Reduced geographic variation in roars in different habitats rejects the acoustic adaptation hypothesis in the black-and-gold howler monkey (*Alouatta caraya*)

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**Abstract**

Vocalizations used for long-range communication must disperse without significant structural changes to be decoded by receivers. The acoustic adaptation hypothesis (AAH) holds that, since acoustic signals are influenced by the habitat in which they disperse, sounds will possess specific structural characteristics to diminish sound degradation. Additionally, vocalizations can also be influenced by genetics, anatomy, and/or cultural aspects. Here, we tested the AAH through quantitative comparisons of roars in four allopatric populations of the black-and-gold howler monkey (*Alouatta caraya*) across an environmental gradient from open to closed, in northeastern Argentina. At each site, we obtained good-quality recordings from three adult males, also between July and November 2013, conducted vegetation surveys (measuring tree density, canopy closure, and vertical structure), evaluated potential masking of roars by gathering environmental sound samples, and assessed sound attenuation of a synthetic tone at three different distances: 10 m (landmark reference distance), 50, and 100 m. We also tested the alternative hypothesis that acoustic properties of roars could be explained by population genetic divergence. Our results did not support the AAH. Although our four study sites were significantly different in vegetation structure, conforming to an open-to-closed gradient, roars of *A. caraya* were not different among populations. Likewise, although environmental sound differed between sites, we found no evidence of environmental sound affecting the acoustic properties of roars. The attenuation of the synthetic tone was only near significant at 100 m distance between both extreme sites from the environmental gradient. The four *A. caraya* study populations grouped into three genetically differentiated clusters. Since roar features were independent from population genetic clustering, we reject the genetic hypothesis too. The combination of high amplitude and low peak frequency of roars, coupled to small home range size and extensive overlap between neighboring groups, allows roars to keep their communication value across habitats without need of specific environmental tuning.

**KEYWORDS**

acoustic adaptation hypothesis, black-and-gold howler monkey, environmental sound, genetics, long-distance calls
**INTRODUCTION**

Vocalizations used by senders for long-distance communication must be able to traverse the medium without significant structural changes in order to be unequivocally decoded by receivers. During its transmission, decomposition of a sound may occur mainly due to natural phenomena such as air turbulence, extreme temperatures, and environmental humidity, as well as due to vegetation absorption and rebounds against it (Wiley & Richards, 1978). Thus, the successful propagation of a sound used for communication depends on a well-balanced combination between signal structure and habitat characteristics. The acoustic adaptation hypothesis (AAH) holds that, since acoustic signals are influenced by the habitat in which they disperse (Morton, 1975; Gish & Morton, 1981; Wilkins, Seddon, & Safran, 2013, reviewed in Ey & Fisher, 2009), sounds will possess specific structural characteristics allowing them to diminish sound degradation over distances (Gish & Morton, 1981). Specifically, the AAH predicts that calls emitted in closed habitats (e.g., dense forest) will concentrate their energy at lower frequencies and will have less frequency modulated and longer elements emitted at lower repetition rates, than sounds broadcasted in more open ones (e.g., grassland; Morton, 1975; reviewed in Boncoraglio & Saino, 2007; Ey & Fisher, 2009). Additionally, a propagating sound can experience interference by other environmental sounds, such as birds, insects, and wind (among others), that could potentially mask the signal of interest (Brenowitz, 1982; Ryan & Brenowitz, 1985; de la Torre & Snowdon, 2002; Waser & Brown, 1986). If environmental sound masking is an important pressure for long-range communication, vocalizations are expected to occur at frequency ranges that are relatively quiet (i.e., not occupied by other sounds; Goodwin & Podos, 2013; de la Torre & Snowdon, 2002).

The AAH has been tested through a wide range of scenarios, either within species by analyzing calls from the same species in two or more different habitats (in birds: Slabbekoorn & Smith, 2002; Kirschel et al., 2011, sea lions: Ahonen, Stow, Harcourt, & Charrier, 2014 and frogs: Velásquez, Moreno-Gómez, Brunetti, & Penna, 2018) or among species living in different habitats (in whistling rats: le Roux, Jackson, & Cherry, 2002; singing mice: Campbell et al., 2010; marmots: Daniel & Blumstein, 1998; felids: Peters & Peter, 2018).

