Vocal communication in the Iberian wolf

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Certifican:

Que la memoria presentada por Vicente Palacios Sánchez con título “Vocal communication in the Iberian wolf” corresponde a su tesis doctoral y ha sido realizada bajo su dirección en el Instituto Cavanilles de Biodiversidad y Biología Evolutiva, autorizando mediante este escrito la presentación de la misma para optar al grado de Doctor en Biología por la Universidad de Valencia.

En Paterna, a 24 de abril de 2015

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Photo courtesy of José Larrosa

CHAPTER 1. PART I. GENERAL INTRODUCTION

1.1 ANIMAL COMMUNICATION

Many of the most fascinating animal behaviours apparently function as communicative signals. This is possibly the reason why the study of communication has traditionally been a key issue in the study of animal behaviour and evolutionary biology in general. Communication provides a vehicle for conveying information and for expressing to others what has been perceived (Hauser 1996). The central point in communication is the relationship between the individual that emits the signal (sender) and the receiver, although different authors view this relationship in a different way, and there is no universally accepted definition of communication or of communicative signal. According to Burghardt (1970), for instance: "*Communication is the phenomenon of one organism producing a signal that, when responded to by another organism, confers some advantage (or the statistical probability of it) to the signaller or his group*". In the same spirit, Slater (1983) defined communication as "*the transmission of a signal from one animal to another such that the sender benefits, on average, from the response of the recipient*". However, more recent works consider that "*In true communication, both sender and receiver benefit (on average) from the information exchange*" (Bradbury & Vehrencamp 2011). Among the numerous definitions proposed, Carazo & Font (2010) provided the most all-encompassing definition of communicative signal, which included a functional concept of information: "*any act or structure that (i) affects the behaviour of other organisms; (ii) evolved (or is maintained) because of those effects; (iii) is effective because it transfers [functional] information to receivers*".

In general, signals used in animal communication can transmit different types of information (Bradbury & Vehrencamp 2011):

- Information related to the environment.
- Information about sender identity.
- Information about sender quality (strength, social status, size, age, etc.) as potential competitor or potential mate.
- Information related to the sender's intention.

Communication involves the transmission and receipt of signals in different sensory modalities, e.g. olfactory, auditory, visual, tactile, etc. (Alcock 1989). Each sensory modality is more or less efficient depending on the properties of the environment (Dusenbery 1992). Specifically, acoustic signals can be transmitted quickly, over long distances, and are efficient even in the presence of some types of obstacles (Barnard 2004).

1.2 ACOUSTIC COMMUNICATION

Communication by means of acoustic signals, or acoustic communication, is widespread among vertebrate and invertebrate animals (Bradbury & Vehrencamp 2011). The terms "acoustic signal" or "vocalization" are used indistinctly for any sound produced by animals, regardless of the mechanisms and physical structures used to generate the sounds (Peters & Wozencraft 1989). Acoustic signals can be produced by oscillations of the vocal chords (vocal sounds) and by other mechanisms (non-vocal sounds). An alternative classification is based on the acoustic structure of sounds, and distinguishes between "tonal" or "harmonic" signals, the acoustic energy of which is concentrated in narrow band frequencies (fundamental frequency and a number of harmonics which are integer multiples of the fundamental), and “noisy”, with the acoustic energy dispersed and not concentrated in narrow bands (Peters & Wozencraft 1989).

The acoustic features of animals sounds that are considered relevant from a biological point of view can be temporal (duration, repetition, temporal sequence of syllables, etc.) or spectral (frequency, distribution of the acoustic energy, frequency range, etc., Beeman 1998). Sound waves are amenable to different types of graphical representation (e.g. waveform, spectrogram, and power spectrum, Figure 1.1). Graphical displays of sounds allow researchers to extract information in both temporal and spectral domains.

Many studies have focused on the type of information transmitted by acoustic signals. For instance, vocalizations contain information about sender identity in baboons, *Papio cynocephalus ursinus* (Fischer et al. 2002), Amazonian manatees, *Trichechus inunguis* (Sousa-Lima et al. 2002), barking foxes, *Alopex lagopus* (Frommolt et al. 2003), and African elephants, *Loxodonta africana* (McComb et al. 2000). Sex-related differences in vocalizations have been reported in many taxa, e.g. zebra finches, *Taeniopygia guttata* (Vicario et al. 2001). Alarm calls contain information about predator type in suricates, *Suricata suricata* (Manser 2001), vervet monkeys, *Cercopithecus aethiops* (Seyfarth et al. 1980), and chicken, *Gallus domesticus* (Gyger et al. 1987). Acoustic signals allow mother-offspring recognition in macaroni penguins, *Eudyptes chrysolophus* (Searby et al. 2004), hyaenas, *Crocuta crocuta* (Holekamp et al. 1999), seals, *Arctocephalus tropicalis* (Charrier et al. 2003), and bats, *Tadarida brasiliensis* (Balcombe 1990; Balcombe & McCracken 1992).

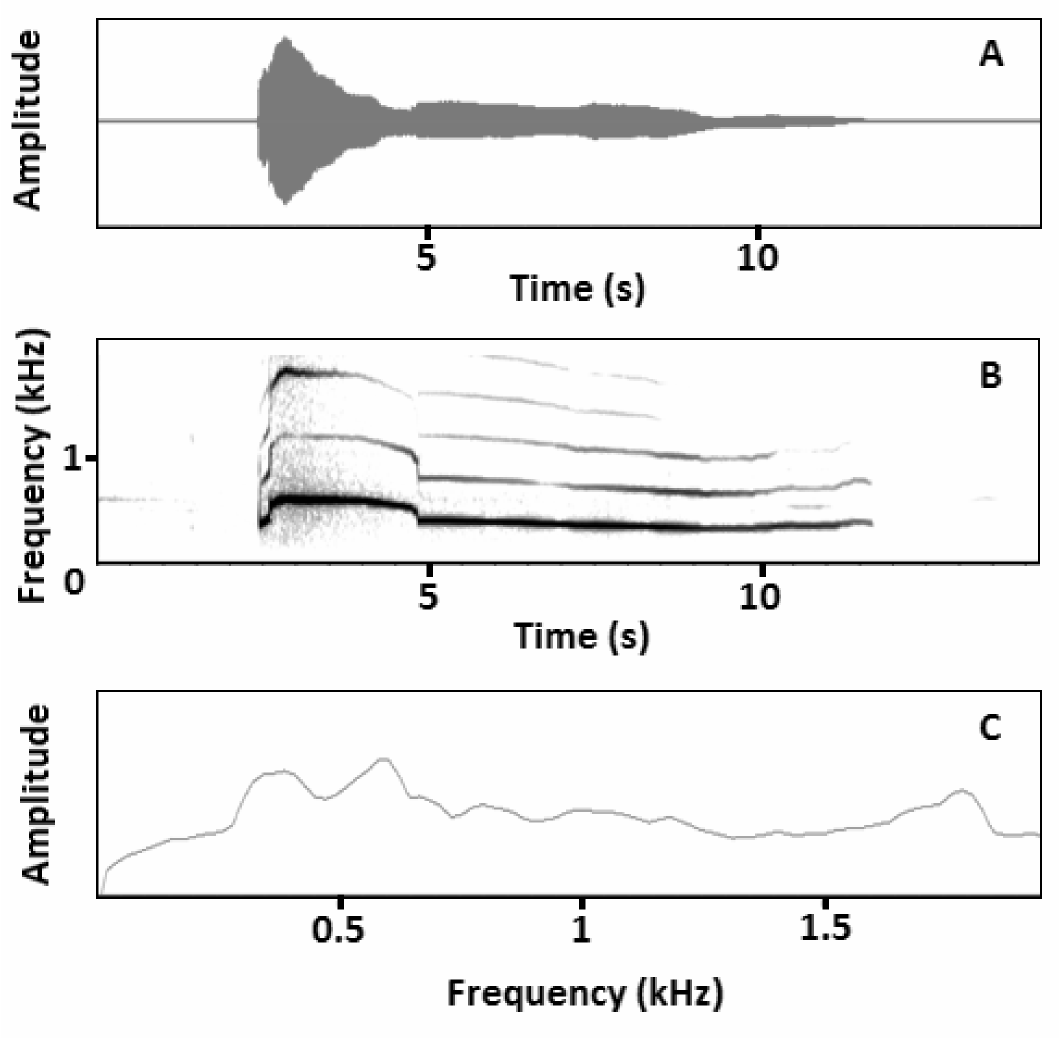


Figure 1.1.- Wolf howl waveform (A), spectrogram (B) and power spectrum (C).

1.3 THE WOLF

Wolves (*Canis lupus*) are social carnivores that live in social units known as packs. In general, packs are comprised by a breeding pair (“alpha” pair) and their descendants, although sometimes unrelated wolves may join the pack (Mech 1970). The traditional view emerging from studies conducted with captive wolves held that the social organization of wolves is based on a hierarchy of competitors vying for dominance (Packard 2003). However, this point of view has been questioned for wild wolves by Mech (1999). For this author the dominance hierarchy is "*a natural reflection of the age, sex, and the reproductive structure of the group*", and the typical wolf pack "*should be viewed as a family with the adult parents guiding the activities of the group and sharing group leadership in a division-of-labour system in which the female predominates primarily in such activities as pup care and defence and the male primarily during foraging and food-provisioning and the travels associated with them*". Pack size ranges between 3 and 11 individuals (Fuller et al. 2003), and depends mainly on the availability of food in natural conditions (Mech & Boitani 2003). Pack size varies seasonally, reaching a maximum after births and a minimum in winter, due to mortality and dispersion (Fuller et al. 2003).

Wolf pups are born in spring, leave the den at 6-8 weeks, and until 20 weeks of age, stay in the so-called *rendezvous* sites, defined by Packard (2003) as *"… areas above ground that include a 'nest' or nests where they [the pups] huddle together, a network of trails, and various play areas." Rendezvous* sites may be close to the natal den or several kilometres from it (Mech 2000). Sometimes a single site is used all summer, whereas in other cases wolves shift sites every few weeks (Mech 2000). The activity of pack mates is focused on the care of the pups during this period (Mech 1970).

Wolves are territorial. Pack mates do not defend individual territories, but cooperate with other pack mates in the defence of a common territory (Mech 1970). Packs compete for resources, and territorial conflicts among packs are frequent (Mech & Boitani 2003). Interactions among wolves belonging to different packs often result in aggression and are an important cause of mortality in wild wolves (Mech 1970). Territory sizes are very variable, from 33 to 6272 km^2 (Mech & Boitani 2003).

1.4 WOLF ACOUSTIC COMMUNICATION

Communication in wolves usually involves multiple sensory modalities simultaneously (Zimen 1978). Schenkel (1947) provided a detailed description of visual communication in the wolf. Facial features, body postures and tail position are important visual signals used during social interactions, particularly at close range (Harrington & Asa 2003). Smell provides an important avenue for communication in wolves. Although several odour sources play a role in wolf olfactory communication (e. g. skin glands, tail, saliva, preputial glands and vagina, see review in Harrington & Asa 2003), the most studied olfactory signals in wolves are the scent marks used for territory maintenance: urine, faeces, and glandular secretions (Peters & Mech 1975; Vilá et al. 1994; Zub et al. 2003; Barja et al. 2005; Martín et al. 2010). Scent marks and some behaviours associated to scent mark deposition can also act as visual signals (Peters & Mech 1975; Bekoff 1979a, 1979b; Harrington & Asa 2003; Barja 2009). However, arguably the most studied communicative signal produced by wolves is the howl (Harrington & Asa 2003). Acoustic and olfactory signals play complementary roles in wolf communication, acoustic communication being especially important at night and in situations where information has to be transmitted through long distances and quickly (Harrington & Asa 2003).

Little is known about the auditory abilities of wolves. It seems safe to assume that hearing in wolves may be similar to hearing in the domestic subspecies of wolves, the dog (*Canis lupus familiaris*). The dog's auditory range at 60 dB extends from 67 Hz to 45 kHz, and best sensitivity is reached between 500 Hz and 32 kHz (Harrington & Asa 2003). The ability to locate the source of sounds (spatial acuity) in dogs (4-8^{o}) is similar to that of cats, but poorer than that of humans (Heffner 1997; Harrington & Asa 2003). Although wolves can hear sounds within the ultrasonic range (above 20 kHz), apparently they do not emit ultrasounds. The capacity for sending and receiving vocal signals in the ultrasonic domain has been described only in a few mammal species (cetaceans, domestic cats, bats, rodents, and nocturnal primates, Ramsier et al. 2012). Due to the inherent transmission limitations of ultrasonic frequencies (high rate of atmospheric attenuation, directionality and vulnerability to scattering), we expect animals using long-distance communication, or communicating in environments with abundant obstacles, to focus their vocal efforts in the human audible frequency range (20 Hz – 20 kHz) to optimize efficacy during transmission from emitter to receiver (Arch & Narins 2008).

Wolf vocalizations have been studied in some detail (e. g. Theberge & Falls 1967; Harrington & Mech 1978a, 1983; Tooze et al. 1990; Schassburger 1993; Holt 1998). The vocal repertoire of wolves is complex and there is no consensus regarding the number and characteristics of the different vocal types comprising it. Thus, vocalizations that constitute well-defined vocal types for some researchers are considered as subcategories of broader types by others. Schassburger (1993) provided the most detailed description of the wolf's vocal repertoire, including 11 different vocal types. Other authors group wolf vocalizations into four broad vocal types (Harrington & Mech 1978a; Coscia 1995; Holt 1998): howls, growls, barks, and whines.

1.5 THE IBERIAN WOLF

The Iberian Peninsula holds the largest wolf population in Western Europe. This population has been isolated from other European wolves for more than a century (Vilá 1993). Some authors have proposed that Iberian wolves belong to a distinct subspecies, *Canis lupus signatus*, based on morphological traits (Fig. 1.2) (Vilá 1993).

Figure 1.2.- Iberian wolves. Photos courtesy of Bob (left) and Paco Perú (right)

The range of the Iberian wolf extends over the Northwestern quadrant of the Iberian Peninsula, covering a total of 140,000 km^2, with a population size estimated between 2000 and 3000 individuals (around 322 packs, Álvares et al. 2005). Iberian wolves occur at low densities, such as 1.7-3.7 wolves/100 km^2 in Gerés-Xurés International Park (Álvares et al. 2000), 2.3 wolves/100 km^2 in Castilla-León (Llaneza & Blanco 2001), 1.36-2.02 wolves/100 km^2 in A Coruña province, and 2.97-4.38 wolves/100 km^2 in Pontevedra (Llaneza et al. 2005a). These densities are similar to those reported for North America, between 0.3 and 4.3 wolves/100 km^2 (Boitani 2000).

Iberian wolves live in a great variety of environments, such as high mountain ranges (Picos de Europa), agricultural areas (Tierra de Campos, Valladolid), coastal lands (Costa da Morte, Galicia), and near large cities such as Santiago de Compostela. In fact, wolf habitat has been defined as any place with food availability where human persecution does not threaten its survival (Boitani 2000). In the Iberian Peninsula, vegetation cover is important for den site and resting site selection (Vilá et al. 1990; Blanco & Cortes 1999; Roque et al. 2001; Llaneza et al. 2012b).

1.6 OBJECTIVES

Acoustic communication in wolves has been the focus of several studies that have analysed, among others, the vocal repertoire (Schassburger 1993), the ontogeny of wolf vocalizations (Coscia 1995), individual signature of howls (Theberge & Falls 1967; Tooze et al. 1990), the importance of howls for territory maintenance (Harrington & Mech 1978b), and the acoustic structure of chorus howls (Harrington 1989). However, most of these studies have been conducted with North American wolves. This is the first study of acoustic communication in the Iberian wolf, a wolf with distinctive morphological traits (and maybe genetic differences as well) that lives in habitats different from those of North American wolves (Boitani 2000).

This thesis includes two parts. In the first part, we describe the vocal repertoire of Iberian wolves and study the role of long-distance communication in individual recognition (chapters 2, 3, and 4). The second part of the thesis deals with a different topic, focusing on the usefulness of howls for monitoring wolf populations (chapters 6, 7, and 8), and the objectives of this part are presented in detail in chapter 5, beginning on page 99.

The specific objectives of the first part of the thesis (chapters 2, 3, and 4) are:

1. To describe the close-range vocalizations comprising the close-range vocal repertoire of Iberian wolves and to make a comparison with published studies of other wolf populations. These are objectives addressed in chapter 2.

2. To describe the long-distance vocalizations (howls) emitted by adult Iberian wolves, to identify different howl types, to compare Iberian wolf howls

with those reported for other populations, and to explore the potential usefulness of howls for individual recognition. These are the objectives of chapter 3.

3. In chapter 4 we conduct playback experiments to investigate the acoustic basis of individual discrimination in wolves based on howling.

Ethical note: For both parts of this thesis we obtained recordings of sounds emitted by captive and wild wolves. All the recordings were obtained with permits from private Parks (captive wolves) and regional governments (wild wolves). All subjects were treated in accordance with Animal Care Guidelines and International regulations (Sikes et al. 2011).

CHAPTER 2. ADULT CLOSE-RANGE VOCAL REPERTOIRE OF IBERIAN WOLVES

2.1 INTRODUCTION

A proper knowledge of the signals emitted by animals is essential for understanding the evolution of complexity in animal communication (Botero et al. 2008). The description and quantification of signals is the first step to investigate their function, geographic variation, social relevance, and cultural transmission (Boisseau 2005). During the last decades, researchers have described the vocal repertoires of many taxa, including fishes (Amorim et al. 2008), amphibians (Capranica 1968; Grafe et al. 2000; Feng et al. 2009), birds (Ficken et al. 1978; Seddon et al. 2002; Bragina & Beme 2007), and mammals (Binz & Zimmermann 1989; Bermejo & Omedes 1999; Hsu et al. 2005).

Methods used to classify vocalizations have changed over time, in part due to the development of accessible computer hardware and software allowing sophisticated digital signal processing algorithms to be applied for sound analysis. Early studies used human audible differences, visual inspection of the spectrograms, and context to classify and identify different vocal types (Cohen & Fox 1976; Lehner 1978a; Brady 1981). More recently, statistical analyses of acoustic features measured from vocalizations have been used to support a priori classifications based on audible differences, spectrogram similarity, or context (Boisseau 2005; Le Roux et al. 2009; Déaux & Clarke 2013).

The study of wolf acoustic communication has focused mainly on the howl, which is a vocalization used to communicate over long distances (Theberge & Falls 1967; Harrington & Mech 1979; Harrington 1989; Tooze et al. 1990; Holt 1998; Gazzola et al. 2002). Close-range vocalizations have been much less studied (Schassburger 1993; Goldman et al. 1995; Feddersen-Petersen 2000).

Schassburger (1993) provided the most detailed catalogue of wolf vocalizations, based on audible differences and visual inspection of spectrograms, which included 11 different vocal classes (10 close-range vocalizations and the howl): whine, whimper, yelp, growl, snarl, woof, bark, whine-moan, moan, growl-moan, and howl. However, there is a lack of consensus with respect to the number and characteristics of the different vocal types comprising the wolf's vocal repertoire. This is a problem endemic to the study of animal signals generally (Font & Kramer 1989, Nowicki & Nelson 1990) and reflects the traditional tension between ”lumpers” and ”splitters” in the segmentation of behaviour (Lehner 1996). Where a researcher claims that certain vocalizations comprise an independent category, others may consider them a subcategory within a wider vocal category. Thus, the ten close-range vocal categories defined by Schassburger (1993) have been pooled by others into growls, barks, and whines (Harrington & Mech 1978a; Harrington & Asa 2003, Table 2.1).

Table 2.1.- Major vocal classes comprising the close-range vocalizations repertoire for adult wolves.

Sound type	Harrington & Mech (1978)	Schassburger (1993)	Harrington & Asa (2003)
Harmonic sounds	Whimper	Whine Whimper	Whine Whimper
	-	Yelp	Yelp
Noisy sounds	Growl	Growl Snarl	Growl Snarl
	Bark	Bark Woof	Bark Woof
Intermediate sounds	-	Whine-Moan Moan Growl-Moan	Moans

On the other hand, many different sounds have been reported in the literature (i.e. barks, howls, yelps, yips, yaps, squeaks, whines, whimpers, moans, squeals, screams) without a detailed description of each vocalization (Goodmann et al. 2002). This makes comparing vocal types identified by different authors an almost impossible task.

There have been only two surveys dealing with European wolf vocalizations. Kappe (1997) studied the threat vocalizations emitted by European wolves when competing over a food item, and Feddersen-Petersen (2000) compared the ontogeny of acoustic communication in European wolves and in various dog breeds. However, the acoustic structure of Iberian wolf vocalizations is completely unknown. The aims of this study are: 1) to identify the vocal types comprising the adult close-range vocal repertoire of Iberian wolves; 2) to provide detailed descriptions and definitions of different vocal types that could be used for comparisons in future studies, and 3) to compare the close-range vocalizations of Iberian wolves with those reported for other wolf populations.

2.2 METHODS

From 2001 to 2010 we recorded vocalizations of wolves held in captivity at seven locations in the Iberian Peninsula: Cañada Real (Madrid, Spain), Carpín (Bilbao, Spain), Parque Grande (Madrid, Spain), Zoológico de Santillana del Mar (Cantabria, Spain), Centro de Recuperação do Lobo Ibérico (Malveira, Portugal), Senda Viva (Navarra, Spain), and Lobo Park (Málaga, Spain). Vocalizations were recorded opportunistically during morning and afternoon peak activity periods in 81 (non-consecutive) days of observation (245 hours of observation). The wolves were habituated to humans, and recordings were made 5–40 m from the wolves, with the researcher often in full sight of the animals. Exceptionally, we recorded the vocalizations emitted by three wild wolves caught in footsnare traps in response to an approaching human. The traps were set for research purposes under Regional Government permission, and vocalizations were recorded opportunistically during the short period until the wolves were injected with a tranquilizer, causing no additional stress to the animals.

The bulk of the recordings were made on TDK SA-60 cassette tapes (TDK Electronics Corp., New York) using a Sennheiser MK 66 unidirectional microphone with K-6 power unit (Sennheiser Electric GmbH & Co. kG, Wedemark, Germany) connected to a Marantz PMD 222 cassette recorder (Marantz America, Inc., Mahwah, New Jersey). Recordings were digitized with 44.1-kHz sampling frequency and 16 bits in the Fonoteca Zoológica, Museo Nacional de Ciencias Naturales (CSIC, Madrid, Spain), using Delta 66 (Irwindale, California) or Digi 001 (Bucks, United Kingdom) digitizer cards connected to Apple Macintosh G4 computers (Cupertino, California). For the remaining recordings we used a Marantz PMD 660 portable solid-state, compact flash audio field recorder, and with a Panasonic DMC-FX10 digital camera (wild wolves only). All recordings were saved in ".WAV" format, at 44.1-kHz sampling frequency and 16 bits.

Recordings were analysed with Raven 1.4 Pro software (www.birds.cornell.edu/raven). We filtered (bandpass filter 50-15000 Hz) and normalized all the vocalizations to generate audiospectrograms (2048-point fast Fourier transform; Hann window; time window: 4 s; frequency range: 8000 Hz). We measured 18 acoustic features, using Praat software (version 5.2.08, http://www.fon.hum.uva.nl/praat/) to obtain fundamental frequency related variables (pitch settings: cross-correlation method, 125 ms time window, 20-2000 Hz, Table 2.2). We measured the duration of sounds from oscillograms. Each vocalization was classified as harmonic (when visual inspection of spectrograms revealed evidence of harmonically related frequency bands -512 points Fast Fourier Transform, 4000 Hz, 4 s) or noisy (no evidence of harmonically related frequency bands, Figure 2.1). Harmonic vocalizations included sometimes a “whistle” part (high pitch sounds with acoustic energy concentrated at 3-6 kHz, and with harmonic structure, Figure 2.1).

Table 2.2.- Acoustic features measured. *: variables that were measured applying a 90% amplitude threshold (i.e. filtering out sounds below 90% of the maximum amplitude in the recording). Amplitude thresholds were applied only to noisy sounds to remove all background noise from the signal. Type: type of variable, categorized as categorical (Categ), quantitative continuous (Q cont), and quantitative discrete (Q disc). Whistle measurements correspond to the fundamental frequency of the whistle.

Variable	Description	Type
Structure	Acoustic structure: harmonic/noisy	Categ
Perc.harm	Percentage of the duration with harmonic structure	Q cont
Perc.whist	Percentage of the duration with whistle part	Q cont
Dur	Duration	Q cont
Freqmin90*	Minimum frequency	Q disc
Freqmax90*	Maximum frequency	Q disc
Fundmin	Minimum fundamental frequency	Q disc
Fundmax	Maximum fundamental frequency	Q disc
Range fundamental	Fundmax – Fundmin	Q disc
Fundmean	Mean fundamental frequency	Q cont
Whistmin	Minimum whistle frequency	Q disc
Whistmax	Maximum whistle frequency	Q disc
Range Whistle	Whistmax – Whistmin	Q disc
Whistmean	Mean whistle frequency	Q cont
Domfreq	Dominant frequency. Frequency at which the maximum of the amplitude occurs	Q disc
Aggent90*	Aggregate entropy: measurement of the disorder by analyzing the energy distribution.	Q cont
Avent90*	Average entropy: average of the entropy for each frame in the selection.	Q cont
Centraltime	Position where the maximum amplitude occurs (values from 0 – beginning – to 1 –end of the signal).	Q cont

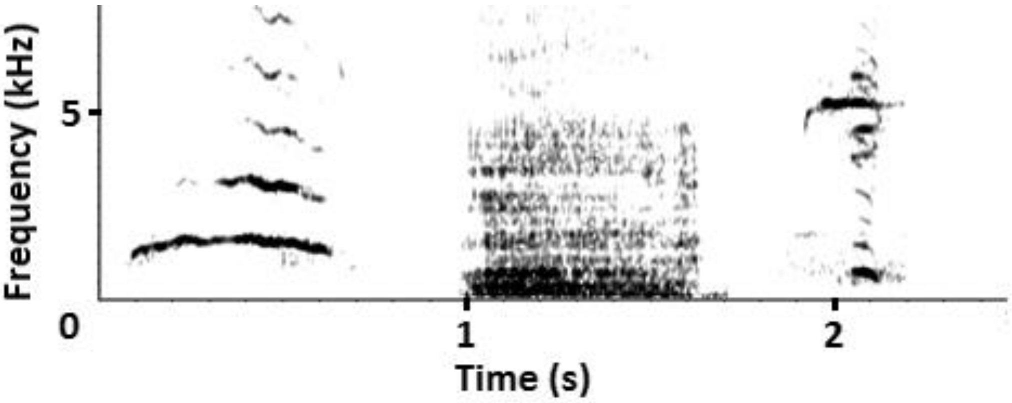


Figure 2.1.- Different vocalization types: harmonic (left), noisy (middle), and harmonic with whistle part at 5 kHz (right). Spectrogram: 512 FFT (Fast Fourier Transform) points, Hann window.

Very often wolves emit several vocalizations consecutively. We used the following terms to describe how consecutive vocalizations are linked in the temporal domain (Figure 2.2):

- *Single vocalization*: the animal emits only one vocalization or, when emitting more than one vocalization, the interval between two consecutive vocalizations is longer than twice the duration of the longest vocalization.

- *Bout of vocalizations*: the interval between two consecutive vocalizations is shorter than twice the duration of the longest vocalization. Bouts can include different vocal types (e. g. barks and growls).

- *Multiple vocalization*: a vocalization is repeated with no obvious silent interval between consecutive vocalizations, and the beginning and end of the acoustic signals comprising the multiple vocalization can be easily delimited. We defined a *mixed vocalization* as two or more vocalizations belonging to different vocal classes emitted together, without an interval of silence between them.

- *Merged vocalization:* a vocalization is repeated without an interval of silence between consecutive vocalizations, and the acoustic signals merge resulting in a vocalization in which the beginning and end of the vocalizations that comprise the merged vocalization are not clearly distinguishable.

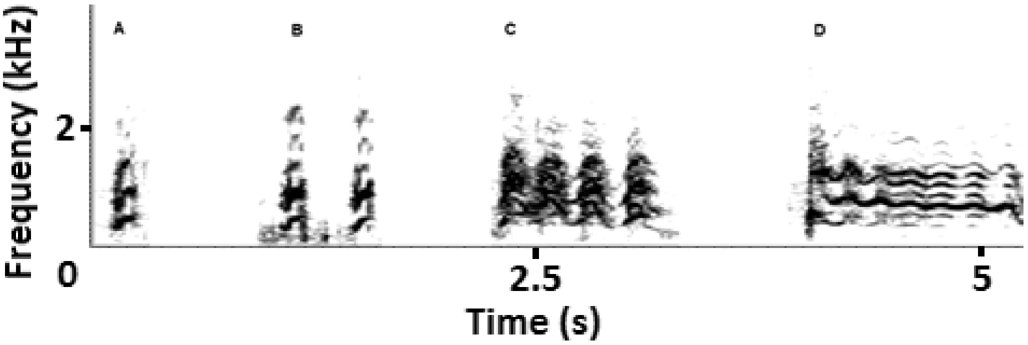


Figure 2.2.- Temporal association of barks: A) single bark; B) bout of barks; C) multiple bark; D) merged bark. Spectrogram: 2048 FFT (Fast Fourier Transform) points, Hann window.

We classified vocalizations based on spectral and audible differences and context, following Schassburger's (1993) description of the vocal repertoire of North American wolves. This classification was validated using discriminant function analysis (DFA). Discriminant function analysis identifies a linear combination of independent variables that best discriminates groups from each other. The assumptions of multivariate normality and equal covariance matrices were not met even with transformed variables, but DFA is robust to violations of these two assumptions (Klecka 1980; Selvin 1995). When the assumptions of multivariate normality and equal covariance are not met the leave-one-out (L-O-O) cross validation results are preferred (Huberty 1994). In this method, each observation is systematically dropped, the discriminant function is re-estimated and then the excluded observation is classified (Huberty 1994). We conducted

Student's-t tests, ANOVA or Kruskal-Wallis tests to compare acoustic features between vocal classes, selecting those variables with significant differences among groups ($p < 0.05$) to reduce the number of variables included in DFA. Our sample comprised several vocalizations per individual per category. To minimize the effect of pseudoreplication we only included in the analyses 1-5 sounds per individual per category (randomly selected), which is less than the number of sounds per individual included in published analyses (e.g. Mundry & Sommer 2007; Déaux & Clarke 2013).

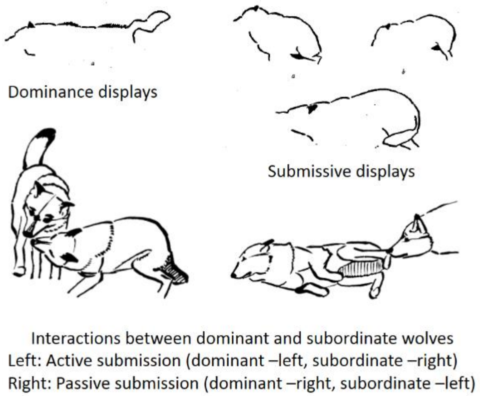
We noted for each vocalization the context in which it occurred and other attending behaviours (Tables 2.3 and 2.4). We also noted whether the vocalization was emitted alone or included in a sequence of vocalizations.

Table 2.3.- Description of the contexts used in this study to classify acoustic signals.

Contexts		Description
Wolf-wolf interaction	**Agonistic**	Fighting and competitive interactions between same or different-sex individuals, including threats and offensive attacks as well as defensive fighting (Johnson 1972).
	Mating	Male-female interactions related to reproduction (e. g. courtship, copulation).
	Other social interaction	Social interactions different from agonistic and mating (e. g. a wolf approaching other pack-mates, play -see below)
Wolf-human interaction		Human close to or approaching the enclosure, entering the enclosure, or approaching a trapped wolf.

Table 2.4.- Description of the behaviours used in this study to classify acoustic signals.

Behaviours	Description
Greeting	To receive or meet other pack mates, in which subordinates nip, lick and smell the mouth of the dominants (Mech, 1970). Characterized by ears oriented backward, muzzle-muzzle contacts, tail wagging and, sometimes, whining (Goodmann et al., 2002).
Play	Play is defined as "repeated, incompletely functional behaviour differing from more serious versions structurally, contextually, or ontogenetically, and initiated voluntarily when the animal is in a relaxed or low-stress setting" (Burghardt 2005). This is a very broad category and can be considerably subdivided. In wolves, play behaviour includes: Play-face (lips horizontally retracted, jaws slightly open, ears pulled straight up and back, or folded flat back), play-run (the wolf runs with its head up, and a play-face expression), and play-solicit (prancing and bowing in front of another wolf) as reported by Goodman et al. (2002).
Escape	A wolf moves quickly away from the stimulus that elicited the behaviour (in this study, a human approaching).
Scratching	A wolf scratches the ground with the paws, moving rapidly the front legs, the hind legs or both backwards, displacing vegetation, soil or other materials.
Dominance displays	Threat, aggression, attack, and assertion of dominance behaviours as reported in the wolf ethogram (Schenkel 1947; Goodmann et al. 2002), such as show teeth, chase other wolf with tail above the back, high body posture, piloerection along the back and the tail, tail held high, arched neck, etc. (Schenkel 1947, 1967).
Submissive displays	Defence and submissive behaviours as reported in the wolf ethogram (Schenkel 1947; Goodmann et al. 2002), such as inguinal offer, tail between hind legs, active and passive submission, etc. (Schenkel 1947, 1967).



Adapted from Schenkel (1947, 1967)

2.3 RESULTS

We recorded 1936 vocalizations emitted by 29 Iberian wolves. Of the 29 wolves, 26 belonged to 11 social groups held in captivity at seven different locations; the other three were wild wolves belonging to three different packs caught in footsnare traps. We identified the following broad vocal types: 1) noisy sounds: bark-like (15.3% of vocalizations recorded) and growl-like (18.1%) vocalizations; 2) harmonic sounds: whine-like (51.5%), moan-like (1.2%) and yelps (9.8%); and 3) non-vocal sounds: scratches (0.1%) and teeth snapping (0.2%). We also identified mixed sounds (3.8%) such as bark-growls. Further analyses were restricted to the subset of best quality (i.e. suitable signal-to-noise ratio) recordings (N = 678 vocalizations emitted by 29 wolves). Most of the vocalizations analysed (94%) were emitted by wolves older than two years (23 individuals). The remaining 6% were vocalizations emitted by yearlings (one year old, 6 individuals).

2.3.1 Noisy Sounds

Bark-like vocalizations

Barks are noisy, short, loud, explosive sounds, emitted alone or with other vocalizations such as growls and howls. We recorded barks in agonistic contexts and wolf-human interactions. The acoustic energy of a bark is distributed giving rise to a Christmas tree-like outline in the spectrogram (Schassburger 1993, Figure 2.3). We identified three bark-like vocal classes (Figure 2.3):

1. **Woofs** are the shortest, lowest-frequency and most noisy (highest values of entropy) bark-like vocalizations (Table 2.5). Almost all woofs (99%) were emitted as a sequence of single calls, and no other type of vocalizations was emitted accompanying woofs. Unlike other bark types, woofs were emitted by one or several individuals simultaneously of any social status and age. Wolves emitted woofs in wolf-human interactions: when humans approached the fence carrying

objects unfamiliar to the wolves (e.g. recording equipment), when humans entered the enclosure and, in one location (Cañada Real), when the caretaker approached the fence with food (APPENDIX I). Woofs apparently acted as alarm signals: when a wolf emitted a woof other pack members begun to woof and displayed escape behaviours (move away from the stimulus triggering the behaviour, Table 2.4). In the case of wolves woofing when the caretaker approached with food, they moved towards the fence in the caretaker's direction, and woofs apparently alerted other pack mates to the presence of food.

2. **Agonistic barks** are short noisy sounds, with the highest values of minimum, maximum and dominant frequencies of all bark types (Table 2.5, Figure 2.3). These barks were emitted in agonistic contexts by the dominant individual (i.e. the individual showing dominance displays, Table 2.4, APPENDIX I). Wolves that emitted agonistic barks were in close proximity to the receiver (≤ 1 m) and often showed dominance displays (e.g. tail held high, and piloerection along the back and the tail, Table 2.4). Occasionally, agonistic barks were directed at a human close to the fence surrounding the wolves' enclosure. Agonistic barks were emitted as single vocalizations or in bouts of vocalizations that included growls, snarls, multiple barks, and mixed vocalizations (bark-growls).

3. **Disturbance barks** are the most harmonic barks, with mean fundamental frequencies around 450 Hz (Table 2.5). Disturbance barks were only recorded in wolf-human interactions towards humans approaching wild wolves caught in traps (APPENDIX I). These barks resembled acoustically the disturbance barks emitted by domestic dogs (calls given in response to disturbances in the external environment, Yin & McCowan 2004). Unlike agonistic barks, disturbance barks identified in this study where emitted when humans where several meters away from the trapped animal, but when humans got closer, the wolf showed escape behaviours (rapid locomotion away from humans, Table 2.4). Only 13% of disturbance barks were emitted as single vocalizations. Most of them were

emitted in sequences of 2-10 barks together with growls, multiple barks, merged barks, and mixed vocalizations such as bark-growls and bark-growl-howls.

We found differences among these bark-like vocal classes in duration, average entropy, and minimum, maximum, and dominant frequencies (Table 2.5). Using stepwise discriminant function analysis, we could assign 80.9 % of barks to the defined type. The cross-validation procedure resulted in 74.5 % of barks correctly assigned, a percentage much higher than expected by chance (33.3%). Minimum frequency and average entropy are the variables that best discriminate between bark types.

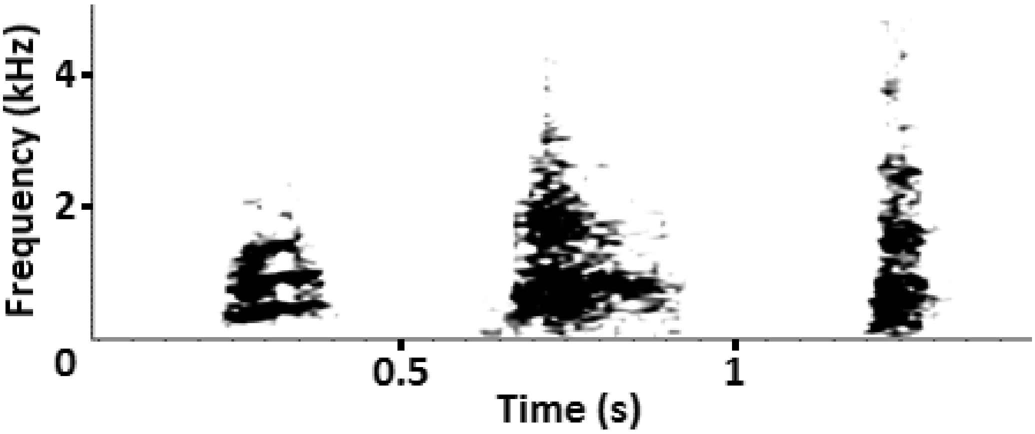


Fig. 2.3.- Bark types: Disturbance bark (left), agonistic bark (middle), and woof (right). Spectrogram: 2048 FFT (Fast Fourier Transform) points, Hann window.

Table 2.5.- Acoustic features of the bark types identified in this study. N: No. of vocalizations/No. of individuals. Differences between vocal types (ANOVA, Kruskal Wallis test). [1]: Only some disturbance barks had harmonic structure and fundamental frequency could be measured. *: significant differences.

Variable	Vocal class	N	Mean ± SD	Statistic	p
Dur	Disturbance bark	78/3	0.19 ± 0.05	$\chi^2 = 69.272$	< 0.001*
	Agonistic bark	19/6	0.15 ± 0.05		
	Woof	55/11	0.12 ± 0.06		
Freqmin90	Disturbance bark	78/3	318 ± 72	$F_{(2,149)} = 74.184$	< 0.001*
	Agonistic bark	19/6	505 ± 205		
	Woof	55/11	170 ± 103		
Freqmax90	Disturbance bark	78/3	1131 ± 312	$F_{(2,149)} = 13.297$	< 0.001*
	Agonistic bark	19/6	1873 ± 443		
	Woof	55/11	1400 ± 852		
Fundmin [1]	Disturbance bark	48/3	361 ± 75		
Fundmax [1]	Disturbance bark	48/3	504 ± 55		
Fundmean [1]	Disturbance bark	48/3	462 ± 43		
Domfreq	Disturbance bark	78/3	634 ± 293	$\chi^2 = 30.883$	< 0.001*
	Agonistic bark	19/6	924 ± 293		
	Woof	55/11	509 ± 395		
Aggent90	Disturbance bark	78/3	3.6 ± 0.8	$F_{(2,149)} = 2.304$	0.103
	Agonistic bark	19/6	4.0 ± 0.8		
	Woof	55/11	3.8 ± 0.9		
Avent90	Disturbance bark	78/3	2.2 ± 0.6	$F_{(2,149)} = 13.966$	< 0.001*
	Agonistic bark	19/6	2.7 ± 0.6		
	Woof	55/11	2.8 ± 0.9		

Growl-like sounds

Growling has previously been defined as "a low-frequency, broadband signal, uttered in sequences of variable duration consisting of growls with interspersed pauses" (Riede & Fitch 1999). We defined four growl-like vocal classes: **growls**, **snarls**, **short exhalations,** and **courtship growls**:

1) Both **growls** and **snarls** are produced during long breathing exhalations. (Fig. 2.4). Growls and snarls are vocalizations with variable duration (from tenths of a second to 4.5 s) and dominant frequencies about 500 Hz, and differ from each other only in the range of frequencies that comprises the acoustic energy distribution, both minimum and maximum frequencies being lower for growls (Table 2.6). Sometimes an individual growled continuously during two or more exhalations, emitting broadband sounds during the inhalation as well. We defined one growl as the sound emitted during the exhalation phase, and the sound produced during a cycle of exhalation-inhalation-exhalation as a bout of two growls, excluding the inhalation from the analyses. These vocal classes were emitted in all the contexts defined (agonistic, mating, other social interactions, and in wolf-human interactions in response to a human approaching the enclosure and towards a human approaching a trapped wolf), usually associated to dominance displays and play behaviours (Table 2.4, APPENDIX I). Only 23% of growls were emitted as single vocalizations. Most of them were included in sequences of vocalizations that included, besides growls and snarls, barks, moans, and mixed sounds such as growl-moans, bark-growls, bark-snarls, bark-growl-howls, multiple bark-snarls, and multiple bark-growls.

2) Growl-like sounds can also be produced by successive short breathing exhalations (pulsed growls). We defined **short exhalations** as pulsed growls emitted accompanying growls and snarls, and **courtship growls** as pulsed growls emitted in a mating context (Figure 2.4). Courtship growls differ from short

exhalations in duration, minimum frequency, dominant frequency, and entropy, but these differences could be an artefact because **short exhalations** were recorded only from one individual. Short exhalations were emitted in other social interactions during play between the reproductive pair outside the mating season, and in wolf-human interactions (addressed to humans close to the enclosure). They were always emitted in sequences of vocalizations, accompanied by growls and snarls. **Courtship growls** were emitted by wolves of both sexes in mating contexts during courtship. Only 19% of courtship growls were emitted as single vocalizations. The remaining 81% comprised bouts of courtship growls. No other vocalizations were emitted together with courtship growls.

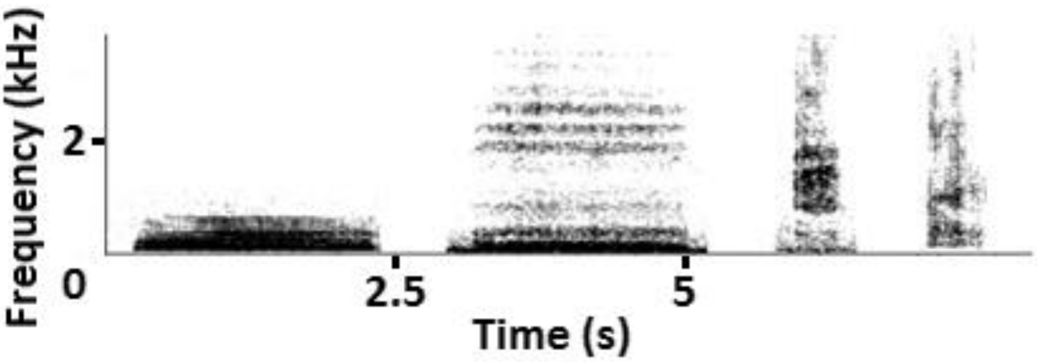


Fig. 2.4.- Growl types identified in this study. From left to right: growl, snarl, short exhalation, courtship growl. Spectrogram: 2048 FFT (Fast Fourier Transform) points, Hann window.

Grouping the four growl-like classes into long growls (growls + snarls) and pulsed growls (short exhalations + courtship growls), DFA correctly classified 85.9% of cases, considering all the cases and with L-O-O procedure. Duration and maximum frequency are the variables that best discriminate between these two categories.

Table 2.6.- Acoustic features of the growl types identified in this study. N: No. of vocalizations/No. of individuals. Differences between vocal classes (Student's-t test). *: significant differences.

