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Wind farm noise shifts vocalizations of a threatened shrub-steppe passerine[☆]

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ABSTRACT

Wind energy has experienced a notable development during the last decades, driving new challenges for animal communities. Although bird collisions with wind turbines and spatial displacement due to disturbance have been widely described in the literature, other potential impacts remain unclear. In this study, we addressed the effect of turbine noise on the vocal behaviour of a threatened shrub-steppe passerine highly dependent on acoustic communication, the Dupont's lark *Chersophilus duponti*. Based on directional recordings of 49 calling and singing males exposed to a gradient of turbine noise level (from 15 up to 51 dBA), we tested for differences in signal diversity, redundancy, and complexity, as well as temporal and spectral characteristics of their vocalizations (particularly the characteristic whistle). Our results unveiled that Dupont's lark males varied the vocal structure when subject to turbine noise, by increasing the probability of emitting more complex whistles (with increased number of notes) and shifting the dominant note (emphasizing the longest and higher-pitched note). In addition, males increased duration and minimum frequency of specific notes of the whistle, while repertoire size and signal redundancy remain constant. To our knowledge, this is the first study reporting multiple and complex responses on the vocal repertoire of animals exposed to turbine noise and unveiling a shift of the dominant note in response to anthropogenic noise in general. These findings suggest that the Dupont's lark exhibits some level of phenotypic plasticity, which might enable the species to cope with noisy environments, although the vocal adjustments observed might have associated costs or alter the functionality of the signal. Future wind energy projects must include fine-scale noise assessments to quantify the consequences of chronic noise exposure.

1. Introduction

Animals widely rely on acoustic communication to convey and receive biologically relevant information (Bradbury and Vehrencamp, 1998; Gerhardt and Huber, 2002; Narins et al., 2006). Overall, vocalizations are subjected to behavioural plasticity (vocal adjustments) and adaptation (evolutionary changes) that optimize acoustic communication and enable species to overcome constraints imposed by a myriad of environmental factors (Brumm and Slabbekoorn, 2005; Ey and Fischer, 2009). In addition to the effect of habitat on sound transmission (e.g.,

vegetation structure, Penna and Solís, 1998; Slabbekoorn and Smith, 2002; Wiley and Richards, 1982), abiotic (e.g., rain, streams or wind: Penna et al., 2005) and biotic noise (e.g., heterospecific choruses; Brumm and Slabbekoorn, 2005) can strongly influence communication, and hence both vocal performance and signal evolution (Wilkins et al., 2013). Besides, the abundance of conspecifics determines the intensity of territory defence, leading to shifts in signal redundancy as well as spectral and temporal characteristics of vocalizations (Barrero et al., 2020; Hamao et al., 2011; Liu, 2004; Perrill and Bee, 1996; Wells and Greer, 1981). In vocal-learning animals, isolation and population

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declines accelerate cultural drift due to the associated fewer number of adult tutors during song learning in juveniles (Laiolo and Tella, 2007; Paxton et al., 2019).

The impact of anthropogenic noise on animal acoustic communication has attracted growing attention in the last decades (Brumm and Slabbekoorn, 2005; Gil and Brumm, 2014; Kunc and Schmidt, 2019). In birds, studies have shown that such noise source potentially cause masking interference, leading to deleterious effects on mating (Habib et al., 2007), breeding (Habib et al., 2007; Halfwerk et al., 2011b; but see Francis et al., 2009), behaviour (Karp and Root, 2009), physiology (Barber et al., 2010), survival (Zhou et al., 2019), and ultimately on population status (Forman et al., 2002; McClure et al., 2013; Reijnen et al., 1995; Shannon et al., 2016). Despite these impacts, bird species persist in noisy environments due to several communicative strategies (Wong and Candolin, 2015). By adjusting their vocalizations, birds can avoid masking interference and improve communication via an increased signal-to-noise ratio (Redondo et al., 2013). Among other behavioural responses (Wong and Candolin, 2015), these vocal adjustments have been observed in birds exposed to multiple noise sources (e.g., abiotic, air or road traffic noise) and include shifts in amplitude (the so-called 'Lombard effect'; Brumm, 2004; Brumm and Zollinger, 2011; Derryberry et al., 2017), spectral (Courter et al., 2020) and/or temporal characteristics (see review in Brumm and Slabbekoorn, 2005), as well as on timing (Dorado-Correa et al., 2016), redundancy (Barrero et al., 2020; Brumm and Slater, 2006), and song structure and repertoire (Juárez et al., 2021; Redondo et al., 2013).

As technology evolves, anthropogenic noise changes, opening new questions about how animals deal with changing acoustic environments. A relatively novel noise source in natural settings is generated by wind energy, which has globally experienced a notable expansion in recent years (Global Wind Energy Council, 2020). A growing body of evidence has documented the effects of these infrastructures on bird populations (Adeyeye et al., 2020), such as collisions with wind turbines (Wang et al., 2015), barrier effects (Cabrera-Cruz and Villegas-Patracá, 2016) and disturbances driving spatial displacements (Winder et al., 2014). However, the potential impact of wind turbine noise has received less attention so far (Szymański et al., 2017; Whalen et al., 2019; Zwart et al., 2015), despite that previous studies have speculated that it might cause alterations on bird populations (Gómez-Catasús et al., 2018). As other sources of anthropogenic noise, wind turbine noise might reduce the likelihood of effective acoustic communication by decreasing detection space (Raynor et al., 2017) and inducing changes on bird vocalizations (Szymański et al., 2017). Although the impact of anthropogenic noise on the animal behaviour has largely examined in recent years, turbine noise has distinct characteristics (e.g., particular temporal and spectral features or the presence of associated substrate-borne vibrations), which may generate specific and complex effects on local populations.

In this study, we explore the potential impact of wind turbine noise on the vocalizations of an oscine passerine, the Dupont's lark *Chersophilus duponti* (Vieillot, 1820), classified as Vulnerable in the IUCN Red List (BirdLife International, 2020). Using directional recordings of calling and singing males in populations exposed to a gradient of turbine noise level, we determined differences in signal diversity (vocal repertoire size), signal redundancy (vocalization rate), signal complexity (number and type of notes per vocalization), and temporal (note duration) and spectral characteristics (frequency 5%, peak frequency and dominant note), while accounting for other confounding factors (hour, date, population size and isolation). We hypothesize that birds in populations exposed to higher level of wind turbine noise might exhibit a poorer vocal repertoire size, since masking interference compromises signal detection and hence song learning in juveniles (Slabbekoorn and Ripmeester, 2008). Conversely, those birds might show increased signal complexity (Klingbeil et al., 2020), signal redundancy (Brumm and Slater, 2006), note duration (Brumm and Slabbekoorn, 2005) and spectral properties of their vocalizations (Bermúdez-Cuamatzin et al., 2009), whether they are able to show behavioural responses to enhance

signal detectability and overcome acoustic masking. Our findings provide a better understanding of the effect of wind farms on birds, specifically assessing complex behavioural responses that enable species to adapt to turbine noise pollution and thus to potentially mitigate the impacts of wind energy development.

2. Material and methods

2.1. Study area

Dupont's lark males were recorded in 11 localities, eight in the absence and three in the presence of wind farms. The study area is located in central Spain (Soria; 2°26'35.1"W, 41°11'28.9"N; c. 1200 m a.s.l.; Fig. 1a) and covers around 200 km². The landscape is a flat, short shrub-steppe dominated by *Genista pumila*, *G. scorpius*, *Thymus* spp. and *Satureja intricata* (Garza et al., 2005). The study area forms a meta-population scenario consisting of a set of patches of suitable habitat for the Dupont's lark (i.e., short shrub with slopes lower than 15%; Garza et al., 2005, Fig. 1a). Patches closer than 1 km are considered as the same locality for Dupont's lark since most adults typically move within this distance (Vögeli et al., 2008). The study area is among the more depopulated regions in Europe with less than 3 citizens/km² and hence other sources of anthropogenic noise are negligible. The distance from the recorded males to the nearest urbanized area ranged from 0.70 to 4.42 km (mean ± SD = 2.23 ± 0.98 km), and to the nearest road from 0.22 to 3.41 km (1.41 ± 0.79 km).

The so-called Medinaceli Wind Resource Area is an extensive node located in the study area (Fig. 1a) that includes nine wind farms, consisting of 10–32 turbines of 1500–2500 kW each. Wind farms were installed between 2007 and 2008, five of them being within historical Dupont's lark localities. Wind turbine sounds are mainly generated by the interaction between the blades and the air or atmospheric turbulence (aerodynamical sound), or by the rotation of the blades (mechanical sound; van Kamp and van den Berg, 2018). The maximum sound pressure level radiated from a turbine is ca. 100–110 dBA in their immediate vicinity, but it drops down to approximately 55 dBA at ground level (van Kamp and van den Berg, 2018), and to 45 dBA at a distance of 300 m away from wind turbines (Kaldellis et al., 2012). Turbine noise concentrates on the low-frequency band up to 2 kHz, but it can reach up to 6–8 kHz (Kikuchi, 2008). Moreover, infra-sound noises (<20 Hz) are also produced due to inflow turbulence or by sideways movements of blades, as well as ground vibrations (van Kamp and van den Berg, 2018).

