used to describe the pectoral musculature of catfishes, and of fish in general, as the names do not always correspond to muscle function (Miano et al. 2013) and some small muscles have been considered as bundles of other muscles (Diogo and Abdala 2007). Table 1 presents the synonyms of the principal muscles from the literature. Muscle names are here based on Miano et al. (2013). More information about the standardization of the terminology of the spines can be found in Ballen and De Pinna (2021).
Table 1 Comparisons of names of the muscles in the pectoral girdle in teleosts in the literature (non-exhaustive).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spine muscles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Musculus exterior anterior</strong></td>
<td>Abductor muscle of the spine</td>
<td>Abductor Streckermuskel</td>
<td><strong>Arrector dorsalis</strong> (dorsal division)</td>
<td><strong>Arrector dorsalis</strong> (dorsal division)</td>
<td><strong>Arrector ventralis</strong></td>
<td><strong>Arrector ventralis</strong> (ventral division)</td>
<td>Spine abductor</td>
<td></td>
</tr>
<tr>
<td>Spine muscles</td>
<td>Ventral arrector dorsal Z part</td>
<td>Ventral arrector dorsal Y part</td>
<td>Ventraler drehermuskel</td>
<td><strong>Arrector dorsalis</strong> (ventral division)</td>
<td><strong>Arrector ventralis</strong></td>
<td><strong>Arrector ventralis</strong> (ventral division)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musculus interioris anterior</td>
<td>Superficial abductor (Part)</td>
<td>Dorsal arrector</td>
<td><strong>Adductor profundus</strong></td>
<td><strong>Adductor profundus</strong></td>
<td><strong>Adductor dorsal</strong></td>
<td><strong>Adductor dorsal</strong> (dorsal division)</td>
<td>Spine adductor</td>
<td></td>
</tr>
<tr>
<td><strong>Ray muscles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial abductor</td>
<td><strong>Abductor superficialis 1</strong></td>
<td>Abductor of the pectoral fin</td>
<td><strong>Abductor superficialis</strong></td>
<td><strong>Adductor superficialis 1</strong></td>
<td>Adductor of the pectoral fin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep abductor</td>
<td><strong>Abductor superficialis 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial adductor (Part)</td>
<td><strong>Adductor superficialis 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep adductor</td>
<td><strong>Adductor superficialis 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Authorisations

The capture of the specimens was achieved under the license 10306-1 from the Brazilian Ministry of the Environment. In the Paraopeba River, the fish were captured with an authorisation n° PT-04/07/2018 issued by Retiro Baixo Energética. In the Araguari River, the fish were captured with an authorisation from Consórcio Capim Branco. The fixed specimens were imported to Belgium with the authorisation of the Federal Agency for the Safety of the Food Chain (COBT/IEC/CMY/1546595), the Federal University of Minas Gerais (MTA/TTM n° 02/2019) and the Conselho de Gestão do Patrimônio Genético of the Ministério do Meio Ambiente (n°R2B8FF7 et n°A57A7E9). The sound recording protocol was approved by the ethical commission of the University of Liège (case 1759).

Results

Sounds

All the 58 specimens produced intense stridulating sounds when gently handled with the pectoral fins free to move (Fig. SP2 and SP3). For all the acoustic features, no statistically significant correlations with the SL were found (Table 2). The detailed features for each species can be found in Table 3. Although it seems unsafe to draw conclusions for species with just one specimen, the acoustic features of such species were in the same range as species with a higher number of specimens studied. In addition, this shows that these species are able to produce sounds.
Table 2. Summary of the features and their comparisons in *Hypostomus*. Acoustic features (mean and standard deviation, SD); Spearman correlation ($r_S$) between the acoustic features and standard length (SL) and associated $P$-values ($P$) adjusted by Holm-Bonferroni method; comparison of the acoustic features among species (Kruskal-Wallis tests, KW: $\chi^2$, df and $P$) and Spearman correlation ($r_S$) and associated $P$-values ($P$) adjusted by Holm-Bonferroni method for the two first principal components (PC1 and PC2). Except for mean and SD, which were calculated for all the species, the other statistical analyses were conducted for the five species of *Hypostomus* from Araguari River only. Significant $P$-values are in bold. For the abbreviations of the acoustic features, refer to the “Sound-analysis” paragraph in the Materials and Methods.