**FIGURE 1** (a) Location of the four study sites where sound recordings of black-and-gold Howler Monkeys (*Alouatta caraya*) and environmental sounds were obtained and sound transmission experiments performed. (b) Plot of ancestry membership proportion in the four populations of *A. caraya*. Individuals are represented with vertical bars, separated into color segments, corresponding to their membership coefficient to each cluster (K = 3). Sampled populations are separated with white dashed lines. Equally color populations share genetic ancestry (modified from Oklander et al. 2017) [Colour figure can be viewed at wileyonlinelibrary.com]
across habitats without the need of specific environmental tuning. Living in different habitats, or whether roars can keep their structure, the fine-scale structure of these vocalizations differs among populations to shape the structure of long-distance signals (AAH), whether the signal would be affected by ecological factors traditionally believed (da Cunha et al., 2015). However, it remains unknown whether this frequency range (emphasized frequencies between 200 and 700 Hz, 5 m from the source; Whitehead, 1989), they occur in the low-frequency (in primates de la Torre & Snowdon, 2009; Briseño-Jaramillo, Estrada, & Lemasson, 2015, and bats Xie et al., 2017), and genetic drift (in primates Wich, Schel, & Vries, 2008 and microhyld frogs: Lee, Shaner, Lin, & Lin, 2016).

Howler monkeys (Alouatta spp.) perform extremely powerful long-distance roars that are used in intergroup communication (Holzmann, Agostini, & Di Bitetti, 2012; Whitehead, 1987) and can travel ca. 1 km (Baldwin & Baldwin, 1976). These potent low-frequency roars are considered among the loudest vocalizations produced by a terrestrial mammal (da Cunha, Oliveira, Holzmann, & Kitchen, 2015; Dunn et al., 2015), and howlers exhibit a highly modified larynx and an enlarged hyoid bone that acts as a resonance chamber thereby amplifying roars (da Cunha et al., 2015; Kelemen & Sade, 1960). Howler roars appear to be well adapted for long-range communication, because in addition to their power (90 dB SPL at 5 m from the source; Whitehead, 1989), they occur in the low-frequency range (emphasized frequencies between 200 and 700 Hz, da Cunha et al., 2015). However, it remains unknown whether this signal would be affected by ecological factors traditionally believed to shape the structure of long-distance signals (AAH), whether the fine-scale structure of these vocalizations differs among populations living in different habitats, or whether roars can keep their structure across habitats without the need of specific environmental tuning.

The black-and-gold howler monkey (Alouatta caraya) occupies a wide geographic range in South America and occupies structurally different habitats that may impose distinctive challenges for communication over long distances, including closed subtropical moist forests, dry forests, and semi-open forest patches surrounded by grasslands (Holzmann et al., 2015). No subspecies have been recognized on morphological and genetic grounds (Cortés-Ortiz, Rylands, & Mittermeier, 2015), although population genetic studies show the existence of geographic structuring in clusters based on nuclear genetic diversity (Oklander, Miño, Fernández, Caputo, & Corach, 2017). Vocalizations in howlers are thought to be genetically determined, and to this day, there is no definitive evidence of vocal learning in non-human primates (reviewed in Egnor & Hauser, 2004; but see Ghazanfar, Liao, & Takahashi, 2019).

In this paper, we quantified the acoustic structure of roars in four allopatric populations of A. caraya across an environmental gradient from semi-open to closed habitats, obtained environmental sound profiles to evaluate environmental masking and performed sound transmission experiments of a synthetic sound to assess whether ecological selection (adaptive process) could drive geographic variation in roars. Also, since data from a previous study (Oklander et al., 2017) are available, we also tested the alternative hypothesis that geographic variation in roars is driven by patterns of neutral genetic divergence between these four howler monkey populations (Lee et al., 2016; Wilkins et al., 2013).

1.1 Hypotheses and predictions

The AAH predicts that in a widespread species using different allopatric habitats, there should be geographic variation in vocalizations, and the vocal features of the different populations would be suited to increase transmission of the sound in the corresponding habitat. If roars of A. caraya show geographic variation due to acoustic adaptation to local conditions (related to habitat structure), we expected to observe a higher peak frequency, higher bandwidth, and frequencies at which the 25% and 75% of the global energy were contained and shorter element duration in the population living in semi-open habitats, in comparison with populations inhabiting in more closed habitats, or alternatively, to find a gradient of differences from the semi-open study site to the more closed ones.

Regarding environmental sound, we expect roars to concentrate their energy in quiet portions of the acoustic space that are not much used by other species for long-range communication or less occupied by abiotic sounds such as wind, in each type of habitat, to avoid sound masking. Regarding the expected attenuation in relation to vegetation structure, we predict higher frequencies of the artificial tone to attenuate more in closed habitats or alternatively, lower frequencies to attenuate less in closed ones.

If roars show geographic variation due to the genetic makeup of different populations, we expect to observe significant differences in roars between populations belonging to different genetic clusters and no differences between populations from the same genetic cluster. If this is the case, expected differences may not be adaptive and roar structure may thus be independent from environmental features (habitat structure and environmental sound).