2.2. Turbine noise map

A noise map was computed in accordance with the procedure established in the international standard ISO 9613 (ISO, 1993, 1996), as recommended by the Directive 2002/49/EC (but see Hansen et al., 2017 for the pros and cons of different national and international standards on noise propagation). Noise mapping tools are based on commonly used prediction models, with high accuracy and validated by national and international standard organisations (Alberola Asensio et al., 2002; Asensio et al., 2011). Noise computation models allow assessing and mapping environmental noise pollution (in terms of sound pressure level) from a diversity of noise sources over a given area and a specific period of time (Iglesias et al., 2012). It must be noted two key concepts: (i) *sound power level* (L_w), which is the general strength of a noise source; and (ii) *sound pressure level* (SPL), which is a noise level measured at the receiver's position. In this case, the actual instantaneous acoustical sound power level depends on the local effective wind speed at the turbine's height (Verheijen et al., 2011). Noise mapping requires L_w input data to be estimated (Appendix A) on the basis of technical specifications published by manufacturers as well as data included in official projects' authorization statement (e.g., turbine's height, model). In total, 162 wind turbines were modelled within the study area, and turbines' height ranged between 67 and 100 m above ground level. We

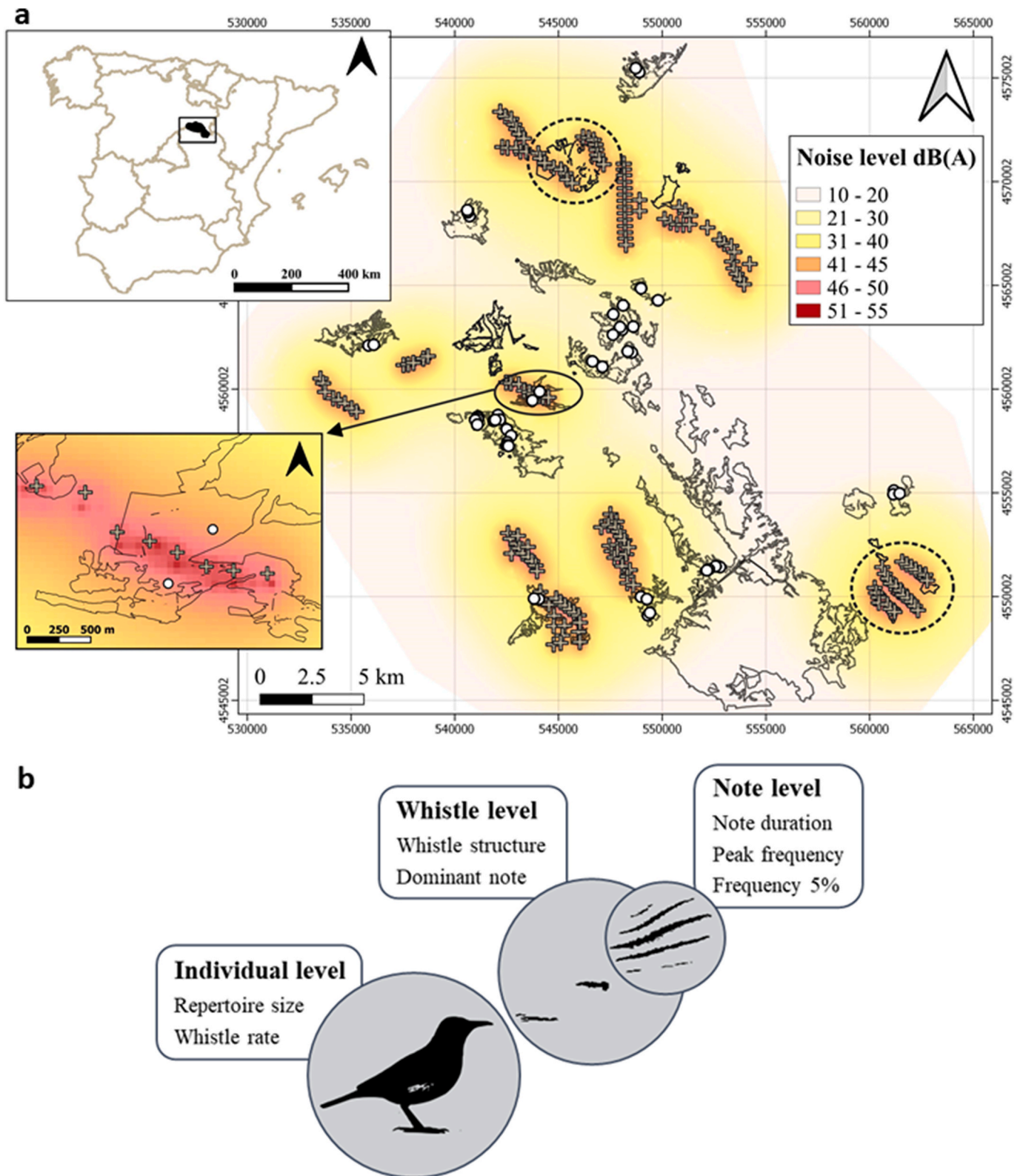


Fig. 1. (A) Map of the study area. Shrub-steppe patches with historical Dupont's lark localities since 2008 (black lines), wind turbines (grey crosses) and recorded Dupont's lark males (white dots) are depicted. Patches with extinct Dupont's lark localities at wind farms are highlighted by circles with dotted line. Moreover, the noise map is shown, representing pure turbine noise in terms of sound pressure level in A-weighted decibels, dBA: from high (dark red) to low (white) turbine noise levels. At bottom left, one Dupont's lark locality at wind farms is expanded (ellipse). Lastly, the map at the top represents the location of the study area in Spain. (B) Scheme on the acoustic parameters calculated at the individual, whistle, and note level. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

modelled the worst-case scenario (i.e., noise emissions are close to the maximum power) which, according to manufacturers, may occur at wind speeds higher than 7–9 m/s (at 10 m height above ground level).

SPL varies with distance from the receiver to the noise source, among other variables (e.g., orography and meteorological variables). A digital elevation model (DEM) was built based on official and digital national topography maps (1:25,000 scale) from the National Centre for Geographic Information (CNIG), which shows contour lines at 5 m intervals. We employed annual meteorological data (temperature and air humidity), instead of weekly or monthly data, to avoid computing multiple maps for each recording period, as well as to search its maximum applicability in fields such as environmental impact assessment of new projects. In any case, changes on these variables have a negligible effect in terms of SPL (Appendix B). We considered a ground factor $G = 1$ which represents porous ground (i.e., the acoustic softness of grass land, farming land). We did not incorporate an additional layer of vegetation in order to avoid an excessive underestimation of SPL, since the screening effect of foliage is produced when the vegetation is so dense that the receiver cannot see through it (Brüel & Kjær 2005), which is far from our case. The vegetation in the study area is comprised of sparse and short shrubs (<10 cm; see Section 2.1 Study area), and noise sources (i.e., wind turbines) are located at a height over 65 m above ground level, so the effect of vegetation of noise propagation is negligible.

We calculated the equivalent continuous sound pressure level (LAeq) in A-weighted decibels (dBA), which characterizes noise pollution when wind turbines are operating. It is also important to note that a noise map of a sound source does not represent the actual level of the acoustic environment, but the contribution in terms of SPL (re 20 μ Pa) of the modelled noise source to the acoustic environment. In our study area, three main sound sources were identified: biophony (mainly birds), geophony (wind) and anthrophony (wind turbines). The noise map represents an estimation of how SPL is distributed throughout the study area due to wind turbine noise during the so-called worst-case scenario. Calculations were carried out over a grid (100 m resolution) of potential receiver's positions (more than 42,000 points), virtually located at a height of 0.10 m above ground level. The noise prediction software package Predictor™ Type 7810 version 5.4 (Brüel & Kjær, 2005) was used for the noise model computation. Finally, we employed a usual spatial interpolations technique, namely inverse distance weighting, IDW (Stoter et al., 2008), to plot 50 m resolution noise maps using ArcGIS 10.4 (ESRI, 2016) since it captures the local surface variation when there is an initial dense set of points (Margaritis and Kang, 2017).

2.3. Noise mapping validation

Whenever possible, noise maps are validated using SPL field measurements (Iglesias-Merchan et al., 2018; Mioduszewski et al., 2011), but unfortunately there is neither standard methods of verification nor a homogeneous criterion (GoŁębiewski and Makarewicz, 2009). The conventional way to make this comparison is by measuring ambient noise levels in the field with a sound level meter that gives SPL in decibel (re 20 μ Pa). However, most noise sources cannot be adequately identified by sound level meter's spectral measurements. On the contrary, audio recordings allow identifying sound sources (Mennitt and Frstrup, 2012). In this sense, our validation relied on field audio recordings that were taken in the closest monitoring location to a wind turbine (i.e., 177 m; hereinafter referred to as the validation point).

First of all, it is worth to mention that feasible and valid calibration of recording equipment (microphone, preamplifier and digitizer as a whole) can be done in different ways in order to estimate SPL (re 20 μ Pa) from audio recorded files (Garg et al., 2019; Maryn and Zarowski, 2015). Calibrated data provide absolute measures of sound levels as well as it enables post hoc analyses to identify components of the acoustic environment (Merchant et al., 2015). Thus, we conducted field calibration of the recording equipment (see 2.6 Recordings and acoustic analyses) by

synchronizing it with a professional audio sound level meter CESVA SC 420, which meets the specifications of IEC 61672 international standard for class 1, and the American standards ANSI S1.4 and ANSI S1.43 as class 1. Both instruments were installed in parallel at approximately 1.5 m above ground level in open country, and outdoor background noise level (in 1-s intervals) was recorded and analysed in 1:1 octave band spectra (ranging from 63 Hz to 8 kHz).