<table>
<thead>
<tr>
<th>Acoustic features</th>
<th>Description</th>
<th>SL</th>
<th>KW</th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean</td>
<td>SD</td>
<td>$r_S$</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>D (ms)</td>
<td></td>
<td>212</td>
<td>96</td>
<td>0.16</td>
<td>&gt; .999</td>
</tr>
<tr>
<td>$F_{\text{AmpMax}}$ (Hz)</td>
<td></td>
<td>170</td>
<td>109</td>
<td>-0.23</td>
<td>&gt; .999</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>15</td>
<td>7</td>
<td>-0.13</td>
<td>&gt; .999</td>
</tr>
<tr>
<td>P (ms)</td>
<td></td>
<td>16</td>
<td>8</td>
<td>0.39</td>
<td>.11</td>
</tr>
<tr>
<td>$F_{Q1}$ (Hz)</td>
<td></td>
<td>205</td>
<td>94</td>
<td>-0.12</td>
<td>&gt; .999</td>
</tr>
<tr>
<td>$F_{Q2}$ (Hz)</td>
<td></td>
<td>447</td>
<td>314</td>
<td>0.04</td>
<td>&gt; .999</td>
</tr>
<tr>
<td>$F_{Q3}$ (Hz)</td>
<td></td>
<td>931</td>
<td>567</td>
<td>-0.02</td>
<td>&gt; .999</td>
</tr>
<tr>
<td>$\text{AmpF}_{\text{AmpMax}}$ (dB re 1 µPa @ 5 cm)</td>
<td></td>
<td>106</td>
<td>8</td>
<td>0.11</td>
<td>&gt; .999</td>
</tr>
</tbody>
</table>

In fishes from the Araguari River, it was not possible to distinguish the different species using the sounds they produced: all the acoustic features were statistically equivalent between the different species (Table 2). The PCA performed on the acoustic features did not allow to differentiate between the species: PC1 (37%), PC2 (23%) and PC3 (19%) were equivalent between the different species (Kruskal-Wallis: $\chi^2 = 7.02, 7.00 \& 3.13; df = 4; P = 0.14, 0.14 \& 0.54$ respectively; Fig. 4A). PC1 was highly correlated ($r_S > 0.60$) with $\text{AmpF}_{\text{AmpMax}}$ and the frequency quartiles (Table 2), PC2 was highly correlated with $F_{\text{AmpMax}}$ (Table 2) and PC3 with the number of peaks and the peak period.
Table 3 Summary of the different acoustic features for *Hypostomus* from Paraopeba River (five species on the left) and from Araguari River (five species on the right).

<table>
<thead>
<tr>
<th></th>
<th><em>Hypostomus</em> alatus</th>
<th><em>Hypostomus</em> francisci</th>
<th><em>Hypostomus</em> aff. paulinus</th>
<th><em>Hypostomus</em> guajupia</th>
<th><em>Hypostomus</em> velhochico</th>
<th><em>Hypostomus</em> margaritifer</th>
<th><em>Hypostomus</em> strigaticeps</th>
<th><em>Hypostomus</em> regani</th>
<th><em>Hypostomus</em> iheringii</th>
<th><em>Hypostomus</em> denticulatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>N fish</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>24</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>N sounds</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>240</td>
<td>50</td>
<td>180</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>mean</td>
<td>133</td>
<td>17</td>
<td>102</td>
<td>22</td>
<td>144</td>
<td>76</td>
<td>85</td>
<td>35</td>
<td>192</td>
<td>61</td>
</tr>
<tr>
<td>SD</td>
<td>17</td>
<td>17</td>
<td>22</td>
<td>22</td>
<td>76</td>
<td>35</td>
<td>35</td>
<td>16</td>
<td>61</td>
<td>30</td>
</tr>
<tr>
<td>D (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F_{AmpMax} (Hz)</td>
<td>115</td>
<td>87</td>
<td>158</td>
<td>42</td>
<td>265</td>
<td>186</td>
<td>409</td>
<td>24</td>
<td>170</td>
<td>197</td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>p (ms)</td>
<td>12</td>
<td>7</td>
<td>12</td>
<td>4</td>
<td>20</td>
<td>3</td>
<td>14</td>
<td>5</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>FQ1 (Hz)</td>
<td>225</td>
<td>44</td>
<td>199</td>
<td>23</td>
<td>263</td>
<td>70</td>
<td>332</td>
<td>38</td>
<td>342</td>
<td>260</td>
</tr>
<tr>
<td>FQ2 (Hz)</td>
<td>434</td>
<td>118</td>
<td>405</td>
<td>99</td>
<td>509</td>
<td>163</td>
<td>448</td>
<td>29</td>
<td>630</td>
<td>289</td>
</tr>
<tr>
<td>FQ3 (Hz)</td>
<td>1584</td>
<td>519</td>
<td>672</td>
<td>44</td>
<td>1020</td>
<td>724</td>
<td>685</td>
<td>142</td>
<td>1085</td>
<td>494</td>
</tr>
<tr>
<td>AmpF_{AmpMax} (dB re 1µPa @ 5cm)</td>
<td>102</td>
<td>4</td>
<td>98</td>
<td>4</td>
<td>88</td>
<td>6</td>
<td>103</td>
<td>6</td>
<td>88</td>
<td>3</td>
</tr>
</tbody>
</table>
Fig. 4  A PC1 and PC2 scatterplot with all the specimens of *Hypostomus* from Araguari River. B Oscillogram of a sound produced by *Hypostomus denticulatus* subsampled at 3 kHz. C Power spectrum of the same sound (evaluation window: Hamming).