2 METHODS

2.1 Study sites

We worked at four sites with naturally occurring populations of A. caraya in northeastern Argentina (Figure 1a). These four study sites were selected because they can be placed in a gradient from semi-open to closed vegetation and because we had genetic information from their respective populations of A. caraya. From semi-open to closed, our sites were as follows: 1—Estación Biológica Corrientes (EBCo, 27°30′S; 58°41′W), composed mainly of open forest patches separated by grasslands. This is the only site where howler monkey roars need to travel through open grasslands to reach groups inhabiting other forest patches, 2—Parque Nacional Chaco (PN Chaco,
26°48′S; 59°36′W), a riverine forest along the Negro River, 3—Reserva privada Guaycolec (Guaycolec 25°54′S; 58°13′W), a tall and dense riverine forest along the Pilagá River, and 4—Reserva de Vida Silvestre Yacutinga (Yacutinga 25°35′S; 54°03′W), a typical dense and closed subtropical forest. The genetic structure of the four *A. caraya* study populations demonstrated three genetically differentiated clusters: (K1) EBCo, (K2) PN Chaco and Guaycolec, and (K3) Yacutinga (Oklander et al., 2017; Figure 1b).

### 2.2 Howler monkey recordings and acoustic analyses

Between June and December 2012, we obtained high-quality recordings from three different adult males (belonging to different groups), calling alone in each site. We obtained all recordings using a Sennheiser ME-67 directional microphone and a Marantz PMD-661 digital recorder, set to record at a sample rate of 44.1 Hz and a resolution of 16 bits. The duration of each recording was at least 1 min, and the distance to the vocalizing male was never more than 15 m (Macej, Fischer, & Hammerschmidt, 2011). Howler monkeys of the same group typically howl together synchronously forming a chorus with other males and females (Holzmann et al., 2012; Kitchen, 2004; Van Belle, 2015); this precluded us from obtaining a higher number of high-quality recordings from males vocalizing alone. Howler monkey density is very low at Yacutinga and in other areas from the argentine Atlantic Forest (Agostini, Holzmann, & Di Bitetti, 2008). Since at Yacutinga we were able to obtain a single recording from an adult male, we completed our dataset by adding recordings of two different males at El Piñalito Provincial Park (26°30′S, 53°50′W), 105 km SE from Yacutinga. Both areas share the same general vegetation type (dense Atlantic Forest, Holzmann et al., 2015), and howler monkeys belong to the same genetic population cluster (Oklander et al., 2017).

Howler monkey roars are composed of respiratory cycles with two sections: an exhalatory section and an inhalatory one (da Cunha et al., 2015, Figure 2a). During a roaring bout, these cycles become shorter in duration, faster, and louder until they reach a “climax” in amplitude (da Cunha et al., 2015). The climax was excluded from our analyses because it is characterized by deterministic chaos which

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**FIGURE 2** (a) Spectrogram of an *A. caraya* roar showing the respiratory cycle composed of the exhalatory (Exh.) section and the inhalatory (Inh.) one as well as the measurements performed in this study: duration (s), peak frequency (Hz), bandwidth (max. frequency-min frequency, Hz), frequency quartile 25% (Hz), and frequency quartile 75% (Hz). (b) Three spectrograms of the artificial tone broadcasted and recorded at the 10 (reference landmark), 50, and 100 m away from the source. The nine frequency bands (arrows from 164 to 2,016 Hz) over which the amplitude was measured can be seen across the three spectrograms. [Colour figure can be viewed at wileyonlinelibrary.com]
makes measurements very difficult even in high-quality recordings (Dunn et al., 2015). For each 1-min recording of howls, we isolated eight good-quality respiratory cycles, and each of these cycles was considered as “elements” for measurements. We were very cautious in selecting typical respiratory cycles (not close to the climax section), because of possible differences in duration of cycles near this section. We took acoustic measurements using the Automatic Parameter Measurement tool in Avisoft-SASLab Pro 5 (R. Specht). Elements were delimited by manually adjusting the element separation threshold for each respiratory cycle until we achieved a complete detection. Within the element separation box, we selected the automatic (two threshold) option using default parameters for the start/end threshold (~20 dB) and the hold time (20 ms). Measurements were taken at the center of each element (respiratory cycle) using spectrograms built with a Hamming window type, FFT length of 1,024 samples, frequency resolution of 43 Hz, and time resolution of 11.6 ms. In each of the eight respiratory cycles, we measured one temporal parameter (duration, s) and four spectrum-based parameters: peak frequency (Hz), bandwidth (max. frequency - min. frequency, Hz), and 25% and 75% quartiles (frequencies at which the 25% and 75% of the global energy were contained, Hz; Figure 2a). All original recordings analyzed here are archived in the Macaulay Library of Natural Sounds (Ithaca NY, http://macaulaylibrary.org).