Variable	Vocal class	N	Mean ± SD	t	p
Dur	Growl	76/6	1.54 ± 0.79	0.748	0.456
	Snarl	44/6	1.43 ± 0.78		
	Short exhalation	26/1	0.33 ± 0.24	3.684	0.001*
	Courtship growl	27/3	0.73 ± 0.47		
Freqmin90	Growl	76/6	146 ± 132	-2.595	0.012*
	Snarl	44/6	229 ± 186		
	Short exhalation	26/1	437 ± 265	-3.240	0.002*
	Courtship growl	27/3	229 ± 197		
Freqmax90	Growl	76/6	994 ± 482	-2.878	0.005*
	Snarl	44/6	1350 ± 722		
	Short exhalation	26/1	1761 ± 340	0.336	0.739
	Courtship growl	27/3	1821 ± 859		
Domfreq	Growl	76/6	476 ± 322	-0.224	0.823
	Snarl	44/6	489 ± 292		
	Short exhalation	26/1	993 ± 291	-2.174	0.034*
	Courtship growl	27/3	788 ± 386		
Aggent90	Growl	76/6	3.7 ± 0.9	1.160	0.248
	Snarl	44/6	3.5 ± 0.9		
	Short exhalation	26/1	4.2 ± 0.6	2.846	0.006*
	Courtship growl	27/3	4.8 ± 0.8		
Avent90	Growl	76/6	2.4 ± 0.7	1.755	0.082
	Snarl	44/6	2.2 ± 0.8		
	Short exhalation	26/1	2.8 ± 0.6	2.063	0.044*
	Courtship growl	27/3	3.2 ± 0.7		

For one individual we recorded the growls emitted in two different contexts: wolf-human interactions towards a human approaching the enclosure, N = 9) and other social interactions during play (N = 24, Fig. 2.5). Growls emitted, towards a human were longer (*Dur*: t = -2.279; df = 31; p = 0.03); with lower values of frequency (*Freqmin90*: t = 3.036; df = 23; p = 0.006; *Freqmax90*: t = 8.827; df = 30; p < 0.001; *Domfreq*: t = 6.747; df = 23; p < 0.001), and with lower values of entropy (*Aggent90*: t = 4.944; df = 31; p < 0.001; *Avent90*: t = 3.498; df = 31; p = 0.001).

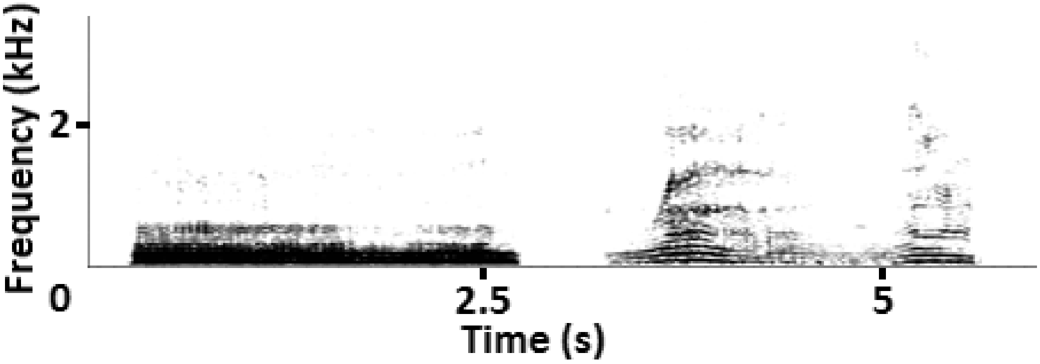


Fig. 2.5.- Growls emitted by the same individual in two different contexts: wolf-human interaction (left) and other social interaction (right). Spectrogram: 2048 FFT (Fast Fourier Transform) points, Hann window.

We also recorded growls emitted by two individuals in the same context (N_A = 9, N_B = 10), in wolf-human interactions (response to a human approaching the fence, Fig. 2.6). There were differences in duration (*Dur*: t = -2.220; df = 17; p = 0.04); maximum frequency (*Freqmax90*: t = 2.641; df = 17; p = 0.017); dominant frequency (*Domfreq*: t = 7.443; df = 10; p < 0.001) and entropy (*Aggent90*: t = 3.3; df = 17; p = 0.004).

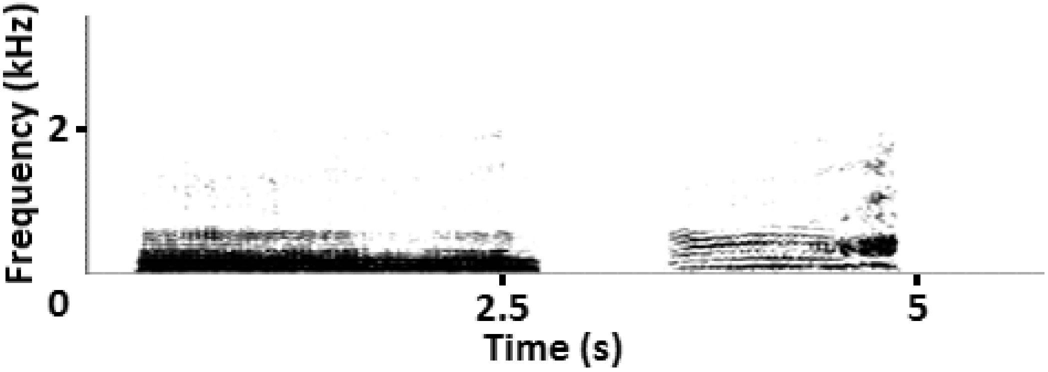


Fig. 2.6.- Growls emitted by two different individuals in the same context, towards a human approaching the fence. Spectrogram: 2048 FFT (Fast Fourier Transform) points, Hann window.

2.3.2. Harmonic Sounds

An important part of Iberian wolf close-range vocalizations comprises sounds with harmonic structure. We identified three broad harmonic vocal types: **whine-like, yelp-like,** and **moan-like**. By means of discriminant analysis 83.8 % of whine-like, moan-like and yelp-like vocalizations were correctly classified, 82.4 % with the L-O-O procedure. The variables that best discriminate among classes are duration and fundamental frequency. Some whine-like and yelp-like vocal classes were accompanied by high-pitched sounds, around 4 kHz, named “whistle” sounds (Figure 2.7). Sometimes, the vocalization only comprises the whistle part of the sound, the tonal (fundamental with lowest frequency) part being suppressed.

Whine-like vocalizations

We defined two whine-like vocal classes, **whines** and **whimpers**. **Whines** are harmonic sounds, from tenths of a second to 1 s in duration, with a mean fundamental frequency about 500-600 Hz, emitted in all social contexts (agonistic, mating and other social interactions), related to submissive displays (Figure 2.7,

Table 2.4, APPENDIX I). **Whimpers** are short whine-like sounds emitted consecutively in bouts and in the same contexts as whines (Figure 2.7, Table 2.4, APPENDIX I). Whimpers differ from whines only in duration and fundamental frequency of whistles, whimpers being shorter and with lower whistle frequencies than whines (Table 2.7). Most of the whimpers and whines were emitted as bouts of up to 25 and 7 consecutive vocalizations respectively, including other whine-like vocalizations, howl-whimpers, moans, yelps and multiple yelps. Most (73%) whines and whimpers incorporated whistles lasting from 20% to 100% of the duration of the vocalization. We defined three different vocal subclasses for both whines and whimpers based on the presence of tonal and whistle parts: **tonal-whines** and **tonal-whimpers** (tonal part present, whistle absent), **tonal-whistle-whines** and **tonal-whistle-whimpers** (both tonal and whistle part present), and **whistle-whines** and **whistle-whimpers** (only whistle part present). **Whistle-whimpers** have been described by other authors as "squeaks" (Harrington & Asa 2003).

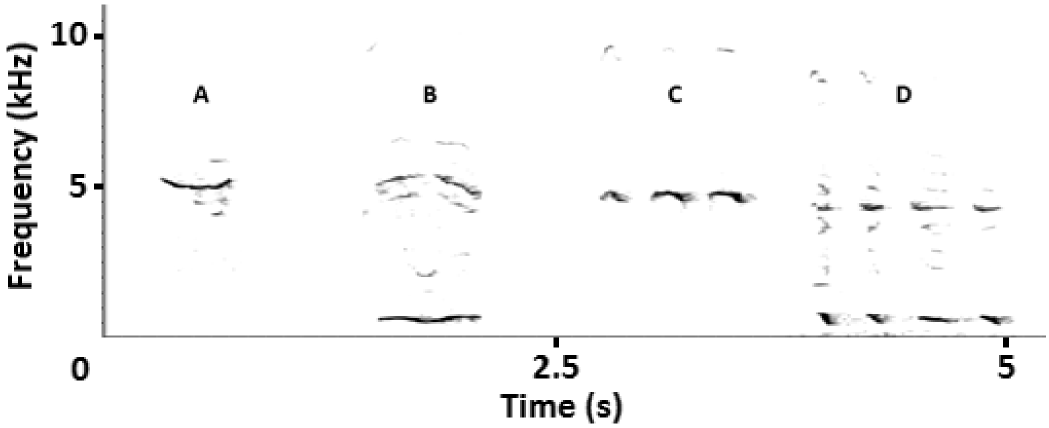


Figure 2.7.- Whine-like vocalizations: A) whistle-whine, B) tonal-whistle-whine, C) whistle-whimpers (squeaks), D) tonal-whistle-whimpers. Spectrogram: 2048 FFT (Fast Fourier Transform) points, Hann window.

Table 2.7.- Acoustic features for the whine-like vocal classes identified in this study. N: (No. of vocalizations/No. of individuals). Differences between vocal classes (Student's-t). *: significant differences.

Variable	Vocal class	N	Mean ± SD	t	p
Dur	Whine Whimper	45/7 111/9	0.54 ± 0.29 0.18 ± 0.09	-8.005	< 0.001*
Fundmin	Whine Whimper	37/7 61/9	493 ± 130 535 ± 166	1.331	0.186
Fundmax	Whine Whimper	37/7 61/9	634 ± 154 635 ± 167	0.045	0.965
Fundmean	Whine Whimper	34/7 57/9	553 ± 128 586 ± 162	1.014	0.313
Whistmin	Whine Whimper	34/7 86/9	4419 ± 825 3956 ± 699	-3.099	0.002*
Whistmax	Whine Whimper	34/7 86/9	5037 ± 1039 4357 ± 805	-3.433	0.001*

Yelp-like vocalizations

Yelp-like vocalizations are short, high pitched, loud, harmonic sounds, emitted in agonistic interactions (Table 2.8, APPENDIX I). Wolves emitted yelps apparently to prevent or stop an ongoing attack (although no systematic data have been collected to support this hypothesis), and always showed submissive displays while yelping (Table 2.4). We considered only one yelp-type vocal class, the **yelp**, but we found great variability in the way yelps are linked in the temporal domain. Wolves emitted yelps as single yelps, in bouts, as multiple, and merged yelps (Fig. 2.8). One particular type of yelp consisted in a sequence of short yelps increasing and decreasing the fundamental frequency, resulting in a call that sounds like laughing hyenas (Mathevon et al. 2010). We called this type of vocalization a laughing-yelp (Fig. 2.8). Most yelps (81%) were emitted in bouts and sequences of up to eight consecutive vocalizations. These sequences included merged yelps, multiple yelps, moan-yelps, moans, whimpers, and whines.

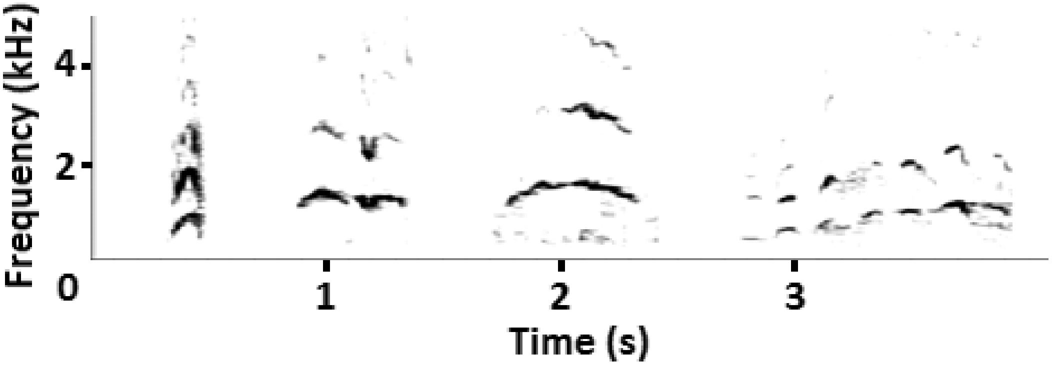


Fig. 2.8.- Different types of yelps emitted by Iberian wolves, from left to right: single yelp, multiple yelp, merged yelp and laughing yelp comprised by a bout of seven yelps. Spectrogram: 2048 FFT (Fast Fourier Transform) points, Hann window.

As in whine-like vocalizations, wolves sometimes emitted yelps with a whistle part. We distinguished three yelp subclasses, **tonal-yelps**, **tonal-whistle-yelps**, and **whistle-yelps**, depending on the presence of tonal and whistle part. The proportions of each subclass were different in whine-like and yelp-like vocal classes ($\chi^2 = 57.967$, d.f. = 4, $P < 0.001$, Table 2.9).

Table 2.8.- Acoustic features for yelps emitted by Iberian wolves. N: No. of vocalizations/No. of individuals.

Variable	N	Mean ± SD
Dur	119/8	0.14 ± 0.09
Fundmin	99/8	1039 ± 283
Fundmax	99/8	1191 ± 292
Fundmean	96/8	1146 ± 277
Whistmin	44/8	3986 ± 690
Whistmax	44/8	4889 ± 809

Table 2.9.- Proportions of different vocal subclasses among vocalizations that can include whistle part.

Subclass	Whine-like vocal class		Yelp-like vocal class
	Whimper	Whine	Yelp
Tonal	18%	20%	60%
Tonal-whistle	41%	62%	24%
Whistle	41%	18%	16%
N	111	45	119

Moan-like vocalizations

Moan-like vocalizations are vocalizations lasting from tenths of a second to several seconds, lower in pitch than whines (fundamental from 50 to 500 Hz), emitted in social interactions (agonistic, mating and other social interactions) (Table 2.10, APPENDIX I). Moans have been described as intermediate sounds (acoustic structure between harmonic and noisy, Schassburger 1993). Some moans, especially those with lower fundamental frequencies, are noisy with the parameters described above (512 FFT points), but increasing the frequency resolution (1024 FFT points) harmonics can be visualized in the spectrogram. We recorded moans as single vocalizations (49%) and as mixed sounds (51%) with growls, barks, whines, and yelps (Fig. 2.9). We identified **moans** and **growl moans** as described by Schassburger (1993). Growl moans have lower values of fundamental frequency and higher entropy than moans (Table 2.10).

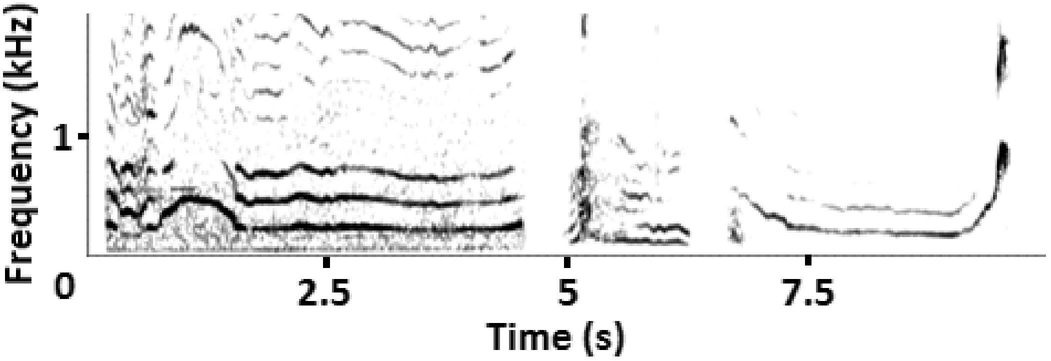


Fig. 2.9.- Moans emitted by Iberian wolves: moan (left), growl moan (middle), and mixed sound moan-yelp (right). Spectrogram: 2048 FFT (Fast Fourier Transform) points, Hann window.

Table 2.10.- Acoustic features for the moans identified in this study. N: (No. of vocalizations/No. of individuals). Differences between vocal types (Student's-t). *: significant differences.

Variable	Vocal type	N	Mean ± SD	t	p
Dur	Moan Growl moan	14/7 6/2	1.2 ± 1.0 1.0 ± 0.4	0.422	0.678
Fundmin	Moan Growl moan	14/7 6/2	188 ± 79 85 ± 24	4.281	0.001*
Fundmax	Moan Growl moan	14/7 6/2	338 ± 173 127 ± 36	4.215	0.001*
Fundmean	Moan Growl moan	14/7 6/2	217 ± 93 99 ± 18	3.843	0.003*
Aggent90	Moan Growl moan	14/7 6/2	3.25 ± 0.66 4.6 ± 1	-3.569	0.002*
Avent90	Moan Growl moan	14/7 6/2	1.97 ± 0.71 2.1 ± 0.39	-0.392	0.7

2.3.3 Non-vocal sounds

We identified two non-vocal sounds: **scratches** and **teeth snapping** (Figure 2.10). We recorded twice **scratches** (the sound produced by wolves scratching the ground, Table 2.4). This behaviour was displayed in agonistic contexts, after urine deposition by the reproductive pair (APPENDIX I). Sometimes the wolves emitted growl-moans simultaneously while scratching the ground (Figure 2.10). The other non-vocal sound recorded was **teeth snapping**, defined by Fox (1971) as a "*warning bite-intention signal .... The animal snaps at the air close to its adversary and does not actually bite*". We only heard teeth snapping in agonistic contexts by a female that was being attacked by the alpha female (APPENDIX I).

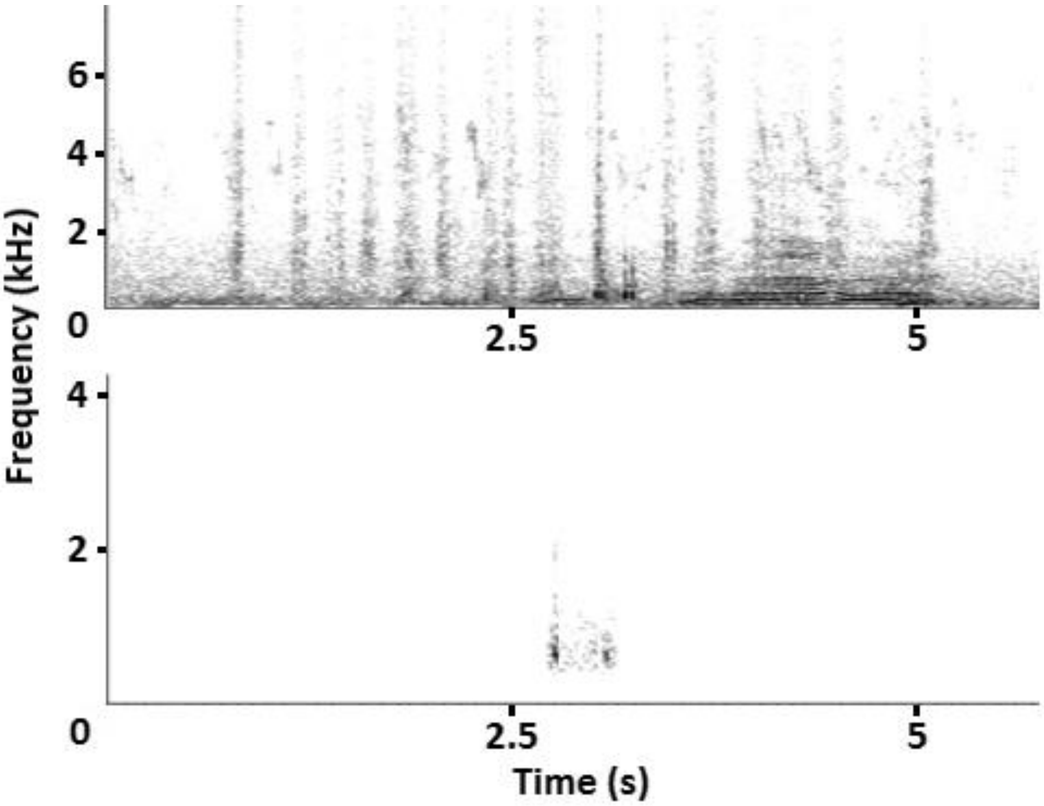


Figure 2.10.- Non-vocal sounds made by Iberian wolves: sound produced by a wolf scratching the ground and emitting a growl moan simultaneously at the end (up), and sound produced by the teeth snapping twice (down). Spectrogram: 2048 FFT (Fast Fourier Transform) points, Hann window.

Comparison with the wolf's vocal repertoire reported by Schassburger (1993)

We identified nine of the ten close-range vocal types described for North American[1] wolves by Schassburger (Table 2.11, Figure 2.11). The main differences between Schassburger's catalogue and the Iberian wolf's repertoire identified in this study are as follows:

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[1] Although Schassburger studied North American and Euro-Asian wolves, North American wolves provided the bulk of his data (Schassburger 1993). We assume that acoustic features published in Schassburger's study correspond to North American wolves.

1. The dominant frequencies of bark-like sounds are higher for Iberian wolves than for North American wolves (no data are available to conduct analyses to test if differences are statistically significant).
2. We identified two types of barks: agonistic and disturbance, and considered woofs as a bark-like vocal class.
3. The dominant frequencies of growls are higher for Iberian wolves. In contrast, the dominant frequencies for snarls are higher for North American wolves (no data are available to conduct analyses to test if differences are statistically significant).
4. Schassburger did not describe pulse growls (short exhalations and courtship growls).
5. The definitions of whine and yelp subclasses are different in the two catalogues. Schassburger defined two forms, full and whistle, based on the sound's quality to the human ear (resonant vs. whistle-like). Both full and whistle forms could include tonal and whistle parts simultaneously. Our definition is based on the presence/absence of tonal and whistle parts.
6. The fundamental frequency of yelps reported by Schassburger for North American wolves is much lower than that found in this study. However, Schassburger considered that the yelps have a "supressed" fundamental and the first visible frequency band corresponds to the third harmonic of the fundamental, whereas in this study we defined the fundamental as the first visible frequency band (we found no reason to consider a supressed fundamental), which may explain this difference.
7. Our sample of moans did not allow us to clearly distinguish between whine-moans and moans according to Schassburger.

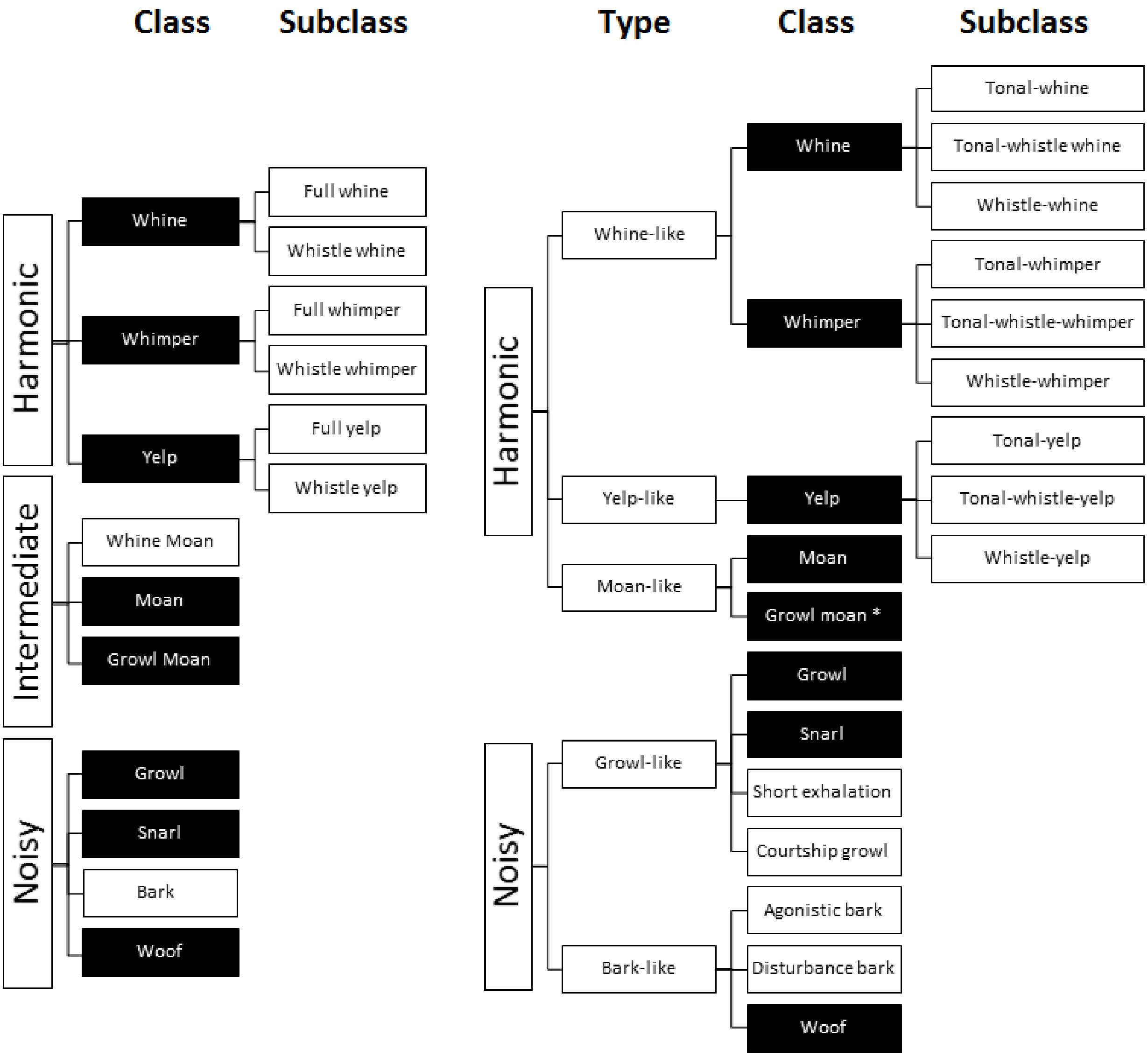

Figure 2.11.- Wolf’s close-range vocalizations described by Schassburger in 1993 (left) and Iberian wolf’s close-range vocalizations (vocal sounds, right). Black cells identify vocal classes described equally in both studies. The asterisk indicates vocalizations with harmonic structure at 1024 FFT points and noisy at 512 FFT (considered intermediate sounds by Schassbuger).

Table 2.11.- Available quantitative data of close-range vocalizations described for North American wolves (Schass: Schassburger 1993), and Iberian wolves (I. W.: this study). Fund: fundamental frequency, F. range: frequency range, Dom freq: dominant frequency, W range: range of the fundamental of the whistle. ± indicates mean ± SD, - indicates minimum – maximum.

Vocal class	Source	Duration (s)	Fund (Hz)	F range (Hz)	Dom freq (Hz)	W range (Hz)
Woof	*I.W.*	0.12 ± 0.06		67-2252	509 ± 395	
	Schass.	0.1-0.15	90-120	90-1080	105	
Disturbance bark	*I.W.*	0.19 ± 0.05	286-559	246-1443	634 ± 293	
Agonistic bark	*I.W.*	0.15 ± 0.05		300-2316	924 ± 293	
Bark	*Schass.*	0.25-0.4	145-170	145-2720	465-480	
Growl	*I.W.*	1.54 ± 0.79		50-1476	476 ± 322	
	Schass.	1-several s	70-145	70-2175	70-580	
Snarl	*I.W.*	1.43 ± 0.78		50-2072	489 ± 292	
	Schass.	1-several s	145-170	145-8000	2635-3565	
Short exhalations	*I.W.*	0.33 ± 0.24		172-2101	993 ± 291	
Courtship growls	*I.W.*	0.73 ± 0.47		50-2680	788 ± 386	
Whimper	*I.W.*	0.18 ± 0.09	369-802			3257-5162
	Schass.	0.1-0.2	575-645	575-7320		3600-3870
Whine	*I.W.*	0.54 ± 0.29	363-788			3594-6076
	Schass.	1-2 +	440-680	440-7500		
Yelp	*I.W.*	0.14 ± 0.09	756-1483			3296-5698
	Schass.	0.3-0.7	380-555	460-9900	1405-1450	
Moan	*I.W.*	1.2 ± 1.0	109-511			
	Schass.	tenths-2 s	75-215	75-2795		
Whine-Moan	*Schass.*	1-5	305-665	305-2205		
Growl-moan	*I.W.*	1.0 ± 0.4	61-163			
	Schass.	1-several s	90-165	90-2340		

2.4 DISCUSSION

The close-range vocal repertoire of the Iberian wolf comprises five broad vocal types (barks, growls, whines, yelps, and moans), mixed sounds resulting from the combination of different vocal types, and non-vocal sounds such as scratches and teeth snapping. These broad vocal types can be broken down into 12 vocal classes, three of which contain three vocal subclasses: woofs, agonistic barks, disturbance barks, growls, snarls, short exhalations, courtship growls, whines (tonal-whine, tonal-whistle-whine, and whistle-whine), whimpers (tonal-whimper, tonal-whistle-whimper, and whistle-whimper), yelps (tonal-yelps, tonal-whistle-yelps, and whistle-yelps), moans, and growl moans (Fig. 2.11).

Close-range communication system

As a first step in the analysis of a communication system, signals must be described and methods developed to classify them. If signals are not correctly identified, important questions in ethology will remain unresolved (Font & Kramer 1989). However, repertoire sizes are not easy to define and estimate in a way that is useful for inter and intra-specific comparisons (Catchpole & Slater 2008). The signals of some birds are highly variable and there is extensive intergradation between signal types (Marler 1967). Mammals, in general, possess graded vocal systems, in which specific call types vary extensively (Marler 1967). The complexity of mammalian vocal expression is achieved by the use of mixed sounds, transitions, and gradations, which occur throughout the vocal repertoire but are especially frequent among noisy sounds (Schassburger 1993). Repertoires composed of intergraded acoustic signals may be expected to have evolved in species in which sender and receiver have visual access to each other, so that information conveyed by means of signals in other sensory modalities (e.g. visual signals) increases the efficacy of the communicative process (Marler 1967). For these graded communication systems, such as the close-range repertoire of

Iberian wolves, it may be difficult to clearly categorize sounds, leading to a “splitter-lumper” dilemma (Font & Kramer 1989). This has been evident in previous studies of wolf vocal communication, with early workers taking the more conservative (i.e. “lumpers”) approach and researchers such as Schassburger (1993) making finer distinctions (i.e. splitters, Harrington & Asa 2003). To identify vocal types in such complex repertoires, researchers support a priori classifications (based on the acoustic structure, context, or previously reported descriptions of vocal classes) with statistical analyses (Gamba & Giacoma 2007). This approach has been applied to the study of acoustic communication in a wide variety of taxa, including fishes (Amorim et al. 2008), birds (Nowicki & Nelson 1990; Gammon & Baker 2004; Bouchet et al. 2010), marine mammals (Boisseau 2005), carnivores (Wong et al. 1999; Le Roux et al. 2009; Mumm & Knörnschild 2014), and primates (Range & Fischer 2004; Hsu et al. 2005; Bouchet et al. 2010). In this study we have classified vocalizations: 1) based on acoustic structure and context, and 2) using discriminant analysis to confirm that our proposed classification corresponded to statistically different acoustic signals. However, this debate will ultimately be resolved when we learn where the animals themselves draw the line between signals.

Vocal communication in animals is thought by some to differ from human language because the former is essentially a system of emotional expression (i.e. affective communication), whereas the latter is a referential system based on the relationship between words and the objects or events they represent (Seyfarth & Cheney 2003). Most animal signals have rudimentary referential properties. Often, the information obtained by the receiver refers to some property of the sender or the sender’s behaviour (e.g. its immediate needs, intentions, or its quality as a mate or as a competitor), but signals tend not to be very informative about other potential referents that could be relevant for receivers (Font & Carazo 2010). It has been claimed that the wolf vocal repertoire consists of affective signals that follow Morton’s motivation-structural (M-S) rules (Schassburger 1993;

Harrington & Asa 2003). Morton postulated M-S rules to explain how selective pressures may have shaped acoustic design features (Morton 1977). According to Morton's model birds and mammals use harsh, relatively low frequency sounds when they are being hostile and higher frequency sounds when they are approaching in an appeasing or friendly manner or when they are frightened. Recent studies have suggested that canid vocalizations such as dog barking could act as referential signals because their acoustic structure varies with context (Feddersen-Petersen 2000; Yin 2002; Yin & McCowan 2004; Molnár et al. 2009). These studies have been criticized by other authors that argue that the variability found in dog barks does not provide evidence of referential function and contextual cases are entirely accounted for by Morton's rules and arousal levels (Lord et al. 2009). However, animal signals should not be treated as either referential or affective, since affective and referential properties reflect different phenomena (the former depends on mechanisms of call production in the signaller whereas the latter depends on the listener's ability to extract information from events in its environment, Seyfarth & Cheney 2003). Referential signals could be stimulated by a signaller's emotions, and affective signals could be elicited by specific stimuli and thus function as referential calls. Although the vocal repertoire of the wolf can be considered as a system mainly based on emotion, we found differences in barks and growls emitted in different contexts, providing the first evidence of context specificity of agonistic vocalizations in the wolf. Further research is needed to investigate the referential properties of the acoustic signals of wolves.

Vocal types

We have identified three bark-like vocal classes: woofs, disturbance barks, and agonistic barks. Schassburger (1993) suggested that woofs could be considered as low intensity barks. Based on our results, however, woofs and barks should be considered different vocalizations. Woofs emitted by Iberian wolves may act as alarm calls and were not accompanied by other vocalizations. Even in conditions of intense arousal (when the caretaker approached with food, with all the wolves jumping and running along the fence) the only vocalizations emitted were woofs. By contrast, barks were more variable, with wolves emitting single, multiple and merged barks depending on arousal intensity. On the other hand, the emission of woofs apparently alerts other pack members, eliciting escape behaviours of pack mates.

Schassburger (1993) distinguished two types of growls: growl and snarl. We found acoustic differences between growls and snarls (growls and snarls differ in frequency range and dominant frequency, which is higher for snarls). However, both are emitted in the same contexts and in bouts comprising both types, suggesting that growls and snarls could reflect different levels of arousal. Pulsed growls have not been described for wolves, but similar vocalizations have been reported for dingoes (nasal sounds: snuffs and snorts, Déaux & Clarke 2013) and coyotes (huffs, Lehner 1978a). We do not know to which extent these vocalizations can be considered the same vocal type.

The mechanisms of production and the function of the whistle sounds that accompany whines and yelps need to be clarified and are still under debate. Schassburger (1993) thought that whistle sounds were harmonically related to the fundamental frequency of the vocalization that they accompany. However, Harrington & Asa (2003), concluded that the low and high frequency components are not harmonically related, and so they must be formed by independent mechanisms, perhaps vocally and nasally, or by a laryngeal whistle mechanism similar to that used by rodents to produce ultrasound. We randomly chose 20 vocalizations (whimpers and whines) and checked if the whistle frequency was in the range of frequencies corresponding to harmonics ((fundamental ± frequency resolution) * number of closest harmonic) and concluded that the tonal and whistle parts are not harmonically related. Schneider & Anderson (2011), studying vocalizations emitted by red wolves (*Canis rufus*) distinguished three vocalizations:

wuhs (tonal-whines and whimpers as described in our study), squeaks (whistle-whines and whimpers), and non-linear units (wuhs and squeaks emitted simultaneously, corresponding to our tonal-whistle-whines and whimpers). These non-linear units would be produced by biphonation (two fundamental frequencies not harmonically related emitted simultaneously, Schneider & Anderson 2011). These authors concluded that biphonation is produced by splitting the airflow between the mouth (tonal sound) and nasal (whistle sound) cavities. They also suggested different functions for tonal and whistle sounds, reporting that red wolves squeaked (whistle) most often when oriented towards others, implying a solicitation function, while wuh vocalizations (tonal) were more common during social interactions. Volodin & Volodina (2002) described similar sounds with two different fundamental frequencies (yap-squeaks) produced by biphonation in the dhole (*Cuon alpinus*) vocal repertoire. Therefore, Iberian wolf vocalizations that include a tonal and a whistle part should be considered non-linear vocalizations, presumably produced by biphonation.

We found that the occurrence of whistle sounds varies with vocal type, being more frequent in conjunction with whines or whimpers. Furthermore, the proportions of each subclass are different in whine-like and yelp-like vocal classes. However, this result has to be interpreted cautiously since the data analysed correspond to the best-quality recordings, and do not reflect the real proportion in which wolves emit different vocal subclasses. Additional research is needed to clarify how the context, individual, or individual's internal state influence the emission of tonal, whistle, and tonal-whistle forms of whines, whimpers, and yelps.

With respect to non-vocal sounds, it has been reported that ground scratching may act as a visual or olfactory signal related to scent-marking (Goodmann et al. 2002; Harrington & Asa 2003). Communication researchers tend to categorize signals by the primary sensory modality involved, but in fact multiple channels are often engaged simultaneously (Partan & Marler 1999). Wolves can obtain visual and olfactory information from scratches long after they are produced as these signals remain on the ground, but they might also get instantaneous information from hearing an individual scratching several meters away. The other non-vocal sound identified, teeth snapping, has been described in other canids such as coyotes (Lehner 1978b) and in wolves as an agonistic display, but previously had not been considered an acoustic signal (Fox 1971).

Comparison with other canids

Comparisons of signal repertoires across species are crucial to understanding the evolution of complexity in animal communication (Botero et al. 2008). During the last decades, the vocal repertoires of 11 canid species have been described (including the dingo, which is a wolf subspecies, Table 2.12). However, comparisons between different studies may be complicated due to several reasons: 1) the same vocal category can be treated as a vocal class or a subclass depending on the researcher (i.e. “splitter-lumper” dilemma), 2) there is no consensus about the names of the vocal classes (e.g. yelps, squeals, and screams described for different canids may refer to the same vocal class), 3) description of contexts, functions and behaviours are sometimes ambiguous, lack enough detail and vary across studies, 4) visual representations of vocalizations (spectrograms) are of different quality depending on publication date, and the parameters used to create them are not consensual, often making visual comparisons practically impossible. Despite these problems, some broad comparisons can be made.

Recent studies have suggested that social complexity may drive the evolution of repertoire size in primates, birds and carnivores such as mongooses (Le Roux et al. 2009). In fact, it has been reported that increased sociality is associated with an increased complexity of the acoustic communication system in canids (Cohen & Fox 1976). However, social complexity does not seem to affect the size of the repertoire of close-range vocalizations: the widest repertoire has been described for swift foxes, a canid in which the social basic unit is the reproductive pair, and the smallest has been described for red wolves, which live in packs (Table 2.12). These differences could be an artefact due to the “splitter-lumper” dilemma, the number of vocal types depending more on the individual researcher’s criteria than on how the animals actually partition the behaviour stream (Font & Kramer 1989). Canids have a highly graded vocal communication system with few vocal classes (3-17), specially compared to birds, which have repertoire sizes ranging from one to several hundred songs (Catchpole & Slater 2008). All canid species for which information is available have vocalizations for appeasement (whines, yelps/squeals/screams) and threat (barks, growl type vocalizations) in agonistic contexts, alarm signals (woofs, coughs), and vocalizations emitted in mother/offspring interactions (whines). One difference between social canids and those living alone or in pairs may be the existence of specific contexts and behaviours associated to group living, such as greeting (Table 2.4). However, the vocal types emitted during greetings are the same as those emitted in other social contexts (mainly whines).

Rather than repertoire size, vocalization rate may be the most important difference relating to sociality. Thus, red foxes (*Vulpes vulpes*), one of the less social canids with a repertoire of 10 vocal classes, rarely vocalize, reaching a frequency peak during the dispersal and mating season, when encounters between individuals are more frequent (Newton-Fisher et al. 1993). By contrast, interactions between individuals and therefore the probability of emitting close-range vocalizations should be more frequent for canids that live in groups throughout the year. In addition, it has been suggested that social species should have a higher proportion of “friendly” vocalizations than solitary species irrespective of repertoire size (Schassburger 1993).

Table 2.12.- Vocal repertoires described for canids. Social basic unit according to (Macdonald & Sillero-Zubiri 2004) excepting Iberian wolf pack size (Llaneza et al. 2012a). Numbers in brackets are minimum and maximum pack size.

Species/Subspecies	Social basic unit	Close-Range vocal classes	References
Iberian wolf (*Canis lupus signatus*)	pack (4-9)	12	This study
Wolf (*Canis lupus*)	pack (5-12)	10	Schassburger 1993
Dingo (*Canis lupus dingo*)	pack (3-12)	10	Déaux & Clarke 2013
Red Wolf (*Canis rufus*)	pack (2-12)	3	McCarley 1978
Coyote (*Canis latrans*)	pair - pack (2-10)	8	Lehner 1978
African wild dog (*Lycaon pictus*)	pack (2-30)	10	Robbins 2000
Dhole (*Cuon alpinus*)	pack (5-10)	11	Volodin et al. 2001
Bush dog (*Speothos venaticus*)	pack (2-12)	6	Brady 1981
Crab-eating fox (*Cerdocyon thous*)	pair + offspring (1-5)	6	Brady 1981
Maned wolf (*Chrysocyon brachyurus*)	pair	7	Brady 1981
Red fox (*Vulpes vulpes*)	pair	10	Newton-Fisher et al. 1993
Swift fox (*Vulpes velox*)	pair	17	Dabelsteen & Darden 2006

Another source of variability in the behavioural repertoire among species is evolutionary history (Slabbekoorn et al. 1999; Range & Fischer 2004; Price & Lanyon 2004). The close-range vocal repertoire of different canids is, in general terms, quite similar. All the canids studied emit barks, whines, yelp/scream type vocalizations and a vocalization used as an alarm/warning call. Growls are emitted by all canids excepting dholes (*Cuon alpinus*), but dholes emit a noisy vocalization similar to growls described as “staccato” (Volodin et al. 2001). However, the variability found in different canid species could be explained to some extent by phylogenetic relationships (Fig. 2.12). All the species belonging to the genus *Canis* share the same vocal types. Even those apparently specific vocal types reported for *Canis* (coyotes’ huffs -Lehner 1978a-, dingoes’ snuffs and snorts -Déaux & Clarke 2013- and Iberian wolves’ pulsed growls) could be considered as the same vocal type based on visual inspection of the published spectrograms. Major differences with *Canis* are found in African wild dogs and red-fox like canids (Figure

2.12). At least three close range vocal types are unique to African wild dogs (twitters, begging cries and rumbles; Robbins 2000). Red fox vocalizations not reported in the genus *Canis* include ratched calls, wow-wow barks, yodel barks and yell whines (Newton-Fisher et al. 1993). Swift foxes emit vocalizations described as whistles and chuckles that are different from those described in *Canis* (Dabelsteen & Darden 2006). Screams emitted by red-fox like canids are noisy sounds while yelps/screams emitted by other canids are harmonic sounds (Newton-Fisher et al. 1993; Dabelsteen & Darden 2006). Differences between the vocal repertoires of foxes and other canids had been reported by other authors (Tembrock 1976; Peters & Wozencraft 1989). Furthermore, non-linear vocalizations (biphonation) are apparently exclusive of wolf-like canids (Figure 2.12). Based on visual inspection of published spectrograms, only the yell-whine emitted by red foxes is a potential candidate for being a vocalization produced by biphonation, although the authors did not report the existence of non-linear phenomena (Newton-Fisher et al. 1993). With respect to the South American canids, there is only one published study describing the vocal repertoire of three different species (bush dog, maned wolf and crab-eating fox; Brady 1981). Four of the six/seven close-range vocal types described are shared by all three species (Brady 1981). Thus, in spite of the difficulties of comparing the vocal repertoires of different species and the scarcity of studied species, it seems that phylogenetic relationships explain part of the variability found in the acoustic signals emitted by canids. This congruence between phylogeny and vocal characteristics has also been reported for friendly close-range vocalizations in Felidae (Peters & Tonkin-Leyhausen 1999).

In canids, large differences in vocalizations are apparent among clades (i. e. there are clear differences among Red-fox-like, South American and Wolf-like clades). However, similarities among closely related species may arise from reasons other than phylogeny, such as genetic or environmental constraints (Harvey & Nee 1999). In addition, studies conducted with closely related species may use the same terminology, producing a "mimetic" effect responsible for this apparent similarity. Due to the existence of comprehensive molecular evolutionary trees for Canidae (Bardeleben et al. 2005; Lindblad-Toh et al. 2005), further research should strive to obtain detailed descriptions of vocal repertoires of other canid species, which could shed light on the evolution of acoustic communication in this group.

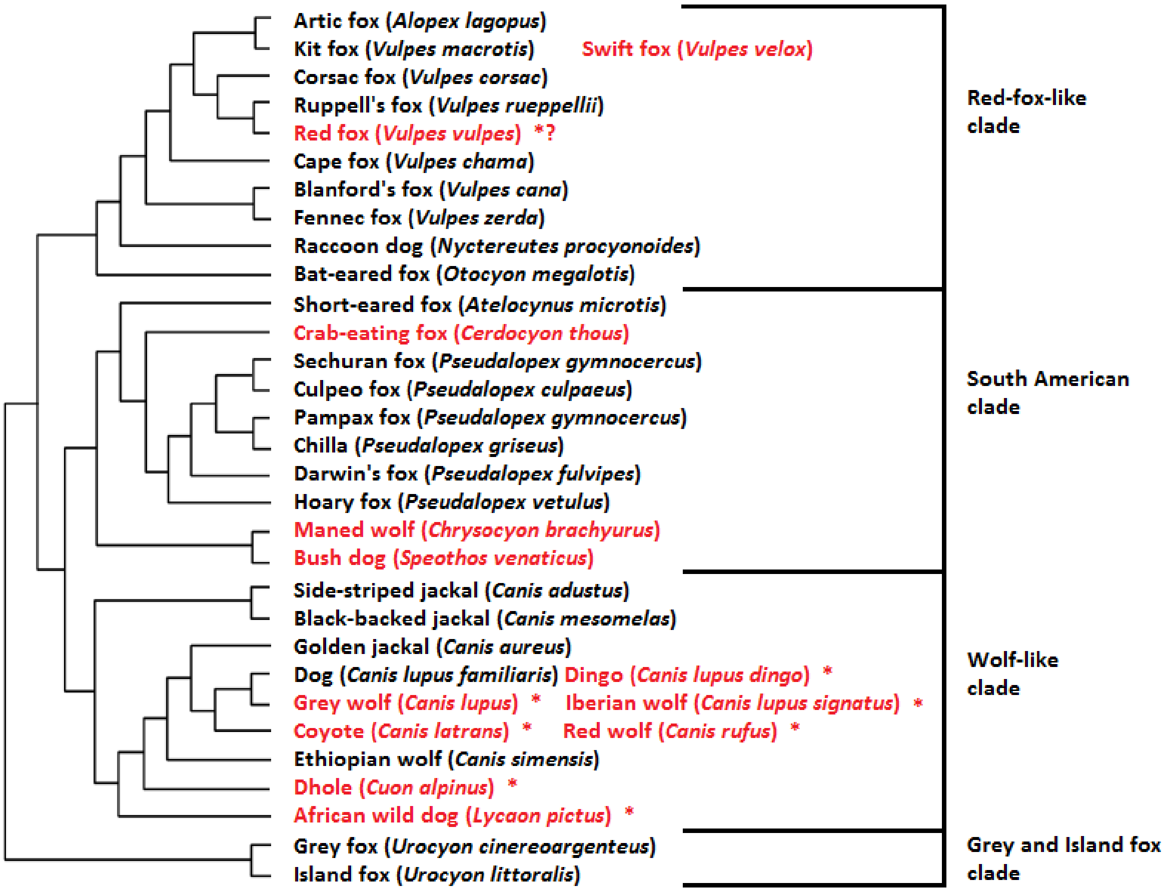

Figure 2.12.- Molecular phylogenetic tree of canids based on Lindblad-Toh et al. (2005). Four species that were not examined in their report were categorized as follows: swift foxes into the red fox-like clade, and dingoes, red wolves, and Iberian wolves into the wolf-like clade. Red colour indicates species with available descriptions of vocal repertoires. *: species that emit vocalizations comprising two different fundamental frequencies (biphonation).

