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Individual variation in the booming calls of captive Horned Guans (*Oreophasis derbianus*): an endangered Neotropical mountain bird

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ABSTRACT

The Horned Guan (*Oreophasis derbianus*) is a cracid restricted to cloud forests in the Sierra Madre of Chiapas in Mexico and the western-central Mountains in Guatemala. It is an endangered species and urgent conservation measures are required, such as non-invasive monitoring techniques. Here, we study individual features in the boom calls of Horned Guans. Boom calls are acoustic signals used by males during courtship and territorial displays. This call is made of seven notes, divided into two parts: an introductory section characterized by low-amplitude notes and a body section characterized by high-amplitude notes. We recorded 10 males during the breeding seasons of 2010 and 2011 in two captive populations and measured 22 acoustic variables of the calls. We used a combination of statistical analyses to test individuality in Horned Guan vocalizations. Our results showed that time-related variables – but not frequency-related traits – varied between individuals, and that individual calls showed no variation between years. Our results suggest that Horned Guan individuals can be distinguished using fine structural characteristics of their calls and that calls remain stable across years. We argue that such vocal signature could be used to track wild populations as a non-invasive technique in order to improve census data in the short and long term.

Introduction

The individual acoustic signature is a widespread phenomenon in birds. For instance, this behaviour has been reported in several non-passerine species such as Wild Turkeys *Meleagris gallopavo* (Dahlquist et al. 1990), King Penguins *Aptenodytes patagonicu*s (Robisson 1992), domestic pigeons *Columba livia domestica* (Abs & Jeismann 1988), Bobwhite Quail *Colinus*

virginianus (Bailey 1978), Corncrake *Crex crex* (Peake et al. 1997), Great Bittern *Botaurus stellaris* (Mcgregor & Byle 1992; Gilbert et al. 1994; Puglisi & Adamo 2004), Hawk Owls *Ninox natalis* (Hill & Lill 1998), Pygmy Owls *Glaucidium passerinum* (Galeotti et al. 1993), Tawny Owls *Strix aluco* (Appleby & Redpath 1997), African Wood Owls (*Strix woodfordii*; Delport et al. 2002), Scops Owls (*Otus scops*; Galeotti & Sacchi 2001), Saw-whet Owls (*Aegolius acadicus brooksi*, Holschuh & Otter 2005), Eagle Owl (*Bubo bubo*, Lengagne 2001; Grava et al. 2008) and Western Screech-owls (*Megascops kennicottii*, Tripp & Otter 2006).

Understanding individual vocal differences is important for conservation purposes. Acoustic tools are especially useful when focal species are rare, threatened, cryptic and elusive, or when species are sensitive to disturbance caused by capturing and handling (Terry et al. 2005; Policht et al. 2009). Special caution should be exercised when declining population species are involved, where a small change in numbers may reflect a relatively large change in the overall population size (McGregor & Peake 1998; González-García 2005; Policht et al. 2009; Budka et al. 2015).

In the Cracidae family, inter-individual variation in Great Curassows's boom calls structure has been studied by Baldo and Mennill (2011), suggesting that this species' vocalizations may be useful to track individuals. The use of vocalizations as a non-invasive census method for secretive or threatened birds is of great relevance when physically marking individuals is impractical for logistical or welfare reasons (Mcgregor & Byle 1992; Gilbert et al. 1994; Peake et al. 1997). Acoustic monitoring also offers advantages in complex environments — like cloud forest — where visual markers are difficult to detect, and call recordings may be obtained from far away without having to disturb the animals (Gilbert et al. 1994; Hartwig 2005; Grava et al. 2008). Furthermore, if calls provide information about individuals, it is possible to estimate important population parameters such as abundance (Terry et al. 2005).

The Horned Guan (*Oreophasis derbianus*) is an endemic cracid restricted to the Sierra Madre of Chiapas in Mexico and the western-central mountains in Guatemala. With a small and severely fragmented population, this species is one of the most threatened birds in the Neotropics. The wild population has been estimated to be fewer than 1,000 individuals (BirdLife International 2016). As a consequence, the Red Lists and the Mexican Government classified the Horned Guan as an endangered species (SEMARNAT 2010; BirdLife International 2016). Deforestation, habitat alteration, global climate change, hunting and trade are some of the several threats that have been thought to contribute to its decline (Peterson et al. 2001; del Hoyo et al. 2014; BirdLife International 2016). However, there is insufficient information to understand the impacts of such threats on Horned Guan wild populations (Gómez de Silva et al. 1999; Abundis 2006). A continuous monitoring programme based on non-invasive methods is needed to ensure the implementation of short- and long-term management strategies in Horned Guan's conservation.