Differences between the four study sites in relation to each of the five acoustic parameters measured were analyzed through two-way repeated-measures ANOVA. We also report partial eta square ($\eta^2_p$) values for each acoustic variable, as a measure of the effect size (magnitude of the difference between compared groups). Partial eta square ($\eta^2_p$) varies from 0 to 1, and its interpretation is similar to the coefficient of determination ($r^2$) and could be interpreted as the proportion of the dependent variable explained by the independent one; for example, 0.04 would indicate a small effect size, 0.25 a medium effect, and 0.64 a large effect (Sullivan & Feinn, 2012).

All statistical tests in this study were performed using GraphPad Prism, at a significance level of 0.05.

### 2.3 Habitat structure and acoustic environment characterization

Following Ey (2008), we characterized three different aspects related to sound degradation over distance at each site: (a) habitat structure (vegetation), (b) environmental sound, and (c) sound transmission.

#### 2.3.1 Habitat structure: vegetation survey

In each study site, we set 12 straight-line transects measuring 50 m long by 20 m wide. In EBCo, six of these transects were placed within forest patches and six within grasslands to capture the heterogeneity of conditions through which roars must disperse. In the rest of the study sites, all vegetation transects were placed within the forest (where roars disperse). We took three measurements to characterize habitat structure: (a) tree density, (b) canopy closure, and (c) vertical structure. We estimated tree density by measuring all trees with DBH ≥ 10 cm on each transect. Canopy closure was measured at four different points along each transect (0, 16, 33, and 50 m) using a spherical densiometer (Paletto & Tosi, 2009). We sampled vertical structure at the same four points (0, 16, 33, and 50 m) on each transect, by holding up a 2-m telescopic rod (marked at 1-m intervals and extensible up to 15 m) and recording the number and height of contacts of woody vegetation on the rod (Lopez de Casenave, Pelotto, Caziani, Mermozand, & Protomastro, 1998; Trigo et al., 2017). Although tree height varied between sites from around up to 20 m in Yacutinga (pers. obs.) to 12 m in EBCo (Brividoro, Kowalewski, Scarry, & Oklander, 2019), howlers use mostly the middle strata (11-18 m in Yacutinga and 8-10 m in the rest of the sites), and so, the height of our vertical structure measurements (up to 15 m) captured the biologically relevant vegetation structure. We chose to measure vertical structure instead of other classic methods such as “visibility” (Ey, 2008; Hedwig, Mundry, Robbins, & Boesch, 2015), because vertical structure provides a better characterization of the vegetation objects (leaves and branches) that can affect sound transmission at heights where arboreal primates move and vocalize. To capture seasonal changes in vegetation, we sampled canopy closure and vertical structure in winter (July–August) and again in spring (October–November) of the same year (2013). Tree density (DBH ≥ 10 cm) is not expected to change through the year and hence was measured only once.

We compared tree density, canopy closure, and vertical structure between the four study sites with Kruskal-Wallis tests. Differences between winter and spring within each site in canopy closure and vertical structure were evaluated using the Wilcoxon matched-pairs tests.

#### 2.3.2 Acoustic environment characterization: environmental sound

We established six line transects to perform sound transmission experiments in each study site (see below). At the beginning of each of these transects, we obtained a 3-min recording of environmental sound composed of three 1-min recordings, each obtained pointing to one of three different directions (north, south, and up). We obtained all recordings at a sample rate of 44.1 Hz and a resolution of 16 bits using the exact same gain (recording volume) in all recordings. We repeated these environmental sound recordings at two different times of the day, during which howlers are more likely to roar: early in the morning (between 6:00 and 9:00) and late in the afternoon (between 16:00 and 19:00). To capture seasonal differences, we performed this procedure in winter (July–August) and again in spring (October–November) of the same year (2013). The aim of these recordings was to build environmental sound profiles (mean peak amplitude vs. frequency graphs; Bradbury and Vehrencamp 2011) of our study sites to assess frequency distribution of background noise and evaluate whether environmental sound can mask a propagating roar. To build these profiles, we first extracted nine 1-s samples from each 3-min recording. We then used Raven 1.4 (Cornell University) to build spectrograms from each 1-s sample (Hamming window type, a FFT length of 1,024 samples, a frequency resolution of 43 Hz, and time resolution of 11.6 ms).
of 43 Hz, and a time resolution of 11.6 ms). In each spectrogram, we applied the Split all selection borders function and split the entire frequency range (from 150 Hz up to 8,000 Hz) in intervals of 50 Hz. For all these 50-Hz intervals, the peak amplitude (dB) was automatically measured. Each environmental sound profile (one per study site) was carefully inspected to detect background noise in overlapping frequencies of a roar. We used the Wilcoxon matched-pairs test to evaluate differences in the mean peak amplitude (dB) between mornings and afternoons within each season, as well as between winter and spring within each site.