2.5 ACKNOWLEDGMENTS

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2.6. APPENDIX I

Iberian wolf vocalizations (vocal and non-vocal classes[1]) recorded in this study by context. W-W: wolf-wolf interactions. W.H.: wolf-human interactions. [2]: trapped wolves barked and growled towards a human approaching, but they did not display dominance behaviours, and when the human was close to the animal, they often exhibited escape behaviours. Possible function according to functions suggested in published studies (e.g. Fox 1971; Schassburger 1993; Goodmann et al. 2002; Harrington & Asa 2003) and our own opinion (no systematic data collected to confirm these hypotheses).

Context		Sender	Receiver	Vocalization	Possible function
W-W	Agonistic interaction	Attacker (dominance displays)	Attacked (submissive displays)	agonistic bark multiple bark growl snarl	Threat
		Attacked (submissive displays)	Attacker (dominance displays)	whine whimper moan yelp multiple yelp merged yelp laughing yelp chattering[1]	Appeasement
		Reproductive pair (after urine deposition)	Pack mates	growl moan scratches[1]	Dominance assertion
	Mating interaction	Pair mate	Pair mate	courtship growl moan	Courtship
		Reproductive female	Reproductive male	growl growl moan	Courtship
		Reproductive male	Reproductive female	whimper whine	Courtship
	Other social interaction	Pack mate (isolated, approaching pack mates, greeting)	Pack mates	whimper whine moan	Contact seeking Strengthening social bonds
		Reproductive male (play)	Reproductive female (play)	growl snarl short exhalation	¿?
W-H		Adult wolf (dominance displays)	Person (approaching the fence)	agonistic bark growl snarl	Threat
		Trapped wolf	Person (approaching)	disturbance bark[2] multiple bark[2] merged bark[2] growl[2]	Threat
		Pack mate	Person (approaching) Pack mates	woof	To alert pack mates

CHAPTER 3. IBERIAN WOLF HOWLS: ACOUSTIC STRUCTURE, INDIVIDUAL VARIATION, AND A COMPARISON WITH NORTH AMERICAN POPULATIONS

3.1 INTRODUCTION

Behaviour, like other phenotypic traits, often exhibits geographic variation within a species (Foster & Endler 1999). In fact, population comparisons provide some of the best insights into the causes and mechanisms of adaptive differentiation. Vocalizations are not an exception. Recent research has revealed that, far from being invariant, vocalizations often show geographic variation at macrogeographic or microgeographic scales (Mundinger 1982). Vocal geographic variation has been documented for American pikas (*Ochotona princeps*—Conner 1982), Gunnison's prairie dogs (*Cynomys gunnisoni*—Perla & Slobodchikoff 2002), leopard seals, (*Hydrurga leptonyx*—Thomas & Golladay 1995), bottlenose dolphins (*Tursiops truncatus*—Wang et al. 1995), sperm whales (*Physeter catodon*—Weilgart & Whitehead 1997), and Barbary macaques (*Macaca sylvanus*—Fischer et al. 1998). Geographic variation in vocalizations can be based on genetic differences, environmental differences, or vocal learning (Janik & Slater 1997).

The wolf (*Canis lupus*) is a wide-ranging social carnivore with a complex spatial organization for which acoustic communication plays an important role (Mech 1970; Harrington & Asa 2003). Wolves are found throughout the northern hemisphere, inhabiting a great variety of habitats. Eurasian and North American wolves have been isolated for 10,000 years, since the closing of the Bering land bridge, and wolf populations show evidence of genetic differentiation on regional and continental scales (Wayne & Vilá 2003). Thus, it is conceivable that the acoustic structure of wolf vocalizations shows geographic variation. However, to our knowledge, no attempt to look for variation in wolf vocalizations among different populations has been made. Most studies of wolf vocalizations have been carried out with North American wolves (Theberge & Falls 1967; Harrington & Mech 1983; Harrington 1989; Tooze et al. 1990; Coscia 1995; Holt 1998). Schassburger (1993) described the vocal repertoire of Eurasian and North American timber wolves kept in captivity, but the bulk of the data in his study belonged to North American wolves and he did not look for geographic differences.

Howls allow wolves to communicate over distances up to several kilometres (Harrington & Asa 2003). Howls have been described as long harmonic sounds with a fundamental frequency from 150 Hz to more than 1000 Hz for adults (Harrington & Asa 2003). Two types of vocalizations involving howls have been reported: solo (lone) and chorus howls. Solo howls are emitted by a single individual (alone or with other pack members that do not howl). Chorus howls have been described as a vocalization in which one wolf begins howling, with other members joining in until several or all members of a pack are howling together (Joslin 1967). Usually, chorus howls include not only howls but also other vocalizations such as growls, barks, squeaks, and howl variations such as ‘‘woa-woa howls’’ (Holt 1998).

Recognizing individuals could be advantageous for social mammals and some long-distance vocalizations do contain information about individual identity (e.g., African lions [*Panthera leo*—McComb et al. 1993], spotted hyenas [*Crocuta crocuta*—Holekamp et al. 1999], African bush elephants [*Loxodonta africana*—McComb et al. 2000], yellow baboons [*Papio cynocephalus*—Fischer et al. 2002], and arctic foxes [*Vulpes lagopus*—Frommolt et al. 2003]). The role of howling in individual recognition in wolves has been explored in some detail (Theberge & Falls 1967; Tooze et al. 1990). Tooze et al. (1990) identified vocal signatures in the solo howls of seven North American wolves. With respect to chorus howling, it has been suggested that the initial howls of choruses may provide signature information about individual or pack identity (Harrington 1989).

In this study, we analysed 176 howls from 11 Iberian wolves held in captivity. Howls were emitted by a single wolf (solo howls) or by two or more wolves howling successively or simultaneously (chorus howls). We present a detailed description of the acoustic structure of howls emitted by wolves belonging to this population and investigate whether howls provide information regarding the individual identity of the emitter. Furthermore, we compare our results with those obtained by Tooze et al. (1990) for seven timber wolves from North America to assess whether the acoustic structure of howls shows differences between these two populations.

3.2 METHODS

Howls were recorded from 2001 to 2003 from wolves held in captivity at three locations in the Iberian Peninsula: Centro de Recuperacão do Lobo Ibérico (CRLI, Malveira, Portugal), La Dehesa (Riopar, Albacete, Spain), and Fauna Ibérica (El Rebollar, Valencia, Spain). Ninety-one percent of the howls included in the analysis were evoked by human imitations of wolf howling, whereas the remaining 9% were howls that were emitted spontaneously. Recordings were made during two seasons: from January to March (corresponding to the mating season of wolves in the Iberian Peninsula, Blanco 2004) and from September to November. There were typically two recording sessions per day during times when the wolves howl regularly and are visually identifiable: 0600–1000 h and 1800–2100 h. The wolves were habituated to humans. Recordings were made 5–40 m from the wolves, with the researcher often in full sight of the animals. We analysed howls of 11 wolves held in captivity in eight different enclosures, each with 1–5 individuals (Table 3.1). We analysed solo howls and howls included in choruses (Table 3.1). Two types of choruses were recorded: choruses that included only howls (n = 20); and choruses that included, in addition to howls, other vocalizations such as growls, barks, squeaks, and woa-woa howls (n = 22).

Audio recordings were made on TDK SA-60 cassette tapes (TDK Electronics Corp., New York, New York) using a Sennheiser MK 66 unidirectional microphone with K-6 power unit (Sennheiser Electric GmbH & Co. kG, Wedemark, Germany) connected to a Marantz PMD 222 cassette recorder (Marantz America, Inc., Mahwah, New Jersey). Recordings were digitized with 44.1-kHz sampling frequency and 16 bits in the Fonoteca Zoológica, Museo Nacional de Ciencias Naturales (CSIC, Madrid, Spain), using Delta 66 (Irwindale, California) or Digi 001 (Bucks, United Kingdom) digitizer cards connected to Apple Macintosh G4 computers (Cupertino, California). Recordings were saved in ‘‘.WAV’’ format. Recordings were subsequently deposited in the animal sounds collection of the Fonoteca Zoológica. Tape recordings, once digitized, were analysed using commercially available software (Spectrogram 7.2, 2002; Visualization Software LLC, Stafford, Virginia). We generated audiospectrograms conducting a fast Fourier transform (2048-point fast Fourier transform; Hann window; time step: 10 ms; frequency range: 9000 Hz; frequency resolution: 21.5 Hz). We used the cursor to measure the fundamental frequency and amplitude at intervals of 0.05 s along the entire length of the howl. For each howl we measured 16 variables (APPENDIX I), 13 of which have been used in previous works (Tooze et al. 1990; Coscia 1995).

Statistical analyses

For statistical analyses we used SPSS (12.0) for Windows (SPSS Inc., Chicago, Illinois) and the R statistical package (R Development Core Team 2010). We used discriminant function analysis to classify 176 howls from 11 known individuals. Discriminant function analysis identifies a linear combination of independent variables that best discriminates groups from each other (see methods in chapter 2). Our data set included cases of temporally close howls, thus violating the independence assumption. We grouped howls recorded in the same session and conducted a one-way analysis of variance for each individual using session as the independent variable and the acoustic variables as dependent variables. We found differences in only one variable (frequency modulation) of howls from one individual recorded in different sessions. Therefore, we assume that such a small amount of temporal autocorrelation should not affect the overall results.

We compared our results with those reported by Tooze and colleagues (Tooze 1987; Tooze et al. 1990) for a sample of 308 howls recorded from seven wolves. Because there are errors in the figures for duration reported in Tooze et al. (1990: table 2; F. Harrington, pers. comm.), we used the original values reported in Tooze (1987: table 1.8). Because of non-normality, the presence of outliers, and the limited sample size, we used the Yuen–Welch test for equality of trimmed (a = 0.2) means to compare 11 variables recorded in both studies (Yuen 1974). We used sequential correction to account for the number of pairwise comparisons made (Rice 1989).

Table 3.1. - Characteristics of the Iberian wolves and number of howls (N) analysed at three wolf recovery centres.

Center	Enclosure area (m^2)	Social group	Wolf	Sex	Weight (kgs)	Age (years)	Howls			
							N	Season (A/B)[1]	S/Ch[2]	S/I[3]
CRLI	310	Adult male and adult female	C-1	♀	28	7	15	3/12	0/15	1/14
			C-2	♂	42	7	19	1/18	0/19	0/19
	1830	Adult male and adult female	C-3	♀	30	11	15	1/14	1/14	0/15
			C-4	♂	39	11	16	9/7	0/16	0/16
	1753	Adult male and adult female	C-5	♀	28	11	26	6/20	16/10	14/12
	8387	Four adult males and one adult female	C-6	♀	27	7	14	0/14	0/14	0/14
			C-7	♂	43	7	15	0/15	0/15	0/15
La Dehesa	500	Adult male, adult female and subadult male	D-1	♂	42	4	25	9/16	19/6	1/24
	1500	Two adult males	D-2	♂	36	2	14	4/10	14/0	0/14
	1000	Adult male and two adult females	D-3	♀	?	3	11	11/0	0/11	0/11
Fauna Ibérica	600	Adult female	F-1	♀	23	2	6	0/6	6/0	0/6
Sample size:			**N=11**				**176**	**44/132**	**56/120**	**16/160**

[1] A/B: Autumn/Breeding
[2] S/Ch: Solo/Chorus
[3] S/I: Spontaneous/Induced

3.3 RESULTS

Iberian wolf howls were long-duration (1.1- to 12.8-s), harmonic sounds (1–18 harmonics), with a mean fundamental frequency between 270 and 720 Hz (APPENDIX II). Fundamental frequencies in howls ranged from 92 to 1116 Hz. The coefficient of frequency modulation ranged from 0.21 to 6.72, and the range of the coefficient of frequency variation was between 2.03 and 44.63. Iberian wolf howls usually had inflexion points (1–15) and discontinuities (1–8) in the fundamental frequency. The maximum fundamental frequency occurred in most cases (79% of the howls analysed) during the 1st quarter of the howl, and the minimum during the last quarter (78%). The fundamental peak amplitude occurred in the 1st half of the howl (83%).

The two acoustic characteristics that best distinguish each howl are the presence of frequency discontinuities and frequency modulations. Thus, howls were arbitrarily assigned to one of four groups based on these two attributes (Fig. 3.1). The 1st group consisted of flat howls, which were relatively constant frequency howls, without frequency discontinuities and with low values of both frequency modulation and variation (Table 3.2). The shape of the audiospectrogram was flat, not wavy. The 2nd group consisted of continuous wavy howls, which were howls without frequency discontinuities and with frequency modulations (i.e., wavy audiospectrograms). The 3rd group consisted of breaking howls, which were howls with large (21 to 250 Hz) frequency discontinuities, and low values of the coefficient of frequency modulation (Table 3.2). The audiospectrogram was not wavy. The 4th group consisted of breaking wavy howls, which were howls with large (21 to 250 Hz) frequency discontinuities and numerous frequency modulations (Table 3.2). The audiospectrogram was wavy.

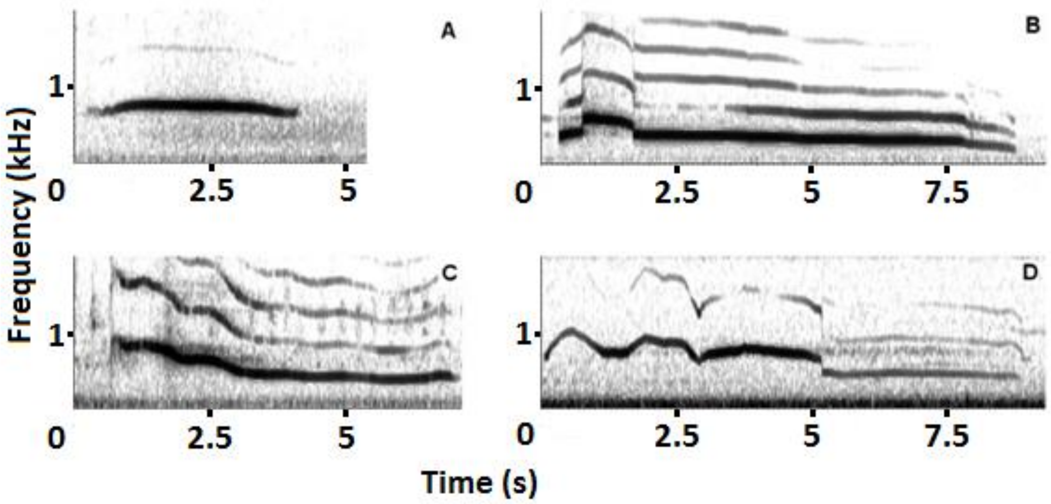

Figure 3.1.- Types of howls recorded from wolves held in captivity at 3 locations in the Iberian Peninsula from 2001 to 2003: A) flat, B) breaking, C) continuous wavy, and D) breaking wavy. Spectrogram: 1024 FFT (Fast Fourier Transform) points, Hann window.

Breaking wavy howls had the greatest coefficients of frequency modulation and variation, and flat howls had the lowest (Table 3.2). Howls with frequency discontinuities had the longest duration. In most cases, both solo howls and howls included in choruses had discontinuities, with breaking howls being the most frequent type of howl (Table 3.3). Solo howls were significantly shorter than howls included in a chorus (t = - 5.734, d.f. = 174, P < 0.001).

The discriminant function analysis identified mean fundamental frequency, maximum frequency of the fundamental, number of harmonics, and frequency modulation as the most important discriminating variables. Using discriminant function analysis with independent variables entered simultaneously, we could assign 84.7% of howls to the correct individual. The cross-validation procedure resulted in 72.7% of howls correctly assigned, a percentage much higher than expected by chance (10.15%). Using only the howls included in chorus howling, 81.7% of howls were assigned to the correct individual, and the leave-one-out cross-validation resulted in 72.5% of howls correctly assigned.

Table 3.2.- Shape variables (mean and range [maximum - minimum value]) for each type of howl. Variables are described in APPENDIX I.

		Howl type			
Variable	**Statistic**	**Flat**	**Breaking**	**Continuous wavy**	**Breaking wavy**
Cofv	$\bar{X}$ ± SD	5.7 ± 2.5	18.3 ± 6.9	19.5 ± 7.1	23.4 ± 8.1
	Range	8.0	32.7	32.5	26.5
Range	$\bar{X}$ ± SD	125 ± 57	279 ± 88	318 ± 137	438 ± 167
	Range	187	425	550	572
Cofm	$\bar{X}$ ± SD	0.7 ± 0.5	1.3 ± 0.6	1.4 ± 1.5	1.9 ± 0.8
	Range	1.8	3.7	6.2	3.3
Abrupt	$\bar{X}$ ± SD	0.0 ± 0.0	2.1 ± 1.1	0.0 ± 0.0	3.3 ± 1.9
	Range	0.0	7.0	0.0	7.0
Changf	$\bar{X}$ ± SD	1.1 ± 1.8	2.2 ± 2.0	1.6 ± 1.7	5.9 ± 3.0
	Range	6.0	9.0	5.0	14.0
Dur	$\bar{X}$ ± SD	5.5 ± 2.3	6.9 ± 2.3	5.3 ± 1.8	6.4 ± 2.6
	Range	8.4	9.7	6.5	10.0

Comparing our results with those reported by Tooze and colleagues (Tooze 1987; Tooze et al. 1990) we found statistically significant differences only for the variable Abrupt (Table 3.4). The Iberian wolf howls analysed have fewer frequency discontinuities than the howls recorded from seven North American timber wolves. Nevertheless, the two data sets are not homogeneous. All the wolves in our study were adults, whereas in the study of Tooze and colleagues (Tooze 1987; Tooze et al. 1990) two individuals were yearlings. However, an age effect seems unlikely because the results are similar when only data from adult wolves are compared (Table 3.4).

Table 3.3.—Frequency of the different types of howls identified in this study. See Table 3.1 for characteristics of individual wolves.

Wolf	**Solo**					**Chorus**				
	Flat	**Breaking**	**Continuous wavy**	**Breaking wavy**	**Total**	**Flat**	**Breaking**	**Continuous wavy**	**Breaking wavy**	**Total**
C-1	0	0	0	0	0	1	13	0	1	15
C-2	0	0	0	0	0	1	13	2	3	19
C-3	0	1	0	0	1	0	4	10	0	14
C-4	0	0	0	0	0	2	7	2	5	16
C-5	1	13	1	1	16	0	9	1	0	10
C-6	0	0	0	0	0	1	10	1	2	14
C-7	0	0	0	0	0	0	13	0	2	15
D-1	1	17	0	1	19	0	4	1	1	6
D-2	0	1	3	10	14	0	0	0	0	0
D-3	0	0	0	0	0	0	1	1	9	11
F-1	4	2	0	0	6	0	0	0	0	0
N. howls	6	34	4	12	56	5	74	18	23	120
%	10.71	60.71	7.14	21.43	100	4.17	61.67	15.00	19.17	100

Table 3.4.- Yuen–Welch test for equality of trimmed ($\alpha = 0.2$) means to compare acoustic features of North American timber wolves (Tooze 1987; Tooze et al. 1990) and Iberian wolf howls. Statistically significant differences are set in boldface. See APPENDIX I for descriptions of variables.

All the wolves	**Meanf**	**Maxf**	**Minf**	**Range**	**Cofv**	**Cofm**	**Dur**	**Changf**	**Abrupt**	**Posmax**	**Narm**
Yuen’s test statistic	0.84	0.25	1.68	0.93	2.90	0.49	1.25	0.07	7.93	1.91	2.40
d.f.	7.73	7.96	7.92	9.99	7.67	9.49	6.96	6.72	10.00	9.51	9.70
p	0.425	0.809	0.132	0.372	0.021	0.638	0.252	0.944	**< 0.001**	0.087	0.038
critical p value (Rice)	0.013	0.025	0.007	0.01	0.005	0.017	0.008	0.05	0.005	0.006	0.006
Adult wolves only	**Meanf**	**Maxf**	**Minf**	**Range**	**Cofv**	**Cofm**	**Dur**	**Changf**	**Abrupt**	**Posmax**	**Narm**
Yuen’s test statistic	0.64	0.15	2.15	1.02	3.77	0.53	1.55	0.67	6.76	1.86	2.64
d.f.	2.65	2.73	3.49	5.29	4.36	7.96	6.69	2.89	6.21	3.74	5.24
p	0.571	0.894	0.107	0.351	0.017	0.609	0.167	0.554	**< 0.001**	0.141	0.044
critical p value (Rice)	0.017	0.05	0.006	0.01	0.005	0.025	0.008	0.013	0.005	0.007	0.006

3.4 DISCUSSION

Iberian wolf howls can be classified into four types (flat, continuous wavy, breaking, and breaking wavy howls) based on the two criteria that best define howl shape in the audiospectrogram: the presence of discontinuities in the fundamental frequency and the pattern of frequency modulation. Before our study, two types of howls had been reported for North American wolves: flat howls and breaking howls. Although these two types were singled out as representing the extremes seen in frequency modulation, there is a fair degree of variation within each type (revised in Harrington and Asa, 2003). It is unclear to what extent the four howl types identified in our study for descriptive purposes represent, to the wolves, natural or functionally distinct vocalizations. Using discriminant function analysis with howl type as the grouping variable (results not shown) we could assign 89% of howls to the correct type, showing that the four howl types have a different acoustic structure. It has been proposed that variation in howls may be related to general arousal or to the sequence of the howl in a chorus, among other factors (Harrington 1989; Harrington & Asa 2003). The fact that we have identified solo howls of the four types suggests that, under certain circumstances, wolves can emit highly modulated howls not necessarily integrated in a chorus. It would be interesting to investigate whether the different howl types reported in this study are functionally distinct and if so, how they are produced, under what circumstances they arise, and what information they might transmit.

Tooze et al. (1990) found individual differences among the solo howls of seven North American timber wolves. Our results show that solo and chorus howls of 11 Iberian wolves are individually distinct. The acoustic structure of wolf howls allowed us to identify individuals, and wolves could use this information for individual recognition. Our results agree with those obtained in Tooze et al. (1990) in emphasizing variables related to fundamental frequency (mean and maximum) and coefficient of fundamental modulation as the variables that best discriminate individuals. Frequency characteristics usually encode individuality because they are mostly determined by the characteristics of an animal's vocal apparatus (Fitch 1997). The fundamental frequency is one of the acoustic features that best discriminate among individuals in other mammal vocalizations, including isolation calls of Amazonian manatees (*Trichechus inunguis*—Sousa-Lima et al. 2002), calls of African bush elephants (*L. africana*—McComb et al. 2003), domestic dog barks (*Canis lupus familiaris*—Yin & McCowan 2004), and the whistle call of dholes (*Cuon alpinus*—Durbin 1998). Frequency modulation also plays an important role in individual recognition as reported in chirps of Belding's ground squirrels (*Spermophilus beldingi*—Mccowan & Hooper 2002), whistles of bottlenose dolphins (*T. truncatus*— Caldwell & Caldwell 1965), and calls of subantarctic fur seal pups (*Arctocephalus tropicalis*—Charrier et al. 2002). Transmission characteristics of the atmosphere impose constraints on acoustic communication, and frequency modulation represents one of the best ways to encode information in long range vocal signals (Wiley & Richards 1978). Although fundamental frequency is highly determined by morphological characteristics, acoustic features related to the shape of the spectrum (e.g., frequency modulation) are determined by details of the opening and closing movement of the vocal folds (Rubin & Vatikiotis-Bateson 1998). Morphological characteristics of the vocal apparatus and the development of an individually specific howling technique could be the basis for individual recognition by means of howling in wolves, as it has been suggested for coyotes (*Canis latrans*—Mitchell 2004). Nevertheless, the fact that acoustic structure of howls is individually specific does not imply that wolves use this information for individual recognition. To confirm this hypothesis would require playback experiments (e.g., McComb et al. 2001; Frommolt et al. 2003; Mitchell 2004).

Animal vocalizations commonly vary over the geographic range of the species. However, our results reveal many similarities between the acoustic structure of howls of Iberian and North American wolves (Theberge & Falls 1967;

Harrington & Mech 1978a; Harrington 1989; Tooze et al. 1990). Furthermore, when comparing our results with those obtained by Tooze and colleagues (Tooze 1987; Tooze et al. 1990), we only found significant differences in the number of frequency discontinuities. This difference could be due to Iberian wolves emitting relatively fewer breaking howls than North American wolves. However, this explanation seems unlikely considering that most howls produced by Iberian wolves have frequency discontinuities (Table 3.3). Alternatively, Iberian and North American wolves could be producing a similar proportion of breaking howls but those of Iberian wolves would have fewer discontinuities per howl. Further data, including a larger sample of howls and individuals, will be required to assess the importance of these interpopulation differences.

Thus, in spite of possible genetic, morphological, or environmental differences, it seems that Iberian wolf howls and North American timber wolf howls show few detectable differences, at least with the variables used in this study. Lack of geographic differences in vocalizations has been reported for other large mammals, such as West Indian manatees (*Trichechus manatus*—Nowacek et al. 2003), and in the songs of gibbons (*Hylobates*—Marshall & Marshall 1976). Genetic differences are not always correlated with variation in vocal signals. For example, on a microgeographic scale, Wright et al. (2005) did not find concordance between vocal dialects and population genetic structure in the yellow-naped parrot (*Amazona auropalliata*). Geographic variation in vocalizations can also arise because of environmental differences. Selection could shape the structure of long-distance acoustic signals to maximize transmission through different environments (Morton 1975; Blumstein & Turner 2005). Both North American timber wolves and Iberian wolves live in mountainous and forested areas. It is possible that the similarities found in their howls are due to selection for acoustic characteristics that maximize their transmission in similar habitats. Further research including samples of vocalizations from wolves living in different environments is needed to clarify this issue.

3.5 ACKNOWLEDGMENTS

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3.6 APPENDICES

APPENDIX I

Description of structural variables used in analysis of wolf howls and units of measure (in parentheses).

Variable type	Abbreviation	Structural variables
Frequency	Meanf	Mean frequency of the fundamental at 0.05 intervals over the duration (Hz)
	Maxf	Maximum frequency of the fundamental (Hz)
	Minf	Minimum frequency of the fundamental (Hz)
	Range	Range of the fundamental (Maxf-Minf) (Hz)
	Cofm	Coefficient of frequency modulation: Cofm = $\frac{\sum_{1}^{n-1} \lvert f(t)-f(t+1) \rvert \big/ (n-1)}{Meanf}$ x 100
	Cofv	Coefficient of frequency variation: Cofv = $\left(\frac{s}{Meanf}\right) \times 100$
	Changf	Number of inflexions of the fundamental
	Abrupt	Number of discontinuities of the fundamental
	Posmax	Position in the howl at which the maximum frequency occurs, (time of Maxf)/Dur
	Posmin	Position in the howl at which the minimum frequency occurs, (time of Minf)/Dur
	Endf	Frequency at the end of the fundamental (Hz)
	Dur	Duration of the howl (measured at the fundamental) (s)
	Narm	Maximum number of harmonics to 2000 Hz
Amplitude	Frecpaf	Fundamental at its amplitude peak (Hz)
	Pospaf	Position in the howl at which Frecpaf occurs, (time of Frecpaf)/Dur
	Coidv	Coefficient of amplitude variation at the fundamental frequency

APPENDIX II. Parameters of Iberian wolf howls analysed. Range denote minimum and maximum values for each variable.

Wolf		Meanf	Maxf	Minf	Range	Cofm	Cofv	Narm	Changf	Abrupt	Dur	Posmax	Posmin	Pospaf
C-1	$\bar{X}$±SD	373±32	430±41	234±55	196±57	1.1±0.5	11.8±5.3	4.5±0.5	3.5±2.9	2.5±1.9	8.3±2.5	0.48±0.34	0.38±0.48	0.31±0.18
	Range	320-431	367-504	108-335	103-274	0.5-2.0	5.2-20.3	4.0-5.0	0.0-10.0	0.0-6.0	3.8-11.0	0.00-0.99	0.00-1.00	0.06-0.63
C-2	$\bar{X}$±SD	474±48	596±60	318±75	278±97	0.8±0.3	15.7±7.1	3.1±0.6	2.3±1.8	2.1±1.9	8.0±1.6	0.04±0.06	0.90±0.17	0.26±0.12
	Range	360-588	496-712	173-524	114-539	0.4-1.2	6.2-38.0	2.0-5.0	0.0-6.0	0.0-8.0	4.6-10.6	0.00-0.02	0.42-1.00	0.10-0.57
C-3	$\bar{X}$±SD	466±25	587±23	346±17	241±29	0.8±0.2	18.1±3.1	2.9±0.8	1.3±1.6	0.6±0.9	5.8±1.3	0.05±0.06	0.88±0.15	0.25±0.27
	Range	417-517	547-647	302-376	205-302	0.5-1.0	12.9-22.4	2.0-5.0	0.0-5.0	0.0-2.0	3.0-7.6	0.00-0.17	0.51-1.00	0.07-0.80
C-4	$\bar{X}$±SD	357±39	472±75	222±53	250±100	1.2±0.3	15.4±5.1	4.1±0.9	3.6±2.8	1.6±1.1	8.3±1.9	0.30±0.28	0.64±0.45	0.39±0.22
	Range	274-405	296-570	114-319	68-456	0.4-1.7	4.4-23.0	2.0-5.0	0.0-10.0	0.0-3.0	4.8-11.7	0.00-0.92	0.00-1.00	0.02-0.80
C-5	$\bar{X}$±SD	395±47	562±74	240±74	324±70	1.3±0.3	21.4±5.1	4.9±0.4	2.6±2.6	1.6±0.9	7.3±1.8	0.08±0.10	0.96±0.07	0.21±0.24
	Range	345-533	389-672	92-349	114-456	0.8-2.1	10.0-32.8	4.0-5.0	0.0-9.0	0.0-3.0	2.7-9.5	0.02-0.52	0.70-1.00	0.01-0.86
C-6	$\bar{X}$±SD	407±34	524±60	294±38	229±78	1.1±0.5	15.1±5.8	2.9±0.4	1.9±2.6	1.9±1.2	6.6±1.7	0.14±0.14	0.75±0.41	0.40±0.28
	Range	340-454	388-615	205-324	64-410	0.3-2.6	2.8-24.4	2.0-3.0	0.0-8.0	0.0-4.0	2.1-8.8	0.00-0.41	0.00-1.00	0.01-0.85
C-7	$\bar{X}$±SD	332±47	459±70	201±19	258±65	1.3±0.4	17.3±5.6	5.1±0.9	2.5±2.6	3.2±1.9	8.2±2.4	0.08±0.21	0.95±0.10	0.21±0.15
	Range	273-424	342-583	173-227	137-367	0.6-2.0	8.3-27.5	3.0-6.0	0.0-8.0	1.0-8.0	4.2-12.8	0.00-0.77	0.63-1.00	0.02-0.51
D-1	$\bar{X}$±SD	409±39	568±65	246±50	322±100	2.0±0.9	21.5±8.2	4.6±0.6	2.2±1.8	1.6±0.7	4.4±1.3	0.13±0.09	0.95±0.19	0.22±0.15
	Range	359-491	456-661	159-342	114-456	0.8-4.7	6.3-31.7	3.0-6.0	0.0-7.0	0.0-3.0	1.1-7.1	0.00-0.41	0.03-1.00	0.04-0.56
D-2	$\bar{X}$±SD	598±52	961±111	340±44	621±138	3.0±1.4	31.6±7.7	3.6±1.0	6.5±3.6	3.1±2.6	4.9±1.6	0.22±0.15	0.62±0.48	0.32±0.20
	Range	524-711	691-1116	281-432	259-777	1.2-6.7	14.4-44.6	2.0-5.0	1.0-15.0	0.0-8.0	1.3-6.5	0.02-0.53	0.00-1.00	0.05-0.68
D-3	$\bar{X}$±SD	570±52	744±79	326±41	418±70	1.6±0.4	21.9±5.7	3.0±0.5	3.0±1.8	2.5±1.4	4.3±1.3	0.18±0.14	0.96±0.10	0.25±0.14
	Range	463-641	615-842	251-388	319-540	1.1-2.2	16.1-30.1	2.0-4.0	1.0-7.0	0.0-5.0	2.8-6.6	0.06-0.53	0.67-1.00	0.04-0.46
F-1	$\bar{X}$±SD	666±60	731±34	543±95	188±78	0.9±0.5	7.0±5.3	2.5±0.6	1.7±2.4	0.5±0.8	4.3±0.9	0.24±0.24	0.51±0.51	0.41±0.10
	Range	565-723	684-775	387-638	91-297	0.2-1.7	2.0-16.9	2.0-3.0	0.0-6.0	0.0-2.0	3.3-5.7	0.00-0.57	0.00-1.00	0.22-0.53

CHAPTER 4. RECOGNITION OF FAMILIARITY ON THE BASIS OF HOWLS: A PLAYBACK EXPERIMENT IN A CAPTIVE GROUP OF WOLVES.

4.1 INTRODUCTION

In animals that use vocalizations for social communication, the selective advantage provided by being able to identify and locate distant individuals could promote the evolution of individual identity acoustic signals (Rendall et al., 1996). This would allow individuals to recognize each other by their vocalizations when the transmission of signals in other sensory modalities is constrained (Falls, 1982). Penguins, for instance, identify their kin in dense colonies using vocal signatures (Searby et al., 2004), domestic sheep (*Ovis aries*) can recognize their ewes based on their calls (Searby & Jouventin, 2003), Seba´s short-tailed fruit bats (*Carollia perspicillata*) recognize their pups based on the individual signature in isolation calls (Knörnschild et al., 2013), and individual recognition has been experimentally demonstrated in dwarf mongooses (*Helogale parvula*) using contact calls (Sharpe et al., 2013).

When individuals belonging to the same group are separated, recognizing and maintaining contact with specific individuals can be challenging. Therefore, the ability to individually recognize group members at a distance is an important adaptation for group-living animals (Bradbury & Vehrencamp, 2011). Wolves (*Canis lupus*) are group-living canids whose basic social unit is the pack, comprised basically of a mated pair and their offspring (Packard, 2003). Wolf packs occupy wide territories, ranging in Europe from 100 to 500 km^2 (Boitani, 2000). Wolves belonging to the same pack often do not travel together (Demma & Mech, 2009), and it has been suggested that maintaining a loose cohesion with pack mates while travelling separately could increase the chances of finding scattered food sources (Palacios & Mech, 2011). Furthermore, maintaining contact with other pack members is also crucial during territory defence as intra-specific aggression is one of the main causes of natural mortality in wolf populations, and each pack actively defends its own territory from neighbouring packs (Mech & Boitani, 2003). Thus, wolf packs live under conditions that would seem to promote the evolution of individual vocal recognition.

Howls are arguably the most conspicuous wolf vocalizations. Functions attributed to howling often involve some sort of communication among individuals belonging to the same pack. For example, it has been proposed that howls can serve to reunite pack mates in situations where they have been separated (Harrington & Asa, 2003). Howls can also be used as territorial displays, conveying information about pack location and minimizing contact between different packs (Harrington & Mech, 1979). Given these proposed functions, we predict that howls are ideally suited to serve as signals allowing individual recognition. In fact, it has been shown that howls contain information on individual identity (Tooze et al., 1990; Palacios et al., 2007). In Iberian wolves, the acoustic parameters that best discriminate among individuals are howl fundamental frequency and frequency modulation (Palacios et al., 2007). However, the fact that the acoustic structure of howls contains information regarding identity does not imply that wolves use these features to discriminate individuals. Although the ability for discriminating vocalizations from different individuals seems widespread, studies that investigate the acoustic parameters involved in discrimination are rare (Bee & Gerhardt, 2001; Charrier et al., 2003; Searby & Jouventin, 2003).

The purpose of this study was to investigate the acoustic basis of individual discrimination in wolves. We used playback experiments to assess the ability of wolves to discriminate between: a) familiar howls before and after manipulation of acoustic parameters previously shown to encode individual information (fundamental frequency and frequency modulation; Palacios et al., 2007), and b) familiar howls to which they had been habituated, unfamiliar howls from a familiar wolf (i.e. the sender of the howl they had been habituated to), and unfamiliar howls from unfamiliar wolves (i.e. no previous exposure to any of its howls). Our experimental design was based on the habituation-discrimination paradigm (Friedman, 1972), which is based on the premise that when subjects habituated to repeatedly presented stimuli respond more intensely to a new stimulus, they perceive it as different from the ones used for habituation (Shettleworth, 2010). This is one of the methods commonly used to evaluate the ability of animals to discriminate among different stimuli and, in particular, it has been used to examine individual recognition in various species such as leopard geckos, *Eublepharis macularius*, (LaDage & Ferkin, 2006), bullfrog, *Rana catesbeiana*, (Bee & Gerhardt, 2001), red deer, *Cervus elaphus* (Reby et al., 2001), yellow-bellied marmots, *Marmota flaviventris* (Blumstein & Daniel, 2004), giant pandas, *Ailuropoda melanoleuca* (Charlton et al., 2009), rhesus monkeys, *Macaca mulatta* (Rendall et al., 1996), and little brown bats, *Myotis lucifugus* (Kazial et al., 2008).

4.2 METHODS

Playback experiments were conducted in 2010 (from November-December) in Senda Viva Park (Navarra, Spain), a privately-owned nature preserve that houses several species of birds and mammals. We studied a pack of seven Iberian wolves (four adult males and three adult females) held in captivity in a 3320 m^2 enclosure. Three wolves (a male and female siblings and an unrelated female) had been hand reared by the park staff, while the remaining four wolves were from a different litter and had not been tamed. All the wolves were habituated to human presence.

The experimental design was based on the habituation-discrimination paradigm (Friedman, 1972): the subject is initially habituated by repeated exposure to stimulus A before a dishabituation stimulus of type B is presented.

During habituation, the response to stimulus A decreases. A restoration in the level of response to stimulus B implies an ability to discriminate between stimuli of type A and B. Finally, a re-habituation stimulus is provided by a re-exposure to a stimulus of type A. A level of response similar to that obtained before the dishabituation stimulus can again be considered as evidence of discrimination between the habituation (A) and the dishabituation (B) stimuli.

Our playback experiments comprised two phases: habituation and habituation-dishabituation (Figure 4.1). The stimulus played during the habituation phase (Familiar Stimulus - FS) was always the same. The response of the wolves determined the duration of the habituation phase. In order to maintain the habituation, during the habituation-dishabituation phase the first two stimuli broadcast in a session were always FS. After the first two FS, four additional stimuli were presented in random order. One of these stimuli was again the FS, while the remaining three stimuli were either modified FS howls, unfamiliar howls from the same FS wolf, or unfamiliar howls from an unfamiliar (i.e. no previous exposure to any of its howls) individual (Figure 4.1).

Playback stimuli

Each acoustic stimulus used for playback consisted of a series of six howls (two howls emitted by the same wolf repeated three times in a randomized order) separated by intervals of 10 s of silence. This interval between consecutive howls is within the range of howl series emitted by wild Iberian wolves (V. Palacios, unpublished data). All the stimuli were created from recordings of 128 howls emitted by six captive wolves unrelated and unknown to the Senda Viva wolves. The amplitude of all the howls was peak normalized using Adobe Audition, version 3.0. We generated nine different acoustic stimuli; three of them included natural howls and the other six were comprised of modified howls.

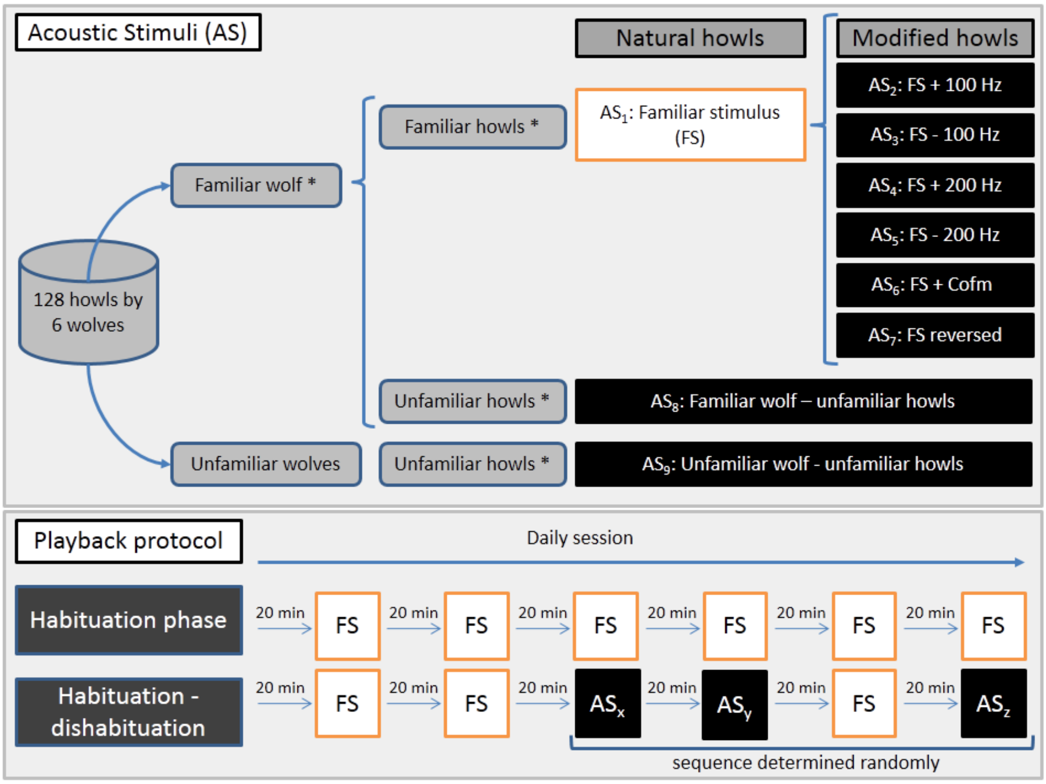

Figure 4.1.- Acoustic stimuli created (upper panel) and playback protocol conducted (lower panel). *: from our sample of recorded howls (emitted by six wolves unrelated to Senda Viva's wolves) we randomly selected a wolf (Familiar Wolf) to create the habituation stimulus (Familiar Stimulus) to habituate the group of captive wolves to its howls.

Natural howls

We created the habituation stimulus (FS) from two natural howls emitted by the same wolf (hereafter 'familiar wolf'), where both individual and howls were randomly selected from our sample of recorded howls (Figures 4.1 and 4.2). The sequence of the howls in FS was randomly selected as well. To investigate whether wolves can distinguish howls emitted by different individuals we also created two different stimulus categories with natural howls: 1) different howls emitted by the familiar wolf (for each stimulus we randomly selected two howls from the familiar wolf different from those included in FS and repeated the howls three times in a random order); and 2) howls emitted by wolves different from the familiar wolf (i.e. each new stimulus created from two howls emitted by a randomly selected wolf different from the familiar wolf; hereafter 'unfamiliar wolves') (Figure 4.1).

Modified howls

To investigate the role of fundamental frequency and frequency modulation in individual discrimination we used stimuli created modifying the howls from FS (Figures 4.1 and 4.2). We used Praat software (version 5.2.08, http://www.fon.hum.uva.nl/praat/) to modify acoustic parameters of howls included in FS. To evaluate the wolves' ability for discriminating changes in the fundamental frequency we created four stimuli, adding or subtracting 100 or 200 Hz to FS (FS+100; FS+200; FS-100; and FS-200) (Praat's procedure: Sound manipulate – To manipulation – Shift pitch frequencies). To determine the magnitude of frequency variation in the modified howls we considered the range of intraindividual variation found in captive Iberian wolves (Palacios et al., 2007). The average range of mean fundamental frequency for howls emitted by the same wolf was 153 Hz. Thus, a 100 Hz variation lies within the intraindividual variation observed, while 200 Hz exceeds the intraindividual variation observed for Iberian wolf howls. We also generated two artificial stimuli that differed from FS in the frequency modulation (Figures 4.1 and 4.2). As with the frequency, we considered the intraindividual variation observed for Iberian wolves (Palacios et al., 2007). The average range for the coefficient of frequency modulation of howls emitted by one wolf found in the same sample was 1.92. To produce stimuli with altered frequency modulation, first, we generated a stimulus increasing the coefficient of frequency modulation of howls but maintaining the same fundamental frequency as the familiar stimulus (FS+cofm). We increased the fundamental frequency (x2) and subtracted the frequency necessary to obtain the same mean fundamental frequency as FS (Praat's procedure: Sound manipulate – To manipulation – Multiply pitch frequencies - Shift pitch frequencies). This yielded stimuli with coefficients of frequency modulation 0.87 and 0.96 larger than the original howls and with the same mean value of the fundamental frequency (Figure 4.2). The second stimulus was generated reversing the original howls (FS Rev) (Praat's procedure: Sound modify - Reverse). Thus, we obtained stimuli with the same fundamental frequency and the same coefficient of frequency modulation, but differing from the original howls in the way the frequency was modulated along the howl (Figure 4.2). These modifications altered only the fundamental frequency and the coefficient of frequency modulation, but duration of howls remained the same.