The sound level meter was measured simultaneously in A and Z frequency weightings, and calibrated (using a 94 dB signal at the frequency of 1 kHz) before and after field recording. On the other hand, Audacity software (version 2.2.1) was used to extract frequency components and amplitude level from the audio file, the latter given in terms of decibels relative to full scale (dBFS). That is a unit of measurement of a digital signal where zero means the loudest level and dB values are expressed as negative numbers (Schroder, 2011). Therefore, a calibration curve was built to know the scaling relationship between dBZ (re 20 μ Pa) from the sound level meter and dBFS from audio recordings in octave bands (63 Hz–8 kHz). Finally, considering octave bands weighting factors, we estimated the corresponding dBA values.

It is worth to notice that noise map validation requires knowing how SPL increases due to wind speed at ground level. The noise map shows predicted SPL at ground level in the worst-case scenario, but wind speed is not the same at hub height (10–12 m/s) than at ground level (where field recordings took place). In any case, background noise levels increase with wind speed, and it is important to understand their correlation. Given the surprising lack of available data in literature and the fact that manufacturers usually refer to the wind speed at hub height or at 10 m above ground (V_{10m}), it is recommendable to conduct accurate SPL measurements instead of assuming a constant baseline value of 45 dBA as typical of rural environments (Hessler and Hessler, 2006; Schneider, 2009). Therefore, we simultaneously measured both background SPL using the sound level meter located on a tripod at approximately 1.5 m above ground in a windy day and wind speed using an anemometer positioned at the same height. Once that background sound level was determined as a function of wind speed (Fig. 2), we were able to estimate SPL due to wind contribution from audio files.

We extracted 9 sections free of biophony from field records at the validation point in order to calculate their SPL (A-weighted and Z-weighted) based on the recording equipment's calibration curve. The logarithmic average of the 9 measurements was calculated obtaining an average background SPL of 49.76 dBA and 59.42 dBZ, due to the simultaneous effect of wind and turbines' noise (Appendix C). According to meteorological data from the nearest meteorological observatory, the highest average wind speed on the date of field recording at the validation point was 3.9 m/s. A wind speed of approximately 4 m/s at a

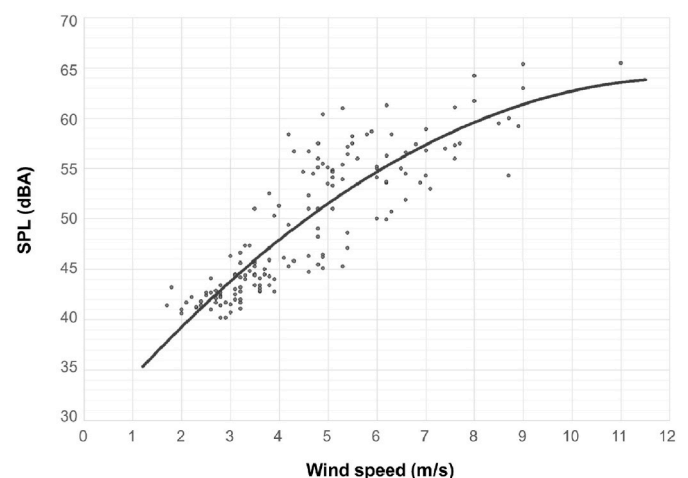


Fig. 2. Background noise level (A-weighted) due to wind speed (m/s) at a height of 1.5 m above ground level.

height of 1.5 m above ground is compatible with a wind speed higher than 10–12 m/s at hub height (the worst-case scenario). Therefore, based on the wind speed-SPL curve (Fig. 2), we considered that theoretical SPL level due to wind speed during field recording at the validation point was 48 dBA. Based on the energetic sum of 48 dBA with the predicted value in the noise map at the validation point (43.59 dBA), this resulted in a total noise level of 49.34 dBA. That is 0.42 dBA less than the total value estimated from the recordings (49.76 dBA). Thus, it can be considered that noise map predictions were in agreement with measured values (Gołębiewski and Makarewicz, 2009). In any case, it should be noticed that the key information supplied by these estimates is not absolute noise levels (in dBA), but its relative variation with respect to the distance from the turbine.

2.4. Bird territories

Dupont's lark territories at the 11 studied localities were mapped (Fig. 1a) using transects and geolocalization (Bibby et al., 2000). Transects were placed through the centre of suitable habitat patches and were walked to search for singing males. The number of transects per locality was proportional to patch size (ranging from 1 to 19 transects per locality), while their length varied between 1 and 3 km. Transects were walked at least 4 times during the breeding season, from the end of March until middle June of 2016. The starting point was alternated in each visit with the aim of surveying both ends of the line transect during the peak of singing activity of the study species. Surveys were carried out approximately 1 h before dawn and lasted around 1 h. The location of each singing male was georeferenced with a GPS and a mapping method was employed to assign each observation to a specific territory (Bibby et al., 2000). By gathering all the observations from the surveys and distinguishing simultaneously contacted neighbouring males, the territory of each Dupont's lark was defined (Pérez-Granados and López-Iborra, 2017).

2.5. Dupont's lark vocalizations

Dupont's lark males emit several vocalizations, the most common being the territorial call and the song (Cramp and Simmons, 1980). The territorial call consists of a varying number of stereotyped vocalizations per individual. Each vocalization is composed of 2–3 notes emitted at increasing frequency (hereafter referred as the whistle; Appendix D). Whistles are relatively simple and preserved throughout the species' western range (Laiolo and Tella, 2005), and highly repetitive within individuals (Laiolo and Tella, 2006), which makes it a suitable signal to be examined for our research purpose.

The song is long, complex and variable, composed of several discrete sequences of notes (Laiolo and Tella, 2007), shared by neighbouring males (Laiolo and Tella, 2005). The last sequence (Cramp and Simmons, 1980) typically ends with 2–3 notes resembling the whistle (Appendix D). Based on the notes included, we classified whistles presented in territorial calls and songs into three categories: 123 structure (notes 1-2-3); 13 structure (notes 1–3); and 23 structure (notes 2–3; Appendix D). In our study area, all whistle structures were presented in songs, whereas only 123 and 13 structures occurred in territorial calls (Appendix D).

2.6. Recordings and acoustic analyses

We recorded vocalizations of a total of 73 Dupont's lark males between 16th March and 6th June, 2016, using a directional microphone (Sennheiser ME 67) connected to a digital audio recorder (TASCAM DR40, sampling frequency 44,100 Hz, 24-bits, Wav format). All recordings were carried out during 5 min, from 1 h before dawn until sunrise, under favourable weather conditions (i.e., absence of precipitation and wind speed below 5 m/s) and without full moon. Humidity and temperature may affect sound transmission and attenuation of

vocalizations (i.e., sound amplitude), but they do not affect acoustic parameters related to temporal or spectral domain, such as the seven parameters measured in this study (see below). During the recordings, special care was taken to avoid altering the behaviour of the focal males and to obtain high-quality recordings, by approaching stealthily and preventing additional disturbances due to noise or lights. Double recording was also avoided using the territory mapping information (see 2.4 *Bird territories*). After a detailed analysis of audio files, recordings from 49 males were selected for further analysis, while 24 males were discarded, to ensure suitable audio quality and prevent any potential double recording. As the vocalizations of the Dupont's lark are highly repetitive, we analysed the 2 min of each recording with the highest audio quality, totaling a set of 98 min of audio files. Such time frame (2 min) has been found to be appropriate to record the full repertoire of the study species (Pérez-Granados et al., 2016).

We measured seven acoustic parameters across three analytic levels (individual, whistle and note; Fig. 1b), using Raven Pro 1.6 (Center for Conservation Bioacoustics, 2019): vocal repertoire size, whistle rate, whistle structure, dominant note, note duration, and minimum and peak frequency of the note. We inspected the whole song and territorial call to measure repertoire size (i.e., number of unique song and call types), whereas we focused on the whistle presented in both songs and territorial calls to measure the other acoustic parameters. The acoustic constancy of whistles makes them a recognizable structure among individuals, while showing certain plasticity that might be related with environmental variables. Firstly, at the individual level, we calculated *repertoire size* as the total number of unique sequences recorded per individual (i.e., song and call types for singing and calling individuals, respectively). Sequences were categorized by audio and visual inspection of all spectrograms by the same researcher to avoid inter-observer bias (Laiolo and Tella, 2005). Secondly, we calculated *whistle rate* as the number of whistles per minute, obtaining a single value per male. At the whistle level, we classified *whistle structure* based on the specific notes of each whistle (123, 13 and 23 structures; Appendix D), and registered the note containing the highest sound energy within the whistle (hereafter *dominant note*). Lastly, we measured *duration* (s), *frequency 5%* (Hz), and *peak frequency* (Hz) in each note of the whistle. The frequency 5% is a measure of energy distribution (the frequency that divides the sound in two parts containing 5% and 95% of the energy; Charif et al., 2010) and it was employed as proxy of minimum frequency. Peak frequency is the frequency at which the maximum power occurs (Charif et al., 2010). Temporal parameters were analysed in oscillograms, while spectral parameters in spectrograms (window size 2046, overlap 90%, Hann window, frequency resolution 21.5 Hz).

2.7. Predictors of vocal behaviour

A total of ten explanatory variables were calculated to examine their relationship with the vocal behaviour of the study species. These variables accounted for effects at three levels: locality, individual and whistle (Appendix E). First, we used two predictors associated with habitat fragmentation at the locality level: isolation and population size. Both variables have been previously associated with impoverishment of call pools and song repertoires on Dupont's lark populations (Laiolo et al., 2008; Laiolo and Tella, 2007) and hence they may also affect other aspects of songs and territorial calls. Moreover, population size is a proxy of male competition, which can produce shifts in bird vocalizations (Barrero et al., 2020; Hamao et al., 2011). Isolation was measured as the distance from the centroid of each locality to the centroid of the territories of the nearest occupied locality in 2016 using the software QGIS 2.14.0 (Quantum GIS Development Team, 2020). Population size was calculated as the number of Dupont's lark territories per locality, using the information derived from the territory mapping (2.4 *Bird territories*). Additionally, we included wind farm occurrence as an indicator of noise disturbance at the locality level.

