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Vocal Individuality and Species Divergence in the Contact Calls of Banded Penguins

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KEYWORDS

bioacoustics, seabirds, source-filter theory, *Spheniscus*

ABSTRACT

Penguins produce contact calls to maintain social relationships and group cohesion. Such vocalisations have recently been demonstrated to encode individual identity information in the African penguin. Using a source-filter theory approach, we investigated whether acoustic cues of individuality can also be found in other *Spheniscus* penguins and the acoustic features of contact calls have diverged within this genus. We recorded vocalisations from two ex-situ colonies of Humboldt penguin and Magellanic penguin (sympatric and potentially interbreeding in the wild) and one ex-situ group of African penguins (allopatric although capable of interbreeding with the other two species in captivity). We measured 14 acoustic parameters from each vocalisation. These included temporal (duration), source-related (fundamental frequency, f_0), and filter-related (formants) parameters. They were then used to carry out a series of stepwise discriminant function analyses (with cross-validation) and General Linear Model comparisons. We showed that contact calls allow individual discrimination in two additional species of the genus *Spheniscus*. We also found that calls can be classified according to species in a manner far greater than that attributable by chance, even though there is limited genetic distance among African, Humboldt, and Magellanic penguins. Our results provide further evidence that the source-filter theory is a valuable framework for investigating the biologically meaningful information contained in bird vocalisations. Our findings also provide novel insights into penguin vocal communication and suggest that contact calls of the penguin family are affected by selection for individuality.

1. Introduction

Penguins are a monophyletic group of pelagic seabirds that for-age at sea and breed on land, where most of the species form dense colonies (Williams, 1995; Schreiber and Burger, 2002). The penguin genus *Spheniscus* comprises four living species collectively known as “banded penguins”. The genus can be further divided in two sister taxa: one formed by the Humboldt (*Spheniscus humboldti*) and Galápagos (*Spheniscus mendiculus*) penguins and the second by the African (*Spheniscus demersus*) and Magellanic (*Spheniscus magellanicus*) penguins. The two sister groups diverged in the Pacific and Atlantic Oceans approximately 4 million years ago (Baker et al., 2006). However, despite their genetic relationship, Magellanic and Humboldt penguins are partially sympatric and their

geographical distributions overlap along the Pacific Ocean coast of South America. For example, in the Chilean islands of Puñihuil and Metalqui both species coexist in significant numbers (Simeone and Schlatter, 1998; Simeone et al., 2009). By contrast, their closest relatives, African and Galápagos penguins are allopatric and breed on the African continent and Galápagos archipelago, respectively.

Penguins use vocalisations for individual localisation, recognition, and to maintain social relationships and group cohesion (Jouventin, 1982; Favaro et al., 2014a). Based on the acoustic properties of their vocalisations and behavioural context of emission, four categories can be distinguished in the vocal repertoire of adult penguins (Jouventin, 1982): contact calls (uttered to maintain cohesion with the group or the partner), agonistic calls (made during fights and in territorial defence), as well as two types of display songs: complex vocalisations made of syllable sequences that can be uttered by single birds (ecstatic display songs), or by pairs (mutual display songs).

Penguin display songs are adapted for mate and parent-offspring recognition (Lengagne et al., 1997; Lengagne et al., 2000; Aubin and Jouventin, 2002) and acoustic cues for individuality have been found in all the penguin species that have been studied (Aubin and Jouventin, 2002; Aubin, 2004; Clark et al., 2006). The mechanisms used to encode the individual identity information in the display songs are exposed to ecological sources of selection and also vary according to breeding ecology and the colonial lifestyles of the different species (Aubin, 2004). In particular, non-nesting species (e.g. King Penguin, *Aptenodytes patagonicus*) use the two-voice system as a principal means of identifying each other (Aubin et al., 2000), while in nesting species (e.g. Adélie penguin, *Pygoscelis adeliae* or Gentoo penguin, *Pygoscelis papua*), the pitch of the song, frequency and relative values of harmonics are the main cues for individual recognition (Jouventin and Aubin, 2002). Despite all the information available on display songs, very limited research has been directed toward studying the other call types, and in particular contact calls.