The aim of this study was to assess vocal individuality in captive Horned Guan males and test whether such individuality remains stable between years. We analysed the calls of 10 Horned Guans and calculated the coefficient of variation within and among individuals. We then tested whether variation among individuals was greater than within individuals. To test whether calls remain stable across years, we analysed the calls of five Horned Guans recorded in 2010 and 2011 and tested differences between years using Discriminant Function Analysis. Our study sheds light into the use of acoustic signals as natural markers for monitoring wild animals with conservation problems. Specifically, we provide a foundation for exploring the use of individual acoustic signals for monitoring Horned Guans

wild populations. To our knowledge, this study is the first to attempt the study of vocal individuality in captive Horned Guans and assess its application in monitoring programmes.

Methods

General field techniques

We recorded 10 Horned Guan adult males during the breeding seasons of 2010 and 2011 in two different captive populations held in Mexican zoos: African Safari Zoo, Puebla (5 males) and El Nido facilities, Ixtapaluca, Estado of Mexico (5 males). All birds were housed either in pairs or individually in contiguous enclosures and sometimes along with other bird species (i.e. parrots, parakeet, pigeons, quails). Recordings were made in the mornings and afternoons when males were vocally more active and from January to June, during the peak of the breeding season (González-García 2005). To record, we used either a directional microphone (Sennheiser MKH 60 or Sennheiser MKH 70) or a wireless microphone system (Sony WCS-990), in combination with a preamplifier (Sound Devices MP-1 or Sennheiser MZA14) and a digital recorder (Sony PCM D50). Every male was recorded in sessions of 5–10 min during 1–3 days. Calls were stored as 16 or 24 bits audio stereo and mono WAV files at a sampling frequency of 44.1 kHz. Most recordings were made from outside of the aviaries. We made some recordings using the wireless microphone system at El Nido facilities, placing the wireless microphone system near the male perch site. Distances between vocalizing birds and microphones fluctuated between 2 and 7 m.

Sound analysis

We selected between 5 and 12 high-quality recordings per male. Since we recorded captive Horned Guans in a zoo, often recordings were overlaid by motor vehicles noise, sounds from other captive bird species or by simultaneous calling of other contiguous Horned Guans males. Thus, we defined high-quality recording based on a high relation signal–noise and with no other sounds overlapping focal male calls. To reduce the non-overlapping background noise, recordings were filtered using 0.3 kHz high-pass filter in Raven Pro 1.5 (www.birds.cornell.edu/raven). We normalized the selected recordings to −3 dB in Adobe Audition 1.5 software (Adobe Systems, San Jose, CA).

The variables were measured directly from the screen using the computer mouse in Raven Pro 1.5. Spectral variables were measured from spectrograms, except bandwidth 90% which is a measure computed directly by Raven Pro 1.5 (see below for the definition), while temporal variables were measured from waveforms. We measured a total of 22 variables that describe call variations in the time and frequency domains. Temporal and spectral variables measured were: (1) length of the introductory portion of the call (defined as the duration from the start of the first note to the end of the third note in the call); (2) length of the body portion of the call (defined as the duration from the start of the fourth note to the end of the seventh note in the call); the length of the (3) first, (4) second, (5) third, (6) fourth, (7) fifth, (8) sixth and (9) seventh note (defined as the duration from the start to the end of each note); the intervals between (10) the first and second note, (11) the second and the third note, (12) the third and fourth note, (13) the fourth and fifth note, (14) the fifth and the sixth note and (15) the sixth and the seventh note (defined as the silence gap between the end of one note to the start of the following note); and the bandwidth 90%
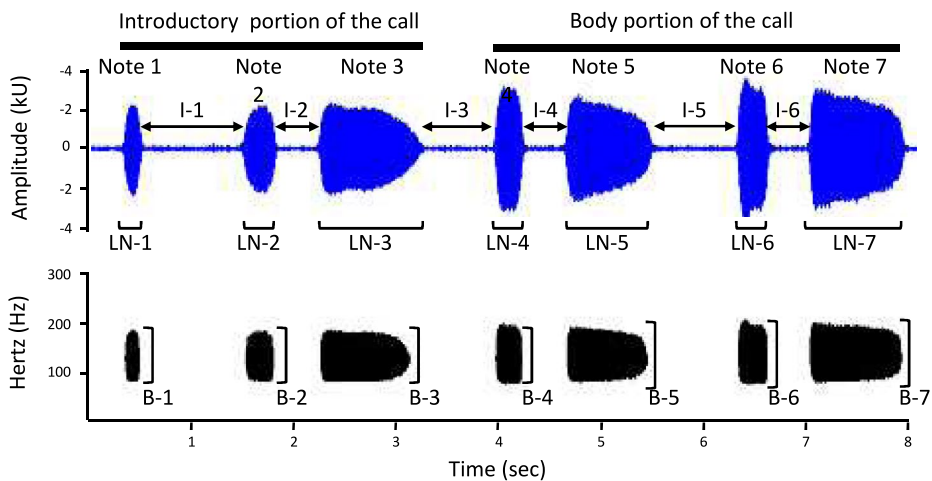
Figure 1. Oscillogram and spectrogram of a typical Horned Guan male boom call. A boom call is composed by an introductory and body section with three and four notes, respectively. The figure depicts the 22 parameter measured to describe the booming call structure. Letters indicate: I, interval between notes; LN, length of note; and B, bandwidth frequency. The numbers after the letters indicate the position of the note within the call.