Second, we considered the date and hour at which each individual

was recorded, since it could potentially affect its vocalizations (Pérez-Granados et al., 2018). Moreover, we recorded the main type of vocalization performed by the individual (singing or calling males; Appendix E). As noise parameters, we measured the distance from the GPS location of the recording to the nearest wind turbine, and we calculated the mean sound pressure level in a 350-m buffer around the recording using the information from the noise map (see 2.2 Turbine noise map; Appendix E). The 350-m buffer was defined in accordance with the maximum home range of the Dupont’s lark (Garza et al., 2005). Both variables were calculated using QGIS 2.14.0 (Quantum GIS Development Team, 2020). Lastly, to account for predictors at the whistle level, we included whistle structure (Appendix D) and the note of the whistle (first/second/third) at which the spectral and temporal variables were calculated (Appendix E).

2.8. Statistical analysis

Fixed covariates were z-standardized (i.e., mean 0 and standard deviation 1), and transformed when necessary to achieve linearity. All predictors were tested for collinearity prior to data analysis, retaining only those with a generalized variance inflation factor ($GVI^{P1/2df}$, where df states for the degrees of freedom) lower than 2 (Fox and Monette, 1992). Wind farm occurrence (locality level), distance to the nearest wind turbine, and sound pressure level from the noise map (individual level) were highly correlated, and thus we used only the predictor noise in subsequent analysis (Appendix F). As decibel measurements are in logarithmic scale, they were transformed to a linear scale (micropascals, μPa) for statistical analysis and then transformed back to dBA for result representation.

To test for the effect of wind turbine noise on acoustic parameters of the Dupont’s lark vocalizations, we used linear mixed-effects models (LMM) and generalized linear mixed-effects models (GLMM). These models are widely used in analysing clustered and hierarchical data, for which the observations within a particular group are expected to be correlated (Casella et al., 2006). Our dataset has a hierarchical structure (note-whistle-individual-locality; Fig. 1b) and hence we expect that observations measured at a given locality, individual or whistle to be correlated, supporting the employment of this analytical tool. For each acoustic parameter, we performed a single model, using turbine noise level and vocalization type as predictors, and recording time, date, locality population size and isolation as covariates (Table 1). For parameters measured at the note level (note duration, frequency 5% and peak frequency), we also included the note as fixed factor (three levels: note 1, 2 or 3) and the interaction term between this and the covariate turbine noise (Table 1), as we expected low-frequency notes to be the most masked ones by noise and hence particularly prone to vocal adjustments (Hu and Cardoso, 2009; Lowry et al., 2013). Lastly, in the analysis addressing the effect of noise on the dominant note, we added whistle structure as fixed factor (Table 1) since we observed that the dominant note differed between whistle structures (Appendix G). Locality, whistle

and individual identity were treated as random effects in all models to control for pseudoreplication (Zuur et al., 2009). All random factors were included in models at the note level, whereas locality and individual were incorporated in models at the whistle level, and only locality was added to models at the individual level (Table 1). Data on captured individuals (unpublished data) revealed that body weight did not differ between Dupont’s lark individuals in localities with and without wind farms ($N = 56$, $\chi^2 0.979$, $df = 1$, p -value = 0.322), and thus it did not influence spectral variables (Brumm, 2009; Mikula et al., 2020).

All models were fitted using Monte Carlo Markov chain (MCMC) techniques as implemented in Just Another Gibbs Sampler (JAGS). We used the ‘rjags’ package (Plummer, 2019) as an interface from R to JAGS library for Bayesian data analysis. Prior distributions were all uninformative, expressing vague or general information on the parameters but not influencing posterior distributions (Appendix H). We ran three MCMC chains for 100,000 iterations, discarding the first 10,000 MCMC samples as a burn-in and retaining one sample every hundredth iterations thereafter (thin parameter = 100). Convergence was assessed using the Gelman-Rubin convergence statistic (Gelman and Rubin, 1992), where values close to 1 indicate convergence. We present the mean (β), standard deviation (SD) and 95% Bayesian Credible Interval (95% BCI) as posterior summaries for each model parameter. We considered predictors to have an effect on the response variable when the 95% BCI did not contain zero.

3. Results

The Dupont’s lark males recorded during singing and calling activity ($n = 49$) were exposed to wind turbine noise levels ranging from 15 to 51.17 dBA (Fig. 1a). Eight males were recorded in localities with wind farms (mean \pm SD = 0.72 \pm 0.54 km to the nearest wind turbine), whereas 41 males in the absence of these infrastructures (3.0 \pm 0.89 km). Noise level around the position of Dupont’s lark males ranged from 25.9 to 44.6 dBA (35.2 \pm 7.1) at localities with wind farms, whereas it ranged from 17.0 to 26.9 dBA (mean \pm SD = 20.7 \pm 2.7) at localities without wind farms. Turbine noise concentrated on the low-frequency band up to 2 kHz, potentially overlapping the first notes of the Dupont’s lark whistle (Fig. 3).

The number of individuals recorded per locality varied between 2 and 4 (mean \pm SD = 2.67 \pm 1.15 males) in localities with wind farms, and between 2 and 15 (5.13 \pm 4.79 males) in the absence of wind infrastructures. The proportion of recorded males out of the total number of males estimated per locality (based on the number of territories surveyed) ranged from 22.2 to 100% (45.0 \pm 22.8%) (Appendix I). Twenty-one Dupont’s lark males (20 in the absence and 1 in the presence of wind farms) only emitted territorial calls during the recording sessions (calling males), whereas the remaining 28 individuals (21 in the absence and 7 in the presence of wind farms) produced songs (singing males). A total of 825 whistles were recorded, out of which 362 were emitted by calling males, whereas 463 were emitted by singing males.

Table 1

Summary of the models fitted in this study to test the effect of turbine noise level on acoustic parameters of the vocalizations of the Dupont’s lark. The model equation is indicated, specifying the fixed and the random (within brackets) factors. “Note:Noise” stands for the interaction term between the factor Note and the covariate Noise.

	Response variable	Statistical model	Model equation
Signal diversity	Repertoire size (Number of unique song sequences and call types)	Poisson Mixed-Effects	Hour + Date + Population size + Isolation + Vocalization type + Noise + (Locality)
Signal redundancy	Whistle rate (Whistles/min)	Gaussian Mixed-Effects	
Signal complexity	Whistle structure (123, 23 or 13 structures)	Multinomial Logit Mixed-Effects	Hour + Date + Population size + Isolation + Vocalization type + Noise + (Locality) + (Individual)
Temporal characteristics	Note duration (s)	Gaussian Mixed-Effects	Hour + Date + Population size + Isolation + Vocalization type + Note + Noise + Note:Noise + (Locality) + (Individual) + (Whistle)
Spectral characteristics	Frequency 5% (Hz)	Gaussian Mixed-Effects	
	Peak Frequency (Hz)		
	Dominant note (Note 1,2 or 3)	Multinomial Logit Mixed-Effects	Hour + Date + Population size + Isolation + Vocalization type + Whistle structure + Noise + (Locality) + (Individual)

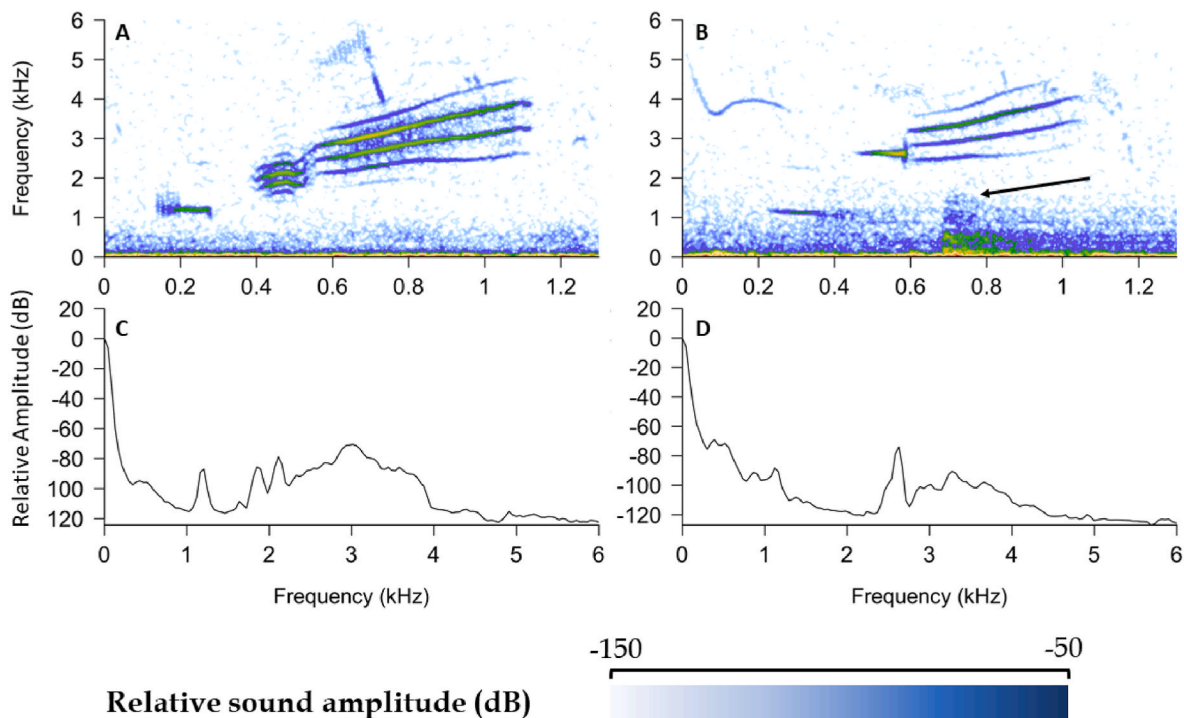


Fig. 3. Spectrograms (A and B) and frequency spectrums (C and D) of two Dupont's lark individuals in the absence (A and C) and in the presence (B and D) of turbine noise. Turbine noise can be observed in panel B concentrated on the low-frequency band up to 2 kHz, and the black arrow corresponds with the mechanical sound associated with the rotation of the blades. Lastly, colour intensity refers to the relative sound amplitude in decibels (dB). Figure created with the R package 'seewave' (Sueur et al., 2008). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.1. Repertoire size