Procedure

We conducted a daily session consisting of six trials interspersed by 20-minute intervals and beginning at 08:00 (Figure 4.1). Acoustic stimuli were played back using a digital sound player (Maxtronics mp5 player 4 Gb) attached to an Anchor Explorer Pro amplified speaker (frequency range = 0.08-16 kHz; output power = 60W RMS). The stimuli scheduled to be played during each playback session were recorded in a .WAV file using Adobe Audition version 3.0. Each session started with 20 min of silence followed by the six stimuli with 20 min of silence intervals. The first 20 min allowed the observer to enter the hide, minimizing the effect of the observer's presence on the wolves' behaviour. The speaker was located 30 m away from the wolves' enclosure (north side), inside a plastic container that protected it from adverse weather conditions (Figure 4.3). The container was installed three days before the beginning of the experiments to habituate the wolves to its presence.

To adjust the equalization of the speaker, FS and an audio file of white noise were played six times with six different equalization settings and recorded 5 m away from the speaker using a unidirectional Sennheiser MK66 microphone with a K6 power unit (Sennheiser Electric GmbH & Co. kG, Wedemark, Germany) attached to a portable solid state recorder Marantz PMD 660 (Marantz America, Inc., Mahwah, New Jersey). We generated spectrograms and conducted spectrogram correlations with the original stimuli using Raven Pro 1.4. The largest correlations were obtained with the bass control set in the middle of the range (set to 5) and treble control at 0, and we used this equalization during the playback experiments. Volume level was adjusted to ensure the stimuli were audible by the wolves anywhere inside the enclosure.

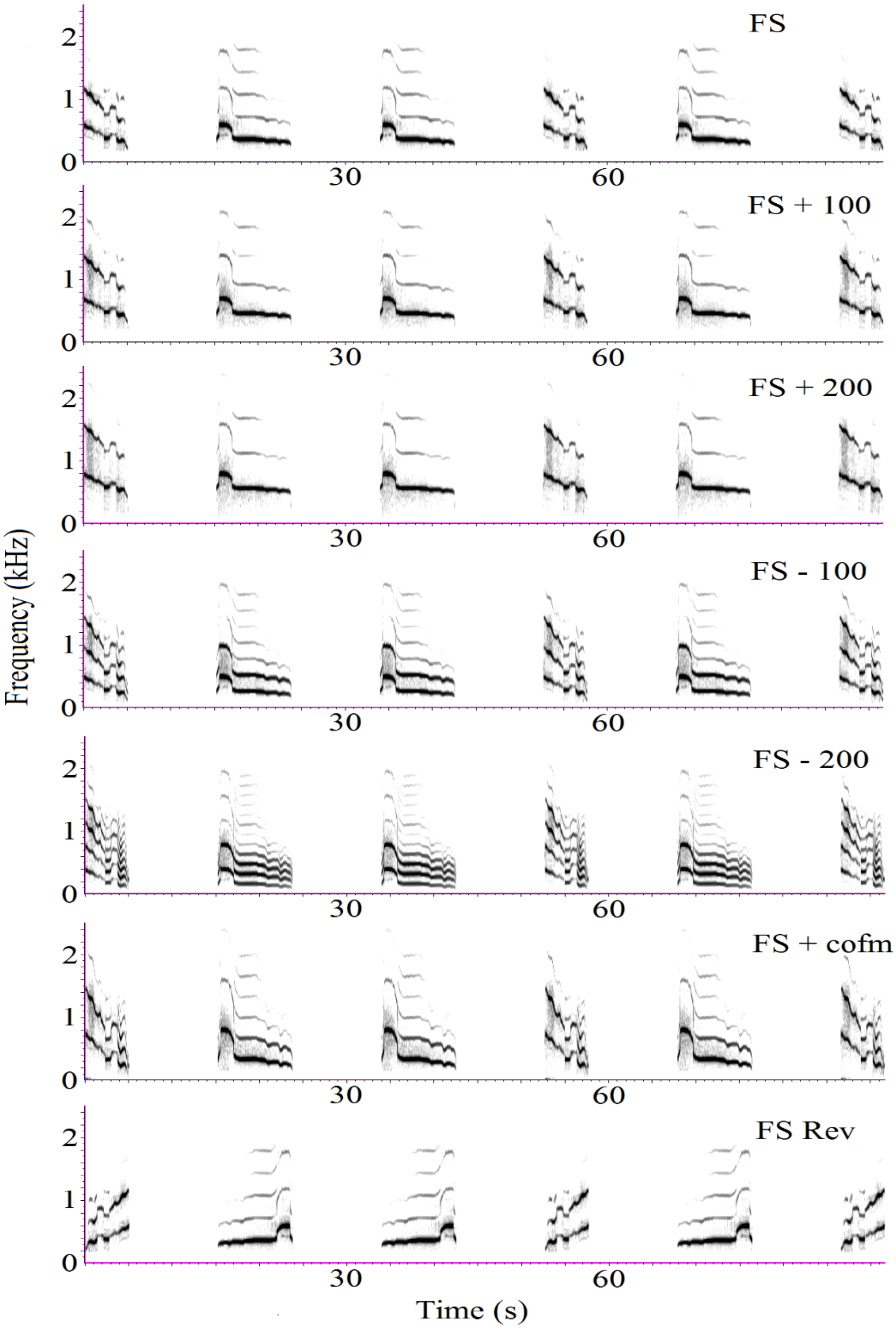

Figure 4.2.- Familiar stimulus and playback stimuli created modifying the acoustic structure of howls. FS: familiar stimulus; FS + 100, FS + 200, FS – 100, and FS - 200: familiar stimulus increasing and decreasing the fundamental frequency by 100 and 200 Hz; FS + cofm: familiar stimulus increasing the coefficient of frequency modulation; FS rev: familiar stimulus reversing the howls. Spectrogram: 2048 FFT (Fast Fourier Transform) points, Hann window.

Behavioural analysis

Behavioural observations were conducted from a hide installed in a vantage observation point that provided visual access to part of the wolves' enclosure (Figure 4.3). We divided the enclosure into three zones: zone 1 (250 m^2, the zone closest to the speaker used to broadcast the experimental stimuli); zone 2 (750 m^2, adjacent to zone 1); and zone 3 (2320 m^2, the rest of the enclosure). When the wolves were in zone 3, they could not be observed from the observation point. The visible area was divided into zones 1 and 2 to assess whether the wolves' position changed with respect to the sound source following exposure to the different acoustic stimuli. The subject in each trial was the group of wolves within sight during the tests, and we included in the analyses only trials in which at least four wolves were in zones 1 or 2. To quantify the wolves' response we scored the behaviour of all observable wolves 5 min before and 10 min after the beginning of the stimulus (ad libitum sampling), using a 1-0 recording rule (Lehner, 1996). In addition, the location of the wolves was recorded at the end of every 30 s interval. During the three days preceding the playback experiments, the observer (VP) learned to recognize the wolves individually based on morphological characteristics such as fur design and the presence of distinctive marks (e.g. ear clips, scars).

We selected 11 behaviours to be recorded based on our prior experience with wolves responding to human imitations of howls or howl recordings (Table 4.1). During the habituation phase we examined the behaviours displayed to determine which followed a habituation pattern and could be considered estimators of the wolves' response to the stimulus. The only behaviour that fulfilled this requirement was *Attention* (a wolf turns its head towards the speaker and remains with its ears raised and eyes and outer ears facing the speaker during at least one second). Therefore, following an exploratory analysis of the behaviours displayed by the wolves during the habituation phase, we defined a 'positive response' to the stimulus as at least one wolf showing the behaviour pattern *Attention* and the 'level of response' in a trial as the number of *Attention* displayed by all the wolves within sight during the 2 min following the onset of the stimulus. For each trial we considered only one value of level of response taking into account the number of *Attention* displayed by all the wolves within sight (instead of a level of response for each wolf) to avoid errors due to a wolf exhibiting *Attention* as a consequence of imitating other pack mates rather than a real interest in the acoustic stimulus.

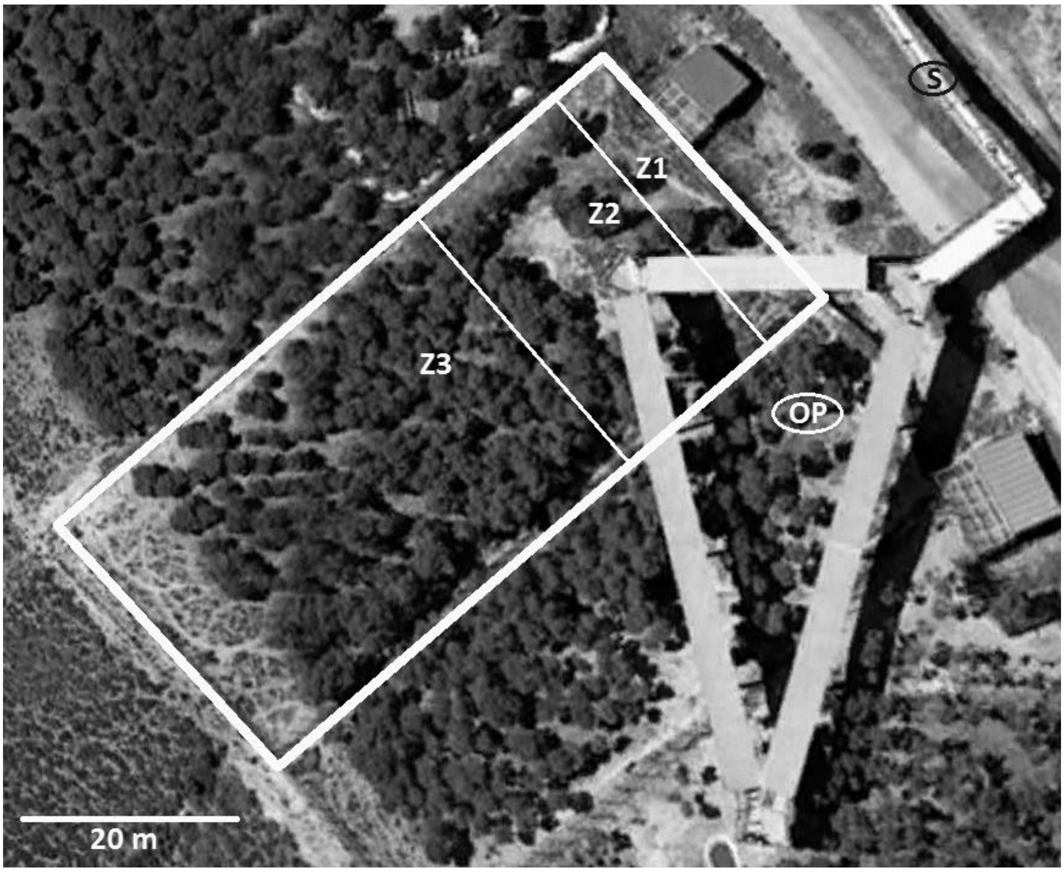

Figure 4.3.- Wolf enclosure sketch. Z1 and Z2: zones where the wolves were visible from the observation point; Z3: area where wolves could not be observed. OP: observation point. S: speaker.

Table 4.1.- Empirical descriptions of the behaviour categories recorded during the playback experiments.

Behaviour	Description
Attention	Wolf turns head towards the speaker and remains with raised ears, eyes and outer ears facing the speaker during at least one second.
Lie down	Wolf lies down without interacting with other pack members.
Approach	Wolf approaches the sound source (i.e. speaker).
Move away	Wolf moves away from the speaker. We define “move away” as to avoid or escape as reported in Goodmann et al.’s wolf ethogram (Goodmann et al., 2002).
Other directions	Wolf moves in directions other than towards or away from the speaker.
Aggressive behaviours	Threat, aggression and attack behaviours as reported in the wolf ethogram (Mech, 1970; Goodmann et al., 2002), such as beat, show teeth, chase other wolf with tail above the back level, etc.
Submissive behaviours	Defence and submissive behaviours as reported in the wolf ethogram (Mech, 1970; Goodmann et al., 2002), such as escape, inguinal offer, submission (tail between hind legs), active and passive submission, etc.
Greeting	Form of active submission involving two or more pack mates, in which subordinates nip, lick and smell the mouth of the dominants (Mech, 1970). Characterized by ears oriented backward, muzzle-muzzle contacts, tail wagging and, sometimes, whining (Goodmann et al., 2002).
Scratching	Wolf scratches ground with the paws, moving rapidly the front legs, the hind legs or both backwards, displacing vegetation, soil or other material.
Raised leg displays	Raised leg urination (RLU) and raised leg display (RLD) (Goodmann et al., 2002; Harrington & Asa, 2003).
Vocalization	Wolf emits a vocalization. We considered the four vocal types reported by Harrington & Mech (1978a): growl, whine, bark, and howl.

Statistical analysis

We conducted binomial tests to analyse the response of wolves to the stimuli. Binomial tests have been used extensively to study discrimination of acoustic stimuli in many taxa such as whooping cranes, *Grus americana* (Fitch & Kelley, 2000), cotton top tamarins, *Saguinus oedipus*, (Weiss & Hauser, 2002), and gibbons (Raemaekers & Raemaekers, 1985). Our null hypothesis was that the probability of response (at least one *Attention* recorded) to a stimulus and lack of response (no *Attention* recorded) are the same (p = 0.5). Thus, if the null hypothesis is rejected, we conclude that the wolves are habituated to the stimulus (absence of response) or, conversely, that the new stimulus leads to a dishabituation (positive response). Response dependence of differences in acoustic parameters was tested by linear regression. To test whether wolves were able to distinguish unfamiliar howls from familiar vs. unfamiliar wolves, we fitted a GLM with a Poisson error distribution and 'group size' (number of wolves within sight during the playback trial) as a covariate to specifically compare how the 'level of response' (i.e. summation of *Attention* displayed by all the wolves within sight) to these two types of unfamiliar howls varied across playback trials.

We conducted complementary analyses in order to control for the potentially important variation in individual responses. We pooled data on individual responses within each trial to calculate, for each trial, the number of times the wolves exhibited a response to playbacks (number of *Attention*). We then fitted a GLMM with number of *Attention* as the response variable (Poisson error distribution), 'group size' and 'treatment' (stimulus) as fixed factors, and trial as a random factor. Finally, we fitted a second GLMM using a binomial error structure on non-pooled data to analyse responses at the individual level by using *Attention* as a binary response variable (i.e. whether a given individual responded or not to a playback in a given trial), 'group size' and 'treatment' as fixed factors, and trial and 'individual' as a random factors. Analyses controlling for the group size effect included all the trials (group size: 1-7). Statistical analyses were made with SPSS (12.0) for Windows (SPSS Inc., Chicago, Illinois) and the R statistical package (R Development Core Team 2010).

4.3 RESULTS

We conducted 178 playback trials, 16 during the habituation phase and 162 during the habituation-dishabituation phase (eight trials were excluded due to adverse weather conditions that we judged could affect the results). Of these, 101 were considered valid trials, i.e. at least four wolves were in zones 1 or 2 (10 trials during the habituation phase and 91 trials during the habituation-discrimination phase).

At the beginning of the habituation phase, during the 2 min following the beginning of the stimulus, the wolves displayed only three behaviours apparently as a response to the stimulus: *Attention*, *Approach* and *Greeting* (see descriptions of behaviours in Table 4.1). Of these, *Attention* was the only behaviour that followed a habituation pattern (Figure 4.4). The frequency of this behaviour decreased during the habituation phase until eventually no wolf showed *Attention* as a response to the stimulus. The habituation phase concluded after three sessions, because at the end of the third session no wolf exhibited any of these three behaviours as a response to the stimulus.

Response to Modified Howls

During the habituation-dishabituation phase, the wolves showed different responses to the different stimuli (Table 4.2, Figure 4.4). There was essentially no response to FS during the last four daily trials (Table 4.2, Figure 4.4). When stimuli with modified fundamental frequency were played, the number of trials in which the wolves responded (at least one *Attention*) was no different from those in which they did not respond ($p = 0.5$) (Table 4.2). However, the first time FS+200 and F-200 were presented, the level of response was greater than for the rest of trials involving stimuli with modified frequency (the behaviour pattern *Attention* was recorded 5 and 9 times respectively). In fact, the first time FS-200 was presented the level of response was similar to that elicited by the first FS trial during the habituation phase. Thereafter, the level of response decreased, probably due to a fast habituation. When stimuli with modified frequency modulation were played, the response was different depending on the type of stimulus (Table 4.2, Figure 4.4). While the response to FS+cofm was largely absent, every time FS Rev was played the wolves showed *Attention*, with the level of response decreasing in successive experiments probably as a result of habituation.

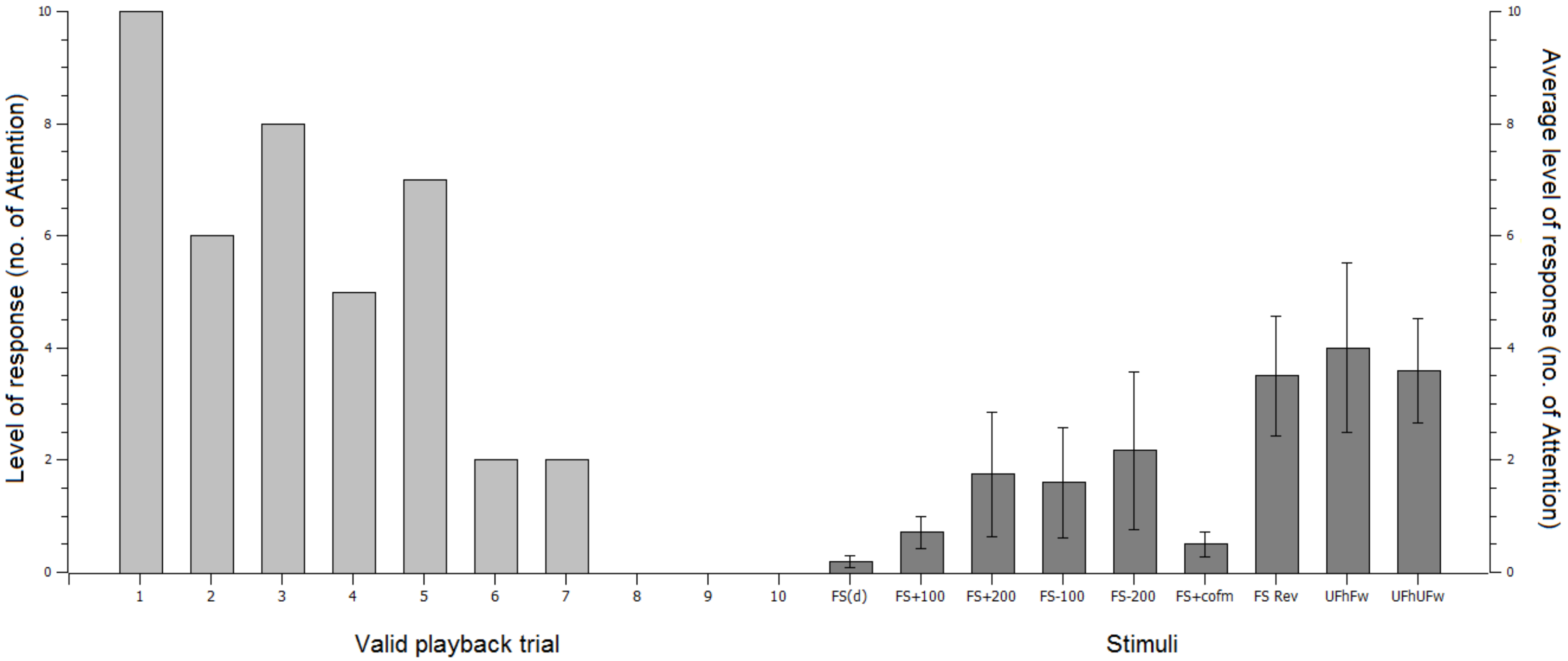

Figure 4.4.- Level of response (number of *Attention* displayed by all the wolves within sight) during the two min following the beginning of the stimulus. Left: level of response obtained to the Familiar Stimulus (FS) in the valid trials (N = 10) during the habituation phase. Right: Average level of response (mean ± SE number of *Attention*) for each stimulus taking into account all the valid trials. FS (d): Familiar Stimulus during the habituation-dishabituation phase; FS + 100, + 200, - 100, - 200: FS adding or subtracting 100 - 200 Hz; FS + cofm: FS manipulating only the coefficient of frequency modulation; FS Rev: FS with the howls reversed; UFhFw: unfamiliar howls by the familiar wolf; UFhUFw: unfamiliar howls by unfamiliar wolves.

Table 4.2.- Response of wolves to different stimuli. FS (h): Familiar Stimulus during the habituation phase; FS (d): Familiar Stimulus during the habituation-dishabituation phase; FS + 100, + 200, - 100, - 200: FS adding or subtracting 100 - 200 Hz; FS + cofm: FS manipulating only the coefficient of frequency modulation; FS Rev: FS with the howls reversed; Positive Response: number of valid trials where the stimulus elicited a positive response (at least one wolf displayed *Attention*); Average level of response: average number of *Attention* displayed by all the wolves within sight taking into account the valid trials; UF: Unfamiliar, F: Familiar.

Stimulus	No. of valid trials	Positive Response	Binomial test p	Average level of response (mean±SD)
FS (h)	7[1]	7	0.008*	5.71±2.98
FS (d)	16[2]	3	0.009*	0.19±0.40
FS + 100	7	4	0.3	0.71±0.76
FS + 200	4	3	0.3	1.75±2.22
FS – 100	5	2	0.3	1.60±2.19
FS – 200	6	4	0.2	2.17±3.43
FS + cofm	6	3	0.3	0.50±0.55
FS Rev	6	6	0.02*	3.50±2.59
UF howls by F wolf	6	6	0.02*	4.00±3.69
UF howls by UF wolves	5	5	0.03*	3.60±2.07

[1]: habituation phase, only valid trials with positive response considered (total no. of valid trials =10).

[2]: only FS included in the last four daily trials because first and second daily stimuli (N = 30), were played to reinforce habituation.

*: $p < 0.05$

Individual Discrimination

The wolves discriminated between FS and unfamiliar howls from both the same familiar individual or from unfamiliar wolves (Table 4.2, Figure 4.4). We found a strong treatment*playback trial interaction (likelihood Chi test between model with and without the interaction term: differential residual deviance = -7.522, p = 0.0061). This shows that the response to unfamiliar howls from the familiar wolf, but not to unfamiliar howls from unfamiliar wolves, decreased across playback trials following a habituation pattern (Figure 4.5). To investigate which acoustic parameter elicited a greater response, we calculated the mean fundamental frequency and the coefficient of frequency modulation of the howls included in the different stimuli. The response to howls from unfamiliar wolves was most intense when the difference between the coefficient of frequency modulation of the first howl of the stimulus and the coefficient of frequency modulation of the first howl of the familiar stimulus was greatest (linear regression: R^2= 0.84, $F_{1,3}$ = 15.71, N = 5, p = 0.03). Regressions of the level of response and differences in the fundamental frequency of howls and the coefficient of frequency modulation for the familiar wolf howls had slopes not significantly different from zero.

Group size effect

GLMMs controlling for the effect of the different number of wolves within sight across trials (group size effect) confirmed the results above. As expected, we found a significant effect of group size (i.e. number of wolves in sight) on the level of response (number of times *Attention* was registered responding to a playback), the level of response increased with the number of wolves within sight (χ^2 = 8.01, p = 0.005). We also found a highly significant treatment effect (χ^2 = 70.28, p < 0.001) on the level of response. *Post hoc* Tukey comparisons showed significant differences in the level of response between the following treatment levels: a lower response to FS playbacks than to unfamiliar wolf howls (Estimate = -2.74, z

value = -4.65, $p < 0.001$), unfamiliar howls from the familiar wolf (Estimate = -2.80, z value = -4.77, $p < 0.001$), FS-200 playbacks (Estimate = -2.25, z value = -3.74, $p < 0.01$) and FS rev playbacks (Estimate = -2.71, z value = -4.61, $p < 0.001$). Tukey comparisons did not detect any significant differences between the latter four treatments.

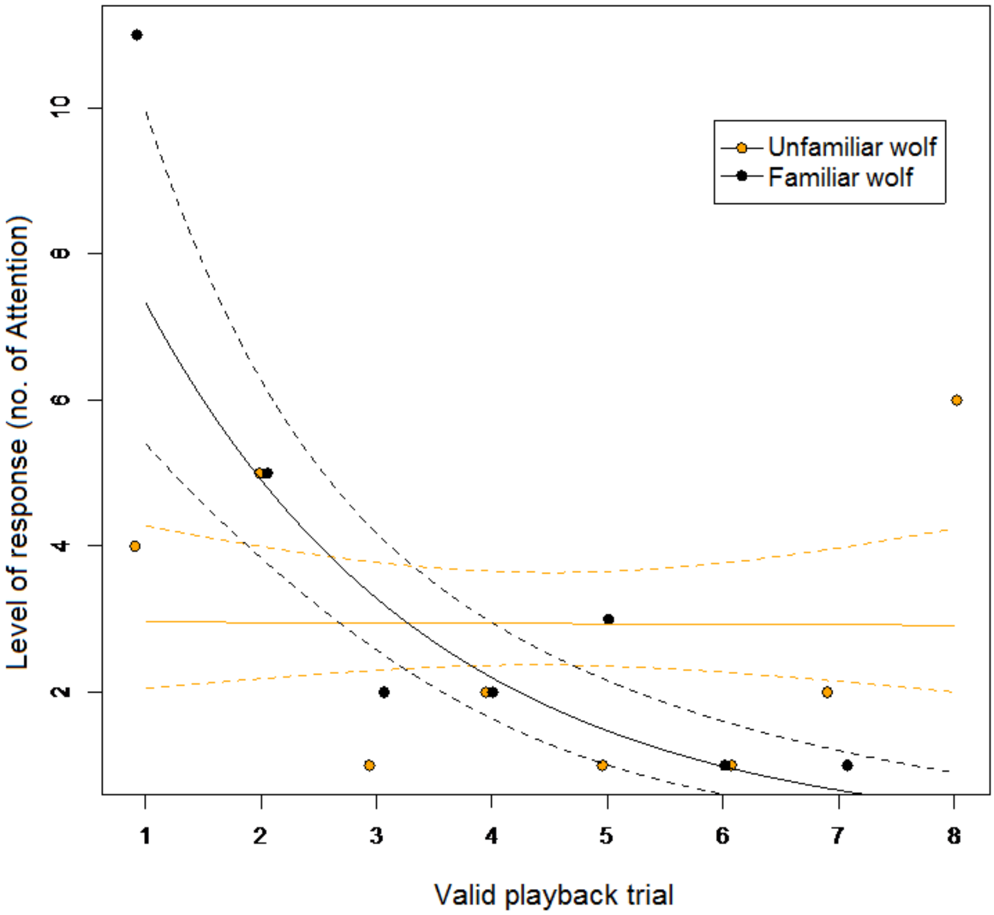

Figure 4.5.- Level of response (i.e. number of *Attention*; see methods) of wolves to unfamiliar howls emitted by a familiar wolf or by unfamiliar wolves. Solid lines reflect predicted values for each treatment, while dashed lines show the standard error interval (GLM model: number of attention~treatment*playback trial+group size; Poisson error distribution). Note that random jitter has been introduced in the x axis to distinguish data points from both treatments.

Our second GLMM examining the probability that a given wolf responded or not to a playback yielded equivalent results. As expected, in this case we did not find a significant group size effect on the probability that a given wolf responded to a playback (χ^2 = 1.48, *p* = 0.223), but we found a highly significant treatment effect (χ^2 = 58.48, *p* < 0.001) that was again driven mainly by a significantly lower probability of responding to a FS playback than to unfamiliar wolf howls (Estimate = -3.27, *z* value = -4.98, *p* < 0.001), unfamiliar howls from the familiar wolf (Estimate = -3.10, *z* value = -4.81, *p* < 0.001) or FS rev playbacks (Estimate = -2.77, *z* value = -4.25, *p* < 0.001). Again, Tukey comparisons did not detect any significant differences between the latter three treatments. In this case, no differences between FS and FS-200 were found (Estimate = -1.99, *z* value = -2.96, *p* = 0.07).

4.4 DISCUSSION

Our results show that wolves detected changes in the fundamental frequency of howls outside their natural range of variability, and changes in the frequency modulation pattern of howls. In contrast, manipulations of fundamental frequency and coefficient of frequency modulation within the natural range of intra-individual variation did not elicit a response. Furthermore, wolves detected differences between different howls, even between unfamiliar and familiar howls emitted by the same individual that they were previously familiarized with. Finally, wolves showed habituation to all new stimuli when presented repeatedly, with the exception of responses to unfamiliar calls of unfamiliar wolves.

Playback experiments are a useful tool to investigate whether animals can distinguish the identity of conspecifics by means of vocalizations, as has been reported in the African elephant, *Loxodonta Africana* (McComb et al., 2000), banded wren, *Thryothorus pleurostictus* (Molles & Vehrencamp, 2001), barking foxes, *Alopex lagopus* (Frommolt et al., 2003), and black-capped chickadees, *Poecile atricapillus* (Wilson & Mennill, 2010). Using this methodology, it has been shown that domestic dogs distinguish between barks by the same individual emitted in two different contexts and between different individuals which barked in the same context (Molnár et al., 2009). In wolves, playback experiments have been used to investigate the ability of pups and juveniles to discriminate between different sounds (Shalter et al., 1977), to determine whether adult wolves distinguish between pup and adult howls (Harrington, 1986), and to study variation in the responsiveness of wild wolves to unfamiliar individuals (Gazzola et al., 2002).

We are aware of the methodological problems inherent to playback experiments, including pseudoreplication and reduced external validity (Searcy, 1989; McGregor, 2000; Kroodsma et al., 2001). Wolf social organization and spatial ecology make it difficult to conduct playback studies in the wild, and there are few captive packs of wolves with appropriate installations and adequate handling and management conditions. Frommolt et al. (2003) carried out playback experiments with eight barking foxes to investigate individual recognition, McComb et al. (2000) conducted playback on 29 independent groups of elephants, and Molnár et al. (2009) conducted his study on 30 dogs. In contrast, the effective sample size in our study is much lower and, to complicate things even further, the levels of response recorded in different trials are not statistically independent. However, we believe the results presented here are suggestive and could be considered a first step in the demonstration of individual discrimination based on howl acoustic structure in wolves.

Our results also show that wolves discriminate manipulations in the acoustic structure of howls. Experimental signal alteration has been previously applied to assess the cues used for recognition (Tibbetts & Dale, 2007). Changes in the fundamental frequency within the observed range of intraindividual variation induced low levels of response, while the response to changes of 200 Hz (exceeding the observed intraindividual variation) was suggestive of dishabituation the first time the stimulus was played. Although this ability for discriminating differences in frequency exceeding natural signal variation has been reported for other species such as bullfrogs, *Rana catesbeiana*, (Bee & Gerhardt, 2001), this is the first time it has been tested with wolves. The fundamental frequency is one of the acoustic parameters that best discriminates individuals in some species such as giant pandas (Charlton et al., 2009), manatees (Sousa-Lima et al., 2002), and wolves (Tooze et al., 1990; Palacios et al., 2007). However, in some species it seems that this variable is less important for individual recognition, possibly because it changes according to the motivational state of the sender (Charlton et al., 2009). In the case of wolves, there is substantial intraindividual variation in the fundamental frequency of howls and it has also been reported that the motivational state of the sender can influence the fundamental frequency of howls (Harrington, 1987). This could explain that changes in the fundamental frequency within the intraindividual range elicited low levels of response.

Increases in the coefficient of frequency modulation within the observed intraindividual range of variation for adult wolf howls did not elicit a response. However, the reversed stimulus induced dishabituation to FS. Frequency modulation does not change during sound propagation, and therefore seems particularly well suited for individual recognition over long distances, or in noisy environments (Slabbekoorn et al., 1998; Charrier et al., 2003). In fact, it has been reported that frequency modulation is important for individual recognition in some species. For example, frequency modulation is crucial for parent-offspring recognition in seals (Charrier et al., 2003), and for individual recognition in macaroni penguins (Searby et al., 2004). In our study, wolves discriminated changes in modulation pattern, suggesting that the frequency modulation pattern may play an important role in wolf acoustic communication and probably in individual recognition.

The results of the playback experiments reported here provide the first evidence that wolves can discriminate among howls according to their acoustic structure. Furthermore, the response to unfamiliar howls emitted by the familiar wolf decreased across trials following a habituation pattern. However, repeated exposure to equally unfamiliar howls emitted by unfamiliar wolves did not result in habituation, and was more intense for wolves emitting howls that differed in the coefficient of frequency modulation with respect to the familiar howls. That wolves discriminate between a familiar wolf and unfamiliar individuals does not necessarily imply that true individual recognition actually exists. Although there is a strong basis for predicting that many species may be able to discriminate between individuals, to determine empirically whether animals are able to identify individuals and which cues they use to do so is difficult (Thom & Hurst, 2004). Wolves produce individually distinct howls (Tooze et al., 1990; Palacios et al., 2007), and the habituation pattern occurred only when unfamiliar howls by the familiar wolf were broadcast, suggesting that wolves treated the familiar wolf uniquely. However, individual recognition is a more complex cognitive capacity than just discrimination, requiring the matching of a specific stimuli with an internally-held template or “representation” of that individual (Tibbetts et al., 2008). Additional experiments may provide crucial insights to understand the role of acoustic communication in wolf’s recognition.

4.5 ACKNOWLEDGMENTS

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PART II. APPLYING BIOACOUSTICS TO MONITOR WOLF POPULATIONS

Photo by Doug Smith, obtained in https://commons.wikimedia.org/wiki/File:Yellowstone_Wolves.jpg?uselang=es#globalusage

CHAPTER 5. PART II. GENERAL INTRODUCTION

Population monitoring plays a critical role in animal ecology and wildlife conservation (Sinclair et al. 2009). Assessing changes in local populations is the key to understanding the temporal dynamics of animal populations, evaluating management practices for harvested or endangered species, documenting compliance with regulatory requirements, and detecting incipient change. Knowledge of the size or density of a population is often a vital prerequisite to managing it effectively (Sinclair et al. 2009). This is especially evident for large carnivores such as wolves (*Canis lupus*), for which management decisions need to reconcile conservation concerns with development in rural communities. Researchers need reliable methods that provide accurate data on the distribution, abundance, and population trends of a species in order to advise wildlife managers. One of these methods is based on the information provided by acoustic signals emitted by animals (Hopp & Morton 1998). The use of bioacoustics in wildlife management has grown extraordinarily during the last decades providing efficient tools for monitoring a great variety of taxa (Table 5.1).

Wolves communicate over long distances by howling, both solo and in groups. The latter are known as chorus howls. Chorus howls are group vocalizations involving several pack mates howling simultaneously, and often include other type of vocalizations, such as barks, whines and howl variations (Holt 1998; Harrington & Asa 2003). Wolves howl in response to unfamiliar howls and even to human imitations of howls, which is the basis of a widely used method for detecting animals by their vocalizations: the elicited-vocalization technique, which in the case of wolves has been named “simulated howling” (Harrington & Mech 1982, Figure 5.1). When pack members travel together or stay near the breeding areas they usually emit chorus howls as a response to simulated howls, making this a suitable technique for locating packs (Harrington & Mech 1982). In the last decades, the elicited-vocalization technique has been widely used as a standardized method for monitoring wild wolf populations, since direct observation of wolves and wolf litters, especially in forested areas, is difficult and inappropriate for surveying wide areas.

Table 5.1.- Some published studies that used bioacoustics to monitor wildlife and ecological variables measured from acoustic signals.

Taxa	Species	Variable	Reference
All	All	biodiversity indexes	Depraetere et al. 2012
Amphibia			
Anura	All	presence	Kaiser 2008
Aves and Amphibia	All	presence	Acevedo & Villanueva-rivera 2006
Amphibia			
Anura	Iberian midwife toads (*Alytes obstetricans, A. cisternasii, A. dickhilleni*), and Iberian tree frogs (*Hyla molleri, H. meridionalis*)	presence/calling activity	Llusia et al. 2013
Anura	All	abundance	Da Silva et al. 2011
Amphibia			
Anura	Three-stripped pond frog (*Rana nigromaculata*)	abundance	Sung et al. 2005
Aves			
Charafriiformes	Woodcock (*Scolopax rusticola*)	abundance	Hoodless et al. 2008
Gruiformes	Corncrake (*Crex crex*)	abundance	Peake & Mcgregor 2001
Strigiformes	African wood owl (*Strix woodferdii*)	abundance	Delport et al. 2002
	Eastern screech-owl (*Megascops asio*)	abundance	Nagy & Rockwell 2012
Mammalia			
Carnivora	Lion (*Panthera leo*) and spotted hyaena (*Crocuta crocuta*)	abundance	Ogutu & Dublin 1998
	Coyote (*Canis latrans*) and wolf (*Canis lupus*)	abundance	Gaines et al. 1995
	Wolf (*Canis lupus*)	reproduction	Mitchell et al. 2008; Llaneza et al. 2014
	African wild dog (*Lycaon pictus*)	abundance	Robbins & McCreery 2003
Proboscidea	Savanna elephant (*Loxodonta africana*)	abundance	Payne et al. 2003
Primates	Golden lion tamarin (*Leontopithecus rosalia*)	abundance	Kierulff & Rylands 2003

Figure 5.1.- Simulated howling at dusk to elicit the wolves response.

Harrington & Mech (1982) proposed two censusing approaches based on the elicited-vocalization technique: the saturation census (an attempt to locate all packs within a limited area), and the sampling census (an estimate of the number of packs surviving in a large area obtained by howling at randomly selected locations). The elicited-vocalization technique has been applied, for instance, to survey a 223 km^2 study area in the Foreste Casentinesi National Park, Italy (Apollonio et al. 2004) and to monitor the wolf population in a 745 km^2 area of the Polish Carpatian Mountains (Nowak et al. 2008). One variation consists of searching for wolf signs (e.g. faeces, scratching, etc.) and emitting simulated howls only in those areas where signs are found at a high concentration. This methodology has been used, for instance, in a 6800 km^2 area of northern Spain (Llaneza et al. 2005b) and in a 1000 km^2 area of northern Portugal (Eggermann et al. 2011).

In addition to detecting the presence of wolf packs, the elicited-vocalization technique has also been used to estimate the number of wolves howling and to determine the presence of pups in a pack (Gazzola et al. 2002; Apollonio et al. 2004; Llaneza et al. 2005b; Nowak et al. 2008; Mitchell et al. 2008). These estimates are usually made by two different methods: 1) acoustic censusing by listening to chorus howls in the field, or 2) by spectrographic analyses. However, the available studies provide no detailed descriptions of the methodologies used (Gazzola et al. 2002; Llaneza et al. 2005b; Nowak et al. 2007) and no attempts to evaluate the reliability of these methods have been made. Due to the complex acoustic structure of choruses, their use for determining the number of wolves and the presence of pups entails a certain level of uncertainty and subjectivity. In particular, use of chorus howls is hindered by the following technical limitations: 1) unknown accuracy of estimates of the number of wolves howling based on acoustic censusing, 2) effect of listener's experience not tested, and 3) lack of detailed descriptions of vocalizations emitted by wolves in choruses.

Here we explore the reliability of chorus howling for obtaining information that could be used for monitoring wild populations. The aims of this study are:

1.- To evaluate the accuracy of estimates of the number of wolves howling in a chorus and the presence of pups obtained by the elicited-vocalization technique. This aim is addressed in chapter 6.

2.- To assess whether there are acoustic features that could provide information regarding group size and to evaluate the suitability of spectrogram analyses for estimating the number of wolves participating in chorus howls (chapter 7).

3.- To assess whether there are acoustic features that could provide information regarding the presence of pups in a chorus howl and to evaluate the suitability of spectrogram analyses to detect the presence of pups. This is the objective of chapter 8.

4.- To compare the accuracy obtained by human listeners with the results obtained from spectrogram analyses to estimate the number of wolves howling and the presence of pups, and to issue recommendations to improve the use of acoustic censusing in wolf surveys (chapters 7 and 8).

CHAPTER 6. HUMAN ESTIMATES OF THE NUMBER OF WOLVES VOCALIZING IN A CHORUS AND THE PRESENCE OF PUPS BY ACOUSTIC CENSUSING

6.1 INTRODUCTION

Human hearing has been widely used to study animal acoustic communication. Naguib & Wiley (2001), for instance, studied human assessment of distance from a sound source to provide a framework for understanding the evolutionary implications of sound degradation in long-distance communication. Human perception has been used to characterize vocal repertoires in species such as yellow mongoose, *Cynitis penicillata* (Le Roux et al. 2009), African wild dogs, *Lycaon pictus* (Robbins 2000), or dingoes, *Canis lupus dingo* (Déaux & Clarke 2013). Caller identity in vocalizations emitted by rhesus monkeys (*Macaca mulatta*) and context differences in domestic cat (*Felis catus*) vocalizations have been studied using the ability of human listeners to discriminate sounds (Nicastro & Owren 2003; Owren & Rendall 2003). The human ability to recognize animal sounds has also been used for monitoring populations or biodiversity. Listening to vocalizations is widely used for species identification in taxa such as birds or amphibians (Gregory et al. 2004).

In the case of wolves, since packs respond to simulated howls emitting chorus howls, the elicited-vocalization technique has been widely used to estimate the number of individuals in wolf packs and to confirm reproduction during the breeding period (Harrington & Mech 1978b, 1982; Dekker 1985; Gaines et al. 1995; Gazzola et al. 2002; Apollonio et al. 2004; Llaneza et al. 2005b; Nowak et al. 2007; Kaczensky et al. 2013). These estimates are usually made by acoustic censusing: researchers listen to chorus howls in the field and estimate how many wolves are howling and whether there are pups vocalizing, without conducting spectrogram analyses (Gazzola et al. 2002; Apollonio et al. 2004; Llaneza et al. 2005b, 2014; Nowak et al. 2008; Mitchell et al. 2008). However, limitations of this method could affect the interpretation of the information obtained from chorus howls. The acoustic structure of chorus howls is extremely complex, as they include, in addition to howls, other vocalizations such as growls, barks, squeaks, and howl variations such as ”woa-woa howls” (Holt 1998, Figure 6.1). Therefore, discriminating *in situ* and in real time, without the benefit of repeated listening to a recording, the number of participants or the presence of pups in a chorus is a difficult task. In fact, Harrington (1989) reported that two or three adult wolves could emit highly modulated choruses that may give the erroneous impression that there are many wolves and even pups vocalizing. In bird species that emit chorus vocalizations, such as laughing kookaburras, *Dacelo novaeguineae*, determining by ear the number of individuals participating in a chorus is considered an impossible task (Baker 2004).

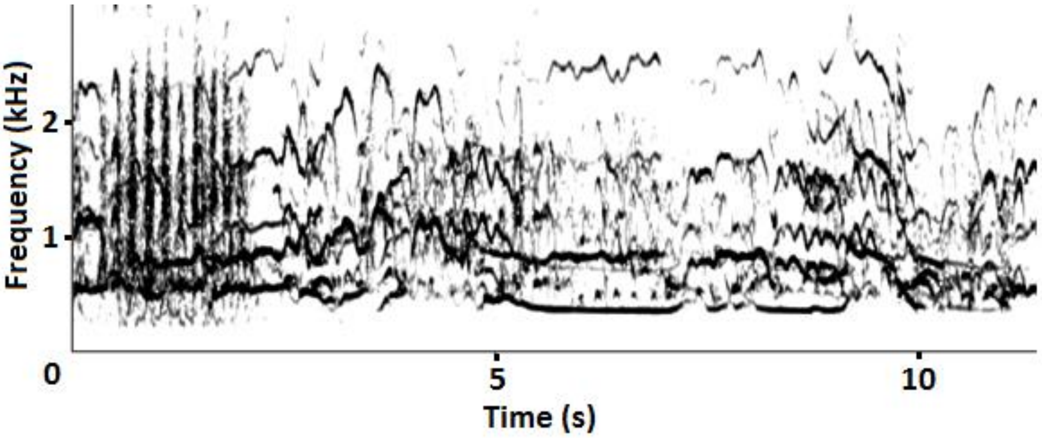

Figure 6.1.- Fragment of chorus howls including several types of vocalizations emitted by four individuals. Spectrogram: 2048 FFT (Fast Fourier Transform) points, Hann window.

Hallberg (2007), as part of a study to assess whether acoustic cues for group size are present in the chorus yip-howl of coyotes, played choruses to human subjects and asked them to estimate the number of animals participating.

The human subjects had no relationship to coyote management or monitoring, and the study concluded that human estimates regarding coyote group size were frequently incorrect. However, Hallberg (2007) considered assessments of coyote group size were fairly accurate because mean differences between actual group size and human estimates were within one or two individuals. To date, no attempt has been made to study the accuracy and reliability of estimates of the number of individuals participating or the presence of pups in wolf chorus howls based on acoustic censusing.

In this study, we assess the accuracy of estimates made by human listeners of the number of wolves participating in chorus howls and the presence of pups. In particular, we compare the accuracy of estimates made by forest rangers, responsible for conducting wolf surveys, and by a pool of subjects with no experience with wolves. Furthermore, we analyse the possible implications for wolf monitoring.

6.2 METHODS

During 2013 and 2014 we ran “chorus tests” on 205 human subjects. The study population included forest rangers from Sweden and Spain and a small group of subjects with no previous experience with wolves or wolf vocalizations (Table 6.1). A test consisted of listening to five complete chorus howls, selected randomly from a sample of 22 recordings of chorus howls (chorus duration: 61 ± 31 s, mean ± SD) emitted by 14 packs of known composition (Table 6.2). The human subjects’ pool included men and women (mostly men), between 30 and 60 years of age, and without hearing problems. In Spain, tests to rangers working in the same location were conducted on the same day in a meeting room for logistic reasons. We provided each ranger with a test sheet that included five numbers (randomly selected) corresponding to the codes of the choruses to be answered. We played the 22 choruses in a random order with an interval of 2-3 minutes of silence between consecutive choruses. During the silence intervals rangers had to answer the questions in their sheet only when the choruses played corresponded to their assigned numbers. All tests were conducted under supervision of the researcher. In Sweden, each human subject conducted the test isolated from other people, using a computer. The choruses were played using a computer and computer speakers (frequency range 20 – 20000 Hz) at a volume ensuring that the chorus was audible in the entire room.