The sounds of all the species were pooled together to provide features to identify specimens from the *Hypostomus* genus in their habitat. These sounds had an average duration of $212 \pm 96$ ms with $15 \pm 7$ peaks and a period of $16 \pm 8$ ms (Fig. 4B). They had a dominant frequency of $170 \pm 109$ Hz with an associated amplitude of $106 \pm 8$ dB re $1 \mu$Pa @ 5 cm (Fig.
However, energy could be found on a broad band of frequencies as shown by frequency quartiles (Table 2, Fig. 4C).

**Morphology**

The general morphology of the pectoral spines is similar among Siluriformes and has been previously described in different species (Fine et al. 1997, 2000; Diogo et al. 2001). In *Hypostomus*, the base of the pectoral spine is composed of three processes that form complex articulations with the cleithrum and scapulocoracoid of the pectoral girdle: the dorsal process, the antero-ventral process (also named anterior process) and the postero-ventral process (also named ventral process) (Fig. 5A, B & C). The dorsal process is the largest one, possesses the shape of one third of a circle (Fig. 5C) and slides into a corresponding depression of the cleithrum, the *Spina fossa* (Fig. 5D). The two other processes are smaller and their bases form together the vertical U-shaped articulating fossa (Fig. 5B, 5C & 6). From a functional point of view, the articulating fossa allows the pectoral spine to turn around the vertical axis, providing abduction and adduction movements being controlled by the dorsal process that follows the *Spina fossa* shape (Fig. 5D, 7C & 8A). Caudally to the articulating process, the medial notch makes place to articulate the first pectoral rays.
Fig. 5 Reconstruction of the left pectoral spine of *Hypostomus velhochico* based on the CT scan. **A** lateral view, **B** medial view, **C** insertion areas of the different muscles, and **D** medial view of the articulation between the spine and the pectoral girdle.
Fig. 6 Dorsal processes of the pectoral spines of eight *Hypostomus* species. A *Hypostomus velhochico* (magnification x 12.5), B *Hypostomus aff. paulinus* (magnification x 12.5), C *Hypostomus regani* (magnification x 10), D *Hypostomus strigaticeps* (magnification x 8), E *Hypostomus iheringii* (magnification x 10), F *Hypostomus guajupia* (magnification x 12.5), G *Hypostomus francisci* (magnification x 8), and H *Hypostomus margaritifer* (magnification x 8).
Fig. 7 Postero-internal reconstruction of the articulation of the pectoral girdle and the pectoral spine of *Hypostomus velhochico* based on the CT scan. A & B with the associated muscles, C without muscles. Given that the radials and the rays of the pectoral fin are not presented in the reconstruction, the muscles inserting on them appear cut.
Fig. 8 Lateral reconstruction of the articulation of the pectoral girdle and the pectoral spine of *Hypostomus velhochico* based on the CT scan; A without muscles and B with the associated muscles. Given that the radials and the rays of the pectoral fin are not presented in the reconstruction, the muscles inserting on them appear cut.
In all studied *Hypostomus* species, the dorsal surface of the dorsal process was convex with a series of ridges that give it a striated appearance (Fig. 5A, B & C). Ridges were parallel between them and perpendicular to the dorsal edge of the process. They were not straight but slightly sinuous and regularly spaced in a “ripple-mark” pattern (Fig. 6). No differences were observed in the organisation of the dorsal process between the different species (Fig. 6).