The number of unique signals emitted per individual was only affected by the type of vocalization, with calling males performing smaller repertoire size than singing males, as shown by the Poisson GLMM ($\beta \pm SD = -1.10 \pm 0.23$, 95% BCI = $[-1.55; -0.73]$). Other factors, such as turbine noise (-0.02 ± 0.11 , $[-0.25; 0.15]$), hour (0.01 ± 0.08 , $[-0.17; 0.14]$), date (-0.15 ± 0.12 , $[-0.38; 0.04]$), population size (0.06 ± 0.14 , $[-0.21; 0.27]$), and isolation (0.09 ± 0.14 , $[-0.17; 0.31]$) did not influence this acoustic parameter.

3.2. Whistle rate

Similarly, the number of whistles emitted per minute only varied in relation to the recording date, decreasing as acoustic displays took place later in the season, as shown by the LMM model ($\beta \pm SD = -0.47 \pm 0.21$, 95% BCI = $[-0.90; -0.05]$). Other factors, such as turbine noise (0.12 ± 0.23 , $[-0.34; 0.59]$), type of vocalization (-0.42 ± 0.40 , $[-1.19; 0.37]$), hour (0.09 ± 0.18 , $[-0.27; 0.43]$), population size (0.27 ± 0.28 , $[-0.27; 0.84]$) and isolation (0.52 ± 0.28 , $[-0.01; 1.09]$), did not affect whistle rate.

3.3. Whistle complexity

Out of the 825 recorded whistles, 512 (62%) had the complete 123 structure (343 and 169 emitted by calling and singing males, respectively), 272 (33%) had the 13 structure (19 and 253, respectively) and 41 (5%) had the 23 structure (all of them by singing males). First, the Multinomial Logit GLMM unveiled that the probability of occurrence of each whistle structure differed between singing and calling males. Under low noise levels, singing males usually displayed 13 structure, followed by 123 and 23 structures, whereas calling males usually emitted the three-note whistles (123 structure), with 13 structure being rare and 23 structure completely absent. Second, turbine noise increased the

probability of males emitting whistles with 123 structure, being the most complex call more commonly used when males were exposed to higher noise level. The probability of occurrence of whistles with two notes (13 and 23 structures) also decreased as population size and isolation increased, where the complete 123 structure was more likely to be recorded. The occurrence of 13 structure decreased with date, and 13 and 23 structures decreased with hour (Table 2; Fig. 4).

3.4. Note duration

The shortest and less common note of the whistle was the second note (mean $\pm SD = 0.13 \pm 0.04$ s), followed by the first (0.17 ± 0.09 s) and third note (0.40 ± 0.12 s). Thereby, males emitted more often longer than shorter notes, according to the structure of the whistles (see previous section). While turbine noise level did not affect the duration of the first ($\beta \pm SD = -0.09 \pm 0.06$, 95% BCI = $[-0.20; 0.02]$) and third note (0.04 ± 0.03 , $[-0.02; 0.09]$), it increased the duration of the second note, the shortest one (0.13 ± 0.03 , $[0.06; 0.19]$; Fig. 5). Overall, notes were longer in calling males as compared with singing males (0.35 ± 0.07 , $[0.22; 0.48]$). No effect of hour (0.01 ± 0.02 , $[-0.04; 0.07]$), date (0.05 ± 0.04 , $[-0.02; 0.12]$), population size (-0.07 ± 0.07 , $[-0.22; 0.08]$) and isolation (-0.06 ± 0.08 , $[-0.23; 0.08]$) on note duration was observed.

3.5. Spectral parameters

The third note, which corresponds with the longest note of the whistle, has higher minimum and peak frequency (mean $\pm SD = 2.94 \pm 0.37$ kHz and 3.52 ± 0.42 kHz, respectively), followed by the second (2.91 ± 0.59 kHz and 2.91 ± 0.59 kHz, respectively) and first note (1.62 ± 0.56 kHz and 1.69 ± 0.58 kHz, respectively). Turbine noise did not influence peak frequency of any of the notes of the whistle, but it increased minimum frequency of the third note (Fig. 5). The minimum and peak frequency of these three notes were lower in calling males as

Table 2

Results of the Multinomial Logit Mixed-Effect model addressing the effect of all predictors on the probability of Dupont’s lark males emitting each whistle structure. The 123 structure is the baseline outcome. For each whistle structure, the coefficient represents the change in the occurrence probability of that whistle structure vs the baseline outcome. Posterior mean (β), standard deviation (SD) and 95% Bayesian Credible intervals (95% BCI) are shown. Important predictors are highlighted in bold.

	13 Structure			23 Structure		
	β	SD	95% BCI	β	SD	95% BCI
Intercept	0.784	0.171	[0.461; 1.123]	-3.809	1.234	[-6.372; -1.604]
Hour	-0.245	0.110	[-0.455; -0.034]	-0.442	0.208	[-0.869; -0.055]
Date	-0.537	0.227	[-0.995; -0.113]	3.348	1.995	[-0.260; 7.431]
Population size	-0.522	0.137	[-0.792; -0.265]	-1.439	0.288	[-2.024; -0.893]
Isolation	-1.003	0.140	[-1.284; -0.735]	-0.630	0.267	[-1.180; -0.110]
Calling males	-4.681	0.544	[-5.848; -3.678]	-27.840	18.004	[-71.893; -4.758]
Noise	-0.482	0.117	[-0.712; -0.252]	-1.111	0.246	[-1.613; -0.648]

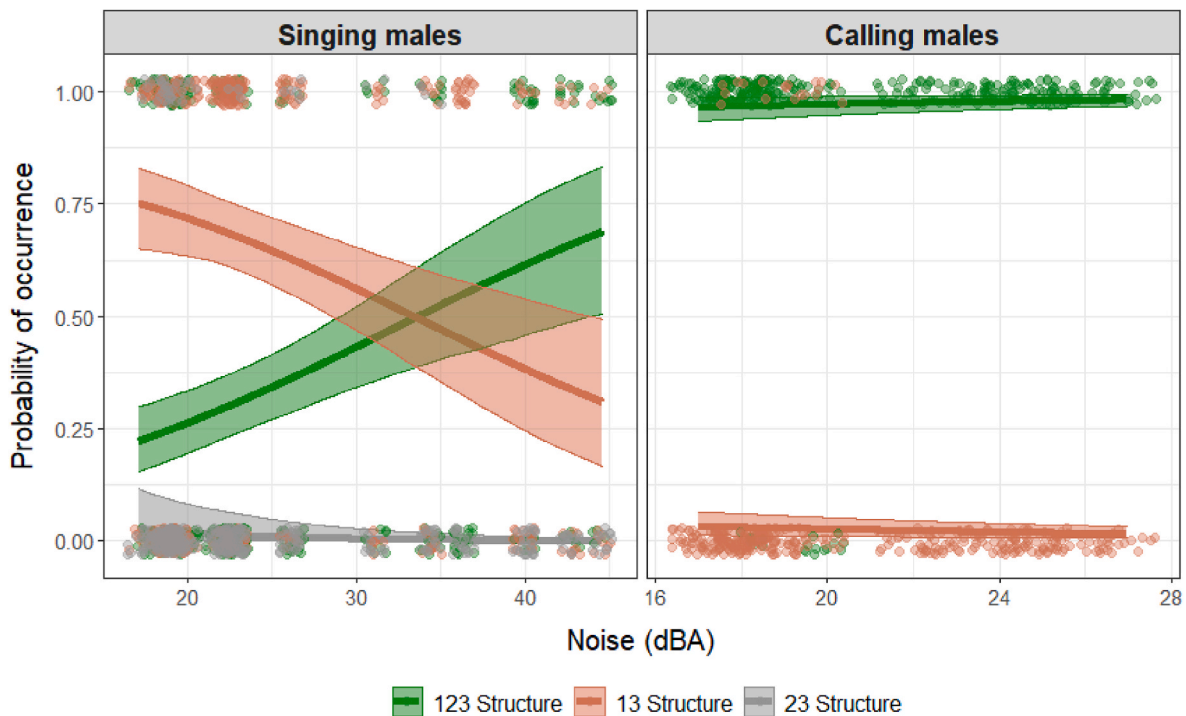


Fig. 4. Effect of turbine noise level on the probability of Dupont’s lark males emitting each whistle structure (see legend) in calling ($n = 21$) and singing ($n = 28$) males recorded in 2016. Mean (lines) and 95% BCI (surfaces) are depicted. Lastly, observed values for each whistle structure (dots) are represented.

compared with singing males. No effect of hour, date, population size and isolation on either of the two variables was observed (Table 3).