of the (16) first, (17) second, (18) third, (19) fourth, (20) fifth, (21) sixth and (22) seventh note (bandwidth 90% is a measure computed directly by Raven Pro 1.5 and is defined as the relative frequency at which 90% of the energy in the selection occurs, see Charif et al. 2010 for further details) (Figure 1). We generated all spectrograms using the following settings: Blackman window, windows size = 1024, overlap 90%.

Statistical analysis

We tested whether boom calls varied among individuals using two different approaches. First, we calculated the coefficients of variation within (CV_w) and among (CV_a) all of the males recorded to describe the intra- and inter-individual variation of each variable. We used the coefficient of variation formula for small samples $CV = 100 \times (SD/mean) \times (1 + 1/4n)$, where SD is standard deviation and *mean* is the average value of a given variable, and *n* is the population sample (Robisson et al. 1993; Sokal & Rohlf 1995). We calculated the coefficient of variation within individuals (CV_w) as the coefficient of variation on a given variable in the boom calls of each individual. We calculated the coefficient of variation among individuals (CV_a) as the coefficient of variation in a given variable in the boom calls of all individuals. We then calculated the potential of individual coding (PIC) as $CV_a/mean\ CV_w$ for each variable to estimate the effect of individuality in the Horned Guan boom calls (Grava et al. 2008; Charrier & Harcourt 2006). A PIC value greater than 1 suggests that this parameter may be useful for individual discrimination, as the intra-individual variability is smaller than the inter-individual variability (Robisson et al. 1993). We calculated *mean* CV_w as the average of all coefficient of variation within individuals (CV_w) for a given variable. We performed a nonparametric analysis of variance, Kruskal–Wallis, to test whether variation among males was significant greater than within males in each of the 22 variables and used Bonferroni-adjusted correction for multiple comparisons (Quinn & Keough 2002).

For the second approach, we performed a combination of Discriminant Function Analysis and MANOVA to test both whether boom calls differ among individuals and whether calls are classified as predicted and to determine the acoustic variables that better explain those differences among individuals. We performed the Discriminant Function Analysis using 12 of the 22 acoustic variables; these 12 variables were those that showed significant differences between individuals in our potential of individual coding analysis (see results; Table 1). The variables were (1) length of the body and (2) introductory portion of the call, length of the (3) first, (4) second, (5) third, (6) fifth, (7) sixth and (8) seventh note, and interval of the (9) first, (10) second, (11) fifth and (12) sixth note. These 12 acoustic variables were input as the dependent variable and individual identity as independent variable. We conducted the analysis using the within-groups covariance matrix and not adjusting the classification coefficients for a priori knowledge of group membership. We reported the classification estimate based on the Jackknife procedure, which calculate group membership of a given sample by omitting it from the analysis and using the remaining observations to calculate the coefficients (Quinn & Keough 2002).