Table 6.1.- Human subjects (rangers and not experienced people) that conducted chorus tests (each test consisted of listening to five chorus howls and estimating the presence of adults, pups and number of adults and pups participating in the chorus).

Subjects		N
Rangers	Sweden	58
	Spain	124
Total rangers		182
Subjects with no prior experience with wolves		23
Total		205

We defined two age classes for wolves participating in a chorus: pups and adults/subadults. We considered pups as wolves from birth to 6/7 months, since it has been reported that at this age wolf pups already sound fully like adult wolves (Harrington & Asa 2003). Our sample of chorus howls included 10 choruses emitted only by adults/subadults, and 12 choruses including pups. The presence of pups in a chorus was visually confirmed in four cases. In the eight remaining choruses we could not watch the pups vocalizing because they were wild wolves and the elicited-vocalization technique was carried out at night. However, we considered that there were pups participating in the chorus howl if the litter had been detected and watched on several occasions, and the chorus elicited included vocalizations corresponding to pup vocalizations as described by Harrington (1989) and in our own pup recordings.

Table 6.2.- Wolves participating in choruses used for the tests. For each chorus we considered two age classes: adults/subadults and pups. ≥ : minimum number confirmed; ≤ : maximum possible number.

Chorus ID	Pack	Location	Composition (No. adults/subadults + No. pups)
1, 2, 6, 8	A	Madrid (captivity)	4 + 0
3, 9	B	Lisboa (captivity)	3 + 0 2 + 0
4, 7	C	Lisboa (captivity)	5 + 0 4 + 0
5	D	Madrid (captivity)	6 + 0
10, 21	E	Zamora (wild)	≥7 + 0 ≥2 + ≥1
11	F	Madrid (captivity	5 + 2
12	G	Lugo (wild)	0 + 3
13	H	A Coruña (wild)	≥1 + ≤3
14	I	Asturias (wild)	≥2 + 4
15	J	Zamora (wild)	≥2 + ≤5
16	K	Asturias (wild)	≥1 + ≤5
17, 18, 19	L	Zamora (wild)	≥2 + 7
20	M	Zamora (wild)	≥1 + ≥1
22	N	Asturias (wild)	0 + ≥3

For each chorus the human subject had to estimate the presence and number of both adult/subadults and pups. We assigned a score (0-5) to each test, depending on the subject's answers (Table 6.3). Rangers had to indicate their experience with wolves (their job included tasks relating to wolf management and conservation such as monitoring or assessment of damage to livestock). Prior experience with wolves was coded as follows: 0 = no experience; 1 = 1-5 years of experience; 2 = 6-10 years of experience; 3 = more than 10 years of experience.

Table 6.3.- Questions that human subjects had to answer for each chorus in the test and score assigned for each question.

Question	Value	Score
Presence of adults	Yes/No	0 : False 1 : True
No. of adults	X	0 : Wrong 1 : Correct
Presence of pups	Yes/No	0 : False 1 : True
No. of pups	Y	0 : Wrong 1 : Correct
No. of individuals	X+Y	0 : Wrong 1 : Correct
Total Score		0-5

We used Generalized Linear Mixed Models (GLMM) to assess which factors affect the accuracy of the estimates made by the human subjects. GLMM analyses are an extension of GLM which allow the incorporation of ”subjects” as a random effect to control for replicated observations (Pinheiro & Bates 2000). We built models for the estimate of the presence (presence/absence of both age classes) and the number of wolves participating (correct/wrong) as dependent variables (binomial error distribution and logit link), including experience of the human subject, group (unexperienced/rangers) and age class (pups/adults) as factors, and chorus and listener as random effects. Analyses for group size included only choruses with exact known composition (Table 6.2). We conducted two analyses for estimates of the presence of pups: including all the choruses (presence of pups = visually-confirmed + not visually-confirmed), and only choruses for which pups were watched howling (visually-confirmed pups), to ensure that the inclusion of choruses where the presence of pups was not visually-confirmed did not affect the results. The significance of terms was determined by a Wald statistic ($P < 0.05$). We fitted subsequent GLMMs removing non-significant variables in a stepwise fashion to find the most parsimonious model that accounted for meaningful variation and we estimated the variance explained by the best model calculating marginal R^2 (variance explained by fixed factors) and conditional R^2 (variance explained by both fixed and random factors, Nakagawa & Schielzeth 2013). GLMMs were conducted in R (R Development Core Team 2010), using the glmer function (in the lme4 library). Differences between rangers and unexperienced subjects were tested with Welch's two-sample t-test with unequal variances. To test for differences among experience classes we used Kruskal-Wallis test.

6.3 RESULTS

We obtained scores for 1023 choruses (answers for two choruses were considered invalid), 910 by rangers and 113 by unexperienced subjects. The global score obtained in a chorus (values from 0 to 5) by the rangers (2.19 ± 1.24, mean ± SD) was not different from the score obtained by the unexperienced group (2.33 ± 1.42, mean ± SD; Welch's test; $t = 1.014$; $p = 0.31$). There were no differences in score depending on the experience of the ranger (Kruskal-Wallis test, $\chi^2 = 0.578$, df = 3; $p = 0.9$, Figure 6.2). We obtained the same results considering only choruses with visually-confirmed pups: scores obtained by rangers were not different from those of the unexperienced group (Welch's test; $t = 0.778$; $p = 0.44$), and there were no differences depending on the experience of the ranger (Kruskal-Wallis test, $\chi^2 = 2.984$, df = 3; $p = 0.394$).

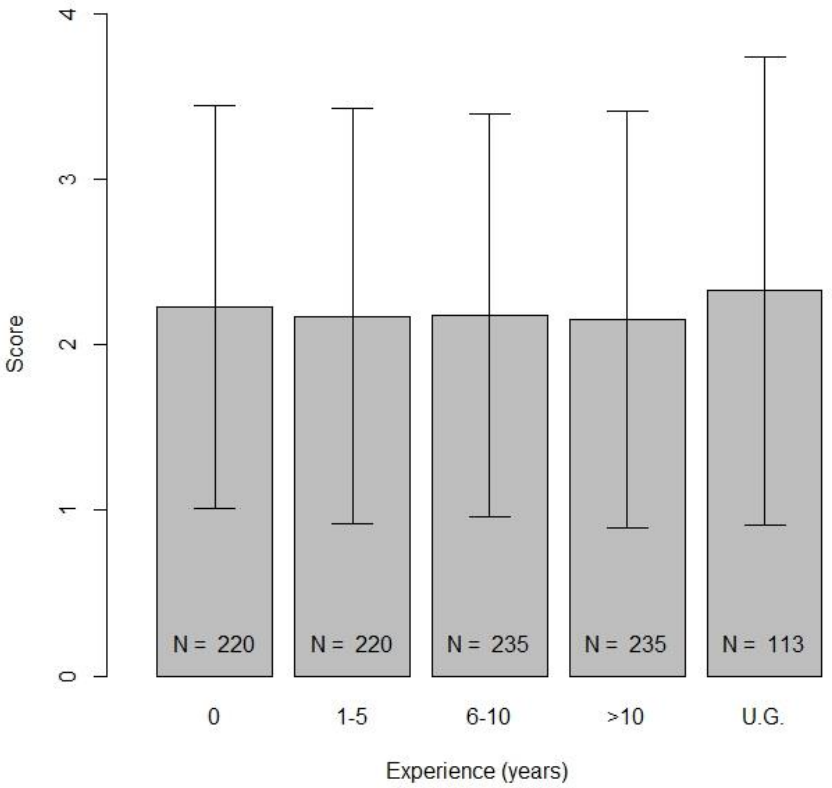

Figure 6.2.- Score obtained per chorus (mean ± SD) by rangers with different levels of experience and by the unexperienced group (U.G.).

We built a model to explore which factors affected the correct determination of the presence of individuals of different age classes (adults/subadults and pups). The experience of the observer, whether ranger or not, had no effect on the correct determination of the presence of pups or adults (Table 6.4). We found a strong interaction between wolf age class and the presence of pups or adults (χ^2: 8.59; df: 1; p = 0.003). The most parsimonious model included an age class by presence interaction and explained 73% of the variance, 21% of this variance explained by chorus and human subject random factors (considering all the choruses: marginal R^2 = 0.52 ; conditional R^2 = 0.73; only with visually-confirmed pups: marginal R^2 = 0.61 ; conditional R^2 = 0.80).

Table 6.4.- GLMM obtained including all the variables for the estimate of the presence of pups and adults in a chorus. *: significant factors.

Fixed effects	Estimate	Std. Error	Z value	Pr(>|z|)
Intercept	-0.467	0.872	-0.536	0.592
Agepups	-1.424	0.892	-1.596	0.111
Presence1	3.296	0.863	3.819	<0.001*
Unexperienced1	-0.082	0.294	-0.278	0.781
Expclass1	-0.163	0.241	-0.677	0.498
Expclass2	-0.152	0.232	-0.658	0.511
Expclass3	-0.230	0.237	-0.970	0.332
Agepups:presence1	3.525	1.005	3.507	<0.001*

Assessment of the presence of adults by rangers was correct 85% of the times (also 85% considering only choruses with visually-confirmed pups), while the proportion of correct answers decreased to 64% (48% considering only choruses with visually-confirmed pups) for estimates of the presence of pups. This difference between correct estimates of the presence of adults and pups is due to an overestimate of the presence of pups. The rangers identified adults howling 88% of times when there were effectively adults vocalizing, and pups in 98% of the cases (90% and 100% respectively for choruses with visually-confirmed pups). However, the rangers wrongly determined that there were adults 49% of times, while this percentage increased to 72% confirming the presence of pups when in fact there were no pups vocalizing (Figure 6.3). Considering only choruses with visually-confirmed pups, both presence of adults and pups were overestimated (rangers estimated the presence of adults 84% of times when there were no adults and they stated wrongly that there were pups 72% of times with no pups vocalizing).

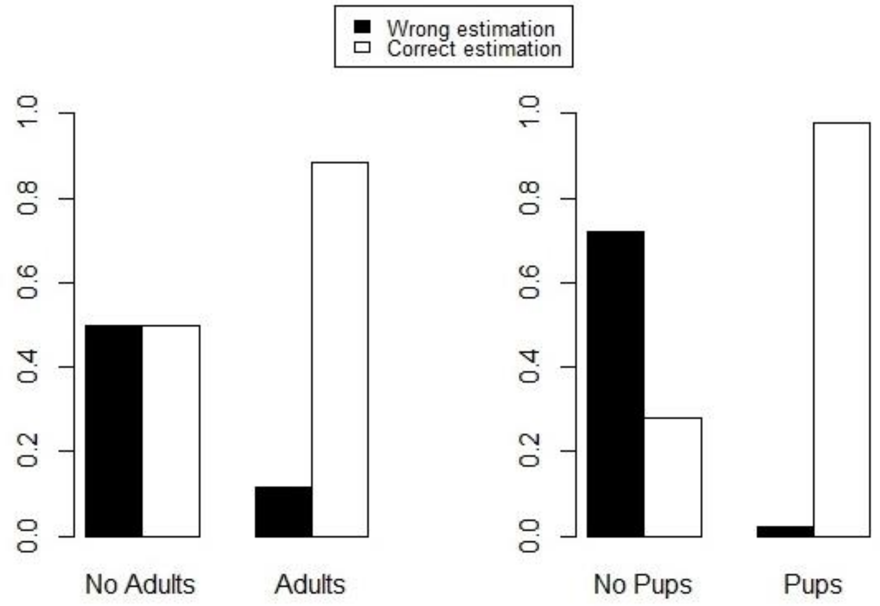

Figure 6.3.- Proportion of correct and wrong estimates of the presence of adults (left) and pups (right) vocalizing in a chorus. Adults: there are adults vocalizing in the chorus; No Adults: there are no adults; Pups: the chorus include pups; No pups: the chorus does not include pups.

With respect to the number of wolves howling, the rangers correctly estimated the number of pups 17% of times, and estimates of the number of adults were correct 13% of times. The overall number of wolves (independently of the age class) estimated by the rangers was correct 31% of times, 38% of times the observer underestimated the number of wolves howling and 30% of estimates were overestimates of the actual number of wolves vocalizing. We built a model to explore which factors affect the correct estimate of the number of wolves howling. Experience and membership in the rangers/unexperienced group did not influence the results (Table 6.5). Only the actual number of wolves affected the correct estimate: as the number of wolves participating in a chorus howl increased, the proportion of correct estimates decreased (Figure 6.4). The interaction between age class and number of wolves was not significant (χ^2:

2.7678; df: 1; p = 0.096). The most parsimonious model included the number of wolves howling and explained 64% of the variance, 13% explained by chorus and human subject random factors (marginal $R^2 = 0.51$; conditional $R^2 = 0.64$). A similar pattern is shown when considering whether the number of wolves is overestimated or underestimated: estimates for choruses emitted by a low number of wolves tend to be overestimates, while choruses emitted by larger group sizes yield underestimates (Figure 6.5).

Alternatively, we conducted all the analyses including *Country* as a covariate. *Country* entered the model for the determination of the presence of adults/pups with a significant effect (p = 0.007) due to differences in the percentage of correct estimates of the presence of adults (90% in Spain vs. 74% in Sweden). However, this variable had no effect in the other models, and test scores, estimates of the presence of pups, and estimates of the number of wolves howling were the same in both countries.

Table 6.5.- GLMM obtained including all the variables for the estimate of the number of pups and adults in a chorus. *: significant factors.

Fixed effects	Estimate	Std. Error	Z value	Pr(>|z|)
Intercept	3.032	1.057	2.868	0.004*
Unexperienced1	-0.393	0.386	-1.017	0.309
Expclass1	-0.258	0.361	-0.715	0.475
Expclass2	-0.511	0.360	-1.418	0.156
Expclass3	-0.464	0.371	-1.251	0.211
Agepups	0.489	0.554	0.882	0.378
Num	-1.422	0.262	-5.427	<0.001*

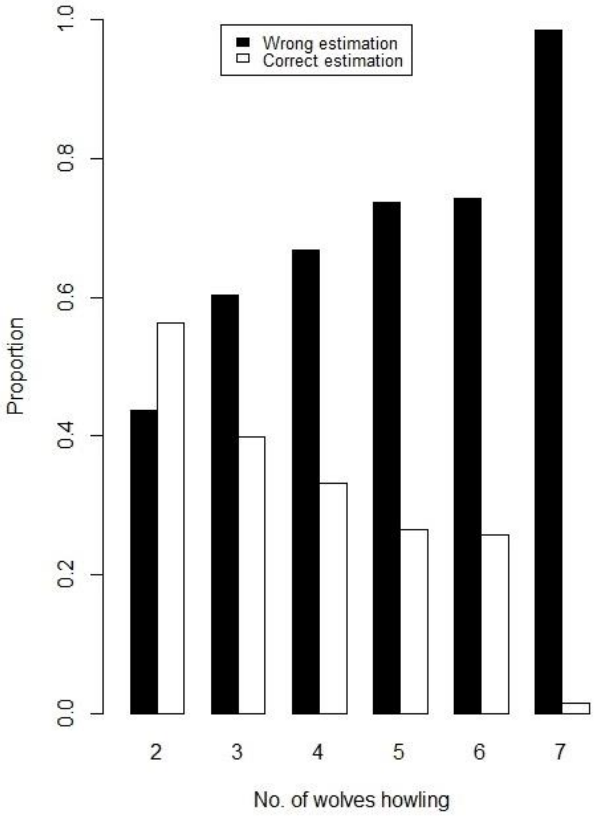

Figure 6.4.- Proportion of correct and wrong estimates of the number of wolves participating in chorus howls.

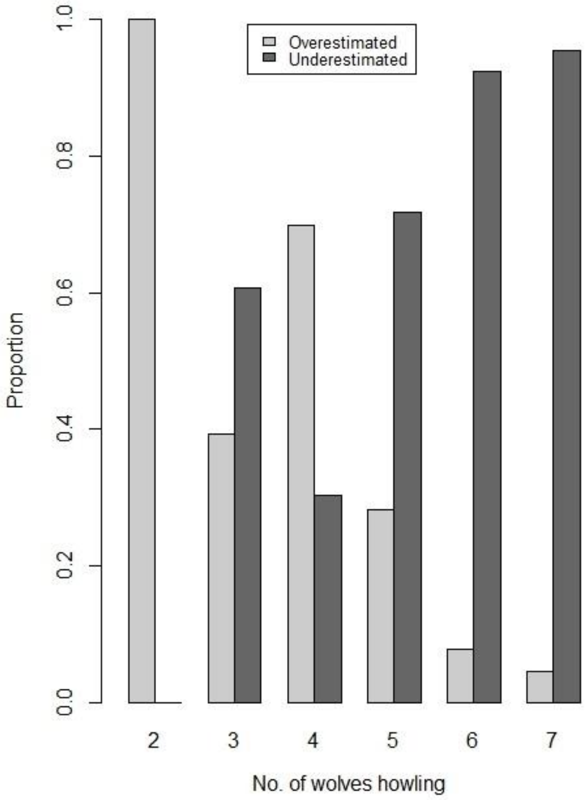

Figure 6.5.- Proportion of over and underestimates of the number of wolves participating in chorus howls.

6.4 DISCUSSION

Our results provide the first evaluation of the accuracy of estimates of the number of wolves howling in a chorus and the presence of pups made by means of acoustic censusing. In our study, 31% of group size estimates made by rangers were correct, 30% were overestimates and 38% underestimates. With respect to the presence-absence of pups 64% of estimates were correct. The experience of the listener had no effect on the accuracy of the estimates, and no differences were found between rangers and a group of unexperienced people.

Joslin (1967) pioneered the use of acoustic censusing from chorus howls to obtain information regarding group size, counting each animal as it first began to howl. However, no systematic data were collected to determine the reliability of this method. Harrington (1989) reported that human estimates of group size from chorus howls overestimate the real size, especially for smaller packs. Although Harrington did not conduct statistical analyses to support this conclusion, our results agree with Harrington’s since overestimates of group size are frequent for small group sizes. Hallberg (2007) carried out a study to evaluate the human estimates of group size from coyote group-yip-howl choruses. Hallberg concluded that human subjects were usually wrong in their estimates of group size, although their estimates were close to the actual number (mean estimates within one or two of the actual numbers). In our study only 31% of the group size estimates were correct. We obtained the same percentage of correct estimates as Hallberg (2007) with a much bigger sample size (205 vs. 21 human subjects) and for wolf chorus howls. This agreement may reflect similarities in the acoustic structure of wolf and coyote chorus howls (Lehner 1978a).

With respect to the ability to determine the presence of pups, it is commonly assumed that pup/adult discrimination by ear is feasible, at least when the pups are young (Apollonio et al. 2004; Llaneza et al. 2005b). However, we found that one third of the estimates of the presence of pups were false positives. Wolf pups can produce the full range of vocalizations used by adults early in the ontogeny, between the third and the ninth weeks of age (Peters & Wozencraft 1989; Harrington & Asa 2003). Therefore, the only difference between the vocalizations emitted by pups and adults in chorus howls are related to the fundamental frequency (higher in pups) and, in the case of howls, duration (longer in adults, Harrington & Asa 2003). Chorus howls can be very complex depending on the number of wolves participating and the vocalizations emitted. Although the human ear can discriminate changes of approximately 3 Hz in a 1000 Hz tone (Sinnott & Brown 1993), the complexity of chorus howls may place constraints on the capacity of the human ear for pup/adult discrimination.

Forest rangers are involved in wildlife monitoring worldwide. For instance, rangers carry out mountain gorillas censuses (Koenig 2008), they participate in field work monitoring African herbivores (Valeix et al. 2009), they survey large carnivores in Norway and Sweden by rangers (Kaczensky et al. 2013), and they participate in wolf surveys in Poland and Spain (Kuijper et al. 2013; Llaneza et al. 2014). Experience and training of personnel responsible for surveys are factors that could affect the accuracy of information obtained in monitoring systems (Bibby et al. 1992; Sutherland 2006). We found that experience had no effect in the accuracy of the estimates made by human listeners. We did not find differences among rangers with more or less experience, or between unexperienced people and rangers. Furthermore, our results agree with those reported by Hallberg (2007) for coyotes and Harrington (1989) for wolves, suggesting that differences in accuracy of estimates may be due to complexity of signals or human auditory capacity rather than to prior experience with the animals. In this sense, this study provides information potentially useful for designing training programs intended for people responsible of wolf monitoring. It would be interesting to investigate whether learning can increase the accuracy of estimates by acoustic censusing of chorus howls. With respect to differences found between countries, these differences do not affect the estimates of the presence of pups and the number of wolves howling, and may reflect minor differences in the methodology used (e.g. all the listeners from the same location in a common room in Spain vs. individually isolated listeners in Sweden).

These results should be taken into account to analyse the information obtained in wolf monitoring systems by means of acoustic censusing of wolf chorus howls. Group size estimates by listening to chorus howls are usually wrong, especially as group size increases. In most cases (53%) differences between estimates and actual group size involve 1-2 individuals. However, 15% of times the listeners overestimated actual group size by more than two individuals. Detection of pups yielded better results than group size estimates. When there really are pups in the chorus howl, humans detect them 98% of the time. However, false positives in the detection of pups were very frequent. In terms of monitoring wolf populations this is a mistake with undesirable consequences, because the presence of pups implies the existence of a reproductive pack. Two choruses emitted by the same pack from two locations a few kilometres apart could be considered as two different packs if the listener concluded that pups were present in both choruses. This underscores the need for improved monitoring methods that allow objective and reliable estimates of group size and the presence of pups from chorus howls.

6.5 ACKNOWLEDGEMENTS

We thank all the rangers and unexperienced people that participated in the chorus tests. Tests were carried out thanks to Dr. J. V. López-Bao, Dr. J. Frank, and Dr. L. Swenson (Sweden), and E. J. García and L. Llaneza (Spain).

CHAPTER 7. BIOACOUSTIC ANALYSIS OF WOLF CHORUS HOWLS (I): ESTIMATES OF GROUP SIZE

Results presented in the previous chapter show that estimates of the number of wolves in a pack based on listening to playbacks of chorus howls are not very accurate. On average, rangers determined the correct number of wolves in only 31% of playbacks. Even of more concern is the fact that listeners often report that pups contribute to a chorus howl when in fact there are no pups in the pack. Such false positives could lead to overestimating reproduction, which may hinder conservation and management efforts. In chapters 7 and 8, we will explore the usefulness of bioacoustics analyses as an objective and reliable method to estimate the number of wolves participating and the presence of pups in chorus howls.

7.1 INTRODUCTION

Some bird and mammal species live in groups of various sizes and defend group territories with group vocalizations (Mech 1970; Baker 2004). In these cases, selection could favour the existence of group vocal signatures. Cues regarding group identity would allow individuals to discriminate between group mates and others, enabling cooperation within the group and exclusion of strangers who compete for the same resources (Boughman & Moss 2003). Group vocal identity has been described, for instance, in laughing kookaburras, *Dacelo novaeguineae*, (Baker 2004), green woodhoopoes, *Phoeniculus purpureus*, (Radford 2005), wild chimpanzees, *Pan troglodytes verus* (Crockford et al. 2004), and free-ranging wolf (*Canis lupus*) packs (Zaccaroni et al. 2012).

Group vocalizations could also encode information regarding group size. Black-breasted wood-quail (*Odontophorus leucolaemus*) choruses, for example, could function in group size assessment (Hale 2006b). Group size information is important because in contests between social groups competitors might be expected to adjust their agonistic behaviour according to the number of individuals in their own and the competing group. In fact, McComb et al. (1994) showed that groups of female lions (*Panthera leo*) adjusted the decision to approach a roaring stimulus played back according to the size and composition of their own group and the number of lions participating in the roaring stimulus. Providing group size information could also be advantageous in predator-prey contexts, as in howler monkeys, where it has been reported that loud calls may serve as an honest indicator of a group's size and thus of its capacity to defend itself against predatory attacks (da Cunha & Byrne 2006). In contrast, chorus songs of other species such as Australian magpie (*Cracticus tibicen dorsalis*) do not appear to reveal group size and composition (Baker 2009).

Besides their importance for intra- and interspecific communication, chorus vocalizations are a potentially useful monitoring/management tool. Chorus howls could provide information about the number of individuals comprising a wolf pack, allowing researchers to estimate population size, which is a critical variable in conservation and management programs (Sutherland 2006; Sinclair et al. 2009). This is especially relevant for elusive species like the wolf (*Canis lupus*), that lives at low densities in forested areas that make it difficult to obtain information regarding group size by other means. It is important to emphasize that although the wolves' basic social unit is the familiar pack, all the wolves in a pack do not always travel together (Demma et al. 2007; Demma & Mech 2009; Palacios & Mech 2011). In addition, sometimes all the present wolves do not participate in a chorus (unpublished data). For these reasons, the number of wolves participating in a chorus howl should be considered the minimum number of wolves integrating a pack.

In this chapter we explore the potential of spectrograms obtained from recordings of chorus howls to extract accurate estimates of the number of wolves participating in them. Filibeck et al. (1982) proposed a method based on the analysis of instantaneous frequencies for counting wolves emitting single howls simultaneously. In Italy, researchers have determined the minimum number of individuals vocalizing at the beginning of a chorus based on the fundamental harmonics (Apollonio et al. 2004). However, no studies have determined the reliability of these methods nor proposed a method to estimate the number of wolves participating in more complex choruses that include other types of vocalizations different from howls. The aims of this chapter are 1) to evaluate whether the acoustic features of chorus howls emitted by wolves contain information regarding group size, 2) to propose a method to estimate the number of wolves participating in choruses from audio recordings that could be used for monitoring wild populations, and 3) to compare the results obtained with human listener's estimates from chapter four.

7.2 METHODS

We collected recordings of 60 chorus howls emitted by 2-9 wolves belonging to 18 different packs recorded at 12 different locations. In all cases the wolves were seen howling, and the exact number of wolves participating in the chorus was known. The sample included chorus howls from several locations worldwide since we wanted to increase the sample size, especially for large pack sizes. The bulk of the recordings were chorus howls emitted by captive wolves at Cañada Real, Madrid (n = 20), Carpin, Bilbao (n = 2), Centro de Recuperação do Lobo Ibérico, Portugal (n = 16), La Dehesa, Albacete (n = 4), Novosibirsk Zoo, Rusia (n = 1), Berlin Zoo, Berlin, Germany (8), Kolmården Wildlife Park, Sweden (n = 1), and International Wolf Center, Minnesota, USA (n = 2). We also used choruses emitted by wild Iberian wolves with known number of wolves vocalizing at four different Spanish locations: Lugo (n = 1); Pontevedra (n = 1); Zamora (n = 2); and Asturias (n

= 2). All the chorus howls were originally recorded in .WAV format, and we standardized the format to sampling rate of 48 kHz, mono, and 16 bits accuracy, using Adobe Audition 3.0 (Adobe Systems, San José, CA, USA). A preliminary analysis revealed that choruses emitted by large packs were underrepresented in our sample: only two recordings of chorus howls emitted by eight and two emitted by nine wolves. Therefore, we decided to remove choruses emitted by eight and nine individuals to avoid spurious results due to small sample size and analysed 56 choruses emitted by 2-7 wolves (Figure 7.1).

For each chorus we measured 16 variables: 13 variables describing the acoustic energy distribution (energy transmitted by sound via the propagating pressure fluctuations, Hopp & Owren 1998), chorus duration, and mean and maximum number of wolves vocalizing simultaneously (Table 7.1). We measured the acoustic energy variables considering only the signal above an amplitude threshold of 75% of the maximum amplitude to reduce influences due to the distance of the emitter to the microphone and different signal-to-noise ratios (Schrader & Hammerschmidt 1997). Acoustic energy variables and duration were measured using Raven Pro 1.5 (Cornell Laboratory of Ornithology, Ithaca, NY, U.S.A.) and custom-made software (APPENDIX I).

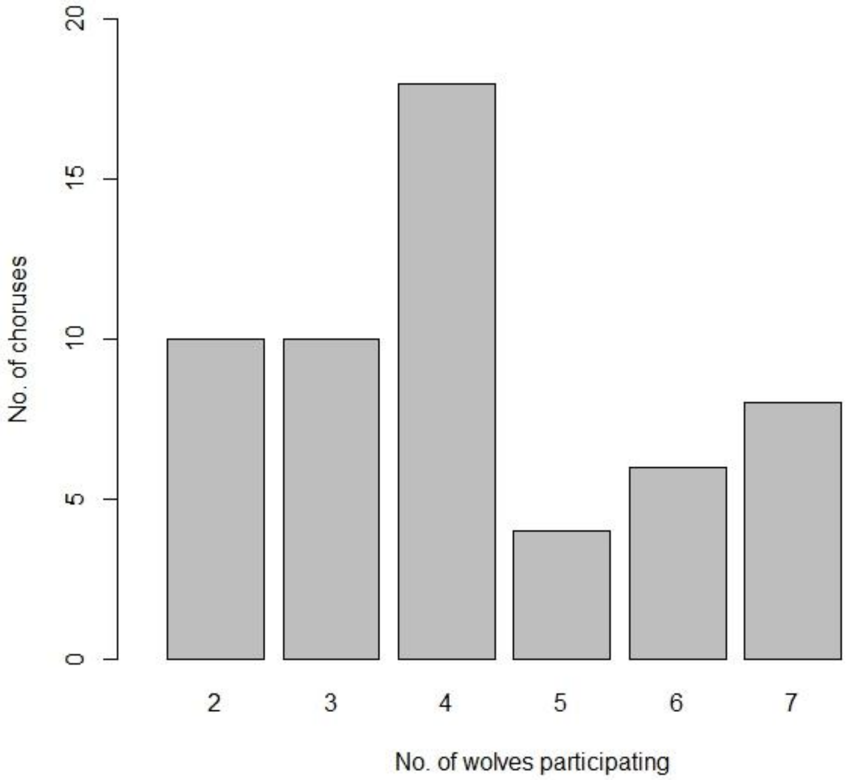

Figure 7.1.- Sample size of choruses used to build the models

To measure the number of wolves vocalizing simultaneously we generated spectrograms (2048-point fast Fourier transform; Hann window; bandpass filter 200-2500 Hz; frequency resolution: 21.5 Hz) using Adobe Audition 3.0 (Adobe Systems, San José, CA, USA). We conducted a blind analysis, recoding the name of the file to be analysed and hiding the number of wolves actually participating in it, because previous knowledge of the number of wolves howling could lead to unintentional biases. To develop a standardized methodology we measured the number of wolves vocalizing simultaneously at 1 s intervals, based on the analyses of instantaneous frequencies (Filibeck et al. 1982). As most of the wolf vocalizations emitted in choruses are harmonic sounds, we can calculate the number of different vocalizations emitted simultaneously because harmonics are multiple integers of the fundamental frequency of harmonic signals (Figure 7.2).

As choruses can be complex signals and include other vocalizations besides howls, sometimes the identification of simultaneous vocalizations is difficult. In case of doubt, we included in the analyses the minimum number of wolves that we could identify. Once we measured the number of wolves vocalizing every second we calculated the average number of wolves vocalizing ($\sum no. of\ wolves\ vocalizing\ at\ each\ 1\ second\ interval\ / no. of\ intervals$) and recorded the maximum obtained for that chorus (Table 7.1).

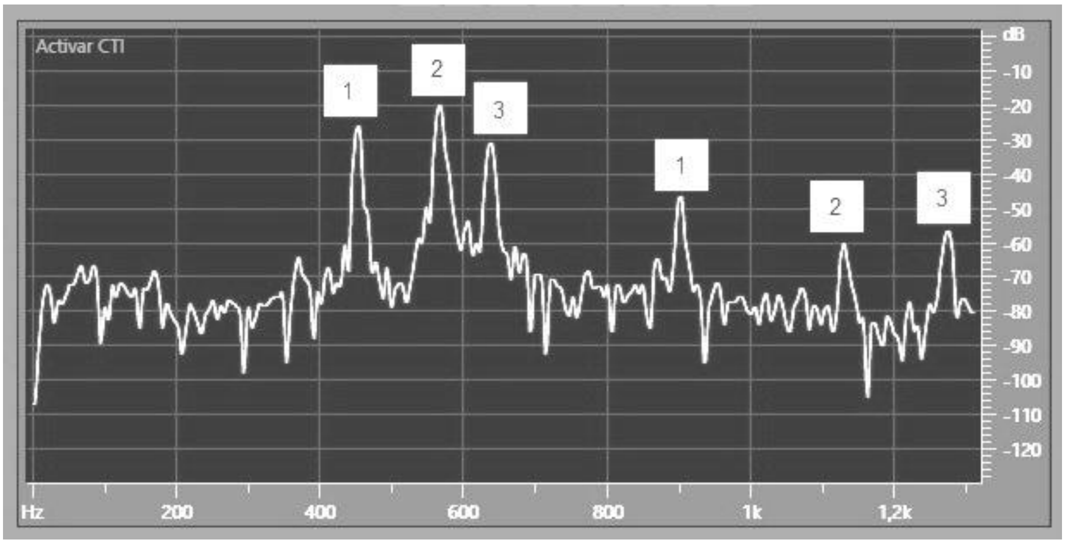

Figure 7.2- Analyses of instantaneous frequencies in a given time of a chorus (Hann window, 2048 FFT points), with three individuals vocalizing: 1) fundamental frequency = 452 Hz, second harmonic = 904 Hz; 2) fundamental frequency = 565 Hz, second harmonic = 1130 Hz; 3) fundamental frequency = 635 Hz, second harmonic = 1270 Hz.

Table 7.1.- Acoustic features measured. AED: acoustic energy distribution.

Variable	Description
Max	Maximum number of wolves vocalizing simultaneously *
Mean	Mean number of wolves vocalizing simultaneously *
Dur	Duration of chorus (s)
Q1Freq	Frequency that divides the spectrum into two frequency intervals containing 25% and 75% of the energy (Hz)
Q3Freq	Frequency that divides the spectrum into two frequency intervals containing 75% and 25% of the energy (Hz)
IQRBW	Difference between the 1st and 3rd Quartile Frequencies (Hz)
AggEntropy	Aggregate entropy: measurement of the disorder by analysing AED
AvgEntropy	Average entropy: average of the entropy for each frame in the selection
CentFreq	Frequency that divides the selection into two frequency intervals of equal energy (Hz)
Freq5	Frequency that divides the selection into two frequency intervals containing 5% and 95% of the energy (Hz)
Freq95	Frequency that divides the selection into two frequency intervals containing 95% and 5% of the energy (Hz)
BW90	The difference between the 5% and 95% frequencies (Hz)
MaxFreq	The frequency at which the maximum of the amplitude occurs (Hz)
AED-M**	Frequency corresponding to the mean energy density (Hz)
AED-SD**	Standard Deviation of the mean energy density (Hz)
AED-P**	Frequency corresponding to the peak of the energy density (Hz)

*: For each chorus we measured the number of wolves vocalizing simultaneously using instantaneous frequencies at intervals of 1 s.

**: variables measured using custom made software (APPENDIX I)

We fitted general linear models (GLM) to predict the number of howling wolves from the variables measured (hereafter acoustic structure based model: AS-based model). First we removed variables with correlations above 0.8 to eliminate problems caused by colinearity. Then we performed a multivariate GLM including all the resulting variables and fitted subsequent GLMs removing non-significant variables in a stepwise fashion to find the most parsimonious model that accounted for meaningful variation. We used Akaike's Information Criterion (AIC) to rank models, selecting the model with lowest AIC values (Burnham & Anderson 2002). We considered models with AIC differences ≤ 2 to have equal empirical support (Burnham & Anderson 2002). We assumed that the number of wolves participating in a chorus is distributed according to a Poisson distribution truncated at two, which is the minimum number of wolves that can emit a chorus. Thus, the formula used to perform GLMs was:

$$Number\ of\ wolves - 2 \sim varA + varB + varC + \cdots$$

And the formula to predict the number of wolves:

$$Number\ of\ wolves = \exp(Intercept + \alpha * varA + \beta * varB + \cdots) + 2$$

Following Aydin (2008) the proportion deviance explained, a generalization of the R^2 value, was calculated as:

$$D^2 = \frac{Null\ deviance - Residual\ deviance}{Null\ deviance} * 100$$

We checked for overdispersion in our data by calculating the residual deviance/residual degrees of freedom (values > 1 indicate overdispersion, Crawley 2007). Our sample included several choruses emitted by the same pack. However, the data had no grouped or hierarchical structure that made it advisable to consider pack as random factor (Zuur et al. 2009). In any case, to check for potential problems arising from pseudoreplication in our data, we conducted the same analyses using GLMMs with pack as a random factor, and the explained variance, Intercept and coefficients were the same as those obtained with GLMs. The statistical procedures were conducted using R (R Development Core Team 2010).

7.3 RESULTS

We analysed 56 chorus howls, lasting from 26 to 175 seconds. The number of wolves participating in a chorus was highly correlated to *Max* (maximum number of wolves vocalizing simultaneously, Spearman´s Rho: 0.98; n = 56; p < 0.001). In most cases (73%) the maximum number of wolves vocalizing simultaneously agreed with the actual group size. The other two variables correlated with group size were *Mean* (average number of wolves vocalizing simultaneously, Spearman´s Rho: 0.78; n = 56; p < 0.001) and *Dur* (duration of the chorus, Spearman´s Rho: 0.38; n = 56; p = 0.0035). As the number of wolves increases, the number of wolves vocalizing simultaneously and the duration of the chorus also increase (Figure 7.3).

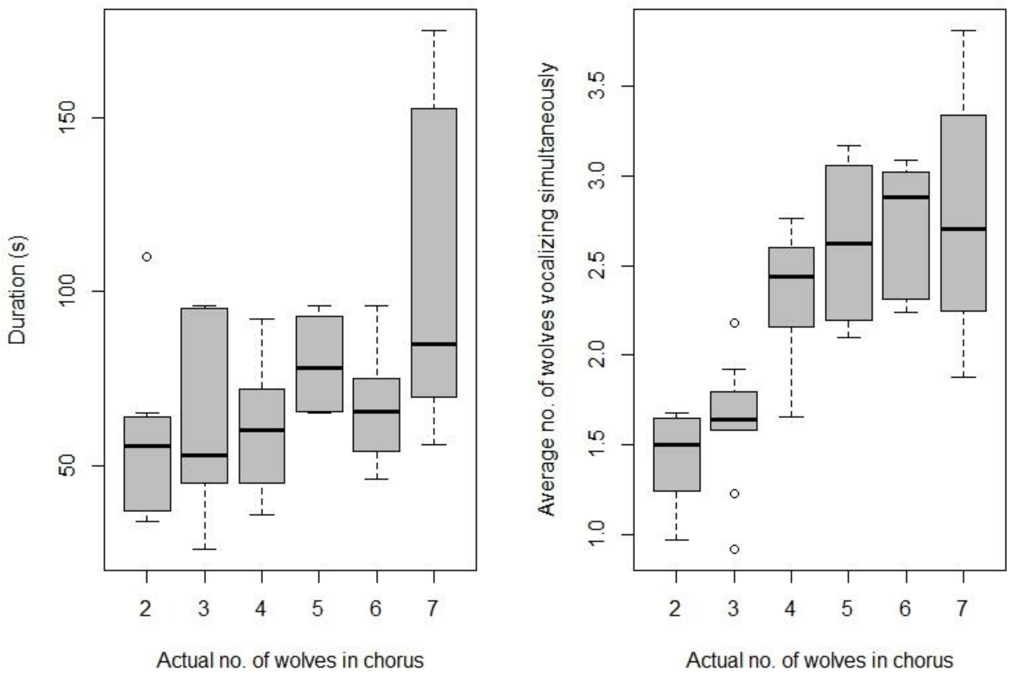

Figure 7.3.- Values of duration and average number of wolves howling simultaneously (*Mean*) according to group size (actual number of wolves vocalizing in the chorus). The horizontal bar shows the median, the edges of the boxes mark the first and third quartile of the data, and the whiskers include all data within 1.5 times the interquartile range. Circles are outliers.

The best AS-based model included *Max* and *Dur* (Table 7.2) and explained 82% of the variability according to the deviance criterion. An alternative model including only *Max* had the lowest AIC value, but the low ΔAIC between both models (0.89) indicate equally strong support. As the model including *Max* and *Dur* had higher R^2 we chose this model to make predictions.

Table 7.2.- Values of AIC corresponding to the GLMs conducted. The best models considering the AIC criteria and the minimum number of parameters are highlighted.

Variables in the model	**AIC**	**ΔAIC**
Max, Mean, Dur, AED-M, AED-SD, AED-P, Q1Freq, Q3Freq, AggEntropy, AvgEntropy, BW90, CentFreq, Freq95, MaxFreq	168.5	21.86
Max, Dur, AED-M, AED-SD, AED-P, Q1Freq, Q3Freq, AggEntropy, AvgEntropy, BW90, CentFreq, Freq95, MaxFreq	166.5	19.86
Max, Dur, AED-M, AED-SD, AED-P, Q1Freq, Q3Freq, AggEntropy, AvgEntropy, BW90, CentFreq, Freq95	164.51	17.87
Max, Dur, AED-M, AED-P, Q1Freq, Q3Freq, AggEntropy, AvgEntropy, BW90, CentFreq, Freq95	162.52	15.88
Max, Dur, AED-M, AED-P, Q1Freq, Q3Freq, AvgEntropy, BW90, CentFreq, Freq95	160.57	13.93
Max, Dur, AED-M, AED-P, Q1Freq, AvgEntropy, BW90, CentFreq, Freq95	158.69	12.05
Max, Dur, AED-M, Q1Freq, AvgEntropy, BW90, CentFreq, Freq95	156.77	10.13
Max, Dur, AED-M, AvgEntropy, BW90, CentFreq, Freq95	154.94	8.3
Max, Dur, AED-M, BW90, CentFreq, Freq95	153.05	6.41
Max, Dur, AED-M, BW90, CentFreq	151.55	4.91
Max, Dur, AED-M, BW90	149.84	3.2
Max, Dur, AED-M	148.38	1.74
Max, Dur	147.53	0.89
Max	146.64	

Thus, the formula to predict the number of wolves from our data includes the *Intercept*, *Max*, and *Dur*:

$$num = \exp\left(-1.88 + 0.67 * \mathrm{Max} - 0.003 * Dur\right) + 2$$

Applying the formula to predict the number of wolves participating in a chorus, the model predicted the correct number of individuals in 79% of our choruses, 12% of estimates overestimated and 9% underestimated the actual number of wolves (Figure 7.4). The estimated number differed in one individual 16% of cases (7.1% underestimated and 8.9% overestimated); two individuals 3.6% of cases (1.8% underestimated and 1.8% overestimated); and the difference between the estimate and the real number was three individuals in only 1.8% of the cases (overestimated).

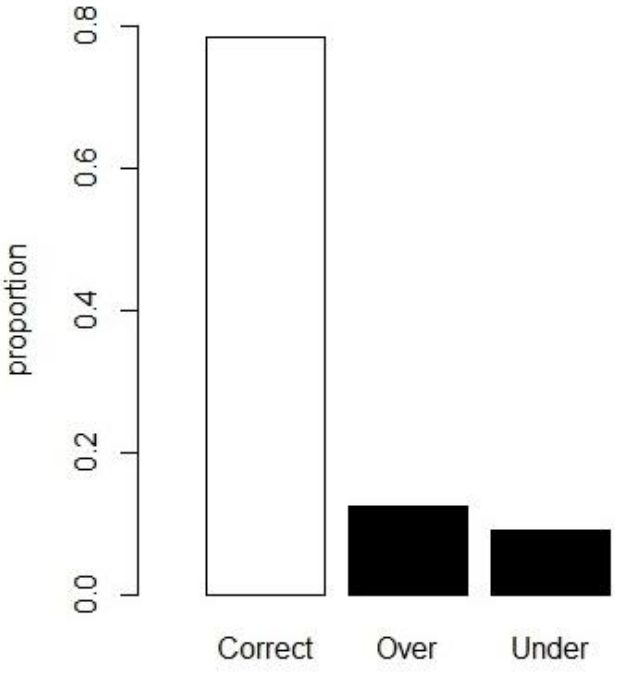

Figure 7.4.- Proportions of correct and wrong estimates made by the model. Over: overestimated; Under: underestimated.

7.4 DISCUSSION

Group vocalizations, also known as choruses, are acoustic displays characterized by simultaneous signalling by many individuals (Foote et al. 2011). Choruses are present in the vocal repertoire of many insects (Oliveira et al. 2001), amphibians (Sun et al. 2000), birds (Hale 2006a; Foote et al. 2011), and mammals (Fischer et al. 2004; Bolt 2013; Mumm & Knörnschild 2014). We found that the number of wolves vocalizing simultaneously increases with group size and that choruses emitted by larger groups last longer, although the correlation between duration and number of wolves howling is much lower than the correlation found for the number of wolves vocalizing simultaneously. The most important variable predicting group size in wolf chorus howls is the maximum number of wolves vocalizing simultaneously.