Contact calls have evolved as social signals to maintain cohesion in stable groups (Cheney et al., 1996; Kondo and Watanabe, 2009; Bergman and Sheehan, 2013), but also to advertise on the identity of the sender (Sharpe et al., 2013; Favaro et al., 2015), which is particularly important in fission-fusion societies (Ramos-Fernández, 2005; Terry et al., 2005; Janik et al., 2006). Moreover, contact calls can encode a great deal of information about the emitter. There is growing evidence that these vocalisations have the potential to provide information on sex (Guillette et al., 2010), age (Favaro et al., 2014b), group membership (Boeckle and Bugnyar, 2012), and even emotional state (Briefer et al., 2015). They can also contain acoustic cues to species (Gamba et al., 2012a; Cinkova and Policht, 2014) and population (Buhmann-Deever et al., 2007; Husemann et al., 2014).

Although the African, Humboldt and Magellanic penguins are closely related, their ecstatic display songs are clearly recognizable even to human listeners (Jouventin, 1982). Indeed, this vocalization has a significant amount of variation among species, and African and Magellanic penguins are more similar to each other than to Humboldt penguins (Thumser et al., 1996). Moreover, Thumser and Ficken (1998) showed that contact calls of *Spheniscus* penguins have very similar acoustic structure among the different species. They are short vocalizations with a clear harmonic structure, and that intraspecific variation only exists for the dominant frequency. However, these observations were based on a very limited number of individuals and vocalisations. A recent study showed that the contact calls of African penguin have the potential to allow individual discrimination (Favaro et al., 2015). In particular, Favaro et al. (2015) used a Discriminant Function Analysis (DFA) to show that contact calls in this species differ systematically between individuals (DFA accuracy = 61.1%) and can be classified according to the emitter in a manner far greater than that attributable to chance. Nevertheless, whether these vocalisations allow individual discrimination also in other penguin species and contain other biologically meaningful information still remains to be tested.

Bird calls are generated by vibrations of membranes in the syrinx (source, determining the fundamental frequency, " f_0 ") and are subsequently filtered by the suprasyringeal vocal tract (filter, resulting in amplified frequencies called "formants"). The source-filter theory of mammal vocal production (Fant, 1969; Taylor and Reby, 2010) has recently been shown to be useful in gaining a far greater understanding of individual identity information contained in seabird vocalisations (Hardouin et al., 2014; Favaro et al., 2015). Following the source-filter theory approach, we determined whether the contact calls of Magellanic and Humboldt penguins have the potential to allow individual discrimination of the callers. Moreover, we tested whether the acoustic features of contact calls have diverged across this genus. To this end, we compared calls of Magellanic and Humboldt penguins (that potentially interbreed in the wild), and the African penguin, which is fully allopatric.

2. Methods

2.1. Ethics statement

The research conforms to the Ethical Guidelines for the Conduct of Research on Animals by Zoos and Aquariums (WAZA, 2005) and complies with all applicable Italian laws. Acoustic recordings were non-invasive and we made every effort to minimize possible disturbance to the penguins.

2.2. Study sites and penguins

We recorded nine Magellanic penguins (5 males and 4 females) that were all the adult members of an ex-situ colony housed at the Acquario di Genova, Italy. The colony was imported in 2006 from the SELWO Marina Delfinarium (Benalmadena, Spain), but was originally from Argentina (wild individuals stranded due to an oil spill). In Genoa, the colony was maintained in a communal indoor exhibit (123 m² including a pond of 66 m², water depth maximum 3 m) with three concrete walls and one facing the visitor corridor made up of glass panels, which allows a combined vision of open air and underwater penguin activity.

Vocalisations of Humboldt penguins were collected from six birds (3 males and 3 females) belonging to a captive colony housed at the Acquario di Cattolica, Italy. The composition of the whole colony was 4 males and 8 females. Penguins were housed in an indoor communal exhibit of 75 m², including a pond of 35 m² (maximum depth 2 m). The colony was established at the Acquario di Cattolica from 2007 to 2009 joining adult penguins from the Schönbrunn Zoo (Austria) and the North of England Zoological Society, Chester (United Kingdom).