We tested whether individuality in the booming calls remained consistent across years by analysing calls recorded in both years 2010 and 2011. In this analysis, we included the boom calls of five Horned Guans from African Safari Zoo because we were certain of each bird's identity between years for these individuals. We used an average of 11.3 ± 2.9 calls per individual per year (for a total of 113 calls for both years). We calculated the individual average values for each of the 12 acoustic variables that showed significant differences between individuals in our potential of individual coding analysis (see results; Table 1). Then, adapting a procedure proposed by Wilson and Mennill (2010), we estimated the average values per individual for calls performed in 2010 and 2011, and both years pooled together, resulting in three categories (i.e.

Table 1. Coefficients of variation among (CV_a) and within (*mean* CV_w) males Horned Guan calls and the potential of individual coding (PIC).

Variable	CVa	*mean* CVw	PIC	Chi-Square
Length of the introductory portion of the call	6.6	3.5	1.9	55.7*
Length of the body portion of the call	6.0	2.8	2.1	54.3*
Length of the first note	17.5	13.4	1.3	30.6*
Length of the second note	14.4	10.4	1.4	30.6*
Length of the third note	13.7	7.0	1.9	53.9*
Length of the fourth note	9.1	7.6	1.2	22.7
Length of the fifth note	17.3	7.2	2.4	56.9*
Length of the sixth note	9.1	6.5	1.4	35.0*
Length of the seventh note	10.9	7.0	1.6	43.6*
Interval of the first note	11.6	7.3	1.6	44.9*
Interval of the second note	12.0	8.0	1.5	39.2*
Interval of the third note	9.5	7.3	1.3	29.2
Interval of the fourth note	8.1	6.4	1.3	23.3
Interval of the fifth note	7.5	6.1	1.2	34.0*
Interval of the sixth note	8.1	5.6	1.5	34.6*
Bandwidth of the first note	17.6	13.2	1.3	6.8
Bandwidth of the second note	22.5	21.2	1.1	8.9
Bandwidth of the third note	23.9	19.7	1.2	11.4
Bandwidth of the fourth note	18.8	13.9	1.4	9.2
Bandwidth of the fifth note	16.6	11.4	1.5	12.6
Bandwidth of the sixth note	16.8	13.5	1.2	6.1
Bandwidth of the seventh note	12.4	7.9	1.6	11.1

Notes: Chi-Square values for Kruskal–Wallis tests are showed. Asterisk indicates acoustic variables that were significant different between individual after Bonferroni correction; alpha levels were adjusted at 0.002 per test ($p = 0.05/22$).

2010, 2011 and 2010–2011 pooled together). We included the last category (both years pooled together) in the analysis to compare how variation between years compares to that present within year. The individual average values did not meet normal distribution; thus, we used a nonparametric Friedman test for repeated measurements to determine whether there were statistically significant differences between the distributions of the average values between years (Quinn & Keough 2002). Descriptive statistics are presented as means ± SD. We performed all statistical analyses in IBM SPSS Statistics (Version 20.0; Chicago, IL, United States).

Results

The *boom* call is a stereotyped, low-frequency vocalization produced only by adult males in long bouts (Figure 1). Boom calls averaged 7.79 ± 0.25 s in duration ($N = 84$ calls). Structurally, the boom call consists of seven notes divided into two parts: an introductory and a body section. The introductory section is composed of three relatively quiet notes, while four, louder notes comprise the body section. Notes varied in length in the introductory and body portion (Figure 2(a)), in the length of each note (Figure 2(b)), in the interval between of notes (Figure 2(c)) and in the bandwidth frequency (Figure 2(d)). The introductory note is the shortest (average = 0.202 ± 0.035 s). The second (average = 0.299 ± 0.043 s), fourth (average = 0.300 ± 0.027 s) and sixth (average = 0.330 ± 0.031 s) notes have similar duration but are shorter than the third (average = 0.931 ± 0.126 s), fifth (average = 0.967 ± 0.162 s) and seventh (average = 1.095 ± 0.122 s) notes. The seventh note is the longest of all notes (Figure 2(b)). Similarly, the bandwidth frequency is lower
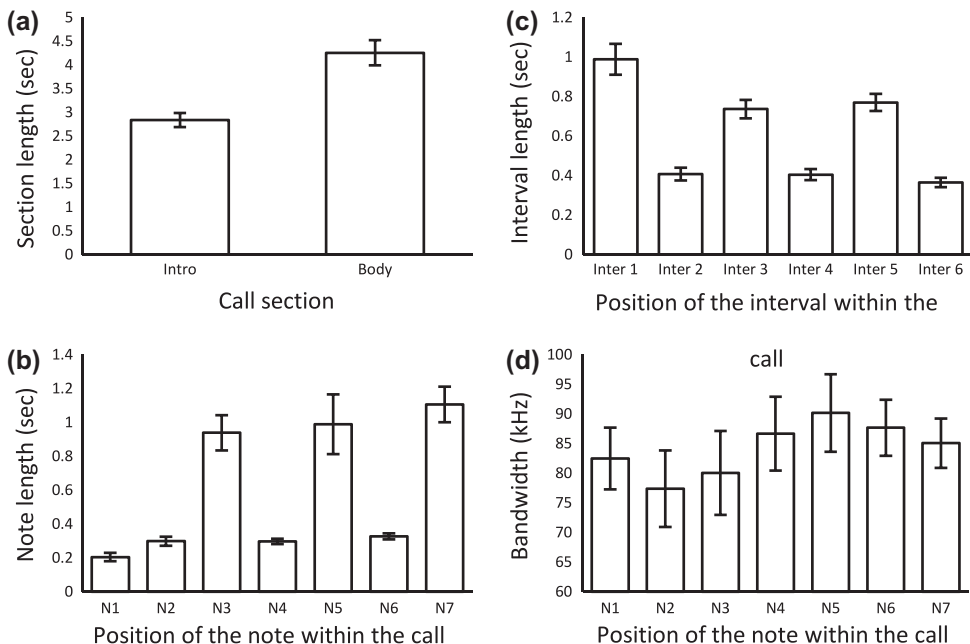
Figure 2. Graphs depicting the average values of the (a) section length of the introductory and body portion, (b) length of the notes, (c) interval length between notes and (d) bandwidth frequency of the boom calls produced by Horned Guan males. The mean ± SE for each acoustic variable is shown.