We also evaluated between-site variation through a Kruskal-Wallis test to assess differences in the mean peak amplitude (dB) of environmental sound within the frequency range of howler roars (150–1,000 Hz).

2.3.3 | Acoustic environment characterization: Sound transmission

We performed sound attenuation experiments (Ey, 2008; Slabbekoorn & Smith, 2002) to learn how an artificial sound changed when traversing each of the four types of habitats used by A. caraya. This artificial sound (built with Sonic Solution Sound Blade Digital Audio Workstation) was composed of nine pure tones (frequency bands from 164 Hz to 2,016 Hz), most of them within the natural frequency range of an A. caraya roar. This artificial sound was built to simulate a real howler monkey roar, both in intensity in order to be detected and recorded at the end of the transect, and in structure, whereby each frequency band possessed its own individual amplitude, so that the artificial sound tone has similar amplitude envelope to a real A. caraya roar.

To measure sound attenuation in amplitude over distance, we set six 100-m-long straight-line transects in each study site. We hung up a speaker at heights frequently used by howlers to move and vocalize and broadcasted our custom-made artificial sound in an amplitude similar to that of a howler monkey roar: 90 dB SPL at 5 m distance from the source (Whitehead, 1989). On each transect, we recorded 1 min of this tone at three different distances from the speaker: 10 m (established as a landmark reference distance without sound attenuation), 50, and 100 m (Figure 2b). We obtained all recordings using a Sennheiser ME-67 directional microphone and a Marantz PMD-661 digital recorder at a sample rate of 44.1 Hz and a resolution of 16 bits, always using the exact same gain (recording volume). We repeated this procedure at two different times of the day, during which howlers are more likely to roar: early in the morning (between 6:00 and 9:00) and late in the afternoon (between 16:00 and 19:00). Also, to capture seasonal differences in sound transmission due to changes in vegetation, we performed these experiments in winter (July/August) and again in spring (October/November) the same year (2013). The aim of these recordings was to obtain sound attenuation profiles (mean amplitude difference vs. frequency graphs) for the artificial sound in each study site. To build these profiles, we first extracted three 1-s segments from each recording, avoiding parts with excessive background noise (generally birds and insects) that could interfere with precise measurements of each frequency band and built a power spectrum (an amplitude vs. frequency graph with the following parameters: Hamming window type, bandwidth: 1,300 Hz, and resolution: 0.67 Hz), using Avisoft-SASLab Pro 5 (R. Specht). Then, in each power spectrum, we measured the amplitude (dB) at each of the nine frequency bands in the recordings at 10, 50, and 100 m from the source (Figure 2b). Finally, the difference between the mean amplitude of a frequency band recorded at 10 m from the loudspeaker and the amplitude of the same frequency band recorded at 50 and 100 m from the loudspeaker was plotted as a function of frequency (Ey, 2008; Slabbekoorn & Smith, 2002).

Differences in sound attenuation (mean amplitude difference, dB) of the artificial sound between the four study sites were evaluated through a one-way ANOVA. We also used paired t tests to evaluate differences between mornings and afternoons within each season as well as between winter and spring within each site.

3 | RESULTS

3.1 | Similar roar structure between sites

Roar structure of A. caraya males was not different between sites, since none of the five acoustic parameters were significantly different between populations (Table 1).

**TABLE 1** Acoustic characterization of roars of black-and-gold Howler (Alouatta caraya) at an environmental gradient from semi-open (EBCo) to closed (Yacutinga) habitats

<table>
<thead>
<tr>
<th></th>
<th>Duration (s)</th>
<th>Peak Frequency (Hz)</th>
<th>Bandwidth (Hz)</th>
<th>Quartile 25% (Hz)</th>
<th>Quartile 75% (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBCo</td>
<td>1.6 ± 0.11 (3)</td>
<td>291.2 ± 29 (3)</td>
<td>919.5 ± 77.6 (3)</td>
<td>336.6 ± 23.3 (3)</td>
<td>768.3 ± 126.4 (3)</td>
</tr>
<tr>
<td>PN Chaco</td>
<td>1.2 ± 0.5 (3)</td>
<td>309.1 ± 28.7(3)</td>
<td>1,028.7 ± 94 (3)</td>
<td>317 ± 48.4 (3)</td>
<td>817.9 ± 134.2 (3)</td>
</tr>
<tr>
<td>Guaycolec</td>
<td>1.4 ± 0.6 (3)</td>
<td>318.3 ± 54.5 (3)</td>
<td>1,025.4 ± 82.8 (3)</td>
<td>340.4 ± 30.1 (3)</td>
<td>813.3 ± 101.1 (3)</td>
</tr>
<tr>
<td>Yacutinga</td>
<td>1.5 ± 0.1 (3)</td>
<td>278.3 ± 41.4 (3)</td>
<td>988.7 ± 61.5 (3)</td>
<td>307.5 ± 28 (3)</td>
<td>666.6 ± 149.1(3)</td>
</tr>
</tbody>
</table>