Different muscles are involved in the movement of the pectoral spine and fin. Five muscles were observed during the dissections (Fig. 7A, 7B & 8B). The first three listed below are inserted on the spine (see Table 1 for synonymy in the literature) and the two latter ones on the rays. (1) The *Spine abductor* originates in the fossa of the cleithrum and inserts on the anterior margin of the dorsal process of the pectoral spine (see the red line in Fig. 5C for the insertion zone and Fig. 8B for the muscle). (2) The *Spine adductor* originates on the caudal face of the vertical wall of the cleithrum, passes below the mesocoracoid arch and inserts on the caudal part of the dorsal process of the pectoral spine (Fig. 7A, 7B & 8B). (3) The *Arrector ventralis* originates at the level of the anterior margin of the fossa of the cleithrum, goes ventrally over the scapulocoracoid bridge and inserts on the postero-ventral process of the pectoral spine (Fig. 8B). (4) The *Adductor radialis* originates on the scapulocoracoid and inserts on the ventral basis of the different rays of the pectoral fin (Fig. 7A, 7B & 8B). (5) The *Abductor radialis* originates on the humeral process of the cleithrum and inserts on the dorsal basis of the different rays of the pectoral fin (Fig. 7A).

**Discussion**

This study described for the first time the sounds produced by ten Brazilian *Hypostomus* species. The number of specimens from each species allowed statistical comparisons between species from the Araguari River only. The sounds produced by the different species had similar features, meaning acoustic features could not be used to discriminate between the species.
Despite the lack of statistical support, the sounds produced by specimens from Araguari River were similar to the ones produced by species from the Paraopeba River. Moreover, sounds produced by the Brazilian species appear to be close to sounds briefly described in two Uruguayan species having sounds that last from 130 to 260 ms with a frequency (F_{AmpMax}) of 348 Hz in *H. commersoni*, and 292 ms and 292 Hz in *H. derbyi* (Tellechea et al. 2013).

Due to the lack of differences between the acoustic features of the studied *Hypostomus* species, it is hard to sustain that their sounds could be involved in conspecific recognition and could act as reproductive barrier. In accordance with Kaatz (1999) and Heyd and Pfeiffer (2000), we hypothesize that these sounds could serve as an aposematic signal to warn the predator of the presence of a defence mechanism. In several species of catfish, the positioning of the pectoral spine in erected position has indeed an antipredator and/or protection function (Alexander 1966; Fine and Ladich 2003), meaning a catfish stridulating warning signal addressed at different predators does not need to be specific (Heyd and Pfeiffer 2000). However, even with an aposematic function, it is possible to find interspecific differences in the highly diverse taxa of Siluriformes since Heyd and Pfeiffer (2000) and Kaatz (1999) have found some species differences related to number of ridges and their widths. Acoustic aposematic signals are found in different clades (Heyd and Pfeiffer 2000) like insects (Ratcliffe et al. 2008), frogs (Santos et al. 2014) and snakes (Caine et al. 2020). Perhaps one of the best-known examples of aposematic sounds can be found in the rattlesnakes of the *Crotalus* and *Sistrurus* genera (Caine et al. 2020). However, the evidence of an aposematic sound function in rattlesnakes is in debate (Caine et al. 2020). Some authors have proposed that the rattling is not used to warn but to direct threatening attention away from the snake’s head (Williams 1966), and the rattling is observed only in defensive contexts (Greene 1988) making unlikely the “caudal luring” hypothesis for rattlesnakes (Schuett et al. 1984; Sisk and Jackson 1997). In addition, some species of birds (Owings et al. 2002) and nonvenomous snakes (Kardong 1980; Sweet 1985)
can mimic rattling, reinforcing the hypothesis of a protective function (Caine et al. 2020). 