The Multinomial Logit GLMM showed that the probability of the third note being the dominant note increased as males were exposed to higher turbine noise level (Fig. 6e–f), implying that they emphasized the longest and higher-pitched note of the whistle in noisy sites, while the probability of the first and lower-pitched note decreased (Fig. 6a–b). Moreover, the probability of each note being the dominant note differed among whistle structures. Under low turbine noise levels, the third note had a higher probability of being the dominant note in 13 structures (Fig. 6e–f). When the note with an intermediate peak frequency was present (123 and 23 structures), this second note had the highest probability of being the dominant note (Fig. 6c–d; Appendix J). The probability of the third note being the dominant note increased with hour, date, population size and isolation, and it was higher in calling males (Table 4).

4. Discussion

The recent expansion of wind energy poses questions about how animals deal with this novel source of acoustic pollution in their natural

habitats. Directional recordings of calling and singing males of an endangered songbird (the Dupont’s lark) over a gradient of wind turbine noise revealed differences in acoustic parameters of their vocalizations, including complex behavioural responses. In addition to fine-scale changes in the notes of the whistle (i.e., increased minimum frequency of the longest and higher-pitched note; increased duration of the less common, shortest and lower-pitched note), Dupont’s lark males varied the structure of this vocalization when subject to wind turbine noise. Specifically, they emitted more complex whistles (with increased number of notes) and whistles with the dominant frequency in the longest and higher-pitched note. These findings demonstrate that the study species adjust their vocalizations in response to wind turbine noise, following a complex strategy to cope with this source of disturbance for acoustic communication. Together with previous studies (Szymański et al., 2017; Whalen et al., 2018; Zwart et al., 2015), our work shed light on the potential alterations caused by a new anthropogenic noise on animal behaviour. To our knowledge, this is the first study reporting a diverse and complex set of vocal responses to wind farms noise.

Anthropogenic noise produced by wind turbines is a relatively new source of noise with unique and novel features. Turbine noise occurs at low frequencies up to 1–2 kHz (van Kamp and van den Berg, 2018),

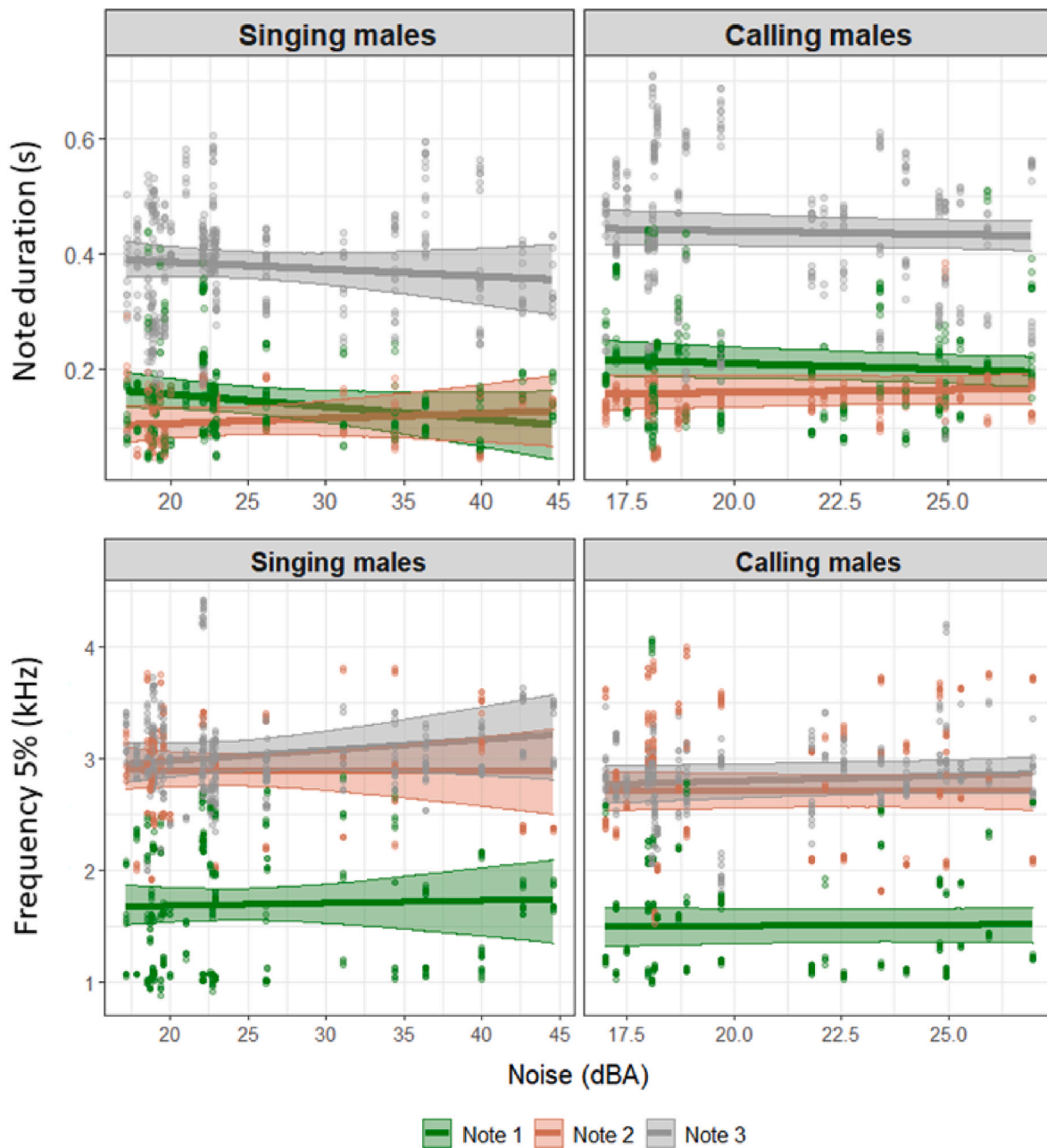


Fig. 5. Effect of turbine noise level on the duration and frequency 5% of each whistle note (see legend) in singing (n = 28) and calling (n = 21) males recorded in 2016. Mean (lines) and 95% BCI (surfaces) are depicted. Lastly, observed values for each whistle structure (dots) are represented.

posing a risk to the acoustic communication of animals conveying information around this frequency band, such as the Dupont’s lark or other bird species (Fig. 3). Our noise map revealed a maximum sound pressure level of 51.2 dBA at 0.1 m of height above ground and in the immediate vicinity of wind turbines, which agree with data previously reported (Szymański et al., 2017; van Kamp and van den Berg, 2018). Some studies have considered turbines as a ‘low noise emission source’ (Kaldellis et al., 2012) because the sound pressure level received at ground level (i.e., ca. 100 m away from the rotor blade) is almost half of the noise levels registered in the immediate surroundings of other sources of anthropogenic noise (e.g., 70 dBA for road traffic or 110 dBA for aircraft noise; Barrero et al., 2020; Gil et al., 2015). However, noise levels are expected to be more intense at heights of 100–150 m (up to 100 dBA; van Kamp and van den Berg, 2018) at which some shrub-steppe passerines typically perform aerial displays during breeding season (Gómez-Catasús et al., 2016).

On the other hand, noise modelling estimates sound pressure level in dBA, intended to reflect the response of the human ear. One common limitation of dBA is that SPL is significantly attenuated at frequencies below 400 Hz, and thus it could underestimate noise levels in the vicinity of turbines by overlooking infra-sound noises (<20 Hz) and ground vibrations (van Kamp and van den Berg, 2018). Indeed, our findings showed that SPL in Z-weighted decibels (dBZ) are expected to be almost 10 dB higher than dBA in areas close to wind turbines (see 2.3 Noise mapping validation). Typically, this difference can rise up to 15 dB due to the presence of low-frequency noise (Lagö and Persson, 2019), which increases actual differences in terms of noise pollution between areas near and far from wind farms. Until noise modelling can be standardly applied with other frequency weighting, the dBA values can be considered suitable estimates as they are highly correlated with the dBZ values. Future studies should address the effect of infra-sounds and ground vibrations on the wildlife beneath turbines (Caorsi et al., 2019).

Table 3

Results of the Gaussian Mixed-Effect models addressing the effect of all predictors on the peak frequency and the frequency 5% of each note. Posterior mean (β), standard deviation (SD) and 95% Bayesian Credible intervals (95% BCI) are shown. Important predictors are highlighted in bold.