We recorded 24 adult African penguins (18 males and 6 females) from a large ex-situ colony (26 males, 27 females, 4 juveniles, and 3 chicks hatched during the study period), maintained at the biopark Zoom Torino, Italy. The colony was established in 2009 by combining several adult African penguins from four different zoological facilities in Europe (Artis Royal Zoo, Amsterdam, NL; Bird Park Avifauna, Alphen an den Rijn, NL; Wilhelma Zoo, Stuttgart, DE; South Lake Wild Animal Park, Manchester, UK). The colony was housed in an outdoor exhibit (1500 m², including a pond of 120 m², water depth maximum 3 m), which reproduces the habitat of "Boulders Beach", a natural nesting site in South Africa.

All penguins recorded in this study were habituated to human presence and used to being audio- and video-recorded during their normal daily activity. Additionally, all birds had a microchip transponder and a flipper band to allow individual identification.

2.3. Recordings of vocalisations

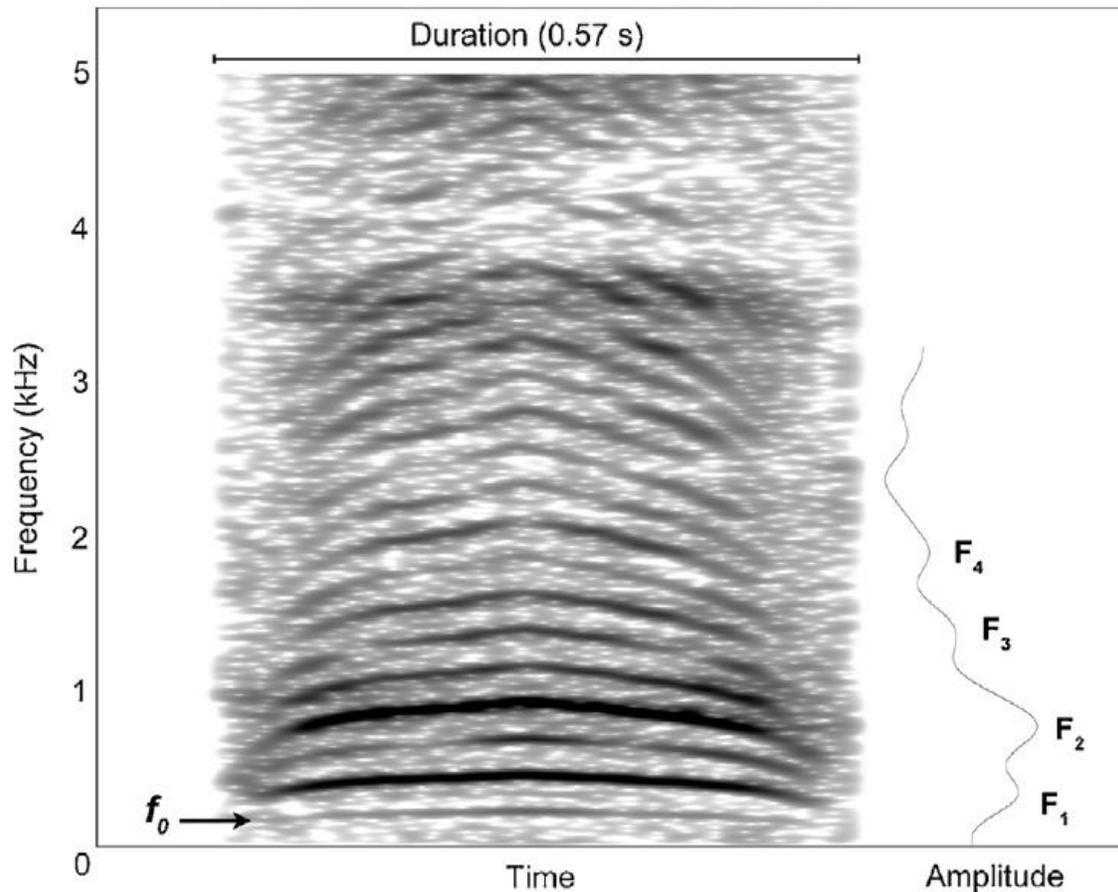
Vocalisations of African penguins were collected over 50 non-consecutive days from September 2014 to March 2015. Recordings of Humboldt penguins were collected over 40 non-consecutive days between May and September 2015. Magellanic penguins were recorded over 30 non-consecutive days from February to April 2015. In all facilities, vocalisations were collected using the all occurrence animal sampling method (Altmann, 1974). Vocalisations were recorded at a distance of between 2 and 10 m from the caller with a RØDE NTG2 Super-Cardioid microphone (frequency response 20 Hz to 20 kHz, sensitivity -36 dB±2 dB re 1 V/Pa at 1 kHz, max SPL 131 dB). In order to reduce recorded noise, the microphone was mounted on a RØDE PG2 Pistol Grip and protected with a windscreen. We also made every effort to orientate the microphone towards the calling bird. The microphone was connected to a TASCAM DR-680 or TASCAM DR-40 digital recorder (44.1 kHz sampling rate) and acoustic data were saved into an internal SD memory card in WAV format (16-bit amplitude resolution). All the files were then transferred to a laptop computer for later acoustic analyses.