These results suggest that wolves could encode information regarding group size in chorus howls. One of the main functions attributed to wolf chorus howls is pack spacing and maintenance of territories (Harrington & Mech 1979, 1983). Game theoretical models predict that where fighting is costly, contestants should assess the value of the resource and the resource holding potential of their opponent, and withdraw without escalation if they would be unlikely to win an ensuing fight (Maynard Smith 1982). Hence, a strong selective advantage to avoiding the costs of fighting with larger groups could have led to the widespread evolution of numerical assessment skills in social species (McComb et al. 1994). It has been proposed that coyotes and possibly other canids that use chorus vocalizations similar to those of wolves are capable of assessing group size from a chorus based on differences in individual voices (Lehner 1978a), but no systematic data have been collected to confirm this hypothesis. Harrington (1989) did not find any relationship among the variables he measured and pack size, and concluded that it is unlikely that packs can assess the size of neighbouring packs using the acoustic information provided by choruses. Furthermore, he suggested that chorus structure could have evolved in order to either withhold information on pack size or exaggerate the apparent size of the pack, proposing that wolf chorus howls might be a mammalian example of the Beau Geste effect. The Beau Geste hypothesis holds that song repertoires in some bird species could have evolved in the context of density assessment, to increase the apparent density of singing residents (Krebs 1977). However, results of studies conducted with birds are inconsistent with some predictions from the Beau Geste hypothesis (e.g. no evidence that prospecting males actually avoided areas which were more densely populated, Catchpole & Slater 2008). On the contrary, other studies conducted with coyotes support the hypothesis that chorus howls might contain information regarding group size. Hallberg (2007) found that target animals tended to move away from a pack chorus and approach stimuli emitted by a pair of coyotes. The most important cues indicating group size in that study were acoustic features relating to individual voice characteristics and the type of vocalizations occurring alternative or simultaneously.

The AS-based model predicts correctly the number of wolves participating in a chorus 79% of the time, with most errors missing the actual number of wolves by only one individual. Thus, analyses of recordings yield accurate estimates of group size that could be used to monitor wild populations. Group size estimates have previously been done by listening to chorus howls (Gazzola et al. 2002; Apollonio et al. 2004; Llaneza et al. 2005b; Nowak et al. 2008; Mitchell et al. 2008). However, none of those studies calculated the accuracy of estimates of group size based on listening to chorus howls. In the previous chapter we analysed the precision of group size estimates made by acoustic censusing. AS-based model estimates are much better than those obtained solely by listening to chorus howls, providing a method more suitable for monitoring wild wolves (Figure 7.5). Model estimates have both advantages and disadvantages with respect to acoustic censusing. The most important disadvantage is that choruses need to be recorded. However, nowadays recording devices are small, portable and relatively cheap.

The main advantages include accuracy and repeatability, both of them crucial for monitoring methodologies.

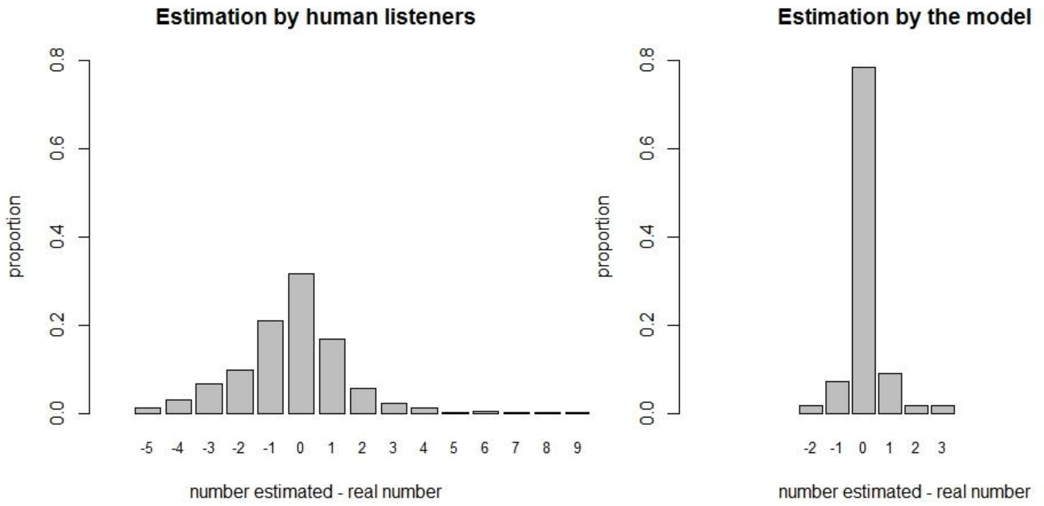

Figure 7.5.- Comparison between estimates made by human listeners (see chapter 6) and estimates made by the AS-based model.

The application of models to estimate the number of wolves participating in a chorus is an improvement over methods currently used. We do not know to which extent the inclusion of choruses belonging to different wolf populations could affect the robustness of the AS-based model. However, variables included in the model (the number of wolves vocalizing simultaneously and duration) are not frequency related variables, which could indeed affect the results, since frequency parameters are the most appropriate to encode information regarding identity in long-range vocal signals (Wiley & Richards 1978). More research is needed to build more accurate models, including choruses emitted by more than seven individuals, and taking into account the possible effect of including chorus howls emitted by wolves belonging to different populations.

The AS-based model estimates accurately the number of wolves that participate in a chorus. Nevertheless, as stated in the introduction, not all the wolves belonging to a pack always travel together and the information obtained from a chorus should be considered the minimum pack size. Several questions need further research: 1) to what extent our estimates of wolves howling in a chorus correspond to the actual pack size? 2) Do all the wolves in a pack participate in chorus howling? 3) How many recordings per pack would be needed to obtain a reliable estimate of pack size? 4) Is it possible to identify individuals by their howls analysing chorus howls? 5) How would individual identification and multi-recordings per pack improve estimates of actual pack size?

7.5 ACKNOWLEDGEMENTS

Recordings from other wolf populations were provided by Dr. Ilya A. Volodin, Dr. Karl-Heinz Frommolt (recordings from The Animal Sound Archive at the Museum für Naturkunde in Berlin), Dr. Linn Swenson, and Lori Schmidt (International Wolf Center, Minnesota). C. Fernández built custom-made software to calculate AED related variables, see APPENDIX I (supported by Project PC0448, Gobierno del Principado de Asturias, Spain).

7.6 APPENDIX I

We used custom-made software to measure three variables related to the AED: AED mean (AED-M); AED standard deviation (AED-SD), and AED peak (AED-P). The procedure to calculate these variables follows the next steps:

1) We calculate the Fourier transform

$$(\Gamma_v f)(\tau,\omega) = \int_{-\infty}^{\infty} f(t)\, v\,(t - \tau) \exp[-it\omega]\, dt$$

where τ is time and ω is frequency.

2) The function $\mathcal{E}(\tau,\omega) = |(\Gamma_v f)(\tau,\omega)|^2$

yields the energy of the signal at time τ and frequency ω

3) In this context, the integral

$$\|f\|^2 = \frac{1}{2\pi} \iint_{\mathbb{R}^2} \mathcal{E}\,(\tau,\omega) d\omega d\tau$$

corresponds to the total energy of the signal.

4) We calculate the average energy of the signal in the time

$$(E_\tau)(\omega) = \frac{1}{\|f\|^2} \int_{-\infty}^{\infty} \tau \mathcal{E}\,(\tau,\omega) d\tau$$

This function only depends on the frequency $(E_\tau f)(\omega)$ and yields the percentage of energy corresponding to frequency ω

5) Then we calculate the mean value of the frequency energy density

$$\mu = \mathrm{E}\,[(\mathrm{E}_\tau f)] = \frac{1}{2\pi} \int_{-\infty}^{\infty} \omega\,(\mathrm{E}_\tau f)(\omega)\, d\omega,$$

and its standard deviation

$$\sigma^2 = \mathrm{V}\,[(\mathrm{E}_\tau f)] = \frac{1}{2\pi} \int_{-\infty}^{\infty} (\omega - \mu)^2\,(\mathrm{E}_\tau f)(\omega)\, d\omega,$$

6) AED-P is the frequency corresponding to the maximum energy density.

Number of wolves howling in a chorus

CHAPTER 8. BIOACOUSTIC ANALYSIS OF WOLF CHORUS HOWLS (II): PRESENCE OF PUPS

8.1 INTRODUCTION

The simulated howling technique is commonly used to survey wolf populations worldwide (Llaneza et al. 2005b; Mitchell et al. 2008; Kaczensky et al. 2013). Often wolves respond to simulated howls emitting chorus howls. These choruses allow researchers to locate wolf packs, estimate the number of wolves howling and determine the presence of pups. In this chapter we focus on the usefulness of chorus howls to determine that pups are present in a pack and therefore to confirm reproduction. Since in general only the alpha pair in a pack breeds (Packard 2003), the presence of pups is taken as evidence of the existence of one pack (Fig. 8.1). Although multiple breeding has been reported in wolves (Mech & Boitani 2003), it seems to be exceptional, and it is therefore assumed for monitoring purposes that two different litters correspond to different packs (Llaneza et al. 2005b). Surveys to confirm the presence of pups by means of howling are conducted in the summer and early autumn. At this time, pups remain at so-called "*rendezvous sites*"[2] and are relatively easy to find (Packard 2003). Two choruses emitted from locations several kilometres apart may be emitted by wolves belonging to the same pack. In these cases, knowledge of wolf spatial and social ecology, the presence of pups howling and other information obtained during surveys such as a high concentration of wolf signs (Llaneza et al. 2014) allow researchers to estimate the existence of one or two different packs. Hence, obtaining reliable information regarding the presence of pups in a chorus is crucial to estimating the number of packs and to manage wolf populations efficiently (Apollonio et al. 2004; Llaneza et al. 2005b).

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[2] *See description of "rendezvous site" in Part I. General Introduction*

Figure 8.1.- Iberian wolf litter with the alpha female. During wolf surveys researchers aim to detect wolf litters to confirm the existence of a reproductive pack. Photo courtesy of José Larrosa.

Information regarding the presence of pups in a chorus can be obtained by means of: 1) acoustic estimate (i.e. listening to chorus howls), or 2) spectrographic analyses of chorus recordings. Determination of the presence of pups by acoustic estimate is commonly used for monitoring/management purposes (Gazzola et al. 2002; Apollonio et al. 2004; Llaneza et al. 2005b; Nowak et al. 2008; Mitchell et al. 2008). In chapter 6 we analysed the reliability of such estimates, concluding that human listeners estimate that there are pups when there are actually no pups vocalizing in a high percentage of cases. This type of error could affect the interpretation of estimates of the number of packs living in an area. Some researchers have determined the presence of pups by means of spectrographic analyses of chorus howls (Gazzola et al. 2002; Apollonio et al. 2004; Nowak et al. 2007). When pup presence is determined through analyses of spectrograms, it is based mainly on the fundamental frequency of vocalizations, specifically howls, but detailed descriptions of the methodology applied for discriminating between adult and pup vocalizations have not been provided (Gazzola *et al.* 2002; Apollonio *et al.* 2004; Nowak *et al.* 2007). Since choruses include different types of vocalizations, an efficient method for discriminating the presence of pups in a chorus by means of fundamental frequency analysis should consider not only howls, but all the types of vocalizations emitted, variation among age classes, etc. To our knowledge, there are no standardized methods to identify wolf pups vocalizing in a chorus that take into account these considerations. Considering only the fundamental frequency of vocalizations may be simplistic and pups may not be detected, for instance, if they emit vocalizations other than howls. Moreover, no attempt to quantify the reliability of such methodologies has been made.

The spectral distribution of acoustic energy (energy transmitted by sound via the propagating pressure fluctuations, Hopp & Owren 1998) provides a potentially useful alternative to detect pups in chorus howls. Since pups emit vocalizations higher in pitch than those emitted by adults (Harrington & Mech 1978a; Coscia 1995), the acoustic energy of chorus howls involving pups should concentrate at higher frequencies than those emitted only by adult wolves. Analyses of chorus howls have to take into account that choruses are long vocalizations (up to several minutes) which include different types of vocalizations of different duration emitted by several individuals. Therefore, different parts of a chorus may have different acoustic structure affecting in turn the overall values of the acoustic energy distribution (hereafter AED) and therefore our ability to detect the presence of pups. Finally, analyses of recorded choruses have to consider the quality of the recording, differences in signal to noise ratio, and distortions due to the distance of the sender to the microphone. The aims of this study are: 1) to explore the usefulness of the AED of chorus howls to predict the presence of pups vocalizing in a chorus, 2) to determine the best procedure to analyse a chorus recording using AED, and 3) to compare our results with estimates obtained by listening to chorus howls (chapter 6).

8.2 METHODS

Data collection

Between 2000 and 2011 we recorded 110 chorus howls both in captivity (n = 74) and in the wild (n = 36). Captive wolves were recorded at four locations in the Iberian Peninsula: Cañada Real (Peralejos, Madrid, Spain), Carpín (Carranza, Bilbao, Spain), La Dehesa (Riopar, Albacete, Spain), and Centro de Recuperação do Lobo Ibérico (Malveira, Portugal). Wild wolves were recorded in five Spanish provinces: Lugo, A Coruña and Pontevedra (Galicia), Asturias, and Zamora (Castilla y León). Most of the choruses (94.8%) were emitted in response to standardized human imitations of wolf howls (Palacios et al. 2007), with only a few choruses being emitted spontaneously. Recordings in the wild were obtained during regional wolf monitoring programs or were specifically approved by the corresponding regional administrations (Regional Governments of Galicia, Asturias and Castilla-León).

The bulk of the recordings (87%) were made on TDK SA-60 cassette tapes (TDK Electronics Corp., New York) using a Sennheiser MK 66 unidirectional microphone with K-6 power unit (Sennheiser Electric GmbH & Co. kG, Wedemark, Germany) connected to a Marantz PMD 222 cassette recorder (Marantz America, Inc., Mahwah, New Jersey). These recordings were digitized with 44.1-kHz sampling frequency and 16 bits in the Fonoteca Zoológica, Museo Nacional de Ciencias Naturales (CSIC, Madrid, Spain), using Delta 66 (Irwindale, California) or Digi 001 (Bucks, United Kingdom) digitizer cards. The rest of the recordings were obtained using a Sennheiser MKH 70 directional microphone attached to a Marantz PMD 670 solid state recorder. These recordings were saved in .WAV files with 44.1 kHz sampling frequency and 16 bits.

Data analyses

For each chorus, we generated spectrograms (2048-point fast Fourier transform; Hann window; bandpass filter 200-2500 Hz; frequency resolution: 21.5 Hz), measured the percentage of time the chorus was comprised exclusively by howls or by other vocalizations in addition to howls, and measured a total of 10 acoustic variables relating to AED (Table 8.1) using Raven Pro 1.4 (Cornell University Laboratory of Ornithology, www.birds.cornell.edu/raven). Furthermore, we used custom-made software to measure three additional variables: mean AED (AED-M), AED standard deviation (AED-SD), and peak AED (AED-P) (Table 8.1; see APPENDIX I of chapter 7).

We classified choruses in two groups: with and without pups. We only considered choruses with pups if the recordings were made between July and December, when newborn wolves are less than 6-7 months old, since from this age wolf pups already sound fully like adult wolves (Harrington & Asa 2003). Choruses recorded outside this period were considered to be produced by yearlings, subadults or adults. For all choruses recorded in captivity, we knew exactly the individuals participating in the chorus. However, most of the recordings obtained in the wild were made at night or in areas with scarce visibility. Only in four out of a total of 36 recordings in the wild was it possible to confirm visually that there were pups vocalizing. As mentioned in chapter 6, in the remaining cases we considered that pups participated in the chorus if the following requirements were simultaneously met: i) visual confirmation of the presence of pups at the location were the recording was obtained less than 12 hours before or after the recording was made; ii) the area was used as a *rendezvous site* according to Mech (2000); iii) the chorus included vocalizations consistent with published descriptions of pup vocalizations (Harrington & Mech 1978a; Coscia 1995) and with the same acoustic structure as vocalizations of pups included in our own recordings of visually-confirmed pups. Thus, we included in the analyses 33 recordings classified as with pups: six with visual confirmation of pups vocalizing (two in captivity and four in the wild) and 27 that fitted the criteria mentioned above. We conducted t-tests to compare all the acoustic features measured and there were no differences between choruses with pups visually confirmed and choruses that fulfilled the criteria. Therefore, we assumed that all the choruses included pups vocalizing.

Table 8.1.- Acoustic features measured. AED: acoustic energy distribution.

Variable	Description
Q1Freq	Frequency that divides the spectrum into two frequency intervals containing 25% and 75% of the energy (Hz)
Q3Freq	Frequency that divides the spectrum into two frequency intervals containing 75% and 25% of the energy (Hz)
IQRBW	Difference between the 1st and 3rd Quartile Frequencies (Hz)
AggEntropy	Aggregate entropy: measurement of the disorder by analysing the energy distribution
AvgEntropy	Average entropy: average of the entropy for each frame in the selection
CentFreq	Frequency that divides the selection into two frequency intervals of equal energy (Hz)
Freq5	Frequency that divides the selection into two frequency intervals containing 5% and 95% of the energy (Hz)
Freq95	Frequency that divides the selection into two frequency intervals containing 95% and 5% of the energy (Hz)
BW90	The difference between the 5% and 95% frequencies (Hz)
MaxFreq	The frequency at which the maximum of the amplitude occurs (Hz)
AED-M*	Frequency corresponding to the mean energy density (Hz)
AED-SD*	Standard Deviation of the mean energy density (Hz)
AED-P*	Frequency corresponding to the peak of the energy density (Hz)

*: variables measured using custom-made software

Chorus howls are long (up to 120 s, Harrington & Asa 2003) and include a variety of vocalizations of different duration emitted by several individuals. Because of this complexity, different parts of a chorus may have different acoustic structure affecting in turn the overall values of the AED and therefore our ability to detect the presence of pups (Figure 8.2). We used two different approaches to analyse each chorus: 1) considering the entire duration of the chorus (CHORUS), and 2) segmenting the chorus into small fragments of 5 s (SEGMENTS), since the longest vocalization is the howl and the majority of Iberian wolf howls are between 4-8 s (Palacios et al. 2007). In the first case, we measured the 13 acoustic variables mentioned above for the entire chorus, whereas when the choruses were divided into segments we calculated mean, minimum, maximum and standard deviation for each of the same 13 acoustic features measured for all the segments comprising one chorus.

To reduce influences due to the distance of the sender to the microphone and different signal-to-noise ratios, we measured the acoustic features for chorus and chorus segments considering the beginning of the signal at two amplitude thresholds, 75% and 90% peak amplitude, following recommendations made by Schrader & Hammerschmidt (1997). We therefore compared the effectiveness of four different approaches for determining the presence of pups using AED: entire choruses applying 75% (CHORUS75-model) and 90% (CHORUS90-model) amplitude thresholds, and choruses divided into segments with 75% and 90% amplitude thresholds (SEGMENTS75-model and SEGMENTS90-model respectively).

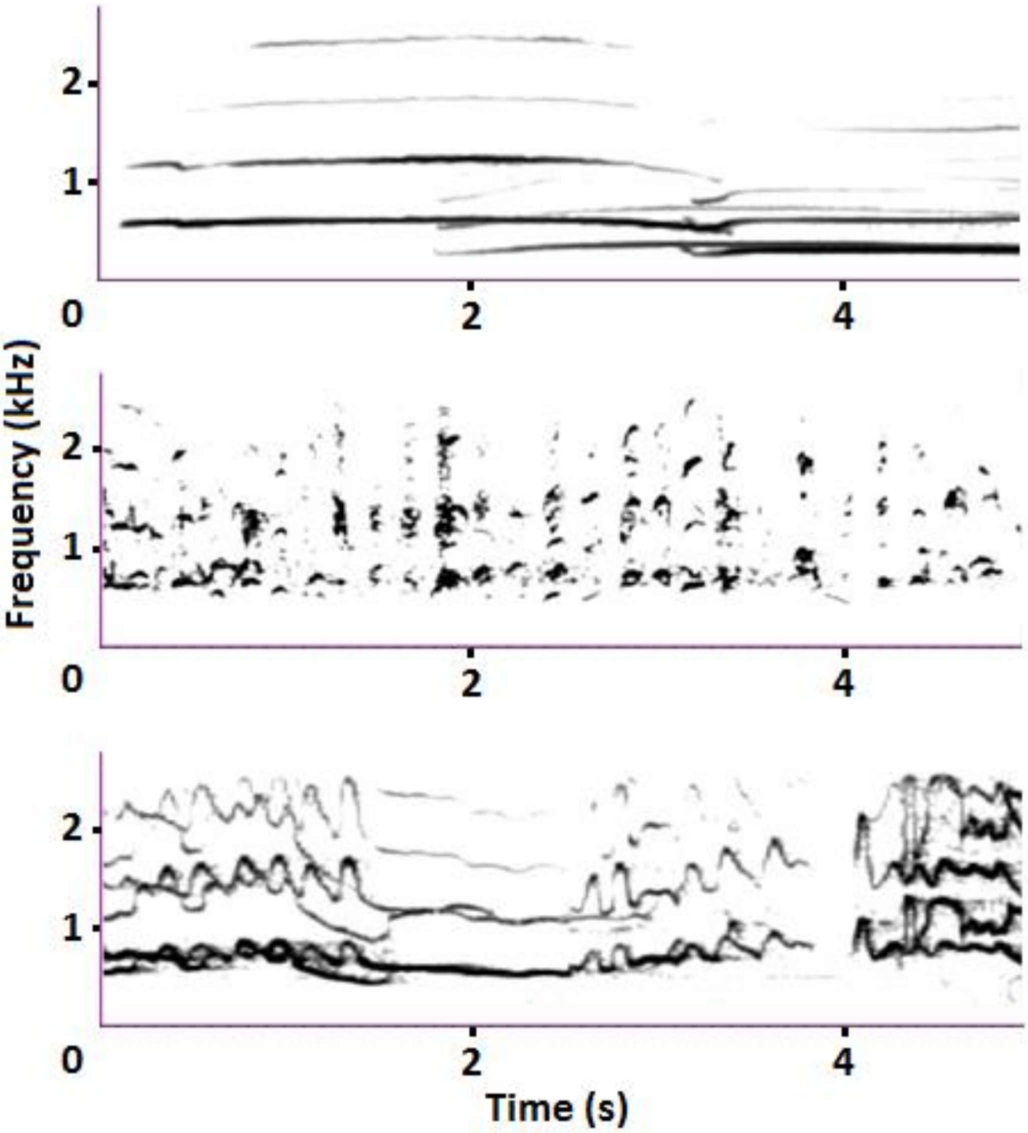

Figure 8.2.- Fragments of choruses including different types of vocalizations: howls (top); short vocalizations (middle); and long highly modulated vocalizations (bottom). Spectrogram: 2048 FFT (Fast Fourier Transform) points, Hann window.

For each data set, we conducted t-tests to select those variables that showed significant differences between choruses with and without pups. Then, in order to obtain the simplest models, we used a two-step modelling approach. We first built univariate generalized linear mixed models (GLMMs) using the selected significant variables from t-tests, with binomial error distribution and logit link to test for differences between choruses with and without pups. Pack was treated as a random effect to avoid pseudoreplication due to the existence of various choruses emitted with and without pups by the same packs. We selected the variables that showed significant differences between choruses with and without pups in the GLMMs and removed those that were highly correlated (Pearson correlation coefficient ≥ 0.9) and had high p values in the GLMMs to eliminate problems caused by colinearity. In a second step, we built a set of competing GLMMs considering all the possible combinations using the selected variables in each dataset (including the null model; i.e. the intercept-only model). We used Akaike's Information Criterion (AIC) to rank models, selecting the model with lowest AIC values (Burnham & Anderson 2002). We considered models with AIC differences ≤ 2 to have equal empirical support (Burnham & Anderson 2002). As we aimed to get the most applicable and simple model, among models with ΔAIC ≤ 2.0 we considered the most parsimonious model the one with the lowest number of parameters (Burnham & Anderson 2002). Moreover, cumulative AIC weights were calculated to evaluate the strength of each model. We calculated the percentage of correct classification of choruses making predictions from the models. All GLMMs were fitted in R software (R Development Core Team 2010) using the Lme4 package (Bates & Sarkar 2007).

8.3 RESULTS

We analysed 110 wolf chorus howls, 77 emitted only by adults and 33 that included pups (Table 8.2). Iberian wolf choruses were long vocalizations (26 to 183 s) with a mean (± SD) duration of 66 ± 27 s. AED was concentrated between 300 and 1900 Hz (minimum and maximum values of the variables *Freq5* and *Freq95* respectively). Howls were the main vocalization (more than 50% of the chorus duration) in 33% of cases, while the remaining 67% included highly modulated sounds, howl variations, barks, etc. (Fig. 8.2). The number of choruses composed mainly by howls was significantly greater for choruses emitted only by adults than those including pups ($\chi^2 = 7.609$; d.f. = 1; $P = 0.006$; Table 8.2).

Table 8.2.- Choruses analysed in this study. N: number of choruses. Main vocal types refer to the vocalizations present in more than 50% of the entire length of the chorus. The code assigned to each pack corresponds to the initials of pack location.

Age class	Pack	N	Main vocal types	
			Howls	Other
Without pups (only adults)	C1*	11	43%	57%
	C2*	2		
	C3*	3		
	C4*	3		
	CA*	2		
	CR1*	23		
	CR2*	6		
	D1*	3		
	D2*	2		
	F	2		
	PO	1		
	S	1		
	T	1		
With pups (adults and pups)	A	1	21%	79%
	F	2		
	F	3		
	PE	1		
	PO	8		
	R	2		
	T	2		
	TI	1		
	TO	1		
	TO	3		
With pups (only pups)	A	1	0%	100%
	C2*	2		
	P	1		
	PI	1		
	PO	4		

*Captivity packs

The acoustic energy of chorus howls with pups was concentrated at higher frequencies and showed values of entropy higher than in choruses without pups (Table 8.3). The values of most variables measured (85%) were different in choruses with and without pups (Table 8.3).

Table 8.3.- Differences between the acoustic energy distribution parameters obtained for choruses with and without pups (Student's t-test). *: significant differences.

Variable	Without pups Mean ± SD	With pups Mean ± SD	t	p
Q1Freq	510 ± 100	693 ± 139	-7.317	< 0.001*
Q3Freq	778 ± 215	1016 ± 237	-4.925	< 0.001*
IQRBW	268 ± 153	323 ± 158	-1.644	0.104
AggEntropy	4.75 ± 0.73	5.26 ± 0.44	-3.601	< 0.001*
AvgEntropy	2.84 ± 0.38	3.69 ± 0.44	-9.787	< 0.001*
CentFreq	606 ± 132	859 ± 189	-6.853	< 0.001*
Freq5	413 ± 66	543 ± 98	-6.832	< 0.001*
Freq95	1141 ± 340	1419 ± 268	-4.342	< 0.001*
BW90	729 ± 316	876 ± 266	-2.379	0.020*
MaxFreq	668 ± 208	955 ± 289	-5.030	< 0.001*
AED-M	851 ± 155	1036 ± 133	-5.778	< 0.001*
AED-SD	434 ± 70	450 ± 43	-1.347	0.181
AED-P	556 ± 132	812 ± 288	-4.793	< 0.001*

The best models considering the entire chorus were obtained for *CentFreq* and *Freq5* for a 75% amplitude threshold, and *CentFreq* for 90% amplitude threshold (Table 8.4). The best models for chorus segments were obtained for the mean value of *Freq5* and the mean value of *AvgEntropy* with a 75% amplitude threshold, and the standard deviation of *IQRBW* and mean and min of *Freq5* for a 90% amplitude threshold (Table 8.4).

Table 8.4.- GLMMs obtained considering the different data sets. The best models considering the AIC criterion and the minimum number of parameters are highlighted. df: number of parameters in the model; AICc: Akaike's information criterion; Delta: Delta AIC value; Weight: Akaike weight; R^2m: marginal R^2; R^2c: conditional R^2.

CHORUS75-models	**df**	**AICc**	**Delta**	**Weight**	**R^2m**	**R^2c**
CentFreq, Freq5	4	65.62	0.00	0.57	0.37	0.89
CentFreq	3	67.14	1.52	0.27		
Freq5	3	68.18	2.56	0.16		
Intercept	2	73.84	8.21	0.01		
CHORUS90-models	**df**	**AICc**	**Delta**	**Weight**	**R^2m**	**R^2c**
CentFreq	3	68.62	0.00	0.47	0.08	0.87
Q1Freq	3	69.42	0.80	0.32		
CentFreq, Q1Freq	4	70.58	1.97	0.18		
Intercept	2	73.84	5.22	0.03		
SEGMENTS75-models	**df**	**AICc**	**Delta**	**Weight**	**R^2m**	**R^2c**
Mean-AvgEntropy, Mean-Freq5	4	59.23	0.00	0.15	0.47	0.91
Mean-AvgEntropy, Min-Freq5	4	60.22	0.99	0.09		
Mean-Freq5	3	60.30	1.07	0.09		
Mean-AvgEntropy, Mean-Freq5, Min-Freq5	5	60.31	1.08	0.09		
Max-AvgEntropy, Mean-Freq5	4	60.88	1.66	0.06		
Mean-AvgEntropy, Mean-Freq5, Min-AED-P	5	61.15	1.92	0.06		
Max-AvgEntropy, Mean-AvgEntropy, Mean-Freq5	5	61.42	2.19	0.05		
Max-AvgEntropy, Min-Freq5	4	61.59	2.36	0.04		
Mean-Freq5, Min-Freq5	4	61.77	2.54	0.04		
Intercept	2	73.84	14.61	0.00		
SEGMENTS90-models	**df**	**AICc**	**Delta**	**Weight**	**R^2m**	**R^2c**
Desv-IQRBW, Mean-Freq5, Min-Freq5	5	41.75	0.00	0.23	0.90	0.94
Desv-IQRBW, Min-Freq5	4	43.08	1.33	0.12		
Desv-IQRBW, Mean-Freq5, Min-Freq5, Min-Q1Freq	6	43.15	1.40	0.12		
Desv-IQRBW, Mean-Freq5, Min-Freq5, Max-Q1Freq	6	43.53	1.78	0.10		
Desv-IQRBW, Mean-AvgEntropy, Mean-Freq5, Min-Freq5	6	43.98	2.23	0.08		
Desv-IQRBW, Min-Freq5, Min-Q1Freq	5	44.21	2.46	0.07		
Desv-IQRBW, Min-Freq5, Max-Q1Freq	5	44.56	2.81	0.06		
Desv-IQRBW, Mean-AvgEntropy, Min-Freq5	5	45.21	3.46	0.04		
Desv-IQRBW, Mean-AvgEntropy, Mean-Freq5, Min-Freq5, Min-Q1Freq	7	45.28	3.53	0.04		
Intercept	2	73.84	32.09	0.00		

Our final selected models using segments classified correctly 86-94% of cases, this percentage being lower for models considering the entire chorus (74-83%; Table 8.5). The best overall results were obtained for SEGMENTS90-model (Table 8.5). This model yielded the best values of R^2, explaining 94% of the variance and only 4% due to random effects (Table 8.4). Applying this model, 93.6% of choruses were correctly classified as with or without pups. The different approaches used showed differences in the accuracy of classification with respect to type I and type II errors (Table 8.5). Classification results for true positives (the model predicts the presence of pups and there were pups) were better for the analyses made dividing choruses into segments (i.e. when the model predicted the presence of pups, 87.9% of times the chorus effectively had pups applying the SEGMENTS90-model and this percentage decreased to 27.3% applying the CHORUS90-model). All the models had low percentages of false negatives; 3.9-6.5% of cases with no pups vocalizing the models wrongly predicted that there were pups.

Table 8.5.- Results obtained (cases correctly classified) applying the best GLMMs. Positive: there are pups vocalizing in the chorus; negative: the chorus is emitted by adults/subadults; true: model's prediction and reality agree; false: model's prediction and reality do not agree.

Model	% correctly classified	True positive	False positive	True negative	False negative
CHORUS75	82.7%	51.5%	48.5%	96.1%	3.9%
CHORUS90	73.6%	27.3%	72.7%	93.5%	6.5%
SEGMENTS75	85.5%	60.6%	39.4%	96.1%	3.9%
SEGMENTS90	93.6%	87.9%	12.1%	96.1%	3.9%

8.4 DISCUSSION

We studied the acoustic energy distribution of chorus howls emitted by wolves to investigate whether the acoustic properties of chorus howls could encode information regarding the presence of vocalizing pups. The acoustic energy of chorus howls was concentrated at lower frequencies when there were no pups vocalizing. Most of the vocalizations included in chorus howls are harmonic sounds, and harmonic signals emitted by terrestrial carnivores undergo a decrease in fundamental frequency, upper frequency limit, frequency range, and harmonic with highest intensity with increasing age (Peters & Wozencraft 1989). It has been reported that some wolf vocalizations show age-related changes in the fundamental frequencies, which probably reflects, at least in part, the growth of the vocal tract (Coscia et al. 1991). For instance, the fundamental frequency of howls drops from an average of about 1100 Hz at two weeks (Coscia 1995) to about 350 Hz by 6-7 months of age (Harrington & Mech 1978a; Harrington 1989). In our study, the frequency that divides the signal in two bands containing 50% of the acoustic energy increases from an average of 600 Hz in choruses without pups to 850 when there are pups vocalizing.

Besides frequency variables, entropy values of choruses with pups were higher than those obtained for choruses without pups. The entropy measures the disorder in a sound by analysing the energy distribution: higher entropy values correspond to greater disorder in the sound whereas a pure tone with energy in only one frequency bin would have zero entropy (Charif et al. 2010). We found that chorus howls with pups vocalizing included a greater proportion of vocalizations other than howls, which could be the reason for the differences obtained related to entropy.

Our results show that, despite their complexity, chorus howls can be used to determinate quantitatively, with a high degree of accuracy, that a pack of wolves contains pups based on the analysis of the acoustic energy distribution. When the aim of the survey is to estimate reproductive rates, population trends, or when management decisions depend on the results of the survey, it is important to apply the most reliable and accurate methodology. Managers need reliable and repeatable methods to obtain information about wildlife population dynamics. Predictive models are a standardized, objective and reliable method to estimate the presence of pups in a chorus. Estimates made by listening to chorus howls (see chapter 6) result in 64% correct assessment of pup presence, far below the 93.6% correct classifications obtained applying the SEGMENTS90-model. On the other hand, the probability of incorrect predictions and the type of error has to be taken into account by managers when decisions deal with endangered or harvested populations. To wrongly claim the presence of the pups when there are in fact no pups is probably the most undesirable mistake, which can be greatly minimized applying predictive models (72% false negatives by listening vs. 3.9% by model prediction, Figure 8.3).

We built different models to predict the presence of pups in a chorus howl. Each approach has advantages and disadvantages. To evaluate which is the best approach to analyse a chorus howl we have to take into account type I and II errors. The major difference between CHORUS and SEGMENTS models is related to true and false positives (Table 8.5). When there are pups vocalizing, the CHORUS75-model detects them (true positive) 50% of the time, whereas this percentage increases to 87.9% applying the SEGEMENTS90-model. False negatives (there are no pups but the model predicts the presence of pups) are quite similar between CHORUS and SEGMENTS models. In addition, CHORUS-based models are less time-consuming than SEGMENTS-based models[3]. Thus, CHORUS models underestimate the presence of pups but can be carried out more quickly than SEGMENTS models. However, when possible, we recommend carrying out the SEGMENTS90-model.

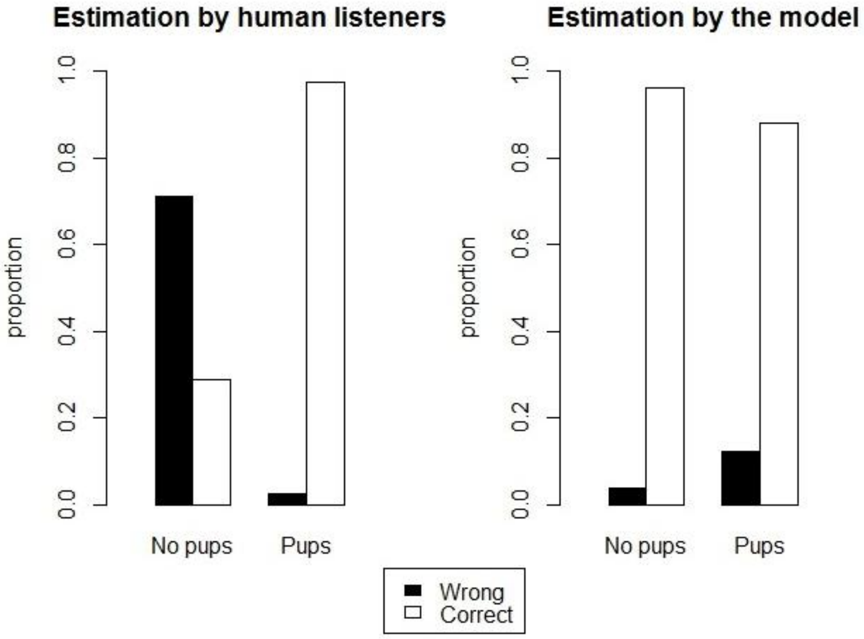

Figure 8.3.- Comparison between estimates of the presence of pups by acoustic censusing and applying predictive models.

We are aware that the inclusion of choruses without visually-confirmed pups vocalizing may compromise the interpretation of our results. However, all choruses with pups included to build the models were chorus howls emitted during the breeding season at *rendezvous* sites by packs of wolves that reproduced successfully. Hence, it seems safe to conclude that the models can indeed be used to estimate the presence of pups in a pack of wolves. Further research is needed to clarify this issue.

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[3] Although no exact measures of time to conduct the analysis of a chorus were made, we estimate that the analysis of a chorus with a CHORUS-based model can be completed in 30 min and the same chorus analysed with a SEGMENTS-based model would require 2-3 hours.

8.5 ACKNOWLEDGMENTS

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Presence of pups

PART III. SYNTHESIS OF RESULTS AND FINAL CONCLUSIONS

Photo courtesy of José Larrosa

CHAPTER 9. SYNTHESIS OF RESULTS AND CONCLUSIONS

PART I. IBERIAN WOLF ACOUSTIC COMMUNICATION

9.1 Adult close-range vocal repertoire of Iberian wolves

We described the close-range vocal repertoire of Iberian wolves based on the analysis of 678 vocalizations emitted by 29 Iberian wolves (26 that belonged to 11 social groups held in captivity, and three wild wolves caught in footsnare traps for research purposes). The close-range vocal repertoire of Iberian wolves comprises five broad vocal types (barks, growls, whines, yelps, and moans), mixed sounds resulting from the combination of different vocal types, and non-vocal sounds. We identified 12 vocal classes (Table 9.1), three of which can be further subdivided into vocal subclasses, and two non-vocal sounds:

1 Noisy sounds, bark-like

- **1.1 Woofs** are the shortest, lowest-frequency and most noisy (highest values of entropy) bark-like vocalizations. Wolves emitted woofs in wolf-human interactions. Woofs apparently acted as alarm signals.

- **1.2 Agonistic barks** are short noisy sounds, with the highest values of minimum, maximum and dominant frequencies of all bark types. These barks were emitted in agonistic contexts by the individual showing dominance displays, and in wolf-human interactions, towards a human close to the fence surrounding the enclosure.

- **1.3 Disturbance barks** are the most harmonic barks, with mean fundamental frequencies around 450 Hz. Disturbance barks were only recorded in wolf-human interactions, directed at humans by wild wolves caught in traps. Animals emitting disturbance barks also displayed escape behaviours when humans approached.

2 Noisy sounds, growl-like

- **2.1 Growls** are vocalizations that last up to several seconds, and dominant frequencies about 500 Hz, produced during long breathing exhalations. Growls were emitted in all the contexts defined (agonistic, mating, other social interactions, as well as in wolf-human interactions).

- **2.2 Snarls** are produced during long breathing exhalations and differ from growls in the range of frequencies that comprise the acoustic energy distribution, both minimum and maximum frequencies being lower for growls. Snarls and growls were emitted in the same contexts.

- **2.3 Short exhalations.** We identified two growl-type sounds emitted during short pulsed exhalations. The first type, the short exhalations, were emitted together with growls and snarls. Short exhalations were emitted in social interactions during play between the reproductive pair and in wolf-human interactions.

- **2.4 Courtship growls.** The second vocal class of pulsed growls defined is the courtship growl, emitted by wolves of both sexes in mating contexts.

3 Harmonic sounds, whine-like

- **3.1 Whines** are harmonic sounds, from tenths of a second to 1 s in duration, with a mean fundamental frequency about 500-600 Hz, emitted in all social contexts (agonistic, mating and other social contexts), associated to submissive displays. Sometimes whine-type vocalizations include two different fundamental frequencies emitted simultaneously, the lowest fundamental about 500 Hz (tonal), and a second fundamental about 4000 Hz (whistle). We defined three different vocal subclasses based on the presence of tonal and whistle part: **tonal-whines** (tonal part present, whistle absent), **tonal-whistle-whines** (both tonal and whistle part present), and **whistle-whines** (only whistle part present).

- **3.2 Whimpers** are short whine-like vocalizations emitted consecutively in bouts in the same contexts as whines. As for whines, we defined three different vocal subclasses based on the presence of tonal and whistle part: **tonal-whimpers, tonal-whistle-whimpers,** and **whistle-whimpers.**

4 Harmonic sounds, yelp-like

- **4.1 Yelps** are short, high pitched, loud, harmonic sounds, emitted during agonistic interactions. Wolves emit yelps apparently to prevent or stop an ongoing attack. One particular type of yelp consists of a sequence of short yelps increasing and decreasing the fundamental frequency, the laughing-yelp. As for whine-type sounds, yelps can also incorporate a whistle part, and we distinguished three yelp subclasses: **tonal-yelps**, **tonal-whistle-yelps**, and **whistle-yelps**.

5 Harmonic sounds, moan-like

- **5.1 Moans** are vocalizations lasting from tenths of a second to several seconds, lower in pitch than whines (fundamental from 100 to 300 Hz), emitted in all social interactions (agonistic, mating and other social interactions).

Table 9.1.- Acoustic parameters of vocal classes identified in this study (mean ± SD). Dur: duration; Freqmin: minimum frequency; Freqmax: maximum frequency; Domfreq: dominant frequency; Fundmin: minimum fundamental frequency; Fundmax: maximum fundamental frequency; Fundmean: mean fundamental frequency; Whistmin: minimum fundamental frequency of whistle sound; Whistmax: maximum fundamental frequency of whistle sound.

Vocal class	Dur (s)	Freqmin (Hz)	Freqmax (Hz)	Domfreq (Hz)	Fundmin (Hz)	Fundmax (Hz)	Fundmean (Hz)	Whistmin (Hz)	Whistmax (Hz)
Woof	0.12 ± 0.06	170 ± 103	1400 ± 852	509 ± 395	-	-	-	-	-
Agonistic bark	0.15 ± 0.05	505 ± 205	1873 ± 443	924 ± 293	-	-	-	-	-
Disturbance bark	0.19 ± 0.05	318 ± 72	1131 ± 312	634 ± 293	361 ± 75	504 ± 55	462 ± 43	-	-
Growl	1.54 ± 0.79	146 ± 132	994 ± 482	476 ± 322	-	-	-	-	-
Snarl	1.43 ± 0.78	229 ± 186	1350 ± 722	489 ± 292	-	-	-	-	-
Short exhalation	0.33 ± 0.24	437 ± 265	1761 ± 340	993 ± 291	-	-	-	-	-
Courtship growl	0.73 ± 0.47	229 ± 197	1821 ± 859	788 ± 386	-	-	-	-	-
Whine	0.54 ± 0.29	-	-	-	493 ± 130	634 ± 154	553 ± 128	4419 ± 825	5037 ± 1039
Whimper	0.18 ± 0.09	-	-	-	535 ± 166	635 ± 167	586 ± 162	3956 ± 699	4357 ± 805
Yelp	0.14 ± 0.09	-	-	-	1039 ± 283	1191 ± 292	1146 ± 277	3986 ± 690	4889 ± 809
Moan	1.2 ± 1.0	-	-	-	188 ± 79	338 ± 173	217 ± 93	-	-
Growl moan	1.0 ± 0.4	-	-	-	85 ± 24	127 ± 36	99 ± 18	-	-

- **5.2 Growl moans** are moan-like vocalizations emitted at lower frequencies than moans (fundamental from 60 to 150 Hz). Growl moans were emitted during courtship and dominance displays by the reproductive pair.

6 Non-vocal sounds

- **6.1 Scratches** (see description of scratching in table 2.4, chapter 2) were displayed in agonistic contexts, after urine deposition by the reproductive pair.

- **6.2 Teeth snapping** were recorded in agonistic contexts by a female that was being attacked by the alpha female.

We identified most of the major vocal types described for wolves by Schassburger (9/10 considering only close-range vocalizations). The main differences between Schassburger’s catalogue and the Iberian wolf’s repertoire identified in this study are as follows:

1. The dominant frequencies of bark-like sounds are higher for Iberian wolves than for North American wolves.
2. We identified three bark-like vocal classes: woofs, agonistic and disturbance barks. Schassburger considered only woof and barks as two different vocal classes.
3. The dominant frequencies in growls are higher for Iberian wolves. In contrast, the dominant frequencies in snarls are higher for North American wolves.
4. Schassburger did not describe pulse growls.

5. Whine, whimper and yelp subclasses are defined differently. Schassburger defined two forms, full and whistle, based on the sound's quality to the human ear (resonant vs. whistle-like). Both full and whistle forms could include tonal and whistle parts simultaneously. We defined three subclasses based on the presence/absence of tonal and whistle parts.
6. The fundamental frequency of yelps reported by Schassburger for North American wolves is much lower than that found in this study. However, Schassburger considered that yelps have a "supressed" fundamental and the first visible frequency band corresponds to the third harmonic of the fundamental, whereas in this study we defined the fundamental as the first visible frequency band (we found no reason to consider a supressed fundamental), which may explain this difference.
7. Our moan's sample did not allow us to clearly distinguish between whine-moans and moans according to Schassburger.
8. Schassburger's classification did not include any non-vocal sounds.

Other conclusions relating to the close-range vocal repertoire of Iberian wolves and the comparison with other canids' vocal repertoires are as follows:

- Iberian wolves use a graded acoustic communication system, comprised by mixed sounds, transitions, and gradations.
- We found differences in barks and growls emitted in different contexts, providing the first evidence of context specificity of vocalizations in the wolf.
- Wolf vocalizations that include a tonal and a whistle part should be considered non-linear vocalizations, presumably produced by biphonation. Tonal and whistle parts are two different sounds emitted simultaneously with a different fundamental frequency.