	Peak frequency			5% Frequency		
	β	SD	95% BCI	β	SD	95% BCI
Intercept	-0.954	0.079	[-1.109; -0.790]	-0.925	0.089	[-1.100; -0.744]
Hour	0.033	0.037	[-0.040; 0.106]	0.024	0.045	[-0.065; 0.115]
Date	-0.084	0.050	[-0.182; 0.015]	-0.048	0.060	[-0.166; 0.066]
Population size	0.026	0.070	[-0.112; 0.167]	0.045	0.084	[-0.125; 0.209]
Isolation	0.035	0.076	[-0.110; 0.193]	0.006	0.083	[-0.155; 0.172]
Calling males	-0.235	0.093	[-0.412; -0.055]	-0.227	0.108	[-0.434; -0.005]
Note 2	1.248	0.022	[1.206; 1.293]	1.512	0.028	[1.458; 1.567]
Note 3	1.902	0.019	[1.864; 1.939]	1.651	0.023	[1.606; 1.696]
Noise	0.006	0.058	[-0.116; 0.117]	0.019	0.069	[-0.131; 0.145]
Noise:Note 2	-0.014	0.023	[-0.059; 0.032]	-0.022	0.028	[-0.076; 0.033]
Noise:Note 3	0.002	0.019	[-0.036; 0.041]	0.057	0.024	[0.010; 0.105]

Our work reveals that turbine noise can drive multiple and complex responses on the vocal performance of the Dupont's lark. First, repertoire size and vocalization rate of the focal males were shown to remain constant regardless the exposure to wind turbine noise. This is in contrast to the selective copying hypothesis (Slabbekoorn and den Boer-Visser, 2006; Slabbekoorn and Ripmeester, 2008) that postulates noise pollution leading to changes in vocal repertoire, as “song types that are not heard well may not be copied”. Thus, it would expect low-frequency signals to first disappear from the repertoire since they are the most vulnerable to acoustic masking by anthropogenic noise. The observed lack of effect might be due to the fact that most of the signals composing the songs and calls of the study species range from 2 kHz up to 5 kHz and are not overlapped with the frequency band of turbine noise (Fig. 3), presumably being detected and copied by juveniles. To our knowledge, this is the first study examining the effect of anthropogenic noise on vocal repertoire size of birds (measured as the unique number of song sequences and call types). At finer scale, a previous study has found a decrease on repertoire size (measured as the number of unique notes or elements) related to the masking effect caused by urban noise on the low-frequency elements of house wrens (*Troglodytes aedon*) vocalizations (Juárez et al., 2021).

As a strategy to increase the chances to convey information (Barrero et al., 2020; Brumm and Slater, 2006; Slabbekoorn and den Boer-Visser, 2006), a higher signal redundancy is expected to favour the likelihood of signal detection in noisy conditions (Wiley, 2006). However, previous studies have found inconsistent results on the changes in signal

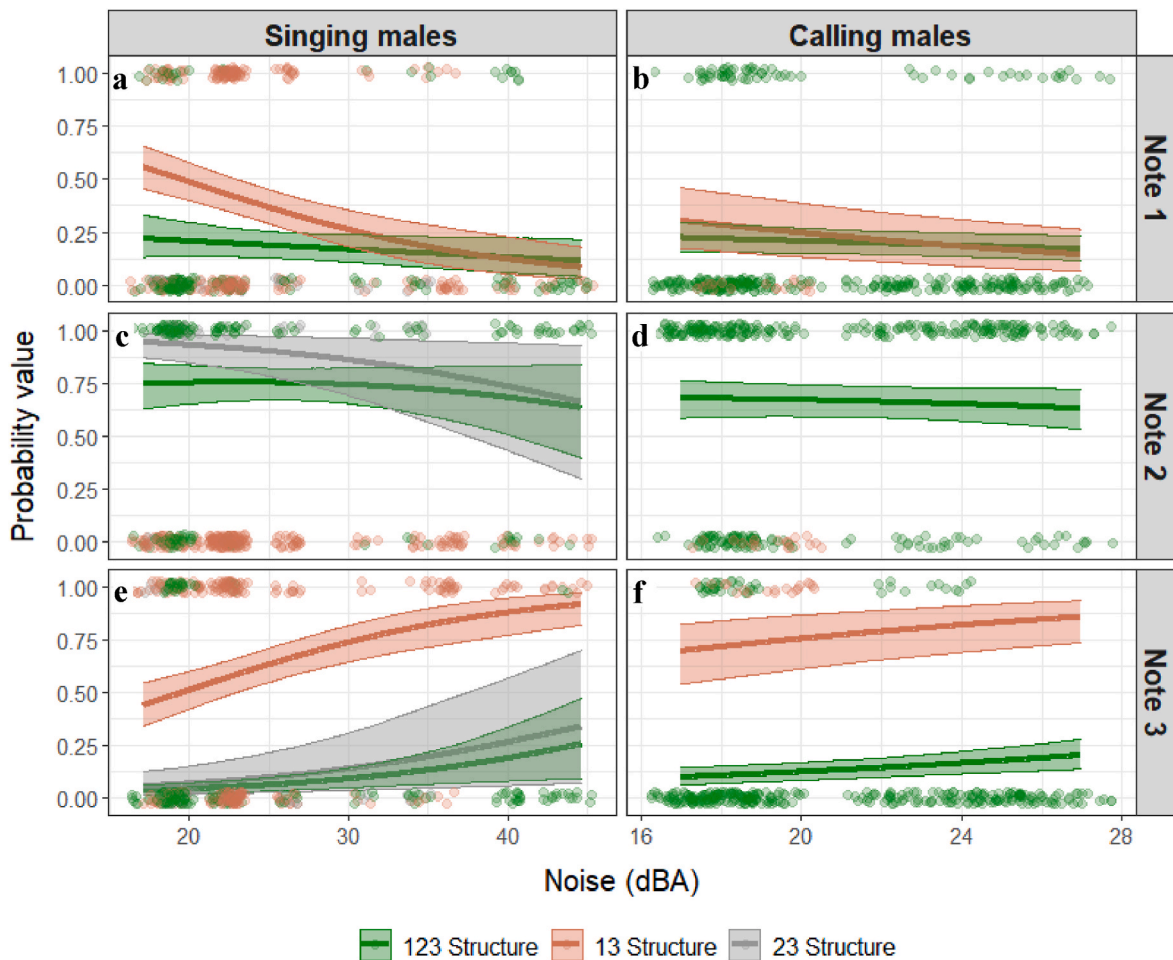


Fig. 6. Effect of turbine noise level on the probability of each note being the dominant note for each whistle structure (see legend) in calling ($n = 21$) and singing ($n = 28$) individuals recorded in 2016. Mean (lines) and 95% BCI (surfaces) are depicted. Lastly, observed values (dots) indicating whether the peak frequency was introduced (1) or not (0) in each note for each whistle structure, is indicated.

Table 4

Results of the Multinomial Logit Mixed-Effect model addressing the effect of all predictors on the probability of each note being the dominant note. The first note is the baseline outcome. For each note, the coefficient represents the change in the probability value for that note vs the baseline outcome. Posterior mean (β), standard deviation (SD) and 95% Bayesian Credible intervals (95% BCI) are shown. Important predictors are highlighted in bold.

	Note 2			Note 3		
	β	SD	95% BCI	β	SD	95% BCI
Intercept	1.370	0.235	[0.932; 1.820]	-1.333	0.327	[-1.991; -0.722]
Hour	0.069	0.138	[-0.200; 0.334]	0.247	0.121	[0.003; 0.488]
Date	-0.218	0.162	[-0.542; 0.093]	0.467	0.161	[0.153; 0.787]
Population size	-0.171	0.174	[-0.528; 0.173]	0.835	0.170	[0.511; 1.179]
Isolation	-0.033	0.184	[-0.410; 0.331]	0.844	0.165	[0.525; 1.181]
13 structure	-29.803	17.970	[-73.354; -7.185]	1.675	0.335	[1.049; 2.329]
23 structure	20.734	13.176	[3.211; 52.239]	20.919	13.171	[3.368; 52.345]
Calling males	-0.124	0.346	[-0.800; 0.533]	1.109	0.400	[0.346; 1.897]
Noise	0.130	0.168	[-0.194; 0.461]	0.646	0.147	[0.364; 0.948]

redundancy related to noise and evidence for such response is weak, suggesting that it may not be an effective adjustment for all signals (Brumm and Slabbekoorn, 2005; Gough et al., 2014). In accordance with these studies, we found that the Dupont's lark used a different strategy to cope with masking interference driven by turbine noise, without increasing signal redundancy (i.e., whistle rate) but adjusting other acoustic aspects of the vocalizations.

In contrast with repertoire size and vocalization rate, the complexity of the most characteristic and preserved acoustic signal of the Dupont's lark (the whistle; Laiolo and Tella, 2005; Laiolo and Tella, 2006) increased in males subjected to higher levels of wind turbine noise. The probability of uttering three-note whistles (123 structure) was higher with wind farm noise, while that of two-note whistles (13 and 23 structures) decreased. A variety of measures are employed to describe acoustic complexity in the birdsong literature, hindering comparative analysis across studies (Benedict and Najar, 2019). The effect of anthropogenic noise on the number of notes per vocalization has been scarcely explored and evidence is still mixed. Some studies found a tendency of bird species towards performing songs with fewer notes as noise exposure increased (Bergman, 1982; Fernández-Juricic et al., 2005; Lehtonen, 1983; Wood and Yezerinac, 2006). This reduction on signal complexity has been considered as a trade-off between other vocal adjustments (e.g., increased song amplitude) and the number of notes uttered per song to optimize energy expenditure (Fernández-Juricic et al., 2005). In agreement with our results, other studies have observed a positive association between noise level and the number of signals within songs (Ríos-Chelén et al., 2013; Slabbekoorn and den Boer-Visser, 2006). Increasing whistle complexity through the introduction of a larger number of notes may increase the probability of one of the notes being detected by neighbours and thus enhancing signal detectability in the presence of anthropogenic noise.