2.4. Acoustic analysis

We used narrow-band spectrograms to visually inspect the overall spectral structure of audio recordings. In particular, the waveform and the FFT (Fast Fourier Transform) spectrogram were generated with the Praat (Boersma, 2001) sound editor window. We used a customised spectrogram setting (view range = 0–8000 Hz, window length = 0.02 s, dynamic range = 50 dB). For the purpose of this study we only selected contact calls (Supplementary material, Fig. S1). However, 355 calls for African penguins, 150 for Humboldt penguins, and 486 for Magellanic penguins were

initially discarded because they showed excessive background noise or because calls were overlapping between different penguins vocalising at the same time. Overall, the spectrographic inspection allowed us to select a total of 392 vocalisations for African penguins, 119 for Humboldt penguins, and 976 for Magellanic penguins. The contribution of each penguin to the recordings is presented in the Supplementary material (Table S1).

Fig. 1. Spectrogram (Gaussian window shape, view range = 0–5000 Hz, window length = 0.05 s, dynamic range = 70 dB, time step = 0.004 s, frequency step = 20 Hz) and LPC spectrum (500 Hz cepstral smoothed) of contact call (African penguin) showing f_0 and formants (F_1 – F_4).



Acoustic measurements on selected vocalisations were carried out using a series of custom scripts (see Gamba et al., 2012b; Favaro et al., 2015) in Praat v.5.4.08. The abbreviations that we use for the various vocal parameters in this study are based on recent advice regarding this terminology (Titze et al., 2015). For each contact call, we measured a series of spectral and temporal acoustic parameters, which were potentially important to vocal distinctiveness. These included both temporal measures, such as call duration (Dur), source-related vocal features (f_0) and filter-related acoustic features (formants; Fig. 1). We also quantified the mean harmonics-to-noise ratio value (Sonority). However, before measuring formants, we estimated the approximate vocal tract length (VTL) for banded penguins, to set a plausible number of formants in a given frequency range (Gamba and Giacomini, 2006; Favaro et al., 2015). In particular, we built computational models of the penguin vocal tract deriving information from cadavers of individuals died from natural causes in different zoological facilities in Italy. We modelled vocal tract resonances using a MATLAB-based computer program for vocal tract acoustic response calculation (VTAR, Vocal Tract Acoustic Response; Zhou et al., 2004). The acoustic response of the vocal tract models and the visual inspection of the spectrograms indicated 5 formants below 3500 Hz for the contact calls.