F value 0.65  1.17  1.09  1.22  0.77

df 21, 56  21, 56  21, 56  21, 56  21, 56

p value .85  .31  .37  .26  .73

η² (effect size) .01  .30  .29  .31  .22

Note: Data reported as x ± SD (sample size: number of recordings per site). Results from the two-factor repeated measures ANOVA between sites are also indicated. Effect size is reported as partial eta square (η²): 0.04, 0.25, and 0.64 (small, medium, and large effect sizes).
3.2 | Geographic variation in habitat structure

The four sites showed significant differences in tree density and canopy closure, shaping the expected gradient from semi-open to closed habitats, from EBCo to PN Chaco, Guaycolec, and Yacutinga (tree density: $H_{(3)} = 39.6; p < .0001$, and canopy closure $H_{(3)} = 37.3; p < .0001$; Figure 3a,b). For both measurements, post hoc Dunn’s multiple test signaled significant differences between EBCo with Guaycolec and Yacutinga as well as between PN Chaco with Yacutinga. Contrary to our expectations, the vertical structure was very similar between the four study sites ($H_{(3)} = 5.2; p = .15$; Figure 3c) and only EBCo differed from PN Chaco by a lower number of vegetation contacts between 7 and 10 m ($H_{(3)} = 7.6; p = .02$; Figure 3d). Habitat structure remained unchanged between seasons at all sites: We did not find significant differences in canopy closure and vertical structure between winter and spring (Table S1 as Supporting information).

3.3 | Geographic variation in environmental sound

Careful inspection of environmental sound profiles revealed similarities and differences between sites (Figure 4). Dogs, thunder, engines, chainsaws, cows, and doves— as well as frogs ($Physalaemus$ sp.), were recorded low in the frequency range, matching the most of the energy from a roar (below 1,000 Hz). Wind was recorded in all study sites (below 1,000 Hz) as well as howler monkey roars (recorded in six different occasions; all in the morning). In the case of Guaycolec, a site located near a highly used national road, the buzz of vehicles was recorded several times (around 500 Hz). However, most biotic sound sources were birds (mainly between 2,000 and 4,000 Hz) and insects (mainly between 4,000 and 7,000 Hz), which produced sounds relatively high in the frequency spectrum (Figure 4). The EBCo was the noisiest site with environmental sound distributed more homogeneously within the acoustic space (only with a “quieter” portion around 1,000–2,000 Hz) while the other three sites showed less environmental sound, with peaks of energy between 4,000 and 7,000 Hz. Within-site results show that mornings in spring at all sites except, PN Chaco, had significantly higher environmental noise than afternoons (Table 2 and Table S2a as Supporting information for statistical tests results). Spring was significantly noisier than winter at all study sites except EBCo (Table 2 and Table S2b as Supporting information for statistical test results).

Between-site comparisons of environmental sounds (mean peak amplitude) within the frequency range of roars (150–1,000 Hz) showed significant differences ($H_{(3)} = 12.8, p < .005$, Figure 5). Specifically, Dunn’s multiple comparison test indicated that EBCo (mean peak amplitude = 70.8 ± SD2.7 dB) and Guaycolec (mean peak amplitude = 66.7 ± SD2.09 dB) were significantly different.

3.4 | Similar sound transmission between different habitats

Transmission of the artificial sound did not differ significantly between sites at 50 m ($F_{(3,32)} = 2.38; p = .08$) or 100 m ($F_{(3,32)} = 2.89; p = .05$; Figure 6) with respect to 10 m. However, we found a borderline case ($p = .05$) in which the artificial tone (particularly at higher frequencies above 469 Hz. Figure 6) was more attenuated in Yacutinga than in EBCo at 100 m from the speaker. This result suggests a potential trend of higher frequencies to attenuate more in the closed habitat, as predicted. Our findings showed no significant differences between mornings and afternoons, as well as between winter and spring, within each site with some exception for PN Chaco (Table S3a–d as Supporting information).