Our results also suggest spectral adjustments of the Dupont's lark whistles in response to wind farm noise. First, the minimum frequency of the third note increased with noise level. Moreover, although peak frequency of the notes remained constant, the distribution of sound energy among notes changed in relation to anthropogenic noise. The note containing the highest sound energy (i.e., dominant note) shifted from low-pitched to high-pitched notes when exposed to wind turbine noise. In other words, focal males emitted each note with the same peak frequency along the noise gradient, but they changed the dominant note of the whistle towards higher frequencies, allocating more energy on (and thus emphasizing) the high-pitched note of their vocalization. The probability of the third note of the whistle (high-frequency note; peak frequency = 3.52 ± 0.42 kHz; Appendix D) being the dominant note increased with turbine noise, while that of the first note (low-frequency note; peak frequency = 1.69 ± 0.58 kHz; Appendix D) decreased. Conversely, the probability of the second note (medium-frequency note, peak frequency at 2.91 ± 0.59 kHz; Appendix D) being the dominant note did not vary with noise level. This shift in the dominant note is likely a strategy to increase communication efficiency, as the first note,

uttered at the lowest frequency, is the most vulnerable to masking by wind turbine noise (minimum frequency 5% registered at 0.88 kHz, 1.62 ± 0.56 kHz; Fig. 3). Our results are in accordance with previous studies describing an increase on the minimum frequency (Bermúdez-Cua-matzin et al., 2009; Redondo et al., 2013) and the peak frequency (Walters et al., 2019) to overcome masking by anthropogenic noise and, in particular, by wind turbine noise (Szymański et al., 2017; Whalen et al., 2018). However, to our knowledge, this is the first study unveiling a shift in the dominant note in response to anthropogenic noise. Future research should address whether these observed shifts in the vocalization are an adaptive response or a side effect of singing at high amplitudes (Brumm and Zollinger, 2011; Nemeth and Brumm, 2010).

In addition to spectral adjustments, temporal parameters of the Dupont's lark whistle also changed, with the duration of the second note increasing when males were exposed to higher noise level. In literature, the effect of anthropogenic noise on signal duration have found to be less consistent, including negative (Slabbekoorn and den Boer-Visser, 2006; Whalen et al., 2018), positive (Brumm et al., 2004) and null effects (Juárez et al., 2021). Negative relationships between noise exposure and vocalization duration have been seen as a response to an increase in amplitude (Whalen et al., 2018). On the other hand, increased duration of vocalizations has been widely described as a potential strategy to reduce masking effects in several taxa (Brumm et al., 2004; Brumm and Slabbekoorn, 2005; Foote et al., 2004; Pittman and Wiley, 2001), since it increases signal detectability (Dooling and Searcy, 1985; Klump and Maier, 1990; Okanoya and Dooling, 1990). The duration-dependent detectability, commonly called as the 'temporal summation' phenomenon, is especially important for brief sounds up to 200 ms (Dooling, 1979). In line with our results, the second note is the shortest of the Dupont's lark whistle and is well below that threshold (130 ± 40 ms), explaining the observed increase on the duration of this note, which would be particularly beneficial in terms of signal detectability. Conversely, increasing the duration of the third note, the longest one (400 ± 120 ms), may lead to small improvements in detectability in relation to the associated costs. Similarly, despite the first note has a duration below the temporal summation threshold (170 ± 90 ms), it is low-pitched note and hence prone to be masked by wind turbine noise (see above), which may reduce the cost-effectiveness of its lengthening. Indeed, contrary to expectations, neither temporal nor spectral adjustments took place at the low-frequency notes of the whistle (i.e., first note), which are often subjected to spectro-temporal modifications (Hu and Cardoso 2009; Lowry et al., 2013). An explanation of this finding could be that the low-frequency notes of the study species may require substantial spectral and temporal shifts to overcome signal masking caused by anthropogenic noise effectively and thus individuals use alternative strategies involving the high-frequency notes (Hu and Cardoso, 2010; Whalen et al., 2018).

A series of confounding factors were included in the analysis to control for their potential effect on the Dupont's lark vocalizations. We found that the probability of males emitting three-note whistles and

whistles with the third note as the dominant note increased with later recording hour and date, as well as larger population size and isolation level. The number of singing con- and hetero-specifics are expected to be proportional, not only to the population size, but also to the period of day and the season, as males progressively join in the bird dawn chorus (Gil and Llusia, 2020). In this context, uttering more complex vocalizations, particularly intense at their higher frequencies, might be an adaptive strategy to increase signal detectability and make itself heard above the background noise. Moreover, the prevalence of simpler whistle structures as population size decreased (i.e., two-note 13 and 23 structures) might be due to the cultural erosion processes, as previously described for the Dupont's lark (Laiolo and Tella, 2007). Contrary to our expectations and previous results, we also found a positive relationship between isolation and whistle structure. This might be caused by other factors not measured in this study, since connectivity seems high throughout the study area (distance to the nearest Dupont's lark locality 4.22 ± 2.15 km) in accordance with data on adult and juvenile movements (García-Antón et al., 2015; Pérez-Granados et al., 2021; Vögeli et al., 2010). The fact that whistle rate decreased with recording date is in agreement with previous studies that reported a fall on song output as the breeding season progresses, suggesting they might serve diverse functions: mate attraction in calls and territorial defence in songs (Pérez-Granados et al., 2018). Lastly, most of the acoustic aspects of the Dupont's lark vocalizations differed between individuals performing territorial calls and songs. In any case, both vocalizations showed spectro-temporal adjustments in the presence of wind turbine noise, in contrast to the argument that the calls may present limited plasticity as compared to songs because they are innate and not learned (Lowry et al., 2013).

Vocalizations are subjected to behavioural plasticity (vocal adjustments) and adaptations (evolutionary mechanisms) that optimize acoustic communication. The changes observed on Dupont's lark vocalizations are interpreted as phenotypic plasticity in a context of turbine noise, since for these changes to become fixed evolutionarily several requirements or situations would have to occur (isolation between populations, sexual preferences for these traits, maintenance of these pressures, etc). Future studies should disentangle the costs and improvements on signal efficiency of the observed behavioural modifications. Vocalization adjustments such as increasing amplitude, duration and frequency, increase the detectability of the signal, but they are also more expensive in terms of energy expenditure (Blickley and Patricelli, 2010; Oberweger and Goller, 2001), with potential consequences on growth, survival and reproductive success (Read et al., 2014). Moreover, even though high-frequency signals avoid noise masking, they attenuate faster, travelling lower distances and reducing the active space for communication (Wiley and Richards, 1982). On the other hand, vocal adjustments can impair the information perceived by the receiver (Halfwerk et al., 2011a; Luther and Magnotti, 2014), with indirect fitness costs (Halfwerk et al., 2011b). For instance, shifts to higher amplitudes or frequencies can lead to misjudging male quality during mate attraction and territory defence, rejecting high-quality males for raising the offspring or mistakenly fighting stronger males (Read et al., 2014). Lastly, vocalization adjustments may also increase conspicuousness to predators or parasites (Dooling and Blumenrath, 2013), which in turn increases the risk of predation or parasitism (Read et al., 2014). Therefore, trade-offs between energy expenditure, conspicuousness to predators, effective information transmission (noise masking avoidance) and the preservation of the functionality of the signal (e.g., information on individual quality), must be playing a key role on vocal adjustments responses to anthropogenic noise (Luther and Magnotti, 2014; Slabbekoorn and Ripmeester, 2008).

These aspects should also be carefully addressed in future studies to have a full understanding of the effects of wind turbine noise (Read et al., 2014). New questions and future lines of research arise: (1) Are there collateral costs to these vocal adjustments?; (2) Is the functionality of the signal altered?; (3) Does turbine noise mask other biological

important cues for prey or predator detection, or communication signals such as begging or alarm calls?; (4) Does noise elevate stress levels in individuals? If so, the Dupont's lark would not be fully compensating for noise impacts by altering its vocalization, justifying the local extinction events observed in the presence of wind farms (Gómez-Catasús et al., 2018). Alternatively, other potential mechanisms might be explaining these trends, such as increases on nest predation rates due to landscapes transformation (Gómez-Catasús et al., 2021), visual disturbance due to aircraft warning lights (Rodríguez et al., 2017) or just behavioural avoidance of wind farms because individuals prefer not to settle onto chronically noisy locations in which their vocalizations propagate ineffectively (McClure et al., 2013; Patricelli and Blickley, 2006).

5. Conclusions

Our results suggest that the Dupont's lark is able to adjust its vocalizations in the presence of wind turbine noise, exhibiting some level of phenotypic plasticity, which might enable the species to cope with noisy environments. Overall, this work reveals that species can develop multiple and complex responses to enhance acoustic communication and overcome the limitations imposed by turbine noise. Particularly novel are the observed changes in the vocal repertoire towards more complex structures, as well as the shift of the dominant note to emphasize the longest and higher-pitched note. These vocal adjustments reveal that turbine noise is a source of behavioural disturbance for the species, and it might have important consequences at the individual and population level. Future research should address the effect of turbine noise on other acoustic parameters such as signal amplitude. In the light of the expected increase on renewable infrastructures, implementing rigorous comprehensive planning and independent environmental assessments is mandatory (Serrano et al., 2020), which, in the case of future wind energy projects, must include a fine-scale noise assessment to quantify the consequences of chronic noise exposure on wildlife (Barber et al., 2010).

Author contributions

Julia Gómez-Catasús: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. Adrián Barrero: Conceptualization, Investigation, Methodology, Writing – review & editing. Diego Llusia: Conceptualization, Writing – review & editing. Carlos Iglesias-Merchan: Methodology, Resources, Writing – review & editing. Juan Traba: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Carlos Iglesias-Merchan applied to participate (non-profit collaboration) in three working groups at the Technical Committee ISO/TC 331 (Biodiversity) through the Spanish Association for Standardization (UNE) in September 2021 and his confirmation was received in January 2022. The International Standard Organization (ISO) has not influenced the work reported in this paper, which was previously conducted.

Data availability

The data that support the findings of this study are openly available in GitHub repository <https://github.com/JGomez-Catasus/Wind-farm-noise-shifts-vocalizations-of-a-threatened-shrub-steppe-passerine>.

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