We extracted the f_0 contour of each call using a cross-correlation method [Sound: To Pitch (cc) command]. Depending on the acoustic characteristics of each vocal type, we used a time step of 0.01 s, a pitch floor of 150 Hz, and a pitch ceiling of 350 Hz. From each extracted f_0 contour, we obtained the frequency value of f_0 at the start (f_0 start) and at the end (f_0 end) of the call; the mean (f_0 mean), minimum (f_0 min) and maximum (f_0 max) fundamental frequency values across the call. We measured the percentage of duration from the beginning of the signal to the time at which the minimum frequency (Time f_0 min) and the maximum frequency (Time f_0 max) occurs. Finally, we extracted the contour of the first four formants (F_1 – F_4) of each call using a Linear Predictive Coding analysis (LPC; Sound: To Formant (burg) command; time step = 0.045 s, maximum number of formants = 5, maximum formant = 3500 Hz) and we calculated the average frequency values. In addition, we calculated the formant dispersion (ΔF) using the methods described by Reby and McComb (2003).

Table 1. Values of the vocal parameters (mean \pm SD) for the contact calls of each penguin species.

Vocal parameter	African ^a (n – 392)	Humboldt ^b (n – 119)	Magellanic ^c (n – 976)
Dur (s)	0.57 \pm 0.12	0.91 \pm 0.20	0.86 \pm 0.16
f_0 mean (Hz)	275 \pm 22	253 \pm 16	270 \pm 23
f_0 max (Hz)	299 \pm 21	302 \pm 22	291 \pm 21
f_0 min (Hz)	248 \pm 25	222 \pm 20	246 \pm 23
f_0 start (Hz)	267 \pm 29	275 \pm 29	267 \pm 25
f_0 end (Hz)	279 \pm 28	278 \pm 27	267 \pm 25
Time f_0 min (%)	69 \pm 34	50 \pm 26	51 \pm 40
Time f_0 max (%)	41 \pm 34	49 \pm 43	39 \pm 35
Sonority	8.63 \pm 5.98	4.9 \pm 3.54	7.43 \pm 3.02
F_1 (Hz)	572 \pm 133	450 \pm 109	599 \pm 80
F_2 (Hz)	1030 \pm 143	976 \pm 96	978 \pm 88
F_3 (Hz)	1668 \pm 200	1660 \pm 123	1625 \pm 118
F_4 (Hz)	2468 \pm 230	2475 \pm 148	2431 \pm 98
ΔF	697 \pm 65	691 \pm 45	683 \pm 31

^a 24 individuals.

^b 6 individuals.

^c 9 individuals.

Table 2. GLM results (ANOVA analysis) for the effects of penguin species on the contact call acoustic variables.

Vocal parameter	df	F	Significance
Dur	2	30.08	p < 0.001
f_0 mean	2	2.73	ns
f_0 max	2	3.90	p < 0.05
f_0 min	2	4.04	p < 0.05
f_0 start	2	1.28	ns
f_0 end	2	5.46	p < 0.05
Time f_0 min	2	4.27	p < 0.05
Time f_0 max	2	1.18	ns
Sonority	2	1.75	ns
F_1 (Hz)	2	14.07	p < 0.001
F_2 (Hz)	2	2.46	ns
F_3 (Hz)	2	2.52	ns
F_4 (Hz)	2	3.51	p < 0.05
ΔF	2	3.27	p < 0.05

Identity was nested into species and included as a random factor. Species was included as a fixed factor.

2.5. Statistical analysis

We performed two separate cross-validated (leave-one-out) discriminant function analyses (DFA) for Humboldt and Magellanic penguins to investigate whether contact calls could be used to discriminate among individuals in these two species. In both analyses, the caller was used as the group identifier and the acoustic variables as discriminant variables. We used a feed forward procedure with default F-values threshold in SPSS v.20 for acceptance or rejection of the discriminant variables. Moreover, the coefficients of classification were corrected according to the group sizes, since the different individuals did not contribute equally to the samples. Finally, for each vocal parameter, we calculated the Potential of Identity Coding (PIC) using the correction for small samples (e.g. Charrier et al., 2004). PIC assesses the ratio between within-individual variation and between-individual variation of an acoustic parameters (Mathevon et al., 2003). If the ratio is >1 , then the parameter has the potential to encode the individual identity information, since its intra-individual variability is smaller than its inter-individual variability. Details for the PIC calculation are presented in the Supplementary material (Table S3).