in the introductory section than the body section (Figure 2(d)). Note that although most Horned Guan boom calls had seven notes, on rare occasions some males emitted boom calls containing eight or nine notes and occasionally with two short introductory notes.

Individual variation of booming calls

Coefficient of variation

We analysed the individual variation in the boom calls of Horned Guan living in captivity. Our coefficient of variation analysis suggests that the values of coefficients of variation within males (CV_w) ranged from 2.8 to 21.2 %, whereas the coefficients of variation among (CV_a) males ranged from 6.0 to 23.9 %. PIC values ranged from 1.2 to 2.4, suggesting that most variables can be used for individual identification. The Kruskal–Wallis test showed significant differences between individuals for 12 of the 22 variables after controlling for multiple comparisons. The variables with significant differences between individuals were all related to the length of the notes and intervals among notes (Table 1).

Discriminant Function Analysis

The Discriminant Function Analysis based on 84 calls produced by 10 male Horned Guans resulted in nine canonical discriminant functions (Table 2). The analysis assigned 89.3% of the 84 songs to the correct individual, which exceeds the assignment expected by chance ($\chi^2 = 5.85$, df = 1, $p < 0.0001$). Overall, the calls differed significantly between individuals in seven of the nine canonical discriminant functions (MANOVA: Wilks' $\lambda < 0.01$, $F_{81,435.49} = 16.46$, $p < 0.0001$; Table 3). The Tukey HSD *post hoc* test revealed significant differences in the calls for nine of the 10 individuals analysed. Such differences were achieved using the first two canonical functions (Figure 3). The first canonical function was heavily associated with the length of the body portion of the call and the length of the fifth note, while the second canonical function was heavily associated with the length of the third note (Table 2).

**Table 2.** Discriminant Function Analysis of 10 males Horned Guan.

Variable[a]	Canonical Discriminant Functions								
	1	2	3	4	5	6	7	8	9
Length of the introductory portion of the call	1.58	0.26	−1.70	0.77	0.65	1.92	−1.43	1.17	−0.47
Length of the body portion of the call	−2.44*	−1.75	−1.95	−3.92	−1.57	−2.10	−1.58	−0.86	−0.90
Length of the first note	−0.04	−0.57	0.76	−0.94	0.17	0.34*	0.27	0.02	−0.02
Length of the second note	−0.62	0.82	−0.62	0.31	−0.35	−0.86	0.34*	−0.06	0.53
Length of the third note	−0.18	−0.95*	1.48	−0.30	−0.77	−1.44	0.75	−1.03	0.28
Length of the fifth note	0.29*	0.65	1.24	2.54	1.20	1.24	1.13	0.31	0.90
Length of the sixth note	0.68	0.88	1.43	1.08	0.79	0.87*	−0.06	0.41	0.52
Length of the seventh note	1.27	1.51	1.46	2.52	0.10*	1.64	0.86	0.58	0.58
Interval of the first note	−1.14	−0.18	1.45	−0.99	−0.88	−1.55	0.94	−0.76	1.40*
Interval of the fifth note	0.35	0.34	0.85	1.60	0.33	0.10	0.62	1.00*	0.24
Interval of the sixth note	1.03	0.65	1.69	0.56	0.65	0.66	−0.15*	0.13	0.12
Eigenvalue	8.94	5.49	4.42	1.94	1.37	0.71	0.7	0.16	0.02
% Variance explained	37.60	23.09	18.57	8.16	5.78	3.01	2.98	0.70	0.11