- Social complexity does not seem to affect the size of the repertoire of close-range vocalizations in canids. Rather, vocalization rate may be the most important difference in relationship to sociality.
- It seems that phylogenetic relationships explain part of the variability found in the acoustic signals emitted by canids. Most differences in vocalizations are between clades (i. e. there are clear differences among Red-fox-like, South American and Wolf-like clades).

9.2 Iberian wolf howls

We analysed 176 howls emitted by 11 wolves held in captivity in eight different enclosures, each with 1–5 individuals. Iberian wolf howls are long duration (1.1-12.8 s), harmonic sounds (1-18 harmonics), with a mean fundamental frequency between 270 and 720 Hz. Fundamental frequencies in howls range from 92 to 1116 Hz. The coefficient of frequency modulation ranges from 0.21 to 6.72, and the range of the coefficient of frequency variation is between 2.03 and 44.63. Iberian wolf howls usually have inflexion points (1-15) and discontinuities (1-8) in the fundamental frequency. The maximum fundamental frequency occurs in most cases (79% of the howls analysed) during the first quarter of the howl, and the minimum during the last (78%). The fundamental peak amplitude occurs in the first half of the howl (83%).

The two acoustic characteristics that best distinguish each howl are the presence of frequency discontinuities and frequency modulations. Thus, howls can be arbitrarily assigned to one of four groups based on these two attributes:

- Flat howls: relatively constant frequency howls, without frequency discontinuities and with low values of both frequency modulation and variation. Audiospectrogram shape flat, not wavy.

- Continuous wavy howls: howls without frequency discontinuities and with frequency modulations (i. e. wavy-shaped audiospectrogram).

- Breaking howls: howls with large (21 to 250 Hz) frequency discontinuities. Low values of the coefficient of frequency modulation. Audiospectrogram shape not wavy.

- Breaking wavy howls: howls with large (21 to 250 Hz) frequency discontinuities and numerous frequency modulations. Wavy-shaped audiospectrogram.

Iberian wolf howls are similar to howls from other populations based on published data. Comparing our results with those reported by Tooze et al. (1990) we found statistically significant differences only in the number of frequency discontinuities. The Iberian wolf howls analysed have less frequency discontinuities than the howls recorded from seven North American timber wolves. Nevertheless, this result has to be cautiously interpreted since all the wolves in our study were adults, whereas Tooze and colleagues (Tooze 1987; Tooze et al. 1990) included individuals belonging to different age classes.

Iberian wolf howls contain information regarding individual identity. Mean fundamental frequency, maximum frequency of the fundamental, number of harmonics, and frequency modulation are the most important variables discriminating among individuals.

9.3 Recognition of familiarity on the basis of howls

We investigated the acoustic basis of individual discrimination in wolf howls by means of a playback experiment (based on the habituation-discrimination paradigm) conducted with a pack of captive wolves. Our results show that wolves detect changes in the fundamental frequency of howls outside their natural range of variability, and changes in the frequency modulation pattern of howls. Furthermore, wolves detect differences between different howls. Wolves show habituation to all new stimuli when presented repeatedly, with the exception of responses to unfamiliar howls of unfamiliar wolves.

The results of the playback experiments reported here provide the first evidence that wolves can discriminate among howls based solely on their acoustic structure. The response to unfamiliar howls emitted by a familiar wolf decreased across trials suggesting a process of habituation. However, repeated exposure to equally unfamiliar howls emitted by unfamiliar wolves did not result in habituation, and the response was more intense for wolves emitting howls that differed in the coefficient of frequency modulation with respect to the familiar howls. Frequency modulation seems to play an important role in acoustic discrimination.

However, that wolves discriminate between a familiar wolf and unfamiliar individuals does not necessarily imply that true individual recognition actually exists. Additional experiments may provide crucial insights to understanding the role of acoustic communication in wolf recognition.

PART II. APPLYING BIOACOUSTICS TO MONITOR WOLF POPULATIONS

9.4 Human estimates of the number of wolves vocalizing in a chorus and the presence of pups by acoustic censusing

The elicited-vocalization technique has been widely used to estimate the number of individuals in wolf packs and confirm reproduction during the breeding period. These estimates are usually made by acoustic censusing: researchers listen to the chorus howls in the field and estimate how many wolves are howling and whether there are pups vocalizing. However, no attempt to investigate the accuracy of these estimates has been made.

We ran “chorus tests” on 205 human subjects (forest rangers from Sweden and Spain and a small group of subjects with no previous experience with wolves or wolf vocalizations). A test consisted of listening to five complete chorus howls, selected randomly from a sample of 22 recordings of chorus howls emitted by 14 packs of known composition. We quantified the accuracy of estimates made by acoustic censusing, and concluded that these estimates are not very accurate.

The overall number of wolves that participate in a chorus howl estimated by the rangers was correct 31% of times, 38% of times the observer underestimated the number of wolves howling and 30% of estimates were overestimates of the actual number of wolves vocalizing. The experience and belonging to the rangers/unexperienced group did not affect the accuracy of the estimates. Only the actual number of wolves affected the correct estimate: as the number of wolves participating in a chorus howl increased, the proportion of correct estimates decreased. The actual number of wolves vocalizing also affects the sign of the difference between estimates and reality: estimates for choruses emitted by a low number of wolves tend to be overestimated, whereas choruses emitted by larger group sizes yield human underestimates.

Assessment of the presence of adults howling in a chorus by rangers was correct 85% of the times, while the proportion of right answers decreased to 64% for estimates of the presence of pups. The experience of the observer and belonging to the unexperienced group vs. rangers had no effect on the correct determination of the presence of pups or adults.

The observers correctly identified adults howling 88% of cases, and in 98% of cases the rangers determined correctly that there were pups howling. In the absence of adults, the observers wrongly determined that there were adults 49% of times, while this percentage increased to 72% determining the presence of pups when there were no pups vocalizing.

The fact that listeners often report that pups contribute to a chorus howl when in fact there are no pups is the most potentially misleading mistake. Such false positives could lead to overestimating reproduction, which may hinder conservation and management efforts.

9.5 Bioacoustic analysis of wolf chorus howls (I): estimates of group size

We analysed recordings of 56 chorus howls emitted by 2-7 wolves belonging to 18 different packs recorded at 12 different locations to study whether we could obtain accurate information about the number of wolves participating in a chorus. Our results show that it is possible to predict accurately the number of wolves howling in a chorus by means of bioacoustic analyses. The number of wolves participating in a chorus is highly correlated to the maximum number of wolves vocalizing simultaneously. In most cases (73%), the maximum number of wolves vocalizing simultaneously coincides with the actual group size. Other two variables correlated with group size are the average number of wolves vocalizing simultaneously and duration of the chorus. As the number of wolves increases, the number of wolves vocalizing simultaneously and the duration of the chorus also increase.

The best predictive model obtained included the maximum number of wolves vocalizing simultaneously (*Max*) and chorus duration (*Dur*), and explained 82% of the variability. The formula to predict the number of wolves from our data includes the *Intercept*, *Max*, and *Dur*:

$$num = \exp(-1.88 + 0.67 * \mathrm{Max} - 0.003 * Dur) + 2$$

Applying the formula, in 79% of our choruses the model predicted the correct number of individuals, 12% of estimates were overestimated and 9% underestimated. The estimated number differed in one individual 16% of cases (7.1% underestimated and 8.9% overestimated); two individuals 3.6% of cases (1.8% underestimated and 1.8% overestimated); and the difference between the estimate and the real number was three individuals in only 1.8% of the cases (overestimated).

The predictive model provides more accurate results (79% of correct estimates) than estimates obtained by acoustic censusing (32%).

9.6 Bioacoustic analysis of wolf chorus howls (II): presence of pups

We analysed 110 wolf chorus howls, 77 emitted only by adults and 33 that included pups to investigate whether we could obtain information about the presence of pups vocalizing in a chorus. Iberian wolf choruses are long vocalizations (26 to 183 s) with a mean (± SD) duration of 66 ± 27 s. Acoustic energy distribution (AED) is concentrated between 300 and 1900 Hz. Howls constitute the main vocalization in 33% of cases, while the remaining 67% are composed by highly modulated sounds, howl variations, barks, etc. The number of choruses composed mainly by howls is significantly greater for choruses emitted only by adults than those including pups.

Chorus howls with pups concentrate the acoustic energy at higher frequencies and show values of entropy higher than choruses without pups. We used four different approaches for determining the presence of pups in a chorus using AED: entire choruses (all the variables were measured for the entire length of the chorus) applying 75% (CHORUS75-model) and 90% (CHORUS90-model) amplitude thresholds, and choruses divided into segments (we measured the acoustic features for each segment of 5 s of duration that comprises a chorus and calculated mean, minimum, maximum and standard deviation for each chorus) with 75% and 90% amplitude thresholds (SEGMENTS75-model and SEGMENTS90-model respectively).

Our final selected models considering the entire chorus classified correctly 74-83% of cases, with this percentage being higher for models using choruses divided into segments (86-94%). The best overall results were obtained for the SEGMENTS90-model. This model yielded the best values of R^2, explaining 94% of the variance and only 4% due to random effects. Applying this model, 93.6% of choruses were correctly classified as with or without pups.

Classification results for true positives (the model predicts the presence of pups and effectively there were pups) were better for the analyses made dividing choruses into segments, and all the models had low percentages of false negatives: the models predicted that there were pups 3.9-6.5% of cases with no pups vocalizing.

As for the models to predict the number of wolves participating in a chorus, the predictive models obtained to determine the presence of pups provide much more accurate results than estimates by acoustic censusing. The best model predicts the presence of pups 94% of times correctly, whereas estimates made by human listeners are correct only 64% of cases. Furthermore, in 72% of cases with no pups howling, the listeners estimated wrongly the presence of pups. This percentage drops to 3.9% applying predictive models.

PART IV. RESUMEN EN IDIOMA OFICIAL

Photo courtesy of Francisco J. Lema

PARTE I. COMUNICACIÓN ACÚSTICA EN EL LOBO IBÉRICO

El lobo (*Canis lupus*) es un carnívoro social cuya unidad básica de organización social es la manada, formada básicamente por una pareja reproductora y sus descendientes de varios años (Mech 1970, 1999). El número medio de lobos que integra una manada (tamaño de grupo) oscila entre 3 y 11 individuos (Fuller *et al*., 2003) y, en condiciones naturales, depende en gran medida de la disponibilidad de alimento (Mech & Boitani, 2003).

Uno de los comportamientos más característicos y llamativos de los lobos es la emisión de aullidos para comunicarse a largas distancias. Quizá por este motivo la comunicación acústica de los lobos ha sido objeto de estudio en un número considerable de publicaciones. En estos estudios se han analizado, entre otros, el repertorio vocal de los lobos (Schassburguer, 1993), la ontogenia de las vocalizaciones (Coscia, 1995), características individuales de los aullidos (Theberge & Falls, 1967; Tooze *et al*., 1990), la importancia de los aullidos en el mantenimiento del territorio (Harrington & Mech, 1978b), y la estructura acústica de los coros de aullidos (Harrington, 1989).

Sin embargo, la mayor parte de estos trabajos han sido realizados con lobos de Norteamérica. En la Península Ibérica vive la población de lobos más numerosa de Europa Occidental. Esta población ha permanecido aislada del resto de los lobos europeos durante más de un siglo (Vilá, 1993). Algunos autores han propuesto la pertenencia de esta población a la subespecie *Canis lupus signatus*, en función de características morfológicas (Vilá, 1993). En este estudio se describen, por primera vez, las señales acústicas que emiten los lobos ibéricos, lobos que presentan unas características morfológicas (y quizá también genéticas) propias y que viven en hábitats distintos a los de los lobos norteamericanos, con un mayor grado de antropización (Boitani, 2000). Además estudiamos el papel de los aullidos en el reconocimiento individual.

Repertorio de vocalizaciones utilizadas en la comunicación a corta distancia

Además de los aullidos (vocalizaciones para la comunicación a largas distancias), los lobos ibéricos emiten distintas vocalizaciones que les permiten comunicarse a corta distancia. Hemos descrito el repertorio vocal del lobo ibérico para la comunicación a corta distancia en base al análisis de 678 vocalizaciones emitidas por 29 lobos ibéricos. Los lobos emiten sonidos con estructura acústica ruidosa (energía distribuida en una amplia franja de frecuencias), armónica (energía distribuida en una estrecha franja de frecuencias y sus armónicos o múltiplos enteros) y sonidos no vocales (producidos por otros mecanismos distintos a la vibración de las cuerdas vocales). En total identificamos 14 clases diferentes de vocalizaciones, 12 vocales y las otras dos no vocales:

1 Sonidos con estructura acústica ruidosa: ladridos (bark-like)

- **1.1 Woofs**, son los ladridos más cortos (0.12 ± 0.06 s), con la energía acústica concentrada entre los 170-1400 Hz. Los lobos ibéricos estudiados emitieron woofs en interacciones lobo-hombre. Estos sonidos supuestamente actúan como señal de alarma, alertando al resto de los componentes de la manada de algún peligro/perturbación.

- **1.2 Agonistic barks,** ladridos con una duración de 0.15 ± 0.05 s, con la energía acústica concentrada entre los 500-1900 Hz. Se registraron este tipo de ladridos en contextos agonísticos, emitidos por el individuo que mostraba comportamientos de dominancia y en interacciones lobo-hombre, ante una persona situada junto a la valla del recinto.

- **1.3 Disturbance barks,** ladridos de 0.19 ± 0.05 s de duración. Son los ladridos que presentan estructura acústica más armónica, con la frecuencia fundamental alrededor de 450 Hz. Este tipo de ladridos sólo se registró al aproximarse una persona a lobos salvajes capturados en trampas (capturas en vivo en el marco de proyectos de investigación). A diferencia de los agonistic barks, cuando las personas se aproximaban al lobo, éste exhibía también comportamientos de huida.

2 Sonidos con estructura acústica ruidosa: gruñidos (growl-like)

- **2.1 Growls,** gruñidos producidos durante largas exhalaciones. Son vocalizaciones de duración variable (desde décimas de segundo hasta varios segundos) y frecuencias dominantes alrededor de 500 Hz (energía acústica concentrada entre 146 y 994 Hz). Los lobos objeto de estudio emitieron growls en todos los contextos definidos, usualmente asociados a comportamientos de amenaza, dominancia y al juego.

- **2.2 Snarls,** son también gruñidos que se producen durante largas exhalaciones. La única diferencia entre growls y snarls es el rango de frecuencias que abarcan: tanto la frecuencia mínima como la máxima es menor en los growls (el rango de frecuencias de los snarls abarca entre 229 y 1350 Hz). También se grabaron snarls en todos los contextos definidos, asociados a comportamientos de amenaza, dominancia y juego.

- **2.3 Short exhalations**. Además de los gruñidos producidos durante largas exhalaciones, hemos definido dos tipos de gruñidos emitidos durante cortos pulsos de exhalaciones consecutivas. Los gruñidos incluidos en el primer tipo, short exhalations, fueron emitidos 1) en interacciones sociales durante el juego entre la pareja reproductora, y 2) en interacciones lobo-hombre hacia personas acercándose al recinto. Son gruñidos cortos (0.33 ± 0.24 s) con la energía concentrada entre los 400 y 1800 Hz.

- **2.4 Courtship growls.** El otro tipo de gruñidos cortos emitidos en pulsos son los courtship growls, emitidos por individuos de ambos sexos durante el cortejo. Son algo más largos que los short exhalations (0.73 ± 0.47 s) y concentran la energía acústica entre los 200 y los 1800 Hz.

3 Sonidos con estructura acústica armónica: gemidos (whine-like)

- **3.1 Whines,** son sonidos armónicos con una duración desde décimas de segundo a un segundo, valores medios de la frecuencia fundamental alrededor de 500-600 Hz y emitidos en todos los contextos sociales, relacionados con comportamientos de sumisión. Los gemidos en ocasiones presentan dos frecuencias fundamentales: sonido tonal (frecuencias fundamentales alrededor de 500 Hz) y otra frecuencia fundamental alrededor de 4000 Hz, que al oído humano suenan de forma parecida a un silbido (whistle). Incluso en ocasiones sólo emiten la parte correspondiente al silbido. Hemos definido tres subclases en función de la presencia de parte tonal y silbido: **tonal-whines** (parte tonal presente y silbido ausente), **tonal-whistle-whines** (partes tonal y silbido presentes), y **whistle-whines** (sólo el silbido presente).

- **3.2 Whimpers,** son gemidos cortos que los lobos emiten en secuencias de hasta 25 consecutivos, en los mismos contextos que los whines. Son más cortos que los whines (0.18 ± 0.09 s) y también tienen frecuencias fundamentales entre 500-600 Hz. Al igual que en el caso de los whines, hemos definido tres subclases en función de la presencia de parte tonal y silbido (whistle): **tonal-whimpers, tonal-whistle-whimpers,** y **whistle-whimpers**.

4 Sonidos con estructura acústica armónica: quejidos (yelp-like)

- **4.1 Yelps,** sonidos armónicos, cortos (0.14 ± 0.09 s), con frecuencias fundamentales entre 1000 y 1200 Hz, emitidos durante interacciones agonísticas. Los lobos emiten quejidos aparentemente para prevenir o detener un ataque. Al igual que en los gemidos, los lobos también pueden emitir quejidos con parte tonal y silbido. De este modo, siguiendo el mismo criterio que para los gemidos, distinguimos tres subclases de quejidos: **tonal-yelps**, **tonal-whistle-yelps**, y **whistle-yelps**.

5 Sonidos con estructura acústica armónica: lamentos (moan-like)

- **5.1 Moan**, vocalizaciones con una duración que oscila entre décimas de segundo y varios segundos, emitidos a frecuencias más graves que los gemidos (fundamental entre 200-300 Hz), en todos los contextos de interacciones lobo-lobo definidos en el presente estudio.

- **5.2 Growl moans,** lamentos de un segundo de duración (1.0 ± 0.4 s), emitidos a frecuencias más graves que los moan (frecuencia fundamental entre 50 y 150 Hz). Este tipo de sonidos fueron emitidos en interacciones agonísticas y durante el cortejo.

6 Sonidos no vocales

- **6.1 Scratches,** sonidos que producen los lobos cuando rascan el suelo con las patas traseras o con las cuatro patas (ver definición en tabla 2.4, capítulo 2). En este estudio sólo los individuos que formaban parte de la pareja reproductora exhibieron este comportamiento.

- **6.2 Teeth snapping,** sonido que producen los dientes al chocar entre sí. Fue registrado sólo en contextos agonísticos por una hembra que estaba siendo atacada por la hembra dominante.

Hemos identificado las principales vocalizaciones del repertorio vocal de los lobos descritas por Schassburger (1993) a excepción del whine-moan. Las diferencias más importantes entre el repertorio de Schassburger (1993) y el repertorio vocal del lobo ibérico descrito en este estudio son:

- Las frecuencias dominantes de los ladridos y los gruñidos son más altas en los lobos ibéricos, mientras que las frecuencias dominantes de los snarls son mayores para lobos norteamericanos. No obstante, no se dispone de datos para poder realizar análisis estadísticos.
- En este estudio hemos identificado tres tipos de ladridos: woofs, agonistic barks, y disturbance barks, mientras que Schassburger sólo distinguió dos tipos de ladridos: el ladrido propiamente dicho y el woof.
- Schassburger no describió gruñidos cortos emitidos en pulsos (short exhalations y courtship growls).
- Los gemidos y quejidos en ocasiones presentan dos sonidos diferentes emitidos simultáneamente (tonal y silbido). Estos sonidos deben considerarse vocalizaciones no lineales compuestas por dos sonidos con frecuencias fundamentales diferentes (bifonación). Schassburger distinguió dos subclases o formas dentro de gemidos y quejidos: full y whistle, basándose en la sonoridad de la vocalización al oído humano (resonante vs. silbido). Ambas formas (full y whistle) según Schassburger pueden incluir partes tonales y silbido simultáneamente. Nosotros hemos realizado una subdivisión más objetiva, basada en la presencia/ausencia de parte tonal o silbido. De este modo distinguimos tres subclases: tonal (sólo parte tonal presente), tonal-silbido (partes tonal y silbido presente), y silbido (sólo silbido presente).

- Nosotros no hemos identificado whine-moans según Schassburger, aunque nuestro tamaño muestral de lamentos quizá es demasiado escaso y puede ser el motivo de no haber identificado todos los tipos posibles de lamentos.

El repertorio vocal de los lobos ibéricos es complejo debido a que existe una alta gradación dentro de una misma clase vocal, existen transiciones entre diferentes clases vocales y también incluye sonidos mixtos formados por la unión de clases vocales distintas. En este estudio hemos encontrado diferencias en ladridos y gruñidos emitidos en diferentes contextos, aportando la primera evidencia de especificidad basada en el contexto en vocalizaciones emitidas por los lobos.

De la comparación del repertorio vocal del lobo ibérico con repertorios publicados de otros cánidos se pueden extraer las siguientes conclusiones:

- En los cánidos, el grado de organización social no parece que influya en el tamaño del repertorio vocal. Especies poco sociales presentan muchas vocalizaciones diferentes y otras muy sociales presentan repertorios menos extensos. Lo más probable es que el grado de sociabilidad de los cánidos se vea reflejado en una mayor tasa de vocalización, más que en el número de vocalizaciones diferentes que conforma el repertorio vocal de la especie.
- A pesar de la dificultad de comparar repertorios vocales entre especies y la escasez de estudios publicados sobre repertorios vocales de cánidos, parece que la filogenia tiene un efecto importante en las señales acústicas que constituyen los repertorios de los cánidos. Las principales diferencias en las vocalizaciones se producen entre clados (hay claras diferencias entre los tres clados principales de cánidos: zorros, cánidos de Sudamérica y lobos).

Los aullidos de los lobos ibéricos. Comunicación a larga distancia

El sonido más característico que emiten los lobos es el aullido, señal acústica para comunicarse a largas distancias. En este estudio se han descrito los aullidos que emiten los lobos ibéricos en base a 176 aullidos emitidos por 11 lobos en cautividad. Los aullidos de los lobos ibéricos son sonidos largos (1.1-12.8 s), de estructura armónica (1-18 armónicos), con una frecuencia fundamental media entre 270 y 720 Hz. Los aullidos de los lobos normalmente tienen puntos de inflexión (1-15) y discontinuidades en la frecuencia (1-8).

Las dos características acústicas que mejor definen cada aullido son la presencia de discontinuidades de la frecuencia y la modulación de la frecuencia. De este modo, arbitrariamente hemos definido los siguientes tipos de aullidos basados en estos dos atributos:

- Aullidos planos: aullidos con frecuencia relativamente constante, sin discontinuidades en la frecuencia y con bajos valores de modulación y coeficiente de variación de la frecuencia. La forma de los audioespectrogramas es plana, no ondulada.

- Aullidos ondulados continuos: aullidos sin discontinuidades en la frecuencia y con modulaciones (i.e. audioespectrograma con forma ondulada).

- Aullidos quebrados: aullidos con discontinuidades en la frecuencia (desde 21 hasta 250 Hz). Bajos valores del coeficiente de la modulación de la frecuencia, audioespectrograma con forma no ondulada.

- Aullidos ondulados quebrados: aullidos con discontinuidades de la frecuencia (21 a 250 Hz) y numerosas modulaciones de la frecuencia. Audioespectrograma con forma ondulada.

Comparando nuestros resultados con los del único trabajo en el que se realiza una descripción detallada de los aullidos de los lobos (Tooze et al. 1990) encontramos diferencias significativas sólo para una variable, el número de discontinuidades de la frecuencia. Los aullidos de los lobos ibéricos analizados tienen menos discontinuidades que los aullidos grabados para siete lobos de Norte América. Sin embargo, estos resultados hay que interpretarlos con cautela, dado que todos los lobos de nuestro estudio eran adultos, mientras que Tooze et al. (1990) incluyeron también en los análisis individuos de un año de edad.

Los aullidos contienen información relativa a la identidad individual, cada lobo aúlla de una forma característica. Los resultados del análisis de funciones discriminantes identificaron la frecuencia fundamental media, la frecuencia fundamental máxima, el número de armónicos y la modulación de la frecuencia como las variables más importantes para la discriminación entre individuos. Sin embargo, el hecho de que la estructura acústica de los aullidos permita discriminar individuos no implica que los lobos usen esta información para el reconocimiento individual.

La utilización de la estructura acústica de los aullidos en el reconocimiento individual. Experimento de playback

En el capítulo 4 investigamos con más detalle la discriminación individual mediante aullidos en los lobos. Para ello llevamos a cabo un experimento de playback con una manada de lobos en cautividad. Este experimento tenía como objetivo determinar la habilidad de los lobos para discriminar entre: a) aullidos manipulados (alterando los parámetros acústicos que mejor discriminan entre individuos (frecuencia fundamental y modulación de la frecuencia), y b) distintos aullidos emitidos por lobos diferentes.

El diseño experimental se basó en el paradigma de habituación-discriminación (Friedman, 1972), utilizando estímulos creados a partir de aullidos de lobos grabados previamente en cautividad. Nuestros resultados mostraron que los lobos detectan cambios en la frecuencia de los aullidos fuera del rango natural de variabilidad intraindividual, así como cambios en el patrón de modulación de la frecuencia. Por el contrario, las manipulaciones de la frecuencia fundamental y el coeficiente de modulación de la frecuencia dentro del rango de variación intraindividual no provocaron ninguna respuesta de los lobos. Además, los lobos detectaron diferencias entre aullidos diferentes, incluso entre diferentes aullidos emitidos por el mismo individuo al cual previamente se habían familiarizado. Los lobos mostraron habituación a nuevos estímulos presentados repetidamente, con la excepción de los aullidos emitidos por individuos desconocidos.

Los resultados del experimento de playback ofrecen la primera evidencia de que los lobos pueden discriminar entre aullidos en función de su estructura acústica. La respuesta a distintos aullidos emitidos por el lobo familiar disminuyó con los ensayos siguiendo un patrón de habituación. Sin embargo, la repetida exposición a aullidos desconocidos pero emitidos por lobos desconocidos no provocó esa habituación, y fue más intensa para lobos emitiendo aullidos con grandes diferencias en el coeficiente de modulación de la frecuencia con respecto a los aullidos a los que estaban habituados. Sin embargo, que los lobos discriminen entre aullidos emitidos por distintos lobos no implica necesariamente que exista realmente reconocimiento individual. Experimentos adicionales permitirán conocer el papel de la comunicación acústica en el reconocimiento individual en los lobos.

PARTE II. APLICACIONES DE LA BIOACÚSTICA PARA EL SEGUIMIENTO DE LAS POBLACIONES DE LOBOS

El seguimiento de las poblaciones desempeña un papel crítico en la conservación de la fauna silvestre (Sinclair et al. 2009). Detectar cambios en poblaciones locales es clave para entender la dinámica de las poblaciones, evaluar las medidas de gestión en especies en peligro o sometidas a algún tipo de explotación, y documentar el cumplimiento de las leyes. Conocer el tamaño o la densidad de una población es con frecuencia un prerrequisito vital para gestionarla eficazmente (Sinclair et al. 2009). Esto es especialmente evidente para especies como el lobo, para el cual las decisiones de gestión necesitan conciliar la conservación con el desarrollo de las comunidades rurales.

Los lobos aúllan en respuesta a otros aullidos e incluso a imitaciones de aullidos hechas por personas, lo cual constituye la base de un método útil para detectar animales por sus vocalizaciones: la técnica de estimular la emisión de vocalizaciones, que en el caso de los lobos se suele llamar técnica de aullidos simulados (Harrington & Mech 1982). En las últimas décadas esta técnica ha sido ampliamente utilizada como un método estandarizado para el seguimiento de poblaciones de lobos, dado que la observación directa de lobos salvajes y la localización de camadas, especialmente en áreas forestales, es difícil y poco práctica para realizar seguimientos en extensas áreas. Además de la detección de la presencia de manadas de lobos, la técnica de estimulación de vocalizaciones ha sido utilizada para estimar el número de lobos aullando y determinar la presencia de cachorros en una manada (Gazzola et al. 2002; Apollonio et al. 2004; Llaneza et al. 2005b; Nowak et al. 2008; Mitchell et al. 2008). Estas estimaciones se realizan generalmente mediante dos métodos: 1) censo acústico escuchando los coros en el campo, o 2) mediante análisis de espectrogramas. Sin embargo, los estudios disponibles no aportan descripciones detalladas de las metodologías utilizadas

(Gazzola et al. 2002; Llaneza et al. 2005b; Nowak et al. 2007) y no se ha intentado evaluar la fiabilidad de estos métodos.

Los objetivos de esta segunda parte de la tesis han sido: 1) evaluar la precisión de las estimas del número de lobos aullando en un coro y la presencia de cachorros que se realizan de oído, mediante censo acústico, 2) determinar si los parámetros acústicos de los coros de aullidos permiten obtener información relativa al tamaño de grupo y evaluar la potencialidad de los análisis de espectrogramas para estimar el número de lobos participando en un coro de aullidos, 3) determinar si los parámetros acústicos de los coros permiten obtener información sobre la presencia de cachorros y evaluar la potencialidad de la detección de cachorros mediante análisis de espectrogramas, 4) comparar los resultados obtenidos mediante censo acústico con los resultados obtenidos de los análisis de espectrogramas respecto a las estimaciones del número de individuos aullando y la presencia de cachorros.

Estimaciones del número de lobos que participan en un coro y de la presencia de cachorros mediante censo acústico

Hemos realizado tests a 182 agentes forestales (responsables del seguimiento de las poblaciones de lobos) y a una muestra de personas sin experiencia con lobos (N = 23) para evaluar la precisión de las estimaciones del número de lobos aullando y la presencia de cachorros hechas mediante censo acústico. Cada uno de los participantes en el estudio tenía que escuchar grabaciones de cinco coros de aullidos seleccionados aleatoriamente de una muestra de 22 y estimar si había adultos aullando, cachorros, y el número de individuos (adultos y cachorros). Para cada coro se asignó una puntuación entre 0 y 5 puntos (0: ningún acierto; 5: todas las respuestas correctas).

No se encontraron diferencias entre la puntuación global obtenida por los agentes forestales y la gente sin experiencia previa con lobos. Del mismo modo, no hubo diferencias según los años de experiencia del agente forestal.

Construimos modelos lineales generalizados mixtos (GLMMs) para explorar qué factores afectan a la determinación correcta de la presencia de individuos de diferente clase de edad (adultos o cachorros). Encontramos que el acierto en una estimación sobre la presencia de adultos o cachorros no depende de la experiencia del oyente ni de la pertenencia al grupo de los agentes o al de los inexpertos. El modelo más parsimonioso incluyó sólo la interacción entre clase de edad y presencia, explicando un 73% de la varianza, 21% debida a los factores aleatorios “coro” y “oyente”. Los agentes forestales estimaron la presencia de adultos correctamente el 85% de las veces, mientras que la proporción de estimaciones correctas descendió al 64% para la estimación de la presencia de cachorros. Esta diferencia se debe a una sobreestimación de la presencia de cachorros. Los oyentes identificaron adultos aullando el 88% de las veces en las que había efectivamente adultos, y cachorros el 98% de las veces en las que había cachorros. Sin embargo, mientras que el 49% de las veces en las que no había adultos estimaron que sí los había, este porcentaje ascendió al 72 % cuando se trata de estimaciones de cachorros en coros en los que no los hay. Los oyentes tienden a estimar que hay cachorros en un coro a pesar de ser coros en los que sólo participen individuos adultos.

Por otro lado, los oyentes estimaron correctamente el número de lobos participando en el coro el 31% de las veces, el 38% este número fue subestimado y el 30% sobreestimado. Construimos un GLMM para explorar qué factores afectan a la correcta estimación del número de lobos aullando. La experiencia y la pertenencia al grupo de agentes o inexpertos no afecta a la precisión en las estimaciones del número de lobos. Sólo el número real de lobos participando afecta a las estimaciones: conforme el número real de lobos aullando aumenta, la proporción de estimaciones incorrectas aumenta. El modelo más parsimonioso incluyó el número real de lobos aullando y explicó el 64% de la varianza, 13% debido a los efectos aleatorios del “coro” y del “oyente”. Un patrón similar se observó al considerar si la estimación era sub o sobreestimación, estimaciones para coros emitidos por un bajo número de lobos tienden a ser sobreestimadas, mientras que, para coros emitidos por muchos lobos, las estimaciones suelen ser menores que el número real de lobos participantes.

Análisis bioacústico de coros de aullidos de lobos (I): Estimaciones del tamaño de grupo

Analizamos 56 coros de aullidos en los que sabíamos exactamente cuántos individuos participaban (entre 2 y 7) con el objetivo de investigar si es posible obtener información sobre el número de individuos aullando mediante el análisis de grabaciones.

Encontramos tres variables correlacionadas con el número real de lobos participando en un coro: 1) el número máximo de lobos vocalizando simultáneamente (Spearman´s Rho: 0.98; n = 56; p < 0.001), 2) el valor medio de lobos vocalizando simultáneamente (Spearman´s Rho: 0.78; n = 56; p < 0.001) y 3) la duración (Spearman´s Rho: 0.38; n = 56; p = 0.0035). A medida que el número de lobos aullando aumenta, el número de lobos que vocaliza simultáneamente y la duración también aumenta.

Se realizaron modelos lineales generalizados (GLM) para predecir el número de lobos participando en un coro. Aplicando el mejor modelo resultante, la predicción coincidió con el número real de lobos participando en el coro en el 79% de los coros, el 12% fueron sobreestimaciones y el 9% subestimaciones. La diferencia entre el número predicho y el número real fue de un individuo en el 16% de los casos (7.1% subestimados y 8.9% sobreestimados); dos individuos el

3.6% de los casos (1.8% subestimado y 1.8 sobreestimado); y sólo en el 1.8% de los casos era de tres individuos (sobreestimado). La aplicación del modelo ofrece unos resultados mucho más precisos que la estimación realizada de oído, por censo acústico (79% de aciertos vs. 31% cuando las estimaciones se realizan de oído). Los resultados de los modelos también son más precisos en cuanto a la diferencia entre las estimaciones y el número real.

Análisis bioacústico de coros de aullidos de lobos (II): presencia de cachorros

En el capítulo 8 estudiamos la potencialidad de la distribución de la energía acústica para predecir la presencia de cachorros en un coro. Analizamos 110 coros de aullidos, 77 emitidos sólo por adultos/subadultos y 33 en los que había cachorros.

Los coros de aullidos analizados son vocalizaciones largas con una duración media de 66 ± 27 s (media ± DE), con la energía acústica concentrada entre los 300 y los 1900 Hz. Los aullidos constituyeron la principal vocalización (más del 50% de la duración del coro) en el 33% de los casos, mientras que el restante 67% de los coros incluía principalmente sonidos altamente modulados, variaciones de aullidos, ladridos, etc. El número de coros compuestos principalmente por aullidos fue significativamente mayor en coros emitidos sólo por adultos que en coros que incluían cachorros ($\chi^2 = 7.609$; d.f.=1; P=0.006).

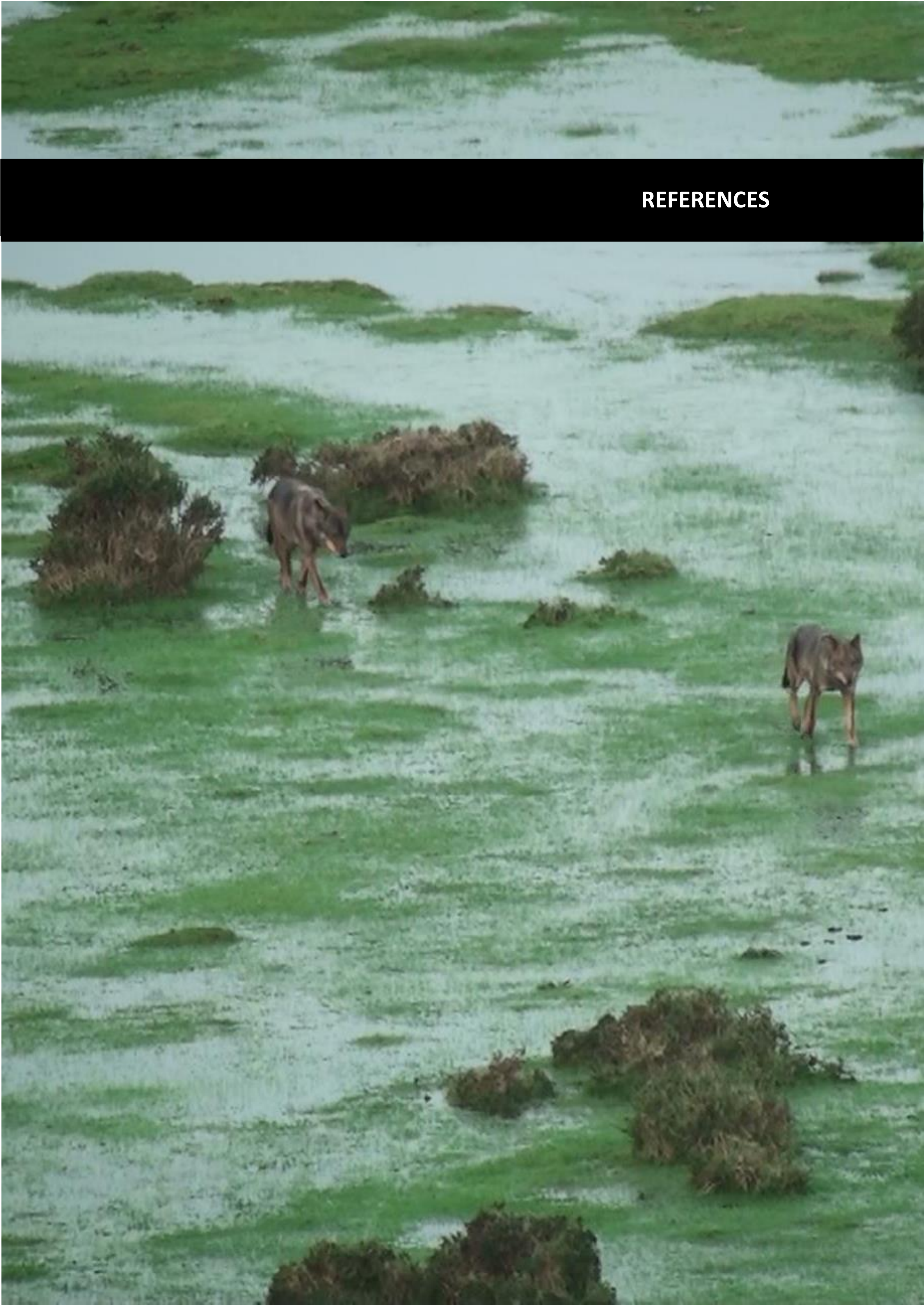
Los coros de aullidos con cachorros concentran la energía acústica a frecuencias más altas y mostraron valores de entropía mayores que los coros emitidos sólo por individuos adultos/subadultos. Los valores de la mayoría de variables medidas (85%) fueron diferentes entre coros con y sin cachorros.

Se realizaron modelos (GLMMs) para estudiar si es posible predecir la presencia de cachorros en un coro analizando los coros según cuatro aproximaciones diferentes: calculando los valores de las variables para todo el coro (CORO), dividiendo el coro en segmentos de 5 s de duración y calculando los valores medio, mínimo, máximo y desviación estándar de cada una de las variables medidas para todos los segmentos de un coro (SEGMENTOS), aplicando un filtro de intensidad del 75% del máximo de la amplitud (sólo se analiza la señal con una intensidad superior al 75% de la intensidad máxima), y aplicando un filtro de intensidad del 90% del máximo de intensidad. De este modo, se obtuvieron cuatro modelos diferentes: CORO75, CORO90, SEGMENTOS75, y SEGMENTOS90.

Los modelos finalmente seleccionados clasificaron correctamente el 86-94% de los casos, siendo menores los porcentajes para los modelos considerando el coro entero (74-83%). El mejor modelo fue el obtenido dividiendo el coro en segmentos de 5 s y aplicando el 90% del umbral de intensidad (SEGMENTOS90). Este modelo explica el 94% de la varianza. Aplicando este modelo, el 93.6% de coros fue clasificado correctamente como con o sin cachorros. Los resultados de clasificación para verdaderos positivos (el modelo predice que hay cachorros y efectivamente los hay) fueron mejores para los análisis dividiendo el coro en segmentos (i.e. cuando el modelo predice que hay cachorros, el 87.9% de veces efectivamente hay cachorros aplicando el modelo SEGMENTOS90, descendiendo este porcentaje a 27.3% para el análisis para todo el coro). Todos los modelos tienen bajos porcentajes de falsos negativos (en el 3.9-6,5% de los casos en los que no hay cachorros vocalizando el modelo predice erróneamente que hay cachorros).

Los resultados de los modelos son mejores que los obtenidos en el capítulo 6 mediante estimación por censo acústico (93.6% de casos clasificados correctamente vs. 64% de oído). Más importante es el hecho de que los modelos disminuyen enormemente la probabilidad de cometer falsos negativos (estimar que hay cachorros cuando en realidad no los hay, error con peores consecuencias para la gestión de la especie). Mientras que el 72% de los casos en los que no hay cachorros los oyentes estiman que sí los hay, este tipo de error supone sólo un 3.9% de los casos aplicando el modelo SEGMENTOS90.

REFERENCES



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Acevedo, M. A. & Villanueva-rivera, L. J. 2006. Using Automated Digital Recording Systems as Effective Tools for the Monitoring of Birds and Amphibians Using Automated Digital Recording Systems as Effective Tools for the Monitoring of Birds and Amphibians. *Wildlife Society Bulletin*, **34**, 211–214.

Alcock, J. 1989. *Animal behaviour: an evolutionary approach.* Sutherland, Massachusetts: Sinauer Associates Publishers.

Álvares, F., Pereira, E. & Petrucci-Fonseca, F. 2000. O lobo no Parque Internacional Gerés-Xurés. Situaçao populacional, aspectos ecológicos e perspectivas de conservaçao. *Galemys*, **12**, 223–239.

Álvares, F., Barroso, I., Blanco, J. C., Correia, J., Cortés, Y., Costa, G., Llaneza, L., Moreira, L., Nascimento, J., Palacios, V., Petrucci-Fonseca, F., Pimenta, V., Roque, S. & Santos, E. 2005. Wolf status and conservation in the Iberian Peninsula. In: *Frontiers of wolf recovery: Southwestern U.S. and the world.*, Colorado.

Amorim, M. C. P., Simões, J. M., Fonseca, P. J. J. & Simoes, J. M. 2008. Acoustic communication in the Lusitanian toadfish, *Halobatrachus didactylus*: evidence for an unusual large vocal repertoire. *Journal of the Marine Biological Association of the UK*, **88**, 1069–1073.

Apollonio, M., Mattioli, L., Scandura, M., Mauri, L., Gazzola, A. & Avanzinelli, E. 2004. Wolves in the Casentinesi Forests: insights for wolf conservation in Italy from a protected area with a rich wild prey community. *Biological Conservation*, **120**, 249–260.

Arch, V. S. & Narins, P. M. 2008. “Silent” signals: selective forces acting on ultrasonic communication systems in terrestrial vertebrates. *Animal Behaviour*, **76**, 1423–1428.

Aydin, D. 2008. A Comparison of the Sum of Squares in Linear and Partial Linear Regression Models Sum of Squares. *World Academy of Science, Engineering and Technology*, **2**, 391–397.

Baker, M. C. 2004. The chorus song of cooperatively breeding laughing kookaburras (Coraciiformes, Halcyonidae: *Dacelo novaeguineae*): Characterization and comparison among groups. *Ethology*, **110**, 21–35.

Baker, M. C. 2009. Information Content in Chorus Songs of the Group-Living Australian Magpie (*Cracticus tibicen dorsalis*) in Western Australia. *Ethology*, **115**, 227–238.

Balcombe, J. P. 1990. Vocal recognition of pups by mother Mexican free-tailed bats, *Tadarida brasiliensis mexicana*. *Animal Behaviour*, **39**, 960–966.

Balcombe, J. P. & McCracken, G. F. 1992. Vocal recognition in Mexican free-tailed bats: do pups recognize mothers? *Animal Behaviour*, **43**, 79–87.

Bardeleben, C., Moore, R. L. & Wayne, R. K. 2005. A molecular phylogeny of the Canidae based on six nuclear loci. *Molecular Phylogenetics and Evolution*, **37**, 815–831.

Barja, I. 2009. Decision making in plant selection during the faecal-marking behaviour of wild wolves. *Animal Behaviour*, **77**, 489–493.

Barja, I., de Miguel, F. J. & Barcena, F. 2005. Faecal marking behaviour of Iberian wolf in different zones of their territory. *Folia Zoologica*, **54**, 21–29.

Barnard, C. 2004. *Animal behaviour: mechanism, development, function, and evolution.* Essex: Pearson Education.

Bates, D. & Sarkar, D. 2007. Lme4: Linear mixed-effects models using S4 classes.

Bee, M. a. & Gerhardt, H. C. C. 2001. Neighbour-stranger discrimination by territorial male bullfrogs (*Rana catesbeiana*): II. Perceptual basis. *Animal Behaviour*, **62**, 1129–1140.

Beeman, K. 1998. Digital signal analysis, editing, and synthesis. In: *Animal acoustic communication: sound analysis and research methods*, (Ed. by S. L. Hopp, M. J. Owren, & C. S. Evans), pp. 59–103. Berlin: Springer-Verlag.

Bekoff, M. 1979a. Ground scratching by male domestic dogs: a composite signal. *Journal of Mammalogy*, **60**, 847–848.

Bekoff, M. 1979b. Scent-marking by free-ranging domestic dogs: olfactory and visual components. *Biology of Behaviour*, **4**, 123–139.

Bermejo, M. & Omedes, A. 1999. Preliminary vocal repertoire and vocal communication of wild bonobos (*Pan paniscus*) at Lilungu (Democratic Republic of Congo). *Folia Primatologica*, **70**, 328–357.

Bibby, C. J., Burgess, N. D. & Hill, D. A. 1992. *Bird Census Techniques.* London: Harcourt Brace & Company.