We performed a cross-validated (leave-one-out) DFA to determine if contact calls of African, Humboldt, and Magellanic penguins could be correctly classified to the correct species. In this case, we used the species as test factor and the acoustic parameters as discriminant variables. Moreover, since we did not record the same number of calls per individual, we also performed a pDFA (Mundry and Sommer, 2007) for nested data with 1000 permutations. We used the species as test factor and individuals as a control factor. Finally, since pDFA classification resulted in a significant discrimination level ($p < 0.001$), we further investigated which vocal parameters significantly differed in their average values among species using a series of univariate General Linear Models (GLM) for ANOVA analysis. Specifically, each vocal parameter was entered in turn as a dependent variable and the species was used as a fixed factor. Individuals were fitted as random factors to control for repeated sampling. Finally, in order to control for individuality, we used a nested design with individual factor nested under the dependent variable (species).

We performed pDFA analysis in R (R Core Team 2014, Version 3.2.2015-04-16, R Foundation for Statistical Computing). All the other statistical analyses were performed in SPSS v.20 (IBM Corp. Released 2013. IBM SPSS Statistics for Macintosh, Version 20.0. Armonk, NY: IBM Corp.).

3. Results

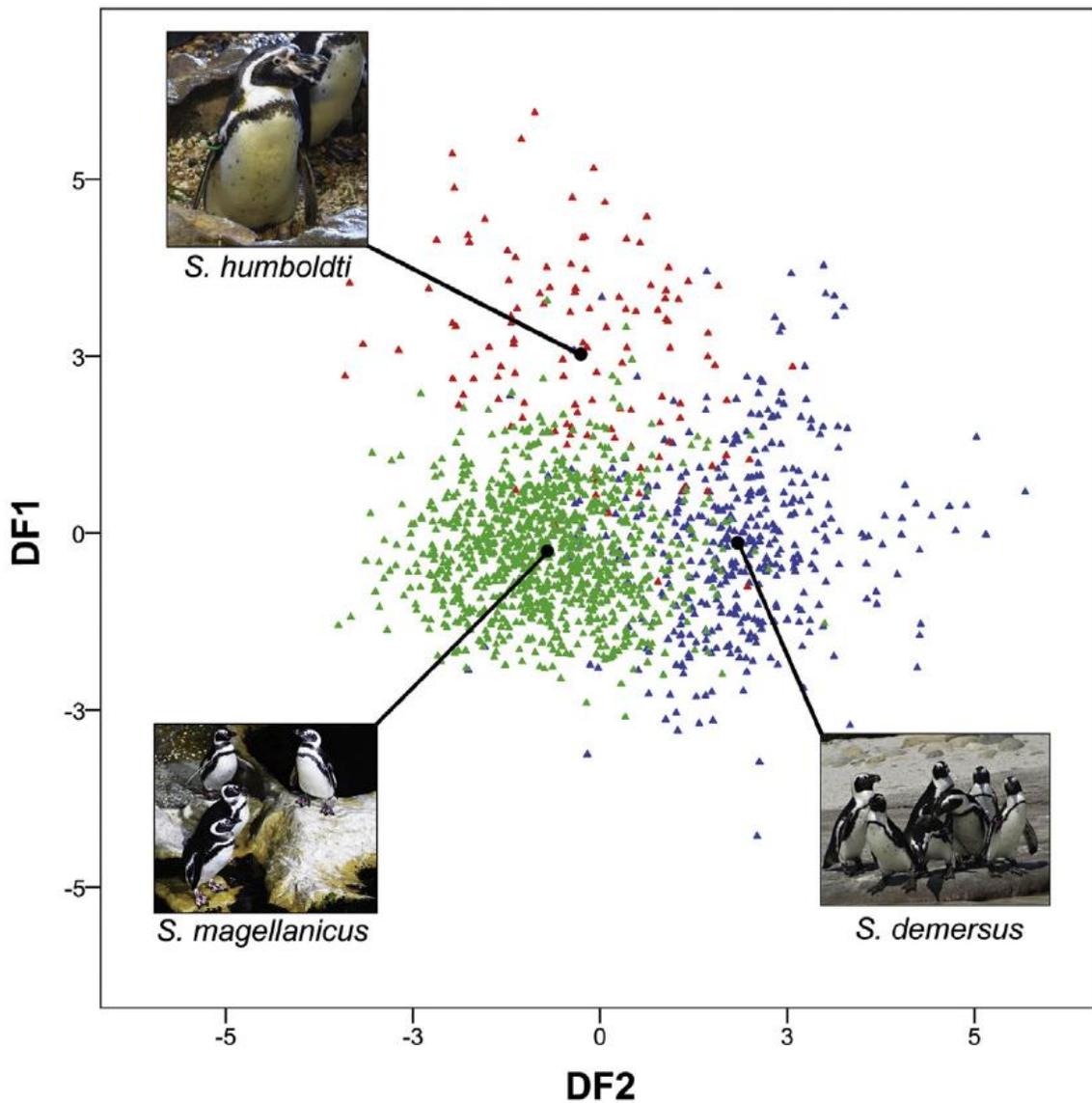
3.1. Individual identity

Using the source and filter vocal parameters as independent variables, the discriminant function analysis (DFA) correctly classified 70.0% of the contact calls to the emitter for Magellanic penguins and 60.5% for Humboldt penguins. The accuracy of the DFA decreased to 69.0% and 55.5% respectively, when the more conservative leave-one-out cross-validation was applied. The statistical significance of this classification and details of the canonical discriminant functions are presented in Supplementary material (Table S2). Overall, our results show that individual identity is encoded also in contact calls of both Magellanic and Humboldt penguins. The PIC analysis confirmed that both source- and filter-related components have the potential to encode individual identity information (Supplementary material, Table S3).