Notes: The variables and their respective standardize canonical discriminant function coefficients, the eigenvalues and the percentage of variation explained by each function are shown. Asterisks indicate largest absolute correlation between variables and canonical discriminant functions.
[a]The variable interval of the second note did not meet the minimum tolerance level and was removed from the DFA.

Table 3. MANOVA results based on the coefficients of the nine canonical discriminant functions of 10 males Horned Guan calls.

Canonical discriminant function	R^2	df	F	P
Function 1	0.88	9	73.58	0.00
Function 2	0.82	9	45.19	0.00
Function 3	0.79	9	36.35	0.00
Function 4	0.61	9	15.98	0.00
Function 5	0.52	9	11.31	0.00
Function 6	0.34	9	5.88	0.00
Function 7	0.34	9	5.83	0.00
Function 8	0.03	9	1.36	0.22
Function 9	0.09	9	0.22	0.99
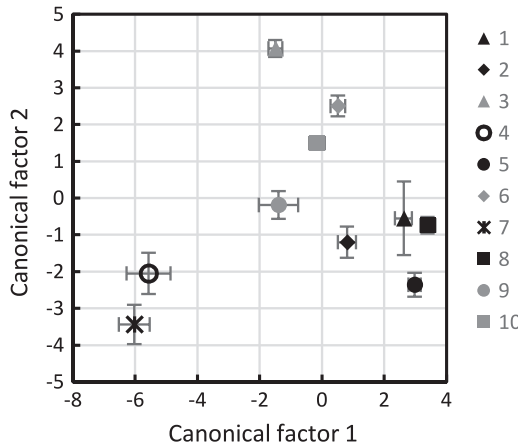
Figure 3. Variation in the booming calls of 10 Horned Guan males described by the first two canonical discriminant factors. The graph shows the mean ± SE for the calls of each individual.

Table 4. Friedman test results comparing variation in calls across years (i.e. 2010, 2011 and 2010–2011 pooled together) for the calls of five Horned Guan males.

Variable	Chi-Square	df	P
Length of the introductory portion of the call	3.6	2	0.16
Length of the body portion of the call	0.4	2	0.81
Length of the first note	0.4	2	0.81
Length of the second note	0.4	2	0.81
Length of the third note	0.4	2	0.81
Length of the fifth note	0.4	2	0.81
Length of the sixth note	0.4	2	0.81
Length of the seventh note	10.0	2	0.007
Interval of the first note	10.0	2	0.007
Interval of the second note	10.0	2	0.007
Interval of the fifth note	10.0	2	0.007
Interval of the sixth note	0.4	2	0.81

Notes: None of the acoustic variables were significant different between years after Bonferroni correction; alpha levels were adjusted at 0.004 per test ($p = 0.05/12$).