*Hypostomus* sounds could also have an alarm or distress function without an aposematic function. Therefore, playback experiments using different predator species should help to clarify these sonic functions (Fine and Ladich 2003).

In *Hypostomus* species, no correlation was found between acoustic features of the produced sounds and SL (standard length), as observed in other clades like adult triggerfishes (Balistidae) (Raick 2015, Parmentier et al. 2017b, Raick et al. 2018). However, when considering both juvenile and adult triggerfishes, a link with SL was found (Parmentier et al. 2017b). Therefore, studies on smaller *Hypostomus* specimens would help to better understand the link between their sounds and SL. In another catfish (*Ictalurus furcatus*), larger individuals generate longer higher amplitude pulses with lower peak frequencies (Ghahramani et al. 2014).

All the studied species had crests on the dorsal process of the pectoral spine, as is the case in numerous catfishes able to produce stridulatory sounds. According to previous studies, the sounds resulted from the friction of the dorsal process ridges against the wall of the Spina fossa (Pfeiffer and Eisenberg 1965; Taverne and Aloulou-Triki 1974; Fine et al. 1997; Ladich 1997b; Heyd and Pfeiffer 2000; Parmentier et al. 2010). At the opposite, silent Siluriformes species (for example, from genera *Ageneiosus*, *Aspidoras*, *Bagrichthys*, *Callichthys* and *Hemibagrus*) lack bony ridges/crests on the dorsal process (Kaatz et al. 2010) and are often phylogenetically nested within vocal clades, supporting the role of microscopic crests in sound production (Parmentier et al. 2010). The high similarity in the crest organisations between the studied *Hypostomus* species reinforces the lack of specific features in the sounds.

The genus *Hypostomus* could have its origin in the Amazon/Orinoco ecoregion before dispersing throughout tropical South America, east of the Andes Mountains. It invaded the Paraná River basin in South-East Brazil approximately 12.5 MYA (Montoya-Burgos 2003; Silva et al. 2016). In a study of fossil fish from Argentina, a new species attributed to genus *Hypostomus*
has been found in a facies that has been dated from late Pleistocene to early Holocene (35.890 – 8.150 years ago) (Vallone et al. 2017). Interestingly, this species showed a well-developed arching dorsal process having parallel surface striations (Vallone et al. 2017). Similarly, Lundberg (1997) attributed a pectoral spine in the Miocene fauna of Colombia to the genus *Hypostomus* and described that the articulating surface was cross-striated (Lundberg 1997). In another non-identified loricariid from the same period, this author also showed the presence of striation on the dorsal process of pectoral spine. Similarities between the dorsal process ornamentation of these fossil species and the different species of this study (Fig. 6) suggest that ancestral *Hypostomus* were already able to make sounds.

Skeletal structures that are involved in sound production have already been identified in different species (Kaatz 1999; Heyd and Pfeiffer 2000; Fine and Ladich 2003). In this study, 3D reconstruction allowed to better understand the relationships between the spine and the pectoral girdle (Fig. 5D, 7C & 8A). Electromyography studies are required to clearly assess the muscle functions during stridulation, but muscle insertion can help to understand them. In catfishes, the spine can move without making sound when it slides within the *Spina fossa* without resistance, highlighting that the sound is intentional and not a simple by-product. According to catfish taxa, the sound production occurs during the abduction or a combination of abduction/adduction (Fine and Ladich 2003) of the pectoral spine (Fine et al. 1996; Ladich 1997b; Heyd and Pfeiffer 2000). The muscles allowing abduction and adduction sweeping movements of the pectoral spine are the spine adductor (adduction movement) and the *Spine abductor* (abduction movement). It could be worth mentioning that this mechanism was also described in Aspredinidae (Gainier 1967), Ictaluridae (Fine et al. 2007; Miano et al. 2013) and Mochokidae (Parmentier et al. 2010), however some muscles and corresponding functions seem to have been, unfortunately, wrongly quoted in this last study. Moreover, in *Hypostomus* species, only three muscles in relation with the spine have been found (instead of four),
supporting that the *Arrector dorsalis* (Miano et al. 2013) could be derived from the spine abductor. This assumption is reinforced by the fact that the spine abductor and the *Arrector dorsalis* as described by (Miano et al. 2013) are considered the same muscle by Diogo and Abdala (2007) named *Arrector ventralis* (and previously named *Arrector dorsalis*, Table 1). The stridulation requires the friction of the dorsal process ridges against the wall of the *Spina fossa* (Fine et al. 1997). The contraction of the *Arrector ventralis* at least could allow it (Schachner and Schaller 1981; Parmentier et al. 2010) by lowering the spine in relation to the sagittal plane. As different pectoral muscles show bundles, it should be possible to describe more refined movements related to sound production.