3.2. Species recognition

Calls were correctly classified to the correct species in 88.8% of cases. The accuracy of the DFA decreased to 88.3% when the more conservative leave-one-out cross-validation was applied. Table 1 shows values of the vocal parameters for contact calls of African, Magellanic and Humboldt penguins. Details of the canonical discriminant functions generated for the discriminant function analysis are presented in Supplementary material (Table S4). Fig. 2 shows that contact calls of the three species form distinctive clusters in space, defined by discriminant functions 1 and 2. The nested pDFA confirmed that when controlling for individuality, contact calls could be correctly classified on the basis of species (cross-validated classification after 1000 permutations: expected = 41.38%, observed = 77.09%, $p < 0.001$). Results from GLMs (nested ANOVA, Table 2) showed that average values of eight acoustic parameters significantly differed among species, and thus are potentially important for vocal distinctiveness. These included call duration, four source- (f_0 min, f_0 max, f_0 end, Time f_0 min) and three filter- (F_1 , F_4 , ΔF) related measures. Therefore, we found evidence that penguin contact calls also encode species-specific acoustic signatures.

Fig. 2. Discriminant scores generated by the discriminant functions 1 and 2 to classify contact calls of African (*S. demersus*), Humboldt (*S. humboldti*), and Magellanic (*S. magellanicus*) penguins. Black dots indicate the centroid of each species.



4. Discussion

We investigated whether banded penguin contact calls encode individual identity information and species-specific vocal signature. We found evidence that contact calls of Spheniscus penguins allow individual discrimination using both source- and filter-related components. Moreover, we showed that contact calls can be classified according to species in a manner far greater than that attributable by chance. Overall, our results provide further evidence that the “source-filter” theory of vocal production (Fitch, 2010; Taylor and Reby, 2010) can be successfully applied to the interpretation of information contained in bird vocalisations (Ohms et al., 2010; Budka and Osiejuk, 2013).