FIGURE 3 Vegetation structure in the four study sites where $A. caraya$ roars were recorded. (a) Tree density, (b) canopy closure, and (c) vertical structure. Tree density (a) and canopy closure (b) showed significant differences between sites conforming the expected gradient from open (EBCo) to close (Yacutinga) habitat. Vertical structure (c) showed no significant differences between sites. (d) Distribution of vertical structure per height in each study site showing only significant differences between EBCo and PN Chaco at heights between 7 and 10 m. [Colour figure can be viewed at wileyonlinelibrary.com]
Results from our study did not support the AAH. Geographic variation in roars was not observed between populations despite strong differences in vegetation structure. Sites were also different in environmental sound in the frequency range that overlapped the roars. Together, these findings reinforce the hypothesis that habitat is not a selective force for this acoustic signal. Likewise, since roar features were also independent from population genetic diversity, we reject the possibility that neutral genetic selection could exert a strong influence on long-distance calls of this howler monkey species.

We are aware that our relatively small sample size per site (three males) could have potentially limited our ability to detect differences in roars between populations of *A. caraya*. However, the small-to-medium effect sizes reported here and the remarkable consistency of the acoustic parameters measured, indicate that roars are highly stereotyped vocalizations suggesting that increasing sample size would not alter our results.

### 4.1 | An invincible cocktail for long-range communication

The AAH has received mixed support in mammals, because results do not backup its predictions in most of the cases (reviewed in Ey & Fisher, 2009; Peters & Peters, 2010). No support for the AAH was found in a comparative study of ten howler monkey species, including *A. caraya* (Dunn et al., 2015). There, the lack of association between hyoid bone volume and habitats with different net primary productivity was interpreted as indicating that the frequency of howler roars would be independent from their habitats (Dunn et al., 2015). Our results showing no geographic variation in the structure of *A. caraya* roars among sites differing in vegetation structure (tree density and canopy closure) and environmental sound, indicate that habitat is not an important selective force for this acoustic signal and opens the door to other interpretations.

Howler roars function in infant, mate, and/or territory defense (reviewed in Kitchen, da Cunha, Holzmann, & Oliveira, 2015) and are mostly directed to conspecifics, solitary individuals, or group residents, especially neighbors. A negative relationship between frequency and home range size has been proposed to exist in primates, showing that species with larger home ranges give comparatively lower-frequency long-distance calls (Mitani & Stuht, 1998). However, howler monkeys may not fit adequately in this general trend, since we now know that howlers own small home ranges in comparison with other Atelinae (reviewed in Barbisan Fortes, Bicca-Marques, Urbani, Fernández, & Silva Pereira, 2015). A recent review from ten different study sites in Brazil and Argentina showed that the mean home range size of *A. caraya* is 11.1 ha ($\overline{SD} = 23.3$ ha; range: 0.7–93.8 ha), and in most of the cases, neighboring groups exhibit high spatial overlap ($\times 38 \overline{SD} = 26\%$; Barbisan Fortes et al., 2015). Although there are no formal quantifications of the distance that a roar can effectively travel in nature, it is for sure at least ca 1 km (Baldwin & Baldwin, 1976). The small home range and the high degree of spatial overlap between neighboring groups suggest that the high amplitude of these signals of *A. caraya* can easily exceed the home range limits and reach distant groups.

The existence of “sound windows” (a range of frequencies that propagates farther in a given habitat) is debated, but a classical study signaled the possibility of a sound window around 200 Hz in forests

**FIGURE 4** Environmental sound profile (mean peak amplitude, dB) of each study site, including all day times and seasons, and frequency (Hz), showing the distribution of environmental sound energy within the entire frequency range (150–8,000 Hz) [Colour figure can be viewed at wileyonlinelibrary.com]
The mean peak frequency in the A. caraya individuals analyzed here was 299.2 Hz (SD ± 42.2, Table 1, see also da Cunha et al., 2015 for similar results) which is among the lowest frequencies reported in primates (Mitani & Stuht, 1998). Thus, most of the energy of roars of A. caraya occurs within a possible sound window which might help roars to propagate farther.

Our sound attenuation experiments show that the transmission of an artificial sound did not significantly vary between study sites at distances of 50 and 100 m from the source, despite marked differences in vegetation structure. Previous studies also have found similar sound transmission in habitats with significant differences in vegetation structure (Shy & Morton, 1986; Slabbekoorn & Smith, 2002), making evident that sound transmission between habitats is complex and not always aligned with what is expected by the theory. However, our artificial sound showed almost statistically significant greater attenuation of higher frequencies at the site with the most closed vegetation (Yacutinga) in comparison with the most open one (EBCo). Thus, it is possible that our habitats may differ in its transmission properties, but that this effect would be more apparent at distances beyond 100 m from the sound source.