In conclusion, a high inter-specific homogeneity was found both regarding the recorded sounds and the associated morphology in *Hypostomus* species. These results suggest that sound production may have an alarm, warning, distress, or acoustic aposematism function in *Hypostomus*.

**Funding**


Freshwater and Oceanic Science Unit of Research, University of Liège (2018)

King Leopold III Fund for Nature Exploration and Conservation (2018/1.12.)

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Acknowledgments**

The authors thank Guilherme Coelho Melazo for the authorization to sample in Amador Aguiar II Hydroelectric Dam and Alessia Huby for the help during fieldwork.
Conflict of interest

The authors declare no competing interest.

Data Availability Statement

All relevant data are within the manuscript and its Supporting Information files. In addition, sounds could be listened at FishSounds and Unicamp (https://www2.ib.unicamp.br). Some specimens are available at the collections of the Functional and Evolutive Morphology from the University of Liège (vouchers n°Hypostomus_001, 002, 003, 004, 005, 006 and 007).

Author contributions

XR designed the study. XR and GK collected the specimens. XR recorded the sounds. AK analysed the acoustic data under the supervision of XR. XR and AK interpreted the acoustic data. EP performed 3D reconstructions and studied the musculature. XR and AK compared dorsal processes. GK and AG provided resources. CZ, XR and AK worked on the species identification. AK, XR and EP wrote the original draft. XR and EP wrote the last version of the manuscript. The manuscript was corrected and implemented by GK, AG and CZ. XR and EP supervised the whole project.

References


http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp


Howard J (2019) Fish Biology and Fisheries. Verlag, Waltham Abbey

Kaatz IM (1999) The behavioral and morphological diversity of acoustic communication in a clade of tropical catfishes (Superfamily Ariodei). College of Environmental Science and Forestry - SUNY, Syracuse, NY


Ladich F (1997b) Comparative analysis of swimbladder (drumming) and pectoral (stridulation) sounds in three families of catfishes. Bioacoustics 8:185–208

Ladich F (2022) Shut up or shout loudly: Predation threat and sound production in fishes. Fish Fish 23:227–238


Owings DH, Rowe MP, Rundus AS (2002) The rattling sound of rattlesnakes (Crotalus viridis) as a communicative resource for ground squirrels (Spermophilus beecheyi) and burrowing owls (Athene cunicularia). J Comp Psychol 116:197–205
Parmentier E, Diogo R, Fine ML (2017a) Multiple exaptations leading to fish sound production. Fish Fish 18:958–966


Sörensen W (1898) Some remarks on Dr. Thilo’s memoir on “Die Umbildungen am den Gliedmassen der Fische.” Morphol Jahrb 25:170–189

Sweet SS (1985) Geographic Variation, Convergent Crypsis and Mimicry in Gopher Snakes (Pituophis melanoleucus) and Western Rattlesnakes (Crotalus viridis). J Herpetol 19:55


Vallone ER, Vezzosi RI, Cione AL (2017) First fossil fish (Teleostei, Siluriformes) from the Late Pleistocene of Santa Fe Province, Argentina. Alcheringa An Australas J Palaeontol 41:369–377


Zawadzki CH, Oyakawa OT, Britski HA (2017) Hypostomus velhochico, a new keeled Hypostomus Lacépède, 1803 (Siluriformes: Loricariidae) from the rio São Francisco basin in Brazil. Zootaxa 4344:560


Zawadzki CH, Tencatt LFC, Britski HA (2020) Taxonomic revision of Hypostomus albopunctatus (Siluriformes: Loricariidae) reveals a new piece of the Hypostomus jigsaw