Penguin contact calls have been poorly studied compared to display songs. However, recent research has found that the contact calls of African penguins are individually distinctive (Favaro et al., 2015). In this species, the morphology and size of the vocal apparatus allow modifications of the energy distribution across the spectrum. Accordingly,

several source- and filter-related vocalisation features were found to exhibit a smaller amount of intra-individual variation when compared to inter-individual variation (Favaro et al., 2015). Our results provide further evidence that individual identity information is also encoded in contact calls of two other species of the genus *Spheniscus*. The results of the PIC and DFA analyses also support the hypothesis that vocal individuality in nesting penguins is determined by both source- and filter- related parameters.

Banded penguins often form flocks at sea for travelling and foraging (Wilson and Wilson, 1990). When in flocks, they utter contact calls to maintain group cohesion (Jouventin, 1982). Penguins at sea have highly synchronised diving behaviour (Siegfried et al., 1975), both when swimming to travel (short and shallow dives of 10–20 s) and when diving for foraging (longest dives up to 2–3 min), (Wilson and Wilson, 1990). When underwater, birds are likely to get out of contact with the other group members and, especially when in large flocks, they can surface asynchronously (Wilson et al., 1986). In such circumstances, they need to call to locate other group members when out of sight. In this fission-fusion context, where the effective distance of visual signals is shorter compared to vocalisations, we expected to find high selective pressures for vocal individuality (Janik and Slater, 1998). In more confined captive settings, juveniles banded penguins swimming alone in ponds emit contact calls to maintain social contact with parents (Thumser and Ficken, 1998). In all the colonies studied, we also observed adult pair members keeping in touch with each other with contact calls when visually isolated and returning to the nest after that the partner had repeatedly emitted contact calls (L. Favaro, personal observation). Overall, our results support the hypothesis that penguin contact calls are social signals that have evolved to facilitate social reunion and maintain group cohesion.

The ecstatic display songs of African, Magellanic and Humboldt penguins have diverged in several spectral and temporal acoustic parameters (Thumser and Ficken, 1998) and it has been suggested that this vocal type can reflect phylogenetic relationships within this genus (Thumser et al., 1996). Our DFA analysis results support the hypothesis, demonstrating that acoustic cues to species are also present in contact calls and are likely to depend on the anatomy of the vocal tract. In addition, the GLM underlined how eight source- and filter- vocal parameters differ among the three species. However, in closely related seabirds, the ability to detect species-specific vocal features is controversial (Bretagnolle and Robisson, 1991; Curé et al., 2010, 2012) and there is no evidence that penguins are capable of using such information from vocalisations. In particular, penguins of the genus *Spheniscus* have limited phylogenetic distance and hybrids have been found where the geographical distributions of Magellanic and Humboldt penguins overlap along the Pacific coast of South America (Simeone and Schlatter, 1998; Simeone et al., 2009). This suggests that the species-specific recognition systems fail to prevent heterospecific confusion. Banded penguins are also known to readily hybridise when grouped together in captivity (McCarthy, 2006). In sympatric closely-related species, differences in signalling characters often evolve to prevent incorrect mate choice (Losos and Leal, 2013). However, although we found acoustic differences in both sympatric and allopatric penguins within the penguin genus *Spheniscus*, we suggest that species-specific acoustic signature in their contact calls is more likely to be a by-product of divergent ecological selection rather than a pre-zygotic mechanism to prevent hybridization. In social animals, not all the acoustic cues encoded in calls are necessarily salient to receivers (Townsend et al., 2011). We recommend additional studies using playback experiments, to further investigate the sensitivity of penguins to acoustic cues of species.

In conclusion, our findings confirm that the source-filter theory of vocal production can be successfully adopted to study bird vocalisations. Using this approach, we provide further evidence that banded penguin contact calls encode individual identity and species-specific signatures. We suggest that the high levels of individuality in these vocalisations are an adaptation to travelling and foraging in large flocks at sea.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.beproc.2016.04.010>.

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