Individual variation of boom calls across year

We analysed individual variation across years in the boom calls of Horned Guan living in captivity. The Friedman test showed that Horned Guans' calls had non-significant variation
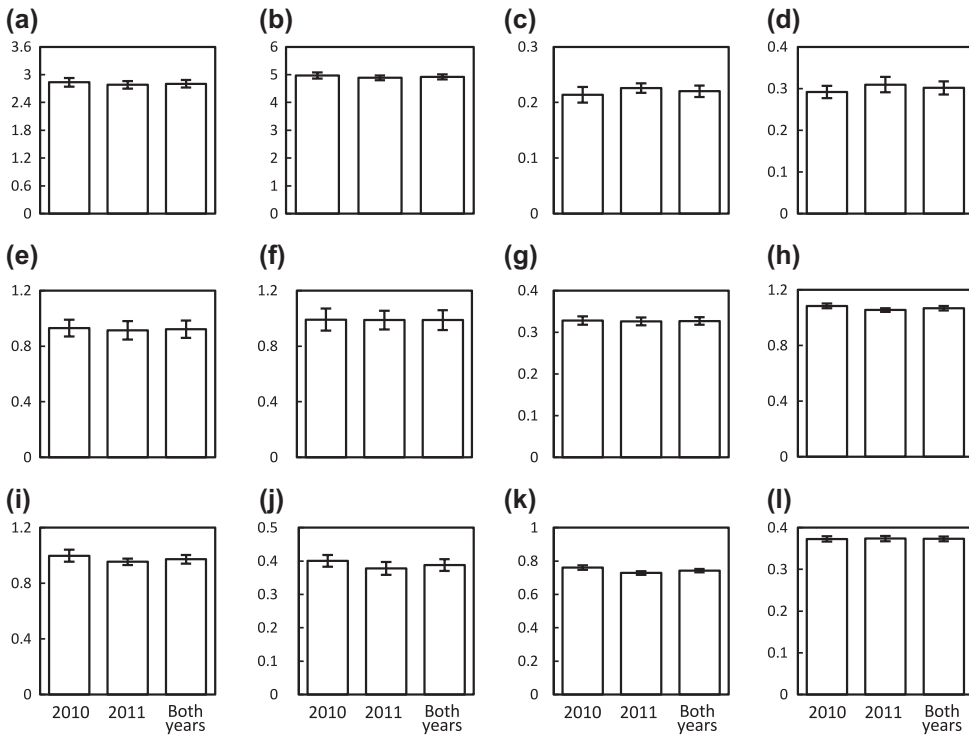
Figure 4. Variation across years in the booming calls of five Horned Guan males. For each individual, we calculated the average values of 12 acoustic variables describing their calls and calculated an overall mean per year, and both years pooled together. The 12 acoustic variables were: (a) length of the body and (b) introductory portion of the call, length of the (c) first, (d) second, (e) third, (f) fifth, (g) sixth and (h) seventh note, and interval of the (i) first, (j) second, (k) fifth and (l) sixth note. The graphs show the mean ± SE for each acoustic variable.

among years for all the 12 acoustic variables tested (Table 4; Figure 4). Thus, our results suggest that individual calls are constant across years.

Discussion

Horned Guans living in captivity emitted low-frequency notes that varied in duration and bandwidth frequency. Structurally, seven notes divided into two sections compose the calls: an introductory section made of three first notes and a body section made of four notes. The notes within the introductory portion of the call are shorter and quieter than the notes within the body portion. Our results suggest that individual features are present in the fine structural characteristics of Horned Guan booming calls, and that such differences are constant across years, suggesting that Horned Guan booming calls have an individual signature.

Two requirements must be satisfied for identifying individuals using vocalizations: acoustic variation within an individual must be lower than variation among individuals, and the acoustic traits unique to an individual should be stable over time (Charrier et al. 2004; Puglisi & Adamo 2004; Fox 2008; Xia et al. 2010). Variables in Horned Guans calls meet these two requirements. All temporal variables with significant differences between

individuals had PIC values that ranged from 1.2 to 2.3. PIC values with >2 (length of the body portion, length of the fifth notes) are considered high because had a superior degree of individuality (Mathevon 1997; Charrier & Harcourt 2006; Cornec et al. 2014). Evidence of individual differences in vocalizations in non-passerines with PIC values >2 have been provided in multiple bird species ranging from colonial species to raptors (Galeotti & Pavan 1991; Robisson et al. 1993; Lengagne et al. 1999, 2001; Grava et al. 2008; Klenova et al. 2011; Cornec et al. 2014) and in some marine mammals (Charrier et al. 2003; Charrier & Harcourt 2006). Thus, our results are consistent with other studies suggesting that variation within individuals is smaller than variation among individuals (Lengagne 2001; Grava et al. 2008; Xia et al. 2010; Cornec et al. 2014).

The Discriminant Function Analysis and the analysis of variance supports our coefficient of variance analysis results, showing significant differences in the calls between Horned Guan individuals and assigning 89.3% of the 84 songs to the correct individual. This outcome is in line with other studies in cracids such as in Great Curassow (*Crax rubra*) (Baldo & Mennill 2011) and in other non-passerine families suggesting individual variation in calls, such as in Accipitridae (Eakle et al. 1989), Spheniscidae (Robisson 1992), Strigidae (Galeotti et al. 1993; Appleby & Redpath 1997; Hill & Lill 1998; Galeotti & Sacchi 2001; Lengagne 2001; Delport et al. 2002; Holschuh & Otter 2005; Tripp & Otter 2006; Grava et al. 2008), Rallidae (Peake et al. 1997), Caprimulgidae (Rebbeck et al. 2001), Ardeidae (Mcgregor & Byle 1992; Gilbert et al. 1994; Puglisi & Adamo 2004), Bucerotidae (Policht et al. 2009), Alcidae (Klenova et al. 2011) and Otididae (Cornec et al. 2014, 2015).