In summary, the combination of high amplitude and low peak frequency of roars coupled to the small distance and extensive overlap between neighboring groups seems to naturally indicate that howlers possess an invincible cocktail for an effective long-range communication despite their occurrence in structurally contrasting habitats.

### 4.2 Environmental sound

Howler monkeys produce roars during group encounters at any moment of the day (Holzmann et al., 2012; Hopkins, 2013), but spontaneous roars (directed to out of view groups) occur most frequently at dawn or during the morning, when air conditions and temperature would improve sound propagation (da Cunha et al., 2015; Wiley & Richards, 1978). Our results indicate that, at almost
all study sites, spring mornings had higher levels of environmental sound than afternoons (mostly from birds and insects). These results are in concordance with other studies reporting noisy mornings in which many species wake up due to acoustical communication (Luther & Wiley, 2009). We captured roars from howlers during our environmental sound sampling in six different opportunities, all of them very early in the morning, reinforcing the idea that A. caraya also uses this time of the day as a time window when atmospheric conditions enhance sound propagation. We found no support for the hypothesis that environmental sound could interfere with propagating roars. Roars did not occupy quiet portions of the acoustic space, less occupy by other environmental sounds. Although it is true that most birds and insects vocalize at much higher frequencies than A. caraya, howler roars extensively overlap their energy with other environmental noises in the lower portion of the frequency spectrum (below 1 kHz, Figure 4; Tobias et al., 2010 for similar results). Previous studies supported an influence of environmental sound when the signal of interest overlapped with quieter portions of the acoustic space, to avoid or reduce sound masking and interference with other surrounding noises (Ryan & Brenovitz, 1985; Slabbekoorn, 2004; Slabbekoorn & Smith, 2002). Probably, amplitude from low-frequency sound sources found in our study (doves, dogs, caws, frogs, etc.) is unable to mask a sound with the amplitude like of the roars examined, even if all signals use similar frequency ranges. Moreover, despite significant differences in the low frequency of environmental sound (below 1 kHz) between sites, roar structure remained stable among populations, indicating that environmental sound does not drive geographic variation in roars. A particular abiotic sound, wind, deserves closer attention. All our study sites had considerable amount of wind and/or noise made by wind, with frequencies below 1 kHz. Since wind is an important source of sound deflection (Ryan & Brenovitz, 1985; Wiley & Richards, 1978), subsequent studies should determine whether wind can mask a propagating roar at relatively long distances. Masking might cause difficulties in the detection and discrimination of crucial information such as the number of roaring individuals and/or group composition, and could reduce the effective range of communication in these primates. Similar attention should be afforded to the buzzing sounds of vehicles in heavily used routes near howler territories.

4.3 | Vocal uniformity despite genetic clustering

Here, we explored the alternative hypothesis that genetic divergence between populations could be responsible for differences in roar structure. Previous studies identified geographic distance and/or the presence of barriers such as rivers and mountains, which can prevent gene flow, as causes promoting geographic variation in calls (Bernal, Guarnizo, & Lüddeke, 2005; Irwin, Thimgan, & Irwin, 2008; Keighley, Langmore, Zdenek, & Heinsohn, 2017; Lee et al., 2016; Wich et al., 2008). In these cases, a clear correlation between genetic distance and vocal divergence between different populations was found. Previous studies in primates found geographic variation in calls, probably due to the lack of gene flow between populations (langurs: Wich et al., 2008 and sportive Lemurs: Méndez-Cárdenas, Randrianambinina, Rabesandratana, Rosoloharjioana, & Zimmermann, 2008). This was not the case in our study system. Instead, although our four study populations can be assigned to three well-differentiated genetic clusters that are connected or isolated by wide rivers (Figure 1a,b; Ascunce, Hasson, Mulligan, & Mudry, 2007; Oklander et al., 2017), roar structure did not vary between these populations. Further research is needed, particularly in mammals with genetically encoded vocalizations, to elucidate the degree to which genetic changes permeate into the acoustic structure of long-distance signals.

To conclude, this is the first study designed to test environmental selection (AAH and environmental sound), as well as neutral selection (genetic diversity between populations), in a howler monkey species, and our analysis reveals no geographic variation in long-distance calls of A. caraya despite differences between sites in vegetation structure (gradient from open to closed habitats) and environmental sound as well as in genetic structure. Further studies needed to deepen our understanding of vocal adaptation for long-range communication in these primates include comparing sound transmission properties of open and closed habitats at distances beyond 100 m, as well as a properly designed assessment of how far a real howler roar can travel and be heard, under different natural situations.

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CONFLICT OF INTEREST

None.

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