Binz, H. & Zimmermann, E. 1989. The Vocal Repertoire of Adult Tree Shrews (*Tupaia Belangeri*). *Behaviour*, **109**, 142–162.

Blanco, J. C. 2004. Lobo - *Canis lupus*. In: *Enciclopedia virtual de los vertebrados españoles*, (Ed. by L. M. Carrascal & A. Salvador), Museo Nacional de Ciencias Naturales.

Blanco, J. C. & Cortes, Y. 1999. *Estudio para la gestión del lobo en hábitats fragmentados por autovías.* Valladolid: Junta de Castilla y León.

Blumstein, D. T. & Daniel, J. C. 2004. Yellow-bellied marmots discriminate between the alarm calls of individuals and are more responsive to calls from juveniles. *Animal Behaviour*, **68**, 1257–1265.

Blumstein, D. T. & Turner, A. C. 2005. Can the acoustic adaptation hypothesis predict the structure of Australian birdsong? *Acta Ethologica*, **15**, 35–44.

Boisseau, O. 2005. Quantifying the acoustic repertoire of a population: The vocalizations of free-ranging bottlenose dolphins in Fiordland, New Zealand. *The Journal of the Acoustical Society of America*, **117**, 2318.

Boitani, L. 2000. *Action Plan for the conservation of the wolves (*Canis lupus*) in Europe.* Estrasburgo.

Bolt, L. M. 2013. The Function of Howling in the Ring-Tailed Lemur (*Lemur catta*). *International Journal of Primatology*, **34**, 157–169.

Botero, C. A., Mudge, A. E., Koltz, A. M., Hochachka, W. M. & Vehrencamp, S. L. 2008. How Reliable are the Methods for Estimating Repertoire Size? *Ethology*, **114**, 1227–1238.

Bouchet, H., Pellier, A.-S. S., Blois-Heulin, C. & Lemasson, A. 2010. Sex Differences in the Vocal Repertoire of Adult Red-Capped Mangabeys (*Cercocebus torquatus*): A Multi-Level Acoustic Analysis. *American Journal of Primatology*, **72**, 360–375.

Boughman, J. W. & Moss, C. F. 2003. Social sounds: vocal learning and development of Mammal and Bird calls. In: *Acoustic communication*, (Ed. by A. M. Simmons, A. N. Popper, & R. Fay, Richard), pp. 404. New York: Springer-Verlag.

Bradbury, J. W. & Vehrencamp, S. L. 2011. *Principles of animal communication.* 2nd edn. Sutherland, Massachusetts: Sinnauer Associates Publishers.

Brady, C. A. 1981. The vocal repertoires of the bush dog (*Speothos venaticus*), crab-eating fox (*Cerdocyon thous*), and maned wolf (*Chrysocyon brachyurus*). *Animal Behaviour*, **29**, 649–669.

Bragina, E. V. & Beme, I. R. 2007. The sexual and individual differences in the vocal repertoire of adult siberian cranes (*Grus Leucogeranus*, Gruidae). *Zoologichesky Zhurnal*, **86**, 1468–1481.

Burghardt, G. M. 1970. Defining “communication.” In: *Advances in chemoreception. Vol. I. Communication by chemical signals*, (Ed. by J. W. Johnston, D. G. Moulton, & A. Turk), New York: Appleton-Century-Crofts.

Burghardt, G. M. 2005. *The Genesis of Animal Play. Testing the limits.* Cambridge: The MIT Press.

Burnham, K. P. & Anderson, D. R. 2002. *Model selection and multimodel inference: a practical information theoretic approach.* New York: Springer-Verlag.

Caldwell, M. C. & Caldwell, D. K. 1965. Individualized whistle contours in bottlenose dolphins (*Tursiops truncatus*). *Nature*, **207**, 434–435.

Capranica, R. R. 1968. Vocal Repertoire of Bullfrog (*Rana Catesbeiana*). *Behaviour*, **31**, 302–&.

Carazo, P. & Font, E. 2010. Putting information back into biological communication. *Journal of Evolutionary Biology*, **23**, 661–669.

Catchpole, C. K. & Slater, P. J. B. 2008. *Bird song. Biological themes and variations.* 2nd edn. Cambridge: Cambridge University Press.

Charif, R. A., Waack, A. M. & Strickman, L. M. 2010. *Raven Pro 1.4 User’s Manual.* Ithaca, NY: Cornell Lab of Ornithology.

Charlton, B. D., Huang, Y. & Swaisgood, R. R. 2009. Vocal discrimination of potential mates by female giant pandas (*Ailuropoda melanoleuca*). *Biology Letters*, **5**, 597–599.

Charrier, I., Mathevon, N. & Jouventin, P. 2002. How does a fur seal mother recognize the voice of her pup? An experimental study of *Arctocephalus tropicalis*. *Journal of Experimental Biology*, **205**, 613–622.

Charrier, I., Mathevon, N. & Jouventin, P. 2003. Vocal signature recognition of mothers by fur seal pups. *Animal Behaviour*, **65**, 543–550.

Cohen, J. A. A. & Fox, M. W. W. 1976. Vocalizations in wild canids and possible effects of domestication. *Behavioural processes*, **1**, 77–92.

Conner, D. A. 1982. Geographic variation in short calls of pikas (*Ochotona princeps*). *Journal of Mammalogy*, **63**, 48–52.

Coscia, E. M. 1995. Ontogeny of timber wolf vocalizations: acoustic properties and behavioural contexts. Halifax, Nova Scotia: Dalhouise University.

Coscia, E. M., Phillips, D. P. & Fentress, J. C. 1991. Spectral analysis of neonatal wolf *Canis lupus* vocalizations. *Bioacoustics-The International Journal of Animal Sound and its Recording*, **3**, 275–293.

Crawley, M. J. 2007. *The R book.* Chichester: John Wiley & Sons.

Crockford, C., Herbinger, I., Vigilant, L. & Boesch, C. 2004. Wild chimpanzees produce group-specific calls: a case for vocal learning? *Ethology*, **110**, 221–243.

Da Cunha, R. G. T. & Byrne, R. W. 2006. Roars of black howler monkeys (*Alouatta caraya*): evidence for a function in inter-group spacing. *Behaviour*, **143**, 1169–1199.

Da Silva, F. R., Gibbs, J. P. & Rossa-Feres, D. D. C. 2011. Breeding habitat and landscape correlates of frog diversity and abundance in a tropical agricultural landscape. *Wetlands*, **31**, 1079–1087.

Dabelsteen, T. & Darden, S. K. 2006. Ontogeny of swift fox *Vulpes velox* vocalizations: production, usage and response. *Behaviour*, **143**, 659–681.

Déaux, É. C. & Clarke, J. A. 2013. Dingo (*Canis lupus dingo*) acoustic repertoire: form and contexts. *Behaviour*, **150**, 75–101.

Dekker, D. 1985. Response of wolves, *Canis lupus*, to simulated howling on a homesite during fall and winter in Jasper National Park, Alberta. *The Canadian field-naturalist*, **99**, 90–93.

Delport, W., Kemp, A. C. & Ferguson, J. W. H. 2002. Vocal identification of individual African Wood Owls *Strix woodfordii*: a technique to monitor long-term adult turnover and residency. *Ibis*, **144**, 30–39.

Demma, D. J. & Mech, L. D. 2009. Wolf Use of Summer Territory in Northeastern Minnesota. *Journal of Wildlife Management*, **73**, 380–384.

Demma, D. J., Barber-Meyer, S. M. & Mech, L. D. 2007. Testing global positioning system telemetry to study wolf predation on deer fawns. *Journal of Wildlife Management*, **71**, 2767–2775.

Depraetere, M., Pavoine, S., Jiguet, F., Gasc, A., Duvail, S. & Sueur, J. 2012. Monitoring animal diversity using acoustic indices: Implementation in a temperate woodland. *Ecological Indicators*, **13**, 46–54.

Durbin, L. S. 1998. Individuality in the whistle call of the Asiatic wild dog *Cuon alpinus*. *Bioacoustics-the International Journal of Animal Sound and Its Recording*, **9**, 197–206.

Dusenbery, D. B. 1992. *Sensory ecology: how organisms acquire and respond to information.* New York: W.H. Freeman & Company.

Eggermann, J., da Costa, G. F., Guerra, A. M., Kirchner, W. H. & Petrucci-Fonseca, F. 2011. Presence of Iberian wolf (*Canis lupus signatus*) in relation to land cover, livestock and human influence in Portugal. *Mammalian Biology*, **76**, 217–221.

Falls, J. B. 1982. Individual recognition by sounds in birds. In: *Acoustic communication in birds. Vol. 2*, (Ed. by D. H. Kroodsma & E. H. Miller), pp. 237–278. New York: Academic Press.

Feddersen-Petersen, D. U. 2000. Vocalization of European wolves (*Canis lupus lupus* L.) and various dog breeds (*Canis lupus f. fam*.). *Archiv fur Tierzucht-Archives of Animal Breeding*, **43**, 387–397.

Feng, A. S., Riede, T., Arch, V. S., Yu, Z., Xu, Z.-M., Yu, X.-J. & Shen, J.-X. 2009. Diversity of the Vocal Signals of Concave-Eared Torrent Frogs (*Odorrana tormota*): Evidence for Individual Signatures. *Ethology*, **115**, 1015–1028.

Ficken, M. S., Ficken, R. W. & Witkin, S. R. 1978. Vocal Repertoire of Black-Capped Chickadee. *Auk*, **95**, 34–48.

Filibeck, U., Nicoli, M., Rossi, P. & Boscagli, G. 1982. Detection by frequency analyzer of individual wolves howling in a chorus: a preliminary report. *Bollettino di Zoologia*, **49**, 151–154.

Fischer, J., Hammerschmidt, K. & Todt, D. 1998. Local variation in Barbary macaque shrill barks. *Animal Behaviour*, **56**, 623–629.

Fischer, J., Hammerschmidt, K., Cheney, D. L. & Seyfarth, R. M. 2002. Acoustic features of male baboon loud calls: influences of context, age, and individuality. *Journal of the Acoustical Society of America*, **111**, 1465–1474.

Fischer, J., Kitchen, D. M., Seyfarth, R. M. & Cheney, D. L. 2004. Baboon loud calls advertise male quality: acoustic features and their relation to rank, age, and exhaustion. *Behavioral Ecology and Sociobiology*, **56**, 140–148.

Fitch, W. T. 1997. Vocal tract length and formant frequency dispersion correlate with body size in rhesus macaques. *Journal of the Acoustical Society of America*, **102**, 1213–1222.

Fitch, W. T. & Kelley, P. 2000. Perception of vocal tract resonances by whooping cranes *Grus americana*. *Ethology*, **106**, 559–574.

Font, E. & Carazo, P. 2010. Animals in translation: why there is meaning (but probably no message) in animal communication. *Animal Behaviour*, **80**, E1–E6.

Font, E. & Kramer, M. 1989. A multivariate clustering approach to display repertoire analysis: headbobbing in *Anolis equestris*. *Amphibia-Reptilia*, **10**, 331–344.

Foote, J. R., Fitzsimmons, L. P., Mennill, D. J. & Ratcliffe, L. M. 2011. Male black-capped chickadees begin dawn chorusing earlier in response to simulated territorial insertions. *ANIMAL BEHAVIOUR*, **81**, 871–877.

Foster, S. A. & Endler, J. A. 1999. *Geographic variation in behavior. Perspectives on evolutionary mechanisms.* Oxford: Oxford University Press.

Fox, M. W. 1971. *Behaviour of wolves, dogs and related canids.* Malabar, Florida, Florida: Robert E. Krieger Publishing Company, Inc.

Friedman, S. 1972. Habituation and recovery of visual response in the alert human newborn. *Journal of Experimental Child Psychology*, **13**, 339–349.

Frommolt, K.-H. H., Goltsman, M. E. & Macdonald, D. W. 2003. Barking foxes, *Alopex lagopus*: field experiments in individual recognition in a territorial mammal. *Animal Behaviour*, **65**, 509–518.

Fuller, T. K., Mech, L. D. & Cochrane, J. F. 2003. Wolf population dynamics. In: *Wolves. Behavior, ecology, and conservation*, (Ed. by L. D. Mech & L. Boitani), Chicago: University of Chicago Press.

Gaines, W. L., Neale, G. K. & Naney, R. H. 1995. Response of coyotes and gray wolves to simulated howling in North-central Washington. *Northwest Science*, **69**, 217–222.

Gamba, M. & Giacoma, C. 2007. Quantitative acoustic analysis of the vocal repertoire of the crowned lemur. *Ethology Ecology & Evolution*, **19**, 323–343.

Gammon, D. E. & Baker, M. C. 2004. Song repertoire evolution and acoustic divergence in a population of black-capped chickadees, *Poecile atricapillus*. *Animal Behaviour*, **68**, 903–913.

Gazzola, A., Avanzinelli, E., Mauri, L., Scandura, M. & Apollonio, M. 2002. Temporal changes of howling in south European wolf packs. *Italian Journal of Zoology*, **69**, 157–161.

Goldman, J. a, Phillips, D. P. & Fentress, J. C. 1995. An acoustic basis for maternal recognition in timber wolves (*Canis lupus*)? *The Journal of the Acoustical Society of America*, **97**, 1970–3.

Goodmann, P. A., Klinghammer, E. & Willard, J. 2002. *Wolf ethogram (Revised 2002).* Battle Ground, IN: Eckhard H. Hess Institute of Ethology.

Grafe, T. U., Steffen, J. O. & Stoll, C. 2000. Vocal repertoire and effect of advertisement call intensity on calling behaviour in the West African tree frog, *Leptopelis viridis*. *Amphibia-Reptilia*, **21**, 13–23.

Gregory, R. D., Gibbons, D. W. & Donald, P. F. 2004. Bird census and survey techniques. In: *Bird Ecology and Conservation; a Handbook of Techniques*, (Ed. by W. J. Sutherland, I. Newton, & R. E. Green), pp. 17–56. Oxford: Oxford University Press.

Gyger, M., Marler, P. & Pickert, R. 1987. Semantics of an avian alarm call system: the male domestic fowl, *Gallus Domesticus*. *Behaviour*, **102**, 15–39.

Hale, A. M. 2006a. Group living in the black-breasted wood-quail and the use of playbacks as a survey technique. **108**, 107–119.

Hale, A. M. 2006b. The structure, context and functions of group singing in black-breasted wood-quail (*Odontophorus leucolaemus*). *Behaviour*, **143**, 511–533.

Hallberg, K. I. 2007. Information in a long-distance vocal signal: chorus howling in the coyote (*Canis latrans*). Ohio: The Ohio State University.

Harrington, F. H. 1986. Timber wolf howling playback studies: discrimination of pup from adult howls. *Animal Behaviour*, **34**, 1575–1577.

Harrington, F. H. 1987. Agressive howling in wolves. *Animal Behaviour*, **35**, 1575–1577.

Harrington, F. H. 1989. Chorus howling by wolves: acoustic structure, pack size and the beau geste effect. *Bioacoustics-The International Journal of Animal Sound and its Recording*, **2**, 117–136.

Harrington, F. H. & Asa, C. S. 2003. Wolf communication. In: *Wolves: behavior, ecology, and conservation*, (Ed. by L. D. Mech & L. Boitani), pp. 66–103. Chicago: University of Chicago Press.

Harrington, F. H. & Mech, L. D. 1978a. Wolf vocalization. In: *Wolf and man: evolution in parallel*, (Ed. by R. L. Hall & H. S. Sharp), pp. 109–132. New York, NY: Academic Press.

Harrington, F. H. & Mech, L. D. 1978b. Howling at two Minnesota wolf pack summer homesites. *Canadian Journal of Zoology*, **56**, 2024–2028.

Harrington, F. H. & Mech, L. D. 1979. Wolf howling and its role in territory maintenance. *Behaviour*, **68**, 207–249.

Harrington, F. H. & Mech, L. D. 1982. An analysis of howling response parameters useful for wolf pack censusing. *Journal of Wildlife Management*, **46**, 686–693.

Harrington, F. H. & Mech, L. D. 1983. Wolf pack spacing: howling as a territory-independent spacing mechanism in a territorial population. *Behavioral Ecology and Sociobiology*, **12**, 161–168.

Harvey, P. H. & Nee, S. 1999. The phylogenetic foundations of behavioural ecology. In: *Behavioural ecology. An evolutionary approach*, Fourth edn. (Ed. by J. R. Krebs & N. B. Davies), pp. 456. Oxford: Blackwell Publishing.

Hauser, M. D. 1996. *The Evolution of Communication.* Cambridge: Mit Press.

Heffner, R. S. 1997. Comparative study of sound localization and its anatomical correlates in mammals. *Acta Oto-Laryngologica*, **Suppl 532**, 46–53.

Holekamp, K. E., Boydston, E. E., Szykman, M., Graham, I., Nutt, K. J., Birch, S., Piskiel, A. & Singh, M. 1999. Vocal recognition in the spotted hyaena and its possible implications regarding the evolution of intelligence. *Animal behaviour*, **58**, 383–395.

Holt, T. D. 1998. A structural description and reclassification of the wolf, *Canis lupus*, chorus howl. Halifax, Nova Scotia: Dalhousie University.

Hoodless, A. N., Inglis, J. G., Doucet, J.-P. P. & Aebischer, N. J. 2008. Vocal individuality in the roding calls of Woodcock *Scolopax rusticola* and their use to validate a survey method. *Ibis*, **150**, 80–89.

Hopp, S. L. & Morton, E. S. 1998. Sound playback studies. In: *Animal Acoustic Communication: Sound Analysis and Research Methods*, (Ed. by S. L. Hopp, M. J. Owren, & C. S. Evans), pp. 323–352. New York, NY: Springer-Verlag Berlin Heidelberg.

Hopp S. L. & Owren, M. J. 1998. *Animal acoustic communication.* Berlin: Springer-Verlag Berlin Heidelberg.

Hsu, M. J., Chen, L. M. & Agoramoorthy, G. 2005. The vocal repertoire of Formosan macaques, *Macaca cyclopis*: Acoustic structure and behavioral context. *Zoological Studies*, **44**, 275–294.

Huberty, C. J. 1994. *Applied discriminant analysis.* New York: John Wiley & Sons.

Janik, V. M. & Slater, P. J. B. 1997. Vocal learning in mammals. *Advances in the Study of Behavior*, **26**, 59–99.

Johnson, R. 1972. *Aggression in man and animals.* Philadelphia: Saunders.

Joslin, P. W. B. 1967. Movements and home sites of timber wolves in Algonquin Park. *American Zoologist*, **7**, 279–288.

Kaczensky, P., Chapron, G., von Arx, M., Huber, D., Andrén, H. & Linnell, J. 2013. *Status , management and distribution of large carnivores – bear , lynx , wolf & wolverine – in Europe.*

Kaiser, K. 2008. Evaluation of a long-term amphibian monitoring protocol in Central America. *Journal of Herpetology*, **42**, 104–110.

Kappe, T. 1997. Subjective resource value and the intensity of threat vocalizations in European wolves (*Canis lupus lupus*). *Mammalian Biology*, **62**, 97–100.

Kazial, K. a., Kenny, T. L. & Burnett, S. C. 2008. Little brown bats (*Myotis lucifugus*) recognize individual identity of conspecifics using sonar calls. *Ethology*, **114**, 469–478.

Kierulff, M. C. M. & Rylands, A. B. 2003. Census and distribution of the golden lion tamarin (*Leontopithecus rosalia*). *American Journal of Primatology*, **59**, 29–44.

Klecka, W. R. 1980. *Discriminant analysis.* Newbury Park, California: Sage.

Knörnschild, M., Feifel, M. & Kalko, E. K. V. 2013. Mother–offspring recognition in the bat *Carollia perspicillata*. *Animal Behaviour*, **86**, 941–948.

Koenig, R. 2008. Rangers Assess Toll of Congo Conflict on Threatened Mountain Gorillas. *Science*, **322**, 1778.

Krebs, J. R. 1977. The significance of song repertoires: The Beau Geste hypothesis. *Animal Behaviour*, **25, Part 2**, 475–478.

Kroodsma, D. E., Byers, B. E., Goodale, E., Johnson, S. & Liu, W.-C. C. 2001. Pseudoreplication in playback experiments, revisited a decade later. *Animal Behaviour*, **61**, 1029–1033.

Kuijper, D. P. J., de Kleine, C., Churski, M., van Hooft, P., Bubnicki, J. & Jędrzejewska, B. 2013. Landscape of fear in Europe: wolves affect spatial patterns of ungulate browsing in Białowieża Primeval Forest, Poland. *Ecography*, **36**, 1263–1275.

LaDage, L. D. & Ferkin, M. H. 2006. Male leopard geckos (*Eublepharis macularius*) can discriminate between two familiar females. *Behaviour*, **143**, 1033–1049.

Le Roux, A., Cherry, M. I. & Manser, M. B. 2009. The vocal repertoire in a solitary foraging carnivore, *Cynictis penicillata*, may reflect facultative sociality. *Naturwissenschaften*, **96**, 575–584.

Lehner, P. N. 1978a. Coyote vocalizations: a lexicon and comparisons with other canids. *Animal Behaviour*, **26**, 712–722.

Lehner, P. N. 1978b. Coyote communication. In: *Coyotes. Biology, behavior, and management*, (Ed. by M. Bekoff), pp. 128–162. New Yotk: Academic Press.

Lehner, P. N. 1996. *Handbook of ethological methods.* Cambridge: Cambridge University Press.

Lindblad-Toh, K., Wade, C. M., Mikkelsen, T. S., Karlsson, et al. 2005. Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature*, **438**, 803–819.

Llaneza, L. & Blanco, J. C. 2001. *Diagnóstico de las poblaciones de lobo ibérico en Castilla y León.* Consejería de Medio Ambiente de la Junta de Castilla y León.

Llaneza, L., Palacios, V., Uzal, A., Ordiz, A., Sazatornil, V., Sierra, P. & Álvares, F. 2005a. Distribución y aspectos poblacionales del lobo ibérico (*Canis lupus signatus*) en las provincias de Pontevedra y A Coruña (Galicia). *Galemys*, **17**, 61–80.

Llaneza, L., Ordiz, a., Palacios, V. & Uzal, a. 2005b. Monitoring Wolf Populations Using Howling Points Combined With Sign Survey Transects. *Wildlife Biology in Practice*, **1**, 108–117.

Llaneza, L., García, E. J., Palacios, V., Sazatornil, V. & López-Bao, J. V. 2012a. Tamaño de manada de lobos en la Cordillera Cantábrica. In: *Resúmenes de las ponencias del III Congreso Ibérico do Lobo*, pp. 103. Lugo.

Llaneza, L., López-Bao, J. V. & Sazatornil, V. 2012b. Insights into wolf presence in human-dominated landscapes: the relative role of food availability, humans and landscape attributes. *Diversity and Distributions*, **18**, 459–469.

Llaneza, L., García, E. J. & López-Bao, J. V. 2014. Intensity of territorial marking predicts wolf reproduction: implications for wolf monitoring. *PloS one*, **9**, e93015.

Llusia, D., Márquez, R., Beltrán, J. F., Benítez, M. & do Amaral, J. P. 2013. Calling behaviour under climate change: Geographical and seasonal variation of calling temperatures in ectotherms. *Global Change Biology*, **19**, 2655–2674.

Lord, K., Feinstein, M. & Coppinger, R. 2009. Barking and mobbing. *Behavioural processes*, **81**, 358–68.

Macdonald, D. & Sillero-Zubiri, C. 2004. *Biology and Conservation of Wild Canids.* New York: Oxford University Press.

Manser, M. B. 2001. The acoustic structure of suricate's alarm calls varies with predator type and the level of response urgency. *Proceedings of the Royal Society of London.Series B: Biological Sciences*, **2001**, 2315–2324.

Marler, P. 1967. Animal communication signals. *Science*, **157**, 769–774.

Marshall, J. T. J. & Marshall, E. R. 1976. Gibbons and their territorial songs. *Science*, **193**, 237.

Martín, J., Barja, I., López, P., Martin, J. & Lopez, P. 2010. Chemical scent constituents in feces of wild Iberian wolves (*Canis lupus signatus*). *Biochemical Systematics and Ecology*, **38**, 1096–1102.

Mathevon, N., Koralek, A., Weldele, M., Glickman, S. E. & Theunissen, F. E. 2010. What the hyena's laugh tells: sex, age, dominance and individual signature in the giggling call of *Crocuta crocuta*. *BMC Ecology*, **10**, 9.

Maynard Smith, J. 1982. *Evolution and the theory of games.* Cambridge: Cambridge University Press.

McCarley, H. 1978. Vocalizations of red wolves (*Canis rufus*). *Journal of Mammalogy*, **59**, 27–35.

McComb, K., Pusey, A., Packer, C. & Grinnell, J. 1993. Female lions can identify potentially infanticidal males from their roars. *Proceedings of the Royal Society B-Biological Sciences*, **252**, 59–64.

McComb, K., Packer, C. & Pusey, A. 1994. Roaring and Numerical Assessment in Contests Between Groups of Female Lions, *Panthera Leo*. *Animal Behaviour*, **47**, 379–387.

McComb, K., Moss, C., Sayialel, S. & Baker, L. 2000. Unusually extensive networks of vocal recognition in African elephants. *Animal behaviour*, **59**, 1103–1109.

McComb, K., Moss, C., Durant, S. M., Baker, L. & Sayialel, S. 2001. Matriarchs act as repositories of social knowledge in African elephants. *Science*, **292**, 491–494.

McComb, K., Reby, D., Backer, L., Moss, C., Sayialel, S. & Baker, L. 2003. Long-distance communication of acoustic cues to social identity in African elephants. *Animal Behaviour*, **65**, 317–329.

Mccowan, B. & Hooper, S. L. 2002. Individual acoustic variation in Belding's ground squirrel alarm chirps in the High Sierra Nevada. *Journal of the Acoustical Society of America*, **111**, 1157–1160.

McGregor, P. K. 2000. Playback experiments: design and analysis. *Acta Ethologica*, **3**, 3–8.

Mech, L. D. 1970. *The wolf: the ecology and behavior of an endangered species.* Garden City, New York: The Natural History Press.

Mech, L. D. 1999. Alpha status, dominance, and division of labor in wolf packs. *Canadian Journal of Zoology-Revue Canadienne de Zoologie*, **77**, 1196–1203.

Mech, L. D. 2000. Wolf numbers and reproduction. In: *The wolves of Minnesota. Howl in the heartland*, (Ed. by L. D. Mech), pp. 73–82. Stillwater, MN: Voyageur Press, Inc.

Mech, L. D. & Boitani, L. 2003. Wolf social ecology. In: *Wolves: behavior, ecology, and conservation*, (Ed. by L. D. Mech & L. Boitani), Chicago: University of Chicago Press.

Mitchell, B. R. 2004. Coyote vocal communication and its application to the selective management of problem individuals. Berkeley: University of California.

Mitchell, M. S., Ausband, D. E., Sime, C. a., Bangs, E. E., Gude, J. a., Jimenez, M. D., Mack, C. M., Meier, T. J., Nadeau, M. S. & Smith, D. W. 2008. Estimation of successful breeding pairs for wolves in the Northern Rocky Mountains, USA. *Journal of Wildlife Management*, **72**, 881–891.

Molles, L. E. & Vehrencamp, S. L. 2001. Neighbour recognition by resident males in the banded wren, *Thryothorus pleurostictus*, a tropical songbird with high song type sharing. *Animal Behaviour*, **61**, 119–127.

Molnár, C., Pongrácz, P., Faragó, T., Dóka, A., Miklósi, A., Molnar, C., Pongracz, P., Farago, T., Doka, A. & Miklosi, A. 2009. Dogs discriminate between barks: the effect of context and identity of the caller. *Behavioural processes*, **82**, 198–201.

Morton, E. S. 1975. Ecological sources of selection on avian sounds. *American Naturalist*, **109**, 17–34.

Morton, E. S. 1977. On the occurrence and significance of motivation-structural rules in some bird and mammal sounds. *American Naturalist*, **111**, 855–869.

Mumm, C. a. S. & Knörnschild, M. 2014. The Vocal Repertoire of Adult and Neonate Giant Otters (*Pteronura brasiliensis*). *PLoS ONE*, **9**, e112562.

Mundinger, P. C. 1982. Microgeographic and macrogeographic variation in the acquired vocalizations of birds. In: *Acoustic communication in birds. Vol. 2*, (Ed. by D. E. Kroodsma & E. H. Miller), pp. 147–208. New York: Academic Press.

Mundry, R. & Sommer, C. 2007. Discriminant function analysis with nonindependent data: consequences and an alternative. *Animal Behaviour*, **74**, 965–976.

Naguib, M. & Wiley, R. H. 2001. Estimating the distance to a source of sound: mechanisms and adaptations for long-range communication. *Animal Behaviour*, **62**, 825–837.

Nagy, C. M. & Rockwell, R. F. 2012. Identification of individual Eastern Screech-Owls *Megascops asio* via vocalization analysis. 1–14.

Nakagawa, S. & Schielzeth, H. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, **4**, 133–142.

Newton-Fisher, N., Harris, S., White, P. & Jones, G. 1993. Structure and function of red fox *Vulpes vulpes* vocalisations. *Bioacoustics-the International Journal of Animal Sound and Its Recording*, **5**, 1–31.

Nicastro, N. & Owren, M. J. 2003. Classification of domestic cat (*Felis catus*) vocalizations by naive and experienced human listeners. *Journal of Comparative Psychology*, **117**, 44–52.

Nowacek, D. P., Casper, B. M., Wells, R. S., Nowacek, S. M., Mann, D. a. & Nowacek, M. 2003. Intraspecific and geographic variation of West Indian manatee (*Trichechus manatus* spp.) vocalizations. *Journal of the Acoustical Society of America*, **114**, 66–69.

Nowak, S., Jedrzejewski, W., Schmidt, K., Theuerkauf, J., Mysłajek, R. W., Jedrzejewska, B., Krzysztof, S. & Myslajek, R. W. 2007. Howling activity of free-ranging wolves (*Canis lupus*) in the Białowieza Primeval Forest and the Western Beskidy Mountains. *Journal of Ethology*, **25**, 231–237.

Nowak, S., Myslajek, R. W., Jedrzejewska, B., Mysłajek, R. W. & Jędrzejewska, B. 2008. Density and demography of wolf, *Canis lupus* population in the western-most part of the polish Carpathian Mountains, 1996-2003. *Folia Zoologica*, **57**, 392–402.

Nowicki, S. & Nelson, D. A. 1990. Defining Natural Categories in Acoustic Signals: Comparison of Three Methods Applied to "Chick-a-dee" Call Notes. *Ethology*, **86**, 89–101.

Ogutu, J. O. & Dublin, H. T. 1998. The response of lions and spotted hyaenas to sound playbacks as a technique for estimating population size. *African Journal of Ecology*, **36**, 83–95.

Oliveira, P. A. P., Simões, P. C. & Quartau, J. A. 2001. Calling songs of certain orthopteran species (Insecta , Orthoptera) in southern Portugal. **1**, 65–79.

Owren, M. J. & Rendall, D. 2003. Salience of caller identity in rhesus monkey (*Macaca mulatta*) coos and screams: perceptual experiments with human (*Homo sapiens*) listeners. *Journal of Comparative Psychology*, **117**, 380–390.

Packard, J. M. 2003. Wolf behavior: reproductive, social, and intelligent. In: *Wolves: behavior, ecology, and conservation*, (Ed. by L. D. Mech & L. Boitani), pp. 35–65. Chicago: University of Chicago Press.

Palacios, V. & Mech, L. D. 2011. Problems with studying wolf predation on small prey in summer via global positioning system collars. *European Journal of Wildlife Research*, **57**, 149–156.

Palacios, V., Font, E. & Márquez, R. 2007. Iberian wolf howls: acoustic structure, individual variation, and a comparison with North American populations. *Journal of Mammalogy*, **88**, 606–613.

Partan, S. & Marler, P. 1999. Communication goes multimodal. *Science*, **283**, 1272–1273.

Payne, K. B., Thompson, M. & Kramer, L. 2003. Elephant calling patterns as indicators of group size and composition: the basis for an acoustic monitoring system. *African Journal of Ecology*, **41**, 99–107.

Peake, T. M. & Mcgregor, P. K. 2001. Corncrake (*Crex crex*) census estimates: a conservation application of vocal individuality. *Animal Biodiversity and Conservation*, **24**, 81–90.

Perla, B. S. & Slobodchikoff, C. N. 2002. Habitat structure and alarm call dialects in Gunnison's prairie dog (*Cynomys gunnisoni*). *Behavioral Ecology*, **13**, 844–850.

Peters, R. P. & Mech, L. D. 1975. Scent-marking in wolves. *American Scientist*, **63**, 628–637.

Peters, G. & Tonkin-Leyhausen, B. A. 1999. Evolution of acoustic communication signals of Mammals: friendly close-range vocalizations in Felidae (Carnivora). *Journal of Mammalian Evolution*, **6**, 129–159.

Peters, G. & Wozencraft, W. C. 1989. Acoustic communication by Fissiped Carnivores. In: *Carnivore behavior, ecology, and evolution*, (Ed. by J. Gittleman), pp. 14–49. Ithaca, N Y: New York University Press.

Pinheiro, J. C. & Bates, D. M. 2000. *Mixed-effect models in S and S-Plus.* New York: Springer.

Price, J. J. & Lanyon, S. M. 2004. Patterns of song evolution and sexual selection in the oropendolas and caciques. *Behavioral Ecology*, **15**, 485–497.

R Development Core Team. 2010. R: A language and environment for statistical computing.

Radford, A. N. 2005. Group-specific vocal signatures and neighbour-stranger discrimination in the cooperatively breeding green woodhoopoe. *Animal Behaviour*, **70**, 1227–1234.

Raemaekers, J. J. & Raemaekers, P. M. 1985. Field playback of loud calls to gibbons (*Hylobates lar*): territorial, sex-specific and species-specific responses. *Animal Behaviour*, **33**, 481–493.

Ramsier, M. A., Cunningham, A. J., Moritz, G. L., Finneran, J. J., Williams, C. V., Ong, P. S., Gursky-Doyen, S. L. & Dominy, N. J. 2012. Primate communication in the pure ultrasound. *Biology Letters*, **8**, 508–511.

Range, F. & Fischer, J. 2004. Vocal repertoire of sooty mangabeys (*Cercocebus torquatus atys*) in the Tai National Park. *Ethology*, **110**, 301–321.

Reby, D., Hewison, M., Izquierdo, M. & Pepin, D. 2001. Red deer (*Cervus elaphus*) hinds discriminate between the roars of their current harem-holder stag and those of neighbouring stags. *Ethology*, **107**, 951–959.

Rendall, D., Rodman, P. S. & Emond, R. E. 1996. Vocal recognition of individuals and kin in free-ranging rhesus monkeys. *Animal Behaviour*, **51**, 1007–1015.

Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Riede, T. & Fitch, T. 1999. Vocal tract length and acoustics of vocalization in the domestic dog (*Canis familiaris*). *The Journal of experimental biology*, **202**, 2859–2867.

Robbins, R. L. 2000. Vocal communication in free-ranging African wild dogs (*Lycaon pictus*). *Behaviour*, **137**, 1271–1298.

Robbins, R. L. & McCreery, E. K. K. 2003. Acoustic stimulation as a tool in African wild dog conservation. *Biological Conservation*, **111**, 263–267.

Roque, S., Álvares, F. & Petrucci-Fonseca, F. 2001. Utilización espaciotemporal y hábitos alimenticios de un grupo reproductor de lobos en el noroeste de Portugal. *Galemys*, **13**, 179–198.

Rubin, P. & Vatikiotis-Bateson, E. 1998. Measuring and modeling speech production. In: *Animal acoustic communication*, (Ed. by S. L. Hopp, M. J. Owren, & C. S. Evans), pp. 251–290. Berlin: Springer-Verlag.

Schassburger, R. M. 1993. Vocal communication in the timber wolf, *Canis lupus*, Linnaeus: structure, motivation, and ontogeny. *Advances in Ethology Series*, **30**,

Schenkel, R. 1947. Ausdrucks-studien an wolfen. *Behaviour*, **1**, 81–129.

Schenkel, R. 1967. Submission : Its Features and Function in the Wolf and Dog. *American Zoologist*, **7**, 319–329.

Schneider, J. N. & Anderson, R. E. 2011. Tonal vocalizations in the red wolf (*Canis rufus*): Potential functions of nonlinear sound production. *Journal of the Acoustical Society of America*, **130**, 2275–2284.

Schrader, L. & Hammerschmidt, K. 1997. Computer-aided analysis of acoustic parameters in animal vocalisations: a multi-parametric approach. *Bioacoustics*, **7**, 247–265.

Searby, A. & Jouventin, P. 2003. Mother-lamb acoustic recognition in sheep: a frequency coding. *Proceedings of the Royal Society of London.Series B: Biological Sciences*, **270**, 1765–1771.

Searby, A., Jouventin, P. & Aubin, T. 2004. Acoustic recognition in macaroni penguins: an original signature system. *Animal Behaviour*, **67**, 615–625.

Searcy, W. A. 1989. Pseudoreplication, External Validity and the Design of Playback Experiments. *Animal Behaviour*, **38**, 715–717.

Seddon, N., Tobias, J. A. & Alvarez, A. 2002. Vocal communication in the pale-winged trumpeter (*Psophia leucoptera*): Repertoire, context and functional reference. *Behaviour*, **139**, 1331–1359.

Selvin, S. 1995. *Practical biostatistical methods.* Belmont, California: Duxbury Press.

Seyfarth, R. M. & Cheney, D. L. 2003. Signalers and receivers in animal communication. *Annual review of psychology*, **54**, 145–173.

Seyfarth, R. M., Cheney, D. L. & Marler, P. 1980. Vervet monkey alarm calls: Semantic communication in a free-ranging primate. *Animal Behaviour*, **28**, 1070–1094.

Shalter, M. D., Fentress, J. C. & Young, G. W. 1977. Determinants of response of wolf pups to auditory signals. *Behaviour*, **60**, 98–114.

Sharpe, L. L., Hill, A. & Cherry, M. I. 2013. Individual recognition in a wild cooperative mammal using contact calls. *Animal Behaviour*, **86**, 893–900.

Shettleworth, S. J. 2010. *Cognition, evolution, and behavior.* 2nd edn. New York: Oxford University Press.

Sikes, R. S., Gannon, W. L. & Mammalogists, T. A. C. and U. C. of the A. S. of. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy*, **92**, 235–253.

Sinclair, A. R. E., Fryxell, J. M. & Caughley, G. 2009. *Wildlife Ecology, Conservation and Management.* Second edn. Oxford: John Wiley & Sons.

Sinnott, J. M. & Brown, C. H. 1993. Effects of varying signal duration on pure-tone frequency discrimination in humans and monkeys. *The Journal of the Acoustical Society of America*, **93**, 1541–1546.

Slabbekoorn, H., Ten cate C & Ten Cate, C. 1998. Perceptual tuning to frequency characteristics of territorial signals in collared doves. *Animal Behaviour*, **56**, 847–857.

Slabbekoorn, H., De Kort, S., Kort, D. & Cate, C. Ten. 1999. Comparative analysis of perch-coo vocalizations in Streptopelia doves. *The Auk*, **116**, 737–748.

Slater, P. J. B. 1983. The study of communication. In: *Animal behaviour 2: communication*, (Ed. by T. R. Halliday & P. J. B. Slater), Oxford: Blackwell.

Sousa-Lima, R. S., Paglia, A. P. & Da Fonseca, G. A. B. 2002. Signature information and individual recognition in the isolation calls of Amazonian manatees, *Trichechus inunguis* (Mammalia: Sirenia). *Animal Behaviour*, **63**, 301–310.

Sun, L. X., Wilczynski, W., Rand, A. S. & Ryan, M. J. 2000. Trade-off in short- and long-distance communication in tungara (*Physalaemus pustulosus*) and cricket (*Acris crepitans*) frogs. *Behavioral Ecology*, **11**, 102–109.

Sung, H.-C., Kim, S.-K., Park, S.-R. & Park, D.-S. 2005. Effectiveness of mating call playbacks in anuran call monitoring: A case study of three-striped pond frogs (*Rana nigromaculata*). *Integrative Biosciences*, **9**, 199–203.

Sutherland, W. J. 2006. *Ecological census techniques. A handbook.* 2nd edn. Cambridge: Cambridge University Press.

Tembrock, G. 1976. Canid vocalizations. *Behavioural processes*, **1**, 57–75.

Theberge, J. B. & Falls, J. B. 1967. Howling as a means of communication in timber wolves. *American Zoologist*, **7**, 331–338.

Thom, M. D. & Hurst, J. L. 2004. Individual recognition by scent. *Annales Zoologici Fennici*, **41**, 765–787.

Thomas, J. A. & Golladay, C. L. 1995. Geographic variation in leopard seal (*Hydrurga leptonyx*) underwater vocalizations. In: *Sensory systems of aquatic mammals*, (Ed. by R. A. Kastelein, J. A. Thomas, & P. E. Nachtigall), pp. 201–221. Woerden, The Netherlands: De Spil Publishers.

Tibbetts, E. A. & Dale, J. 2007. Individual recognition: it is good to be different. *Trends in ecology & evolution*, **22**, 529–37.

Tibbetts, E. a., Sheehan, M. J. & Dale, J. 2008. A testable definition of individual recognition. *Trends in Ecology & Evolution*, **23**, 356.

Tooze, Z. J. 1987. Some aspects of the structure and function of long-distance vocalizations of timber wolves (*Canis lupus*). Halifax, Nova Scotia: Dalhouise University.

Tooze, Z. J., Harrington, F. H. & Fentress, J. C. 1990. Individually distinct vocalizations in timber wolves, *Canis lupus*. *Animal Behaviour*, **40**, 723–730.

Valeix, M., Loveridge, A. J., Chamaillé-Jammes, S., Davidson, Z., Murindagomo, F., Fritz, H., Macdonald, D. W. & Chamaille-Jammes, S. 2009. Behavioral adjustments of African herbivores to predation risk by lions: Spatiotemporal variations influence habitat use. *Ecology*, **90**, 23–30.

Vicario, D. S., Naqvi, N. H. & Raksin, J. N. 2001. Sex differences in discrimination of vocal communication signals in a songbird. *Animal Behaviour*, **61**, 805–817.

Vilá, C. 1993. Aspectos morfológicos y ecológicos del lobo ibérico (*Canis lupus L.*). Sevilla: Universidad de Sevilla.

Vilá, C., Urios, V. & Castroviejo, J. 1990. Ecología del lobo en la Cabrera (León) y la Carballeda (Zamora). In: *El lobo en España*, (Ed. by J. C. Blanco, L. Cuesta, & S. Reig), pp. 95–108. ICONA.

Vilá, C., Urios, V. & Castroviejo, J. 1994. Use of faeces for scent marking in Iberian wolves (*Canis lupus*). *Canadian Journal of Zoology*, **72**, 374–377.

Volodin, I. A. & Volodina, E. V. 2002. Biphonation as a prominent feature of dhole (*Cuon alpinus*) sounds. *Bioacoustics-the International Journal of Animal Sound and Its Recording*, **13**, 105–120.

Volodin, I. A., Volodina, E. V & Isaeva, I. V. 2001. Vocal repertoire in the dhole *Cuon alpinus* in captivity. *Entomological Review*, **81**, 346–361.

Wang, D., Wursing, B. & Evans, W. E. 1995. Whistels of bottlenose dolphins: comparisons among populations. *Aquatic Mammals*, **21**, 65–77.

Wayne, R. K. & Vilá, C. 2003. Molecular genetic studies of wolves. In: *Wolves: behavior, ecology, and conservation*, (Ed. by L. D. Mech & L. Boitani), pp. 218–238. Chicago, Illinois: University of Chicago Press.

Weilgart, L. & Whitehead, H. 1997. Group-specific dialects and geographical variation in coda repertoire in South Pacific sperm whales. *Behavioral Ecology and Sociobiology*, **40**, 277–285.

Weiss, D. J. & Hauser, M. D. 2002. Perception of harmonics in the combination long call of cottontop tamarins, *Saguinus oedipus*. *Animal Behaviour*, **64**, 415–426.

Wiley, R. H. & Richards, D. G. 1978. Physical constraints on acoustic communication in the atmosphere: implications for the evolution of animal vocalizations. *Behavioral Ecology and Sociobiology*, **3**, 69–94.

Wilson, D. R. & Mennill, D. J. 2010. Black-capped chickadees, *Poecile atricapillus*, can use individually distinctive songs to discriminate among conspecifics. *Animal Behaviour*, **79**, 1267–1275.

Wong, J., Stewart, P. D. & Macdonald, D. W. 1999. Vocal repertoire in the European badger (*Meles meles*): Structure, context, and function. *Journal of Mammalogy*, **80**, 570–588.

Wright, T. F., Rodriguez, A. M. & Fleischer, R. C. 2005. Vocal dialects, sex-biased dispersal, and microsatellite population structure in the parrot *Amazona auropalliata*. *Molecular Ecology*, **14**, 1197–1205.

Yin, S. 2002. A new perspective on barking in dogs (*Canis familiaris*). *Journal of Comparative Psychology*, **116**, 189–193.

Yin, S. & McCowan, B. 2004. Barking in domestic dogs: context specificity and individual identification. *Animal Behaviour*, **68**, 343–355.

Yuen, K. K. 1974. The two-sample trimmed t for unequal population variances. *Biometrika*,

Zaccaroni, M., Passilongo, D., Buccianti, A., Dessi-Fulgheri, F., Facchini, C., Gazzola, A., Maggini, I. & Apollonio, M. 2012. Group specific vocal signature in free-ranging wolf packs. *Ethology Ecology & Evolution*, **24**, 322–331.

Zimen, E. 1978. *The wolf: his place in the natural world.* London: Souvenir Press.

Zub, K., Theuerkauf, J., Jedrzejewski, W., Jedrzejewska, B., Schmidt, K. & Kowalczyk, R. 2003. Wolf pack territory marking in the Bialowieza primeval forest (Poland). *Behaviour*, **140**, 635–648.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. & Smith, G. M. 2009. *Mixed effects models and extensions in ecology with R.* New York, NY: Springer.