The analysis across years suggests that a particular Horned Guan can be statistically identified using its calls for a period of two years. Call consistency across years have been reported in some non-passerines bird species. Wooller (1978) established consistency of calls over years by visual comparison of spectrograms in Black-legged Kittiwake (*Rissa tridactyla*). Peake et al. (1998) demonstrated that vocalizations of corncrake *Crex crex* were constant over a two-year period. Lengagne (2001) and Grava et al. (2008) demonstrated that calls of eagle owls were consistent over the years as well. Thus, our findings meet the second requirement for identifying individuals using vocalizations suggesting that features unique to an individual should be stable over time (Charrier et al. 2004; Puglisi & Adamo 2004; Fox 2008; Xia et al. 2010). We recognize that the sample size in our study is small due to a combination of low vocal activity and the shortage of recordings with a high signal quality. However, wild Horned Guans males in the Mexican Reserve El Triunfo vocalize often and continuously during the breeding season (González-García 1995) and getting recordings from wild individuals should be relatively easy (FGG personal observations). Furthermore, many studies have proved bird acoustic individuality based on comparable sample sizes (e.g. Lengagne 2001; Delport et al. 2002; Grava et al. 2008; Policht et al. 2009; Baldo & Mennill 2011).

Horned Guans have low frequency and stereotyped calls that are used among male adults during the breeding season and are thought to serve in both attractions of potential mates and male–male interactions (González-García 1995, 2005). A study conducted in Crested Auklet (*Aethia cristatella*) suggested that a single acoustic signal could contain information used in different functions such mate attraction, individual recognition and social status (Klenova et al. 2011). Similarly, the call of the Horned Guan could be used in different functions. Both call sections in the Horned Guan call have similar low frequencies, and thus they can be effective for long-distance communication(Morton 1975; Baldo & Mennill 2011; Cornec et al. 2014); however, our observations suggest that both sections differ in the energy

that they contained. We noticed during field expeditions that the introductory section is quiet and difficult to detect at long distances, while the body section is louder and audible at large distances (Figure 1). It is known that signals produced at high amplitudes could facilitate the propagation of the signal travelling further through complex environments (Bradbury & Vehrencamp 2011; Cornec et al. 2014, 2015). Thus, while the introductory section could be used for short communication range, the body section could be used in long-range communication. Further analyses studying the propagation of Horned Guan calls could help better understand the function of this signal.

Another interesting observation during our study suggests that booming calls are produced in bout sequences. Bout sessions last extended periods of up to one hour (González-García 1995). This behaviour, where a signal is repeated several times, is also found in other non-passerine birds and could be useful in individual distinction and mate choice (Jouventin et al. 1999; Lengagne et al. 1999; Klenova et al. 2011). By repeating the same signal, signallers could maintain the integrity of the information content despite the degradation of some acoustics features during propagation (Lengagne et al. 1999; Price 2013; Cornec et al. 2014). Thus, the redundancy and low frequency calls should allow the Horned Guan to maximize the propagation of the information content on its calls (Cornec et al. 2014, 2015; Price 2013). Future experimentation using a playback approach is needed to test whether the repetition of a signal helps to convey information such as individuality.

We have documented the individual variation in the calls of the Horned Guan under captive conditions. Our results suggest that Horned Guan's booms can be used as an individual marker with little or no variation across years, fulfilling the requirements for identifying individuals using vocalizations (Terry et al. 2005). Given that the Horned Guan lives in dense cloud forest where capture, handling and tagging are difficult for ethical, logistic and welfare reason (Lengagne 2001), identifying individuals by vocalizations may be an alternative tool for monitoring wild populations. Moreover, Horned Guans conservation measures in Mexico and Guatemala are urgent (Peterson et al. 2001; del Hoyo & Kirwan 2015; BirdLife International 2016) and the implementation of acoustic monitoring programmes based on individual identification could generate information on life history and improve conservation models, improving management decisions.

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